Hepatitis C Virus (HCV) and HIV/HCV Coinfection: A Critical Review of Research and Treatment

July 2004

By Tracy Swan & Daniel Raymond

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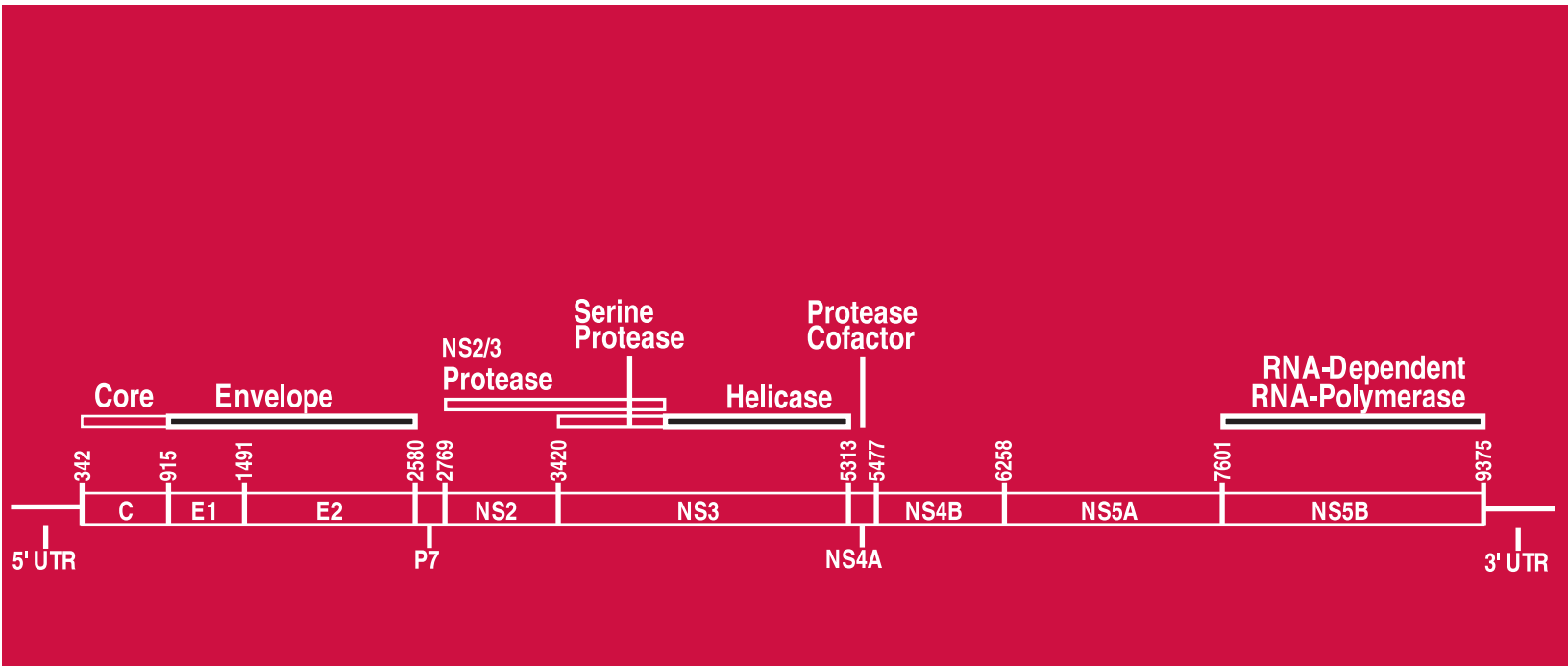

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The Treatment Action Group (TAG) fights to find a cure for AIDS and to ensure that all people living with HIV receive the necessary treatment, care, and information they need to save their lives TAG focuses on the AIDS research effort, both public and private, the drug development process, and our nation's health care delivery systems. We meet with researchers, industry, and government officials, and resort when necessary to acts of civil disobedience, or to acts of Congress. We strive to develop the scientific and political expertise needed to transform policy. TAG is committed to working for and with all communities affected by HIV.

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This report is dedicated to:

Jen Curry and Linda Viens

&

in recognition of:

Carlton Hogan 1961–2003

Tony Tigno 1965–2004

Concepts of health and illness, well-being and disease are cultural constructs —they vary with time and place, with ideology and belief. Over the course of history our views about health and illness have changed. We have moved, or so most of us would like to believe, away from a paradigm where the causes of illness being unknown, were ascribed to divine judgment or intervention, to a paradigm where we have started to understand the biological, physical and psychosocial origins of our personal and community maladies.

However, every now and again, along comes some new infection, some new threat to public and personal health, something where the origins are initially uncertain and obscure. In such circumstances it often appears that it does not take long for us to revert to a more primitive reaction to these new challenges and, in particular, for us to exhibit an irrational degree of prejudice and discrimination against those who suffer from the new infection. This is particularly manifest when the new infection is somehow linked with aspects of personal behavior which depart from the prevailing contemporary norm.

—Chris Puplick
Introduction to C-Change: Report of the enquiry into hepatitis C related discrimination

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FOREWORD

Among the greatest challenges of modern medicine is the need to intermittently stop and synthesize the mass of data that flows from electronic and print media, formal scientific conferences, and small gatherings of focused researchers. In the last decade, few areas have moved forward as quickly as our knowledge of the significance, epidemiology, diagnosis, virology, and treatment of hepatitis C virus infection. A relatively obscure field in the early 1990s has grown up and now holds center stage in HCV-specific symposia and conferences, specialty journals, and even the popular press. Thousands of articles have now been published in peer-reviewed journals, and "experts" abound. It is in this context that TAG's report, *Hepatitis C and HCV/HIV Coinfection: A Critical Review of Research and Treatment*, was born and has matured. The first edition of this treatise (Marco 2000) represented an admirable effort to summarize the prevailing views of the time, and to "push the envelope" in terms of future research and management of HCV-infected patients. The latest effort was monumentally more difficult. Review articles and consensus statements (often conflicting and sometimes self-serving) are ubiquitous. One might ask why anyone would take on the seemingly thankless task of trying to bring order and perspective out of this chaos. It is a credit to Tracy Swan and her research and writing team that she constantly sought fact versus opinion and tempered controversy with balanced and thoughtful commentary. The outcome is a document that can be understood by the uninitiated and used as an important reference by those experienced in the field.

Each section of the document has a brief summary that catalogs its high points. Within each section is a detailed, reference-rich analysis of the current literature. The key sections of the document include Natural History, Diagnosis, Molecular Virology, Pathogenesis, and Treatment. All sections have been written following consultation with key experts and then validated for accuracy and fairness by others. Therefore, the TAG report on hepatitis C and HIV/HCV coinfection provides an overview with a detailed body of text depending on the level of interest and knowledge of the individual reader.

The Natural History section reveals the wide variety of clinical pathways that infected patients may follow. We now recognize that many infected patients will spontaneously clear this infection without treatment intervention, particularly in the early months and years after infection. Others will maintain a chronic viral infection, but may not develop significant fibrosis in their liver. Unfortunately, many will continue in stepwise fashion from portal fibrosis to portal bridging and finally to cirrhosis. Some who develop cirrhosis will find their course complicated by development of hepatocellular carcinoma, whose incidence seems to be increasing in countries most affected by this epidemic.

In Diagnostics, we are updated on the latest generation of serological and virological assays used to evaluate the hepatitis C virus. Over the last few years, commercial viral load assays for HCV have become more sensitive and their performance better standardized. The introduction of a standardized unit—one that is not dependent upon an arbitrary designation of copies—has helped bring interchangeability to these assays; however, patients with very low or very high titers may still not exhibit high degrees of correlation between assay methods. Genotype analysis has finally become more than an epidemiological tool. It clearly identifies those more or less likely to respond to interferon-based therapies. While considerable interest has developed in models that use

common biochemical and/or radiological parameters to evaluate fibrosis, liver biopsy remains the gold standard.

The Treatment of HCV section documents the rapid changes in treatment paradigms over recent years. Pegylated interferon use has moved front and center and essentially replaced use of standard interferon products. The goal of pegylation, namely, the alteration of pharmacokinetics leading to increased area under the curve (AUC), has been achieved in two commercially available products that appear to have similar levels of effectiveness in large-scale Phase III clinical trials. Ribavirin is a mainstay of therapy now, though the exact mechanisms of its action remain uncertain. The duration of therapy has now been clearly delineated in terms of genotype. Genotype 2 and 3 patients have high rates of sustained viral response associated with 24 weeks of continuous interferon/ribavirin therapy. Viral kinetic modeling suggests that even shorter treatment cycles may be possible. In contrast, genotype 1 and probably genotype 4 patients need longer therapy and still fail to achieve the optimal outcome more than 50% of the time. These patients remain the great challenge for newer therapeutic interventions. Potent antiviral agents directed against the HCV serine protease, helicase, and RNA-dependent RNA polymerase are under investigation, and some are in human trials. The timeline of development for antiretroviral agents serves as an important guidepost. We must not forget the lessons learned from early monotherapy interventions that led to rapid mutant virus emergence.

HCV/HIV coinfection represents an important and relatively uninvestigated area for research. The rapid progression to cirrhosis in this group leads to a feeling of great urgency. Unfortunately, the research effort invested in this important subgroup—which may represent 10% of the HCV burden in the United States—has been blunted by a variety of factors. Pharmaceutical companies did not pursue large trials in coinfected subjects until HCV monoinfection trials leading to licensure were complete. Hepatologists and gastroenterologists remain unfamiliar and uncomfortable with the management of HIV-infected patients, and infectious disease experts remain woefully weak in their understanding of liver disease. Interactions between drugs used for the two diseases are not well characterized; adherence in many clinical trials and in clinical practice is abysmal. Finally, there is a sense of therapeutic nihilism vis-à-vis HCV in the infectious disease community that quickly affects patients. Decrements in sustained viral response in coinfected patients lead many to declare that HCV treatment in genotype 1 patients makes therapeutic intervention a nonviable alternative. It is important, however, to note that FDA has approved interferon monotherapies at rates of efficacy far lower than that observed for coinfected subjects in current treatment trials, and that "cure" rates of 10–20% are still far better than those achieved for other chronic virus infections.

It has been a privilege to participate in the review process during the development of this monograph. I applaud the tireless efforts of the authors to seek data in place of opinion, and to temper opinion with a call for definitive research. It is time for the next jump forward, as the years of directed antiviral therapy for HCV approach.

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Volume I. Clinical Science

by Tracy Swan

EXECUTIVE SUMMARY

Hepatitis C is a serious global health problem. Worldwide, an estimated 170 million people have been infected with the hepatitis C virus (HCV). In the United States, an estimated 4 million people have been infected with HCV; 2.7 million of them have developed chronic hepatitis C. The U.S. estimate may be low, as many high-prevalence populations were excluded from HCV surveillance. Up to one quarter of HIV-positive individuals in the U.S. are coinfected with HCV.

Since viral inactivation procedures and effective donor screening have almost completely eliminated the risk of hepatitis C infection from blood transfusions and blood products, the majority of new HCV infections occur via injection drug use with shared, unsterilized equipment. HCV is prevalent among current and former injection drug users; up to 90% have been infected.

Many questions about HCV transmission remain unanswered. HCV can be transmitted through sex, from mother to infant, and via dialysis at centers with inadequate infection control procedures. Men who have sex with men, sex workers, people with multiple sex partners, and partners of HIV/HCV-coinfected persons have higher-than-average HCV prevalence rates, yet the routes and risks of sexual transmission are unclear. The risk of HCV transmission from mother to infant is about 5%; the risk increases if the mother is HIV/HCV coinfected. Interventions to reduce the risk of mother-to-infant transmission have yet to be identified. While it has been speculated that HCV may be transmitted from shared tubes used for intranasal drug use and from crack pipes, the risk of HCV transmission from non-injection drug use has not been quantified. Current HCV prevention strategies for injection drug users are inadequate, and education about HCV transmission and prevention is lacking.

Recommendations:

- **Implement national surveillance for chronic HCV infection.**
- **Provide HCV testing and education for high-risk and high-prevalence populations.**
- **Increase access to sterile injection equipment.**
- **Increase access to drug treatment and methadone maintenance programs.**
- **Institute CDC's recommendations for prevention of HCV transmission in hemodialysis facilities.**
- **Clarify routes and risks of HCV sexual transmission.**
- **Clarify the risk of non–injection drug use behaviors associated with HCV transmission, such as smoking or sniffing.**
- **Research mechanisms and interventions to decrease mother-to-infant transmission.**
- **Develop protocols for HCV counseling and testing for pregnant women, and offer voluntary HCV counseling and testing to pregnant women.**
- **Develop and implement HCV prevention strategies for the developing world.**

Hepatitis C is not invariably chronic; 15% to 45% of those infected achieve spontaneous viral clearance. Chronic HCV infection can result in fibrosis (mild liver scarring) and cirrhosis (serious liver scarring). Symptoms may include fatigue, depression, and confusion ("brain fog").

The course of chronic HCV varies widely and depends on a number of factors, including age, race, sex, and alcohol consumption. People living with HCV and their health care providers need better information about prognostic factors and risk of disease progression to make informed decisions about care and treatment. The most serious clinical consequences of HCV infection are cirrhosis, hepatocellular carcinoma, and hepatic decompensation (liver failure). HCV-related end-stage liver disease is the leading indication for liver transplants in the United States, where there is a critical shortage of donor livers. Potentially life-saving vaccinations for hepatitis A and B are recommended by CDC, but have not yet been universally incorporated into HCV care.

Recommendations:

- **Investigate the role of genetic and ethnic factors in susceptibility to HCV infection, disease progression, and response to treatment.**
- **Investigate the role of sex differences in HCV disease progression.**
- **Investigate the role of light-to-moderate alcohol consumption on HCV disease progression.**
- **Identify possible causes of and interventions for HCV-related "brain fog."**
- **Promote screening and vaccination for hepatitis A and hepatitis B among individuals infected with HCV or coinfected with HIV/HCV.**
- **Create an "opt-out" system for organ donation in the United States and include discussion of organ donation as part of school health education programs and regular medical care.**

Acute HCV is usually asymptomatic and often goes undiagnosed. A series of blood tests is available to diagnose chronic HCV infection, predict and monitor the effects of treatment, and measure other complications of HCV infection. Liver biopsy is still considered the "gold standard" for assessment of liver damage and determining the need for treatment, although it is expensive, invasive, unpopular with patients, and subject to substantial interpretive variation. Hepatocellular carcinoma (HCC) occurs in 1–4% of cirrhotics annually. Current surveillance techniques are suboptimal for early diagnosis of hepatocellular carcinoma.

Provider education on HCV is limited. Many high-risk and high-prevalence populations lack access to regular medical care. Outreach strategies for diagnosis of HCV and linkage of newly diagnosed HCV-infected persons to care programs are needed.

Recommendations:

- **Educate primary care providers about diagnosis of acute and chronic HCV infection.**
- **Develop and market oral fluid test kits for HCV-antibody testing.**
- **Promote use of a standardized system for evaluation of liver biopsy.**
- **Continue research on non-invasive testing methods to replace or reduce the need for liver biopsy.**
- **Identify and validate prognostic markers and more effective screening methods for early diagnosis of hepatocellular carcinoma.**

Treatment for HCV—a 6-to-12 month course of pegylated interferon and ribavirin—has many drawbacks, including potentially severe side effects. On average, treatment eradicates HCV in about half of patients, but various factors can dramatically increase or decrease the likelihood of a sustained virological response. Initial reports suggest that HCV treatment may be particularly effective during acute infection. Treatment may also improve the condition of the liver, even in people who do not have a sustained virological response.

Primary care providers often do not receive adequate information about efficacy and side effects of HCV treatment. Moreover, even for expert clinicians, numerous questions about treatment remain: Can treatment success rates be improved by optimizing dosing regimens? Is treatment safe and effective across all populations with high HCV rates—especially drug and alcohol users and people with psychiatric co-morbidities, who have often been excluded from clinical trials? Is there a role for treatment even in people who don't clear the virus? Can improvements in the management of side effects make treatment more tolerable? When and how should people acutely infected be treated? How effective are herbs such as milk thistle as complementary and alternative therapies for HCV? These questions are relevant not only for HCV monoinfection but also for HCV/HIV coinfection.

Recommendations:

- **Increase knowledge of treatment and care for hepatitis C patients among primary care providers.**
- **Identify optimal dosing strategies.**
- **Increase research on treatment safety and efficacy in understudied populations.**
- **Increase research on strategies to manage side effects of HCV treatment.**
- **Identify when and in whom treatment for acute HCV should be initiated; optimal regimen; and duration of treatment.**
- **Establish prospective, long-term follow-up studies to assess the durability and clinical benefit of histological responses in virological responders, relapsers, and non-responders.**
- **Investigate safety and efficacy of alternative therapies for HCV infection.**

In 1999, CDC added hepatitis C infection to the list of opportunistic infections (OIs) associated with HIV. HIV accelerates HCV disease progression, and HCV-related end-stage liver disease has become a leading cause of death among HIV-positive people. Although potent antiretroviral therapy has significantly increased survival, some studies have reported a blunted immune response to antiretroviral therapy among HCV-coinfected individuals. HCV coinfection increases the risk of hepatotoxicity from antiretroviral therapy. Vaccination for HAV and HBV may be less immunogenic in individuals with advanced HIV disease.

HCV treatment is less effective for HIV/HCV-coinfected people, and they tend to experience more severe side effects. Although promising data have emerged on the survival of coinfected liver recipients, restrictive policies at transplant centers and refusal to reimburse for transplantation have limited access to transplantation for HIV-positive candidates. For these reasons, people coinfected with HIV and HCV and their health care providers face complicated decisions about care and treatment for both infections.

Recommendations:

- **Establish prospective, longitudinal cohort studies of the natural history of HIV/HCV coinfection in the era of HCV treatment and HAART.**
- **Develop guidelines for the care and treatment of coinfected individuals.**
- **Establish a universal definition of hepatotoxicity and characterize its severity.**
- **Explore pharmacokinetics and drug levels of antiretroviral agents and other drugs commonly used by coinfected individuals.**
- **Include HIV/HCV-coinfected individuals in early-phase HCV treatment trials.**
- **Explore strategies to optimize HCV treatment for HIV/HCV-coinfected persons.**
- **Support access to and research on liver transplantation for HIV-positive and HCV/HIV-coinfected individuals.**

HCV is prevalent among African Americans, the incarcerated, the poor, current and former injection drug users, people with psychiatric disorders, and HIV-positive persons. These groups face significant barriers to care and treatment. Incarcerated individuals have had to resort to legal action to obtain HCV treatment, and most still do not receive it. Inadequate, decreasing funding of AIDS Drug Assistance Programs (ADAPs) has limited access to HIV and HCV treatment for HIV-positive individuals. Expected cuts in Medicaid threaten access to HCV treatment for many more.

Recommendations:

- **Provide full access to hepatitis C care and treatment for all of those in need.**
- **Do not withhold treatment from active drug users; decisions should be made on an individualized basis.**
- **Strengthen linkages among substance abuse treatment programs, methadone maintenance programs, medical and mental health providers, and HIV/HCV prevention programs.**
- **Increase capacity to provide individualized medical care and treatment to coinfected active drug users.**
- **Develop integrated, multidisciplinary systems of care for individuals with multiple co-morbidities (HIV, HCV, psychiatric disorders, addiction).**

It is in this environment that TAG is releasing Hepatitis C and HIV/HCV Coinfection: A Critical Review of Research and Treatment. This report is meant to serve as a blueprint for activism as well as a source of information.

TAG's Coinfection Project closely monitors new data on the epidemiology and natural history of HIV/HCV coinfection, as well as the development of new diagnostics, prophylaxes, and treatments for hepatitis C in both the pre- and postmarketing stages. We advocate for the expeditious development, proper clinical research and regulation, ease of access, and optimal use of these drugs. We work with the pharmaceutical companies, NIH, FDA, researchers, and other treatment activists to achieve these objectives. TAG also educates members of the HIV community about coinfection with hepatitis C.

INTRODUCTION

TAG's Hepatitis C and HIV/HCV Coinfection Report is a comprehensive review of basic and clinical science accompanied by recommendations for research and policy. It was written for people living with hepatitis C or HIV/HCV coinfection, clinicians, researchers, activists, educators, and advocates.

The Hepatitis C and HIV/HCV Coinfection Report reflects my experience. I have worked for more than ten years as a direct service provider to people at risk for, or living with, HCV and HIV, which has contributed to my understanding of their needs. While I was writing this report, I reviewed more than a thousand journal articles and hundreds of conference abstracts, and interviewed researchers, clinicians, coinfected individuals, and advocates.

TAG's original hepatitis report was released in July of 2000. Since then, HCV-related end-stage liver disease has been recognized as a leading cause of death among people with HIV. For people with HCV monoinfection, liver-related deaths are projected to increase by 180% over the next 20 years. Meanwhile, a more effective therapy, pegylated interferon, has been approved for treatment of HCV, and researchers are moving closer towards an understanding of HCV pathogenesis. The Hepatitis C and HIV/HCV Coinfection Report is a synthesis of current knowledge; key issues are highlighted in the Recommendations section at the end of each chapter.

Despite advances in treatment, many barriers to optimal care and treatment of people with HCV and HIV/HCV remain. As with HIV, HCV is a lens that magnifies the intersection of medical and social inequities. In the U.S., both viruses disproportionately affect African Americans and other groups who have traditionally had poor access to care and treatment, including the incarcerated, the poor, people with psychiatric disorders, and injection drug users. Up to 90% of injection drug users are infected with HCV. Injection drug users in particular are regarded with an astounding degree of contempt by most of society. Draconian drug laws and inadequate access to sterile syringes have created penalties for drug use that exceed incarceration alone by costing people their health and, ultimately, their lives. Until we acknowledge that HCV is a disease that is prevalent among injection drug users and demand expansion of needle exchange programs, universal availability of syringes through legalized pharmacy sale, access to methadone maintenance therapy, and drug treatment upon request, we will not be able to mitigate the spread of hepatitis C.

Access to care and treatment is not just an issue for injection drug users. In the United States, access to health care for the poor is restricted; more than 43 million Americans are uninsured. Cuts in federal funding for programs such as AIDS Drug Assistance Programs and Medicaid threaten access to health care for the more than 42 million Americans who rely on them. Drug prices are exorbitant; the 48-week course of treatment for HCV costs as much as $40,000.

Even individuals with access to health care suffer from the constraints of managed care and a lack of provider and patient education about HCV; these factors collectively make it difficult to insure that people with HCV receive optimal care. Many clinicians work in health care settings ill-suited to providing care for dually or multiply diagnosed individuals. Successful models of service delivery to injection drug users and people with psychiatric co-morbidities must be imported into the

clinic. Clinicians must take a proactive approach to managing drug-related adverse events by educating people about them before initiating treatment, providing options to ameliorate them and offering access, where indicated, to mental health care. While pressuring the medical system to meet these needs, people must receive clear and direct education about transmission, prevention, diagnostics, care, and treatment of HCV and HIV/HCV.

Preparation and implementation require resources. Very little funding is specifically designated for HCV prevention and education services, nor are there any validated and established models for such programs. HCV prevention and education must be integrated into programming at AIDS service organizations and available to staff and participants at methadone clinics, syringe exchanges, detoxification facilities, residential drug and alcohol treatment programs, homeless shelters, and correctional facilities. Information and services must reach those who need them most, not merely those who are the easiest to reach.

Progress toward new treatments and improvements in efficacy of existing HCV therapy has been hampered by the absence of a coordinated research agenda for hepatitis C. HCV research is currently spread across different NIH institutes without the oversight that exists for HIV research from the NIH Office of AIDS Research (OAR). Currently, there is no mechanism for meaningful community participation in the development of a research agenda for HCV.

The current situation demands intensified, focused action: members of the HCV and HIV communities need to work together to advocate for prevention initiatives, broadened access to care and treatment for HCV and HIV/HCV, and a comprehensive research agenda.

I. Epidemiology and Transmission of Hepatitis C

Summary

Hepatitis C is the most common bloodborne infection in the United States. The Centers for Disease Control's Third National Health and Nutrition Examination Study (NHANES III), conducted from 1988 to 1994, estimated that 1.8% of the non-institutionalized United States population, or 4 million people, have been infected with HCV. Liver damage resulting from HCV infection is the leading cause of liver transplants in the United States (CDC 1998). End-stage liver disease (ESLD) and hepatocellular carcinoma (HCC; liver cancer) resulting from HCV infection cause between 10,000 and 12,000 deaths per year in the United States. Global HCV infections are estimated at 170 million, or 3% of the world's population (World Health Organization 1999).

Decades before the hepatitis C virus (HCV) was identified, many transfusion recipients developed a post-transfusion viral hepatitis infection that was not caused by the hepatitis A or hepatitis B viruses. Non-A, non-B (NANB) hepatitis was originally thought to be a mild infection, but over the years doctors began to notice that some NANB hepatitis patients developed serious liver damage.

In 1988, a small RNA virus, designated hepatitis C, was identified, and an antibody test was quickly developed (Choo 1989; Kuo 1989). Testing of stored blood samples revealed that 70% to 90% of NANB hepatitis infections were hepatitis C infections.

Approximately one-third of the estimated 900,000 HIV-positive people in the United States are coinfected with HCV. The rate of coinfection is much higher among people who acquired HIV from injection drug use. HCV infection rates in injection drug users (IDUs) range from 70% to 90% (M. J. Alter 1998; Donahue 1991; Garfein 1996; Mao 2001; Mendel 1995; Sherman 2002a; Sulkowski 2002; Thomas 1996).

Most new HCV infections in the United States are from injection drug use with shared, unsterilized equipment. People who are on kidney dialysis are at risk for HCV infection if dialysis centers do not practice proper infection control procedures. The other two main sources of HCV infection, blood transfusions and contaminated blood products, have been almost completely eliminated (Donahue 1992). Viral inactivation techniques for clotting factors were introduced in 1985 (Factor VIII) and 1987 (Factor IX). Effective screening for HCV in the United States blood supply began in July 1992 and has improved steadily since then.

Mother-to-infant transmission of HCV occurs among approximately 5% of infants born to mothers with HCV. The risk of HCV transmission increases if the mother is coinfected with HIV (Thomas 1998; Yeung 2001).

HCV can be sexually transmitted, but so far research has yielded conflicting results about how likely sexual transmission of HCV actually is, and exactly how it takes place. HCV is more prevalent among men who have sex with men, people with multiple partners, sex workers, and partners of HIV/HCV coinfected individuals (M. J. Alter 1988; M. J. Alter 2002; Bodsworth 1996; Buchbinder 1994; Eyster 1991).

Tattooing with shared needles and/or ink receptacles may result in HCV infection, though incidence is low and data are scarce.

It has been speculated that HCV infection can be spread by sharing tubes for intranasal (snorting or sniffing) drug use as well as sharing personal implements with infected blood on them (such as nail clippers, manicure sets, razors, and toothbrushes). Again, however, incidence is low and data are scarce.

Epidemiology of Hepatitis C

Hepatitis A and hepatitis B have long been known, and vaccines have been developed to prevent infection with both viruses.

In 1965, Baruch S. Blumberg discovered an antigen that was later named hepatitis B (HBV). In 1968, the hepatitis B antigen was associated with post-transfusion hepatitis (Sherlock 1984). More than 350 million people worldwide are infected with hepatitis B; the Centers for Disease Control and Prevention (CDC) estimates that 4.9% of Americans have been infected with HBV; 1.25 million have developed chronic HBV infections. A vaccine to prevent infection with hepatitis B has been available since 1982 (W. M. Lee 1997). Since 1991, the American Council on Immunization Practices has recommended universal hepatitis B vaccination of newborns and adolescents.

In 1973, hepatitis A (HAV) was first identified; by 1979 it could be grown in tissue cultures (Sherlock 1984). In 1995, the Food and Drug Administration (FDA) licensed a vaccine to prevent infection with hepatitis A. In 2001, FDA approved a combined vaccine to protect against infection with hepatitis A and hepatitis B. Hepatitis A infection is endemic in many parts of the world; CDC has estimated that 31% of Americans were infected with HAV during their lives. Once a person is infected with HAV, re-infection does not occur, but acute HAV can be fatal among people with chronic HBV or HCV infections.

Before the hepatitis C virus was definitively identified, hepatitis infections were frequently seen in recipients of blood transfusions. Screenings for hepatitis A were negative, and only about 25% of transfusion-associated hepatitis infections were caused by hepatitis B (H. J. Alter 1999). The remaining 75% of these infections were attributed to a virus designated non-A, non-B (NANB) hepatitis, which was initially thought to be fairly harmless. Although most NANB infections were identified by liver enzyme elevations as high as 21 times the upper limits of normal soon after infection, only a small group of people developed symptoms during acute infection (M .J. Alter 1992; Koretz 1993). These symptoms—fatigue, low-grade fever, appetite loss, nausea, vomiting, and jaundice (yellowed skin and eyes)—resolved in a few weeks.

Over time, physicians became alert to the possibility of more serious consequences as they began to see many NANB patients with persistently elevated liver enzymes and some with serious liver damage.

In 1988, a group of researchers at Chiron identified an RNA virus (from the flaviviridae family) that they suspected might be the agent causing NANB infections. The virus was named hepatitis C

(Choo 1989). An antibody test was rapidly developed (Kuo 1989). When researchers at the National Institutes of Health (NIH) tested the new antibody on archived blood samples, they discovered that 70% to 90% of NANB hepatitis cases were actually HCV infections.

Hepatitis C Infection in the United States

Early epidemiological studies did not provide an accurate picture of HCV prevalence in the general population because most participants were volunteer blood donors who had already been screened for infectious diseases. The CDC's Third National Health and Nutrition Examination Survey (NHANES III) provided the best available estimate of HCV infection in the United States, finding that 1.8% of Americans, or roughly 4 million people, have been infected with HCV; 2.7 million of them remain chronically infected (see Chapter II, Natural History of Hepatitis C). Hepatitis C is the most common bloodborne infection in the United States.

In NHANES III, blood samples from 21,241 people were screened for antibodies to hepatitis C. Samples with a positive antibody test result were then tested for HCV RNA (viral load) to distinguish between chronic and resolved (past) HCV infections. Three-quarters (74%) of the antibody-positive samples were also HCV-RNA-positive, indicating chronic HCV infection (M. J. Alter 1999). The NHANES III data have been widely cited in media reports and policy discussions; however, there are several limitations to the study. It may significantly underestimate the true prevalence of HCV in the United States, because NHANES III did not survey incarcerated or homeless persons. Between 30% and 40% of the 1.8 million people incarcerated in the U.S. are infected with HCV (Reindollar 1999). A 1994 study of HCV prevalence among 4,513 inmates (87% male; 13% female) in California revealed that 39.4% of the men and 53.5% of the women were HCV-antibody-positive (Ruiz 1999). HCV is prevalent among the homeless; in one study almost 42% of 597 homeless veterans were anti-HCV positive (Cheung 2002).

Limitations also exist with the NHANES III data on drug use; although participants were asked about their drug-use history, they were not asked if they had ever injected drugs. A multivariate analysis revealed that the strongest factors independently associated with HCV infection were drug use (defined as the use of marijuana more than 100 times, or any cocaine use ever) and, in the absence of drug use, "high-risk" sexual behavior (more than 50 sexual partners and/or early age at first intercourse). Because marijuana use does not involve blood-to-blood contact, it presumably serves here as a proxy for other drug use or sexual behaviors. On the other hand, intranasal cocaine use with shared straws or other implements might involve contact with infected blood. Yet because modes of administration—snorting vs. injecting—among cocaine users in NHANES III is unknown, the incidence of HCV transmission via intranasal drug use could not be assessed.

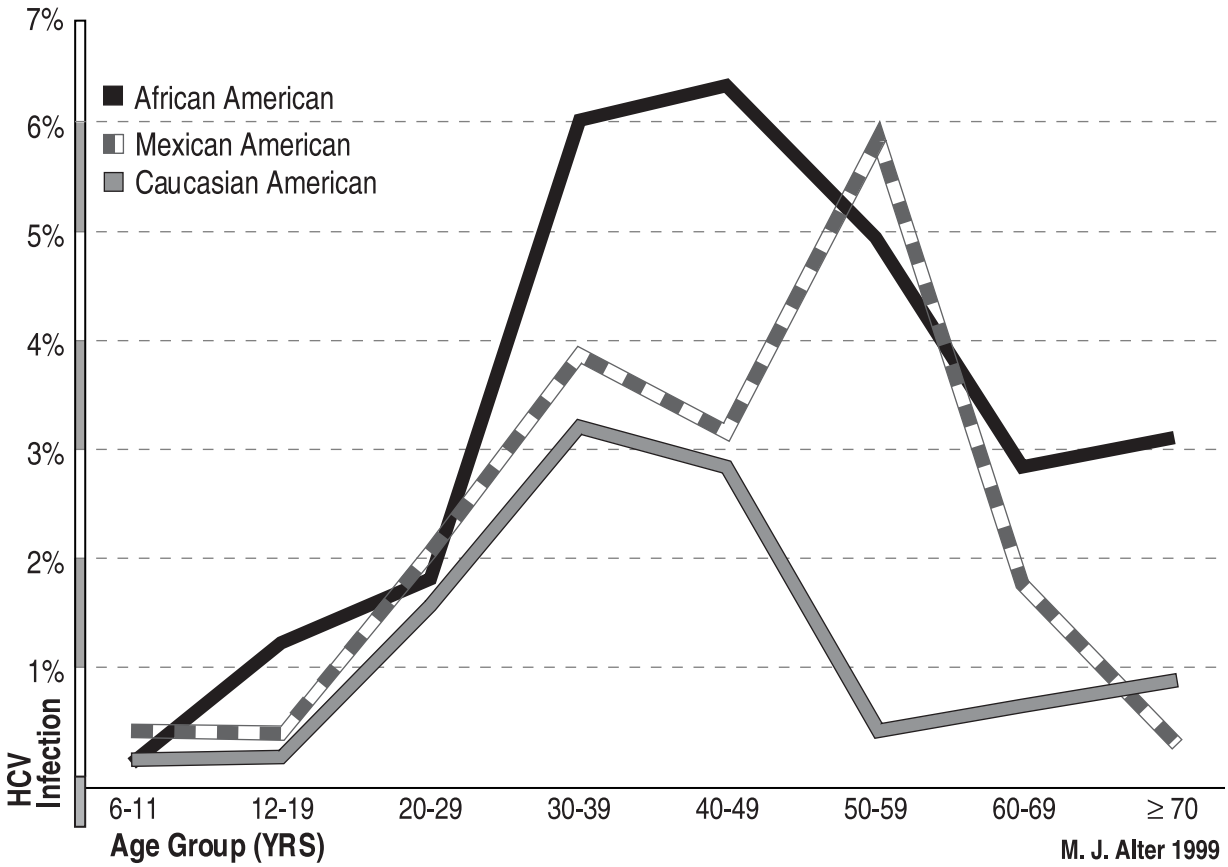
Sixty-five percent of HCV infections identified in NHANES III were found in persons between the ages of 30 and 49. The highest observed prevalence was among black males aged 40 to 49—a shocking 9.8%. This is especially alarming in light of data indicating a poorer response to HCV treatment in African Americans (see Chapter V, Hepatitis C Treatment).

Table 1. Prevalence of Antibody to HCV (Anti-HCV+) According to Demographic Characteristics in NHANES III

Characteristic	N tested	HCV+ %	Nationwide estimate
All Subjects	**21,241**	**1.8%**	**3,875,000**
Non-Hispanic White	7,965	1.5%	2,359,000
Non-Hispanic Black	6,119	3.2%	762,000
Mexican American	6,268	2.1%	261,000
Other	889	2.9%	493,000
Male	10,076	2.5%	2,586,000
Female	11,165	1.2%	1,289,000

M. J. Alter 1999

Figure 1. Prevalence of HCV infection by age and race/ethnicity United States, 1988-1994



Veterans and Hepatitis C Infection

Several studies have found shockingly high rates of HCV among United States veterans. In March of 1999, the Veteran's Health Administration (VHA) did a one-day serosurvey to estimate HCV prevalence in order to forecast future health care costs. Blood was drawn from 26,102 veterans. HCV antibodies were detected in 1,724 (6.6%) veterans. The mean age among HCV antibody-positive individuals was 53.8 years. Most were male (97.4%) and served during the Vietnam era (58.7%); 29% identified themselves as black, non-Hispanic, and 46% identified themselves as white, non-Hispanic (Roselle 2002).

A study of 1,032 veteran outpatients at San Francisco's Veteran's Affairs Medical Center reported an HCV seroprevalence rate of 17.7% (Briggs 2000). Study participants were screened for HCV and given a detailed questionnaire on sociodemographic information and risk factors. Over 90% of veterans surveyed had at least one risk factor not directly related to military service. Risk factors related directly to military service included rank (enlisted vs. officer), exposure to blood during combat, combat job as a medical worker, and history of a needlestick during military deployment.

A retrospective analysis of blood samples and risk histories provided by 597 homeless veterans admitted to a VA shelter found an HCV seroprevalence of 41.7%; intravenous drug use was identified by multivariate analysis as an independent risk factor (Cheung 2002).

A 2001 study found a low HCV prevalence among active-duty personnel in the U.S. military—just five of one thousand troops (0.5%). HCV prevalence increased with age to 3.0% among troops over 40 years old, a prevalence rate similar to that of a matched age cohort (Hyams 2001).

Gathering Data: Hepatitis C Surveillance

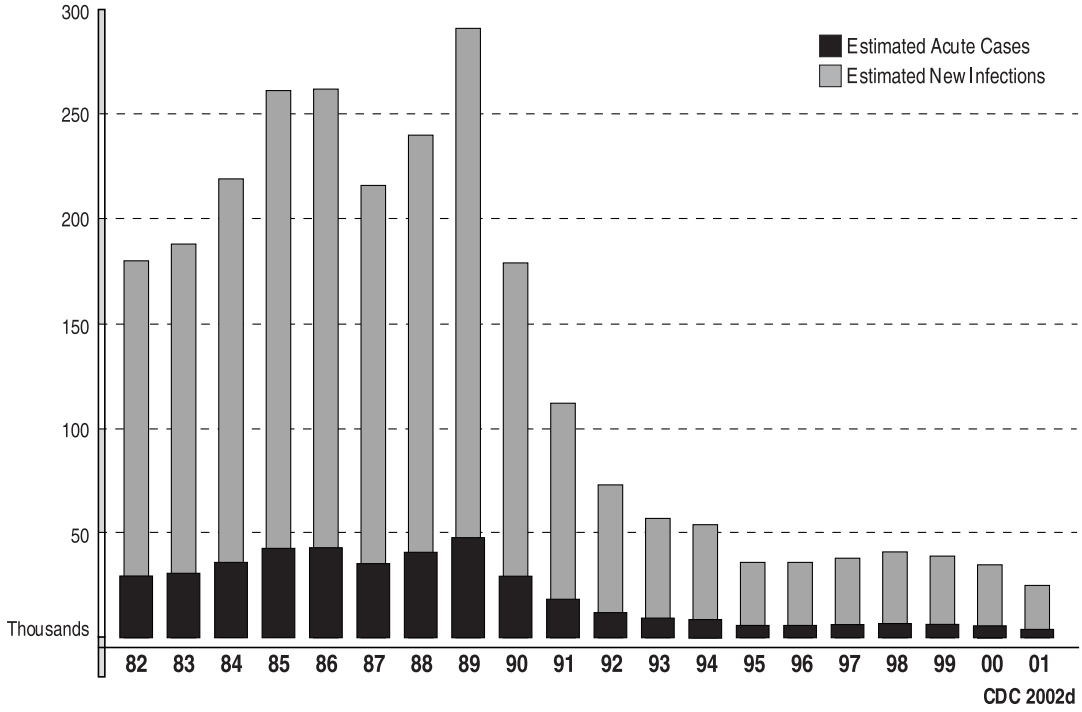
The yearly incidence of hepatitis C infections rose from approximately 45 per 100,000 in the early 1960's to 100–200 per 100,000 in the late 1980's, when they reached their peak (Armstrong 2000). Age-specific prevalence data have been used to identify transmission patterns in the United States (Wasley 2000). Because HCV prevalence is highest among people 30 to 49 years of age, HCV incidence is thought to have peaked among young adults 10 to 30 years ago.

According to CDC estimates, new HCV infections have decreased by 80% (M. J. Alter 1998). CDC estimated that 291,000 new HCV infections occurred in 1989; the estimate of new infections for 2001 dropped to 25,000 (CDC 2002d). The extent of this drastic reduction in new HCV infections is questionable, although effective screening of blood products and the blood supply and saturation of HCV infection among injection drug users shrunk the pool of vulnerable people. Most acute HCV infections are not picked up by surveillance systems because the majority of acutely infected people are asymptomatic (Di Bisceglie 1998; Hagan 2002).

Acute hepatitis C is subject to mandatory reporting requirements by the Council of State and Territorial Epidemiologists (CTSE). The acute hepatitis C surveillance system is inherently limited due to lack of a single, definitive test for acute vs. chronic HCV infection and inadequate resources at state health departments to test for both HCV antibodies and HCV RNA. Some people will achieve spontaneous viral clearance of acute hepatitis C, although they remain antibody-positive,

thus some reported cases of acute HCV may be false positives. In addition, it is not possible to distinguish how recent an HCV infection actually is. Surveillance data are corrected for underreporting and asymptomatic infections, although the method/s of correction is/are unspecified.

Figure 2. Acute Hepatitis C Infections in the United States 1982–2001



Only acute cases of hepatitis C are reported, although a small, pilot program—sentinel surveillance of physician-diagnosed chronic liver disease—has begun tracking both acute and chronic hepatitis C infections. The updated CDC case definition for acute hepatitis C includes clinical and laboratory criteria. Laboratory criteria include ruling out hepatitis A and B; alanine aminotransferase (ALT; a liver enzyme) levels over seven times the upper limits of normal; and confirmatory HCV-RNA testing if HCV antibody testing is reactive. Accurate identification of acute HCV infections will increase by combining laboratory reports of reactive HCV-antibody tests, ALT levels, and clinical information. Chronic hepatitis C infections can be distinguished from acute infections by laboratory reporting of reactive HCV-antibody tests and confirmatory antibody testing by RIBA or HCV-RNA (viral load) detection by RT-PCR. Reporting of HCV infections includes demographic and risk information.

CSTE is considering inclusion of chronic and resolved HCV infections in reporting systems. In June 2002, CDC issued guidelines for viral hepatitis surveillance and case management (CDC 2002). Two different surveillance systems, the National Notifiable Disease Surveillance System and the Viral Hepatitis Surveillance Program have been consolidated into the National Electronic Telecommunications System for Surveillance (NETSS). Upcoming changes in the structure and function of NETSS, will increase the capacity for surveillance of both acute and chronic hepatitis C infections, but must be supported by funding for adequate staffing and laboratory resources.

Global Hepatitis C Prevalence

Although prevalence data are not available from every country, it is estimated that 170 million people are infected with HCV worldwide (WHO 1999). The true number may be much higher due to missing data and differing data collection and interpretation methods.

Table 2. Global Hepatitis C Prevalence

WHO Region	Population (Millions)	HCV Prevalence	Infected Pop. (Millions)	# Countries Not Counted
Africa	602	5.30%	31.9	12
America	785	1.70%	13.1	7
Eastern Mediterranean	466	4.60%	21.3	7
Europe	858	1.03%	8.9	19
SouthEast Asia	1,500	2.15%	32.3	3
Western Pacific	1,600	3.90%	62.2	11
Total	**5,811**	**3.10%**	**169.7**	**57**

WHO 1999

The HCV epidemic in the developing world is largely driven by transfusions, unsterilized medical and dental equipment, unsterilized instruments used for circumcision, scarification, tattooing, traditional medicine, and, in some regions, injection drug use.

The World Health Organization (WHO) developed conservative estimates of the transmission of bloodborne pathogens (hepatitis B, hepatitis C, and HIV) via unsafe injections. This model estimates that 2.3–4.7 million new HCV infections may result from unsafe injections annually (Kane 1999).

Hutin and colleagues estimated the safety and frequency of injection practices at healthcare facilities in ten regions comprised of developing and transitional countries by reviewing literature, including unpublished data from WHO. They reported that at least 16 million injections were administered annually; unsterilized injection equipment was reused in approximately one of three injections (Hutin 2003). Based on this data, Hauri and colleagues estimated that there were two million HCV infections—40% of all new infections—from unsafe injection practices in health care settings in the year 2000 (Hauri 2004).

HIV/HCV Coinfection Prevalence in the United States

CDC estimates that 800,000–900,000 people in the United States are HIV-positive; about 16–25% of them are also infected with HCV (Sherman 2002a; Tedali 2003b; Thomas 2002). Coinfection rates vary according to mode of transmission; 50–90% of HIV-positive people infected from shared injection equipment are coinfected with HCV (Sulkowski 2000). Over 50% of HIV-positive people with hemophilia are HCV coinfected due to receiving clotting factor concentrates before viral inactivation procedures were initiated in 1985.

A cross-sectional analysis of 213 Adult AIDS Clinical Trials Group (AACTG) study participants found an overall HCV seroprevalence of 16.1% (Sherman 2002a). Participants were divided into an at-risk group (those with a history of drug use or people with hemophilia) and a low-risk group. Those in the at-risk group had an HCV seroprevalence of 72.7%. HCV seroprevalence in the low risk group was 3.5%—nearly twice what NHANES III found in a broader population sample.

The Terry Beirn Community Programs for Clinical Research on AIDS (CPCRA) assessed HCV prevalence among 2,705 HIV-positive trial participants. Data were collected from September 1998 through September 2001. Antibodies to HCV were detected in 16.6% of participants (449/2,705). A subgroup analysis of an all-male cohort found a significantly greater odds ratio for coinfection among males with a history of IDU (OR, 69.77; 95% CI, 43.93–110.81; $P<0.001$) and IDU plus male-male sex (OR, 11.86; 95% CI, 7.28–19.32; $P<0.001$) (Tedali 2003b).

A serosurvey of HIV-positive patients receiving care in the San Francisco Community Health Network found that 39.4% of 2,859 people tested were anti-HCV-positive (Hare 2002). Another study reported that 28% (394) of 1403 HIV-positive patients from an urban clinic in Richmond, Virginia, were coinfected with HCV (Tsogas 2002).

A serosurvey of 557 active and former injection and non-injection drug users in New York City found HCV RNA in blood samples from 74.2% (170/229) of HIV-positive participants (Klein 2003).

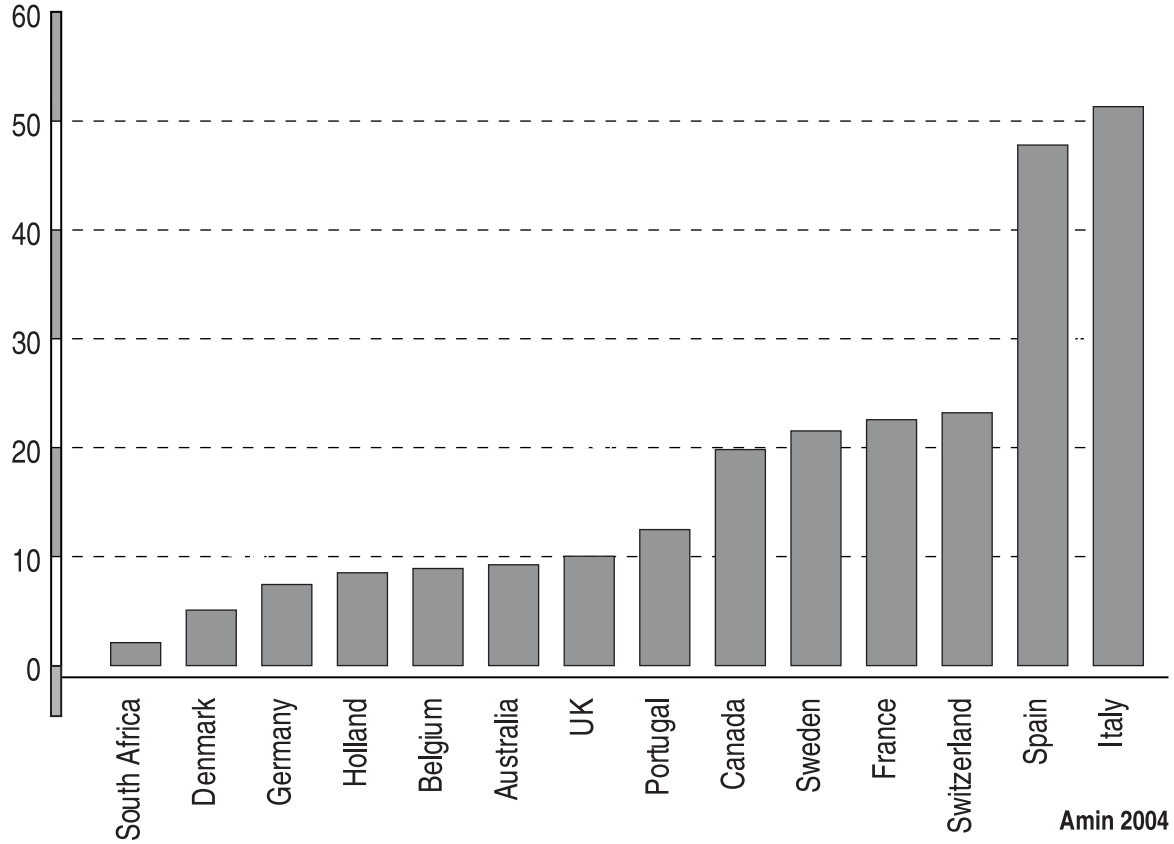
HIV/HCV Coinfection Prevalence in Europe

EuroSIDA is a prospective observational cohort study of more than 9,800 HIV-positive persons from 26 European Countries and Argentina. HCV prevalence was assessed in a subset of 4,957 EuroSIDA cohort participants. Overall, 34% (1685/4.957) were anti-HCV-positive (Rockstroh 2004). Viksna and colleagues assessed regional differences—including HCV serostatus—among a subset of 5,708 EuroSIDA participants from Eastern Europe (907), Southern Europe (1580), Central Europe (1583), Northern Europe (1538) and Argentina (100). Anti-HCV positivity was most prevalent in Eastern Europe (41%), followed by Southern Europe (34%), Argentina (24%), Central Europe (20%) and Northern Europe (13%) (Viksna 2003).

Table 3. Coinfection Prevalence from Five European Studies

Study	Cohort	HIV+	HIV+ & HCV+	% Coinfection
Soriano 2000	Euro SIDA	4,034	1,350	33.5%
Greub 2000	Swiss HIV	3,111	1,157	37.2%
Pailoux 2000	France	1,746	465	27.0%
Mendel 1995	Normandy, France	161	55	34.2%
J. Martín 2001	Madrid, Spain	902	649	72.0%

Figure 3. Prevalence of HCV coinfection by country: CAESAR study



Transmission of Hepatitis C

Transfusion and Blood Products

HCV is most efficiently transmitted through transfusion of infected blood, transplantation of infected organs, and sharing injection drug equipment (M. J. Alter 1994).

In 1985, screening of donor blood for antibodies to HIV, hepatitis A (HAV), and hepatitis B (HBV) was instituted. A study of 912 people who received transfusions between 1985 and 1991 found that the risk for HCV infection was 0.45% per unit transfused before donor blood screening began (Donahue 1992); after screening for HIV, HAV, and HBV was instituted, the risk dropped to 0.19% per unit. The U.S. blood supply has been screened for HCV antibodies since May 1990. This screening has reduced the risk per unit to 0.03%, or about 3 per 10,000 units transfused. The risk of HCV infection from a blood transfusion has continued to decrease since July 1992, when the EIA-2 antibody test replaced the less sensitive EIA-1.

Additional advances in screening technology have further reduced the risk from transfusions; the EIA-2 was replaced by the EIA 3.0 in 1996. HCV nucleic acid amplification (RNA) testing was introduced in April 1999 and approved by FDA in February 2002 (Glynn 2000). Because nucleic

acid amplification testing measures viral RNA rather than antibody, the "window" (the amount of time between infection and detection) for HCV donor blood is reduced from an average of 82 days to 25 days (FDA 2002). It is estimated that 90% of HCV infections due to transfusions result from donations made during the "window" period (CBER 2002). Thus, by shortening the window period, nucleic acid amplification testing will decrease the likelihood of HCV infection from transfusions and blood products. The incidence of HCV infection from a blood transfusion in the U.S., before widespread implementation of nucleic acid amplification testing, was estimated to be about 1 in 103,000 (Schreiber 1996). It is estimated that the use of nucleic acid amplification testing reduces the risk of HCV infection from donor blood by an additional 27–72%.

Before 1985, clotting factor concentrates made from pooled donor blood infected up to 90% of the hemophiliacs who had used them with HCV. Effective viral inactivation techniques were instituted in 1985 with Factor VIII and in 1987 with Factor IX. Viral inactivation procedures have almost completely eliminated the risk of infection with HCV, HIV, and other viral infections from clotting factors.

Since December 1994, all immunoglobulin products made in the U.S. are either screened for HCV or put through a viral inactivation procedure.

Organ donors are screened for HCV antibodies.

Hemodialysis

In a December 2000 study, dialysis recipients at 40% of U.S. dialysis centers were tested for HCV antibody; 8.4% were antibody-positive (Tokars 2002). Other studies of dialysis recipients have reported anti-HCV prevalence of 10–36% among adults and 18.5% among children (CDC 2001).

A majority of these hepatitis C infections result from inadequate infection control procedures at hemodialysis centers. In April of 2001, CDC issued recommendations for preventing transmission of infections among chronic hemodialysis patients which include stricter infection-control practices and regular monitoring of ALT levels and HCV testing of dialysis recipients.

Occupational Exposures

The NHANES III study did not detect a difference in the anti-HCV prevalence of health care workers (1–2%) and that of the general population (1.8%) (H. J. Alter 1991). A serosurvey examining a group of dentists and oral surgeons found anti-HCV in 2.0% of the oral surgeons and 0.7% of the dentists (Thomas 1996). A five-year surveillance program in Italy followed 245 health care workers with occupational exposures to blood. Health care workers and source patients were tested for HCV antibody and RNA. Although 27.8% of the source patients had HCV, there were no seroconversions (development of antibodies in the blood) among the health care workers (Baldo 2002). One review of prospective studies of health care workers has estimated that the risk of HCV infection after a needlestick exposure from an HCV-infected source patient is about 1.8%, with a range of 0–7% (Beltrami 2000). Other studies found risks ranging from 1% to 6% (Lamphear 1994; Puro 2001). It is possible that the risk of needlestick infection increases with higher HCV viremia (McDonald 1996). Needle size, inoculum amount, and inoculation depth may influence

the likelihood of HCV infection. A crude estimate of the comparative risks of transmission from needlestick uses the rule of threes: HBV is transmitted in 30% of exposures, HCV in 3%, and HIV in 0.3% (Lauer 2001).

Injection Drug Use

Because viral inactivation techniques have dramatically reduced the risk of HCV infection from transfusions and the use of clotting factors in the United States, the majority of new HCV infections result from injection drug use (IDU). Before 1990, 60% of HCV infections were attributed to IDU and 10% to blood transfusions. Since 1995, newly acquired HCV infections from transfusions have become extremely rare, while HCV infections from IDU increased to 68% (M. J. Alter 2002). A seven-year CDC surveillance program of acute HCV infections in four counties showed a drop in HCV incidence among transfusion recipients from 17% to 6%, while infections in IDUs rose from 21% to 42% (M. J. Alter 1990).

Hepatitis C is very efficiently transmitted via contaminated syringes. Shared injection equipment, such as cookers, cotton filters, and water can also be a source of infection (Hagan 2001; Thorpe 2002; Vidal-Trecan 2002). Although CDC does not recommend that syringes or injection equipment ever be used more than once, or shared, their *Guidelines for Prevention and Control of Hepatitis C Virus Infection* (1998) and *Guidelines for the Prevention of Opportunistic Infections Among HIV-Infected Persons* (2002) recommend that shared injection equipment should be cleaned with bleach and water. The efficacy of this intervention, however, is not certain; Hagen and colleagues found no difference in the incidence of HCV infection between individuals who almost always used bleach to clean their syringes and those who reported inconsistent or no use of bleach (Hagen 2001). Between 70% and 90% of injection drug users may be infected with HCV (M. J. Alter 1998; Donahue 1991; Garfein 1996; Thomas 1995). Because 55–85% of HCV infections become chronic and remain infectious, there is constant and abundant potential for HCV transmission via shared injection drug equipment.

Although HCV prevalence increases with duration of IDU, new injectors have a higher rate of HCV infection than longer-term IDUs. Garfein and colleagues found that 64.7% of a cohort with less than one year of injection drug use were anti-HCV-positive, and anti-HCV prevalence among a cohort of long-term users increased to 85%. Daily use of injection drugs and cocaine injection was associated with an increased risk of HCV infection. In the same cohorts, the rate of HIV infection during the first year of injection drug use was 13.9%, rising to 20.9% in long-term IDUs (Garfein 1996).

It is difficult to estimate the number HCV infections that result from IDU. A study of blood donors with HCV infection revealed a significant reluctance to disclose even one-time use of injection drugs. Forty-two percent (103) of 248 HCV-positive donors disclosed IDU during a self-administered questionnaire about recreational drug use, although they had denied any injection drug use during their initial blood donation screening (Conry-Cantilena 1996).

In the United States, 9% of HCV infections are from unknown sources, also referred to as sporadic transmission (M. J. Alter 2002).

Other Drug Use

Many people have speculated about the risk of HCV infection from sharing straws for intranasal drug use. In one study of HCV-infected blood donors, HCV infection was significantly linked by multivariate analysis to intranasal cocaine use (Conry-Cantilena 1996). Intranasal cocaine use—but not injection drug use—was reported by 68% of HCV-positive blood donors. Further follow-up of 137 donors reporting intranasal cocaine use found that 115 (84%) shared straws, 60 (44%) used intranasal cocaine more than three times per day, and 40 (29%) had nosebleeds during cocaine use. The authors hypothesized that HCV transmission could result from infected blood in a shared straw entering "denuded nasal mucosa."

An alarming study of female non-injection drug users in East Harlem, New York, found HCV antibodies among 26% (18/70) of heroin users and 11% (11/101) of crack and powder cocaine users (Tortu 2001). The same study reported that 61% of women with a history of IDU had antibodies to HCV. Study participants were screened for past IDU by a detailed questionnaire and an examination of their arms for track marks. Unfortunately, the study did not provide any information about HIV status, number of sexual partners, history of sexually transmitted diseases, or specific sexual practices.

Conflicting data on the likelihood of HCV infection from intranasal drug use has come from the Retrovirus Epidemiology Donor Study (REDS). The REDS has gathered infectious disease and demographic data from blood donors in five urban centers since 1990. After controlling for injection drug use, the REDS found no association between intranasal drug use and HCV infection (E. L. Murphy 2000). According to the CDC's *Guidelines for Prevention and Control of Hepatitis C Virus Infection*: "Currently, the strength of the association between intranasal cocaine use and HCV infection does not support routine testing based solely on this factor." (CDC 1998).

Miscellaneous Exposures to Blood

According to data from NHANES III, three percent of reported acute hepatitis C cases were attributed to "household contact." Murphy and colleagues reported an odds ratio of 1.6 (95% CI, 1.0–2.5) from sharing a toothbrush or razor; although biologically plausible, these are unlikely modes of HCV transmission (E. L. Murphy 2000).

Acute HCV surveillance by CDC over the last 20 years has found that less than one percent of reported acute HCV infections were associated with tattooing. According to CDC, "no data exist in the United States indicating that persons with exposures to tattooing alone are at increased risk for HCV infection" (CDC 2001).

Sexual Transmission of HCV

HCV can be sexually transmitted. Hepatitis C virus RNA has been detected in semen, vaginal fluid, and cervical smears (Caldwell 1996; Gameiro 2001; Leruez-Ville 2000; Manavi 2002; Nyamathi 2002; Pasquier 2003; Tang 1996). Questions about the likelihood of sexual transmission of HCV continue to bedevil researchers, public health educators, doctors, epidemiologists and, most importantly, HCV-infected people, their partners, and sexually active individuals. The risk

associated with specific sexual acts, and the role of possible cofactors that may facilitate sexual transmission of HCV, have not been fully explored. Unfortunately, many of the existing studies have failed to question people in sufficiently extensive detail about their sexual behaviors. Specific sexual practices may have a greater influence on HCV transmission than we are currently aware.

The National Institute of Health's *Consensus Development Conference on Management of Hepatitis C* estimates that the prevalence of HCV is between two to three percent among long-term, monogamous sexual partners of HCV-infected individuals; the estimated risk of transmission is 0–0.6% annually, though in heterosexual couples the risk of male-to-female transmission is three-fold greater. They suggest that "because of the low risk of HCV transmission, [these heterosexual] couples need not use barrier protection (condoms); however, couples should be advised that the use of condoms may decrease the risk of HCV transmission." Because HCV prevalence is higher among people with multiple sex partners, men who have sex with men and sex workers, condom use is "advised…to prevent transmission of HCV and other sexually transmitted diseases."

Studies of long-term, monogamous serodiscordant heterosexual partners have shown a low rate of male-to-female HCV transmission. Three of 106 women (2.7%) from a cohort of long-term sex partners of anti-HCV-positive male hemophiliacs were anti-HCV-positive (Brettler 1992). However, these three women had other potential exposures to HCV: one had a former partner who was an injection drug user; another had jaundice and a prior blood transfusion; the third had worked as a nurse.

In the late 1970s, a group of Irish women were infected with hepatitis C from a batch of anti-D immunoglobulin. In March 1994, they were notified. Since then, two studies of these women and their male partners have examined the possibility of female-to-male sexual transmission of HCV. Neither study found any cases of female-to-male HCV transmission (Meisel 1995; Sachithanandan 1997).

Sometimes, HCV infections from other sources can be attributed to sexual transmission. Zylberberg and colleagues studied 24 HCV-infected couples. All 48 participants provided risk information and blood samples that were tested for HCV RNA, genotype, and in some cases, phenotype. Twelve pairs of partners had matching genotypes; virus from the other twelve couples differed in genotype between partners. A phylogenetic analysis of virus from seven couples showed that four pairs were infected with different viral strains, suggesting that each partner had acquired HCV independently. The remaining three couples with similar strains each had at least one other parenteral risk factor (Zylberberg 1999).

Certain factors may increase the risk of sexual transmission of HCV. A study of the presence and predictors of HCV RNA in semen from 80 men reported that higher HCV virus loads in blood were associated with the presence of HCV RNA in semen, as well as current use of alcohol (Nyamathi 2002). NHANES III found an association among HCV infection and sexual intercourse prior to age 18, multiple sex partners (especially more than 50 partners), and herpes simplex infection. Fifteen percent of acute HCV infections in the U.S. between 1992 and 1995 were attributed to sexual risk factors—a rate that has increased to 18% since 1995 (M. J. Alter 1998; M. J. Alter 2002).

Sexual Transmission of HCV from an HIV-Coinfected Partner

A cross-sectional study at ten hemophilia centers evaluated HCV and HIV transmission rate from 231 male hemophiliacs to their female sex partners. Just 2.6% of the female partners were HCV-antibody positive. No woman with an HCV monoinfected partner had antibodies to HCV. Every anti-HCV-positive female partner had an HIV/HCV coinfected partner. In contrast, 12.8% of the women with HIV-positive male partners and 13% of those with HIV/HCV coinfected partners were also HIV-positive (Eyster 1991). Although sexual transmission of HCV is less efficient than sexual transmission of HIV, coinfection with HIV may increase the risk of sexual transmission of HCV.

Another study found an almost two-fold greater increase in HCV transmission from coinfected partners. Nine of 98 (9.2%) individuals with coinfected partners were anti-HCV-positive; anti-HCV prevalence dropped to 2 of 49 (4.1%) among partners of individuals with HCV alone (P=0.2) (Lissen 1993). Higher HCV RNA levels may increase the risk of HCV transmission. HCV RNA levels increase after HIV infection, and may remain up to ten times higher than in HCV mono-infection (Bonacini 2000, Collier 1998).

One study has looked at factors influencing the presence of HCV RNA in the semen of 35 HIV/HCV coinfected men. HCV RNA was detectable (intermittently) in the semen of 25.7% (9). No correlation between the presence of HCV RNA in semen and the amount of HCV RNA in blood was found, nor was there any correlation with the presence of seminal HCV RNA and duration or treatment of HIV infection, CD4 cell count, HIV viral load, or presence of HIV RNA in semen (Pasquirer 2003).

The Multicenter Hemophilia Cohort study found a three-fold increase in HCV viral load of coinfected men, although there was no significant association between higher HCV RNA levels and sexual transmission. One of 42 males with HCV monoinfection transmitted hepatitis C to his female partner; 20 of the 343 coinfected men transmitted HCV to their female partners (2.3% vs. 5.8%). While HCV viral load was significantly higher among coinfected men, men with HCV-positive partners did not have significantly higher HCV viral loads than those with HCV-negative ones. Each one-log increase in HIV viral load increased HIV transmission risk by 1.37-fold, although higher HCV viral loads were not significantly associated with increased risk of HCV transmission (Hisada 2000).

The presence of other sexually transmitted diseases (STDs) may be a possible cofactor for HCV transmission. An analysis of non-IDU patients at an STD clinic found HCV antibodies among 7% of men and 4% of women. Females with an HCV-positive male sex partner were 3.7 times more likely to be positive than those with HCV-negative male sex partners (Thomas 1995). The presence of HCV antibodies was associated with greater numbers of sex partners and with other STDs (HIV and trichomonas).

Sexual Transmission Among Men Who Have Sex With Men (MSM)

Higher-than-average HCV prevalence has been documented among men who have sex with men (MSM) in a number of studies, but controversy about the risk of sexual transmission due to specific sexual acts remains unresolved, as information about particular sexual acts was not collected

consistently or with sufficient detail. More detailed information on sexual behavior is needed to help guide prevention initiatives and risk-reduction strategies for MSM.

One study associated specific sexual practices with HCV infection in a group of MSM. Craib and colleagues studied blood samples and self-administered questionnaires from 662 MSM. The questionnaires were completed between 1982 and 1985, and the serum samples were collected between 1982 and 1998—therefore, some of the samples were collected years after the completion of questionnaires, and may not have reflected changes in sexual behaviors over time. HCV antibodies were detected in 39 (5.9%) study participants. HIV-positive men were significantly more likely to have HCV antibodies than HIV-negative men (31/352, or 8.8% vs. 8/310, or 2.6%; $P<0.001$). Nineteen HCV-positive MSM reported no history of IDU. Questionnaire data from these 19 men was compared to data from 589 HCV-antibody negative men with no IDU history. Oral-anal contact (rimming) and insertive fisting were significantly associated with HCV ($P=0.029$ for rimming; $P=0.012$ for insertive fisting) (Craib 2001).

Other studies failed to significantly correlate specific sexual acts or number of sex partners and HCV infection. Bodsworth and colleagues studied 1,075 homosexual or bisexual men in Sydney, Australia, and found a 7.6% HCV seroprevalence. Although more HIV-positive men had HCV antibodies than HIV-negative ones (OR, 3.14; $P<0.0001$), no significant difference was reported in the number of sex partners between HCV-negative and HCV-positive men. HCV-negative men reported engaging in anal receptive intercourse without ejaculation, unprotected oral-anal sex (rimming), and insertive fisting more frequently than HCV-positive men. The only factors significantly associated with anti-HCV-positivity were IDU in the previous six months (OR, 7.24; $P<0.001$) and HIV infection (Bodsworth 1996).

Buchbinder and colleagues recruited 435 homosexual men from an STD clinic in 1983–1984. When stored plasma samples were tested for anti-HCV antibodies and behavioral data were analyzed, a history of IDU was the only variable independently associated with anti-HCV-positivity. After controlling for IDU, no significant association was found between sexual practices and anti-HCV-positivity. Overall HCV seroprevalence was 9.2%; among men with a history of IDU it was 25%, vs. just 5% among non-IDUs, making it harder to assess sexual risk factors due to the small sample size.

Donahue and colleagues found HCV seroprevalence of 1.6% in a cohort of 926 homosexual men. There was no association between anti-HCV seroprevalence and the number of sex partners, anal or oral receptive sex, HIV status or history of STD infection. The only associations found were with history of IDU and prior hepatitis A infection (Donahue1991).

Danta and colleagues identified 23 cases of acute HCV in a group of HIV-positive MSM between 2001 and 2003. All had engaged in unprotected anal intercourse; 70% identified fisting and group sex as risk factors and 43% had other sexually transmitted infections, while only 17% had injected drugs (Danta 2003).

Sexual transmission of HIV between men is far more efficient than male-to-male sexual transmission of HCV. A cohort analysis of European homosexual men estimated a cumulative incidence of anti-HCV positivity of 4.1% between 1981 and 1984, with no seroconversions between 1984 and

1989 (Melbye 1990). In the same cohort, the cumulative incidence of HIV from 1981 to 1984 went from 8.8% to 24%; between 1984 and 1989, incidence rose to 30.1%.

Mother-to-Infant Transmission

One hundred and seventy million people worldwide are infected with hepatitis C; of these, 35% are women in childbearing years. Using a conservative estimate of the likelihood of mother-to-infant transmission, with an annual fertility rate of 2%, between 10,000 and 60,000 babies will be infected with HCV each year (Yeung 2001). Mother-to-infant transmission rates vary widely; in their meta-analysis of 77 mother-to-infant transmission studies, Yeung and colleagues found HCV transmission rates ranged from zero to 35.3%. Roberts and colleagues found higher weighted rates of mother-to-infant transmission among viremic Japanese and Italian mothers (6.9% and 5.6%, respectively) than among other viremic women studied (3.1%) (Roberts 2002).

Careful follow-up and repeat testing of babies born to mothers with HCV infection is necessary to ensure proper diagnosis or exclusion of HCV infection. Maternal antibodies to HCV can be found in uninfected infants for up to 18 months after birth, and RNA levels can seesaw from undetectable to detectable while liver enzymes stay normal (Thomas 1999). In an analysis of 441 HCV-positive mothers and their infants, 50% of the uninfected infants became anti-HCV-negative by eight months, and 95% were anti-HCV-negative by 13 months. PCR sensitivity increased with age; in the infant's first month, sensitivity was 22%; after one month of age, sensitivity rose to 97% (Gibb 2000).

The specific mechanisms involved with mother-to-infant transmission are unclear; studies suggest that multiple factors may be involved. Maternal HCV transmission may occur in utero, intrapartum, or postpartum. Resti and colleagues found that 6 of 13 infants who acquired HCV infection had detectable HCV RNA directly after birth, which argues for in utero transmission of HCV (Resti 1998). The presence of HCV RNA in cord blood is difficult to interpret because of potential contamination with maternal blood; therefore it cannot be interpreted definitively as evidence of mother-to-infant transmission in utero. Although HCV RNA has been detected in amniotic fluid, its presence does not offer de facto proof of in utero HCV transmission (Delamare 1999). Azzari and colleagues have observed a greater likelihood of mother-to-infant transmission when active viral replication in peripheral blood mononuclear cells (PBMCs) is present. Replicating HCV was found in PBMCs from 5 of 13 mothers who had HCV-infected infants; none was detected in the mothers who had uninfected infants ($P=0.0001$) (Azzari 2000).

The effect of mode of delivery remains controversial. Some research has found a greater likelihood of mother-to-infant transmission of HCV with vaginal delivery; other research has failed to support this conclusion (Conte 2000; Granovsky 1998; Okamoto 2000; Paccagnini 1995; Resti 1998; Roberts 2002; Tajiri 2001). Delivery via elective caesarean section may lower the risk of mother-to-infant transmission of HCV. In a meta-analysis of 363 cases of mother-to-infant transmission, the rate of HCV transmission was 4.3% for vaginal deliveries vs. 3.0% for elective caesarean deliveries (Yeung 2001).

The single factor that has been linked to mother-to-infant transmission across many studies is the level of maternal HCV RNA. Although there is no known transmission threshold for HCV RNA,

a lower viral load should reduce the likelihood of transmission. A meta-analysis of 77 mother-to-infant transmission studies, using consistent criteria for identification of infected infants, identified a rate of transmission of 1.7% for mothers with antibodies to HCV; when this analysis was restricted to viremic mothers, the rate rose to 4.3% (Roberts 2002). A study of 105 HCV-infected mothers and their infants found a 6.6% rate of transmission; only viremic mothers transmitted HCV (Dal Molin 2002). Other mother-to-infant transmission studies of 63, 30, and 22 HCV-monoinfected women reported that all infected infants had viremic mothers (Sabatino 1996; Spencer 1997; Resti 1995). In an examination of potential risk factors among seven HCV-infected infants, Ohto and colleagues found that non-transmitting mothers had lower HCV titers than transmitting mothers (Ohto 1994). HCV RNA levels may increase during the second and third trimesters of pregnancy (Gervais 2000; Paternoster 2001).

Women coinfected with HIV generally have higher HCV RNA levels, which are associated with a greater risk of mother-to-infant transmission of HCV (Okamoto 2000; Roberts 2002; Tajiri 2001; Yeung 2001). In a meta-analysis of eight studies on HCV transmission among mothers with and without HIV, the rate of hepatitis C transmission from coinfected mothers to their infants was 19.4% vs. just 3.5% in mothers with HCV alone (Yeung 2001). The Women and Infants Transmission Study (WITS) followed 155 infants born to HIV/HCV coinfected mothers for 36 months after birth. Overall, 8.4% (13) of the infants were infected with HCV. The incidence of HCV infection among the HIV-positive infants was 3.2-fold higher than that of HIV-negative infants. While 17% (7/41) of the HIV-positive infants were coinfected with HCV, only 5% (6/112) of the HIV-negative infants were infected with HCV (Thomas 1998). In the Mothers and Infants Cohort Study, incidence of mother-to infant transmission of hepatitis C was 4% among HCV-infected mothers and 7% among HIV/HCV coinfected mothers (Granovsky 1998).

The hepatitis C virus has been detected in genital secretions from HIV/HCV coinfected women (Nowicki 2003; Rakela 2003); this may be involved with mother-to-infant transmission of hepatitis C. Rakela and colleagues found HCV RNA in the cervicovaginal lavage (CVL; a washing technique) from 18/62 (28%) HIV/HCV coinfected women. The presence of HCV RNA in CVL was significantly associated with the presence of HIV RNA in CVL ($P=0.03$) (Rakela 2003).

Researchers have found an increased risk of mother-to-infant transmission among women with a history of IDU or current IDU. In an analysis of six studies which included mothers with and without past or current IDU, the rate of HCV transmission among mothers with a history of IDU was 8.6% vs. 3.4% among mothers with no IDU history (Roberts 2002). A multisite study of 1,372 HCV-infected mothers and their infants found increased rates of mother-to infant transmission in current and former IDUs; no difference in the rate of hepatitis C transmission by HIV status was observed. The overall rate of mother-to-infant transmission of HCV was 7.1% (98/1,372). Among a subgroup of 1,178 HIV-negative mothers, transmission from those with a history of IDU was 10.8% (33/305) vs. 4.8% (42/873) from those without. A few mothers were active IDUs during pregnancy (23/ 461); 13% of this group transmitted HCV to their infant (Resti 2002). In another study, Resti and colleagues followed 403 mother-infant pairs for over two years. Transmission rates were significantly lower among women with no known risk for hepatitis C than women with a history of transfusion or IDU ($P=0.0063$). Mother-to-infant transmission of hepatitis C occurred in 8% of infants born to women with a history of IDU and 10% born to mothers who had received a transfusion, vs. 1% transmission from mothers with no known risk.

Although HCV RNA has been found in breast milk and colostrum, breastfeeding does not appear to pose a significant risk for mother-to-child transmission of HCV, so long as the mother's nipples are intact (Kumar 1998; Roberts 2002). In an analysis of ten studies of mother-to-infant transmission of HCV, the rate of transmission among breast-fed infants was 3.7% vs. 3.9% for non-breast-fed infants (Yeung 2001). The risk of mother-to-infant transmission from breastfeeding may increase when mothers have higher virus loads or postpartum flares of hepatitis C.

There are no known interventions to decrease the risk of mother-to-infant HCV transmission. Elective caesarean sections may slightly decrease mother-to-infant transmission of HCV, but this remains controversial. Treatment of HCV during pregnancy is contraindicated. Ribavirin is known to be teratogenic. Interferon is contraindicated in infants less than two years old because of neuro-toxicity. A large observational study utilizing state-of-the-art, standardized diagnostic guidelines for mothers and infants is needed to identify routes of transmission and risk reduction strategies. In addition, all pregnant women should be offered HCV testing as part of their routine prenatal care.

Recommendations

Implement national surveillance for chronic hepatitis C infection.

The Center for Disease Control's (CDC's) Third National Health and Nutrition Examination Study (NHANES III), conducted from 1988 to 1994, estimated that 1.8% of the United States population—or 4 million people—have been infected with HCV; 2.7 million remain chronically infected. NHANES III may have significantly underestimated the true prevalence of HCV infections in the United States since incarcerated and homeless individuals were not included in the populations surveyed. HCV prevalence among this country's 1.8 million incarcerated persons is estimated at 30% to 40% (Reindollar 1999). A 2002 survey of 597 homeless veterans found an HCV seroprevalence of 41.7% (Cheung 2002). Epidemiological studies need to include high-risk and high-prevalence populations to obtain accurate estimates of hepatitis C prevalence.

The CDC's Sentinel Counties Study of Viral Hepatitis provides data on the incidence of acute HCV infections. At present, only a pilot program—sentinel surveillance for physician-diagnosed chronic liver disease—tracks both acute and chronic HCV infections. National surveillance of chronic hepatitis C infections is necessary to forecast disease burden and provide a sound basis for planning allocation of adequate resources for prevention, education, care, and treatment programs. Funding for implementation of the CDC's 2002 *Guidelines for Viral Hepatitis Surveillance and Case Management* must be allocated by Congress and the Administration.

Clarify the risk of non-injection drug use behaviors associated with HCV transmission.

Conflicting data have emerged about the risk of HCV infection from intranasal drug use (i.e., snorting or sniffing)(Conry-Cantilena 1996; Murphy, 2000). There has also been speculation about HCV transmission from shared crack pipes, since frequent users often have burned or split lips from heated glass crack pipes. NHANES III participants were asked about drug-use history, although they were not specifically asked whether they had ever injected drugs. Because drug-taking modes—snorting or smoking vs. injecting—were not recorded in NHANES III, no estimate of the actual incidence of HCV transmission via intranasal drug use can be made from those data.

Research on the risk of intranasal drug use must clarify questions about this mode of drug administration as a potential route of transmission. Further investigation of the risk of HCV infection from smoking crack or other drugs is also needed. Studies must be designed to elicit accurate information about drug use. Pending more definitive data, educators and medical providers should incorporate appropriate and responsible messages on intranasal transmission risk. The National Institutes of Health and the Centers for Disease Control must fund research on HCV transmission from non-injection drug use.

Clarify routes and risks of sexual HCV transmission.

HCV can be sexually transmitted, although the relative risk and mechanism of sexual transmission remain controversial. A number of studies have documented higher-than-average anti-HCV prevalence among men who have sex with men (MSM), sex workers, individuals who have had multiple partners, and partners of HIV/HCV-coinfected individuals (M. J. Alter 1988; M. J. Alter

2002; Bodsworth 1996; Buchbinder 1994; Eyster 1991). Most research on sexual transmission of HCV has not collected information about specific sexual acts.

Research on HCV transmission must employ direct questions about sexual behaviors. Mucosal transmission by oral, penile, vaginal, and anal routes must be investigated as well as sexual practices that may involve the exchange of blood. Information about the risk associated with specific sexual practices is needed to inform prevention program messages and individual decision-making about risk reduction to prevent HCV transmission. NIH and CDC must fund research on routes and rates of sexual transmission of HCV among MSM, individuals with multiple partners, and partners of HIV/HCV-coinfected individuals.

Research mechanisms and interventions to decrease the rate of mother-to-infant HCV transmission.

If 35% of the 170 million people infected with hepatitis C worldwide are women in childbearing years with an annual fertility rate of 2%, 10,000–60,000 newborns will be infected with HCV each year (Yeung 2001). At present, no interventions have been identified to prevent mother-to-infant transmission of HCV, although some data suggest that transmission may be reduced by elective caesarean (Okamoto 2000; Paccagnini 1995; Yeung 2001). Well-designed studies using standardized diagnostic guidelines for mothers and infants will elucidate factors involved in mother-to-infant transmission of HCV and assist in development of strategies for risk reduction and prevention of mother-to-infant transmission. This research should be funded by NIH and CDC.

Develop and implement HCV prevention strategies for the developing world.

Globally, an estimated 170 million people, or 3% of the world's population, may be infected with hepatitis C (World Health Organization in collaboration with the Viral Hepatitis Prevention Board 1999). HCV infections in the developing world are mainly acquired from unscreened, contaminated blood transfusions, unsterilized medical and dental equipment, and unsterilized instruments used for circumcision, scarification, tattooing, and traditional medicine. In some regions, injection drug use is also a major mode of HCV transmission. In developing and transitional countries, approximately 16 million injections are given each year in formal and informal medical settings; one-third are with reused, unsterilized injection equipment (Hutin 2003). An estimated 2.3 to 4.7 million new HCV infections occur each year in the developing world as a result of unsafe injections (Kane 1999).

In resource-poor settings, prevention of new HCV infections must be a priority. This will include implementing screening of donor organs, blood, and blood products; offering training on viral inactivation techniques, infection control procedures, and proper methods of sterilizing medical equipment (including injection equipment); promoting harm reduction; and providing access to sterile injection equipment for injection drug users. Prevention interventions need to be adapted to specific regions, cultures, and settings.

As antiretroviral scale-up occurs, strategies for prevention and treatment of HCV should be implemented in regions where HCV is prevalent, such as the former Soviet Union. The World Health Organization (WHO) must fund HCV prevention initiatives.

Provide HCV testing and education for high-risk and high-prevalence populations.

Less than half of United States city and county health departments provide hepatitis C counseling, and only 23% provide testing for HCV (National Association of City and County Health Officers. Hepatitis C/HIV Needs Assessment 2000). CDC must provide funding to make free, voluntary HCV testing available to high-risk and high-prevalence populations through city and county health departments.

In the United States, 94,000 injection drug users are between the ages of 12 and 17 (National Household Survey on Drug Abuse, 2000 and 2001). The incidence of HCV infection is highest among new injectors, with an estimated 50% to 80% of injection drug users (IDUs) becoming infected within a year of initiating injection drug use (Garfein 1996). Education about HCV transmission must be provided to young people before they become sexually active or begin injecting drugs. Information about prevention, transmission, diagnosis, natural history, and treatment of HCV must be provided and integrated within program activities for staff and clients of detoxification facilities, drug treatment programs, shelters, methadone maintenance programs, correctional facilities, and AIDS service organizations. Hepatitis C advocacy organizations can provide HCV educational materials and information; AIDS service organizations can be an important source of HIV-related information for clients of hepatitis C organizations. Collaboration among these entities will benefit people with HCV, the coinfected, active and recovering drug users, the homeless, and others who are infected or at risk. Public health funding from Congress and the Administration must be made available to support education, and state-contracted agencies must provide these services.

Institute CDC's recommendations for prevention of HCV transmission in hemodialysis facilities.

People receiving kidney dialysis are at risk for acquiring HCV infection when dialysis centers do not practice proper infection control procedures. A study that screened dialysis recipients for HCV antibodies at 40% of U.S. dialysis centers during December of 2000 reported that 8.4% tested positive (Tokars 2002). Other studies of dialysis recipients have reported anti-HCV prevalence ranging from 10% to 36% among adults and 18.5% among children (CDC 2001).

In April of 2001, CDC issued recommendations for preventing transmission of pathogens among chronic hemodialysis patients that included stricter infection control practices, regular monitoring of ALT levels, and HCV testing of dialysis recipients (CDC 2001). All dialysis facilities must implement these recommendations and be monitored by the appropriate licensing and regulatory bodies.

Increase access to sterile injection equipment.

More than three million people in the United States are injection drug users (National Household Survey on Drug Abuse, 2000 and 2001). Although a majority of new HCV infections in the United States result from drug injection using shared, unsterilized equipment, it has not been widely and openly acknowledged that hepatitis C is a disease of drug users. HCV prevalence among IDUs is estimated at 70% to 90% (M. J. Alter 1998; Donahue 1991; Garfein 1996; Thomas 1995). There is

ample potential for HCV transmission among new IDUs via shared syringes and other injection drug equipment (Thorpe 2002; Vidal-Trecan 2002).

Inadequate access to sterile syringes and injection equipment and restrictive one-for-one syringe exchange policies continue to fuel both the HCV and HIV epidemics. Federal, state and local barriers to syringe exchange programs must be removed; program expansion will require an increased commitment of resources and hence the overturning of the laws against syringe exchange funding. Legislation must be enacted to legalize pharmacy sale of syringes in the remaining states that prohibit over-the-counter sales without prescriptions, and all state and local public health programs must ensure that pharmacy sale of syringes is accessible and affordable.

Research on the efficacy of bleach as a disinfectant and identification of optimal disinfection practices for injection drug equipment should be funded by NIH and CDC.

Increase access to drug treatment and methadone maintenance programs.

Policies that create barriers to risk reduction must be changed. Currently, access to methadone is limited; of an estimated 810,000 opiate-dependent persons, only about 40,500 are known to be receiving methadone maintenance treatment (MMT) (Office of National Drug Control Policy 2000). According to the 1997 NIH Consensus Statement, *Effective Medical Treatment of Opiate Addiction*, "Of critical importance in improving MMT of opiate dependence is the recognition that, as in every other area of medicine, treatment must be tailored to the needs of the individual patient. Current Federal regulations make this difficult if not impossible. However well intended the FDA's treatment regulations were when written in 1972, they are no longer helpful. We recommend that these regulations be eliminated."

Methadone should be available by prescription to all those who need it, and coverage should be provided by private and public insurers.

In 2001, 6,096,000 Americans needed treatment for drug addiction, yet only 17.3% (1,054,000) received treatment at a facility specializing in addiction (National Household Survey on Drug Abuse 2001). Drug treatment must be available on demand.

Develop protocols for HCV counseling and testing for pregnant women, and offer voluntary HCV counseling and testing to pregnant women.

Infection through mother-to-infant transmission of HCV occurs in approximately 5% of children born to mothers with HCV (Yeung 2001). Screening pregnant women for hepatitis C is not a routine part of prenatal care, yet some pregnant women want to be tested for hepatitis C. The draft guidelines from the National Institute of Health's *Consensus Development Conference on Management of Hepatitis C: 2002* do not offer any guidance for HCV testing of infants or pregnant women. We urge CDC to develop guidelines for voluntary HCV counseling and testing of pregnant women and to recommend their incorporation as a routine part of prenatal care.

List of Terms Used in This Chapter

Anti-HCV: antibodies to hepatitis C.

Anti-HCV negativity: no antibodies to hepatitis C detected in the blood.

Anti-HCV-positivity: antibodies to hepatitis C detected in the blood.

Anti-HCV prevalence: the percentage of a population or group that has antibodies to hepatitis C at a given time.

Genotype: the genetic makeup of an organism.

HCV-negative: no antibodies to hepatitis C detected in the blood.

HCV-positive: antibodies to hepatitis C detected in the blood.

Incidence: the rate of occurrence of new cases of a particular disease in a population or group being studied.

Phenotype: visible characteristics of an organism created by the interaction of the genotype and the environment.

Phylogenetic: the evolutionary history of a virus.

Prevalence: the number of individuals with a condition in a specific population or group.

Seroprevalence (also HCV seroprevalence): the frequency of individuals in a population or group that have a particular element (antibodies to hepatitis C) in the serum of their blood.

Serostatus: the presence or absence of antibodies to an organism.

Serodiscordant: having a different serostatus than another person; used to describe a couple in which one person is anti-HCV positive and the other is anti-HCV negative.

II. Natural History of Hepatitis C

Summary

The ultimate outcome of infection with hepatitis C can vary dramatically. While some people will clear the virus at the outset, others will develop a chronic infection that, over years and decades, may remain benign, progress in severity, or turn deadly. In chronically infected persons, the disease usually advances very slowly but in rare cases can prove fatal within a few years. One individual may sustain only mild-to-moderate liver scarring after twenty years, while another during that time will develop serious liver damage, such as cirrhosis (severely scarred liver tissue), hepatocellular carcinoma (HCC; liver cancer), or hepatic decompensation (liver failure).

Most people are asymptomatic during acute HCV infection. When symptoms develop, they appear approximately six weeks after exposure to HCV and can be described as flu-like, with the exception of jaundice (Koretz 1993). These symptoms resolve a few weeks after their onset. In very rare instances, acute HCV infection has caused fulminant hepatitis (sudden and severe; often liver failure is involved) (Chu 1999; Farci 1996).

Not everyone with acute hepatitis C infection develops chronic HCV infection. Studies show that a wide range—15% to 45%—of acutely infected people develop antibodies to hepatitis C, but do not progress to chronic HCV infection (Alberti 1999; M. J. Alter 1992; M. J. Alter 2002; Gerlach 2003; Kenny-Walsh 1999; Seeff 2000). Viral clearance is the spontaneous elimination of hepatitis C virus from the bloodstream, although evidence of previous infection—antibodies to hepatitis C—usually remains. Viral clearance may be partially attributed to a combination of viral factors and host factors such as age, sex, race, history of a prior cleared hepatitis C infection, and HIV status.

For the 55% to 85% of individuals who do not achieve spontaneous viral clearance, hepatitis C infection is diagnosed as chronic when HCV-antibody testing is positive and HCV RNA (viral load) is detectable on at least two occasions over a six month period. Most people with chronic HCV infections remain asymptomatic for years, although some individuals will experience fatigue, depression, and other extrahepatic manifestations of hepatitis C infection. Not all chronically infected people will develop liver damage; if liver damage does develop, it may take from 10 to 50 years to do so. No clinical, serologic, or virologic feature—such as the HCV viral load, liver enzyme level, or HCV genotype (the particular strain of the hepatitis C virus)—can reliably forecast the outcome of untreated HCV infection, although some prognostic factors have been identified.

The clinical complications of hepatitis C may include fibrosis (mild scarring of liver tissue), cirrhosis and hepatocellular carcinoma. Fibrosis may be accelerated by several factors, including older age (>40 years) at infection, moderate-to-heavy alcohol consumption, male sex, concomitant hepatitis B infection, and coinfection with HIV. Fibrosis progression may be more rapid in individuals who were infected with hepatitis C from transfusions (S. C. Gordon 1998).

Some individuals who have been diagnosed with compensated cirrhosis (severe liver scarring without clinical complications; the liver is still functioning) will remain stable for years. Others develop hepatic decompensation or hepatocellular carcinoma. An estimated 1–4% of cirrhotics per year will develop hepatocellular carcinoma.

Liver transplantation is the only intervention for hepatic decompensation. Hepatitis C-related liver damage is the leading indication for liver transplantation in the United States (CDC 1998). Between 10,000 and 12,000 deaths each year are attributed to hepatitis C-related hepatocellular carcinoma and end-stage liver disease.

Many studies have tracked the natural history of hepatitis C infection. Although much has been learned about what can take place during the first 20 years of infection with hepatitis C virus—prognostic factors have been identified and disease progression models developed—outcomes after the second decade of infection remain largely uncharted. To date, only one small (n=17) study has evaluated outcomes 45 years postinfection (Seeff 2000). Yet, despite the lack of longer-term data, observations from several existing natural history studies can illuminate the risk and rate of disease progression.

Acute HCV Infection

The acute stage of hepatitis C infection remains clinically silent for most infected people, with only 15% to 20% of individuals developing symptoms (Koretz 1993). When they occur, symptoms such as low-grade fever, fatigue, appetite loss, abdominal pain, nausea, and vomiting usually appear during the sixth or seventh week after infection and resolve within a few weeks (CDC 1998; Koretz 1993). Jaundice, a classic sign of hepatitis, appears in only 15% to 20% of acutely infected individuals (Marcellin 1999; Villano 1999); yet, in rare instances, fulminant hepatitis may develop during acute HCV infection, leading to liver failure and death (Farci 1996). The risk of developing fulminant hepatic failure during acute HCV infection may be increased in those with concomitant chronic hepatitis B infection (Chu 1999).

Study of the course of acute HCV infection has proven to be extremely difficult for several reasons. To begin with, identification of people with acute hepatitis C is challenging, since many individuals do not have symptoms or, when they do, they are easily mistaken for those of other common viral infections. Moreover, there is no specific test that will distinguish acute stage hepatitis C from chronic hepatitis C infection (see Chapter V, Diagnostics).

Hepatitis C infection is often not suspected in the clinic. One study reported that health care providers recognized none of 32 acute HCV infections during routine visits among a group of injection drug users (Villano 1999). Prospective studies have been performed in relatively homogenous populations. Although these studies have provided important insights, their relevance may be limited.

It is well established that not all acutely infected people will develop chronic HCV infection, although it is far from clear how many are spared. Published studies suggest that the likelihood of spontaneously clearing hepatitis C ranges broadly, from 15% to 45% (Alberti 1999; M. J. Alter 1992; M. J. Alter 2002; Gerlach 2003; Kenny-Walsh 1999; Seeff 2000). Viral clearance or resolved hepatitis C infection is usually recognized by the presence in the blood of antibodies to HCV and the absence, at least twice, of HCV genetic material (RNA).

The determinants of HCV clearance are multifactorial. There is evidence that the younger a

person is at the time of HCV infection, the less likely they are to become chronically infected. Seventeen years after a cohort of 67 children had been infected with hepatitis C from blood transfusions during cardiac surgery, Vogt and colleagues found that only 37 of them (55%) had detectable HCV RNA. In another study of viral clearance of nosocomically acquired (an infection originating in the hospital) acute HCV, Larghi and colleagues followed 14 people, aged 21 to 45. Seven spontaneously recovered within 13 months of infection, and at month 24, an eighth person cleared HCV infection, reflecting a clearance rate of 57% (Larghi 2002).

Symptomatic individuals appear to be far more likely to achieve spontaneous viral clearance of hepatitis C than asymptomatic persons (Gerlach 2003; Ross 2004; Villano 1999). Gerlach and colleagues identified 60 individuals with acute hepatitis C between January 1993 and August 2000. They reported that all 24 (52%) who achieved spontaneous viral clearance were symptomatic, while none of nine asymptomatic persons cleared their HCV infections ($P=0.007$ for symptoms vs. no symptoms). With the exception of sex (women were more likely to clear HCV than men; $P=0.034$), there were no significant differences in age, mode of acquisition, HCV genotype or viral load between those who achieved spontaneous viral clearance and those who developed chronic HCV.

Young women may have a high likelihood of clearing hepatitis C infection (Kenny-Walsh 1999; Koretz 1992; Wiese 2000). Seventeen years after a cohort of 704 young Irish women were exposed to hepatitis C from contaminated anti-D immunoglobulin, only 390 (55%) had detectable HCV RNA (Kenny-Walsh 1999). The remaining 45% were HCV-antibody-positive, but had no detectable viremia. Wiese and colleagues observed similar rates of viral clearance among young women in another cohort ($n=917$) exposed to contaminated anti-D immunoglobulin. Of the 85% (779/917) with antibodies to hepatitis C, only 55% (428/779) had detectable HCV RNA (Wiese 2000).

Two studies have reported a decreased likelihood of spontaneous viral clearance among African Americans. Villano and colleagues recruited 142 HIV-negative members from the AIDS Linked to the Intravenous Experience (ALIVE) cohort in Baltimore (a group of 2,921 current and former injection drug users, enrolled between 1988 and 1989) and subsequently characterized 43 cases of acute HCV infection, 28 (65%) among African Americans. Participants were tested repeatedly for HCV RNA over a median follow-up period of 72 months. Spontaneous viral clearance occurred in 6 of 43 (14%) individuals, 4 of whom (67%) were white. Those who cleared their HCV infection were more likely to be white ($P=0.004$), to have experienced jaundice ($P=0.03$), and to have a lower peak viral load ($P=0.003$) (Villano 1999). Thomas and colleagues also observed less frequent viral clearance among African Americans during observation of a cohort of 919 HCV-antibody-positive injection drug users, 729 of whom were African-American. At least five serum samples were collected from each participant over a period of 61 to 92 months, and HCV RNA testing was performed repeatedly to identify individuals who had cleared HCV. Viremia was persistent in 812 individuals, while 90 individuals achieved spontaneous clearance of HCV. Only 9% (10/90) of the individuals who achieved spontaneous viral clearance were African-American (Thomas 2000a).

The risk for developing chronic hepatitis C infection is dependent on the size of the viral inoculum at the time of exposure. Transfusion recipients appear to have a greater risk for developing chronic hepatitis C than individuals with other sources of infection (Alberti 2002).

It is possible to become re-infected with hepatitis C, or to have a mixed infection (identified by the presence of two or more genotypes of HCV). Cases of re-infection with HCV and HCV superinfection have been documented among hemophiliacs, injection drug users, and HCV-infected individuals with documented, nosocomially acquired re-infection (Accapezzato 2002; Jarvis 1994; Proust 2000). This indicates that neither having a prior, resolved infection nor possessing antibodies to HCV confers full protection against a subsequent HCV infection; however, some evidence suggests that having had a prior resolved infection may increase the chances of clearing a new HCV infection.

Mehta and colleagues studied a cohort of 1,344 injection drug users, identifying two sub-groups. One group of 98 individuals had evidence of previous, resolved HCV infection (antibody-positive, but undetectable HCV RNA on two occasions). The other group of 164 had no evidence of exposure to HCV. After a median follow-up of more than two years, 12% (12/98) of previously infected participants were newly infected with HCV. Among the group with no evidence of prior exposure, 21% (35/164) became infected with HCV, although this difference was not statistically significant. In a multivariate analysis, people with a previous, cleared HCV infection were 12 times less likely to develop chronic HCV infection (OR, 0.08; 95% CI, 0.01–0.46; P=0.02) (Mehta 2002).

Aitken and colleagues reported on evidence suggestive of immunity to hepatitis C in a cohort of 198 Australian injection drug users. Each IDU in the study referred up to ten others that they had injected with to investigators. All study participants were interviewed about their drug use, and provided a blood sample for HCV testing. Antibodies to HCV were detected in 86.9% (172/198), and HCV infection was confirmed by testing for HCV-RNA; 69.7% (138/198) had detectable HCV RNA.

Despite the probability of multiple exposures to HCV, 10.6% (21/198) had no evidence of HCV infection (antibodies or HCV-RNA). Five of them had been injecting drugs for at least nine years; their median duration of injection drug use was eleven years. During the preceeding six months, all five reported sharing injection equipment (including spoons, water, filters and drug solutions) with another study participant who had HCV. Two of the five had injected with a needle previously used by a person with HCV infection (Aitken 2004). The authors speculate that these injectors possessed an inherent or acquired immunity to HCV infection.

Chronic Hepatitis C Virus Infection

Symptoms of Hepatitis C

While some people with chronic hepatitis C infection are asymptomatic, others may suffer from a constellation of symptoms. Fatigue, arthralgia (joint pain), myalgia (muscle pain), and depression are the most commonly reported symptoms of chronic hepatitis C; however, the relationship between the presence of symptoms and the severity of HCV disease is unclear (Barkhuizen 1999; Goh 1999; Kenny-Walsh 1999).

Fatigue and musculoskeletal pain are characteristic symptoms of liver disease, especially chronic hepatitis C. Barkhuizen and colleagues reviewed the charts of 239 hepatology outpatients,

comparing the incidence of fatigue and musculoskeletal pain among individuals with HCV infection, individuals with HBV infection and individuals with alcoholic liver disease. Significant associations between HCV infection and musculoskeletal pain (P=0.0001) and fatigue (P=0.001) were identified, with 81% of the HCV-infected participants reporting musculoskeletal pain as compared to 56% of those with other liver diseases. Fatigue was reported by 66% of HCV-infected individuals, 30% of those with alcoholic liver disease, and 29% of individuals with hepatitis B infection. No associations were identified between musculoskeletal pain and the extent of liver disease, aminotransferase levels, or mode of acquisition.

There is some evidence that hepatitis C-related fatigue may be linked with psychological factors. During psychiatric interviews with 50 people with chronic HCV, Dwight and colleagues found fatigue to be more closely related to the degree of depression than to the severity of liver disease (Dwright 2000). An Australian study of 115 HCV-infected liver clinic patients also found a strong correlation between fatigue and the psychological realms of depression, anxiety, somatization, interpersonal sensitivity, and hostility (McDonald 2002). In another study, the fatigue reported by 53% of 1,614 HCV-infected individuals was independently associated with depression (Poynard 2002a). Obhrai and colleagues evaluated fatigue and psychological disturbances in the following five patient groups: chronic HCV infection, chronic HCV infection with chronic alcohol abuse, alcoholic liver disease, chronic nonliver diseases, and healthy controls. This study identified HCV-related fatigue as more severe and intractable than fatigue related to the other conditions. In addition, those with chronic HCV were more depressed and experience more feelings of anger and hostility than those with other, nonliver-related chronic diseases (Obhrai 2001).

The interpretation of rates of depression among people with chronic hepatitis C, however, is typically complicated by a lack of comparison with similar populations. For example, injection drug users experience high rates of preexisting depression and mental illness; HIV infection has also been independently associated with high rates of depression (Brienza 2000; J. G. Johnson 1999). Psychiatric disorders including depression, post-traumatic stress disorder, and anxiety are common in veterans with hepatitis C (el-Serag 2002; Lehman 2002; Muir 2002; Yovtcheva 2001).Therefore, injection drug use history, military service, and HIV coinfection present potentially confounding variables in examining the contribution of HCV infection to depression.

Extrahepatic Manifestations of Hepatitis C

There are several extrahepatic manifestations of chronic hepatitis C infection, the majority of which take the form of immunologic disorders.

Table 1. Some Extrahepatic Manifestations of Chronic Hepatitis C Infection

Name of condition	Symptoms
Keratoconjunctivitis sicca	Dryness of the mucous membrane lining the eyelids and outer eye surface due to insufficient secretion of tears.
Lichen planus	Flat, itchy patches of skin, usually found on the wrists, shins, lower back, genitalia and sometimes, the scalp, where it can lead to hair loss.
Glomerulonephritis	Inflammation of the kidney's tiny blood vessels used for filtering waste.
Essential mixed cryoglobulinemia	Abnormal proteins in the blood that can cause blood to thicken and blood vessels to become inflamed; essential mixed cryoglobulinemia involves a mixture of different antibodies that can cause joint pain and swelling, spleen enlargement, and nerve, kidney and heart disease.
Sjögren's syndrome	Keratoconjunctivitis combined with inflammation of the joints and mouth dryness.
Porphyria cutanea tarda	Photosensitivity resulting in blisters and ulcerations of the skin in areas commonly exposed to sunlight, such as the face, ears and the backs of the hands. The skin in these areas may become fragile, with excessive pigmentation and excess hair.

Extrahepatic manifestations include keratoconjunctivitis sicca, lichen planus, glomerulonephritis, and essential mixed cryoglobulinemia. Between 42% and 70% of cryoglobulemic individuals are infected with hepatitis C (R. J. Johnson 1993). Kayali and colleagues did a meta-analysis of data on cryoglobulinemia and liver disease; their analysis suggests that the presence of cryoglobulins may be a prognostic indicator for increased risk of cirrhosis, although referral bias may have been a factor in their findings. Cryoglobulinemia can be treated with drugs that decrease inflammation and suppress the immune system or, in extreme cases, by plasmapheresis (removal of antibody-containing fluids from the blood). Successful HCV treatment (when virus is undetectable six months after completion of treatment) may decrease or eliminate symptoms of cryoglobulinemia. Some HCV-infected persons with long-term cryoglobulinemia appear to be at risk for developing B-cell non-Hodgkin lymphoma (Di Bisceglie 1998); however, hepatitis C infection has also been associated with the development of non-Hodgkin's B-cell lymphoma in the absence of cryoglobulin production (Silvestri 1997). The mechanism involved in HCV-associated lymphoma is unknown (Zuckerman 2002).

Keratoconjunctivitis combined with inflammation of the joints and mouth dryness is called Sjögren's syndrome. An association between chronic HCV and Sjögren's syndrome has been reported (Haddad 1992; Pawlotsky 1994). Sjögren's syndrome, an immunologic disorder, involves the progressive destruction of sweat, saliva, and tear glands (exocrine glands).

Porphyria cutanea tarda (PCT) is another complication of HCV infection. It is not clear if hepatitis C itself causes PCT, or if the damage HCV does to the liver results in PCT (Di Bisceglie 1998). PCT can be treated by the periodic removal of blood to reduce the iron level; medication; or successful treatment of the underlying HCV infection.

Autoimmune thyroid dysfunction, a cluster of conditions in which the immune system can either attack or stimulate thyroid tissue, has been associated with HCV infection (Hadziyannis 1997; Rocco 2001; Tran 1993). The prevalence of thyroid autoantibodies in persons with HCV infection ranges from 4.6% to 15% (Broussolle 1999). Among people infected with HCV, as in the general population, thyroid autoimmune dysfunction is more common among females (Broussolle 1999; Ganne-Carrie 2000; Huang 1999; Rocco 2001).

Hepatitis C infection may be a factor in the development of diabetes. An analysis of the data from the Third National Health and Nutrition Examination Study (NHANES III) found anti-HCV-positive people 40 years of age or older were over three times more likely to have type 2 diabetes than those without HCV infection (Mehta 2000). In a retrospective analysis of 1,117 individuals with chronic viral hepatitis (including hepatitis B infection), Mason and colleagues diagnosed diabetes in 21% of those with HCV as compared to 12% of those with hepatitis B ($P=0.0004$). A multivariate analysis found that hepatitis C infection ($P=0.02$) and age ($P=0.01$) were independent predictors of diabetes.

"Brain fog" (confusion and impaired memory) has been reported by many people with HCV. One study compared 27 individuals with detectable HCV RNA and no biopsy-proven evidence of serious liver damage with 16 individuals who had cleared HCV by administering a computer-based assessment instrument to measure cognitive function. Those with chronic HCV demonstrated greater cognitive impairment than those who had cleared HCV, even after controlling for depression, fatigue, injection drug use history, and severity of symptoms. In an attempt to identify a potential biological basis for cognitive impairment in chronic HCV infection, the researchers also looked at choline/creatine ratios using proton magnetic-resonance spectroscopy (MRS) in a subgroup of 17 persons infected with HCV. The choline/creatine ratio was higher in individuals assessed with cognitive impairment than in unimpaired participants, which suggests an underlying mechanism for the cognitive abnormalities reported in people with HCV (Forton 2002). In another study examining choline/creatine ratios, 30 HCV-infected persons with mild liver inflammation as confirmed by liver biopsy, 29 HCV-negative controls, and 12 people with chronic hepatitis B infection, underwent MRS. Significantly higher ratios of choline/creatine were found in the white matter ($P=0.001$) and basal ganglia ($P=0.01$) of the HCV-infected group as compared to the negative controls or the hepatitis B group ($P=0.009$ and $P=0.02$) (Forton 2001).

HCV was found in the cerebrospinal fluid of 8 of 13 individuals with chronic HCV infection (Laskus 2002a). As further support for the direct effect of HCV on the brain, the same group reported evidence of HCV replication in autopsied brain tissue from samples of 3 out of 6 individuals (Laskus 2002b). More research is needed to illuminate potential mechanisms of "brain fog" and identify possible interventions.

Liver Damage: Fibrosis and Cirrhosis

The liver is involved with processing nutrients, hormones, and medications, filtering waste and toxins, producing bile, proteins, and other substances and controlling the amount of sugar, fat, and protein that enter the bloodstream. The damage that HCV does to the liver results in scarred liver tissue. Mild liver scarring, known as fibrosis, often develops without any notable symptoms. There are different grades of fibrosis (see Chapter IV, Diagnostics), reflecting mild to severe liver

damage. Serious liver scarring, or cirrhosis, can obstruct the flow of blood through the liver and damage the actual structure of the liver itself. As liver tissue becomes scarred, liver functioning slows down.

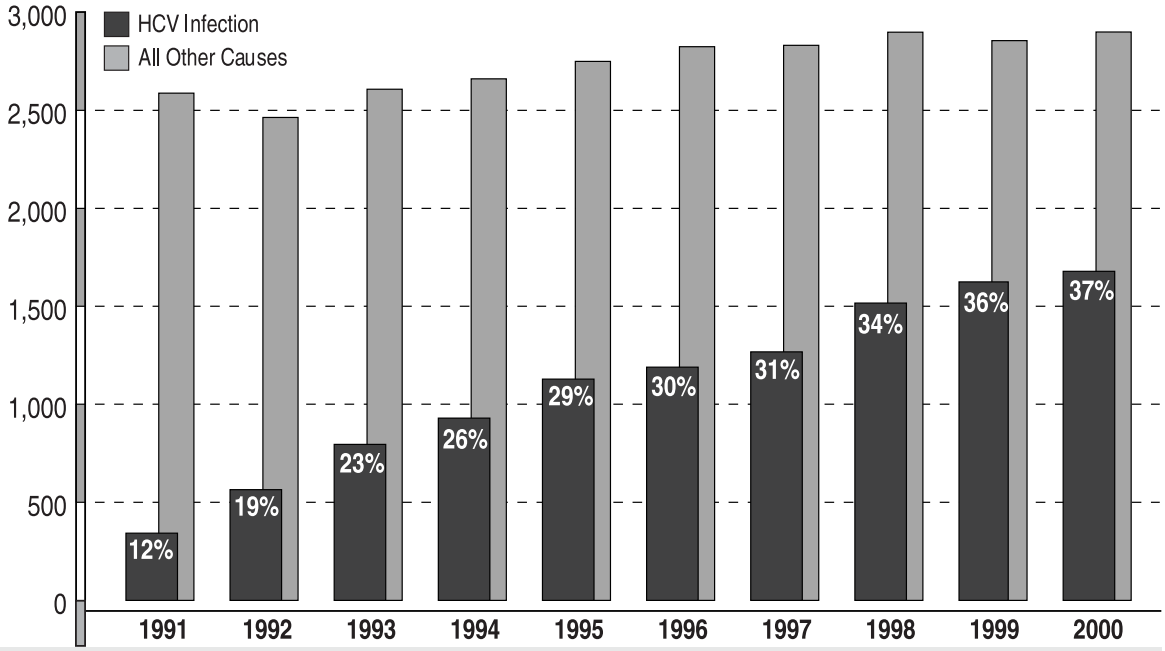
Some experts believe that fibrosis will steadily and inevitably progress to cirrhosis over time; others think that the degree of liver inflammation plays a crucial role in determining the risk of progressive liver injury. Individuals with more advanced fibrosis were found to have a more rapid progression to cirrhosis by Yano and colleagues. Initial biopsy samples from 70 Japanese HCV-infected individuals grouped as stage A (little or no portal fibrosis; less than or equal to liver damage staged as 1.9; and liver disease activity graded as less than or equal to 3.4), stage B (portal/periportal fibrosis with or without portal-bridging fibrosis; liver damage staged as 2.0–2.9; liver disease activity graded as 3.5–4.9), and stage C (septal fibrosis with regions of incomplete nodular regeneration; liver damage staged as 3.0–3.45; liver disease activity graded as greater than or equal to 5.0).

Every individual with stage C liver disease became cirrhotic within five to ten years of their initial biopsy. After 17 years of follow-up, 96% of participants with stage B liver disease developed cirrhosis. Of the stage A group, only 30% progressed to cirrhosis within 13 years of initial biopsy. It is worth noting that much higher rates of HCV-related cirrhosis and hepatocellular carcinoma have been seen in Japan than in the United States (Di Bisceglie 1997; Ikeda 1993; Ikeda 1998; Seeff 1997; Takahashi 1993; Yano 1996).

Cirrhosis can be asymptomatic at first, but symptoms often occur as liver damage increases. Early symptoms of cirrhosis can include fatigue, muscle weakness, loss of appetite, nausea, and weight loss. Some individuals experience loss of sexual desire, amenorrhea (menstrual irregularities), impotence, and breast enlargement (in men). A damaged liver is not able to process medications quickly, so drug levels may accumulate to higher-than-needed levels in the bloodstream, increasing side effects and toxicities. As liver function decreases, more symptoms may occur: edema (fluid retention in the ankles and legs) and ascites (fluid buildup in the abdomen), spontaneous bacterial peritonitis (infected ascites), more frequent bruising and bleeding, visible spider-like blood vessels, jaundice, dark urine, gallstones, and itching.

The most serious complications of cirrhosis are varices (development of abnormal veins, which can rupture and cause life-threatening internal bleeding if untreated) and hepatic encephalopathy (toxic build-up in the blood that can enter the brain and result in mental confusion and coma). People with advanced cirrhosis are at risk of hepatic decompensation (liver failure). The only intervention for hepatic decompensation is liver transplantation.

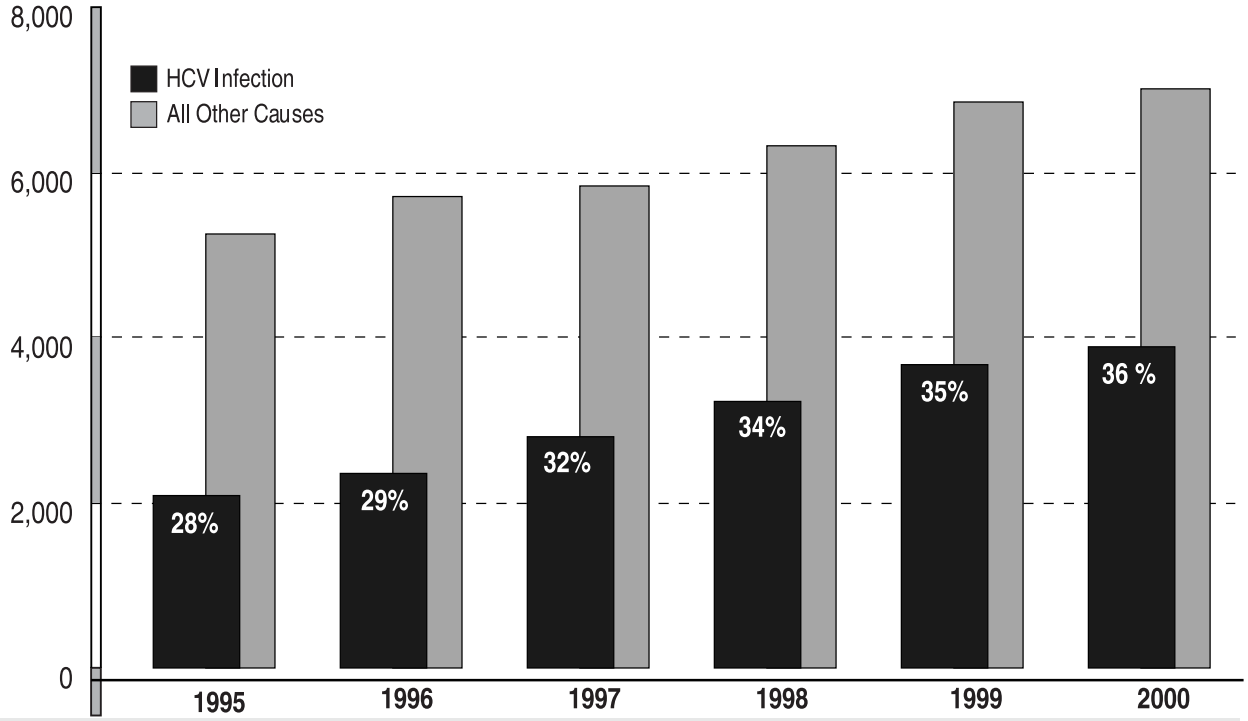
Figure 1. Number of Liver Transplants in the United State



	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
HCV Infection	343	565	796	930	1,129	1,190	1,268	1,517	1,625	1,679
Other Causes	2,588	2,464	2,608	2,661	2,750	2,825	2,832	2,899	2,856	2,900
Total	2,931	3,029	3,404	3,591	3,879	4,015	4,100	4,416	4,481	4,579

Kim 2002b

Figure 2. Number of Individuals Registered to Liver Transplant Waiting List



	1995	1996	1997	1998	1999	2000
HCV Infection	2,086	2,354	2,798	3,225	3,670	3,886
Other Causes	5,251	5,704	5,832	6,318	6,848	7,007
Total	7,337	8,058	8,630	9,543	10,518	10,893

Kim 2002b

Hepatocellular Carcinoma (HCC)

The United States incidence of hepatocellular carcinoma (HCC) in the general population has increased from 1.4 cases per 100,000 between 1976 and 1980, to 2.4 cases per 100,000 during the period between 1991 and 1995 (El-Serag 1999). Since HCC is a known complication of hepatitis C, this rise may be attributable in part to the increased incidence of hepatitis C infection that began decades earlier. Given what we know about the slow progressive liver damage associated with HCV infection, significant numbers of persons infected with HCV during the 1960s and 1970s may have developed hepatocellular carcinoma by the 1980s and 1990s.

The incidence of HCC in the United States reflects the demographics of U.S. hepatitis C infections, where incidence of hepatitis C is higher among African Americans than Whites (3.2% of African Americans as compared with 1.5% of Whites) (M. J. Alter 1999). From 1991 until 1995, the incidence of HCC among African-American males was 6.1 per 100,000; HCC incidence among white males was 2.8 per 100,000. Currently, annual incidence of HCC in hepatitis-C-infected cirrhotics ranges from 1% to 4% (Di Bisceglie 1997; Lauer 2001). Mortality from hepatocellular carcinoma is extremely high, with five-year survival rates of less than 5% (El-Serag 1999).

Although chronic hepatitis C infection causes inflammation, cell injury, and increased turnover of hepatocytes (liver cells)—processes that have also been associated with the pathogenesis of HCC—the specific mechanism by which HCV promotes hepatocellular carcinoma is unknown. Several prognostic factors have been identified, including the presence of cirrhosis, older age, and hepatitis B coinfection. In hepatitis C infection, HCC is almost always preceded by cirrhosis; therefore, cirrhotics are at an increased risk of hepatocellular carcinoma (Colombo 1991; Di Bisceglie 1997; Macias Rodriguez 2000; Tong 2001; Tsukuma 1993). Because alcohol consumption can accelerate HCV disease progression and increase the risk of cirrhosis, alcohol consumption may also indirectly increase the risk of HCC (Schiff 1997).

Aging has been associated with increased risk for HCC, although it is not clear whether this is a function of age itself, duration of infection, or a combination of the two (Aizawa 1999; Ikeda 1993). Two studies of HCV disease progression—one tracking hemophiliacs, the other transfusion recipients—found that an older age at time of infection increased the likelihood of developing HCC (Murakami 1999; Tradati 1998); another study of HCV-infected cirrhotics identified age as the main risk factor for development of HCC, with the relative risk of HCC increasing by 8% per year (Macías Rodriguez 2000). Chiaramonte and colleagues found a 4.5-fold increase in HCC risk among cirrhotic persons over 50 years of age (Chiaramonte 1999). In a longitudinal observational study of 967 cirrhotics, age was the only risk factor for HCC identified by a multivariate analysis (del Olmo 1998).

The risk of HCC is higher in people who are infected with both hepatitis C and hepatitis B (Alberti 1995; Benvegnù 1994; Chiaramonte 1999). Tsai and colleagues reported the annual incidence of HCC during a follow-up of 1185 person-years in four groups of cirrhotics distinguished by type of infection (total n=400). They found annual HCC incidences of 2.0% in those with no viral infection, 6.6% in persons with HBV alone, 7.0% among individuals with HCV alone, and a startling 13.3% in HCV/HBV-coinfected persons (Tsai 1997).

Male sex may be associated with the risk of developing HCC, but some controversy remains about this. Although some research has found an increased risk for HCC in hepatitis C-infected cirrhotic males, other research has found no statistically significant differences according to sex (Chiaramonte 1999; del Olmo 1998; Miyakawa 1996; Tsukuma 1993). In the United States, the incidence of HCC (from all causes) among males is three times higher than among females (El-Serag 1999). This discrepancy may simply reflect higher rates of HCV infection among males (2.5% as compared with 1.2% for females) (M. J. Alter 1999).

Factors That May Accelerate Hepatitis C Disease Progression

HIV Coinfection

See Chapter III, Natural History of HIV/HCV Coinfection.

Superinfection with Hepatitis A and/or Hepatitis B

Individuals who are infected with hepatitis C are at risk for severe, potentially fatal disease if they become superinfected with hepatitis A (Koff 2001; Pramoolsinsap 1999; Vento 1998; Vento 2000). Superinfection with hepatitis B may accelerate progression of an existing hepatitis C infection or cause liver failure and death (Koff 2001; Liaw 2000); therefore, the Centers for Disease Control recommends that all individuals who are infected with, or at risk for, HCV infection be vaccinated to protect against infection with HAV and HBV if they are susceptible to these infections.

There is evidence of decreased immunogenicity of HBV vaccination in individuals with chronic HCV. Researchers have observed significantly reduced responses to HBV vaccination in individuals with chronic HCV. One study identified the main predictor of response to HBV vaccination as the absence of antibodies to HCV (OR, 7.65; $P<0.0001$) (Leroy 2002). Wiedman and colleagues observed non-response to HBV vaccination among 31% (18/59) of those with HCV, vs. 9% (5/58) of uninfected controls ($P<0.005$). Response rates increased after a high-dose booster (40 μg) of recombinant hepatitis B vaccine, with 12 of 15 previous non-responders achieving seroconversion (development of antibodies) (Wiedman 2000). Another strategy for enhancing HBV vaccine immunogenicity is short-course, high-dose vaccination with 40 μg per month for three months—with an 80 μg booster at month four for non-responders. This elicited response rates from 72% (109/152) of those with chronic hepatitis C vs. 92% (24/26) of HCV-negative controls (Idilman 2002).

Although persons with chronic hepatitis C seroconvert after HAV vaccination, their response to HAV vaccination may be less durable than those reported in HCV-negative persons. Keeffe and colleagues reported that seroconversion after HAV vaccination occurred at a similar rate among controls and individuals with chronic hepatitis C (98.2% in controls vs. 94.3% in persons with chronic hepatitis C). Another measure of vaccine-induced immunogenicity (the geometric mean concentration of antibodies to HAV) was significantly lower in persons with chronic HCV than in negative controls (467 vs. 1315 among controls; $P=0.0001$) (Keefe 1998). Anti-HAV antibody

levels of at least 20 MIU/mL confer protection against infection with HAV (Bovier 2002). The higher the anti-HAV level after vaccination, the more durable the response to vaccination will be (Totos 1997). In a group of 120 HCV-negative individuals, the geometric mean concentration level of anti-HAV at 5½ years after HAV vaccination was 522, and the average decrease in anti-HAV was 15–20% (Van Herck 2001).

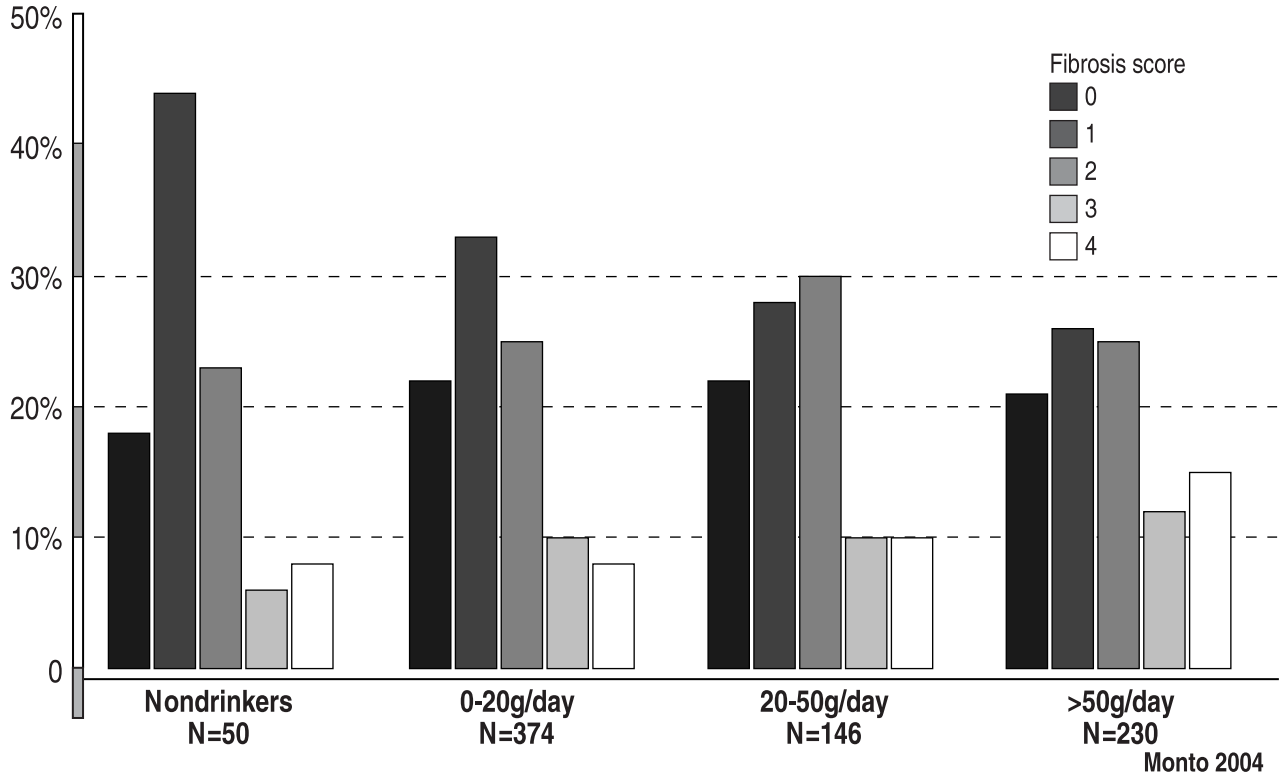
Alcohol Consumption

A large body of data has confirmed that alcohol consumption of more than 50 grams per day (the equivalent of four or five glasses of wine) accelerates the progression of HCV-related liver disease (Harris 2002; Poynard 1997; Thomas 2000a). Thomas and colleagues followed a cohort of 1,667 HCV-infected individuals for a median interval of 8.8 years. The relative risk of ESLD among individuals with alcohol consumption of more than 260 grams per week was 3.60 (95% CI, 1.73–7.52). In a cohort of 176 HCV-infected individuals, alcohol intake of over 60 grams per day in males and over 40 grams per day in females for a period of at least five years resulted in a 2- to 3-fold greater risk of cirrhosis and ESLD (Wiley 1998).

Monto and colleagues performed a cross-sectional study of the effect of alcohol intake on fibrosis progression among 800 individuals with chronic hepatitis C. The duration, frequency and quantity of individual alcohol intake were assessed by a detailed questionnaire, and each participant had a liver biopsy. Lifetime alcohol consumption (in grams per day) was determined by multiplying the number of drinks by the alcohol content of each drink, which was estimated at 10 grams per drink. The result was divided by the length of time since drinking was initiated. Fibrosis progression was estimated by dividing the fibrosis score by the duration of infection.

Alcohol intake was not correlated with fibrosis progression, although it was associated with the presence of fibrosis in a multivariate analysis. The odds of fibrosis increased among those with alcohol consumption ≥ 80 grams/day vs. non-drinkers (OR, 1.76; 95% CI, 0.99–3.12; P=0.05). Yet after controlling for other factors, only age, histological inflammation and alanine aminotransferase level were independent predictors of fibrosis ($P<0.0001$, $P<0.003$ and $P<0.0001$, respectively). Although alcohol intake was not an independent predictor of fibrosis, varying degrees of fibrosis and cirrhosis were present in non-drinkers, and at every threshold of alcohol consumption (Monto 2004).

Figure 3. Fibrosis Score by Daily Alcohol Intake



Although one might leap to the conclusion that light to moderate alcohol intake does no harm to the liver, the relationship between alcohol intake and fibrosis progression is more complex. Westin and colleagues reported that fibrosis progressed among individuals who drank more than a median of 5.7 grams/day; those with median alcohol consumption of >2.6 grams/day did not have fibrosis progression on sequential biopsies (Westin 2002).

Alcohol consumption may be more deleterious for some individuals than others. HCV-infected women with light to moderate alcohol intake may be at a greater risk for fibrosis than men with a similar alcohol intake, but there is little data—and some controversy—regarding the relationship of alcohol intake to fibrosis progression in women with hepatitis C. Monto and colleagues did not observe a difference in fibrosis among 187 women with chronic hepatitis C by alcohol intake, although only 23 of them drank more than 50 grams of alcohol per day. Even the women who drank heavily (>50 grams/day) did not have more fibrosis than those who did not drink at all, or who drank less (Monto 2004). Hezode and colleagues reported that fibrosis and cirrhosis were more prevalent among people with chronic hepatitis C who drank 31–50 grams/day. They suggested that moderate alcohol intake (21–50 grams/day) might worsen fibrosis among women with hepatitis C (Hezode 2003b).

More research on the effects of light or moderate alcohol consumption on HCV disease progression—especially that which considers sex and/or genetic and ethnic factors— is needed.

Smoking

The role of smoking as a potential cofactor for developing HCC is controversial. In a large study of people with chronic liver disease, many of whom were HCV-antibody-positive (433/731), Tsukuma and colleagues found an increased risk of HCC among cirrhotic smokers (adjusted rate ratio of 7.96 for current smokers and 3.44 for former smokers) than cirrhotic nonsmokers (Tsukuma 1993). Interestingly, non-cirrhotic smokers were not at higher risk of developing HCC. While additional research has reported a correlation between smoking and HCC, smoking has not been identified as a promoter of hepatitis C-related hepatocellular carcinoma in particular; however, one study has linked smoking with severity of HCV-related liver damage. In a multivariate analysis, Pessione and colleagues linked smoking with increased fibrosis scores ($P=0.03$) and increased histologic activity scores ($P=0.04$). Since smoking often accompanies alcohol use, and in this study smokers were more likely to drink alcohol than nonsmokers ($P=0.001$), it is not possible to isolate the contribution of smoking to the development of HCC.

Genotype

There are at least six different genotypes (viral strains) of hepatitis C. The prevalence of HCV genotypes varies among countries and regions. Genotypes 1, 2, and 3 are globally distributed; in the United States, HCV genotypes 1a and 1b are the most prevalent, accounting for at least 75% of HCV infections (Blatt 2000; Zein 1996a). In the United States, infection with HCV genotype 1 is more prevalent among African Americans ($P<0.001$) and individuals in the northeastern, southeastern, and midwestern regions (Blatt 2000). Before 1955, genotype 1b was predominant in the United States (Zein 2000). Genotype 1a arrived in the late 1950s and quickly became the most prevalent. Genotypes 1a and 1b were joined by genotype 2 in the 1960s and genotype 3 in the 1970s (Zein 2000). In Japan, as many as 73% of HCV infections are genotype 1b (Takada 1993). Genotype 2a and 2b are fairly prevalent in North America, Europe, and Japan (Zein 2000). Subtype 2c has been observed most often in Northern Italy, and genotype 3a is prevalent in injection drug users in Europe and the United States (Pawlotsky 1995). HCV genotype 4 infections occur most frequently in the Middle East and North Africa; genotype 5a is predominant in South Africa (Abdulkarim 1998; Chamberlain 1997a; Chamberlain 1997b; Nousbaum 1998). Genotype 6 infections are prevalent in Southeast Asia (Adams 1997). Researchers have not yet agreed whether or not five different viruses, three found only in Vietnamese individuals, the remaining two seen in Indonesians, are actually subtypes of genotype 6 or ought to be designated as genotypes 7 through 11.

Genotyping of HCV is clinically significant because genotype is the single most important predictor of response to HCV treatment. Individuals with genotypes 1a, 1b, 4, and 5 have poorer responses to interferon (Fried 2002; Germer 2001; Mondelli 1999; A. U. Neumann 2000; Nousbaum 1998; Poynard 1998; Rosenberg 2001; Zein 1996b). Forty-eight weeks of HCV treatment is recommended for individuals with genotype 1; for individuals with genotypes 2 and 3, the recommended duration of treatment is only 24 weeks (McHutchinson 2002; Poynard 2000; Soriano 2002). Infection with genotype 1 has also been associated with high HCV RNA levels (Kobayashi 1996; Pageaux 1997). In their analysis of 6,807 HCV-infected individuals, Blatt and colleagues discovered significantly higher HCV RNA levels among individuals with genotype 1 as compared with genotypes 2 and 3 ($P<0.001$) (Blatt 2000).

Infection with hepatitis C genotype 3 and subtype 3a has been associated with hepatic steatosis (fat in liver cells), which contributes to HCV disease progression (Hofer 2002; Kumar 2002; Serfaty 2001). The amount of hepatocellular steatosis has been linked to the extent of fibrosis in the liver tissue (Adinolfi 2001; Hourigan 1999; Monto 2002a; Monto 2002b). Among 55 HCV-infected individuals with hepatic steatosis, Hwang and colleagues found higher fibrosis scores in those with steatosis (1.9 ± 1.2 vs. 1.3 ±1.0 without steatosis; P=0.016) (Hwang 2001); however, when HCV treatment is successful in genotype-3- and genotype-3a-infected individuals, reductions in steatosis are often seen. Kumar and colleagues observed significantly reduced hepatic steatosis ($P<0.001$) in individuals with HCV genotype 3a and sustained virologic responses (SVR; no detectable HCV RNA six months after completion of HCV treatment). No improvements in hepatic steatosis were observed in non-responders to interferon (Kumar 2002).

Infection with genotype 1b may increase the likelihood of chronic infection, although more information from larger studies is needed. Among 42 individuals with acute HCV infection, Amoroso and colleagues found a higher rate of chronic infection among those with genotype 1b. A majority of the participants were infected with a subtype of genotype 1. Thirty-eight percent (16/42) of the participants were infected with subtype 1a, and 33.9% (14/42) with subtype 1b. The overall chronicity rate after more than a year of follow-up was 59.5% (25/42). Ninety-two percent (23/42) of the individuals who developed chronic infection had genotype 1b. No significant association between mode of transmission—which included injection drug use, transfusion, nosocomial, and unknown factors—and chronic infection was identified (Amoroso 1998).

HCV genotype 1b has been associated with more aggressive and serious liver disease, but this association remains controversial. In Japan, where genotype 1b is very common, research has found a more frequent and a more rapid development of HCC in individuals with hepatitis C (Takahashi 1993; Yano 1993); however, when the HCV genotypes of 72 Japanese with HCC and 131 without HCC were examined, there was no significant difference in the prevalence of HCC by genotype (Yotsuyanagi 1995). The controversy about the association of genotype 1b with HCV disease severity has carried over into the United States, where Zein and colleagues saw an HCC prevalence of 28% among those with HCV genotype 1b vs. a 3% HCC prevalence among those with all other genotypes (Zein 1996c). However, when Reid and colleagues looked at 28 HCV-infected individuals with HCC and 38 HCV-infected cirrhotics, they did not find a significant association between HCV genotype and the development of HCC (Reid 1999).

Factors influencing the prevalence of genotype 1b among individuals with advanced liver disease might include the duration of infection, age at time of infection, or mode of transmission. In the United States and France, many individuals with genotype 1b are older than those with other genotypes, and many genotype 1b infections resulted from blood transfusions (Pol 1995; Rosen 1999). Both older age at infection and infection via transfusion are poor prognostic factors. It is therefore possible that genotype 1b may be an indirect marker of more severe disease—due to mode of transmission or age at infection—rather than a cause of it.

Some research suggests that recurrent HCV infection among transplant recipients is more aggressive in individuals with genotype 1, especially genotype 1b (Feray 1995; Gane 1996; F. D. Gordon 1997; Pageaux 1997; Shuhart 1997). During a median follow-up interval of 40.4 months, Shuhart and colleagues found that individuals with genotypes 1a and 1b had a risk of recurrence

or death 3.47 times greater than that of individuals with genotypes 2 and 3 combined (95% CI, 1.15–10.56; P=0.02). Only those with genotypes 1a and 1b developed post-transplant fibrosis or cirrhosis. More people in this study had genotypes 1a and 1b (46.9% genotype 1a; 28.6% genotype 1b; 20.4% genotype 2b; and 4.1% genotype 3a). Gane and colleagues followed transplant recipients for a median of 36 months. Twenty of 43 individuals with genotype 1b (46%) developed chronic hepatitis C or cirrhosis compared with 13 of 53 (24%) with other genotypes (P=0.02). Genotype 1b infection was associated with a greater frequency of damage to the transplanted organ than infection with other genotypes (odds ratio, 3.4; 95% CI, 1.4–8.5; P=0.01) (Gane 1996).

Other research, however, does not support a correlation between genotype 1/1b infections and more aggressive post-transplant HCV disease (Boker 1997; Charlton 1998; Crespo 1997b; Zhou 1996). Zhou and colleagues found no significant association between HCV genotypes 1 and 1b and disease severity or graft survival among 124 transplant recipients, despite a significantly longer median follow-up of individuals with genotype 1b (31.1 months vs. 24.5 months; P=0.02). Charlton and colleagues found a significant association between HCV RNA levels before transplant and length of post-transplant survival (cumulative survival at five years was 57% in individuals with pre-transplant HCV RNA ≥ 1 X 10^6 vEq/mL, increasing to 84% in those with HCV RNA <1 X 10^6 vEq/mL), but they did not find an association between the genotype of HCV and post-transplantation patient or graft survival. Crespo and colleagues did not find a relationship between genotype and of post-transplant HCV RNA level or disease severity (Crespo 1997b). It is difficult to reach a conclusion about the role of genotype in post-transplant disease; in addition to conflicting results, a lack of consistent data collection, differing methodology across studies, and uneven duration of follow-up are confounding, as with most other HCV studies conducted to date.

Cases of re-infection and coinfection with more than one genotype of hepatitis C have been documented, although the prognostic impact of mixed infection is unclear (Accapezzato 2002; De Socio 1996; García-Samaniego 1997; Jarvis 1994; Tuveri 1997). Although Benvegnù and colleagues did not find a correlation with a particular genotype and the course of HCV-related cirrhosis, they observed that individuals with mixed infections had more rapid worsening of cirrhosis, and increased mortality (P<0.05). However, only six of the 109 individuals (5.5%) in this study had mixed infections (Benvegnù 1997).

The effect of mixed genotype infection on HCV viral load merits investigation, because a high hepatitis C virus load (≥ 2 million copies/mL or ≥ 800,000 IU) decreases the likelihood of response to HCV treatment. Schijman and colleagues examined HCV viral loads in 257 people with hepatitis C; twelve were infected with two different genotypes. Their median HCV viral loads did not differ significantly from those infected with a single genotype (356,000 IU/ml for mixed-genotype infections vs. 344,000 IU/ml for single-genotype infections) (Schijman 2004).

HCV genotyping also provides valuable epidemiological information. It has been used for tracing the source outbreaks of infection and to establish evidence of transmission from one individual to another. HCV genotyping can identify transmission networks and provide guidance for targeted prevention, education, and treatment initiatives.

Hepatic Steatosis

Hepatitis steatosis, a condition in which the deterioration of liver tissue is accompanied by deposits of fat in liver cells, is a common feature of chronic hepatitis C. The presence of steatosis has been associated with increased fibrosis progression and advanced liver damage (Adinolfi 2001; Hu 2003; Lonardo 2004; Vadan 2003; Walsh 2004) and decreased response to treatment for hepatitis C (Poynard 2003; Ratziu 2004).

Hepatic steatosis is especially prevalent in genotype 3, which may have a direct cytopathic effect (Castéra 2004; Cholet 2004; Hezode 2003a; Kumar 2002; Rubbia-Brandt 2004; Sharma 2004; Westin 2002). Genotype 3-associated steatosis has been associated with higher HCV RNA levels and impaired response to hepatitis C treatment (Hezode 2003a; Patton 2003; Zeuzem 2003).

Steatosis may originate from metabolic abnormalities as well; in hepatitis C genotype 1, there is a strong correlation between steatosis, body mass index and metabolic abnormalities such as visceral adiposity, and insulin resistance, which is a predictor of diabetes (Conjeevaram 2003; Hezode 2003a; A. Gordon 2003; Qadri 2004). Insulin resistance and high levels of serum glucose have been associated with an accelerated fibrosis progression rate in persons with HCV (Hui 2003; Lecube 2004; Ratziu 2003).

Mapping the Natural History of Hepatitis C

Because the data available to assess the risk and rate of HCV disease progression come from widely different patient populations with varying lengths of follow-up, estimates of disease progression, morbidity, and mortality can differ dramatically from one study to the next. Comparisons of progression rates across studies are also difficult to perform due to differences in study design. Not all studies can establish the duration of infection, and uninfected control groups are often not identified. In addition, the use and frequency of biopsy to stage liver disease has been inconsistent, with researchers using various endpoints that preclude simple comparisons. Nevertheless, some distinctive trends emerge within clusters of studies.

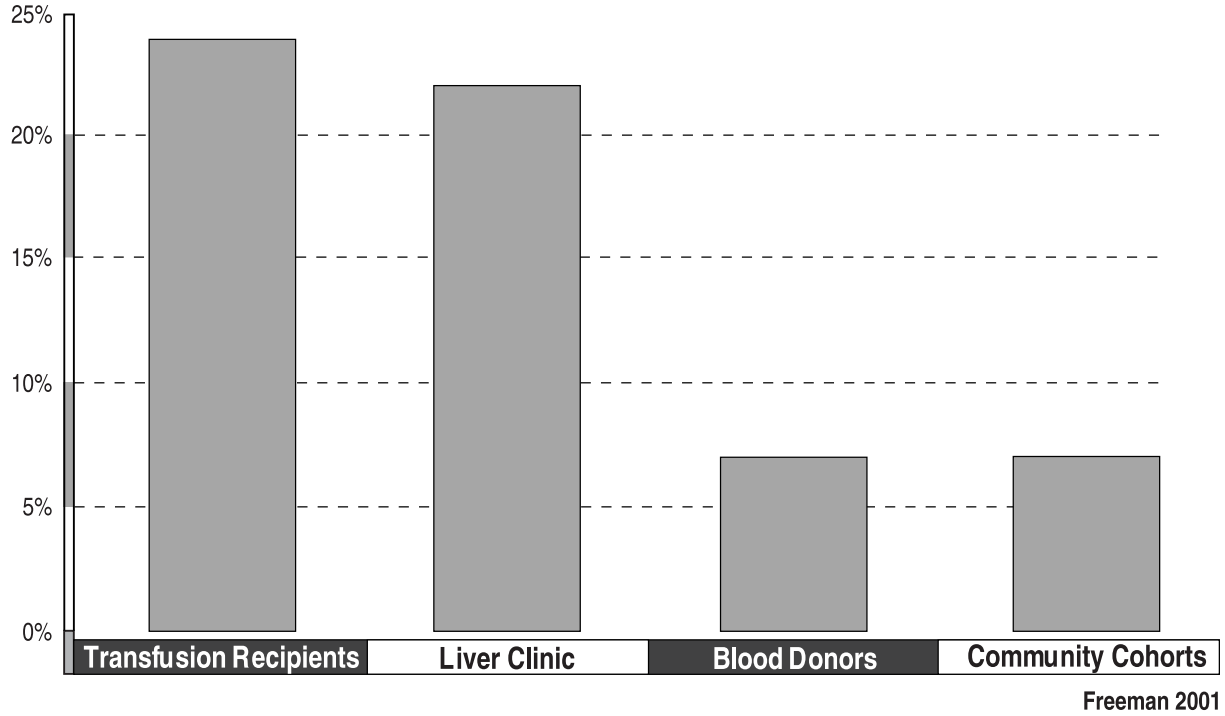
One group of studies has identified individuals with liver disease, often recruited from liver disease clinics, and attempted to correlate disease severity with duration of infection. These studies typically present worst-case scenario data, with high rates of cirrhosis (30–46%) and hepatocellular carcinoma (11–19%) (Seeff 1997).

Another typical group of studies has tracked cases of transfusion-related non-A, non-B hepatitis from the onset of acute infection. These studies support a low rate of morbidity and mortality during the first decade of hepatitis C infection, with cirrhosis appearing in 8% to 24% of individuals. Hepatocellular carcinoma was rare, and death from liver disease occurred in 1.6% to 6% of those observed (Seeff 2000).

Different methods have attempted to create a timeline of the natural history of hepatitis C infection. Freeman and colleagues shed some light with their analysis of 57 studies of HCV disease progression. They grouped the natural history studies used in their evaluation into one of four categories: 1) liver clinic patients; 2) transfusion recipients; 3) blood donors (people whose

infections were detected when they donated blood); and 4) longitudinal community-based cohorts. Twenty years postinfection, estimated progression to cirrhosis among liver clinic patients was 22% (95% CI, 18–26%); progression among transfusion recipients was 24% (95% CI, 11–37%); progression to cirrhosis among blood donors was 7% (95% CI, 1–7%), and progression among community-based cohorts was 7% (95% CI, 4–10%) (Freeman 2001).

Figure 4. Risk of Developing Cirrhosis in 20 Years



Dore and colleagues developed a mathematical model to estimate progression to cirrhosis based on data from natural history studies. They concluded that people with hepatitis C infection have on average a 7% risk of progressing to cirrhosis after 20 years of infection, and a 20% risk of cirrhosis after 40 years (Dore 2002).

Salomon and colleagues reviewed data from various sources on HCV prevalence, natural history, and mortality, examining a range of values to estimate a rate of progression to cirrhosis, and compared these models to existing epidemiological statistics. They found that the model that best fit the available data predicts that males infected at age 25 would have a median time to cirrhosis of 46 years, while less than 30% of females infected at the same age would progress to cirrhosis in 50 years (Salomon 2002).

According to the National Institutes of Health's most recent Consensus Development Conference Statement on Hepatitis C, when individual prognostic factors are controlled for, the actual overall risk of developing cirrhosis within 20 years is estimated to lie between 10% and 15% (NIH 2002). Since these estimates used the total number of people who have ever been infected with HCV—including those who cleared the virus—the risk of developing cirrhosis may be higher among chronically infected individuals.

Fibrosis progression may be linear or, to some extent, exponential (de Torres 2003; Dore 2003; Poynard 2001; Ryder 2004). Poynard and colleagues propose dividing individuals with HCV disease into three groups: rapid fibrosers, intermediate fibrosers, and slow fibrosers. Two thousand two hundred and thirty-five people from the French OBSVIRC, METAVIR, and DOSVIRC groups—chronic hepatitis C patients from different retrospective and prospective studies—were evaluated to determine the effect of nine factors on fibrosis progression: age at time of infection; duration of infection; age at biopsy; gender; alcohol consumption; HCV genotype; HCV viremia; cause of infection; and grade of histological activity. Only three of these factors—older age (above 40) at time of infection, alcohol consumption over 50 grams/day, and male gender—were associated with fibrosis progression. Using this data, projected estimates for median time to cirrhosis were developed. The estimated median time (without treatment) to cirrhosis was 30 years, although 33% had an estimated median projection to cirrhosis of less than 20 years, and 31% were projected to develop cirrhosis after at least 50 years, if ever (Poynard 1997).

Forty-five Years Later

A study that provides documentation of the earliest HCV infection in the United States is also the only study that provides data on individuals who have been infected with hepatitis C for longer than 25 years. Although the number of HCV-positive people in this study is small, it is important for what is suggested about the lifetime consequences of HCV infection.

Seeff and colleagues screened 8,568 frozen blood samples from military recruits who were tested between 1948 and 1954 for group A streptococcal infection and acute rheumatic fever. The stored samples were tested for HCV antibodies and, if repeatedly reactive, for HCV RNA. Seventeen (0.2%) of 8,568 samples were anti-HCV-positive. Twelve of the HCV-positive individuals were African-American (1.8% of 684), four were white (0.07% of 5902), and one was of unknown race/ethnicity. Mean age at the time of the original blood draw was 21.5 in the HCV-positive group and 20.7 in the HCV-negative group.

During the 45 years since the samples were taken, 2 of the 17 HCV-positive persons (11.8%) and 205 of the 8,551 HCV-negative persons (2.4%) had developed liver disease (ethnicity-adjusted relative risk, 3.56; 95% CI, 0.94–13.52). One HCV-positive individual died from liver disease 42 years later; 119 HCV-negative individuals (1.4%) died from liver disease. Death from all causes was more frequent in the HCV-positive group (41%) than in the HCV-negative group (26%), although the difference in age at death between the two groups was not significant (Seeff 2002). The low rates of hepatitis C-related morbidity and mortality seen in this group and in the two women's cohorts suggest that progression to cirrhosis and end-stage liver disease is not inevitable for all persons with HCV infection.

HCV Infection Acquired at Birth or During Early Childhood

HCV infections acquired at birth or in early childhood usually progress slowly (Casiraghi 2004; Guido 1998; Kage 1997), but these infections are not always mild. Badizadegan and colleagues found significant fibrosis in 58% (23/40) and cirrhosis in 8% (3/40) of biopsy samples from children aged 2 to 18.6. The average duration of infection was 6.8 years (Badizadegan 1998).

Disease progression may accelerate as the duration of infection lengthens. Guido and colleagues reported that fibrosis progression was not linear in 13 children with paired liver biopsies; all were infected with hepatitis C during infancy. Age at biopsy and duration of infection were significantly associated with fibrosis stage ($P<0.002$ and $P<0.0005$, respectively). The fibrosis stage differed significantly between individuals who had been infected for less or more than a decade ($P<0.0006$) (Guido 2003). Aging may accelerate fibrosis progression, as liver damage increases in adolescence and young adulthood (Jara 2003).

Casiraghi and colleagues reported on the long-term outcomes of 31 individuals infected with HCV at birth. In 1968, 43 infants were given mini blood transfusions (21–30 mL) from 29 donors. Years later, when HCV antibody testing became available, 15 of the donors were tested for hepatitis C; one donor was infected with HCV. In 1998, a follow-up study began with 31 of the 43 individuals who received mini transfusion from the infected donor plus 31 controls (mini transfusion recipients from anti-HCV negative donors). Eighteen mini transfusion recipients were anti-HCV positive; 16/18 (88.9%) had chronic HCV, confirmed by HCV-RNA testing. Phylogenetic analysis linked the virus of mini transfusion recipients to that of the infected donor.

Liver biopsy samples were available from 11 participants; a majority had no fibrosis or mild fibrosis (Ishak stage 0–1). Only two had moderate to marked fibrosis (Ishak stage 3–4). Disease activity was minimal to mild (Ishak grade 3–6) in ten of eleven. Five years after the initial biopsy, five individuals had a second biopsy. Only one had any significant change in liver histology, progression from stage 0 to stage 1 (Casiraghi 2004).

Transfusion Cohorts

Transfusion recipients often became infected at older ages than those infected through injection drug use or sexual contact. Age at infection may not be the only factor distinguishing transfusion recipients from those with community-acquired HCV infection. Gordon and colleagues compared disease progression by mode of transmission and found that people with transfusion-associated HCV infection were more likely to develop liver decompensation than those infected in other ways (relative risk: 3.921; 95% CI, 2.205–7.015), even after controlling for duration of infection and age at infection (S. C. Gordon 1998).

Seeff and colleagues looked at long-term mortality from groups of non-A, non-B (NANB) hepatitis-infected transfusion recipients and two matched control groups of uninfected transfusion recipients. The 568 NANB-infected transfusion recipients from five different studies were matched with 526 first controls and 458 second controls. While, over an 18-year period, the overall frequency of liver-disease-related mortality was low, the number of deaths from liver disease among those with non-A, non-B hepatitis was significantly higher than among the uninfected transfusion recipients. Nineteen NANB-infected persons died from liver disease (3.3%) compared to six first controls (1.1%) and nine second controls (2 %) (Seeff 1992).

Tong and colleagues studied the clinical outcomes of 131 people with transfusion-associated hepatitis C who had been referred to liver specialists. The mean age at the time of transfusion was 35, and mean age at follow-up was 57. Fifty-one percent (67/131) had cirrhosis and 5.3% (7/131) had hepatocellular carcinoma. Of the cirrhotics, 10% (7/67) developed HCC over an average

time of 36 months. The estimated time from transfusion to cirrhosis was 20.6 ± 10.1 years. The estimated time from transfusion to HCC was 28.3 ± 11.5 years (Tong 1995).

Kiyosawa and colleagues found similar results in a study of 231 people who were infected with non-A, non-B hepatitis mainly through transfusions. Eighty-six point four percent of the 81 cirrhotics had antibodies to HCV; 94.4% of the 54 who were diagnosed with hepatocellular carcinoma were anti-HCV-positive. The mean interval between transfusion and the development of HCV-related cirrhosis was 21.2 years; the mean interval between transfusion and the development of HCV-related hepatocellular carcinoma was 29 years (Kiyosawa 1990).

Female Transfusion Cohorts Infected Through Anti-D Immunoglobulin

Two different studies have identified cohorts of women infected from a single source and have tracked them over a period of time. These studies delineate a very slow disease progression, with few serious complications developing; in the 17 to 20 years after infection, the rates of cirrhosis in these cohorts were 0.4% and 2% (Kenny-Walsh 1999; Wiese 2000).

Liver biopsies were performed on 182 of the cohort of young German women who had been infected with HCV from anti-D immunoglobulin 20 years earlier. While 94% of the women had mild-to-moderate liver inflammation, only four (0.4%) of the women had cirrhosis. Two women had died, one from fulminant hepatitis B infection and the other from alcoholism and cirrhosis (Wiese 2000). Similar findings were obtained from liver biopsies of 363 women in the Irish cohort who had been infected 17 years earlier. Although 98% had liver inflammation, only 16 (4%) of the women had serious inflammation; 177 (49%) of the women had no fibrosis, and only 7 (2%) had cirrhosis. Two of the cirrhotic women reported heavy drinking (Kenny-Walsh 1999).

Natural history studies and estimates of disease progression can provide information, but they cannot clarify uncertainty about disease progression in individuals. People with hepatitis C and their doctors look to such data in order to assess the risk of liver disease and evaluate the need for treatment. Treatment decisions may be guided in part by estimates of the risk of disease progression, giving particular consideration to prognostic factors such as age at infection and gender. The good news is that chronic infection with hepatitis C does not necessarily predict serious liver damage; the bad news is that some people—Poynard's "rapid fibrosers"—will likely develop cirrhosis sooner rather than later.

Recommendations

Investigate the role of genetic and ethnic factors in susceptibility to HCV infection, disease progression, and response to treatment.

Hepatitis C infection is twice as prevalent among black Americans as white Americans. The highest observed prevalence of hepatitis C in the United States—a shocking 9.8%—occurs among black males aged 40 to 49 (M. J. Alter 1999). African Americans appear less likely to achieve spontaneous viral clearance of HCV (Thomas 2000; Villano 1999). In addition, race appears to have a substantial impact on the efficacy of interferon. Significantly lower treatment response rates have been observed in Blacks than in Whites, Latinos, or Asian Americans (Jeffers 2002; McHutchison 2000; Reddy 1999). Research is needed to understand the mechanisms that account for these disparities and to identify strategies to improve treatment response. The National Institutes of Health and the Centers for Disease Control must support this research.

Investigate the role of sex differences in HCV disease progression.

High rates of spontaneous viral clearance have been observed in two cohorts of premenopausal women, and some evidence suggests that the course of hepatitis C disease in this population may be less severe (Benhamou 1999; Kenny-Walsh 1999; Poynard 1997; Weise 2000). No research has explored why female sex appears to be a favorable prognostic factor. The role of hormones, and the immunological differences between males and females warrant further investigation from the National Institutes of Health and the Centers for Disease Control.

Identify possible causes of, and interventions for, HCV-related "brain fog."

Confusion, memory loss, and an inability to concentrate have been reported by many individuals with chronic HCV. HCV is present in the cerebrospinal fluid of some individuals with chronic HCV infection (Laskus 2002a). Evidence to support the direct effect of HCV on the brain was discovered by the same group who detected HCV replication in autopsied brain tissue from samples of three out of six individuals (Laskus 2002b). More research should be supported by the National Institutes of Health to illuminate potential mechanisms of "brain fog" and identify possible interventions.

Promote screening and vaccination for hepatitis A and hepatitis B among individuals infected with HCV or coinfected with HIV/HCV.

Individuals infected with HCV are at risk for severe, potentially fatal, disease if they become superinfected with hepatitis A (Koff 2001; Pramoolsinsap 1999; Vento 1998; Vento 2000). Coinfection with hepatitis B may accelerate progression of an existing hepatitis C infection or even cause liver failure and death (Koff 2001; Liaw 2000). Because of these risks, CDC recommends vaccination against HAV and HBV for all individuals infected with or at risk for HCV infection; yet many are not receiving vaccinations. A survey of primary care physicians found that only 1.6% of their HCV patients were vaccinated against HAV, and only 3% had been vaccinated against HBV (Nicklin 1999).

Physicians, health educators, and direct service staff need to be educated about the importance of vaccination against HAV and HBV. Screening and vaccination initiatives are needed. Vaccination should be available in correctional facilities and outside of clinic and hospital-based settings, especially in venues such as syringe exchange programs, substance abuse treatment programs, shelters, and methadone maintenance clinics, where high-prevalence and high-risk groups receive services. Screening and vaccination should be provided free of charge. Congress and the administration must provide public health funding for these services.

Investigate the influence of light-to-moderate alcohol consumption on HCV disease progression.

A large body of data has confirmed that alcohol consumption of more than 50 grams per day accelerates the progression of HCV-related liver disease (Harris 2002; Poynard 1997; Thomas 2000). Less is known about the effect of light to moderate alcohol consumption on hepatitis C disease progression. Some studies have associated light alcohol intake with fibrosis progression, while others have reported that light to moderate alcohol consumption is not significantly associated with fibrosis (Monto 2004; Westin 2002). The effect of alcohol on HCV progression may vary by sex and/or genetic and ethnic factors. Without more specific information, most clinicians simply recommend abstinence from alcohol. Data to support or modify recommendations of abstinence are needed. The National Institutes of Health should provide funding for this research.

III. The Natural History of HIV/HCV Coinfection

Summary

In the United States, an estimated 16–25% of HIV-positive individuals are coinfected with HCV (Sherman 2002a; Tedali 2003b; Thomas 2002); as many as 90% of the people who acquired HIV through injection drug use are coinfected with HCV. In 1999, hepatitis C was classified as an opportunistic infection of HIV disease.

Before the use of effective prophylactic drugs and the advent of highly active antiretroviral therapy (HAART), many coinfected people died from other opportunistic infections before serious hepatitis-C-related liver damage developed. Now that AIDS-related mortality has decreased dramatically, end-stage liver disease (ESLD) has become a leading cause of death for HIV-infected individuals (Bica 2001; Martín-Carbonero 2001; Monga 2001; Quintana 2002; Rosenthal 2003).

Cohort studies of coinfected hemophiliacs and injection drug users have reported that coinfection with HIV accelerates HCV disease progression (Eyster 1993; Rockstroh 1996; Sánchez-Quijano 1995; Telfer 1994). Coinfected persons with advanced HIV disease (CD4 cell counts <200/mL) are at greater risk of developing cirrhosis (Allory 2000; Goedert 2002; Lesens 1999; Ragni 2001).

While it is clear that HIV infection accelerates HCV disease progression, the effect of HCV on HIV disease progression is controversial. A majority of pre-HAART-era studies did not find any differences according to HCV serostatus in HIV progression or survival (Dorrucci 1995; Macías 1998; Wright 1994). In the HAART era, however, there are new questions; conflicting data about the efficacy of HAART in coinfected individuals have emerged. Some studies have reported that immune responses to HAART are blunted in coinfected people, but the effect of HCV coinfection on the immunological response to HAART remains controversial, as this finding has not been consistent across studies (Chung 2002a; Greub 2000; Rockstroh 2004; Sulkowski 2002; Torriani 2001; Zala 2004).

Although HAART increases survival of HIV-positive people, coinfection with HCV increases the risk of liver-related death (Backus 2004; Fultz 2003a; Rimland 2004; Rockstroh 2004). Coinfected people and their medical providers are left with questions and uncertainties. If HIV disease is controlled with HAART, will hepatitis C be less aggressive as well? Will coinfected individuals fully reap the benefits of HAART? Is there a tipping point when the amount of damage to the immune system will result in acceleration of HCV disease? Information from randomized clinical trials will help inform treatment decisions about which disease to treat first and when to start treatment. In the meantime, balancing the immunological benefit of HAART with its potential hepatotoxicity is a huge concern.

HCV and Immunosuppression

Indirect evidence that HIV may influence HCV disease progression through immune suppression can be found in studies of HCV in immunocompromised people, such as people with congenital immune deficiencies and individuals taking immunosuppressive drugs following transplants. In people with immune deficiencies unrelated to HIV infection, accelerated HCV disease progression

has been observed (Bjorkander 1988; Collier 1997). One study followed a cohort with primary hypogammaglobulinemia who were infected from contaminated intravenous immune globulin treatments between 1982 and 1986. Fifteen of 17 persons were biopsied within 10 years of infection. All 15 had abnormal liver histology; 6 had cirrhosis (Bjoro 1994).

End-stage liver disease from hepatitis C is the leading indication for liver transplantation in the United States (Charlton 1998; Shuhart 1997). Hepatitis C infection almost always returns after transplantation. In the setting of post-transplant immunosuppression, hepatitis C can become more aggressive and have a more rapid course, although disease severity varies among recipients. In one study of transplant recipients, moderate-to-severe hepatitis or cirrhosis was identified in 25% of transplant recipients during a median follow-up period of 25 months (Zhou 1996). Gane and colleagues looked at 130 persons who survived for at least 30 months after transplantation. In this group, 10 (8%) developed cirrhosis during a median follow-up interval of 51 months (range: 24–138 months), and 35 (27%) developed moderate chronic hepatitis over 35 months of follow-up (range: 6–127 months). Seventy individuals (54%) developed mild chronic hepatitis after a median interval of 35 months (range: 6–103 months), and 15 individuals had no evidence of chronic hepatitis after a median follow-up period of 20 months (range: 6–103 months) (Gane 1996).

Acute HCV in HIV-Positive Individuals

HIV-positive people are less likely to achieve spontaneous viral clearance of HCV infection (Augenbraun 2003; Bhagani 2003; Danta 2003; Mehta 2002; Thomas 2000a). Mehta and colleagues evaluated the incidence of spontaneous viral clearance of HCV among 98 injection drug users with previous, resolved HCV infection (three HIV-positive) and 164 with no evidence of HCV exposure (three HIV-positive). They reported that individuals with a previous, resolved HCV infection were 12 times less likely to develop chronic hepatitis C after a new HCV infection than those with no previous exposure to HCV, although none of the three HIV-positive participants who became infected with HCV achieved spontaneous viral clearance despite having a prior, resolved infection (Mehta 2002).

Thomas and colleagues also found an increased likelihood of HCV chronicity among a group of HIV-positive injection drug users; the odds of chronicity increased with lower CD4 cell counts. For those with more than 500 CD4 cells, the odds ratio was 0.64 (95% CI, 0.26–1.36). In individuals with CD4 cell counts between 200 and 499, the odds ratio was 0.64 (95% CI, 0.36–1.14) and for those with less than 200 CD4 cells, the odds ratio was 0.31 (95% CI, 0.13–0.73). The adjusted odds ratio for spontaneous viral clearance of HCV among HIV-negative persons was 2.19 (95% CI, 1.26–3.47) (Thomas 2000a).

HIV-positive women and individuals with higher CD4 cell counts may be less likely to develop chronic HCV infection. Augenbraun and colleagues studied the incidence of HCV infection among members of the Women's Interagency HIV Study (WIHS) by testing samples from 2,628 women, 2,059 HIV-positive. Overall incidence of new HCV infections was low; it was slightly higher among HIV-negative women (3.3 cases per 1,000 person-years vs. 2.7 cases per 1,000 person-years for HIV-positive women). All participants were screened for anti-HCV at enrollment; subsequent samples were stored. The last available sample from women with no evidence of HCV was tested

for anti-HCV; if anti-HCV was detected, samples stored between the enrollment visit and the last visit were tested for anti-HCV. Samples from women who developed anti-HCV were tested for HCV RNA. Fourteen new HCV infections were identified; ten in HIV-positive women. While five women—two HIV-positive—achieved spontaneous viral clearance, chronic HCV developed in nine women, eight of them HIV-positive (Augenbraun 2003).

Danta and colleagues identified 23 cases of acute HCV infection in a cohort of HIV-positive men who have sex with men (MSM). The mean CD4 cell count at the time of HCV diagnosis was 600/μL. Spontaneous viral clearance occurred in 17% (4/23). The mean CD4 cell count at diagnosis was significantly higher among those who achieved spontaneous viral clearance (801 vs. 556; $P<0.05$) (Danta 2003).

Hemophiliacs with HIV/HCV Coinfection

HIV/HCV coinfection is a significant problem among hemophiliacs who were treated with clotting factors prior to the institution of viral inactivation techniques; an estimated 70% to 90% became infected with HCV, and 60% to 95% of the hemophiliacs who received clotting factor before 1985 were infected with HIV (Eyster 1993; Eyster 1994; Ragni 1993). Cohort studies of coinfected hemophiliacs have been a rich source of natural history data, as dates of infection can usually be estimated from records of when clotting factors were received, and several cohorts were followed for over 15 years.

There are some limitations to the information from cohorts of coinfected hemophiliacs. Since the majority of hemophiliacs are white males, the influence of race and sex on HCV disease progression cannot be determined from these studies. The course of disease in hemophiliacs infected with HCV and HIV may differ from that in people who acquired these infections through other routes, due to possible differences in the volume of inoculum and the potential for repeated infections. A majority of hemophiliacs were infected with HCV before they contracted HIV, so it is not possible to analyze potential differences in the natural history of coinfection according to the sequence of acquiring each infection (Eyster 1993; Ragni 2001). Due to the risk of bleeding from liver biopsy on hemophiliacs, assessment of HCV-related liver disease progression in hemophiliac cohorts has relied on biochemical testing and clinical features; information about liver histology is usually unavailable. Additionally, information about alcohol consumption was not consistently collected across studies. Despite these limitations, these studies have produced evidence of accelerated HCV disease progression among coinfected hemophiliacs.

Eyster and colleagues followed a cohort of hemophiliacs from 1973 until 1992. They found a higher incidence of liver failure among hemophiliacs who were coinfected; 8.8% (8/91) developed liver failure vs. none of the 58 hemophiliacs with HCV alone. In another cohort of 181 HCV-infected hemophiliacs (40% coinfected with HIV), hepatic decompensation occurred in 11 individuals; 10 of the 11 were HIV-coinfected. HIV-coinfected individuals were 21 times more likely to develop hepatic decompensation than those with HCV alone, and the median time from first exposure to clotting factor to hepatic decompensation was 16.5 years (range: 7.7–22.9 years) (Telfer 1994).

A low CD4 cell count appears to be an independent risk factor for HCV disease progression in

coinfected individuals. Goedert and colleagues followed 1,816 HCV-infected hemophiliacs, 1,192 (65.6%) of whom were coinfected with HIV. The estimated 16-year cumulative incidence of ESLD in coinfected individuals was 14.0% (95% CI, 11.6–16.4%). vs. 2.6% in those with HCV alone (95% CI, 1.0–4.3%). Coinfection with HIV increased the risk of developing ESLD by eight-fold (relative hazard 7.9; 95% CI, 4.2–15.2). The risk of developing ESLD among coinfected individuals with CD4 cell counts under 200 was doubled (relative hazard 2.1; 95% CI, 1.3–3.3). Ragni and colleagues found that ESLD in coinfected hemophiliacs occurred only at very low CD4 cell counts. A group of 157 HCV-infected hemophiliacs, 85 of whom were coinfected, was followed from 1978 until 1999. By 1999, ESLD was the second leading cause of death in coinfected individuals; 16% of coinfected individuals progressed to ESLD after a mean 18 years' (estimated) duration of HCV infection. No ESLD was reported among coinfected hemophiliacs with CD4 cell counts >150/mL.

Injection Drug Users with HIV/HCV Coinfection

Cohort studies of people who acquired HCV infection from injection drug use have provided important information about coinfection, although cofactors including HCV re-infection, regular use of street drugs, and nonsterile injection practices may also contribute to hepatitis C disease progression.

This research, too, has reported more rapid progression to cirrhosis among coinfected individuals than among those with HCV alone. In a study of 547 individuals who acquired hepatitis C from injection drug use (116 of whom were coinfected with HIV), Soto and colleagues observed stark differences in HCV disease progression by HIV status. Ten years after acquiring HCV, 14.9% (13/87) of coinfected individuals developed cirrhosis versus 2.6% (7/272) of those with HCV alone. The mean interval from HCV infection to cirrhosis was 23.2 years among individuals with HCV alone vs. 6.9 years in coinfected individuals ($P<0.001$) (Soto 1997).

Based on data from a pre-HAART-era, long-term retrospective cohort study of 160 HCV-infected IDUs (80 of whom were HIV-coinfected), Di Martino and colleagues developed a model that projected a higher risk of cirrhosis among coinfected individuals. Over 12 to 180 months, a higher prevalence of cirrhosis among coinfected people was recorded: 17.5% were cirrhotic vs. 7.5% of those with HCV alone. Alcohol consumption of more than 80 grams per day was associated with an eleven-fold increase in the risk of mortality from cirrhosis among coinfected individuals. Based on these findings, they calculated a significantly higher actuarial rate of cirrhosis in coinfected persons. The projected risk of cirrhosis among coinfected individuals at 10 years after HCV infection was 7% as compared to 3% in those with HCV alone; at 20 years, the risk increased to 37% among coinfected individuals as compared with 10% among those with HCV monoinfection. The cirrhosis risk at 25 years jumped to 69% for coinfected individuals, but remained stable at 10% in those with HCV alone. As in the hemophiliac cohorts, the risk of cirrhosis increased in coinfected individuals with CD4 cell counts below 200 (Di Martino 2001).

Although exact rates and outcomes vary, these studies present a consistent picture of accelerated hepatitis C disease progression in HIV-positive individuals. The variations may be attributable in part to high rates of HIV-related mortality before HAART, when HIV-related deaths preceded cirrhosis and ESLD in these cohorts. For example, Goedert and colleagues reported that in up

to 16 years of follow-up of 1,167 coinfected hemophiliacs, AIDS-related deaths reached a cumulative incidence of 45% (95% CI, 6–48.3%), compared to an estimated 16-year cumulative incidence of ESLD in coinfected individuals of 14.0% (95% CI, 11.6–16.4%).

Survival of Coinfected Injection Drug Users in the HAART Era

Injection drug use has been associated with an increased risk of death for coinfected people in the HAART era. Voirin and colleagues analyzed data from a cohort of 2,710 HIV-positive individuals, 469 coinfected, in Lyon, France. They evaluated the effects of HAART, HCV coinfection and injection drug use on survival in the pre-HAART (prior to 1996; N=1,240) and the post-HAART era (1996–2002; N=1,470). After HAART became available, three-year and five-year mortality rates decreased among all groups (HIV only, HIV/HCV and HIV/HCV plus injection drug use). After controlling for age and baseline CD4 cell count, the risk of death after 1996 HAART era was not substantially different for HIV/HCV coinfection (HR, 0.76; 95% CI, 0.28–2.08; P=0.59) than for HIV alone. The risk of death was substantially higher for HIV/HCV coinfection plus injection drug use (HR, 2.92; 95% CI, 1.63–5.23; P<0.001) (Voirin 2003).

Rapidly Progressive HCV in HIV-Positive Individuals: Pre-HAART

Most data on disease progression in coinfected persons come from research on individuals believed to have acquired HCV before becoming infected with HIV. A small body of data from the pre-HAART era provides information about rapidly progressive HCV acquired subsequent to, or at the same time as, HIV infection. These reports suggest rare scenarios in which underlying HIV infection can dramatically accelerate the course of HCV disease.

Martin and colleagues reported three cases of post-transfusion non-A, non-B hepatitis infections among HIV-positive men who were at least 60 years of age; all three developed cirrhosis within three years of the onset of hepatitis (P. Martin 1989). In another case, a 48-year-old health care worker acquired HIV and HCV from a needlestick accident in July 1990. At the time, additional exposure to blood occurred from a spill; blood from the collection tube seeped under the cuffs of the health care worker's gloves and onto chapped skin with open cracks, increasing the total amount of blood that the health care worker was exposed to. The source patient was HIV-positive and had a history of injection drug use, but had not been tested for hepatitis C. The health care worker developed antibodies to HIV between 8 and 9 months after this exposure. HCV seroconversion occurred between 9 and 13 months after the exposure. Less than three years after the needlestick, the health care worker died as a result of complications from hepatic failure. Testing of stored samples from the source patient and the health care worker provided phylogenetic evidence of transmission. The causes of such lengthy incubation periods (her HIV seroconversion at 9 months was one of the longest ever recorded, and her HCV seroconversion also occurred after an unusually long interval after the exposure) and such rapid disease progression remain unclear, but the authors suggest that the simultaneous acquisition and potential interaction between the two viruses might be involved (Ridzon 1997).

Did HCV Worsen HIV in the Pre-HAART Era?

Pre-HAART-era studies generally found no differences in HIV progression or survival between coinfected individuals and those with HIV alone. These studies have certain limitations; for instance, follow-up generally lasted only a few years, and detailed information about trends in CD4 cell counts and viral load was not available. On the whole, however, the data from these studies suggest that HCV infection does not dramatically alter the course of HIV disease over short periods of time (Dorrucci 1995; Macías 1998; Wright 1994). For example, a longitudinal study of 416 HIV-positive individuals (with known dates of seroconversion) compared the rate of clinical and immunologic progression of HIV disease between coinfected individuals (214/416) and those with HIV alone (202/416). During a follow-up period of 30 months, no statistically significant differences were observed between the two groups; endpoints were progression to AIDS and reaching a CD4 cell count below 100 (Dorrucci 1995).

Piroth and colleagues did not find an association between HCV coinfection and HIV clinical progression in a study of 238 HIV-infected individuals, half of whom were coinfected with HCV. Accelerated CD4 cell count decline was observed among a subset of the 27 coinfected persons with CD4 cell counts above 600 ($P=0.05$), but no difference in CD4 cell count changes or clinical progression was noted for individuals with lower CD4 cell counts (Piroth 1998). While hepatitis C itself may not significantly worsen the course of HIV disease, HCV-related liver disease might be a prognostic factor for accelerated HIV disease progression. A study by Lesens and colleagues evaluating a cohort of 147 HCV-infected hemophiliacs, 81of whom were coinfected with HIV, reported a more rapid progression to AIDS in coinfected individuals who developed liver disease ($P<0.03$) (Lesens 1999).

Prognostic Factors Among Coinfected Individuals

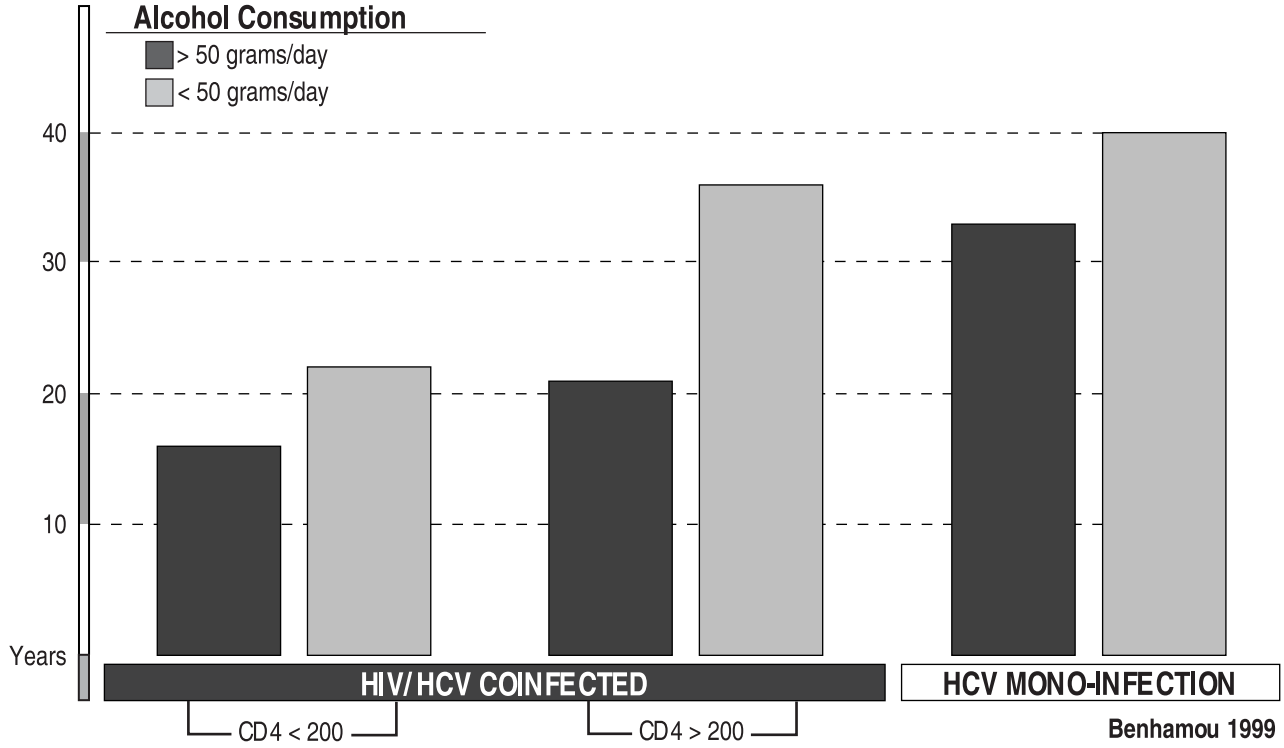
Benhamou and colleagues matched 122 coinfected individuals to 122 individuals with HCV alone and analyzed prognostic factors known to be involved in fibrosis progression. HIV status, presence of severe immunosuppression (defined as a CD4 cell count under 200), gender, age at infection with HCV (over or under 25), and alcohol consumption (>50 or ≤ 50 grams/day) were considered in a multivariate analysis of fibrosis progression. The median CD4 cell count in coinfected persons was 305/μL. Seventy-four of 122 coinfected persons were receiving antiretroviral therapy, although treatment regimens and durations varied widely. Among the coinfected participants, alcohol consumption over 50 grams/day, a CD4 count $\leq 200/\mu$L, and age at HCV infection >25 were independently associated with an accelerated fibrosis progression rate. Notably, HIV status was not associated with fibrosis progression in this analysis after controlling for CD4 cell count (Benhamou 1999).

To determine the rate of fibrosis progression, Benhamou and colleagues then examined 24 paired liver biopsies from 12 coinfected individuals. The observed progression rate was the difference in METAVIR score (see Cahpter IV, Diagnostics) between the two biopsies divided by the amount of time that had elapsed (in years) between them. To estimate the rate of fibrosis progression using results from only one liver biopsy, a model from Poynard and colleagues was used. In this model, the estimated rate of fibrosis progression per year was calculated as the ratio between the fibrosis stage at the time of biopsy (using the METAVIR system) and the estimated duration of infection in

years (Poynard 1997). (The model from Poynard and colleagues is based on cross-sectional data, estimated duration of infection, and an assumption of linear fibrosis progression, which may affect the accuracy of these estimates.) To validate their estimate of fibrosis progression, Benhamou and colleagues compared progression rates from the paired biopsies with estimated rates.

Overall, liver fibrosis progressed at a faster rate in coinfected individuals. Without HCV treatment, the median time to cirrhosis in HIV-coinfected individuals was 26 years vs. 34 years in those with HCV alone. The authors cited two examples: a coinfected individual with a CD4 cell count of ≤ 200/μL and alcohol intake of >50 grams/day would have a liver fibrosis progression rate of 0.250 fibrosis units per year (median expected time to cirrhosis of 16 years), while another coinfected individual with a high CD4 cell count and alcohol intake <50 grams/day would have a fibrosis progression rate of 0.111 fibrosis units per year (median expected time to cirrhosis of 36 years) (Benhamou 1999).

Figure 1. Median Expected Time to Cirrhosis According to CD4 Count and Alcohol Consumption



A direct look at biopsy samples from 492 coinfected individuals with an estimated median duration of infection of 14 years found more advanced liver damage than that seen in those with HCV monoinfection of similar estimated duration. Liver fibrosis was evaluated using the METAVIR system. No fibrosis (F0) was found in 13.2%, while 35% had a METAVIR score of F1, 19% had a METAVIR score of F2, 21% were scored as F3, and 12% as F4 (cirrhosis). The three strongest predictors of severe liver fibrosis (F3 or F4) were duration of HCV infection of over 15 years (OR, 3.6; 95% CI, 1.5–4.4), infection with HCV at over 20 years of age (OR, 3.3; 95% CI, 1.9–5.6), and a history of alcohol consumption of more than 80 grams/day (OR, 2.5; 95% CI, 1.5–4.4). No association of fibrosis with CD4 cell count, the HCV genotype or virus load, sex, or use of HAART was

observed, although 57% of participants had never received antiretrovirals (Martín-Carbonero 2003).

Martín-Carbonero and colleagues examined biopsy specimens from 914 coinfected individuals with elevated alanine aminotransferase (ALT, a liver enzyme; see Sidebar, Chapter IV, Diagnostics) levels. Overall, 57% had moderate-to-serious liver damage (METAVIR score of F2, F3 and F4), while only 10% had a METAVIR score of F0. They reported that predictors of severe liver fibrosis were age of >35 years (OR, 2.95; 95% CI, 2.08–4.18), consumption of >50 grams of alcohol/day (OR, 1.61; 95% CI, 1.1–2.35) and a CD4 cell count <500/mm^3 (OR, 1.43; 95% CI, 1.03– 1.98.) The median duration of HCV infection was 16 years; age at liver biopsy ranged from 33 to 41. Duration of HCV infection was not included in the multivariate analysis due to a strong correlation with age (Martín-Carbonero 2004).

Because data on duration of HIV infection and nadir CD4 cell counts were not included in this analysis, it is difficult to assess the influence of HIV disease on fibrosis progression. Severe liver fibrosis is more common among coinfected persons with advanced HIV disease; in this study, it was significantly more common among individuals using antiretroviral therapy than among the untreated (39% vs. 28%; $P<0.05$). The greater prevalence of severe liver disease among those on antiretroviral therapy could be due to advanced HIV disease requiring treatment, antiretroviral-induced hepatotoxicity, use of alcohol and drugs, or a combination of these and other factors.

Important questions persist in interpreting the increased HCV disease progression rates observed in coinfected individuals: does the presence of HIV itself accelerate HCV disease progression, or are higher rates of fibrosis progression associated primarily with immune deficiency? In a retrospective assessment of fibrosis progression in coinfected individuals, Puoti and colleagues found an association between CD4 cell counts <500/mm^3 and more severe fibrosis (OR, 3.2; 95% CI, 1.1–9.2). After controlling for CD4 cell depletion, HIV infection itself was not associated with severe fibrosis (Puoti 2001); however, in a case-controlled study of 116 HCV-infected individuals, 56 of whom were coinfected with HIV, Allory and colleagues found increased necroinflammatory activity in coinfected individuals, especially in those with high CD4 cell counts (>500/ml) (Allory 2000). This would support the theory that HIV infection itself, even prior to significant immune depletion, may accelerate the progression of liver fibrosis. The potential influence of HAART on these findings is not clear, as participants who were taking more than two antiretroviral drugs were excluded by Puoti and colleagues, and the use of HAART was not reported by Allory and colleagues.

Additional Screenings and Vaccinations

Vaccinations for hepatitis A and hepatitis B are recommended for coinfected individuals (Centers for Disease Control, *Guidelines for Preventing Opportunistic Infections Among HIV-Infected Persons, 2002*). HCV-infected individuals are at risk for fulminant hepatitis A, which can be life-threatening (Koff 2001; Pramoolsinsap 1999; Vento 1998; Vento 2000). Hepatitis B infection in combination with HIV and/or HCV has been associated with more aggressive liver disease, as well as with increases in morbidity and mortality (Cropley 2000; Liaw 2000; Ockenga 1997; Piliero 2002). Although vaccinations for hepatitis A and B are safe in HIV-positive individuals, their immunogenicity is decreased among HIV-positive individuals, especially those with low CD4 cell counts (Bruguera 1992; Hess 1995; Neilson 1997; Puoti 2002). CDC estimates that 66–75% of HIV-positive individuals will experience protective antibody responses after vaccination for HAV.

The CDC estimate may be high. Weissman and colleagues assessed the immune response to HAV vaccination in 123 HIV-positive individuals. Most were receiving antiretroviral therapy at the time of vaccination (102/123). Only half of those vaccinated (61/123) had an antibody response (positive IgG titer). Responders had significantly higher CD4 cell counts at vaccination than non-responders (486/mm^3 vs. 358/mm^3; P=0.02), and were more likely to be female than male (40% vs. 13.5%; P=0.001). Those with a CD4 cell count of <200/mm^3 at vaccination were less likely to respond (14% vs. 35%; P=0.02). Although nadir CD4 cell count was slightly higher among vaccine responders, the difference was not significant (Weissman 2004).

The efficacy of HBV vaccination in HIV-positive individuals is unclear, Tedali and colleagues reported that response to HBV vaccination among 51 of 198 HIV-positive individuals who received ≥ 1 dose of HBV vaccine. More than 70% were receiving antiretroviral therapy at vaccination. Only 37.2% (19/51) were responders. Those who had a CD4 cell nadir of >200/ mm^3, undetectable HIV RNA and high CD4 cell counts at vaccination were more likely to respond. Most responders had a CD4 cell nadir of >200/ mm^3 (84.2 % vs. 46.9%; P=0.008) and undetectable HIV RNA at vaccination (63.2% vs. 33.3%; P=0.04).The median CD4 cell count at vaccination was higher among responders (584 vs. 384/mm^3), although the difference did not reach statistical significance. Although use of HAART was not significant, those with undetectable HIV RNA and higher CD4 cell counts were more likely to be receiving HAART (Tedali 2004).

Strategies for optimizing the immunogenicity of HBV vaccination in HIV-positive people are necessary. Additional boosters or higher dosing have been suggested as possible interventions (Centers for Disease Control and Prevention, Recommendations of the Advisory Committee on Immunization Practices (ACIP): *Use of Vaccines and Immune Globulins in Persons with Altered Immunocompetence; 1993*).

Coinfection at Birth or in Early Childhood

HAART has substantially increased the life expectancy for those infected with HIV at birth (de Martino 2000; Gortmaker 2001), and HCV acquired at birth or early in childhood usually progresses slowly (Casiraghi 2004; Guido 1998; Kage 1997). The effect of untreated HIV on HCV disease progression in adolescents who were HIV/HCV coinfected at birth or during early childhood is unknown.

Thuret and colleagues evaluated biopsy samples from seven coinfected adolescents, six of whom received antiretroviral therapy for 9–14 years. All had CD4 cell counts >200/mm^3; CD4 cell counts ranged from 275 to 1,100 cells/mm^3. Six of seven had detectable HIV RNA, ranging from 3,162 to 54,954 copies/mL . HCV RNA levels ranged from 316,227 to 794,328 copies/mL.

Overall, METAVIR fibrosis and inflammation scores (see Chapter IV, Diagnostics) reflected mild liver disease. The authors found that one feature—intralobular inflammation—which is common in coinfected adults, was more marked in these coinfected children and adolescents than their HCV monoinfected peers (Thuret 2003).

Table 1. Mode of, and Age at Acquisition, CD4 count at Biopsy, Liver Histology & Disease Activity in Seven Coinfected Adolescents

Mode of transmission & age at infection	Sex/age at biopsy	CD4 count at biopsy	Disease activity	Fibrosis
Transfusion at birth	M/17.5	512	Mild	Moderate
Transfusion at 4 weeks	M/16.5	377	Mild	Mild
Transfusion at birth	F/16.5	1078	Mild	Moderate
Mother-to-infant at birth	F/16	275	Mild	Mild
Mother-to-infant at birth	F/13	1061	Mild	Mild
Clotting factor; 4 years	M/17.5	470	Mild	Moderate
Clotting factor; 4 years	M/17.5	1100	Mild	Moderate

Thuret 2003

Coinfection in the Era of Highly Active Antiretroviral Therapy (HAART)

Data collected during the HAART era provide a description of the current impact of coinfection and antiretrovirals on HIV-related hospital admissions. The benefits of HAART have been reflected in a survey of 327,306 HIV-related hospitalizations in 1996, 1998, and 2000. While hospital admissions for opportunistic infections decreased significantly (from 41% to 29% of all HIV-related hospitalizations; $P<0.001$), admissions for liver-related complications rose significantly (from 13% to 18%; $P<0.001$) (Gebo 2003).

Data on the impact of HAART on survival of coinfected people are emerging. Fultz and colleagues analyzed data from 36,419 HIV-positive participants in the Veteran's Aging Cohort Study (VACs) and 35,708 HIV-negative participants. Almost 20% of HIV-positive participants were coinfected with HCV (7,138/36,419), while 9% (3,214/35,708) HIV-negative participants were infected with HCV. The risk of death from HIV disease before 1996 was greater than the risk of death from coinfection after 1996 (Fultz 2003a).

Table 2. The Risk of Death From HIV, HCV, and HIV/HCV Before and After 1996

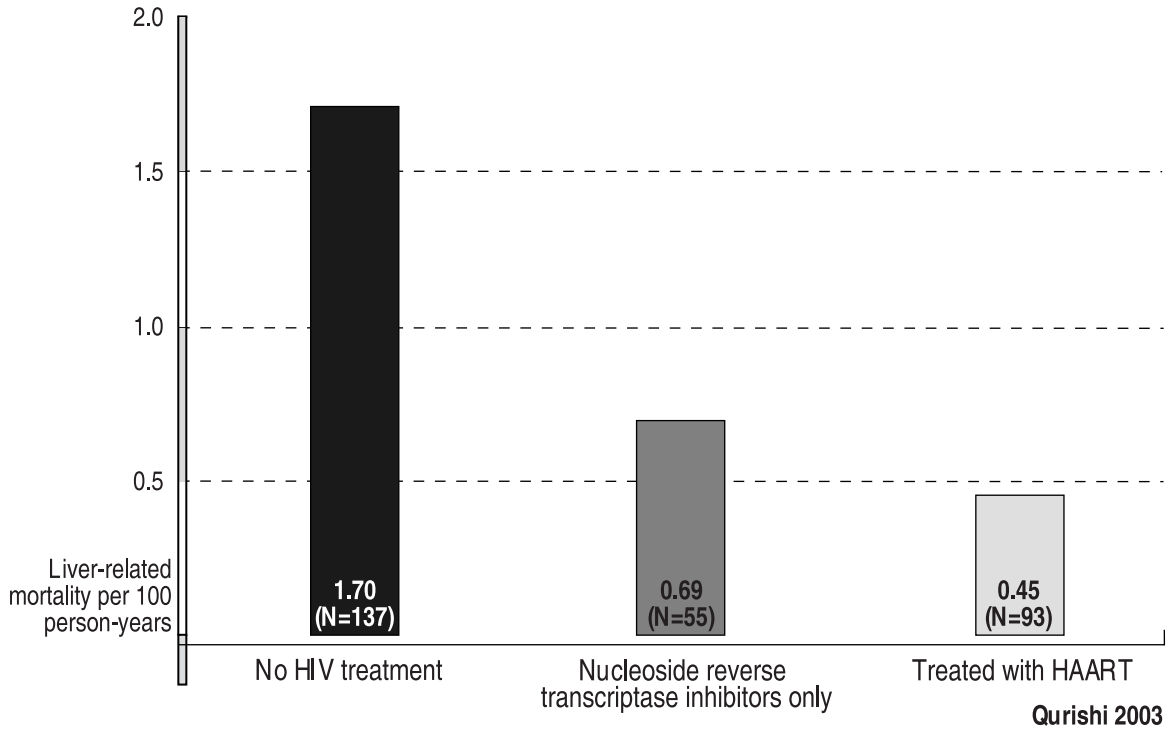
Infections	Pre-1996	Post-1996
HIV	10.2	2.7
HCV	0.3	1.2
HIV/HCV	2.2	2.6

Fultz 2003a

Although HCV coinfection increases the risk of liver-related death in people with HIV, HAART has significantly decreased AIDS-related and liver-related deaths (Backus 2004; Rimland 2004; Rockstroh 2004; Qurishi 2003; Voirin 2003). Qurishi and colleagues looked at the effect of antiretroviral therapy on liver-related mortality in a group of 285 coinfected persons treated for

HIV between 1990 and 2002. A total of 25 people died from liver disease during the study period; two treated with HAART, five with nucleoside analog reverse transcriptase inhibitors only, and 18 were untreated. Antiretroviral therapy significantly decreased liver-related mortality (P=0.018), and use of HAART was a significant predictor of liver-related survival (P<0.005) (Qurishi 2003).

Figure 2. Effect of HIV Treatment on Liver-related Mortality Rates



Rockstroh and colleagues evaluated data from 4,957 EuroSIDA cohort participants, 1,685 coinfected with HCV. HCV coinfection did not have a significant effect on progression to AIDS or on immunological or virological response to HAART. However, the risk of liver-related mortality was significantly greater among coinfected persons (IRR [incidence rate ratio] for liver-related mortality 3.18; 95% CI, 1.23–6.18; P<0.014) (Rockstroh 2004).

Backus and colleagues found that coinfection with hepatitis C did increase the risk of death in people with HIV, although the risk was far greater among those who were not taking HAART, regardless of HCV status. They evaluated Veteran's Administration (VA) National Immunology Case Registry (ICR) records from 12,216 HIV-positive individuals, 4,668 (37%) coinfected; all initiated HAART between January 1997 and February 2003. The mean length of follow-up was 3.5 years. Records included history of AIDS-defining conditions; use of antiretroviral therapy prior to HAART; CD4 cell count and HIV RNA within one year of initiation of HAART; measurement of exposure to HAART from prescription records; HCV treatment; and substance abuse and psychiatric diagnoses. Three sources were used to collect death data (Social Security Administration, VA beneficiary records and ICR).

Psychiatric diagnosis, alcohol abuse and drug use were significantly more common among those coinfected with HCV (P<0.001 for each). Coinfected people were more likely to have been

treated with nucleoside analog reverse transcriptase inhibitors (NRTIs) prior to initiation of HAART (48.8% vs. 38.8%; P<0.0001) and had a shorter duration of HAART (21.3 months vs. 24.9; P<0.0001).

Although HCV coinfection increased the risk of death (HR, 1.38; 95% CI, 1.26–1.51; P<0.0001), being off HAART was associated with a greater risk of death regardless of HCV status (HR, 3.91; 95% CI, 3.53–4.33; P<0.0001) (Backus 2004). This analysis controlled for variables including age; HIV RNA and CD4 at baseline; prior AIDS; pretreatment with NRTIs; HCV treatment; psychiatric diagnoses and use of alcohol and drugs; and HIV caseload of the facility.

Rimland and colleagues reported mortality data from The HIV Atlanta VA Cohort Study (HAVACS) a group of 2,506 HIV-positive individuals, 30–35% coinfected. Prospective data collected from 1981 until the end of 2003 were analyzed for rates and causes of death prior to and during the HAART era. In 1997, the mortality rate decreased from 26.1 per 100 to 6.7 per 100; it has remained stable since then. Death rates from AIDS and opportunistic infections decreased significantly (58.1% prior to 1996 vs. 38% after 1996; P=0.00002). Although deaths from HBV and HCV-related end-stage liver disease (ESLD) increased from 3.8% (27/707) to 6.7%(29/435) after 1996, the ESLD death rate per 100 patients did not change significantly (1.77 before 1996; 1.75 after 1996). In total, 19 deaths were attributed to HCV; four occurred before 1996 (Rimland 2004). Given the high prevalence of HCV coinfection in this cohort, the increase in ESLD-related deaths was not substantial.

Although antiretroviral therapy has significantly reduced AIDS-related mortality and liver-related mortality among coinfected persons, all 20 approved antiretroviral agents have been associated with severe and life-threatening events (DHHS 2004; Qurishi 2003). Reisler and colleagues estimated the incidence and predictors of non-AIDS defining grade 4 (serious or life threatening) events, AIDS events and death among a cohort of 2,947 individuals receiving antiretroviral therapy from December 1996 until the end of December 2001. A subset of 1,628 participants was tested for hepatitis B and C; 17.9% (291/1,628) were HCV coinfected. The risk of death after the first grade 4 event was similar to that of the first AIDS-defining event (5.68 and 6.95, respectively).

Serious and life-threatening liver events—liver enzyme elevations to >10 times the upper limit of normal (ULN); bilirubin elevations >5 times ULN, clinical or fulminant hepatitis, toxic hepatitis, fatty liver, cirrhosis, and hepatic encephalopathy—were the most common grade 4 events in this cohort. Any grade 4 liver event significantly increased the risk of death (HR, 3.49; 95% CI, 2.38–5.12; P=0.0001). HCV coinfection significantly increased the risk of developing a grade 4 liver event (HR, 2.74; 95% CI, 1.29–5.84; P=0.009) (Reisler 2003).

Impact of HAART on Fibrosis Progression

The impact of highly active antiretroviral therapy on fibrosis progression is controversial. Teasing out the contribution of a particular antiretroviral agent, or class of agents to fibrosis progression is difficult. Without sequential biopsy samples, individual fibrosis progression rates cannot be pinpointed. In most studies, fibrosis progression rates are estimated with a model that assumes linearity; this assumption is not always correct. The duration of a person's HIV and their HCV, the severity of their HIV disease, their age, use of alcohol and other drugs and variations in individual

fibrosis progression rates may make conclusions about HAART's effect(s) on fibrosis difficult.

Benhamou and colleagues evaluated the impact of protease inhibitors on fibrosis progression in a retrospective analysis of data from 182 coinfected people; 63 of them were treated for HIV with a protease inhibitor. They considered age, alcohol consumption, CD4 cell count and HIV RNA. For HCV, mode and duration of infection, age at infection, genotype and liver biopsy specimens were evaluated. Fibrosis progression rates were estimated with a model that assumed linear progression from the time of infection until biopsy. Use of a protease inhibitor was significantly associated with a lower stage of liver fibrosis ($P=0.03$). The authors suggested that fibrosis progression might be stabilized by reducing alcohol intake (from >50 grams/day to <50 grams/day) and maintaining a high CD4 cell count with combination therapy including a protease inhibitor (Benhamou 2001).

Macías and colleagues evaluated data and biopsy samples from 152 HIV/HCV coinfected people with a known or estimated duration of HCV infection. A majority had been treated for HIV (105/152) (Macías 2004). They reported that HIV therapy that included nevirapine (a non-nucleoside reverse transcriptase inhibitor) was significantly associated with a more rapid fibrosis progression rate, although the drug's actual contribution to accelerated fibrosis progression is unclear. Recipients of nevirapine were older, and more likely to have clinical AIDS (25% vs. 6% of the non-HAART group). Both aging and advanced HIV disease have been associated with accelerated fibrosis progression.

For more information on antiretroviral agents, see Chapter V, HIV Treatment in HIV/HCV Coinfection.

HCV and HAART: New Questions about Immune Reconstitution

While the introduction of HAART has provided the opportunity for controlling HIV, concerns have been raised about the immunologic benefits of antiretroviral therapy for coinfected persons. In research conducted before the HAART era, HCV was not generally shown to have a significant influence on HIV disease progression. In the post-HAART era, coinfected individuals appear to respond well to therapy as measured by HIV RNA levels (Chung 2002a), although conflicting results have emerged from more recent studies examining whether hepatitis C coinfection affects the likelihood and extent of immunologic response to antiretroviral therapy. One large cohort study showed a blunted CD4 cell response to HAART in coinfected persons as compared to those with HIV alone (Greub 2000); another showed no significantly diminished CD4 cell responses to HAART among coinfected persons (Sulkowski 2002).

The Swiss Cohort Study

The Swiss Cohort Study provided the first indications of reduced immune reconstitution among coinfected persons taking HAART. A cohort of 3,111 HIV-positive individuals, 1,157 (37.2%) of them HCV-antibody-positive, began HAART regimens containing two nucleoside reverse transcriptase inhibitors and a protease inhibitor between June 1, 1996, and May 31, 1999. The median follow-up time was 28 months. There were some small but significant differences between the HCV-seropositive and HCV-seronegative groups. While 58.9% of coinfected individuals were treatment naïve at baseline, only 52.3% of those with HIV alone were treatment naïve ($P<0.001$).

The HCV-seropositive participants were more likely to have had an AIDS diagnosis at baseline (27.7% vs. 23.5%; P=0.009) and lower median baseline CD4 cell counts (172 [range: 70–322] vs. 222 [range: 90–373]; P<0.001). The presence of antibodies to HCV was also associated with active drug use, lower income, less education, female sex, and younger age.

While more than half of the entire cohort maintained undetectable HIV viral loads (<400 copies/mL), there were significant differences in CD4 cell increases after initiation of HAART. HCV-positive individuals had smaller increases in CD4 cell counts; this group was 21% less likely to see CD4 cell count increases of at least 50/μL. Blunted CD4 increases were seen in coinfected individuals at all baseline CD4 strata, as well as in those with undetectable HIV RNA levels; however, no association was found between anti-HCV positivity and either the likelihood of achieving an undetectable HIV viral load after initiation of antiretroviral therapy or the time to subsequent virological failure (two consecutive viral load measurements >400 copies/mL).

The risk of clinical progression to an AIDS-defining event or death was higher among HCV-seropositive individuals. The estimated probability for clinical progression at two years was 6.6% (95% CI, 5.6–7.9) with HIV alone and no active injection drug use (IDU). The probability of clinical progression increased to 9.7% (95% CI, 7.4–12.7) for HCV-seropositive individuals without active IDU, and rose to 15.0% (95% CI, 12.2–18.4) for HCV-seropositive individuals with active IDU. The hazard ratio for clinical progression was 2.07 (95% CI, 1.40–3.06) for anti-HCV-positive individuals as compared to those with HIV alone.

HCV seropositivity was strongly associated with active injection drug use in this cohort (odds ratio 45.4; 95% CI, 30.8–66.8). The overwhelming majority of HCV-seropositive individuals had a history of IDU (87.7%, vs. 4.8% in the HCV-seronegative group; P<0.001) (Greub 2000). Data from other research suggest that IDU—either through direct effects on the immune system or by influencing treatment adherence and utilization of medical care—can play a role in HIV disease outcomes. Opiates may have an effect on the immune system (Carr 1995; G. Nunez 1999; Peterson 1998; Rouveix 1992; Roy 1996). In one study, IDUs reported a 35% rate of nonadherence to HAART as compared to a 24% rate in nonusers and 17% in former users. Smaller CD4 cell increases were observed in the active users (65 CD4 cells vs. 122 in former users and 116 in nonusers) (Lucas 2001).

Although HCV prevalence was not reported in the Lucas study, it would presumably be comparable to that between active and former IDUs, given that most IDUs acquire HCV within a year of initiating injection drug use. This would suggest that current IDU may independently reduce CD4 cell gains for those on HAART, either directly through the immune system or through poorer adherence. The Swiss Cohort Study attempted to discount the potentially confounding influence of IDU in its evaluation of treatment outcomes for HCV-seropositive individuals. It reported that differences in CD4 cell gains and clinical outcomes persisted even when active IDUs were excluded from the analysis; however, determination of active injection drug use was based on patients' reports and physicians' observations, which may have underestimated the extent of injection drug use in the HCV-seropositive group.

The Baltimore Study

Sulkowski and colleagues analyzed data from a cohort of 1,955 HIV-positive individuals, 873 of them anti-HCV-positive. HAART was given to 54% of the HCV-seropositive participants and 67% of the HCV-seronegative participants (total n=1,199). The median length of follow-up for HCV-seropositive individuals was 2.19 years; for those with HIV alone, median follow-up was 2.00 years.

HCV status did not influence the response to HAART, despite differences in baseline characteristics between HCV-seropositive and HCV-seronegative participants. Eighty-five percent of HCV-seropositive individuals had a history of IDU, compared with 13% of the HCV-seronegative participants ($P<0.001$). The HCV-seropositive group also had lower absolute CD4 cell counts at entry (median, 237 vs. 266; $P=0.02$) and were less likely to have received prior antiretroviral therapy ($P<0.001$). Virological response to HAART was equivalent among the HCV-seropositive and HCV-seronegative participants; 29% of both groups achieved well-controlled HIV RNA levels (defined as < 400 copies/mL on at least 75% of clinic visits). Among those with well-controlled HIV RNA, no detectable difference was observed between HCV-seropositive and HCV-seronegative participants in immunologic responses to HAART at one, two, and three years after initiation of anti-HIV treatment.

In HAART recipients, there were no significant HCV-serostatus-related differences in the risk of acquiring an AIDS-defining illness (RH, 1.09; 95% CI, 0.88–1.34) or in the risk of death (RH, 1.22; 95% CI, 0.22–1.61). In a subgroup of HCV-seropositive individuals with CD4 cell counts between 50/μL and 200/μL, the risk of death was higher than that for HCV-seronegative individuals, especially for those who had received HAART. After multivariate analysis, however, death was independently associated with total exposure (in years) to HAART, percentage of clinic visits with detectable HIV RNA levels, older age, and baseline CD4 cell count—not with HCV seropositivity (RH, 1.01; 95% CI, 0.65–1.56) (Sulkowski 2002).

In the Baltimore cohort, there were 187 deaths among the 1,199 individuals who received HAART (15.6%). There were 6 deaths in the subgroup of 208 individuals with durable viral control (2.9%). After controlling for use of HAART, there was no difference in the mortality rate between groups by HCV serostatus. The Baltimore cohort had a higher mortality rate than the Swiss Cohort Study (181 deaths [5.8%] among 3,111 participants). In the Swiss Cohort Study, there were 20 deaths among the subgroup of 1,596 with well-controlled HIV RNA (1.3%). A greater frequency of non-AIDS-related deaths (such as liver disease, drug overdose, and violence) among HCV-seropositive individuals was noted in both cohorts.

The demographic differences between the Baltimore cohort and the Swiss cohort are significant: in the Swiss cohort, IDUs were more likely to be female, white, and young; in the Baltimore study, IDUs were more likely to be male, African-American, and in their late thirties. Information about adherence was not provided for either study, and virological control has been used as a surrogate. There are many confounding variables involved and it may not be possible to elucidate the many external factors that can influence individual disease progression such as poor nutrition, host and virological differences, other co-morbid conditions, variable HIV treatment histories, differing stages of HIV disease, pre-existing liver damage, and other individual prognostic factors.

The Aquitaine Cohort

A French HIV cohort study found an association between severely elevated aspartate aminotransferase levels (AST) and poorer survival on antiretroviral therapy, although the presence of antibodies to HCV was not associated with mortality. In this prospective study, 995 HIV-positive individuals—576 of whom were HCV-antibody-positive—were treated with antiretroviral therapy (consisting of dual nucleosides or triple combinations with a protease inhibitor) and followed for a median of 35 months. At baseline, median CD4 cell counts did not differ significantly between the HCV-positive and HCV-negative groups (189 vs. 213; P=0.3), although mean HIV RNA was slightly lower in anti-HCV-positive individuals (4.07 $\log_{10}$ vs. 4.34 $\log_{10}$; P=0.02; based on 366 available samples). At 24 months, HCV serostatus was not associated with a significant difference in median CD4 cell increases (+82/mm^3 for HCV/HIV vs. +105/mm^3 for HIV alone) or mean HIV-RNA changes (-1.35 $\log_{10}$ vs. -1.60 $\log_{10}$). At 48 months, the overall median CD4 cell increase was +146 mm^3. Those taking a protease inhibitor had significantly higher median CD4 cell increases (+191/mm^3, compared with +81/mm^3 in those taking only dual nucleosides) and larger HIV-RNA decreases (-1.89 $\log_{10}$ vs. -1.27 $\log_{10}$).

No association between HCV seropositivity and poorer survival was detected. After three years, the probability of survival among HCV-seropositive individuals was 89.7% (95% CI, 86.8–92.6), while the survival probability for those with HIV alone was 92.0% (95% CI, 88.7–95.4); however, large elevations in AST levels—seen in 15% of HCV-seropositive individuals and in 7% of those with HIV alone—were significantly associated with poorer survival (HR for elevations >200 IU/l of 2.30; 95% CI, 1.32–4.03; P=0.004) (Rancinan 2002). This is cause for concern, since coinfected individuals often have elevated AST levels, which may be due to both liver disease and hepatotoxicity of anti-HIV medications (Staples 1999).

The AIDS Clinical Trials Group Analysis

Chung and colleagues retrospectively compared the virological and immunologic responses to 16 weeks of HAART by looking at stored plasma samples from 40 coinfected individuals and 129 with HIV alone who participated in two antiretroviral treatment trials, ACTG 348 and ACTG 320. There were no significant differences by HCV status in baseline CD4 cell counts, HIV-RNA levels, or the initial antiretroviral regimen. Stored plasma samples from 40 coinfected participants and 129 with HIV alone were evaluated. At week 16, there were no significant differences in mean CD4 cell increases between the two groups. Coinfected individuals with baseline CD4 cell counts >350/mm^3 had greater CD4 cell increases than did HCV-seronegative individuals in the same CD4 strata (+145/mm^3 vs. + 82/mm^3; P=0.0331). They also had significantly larger decreases in HIV RNA (-2.55 $\log_{10}$ vs. -2.02 $\log_{10}$; P=0.02) and a higher rate of HIV suppression to <500 copies/mL (97.4 vs. 84.4%; P=0.04) (Chung 2002a).

Although 48-week data were provided—showing no significant differences in virological and immunologic response between groups—treatment regimens diverged after week 24 and samples were not uniformly available. A longer, prospective study with a larger sample size is needed to determine if disparities in immunologic responses to HAART by HCV status are consistent in each CD4 cell strata.

Making Sense of Conflicting Results

The contrasting results from these studies will need to be resolved by additional research. The constraints and limitations in the study designs and data collected make definitive answers elusive. For example, HCV-RNA data were not consistently gathered across studies; the reliance on HCV-antibody results without confirmatory testing for the presence of the virus may not have accurately represented the distribution of chronic hepatitis C infection. In the Baltimore cohort, participants with antibodies to HCV were not tested for HCV RNA. This decision was based on previous research by one of the study authors, in which 90% of HIV-positive individuals with antibodies to HCV also had detectable HCV RNA, and results from a similar cohort where the HCV antibody test was found to be more than 99% accurate in HIV-positive individuals (Thio 2000; Thomas 2000a). In the Swiss Cohort, only a small subgroup of HCV-seropositive individuals was tested for HCV RNA; of the 56 individuals tested, 14 (25%) had undetectable HCV RNA (Greub 2000). Without a second HCV-RNA test, it is not possible to distinguish a current HCV infection from a prior, resolved infection, as people with chronic HCV may be intermittently viremic (see Chapter IV, Diagnostics).

In addition, individuals with advanced HIV disease may not produce antibodies to HCV; HCV-RNA testing may be necessary in this population to confirm or rule out active HCV infection (Busch 2001). There were 460 HCV-seronegative participants in the Swiss cohort with AIDS at baseline; a portion of this group may have been infected with HCV but undiagnosed because they did not produce antibodies to HCV.

Because these studies examined the effect of HCV on HIV, information about the severity of HCV disease was not collected. Without data on HCV-RNA levels, liver enzyme levels, and actual liver histology, it is impossible to determine the severity of HCV disease, which may in itself have had an effect on response to HAART.

The long-term effects of HAART on HCV disease progression require further study. HAART increases HCV-RNA levels, especially in individuals with CD4 cell counts below 350 (Chung 2002a; Rutschmann 1998). Coinfected individuals with normal baseline ALT levels have seen ALT flares after immune reconstitution from initiation of HAART (Chung 2002a; Torre 2001; Torriani 2002b). Does the degree of HAART-mediated immune reconstitution influence long-term HCV disease progression? Clinical trials that incorporate assessment of HIV and HCV disease progression are needed to increase our understanding of the complex interrelationship between these two viruses. In the meantime, developing treatment strategies for coinfected individuals must become a priority.

Recommendations

Establish prospective longitudinal cohort studies of the natural history of HIV/HCV coinfection in the era of HCV treatment and HAART.

In the HAART era, new questions and conflicting data about the efficacy of HAART in coinfected individuals have emerged (Greub 2000; Law 2002; Sulkowski 2002). We need to increase our understanding of the complex interrelationship between these two viruses, and the impact of HAART and immune restoration on HCV progression. So far, most studies that have examined the effect of HCV on responses to HAART and clinical progression of HIV disease have not collected information about the progression or severity of underlying HCV disease. Without data on actual liver histology, it is impossible to determine the severity of HCV disease, which may in turn affect an individual's ability to respond to HAART.

Well-designed, prospective longitudinal cohorts will be essential to following infected populations; defining prognostic factors and other cofactors for progressive disease; observing changing treatment outcomes over time; and generating productive hypotheses for pathogenesis, prevention, and treatment studies. Because treatment modalities for HIV and HCV will continue to evolve, cohort studies must be large enough and long enough to measure and account for variations in treatment, as well as other cofactors such as access to health care, drug and alcohol use, race/ethnicity, and sex. Barriers to enrollment such as invasive needle biopsies can be addressed by being restricting those procedures to intensified substudies, if necessary. In any case, full participation of coinfected persons and advocates will be essential in planning and implementing such cohort studies. The National Institutes of Health and the Centers for Disease Control and Prevention must support these studies.

Develop strategies to enhance HAV and HBV vaccine immunogenicity in HIV-positive individuals.

Although vaccinations for hepatitis A and B are safe in HIV-positive individuals, vaccine immunogenicity is decreased, especially in persons with low CD4 cell counts (Bruguera 1992; Hess 1995; Neilson 1997; Puoti 2002; Tedali 2004; Weissman 2004). CDC estimates that only 66–75% of HIV-positive individuals develop protective antibody responses after vaccination for HAV. Response rates to HAV vaccination in HIV-positive people may be lower than the CDC estimate; one study reported response to HAV vaccination in only 49% of HIV-positive individuals (Weissman 2004).

The efficacy of HBV vaccination in HIV-positive individuals is suboptimal; a strategy to enhance immunogenicity is needed. Additional doses of HBV vaccine may improve HBV vaccine response in people with HIV (CDC 1993).

Research on interventions to enhance the immunogenicity of HAV and HBV vaccines in HIV-positive persons should be made a priority by the National Institutes of Health.

List of Terms Used in This Chapter

Actuarial rate: a model projecting future risk.

Aspartate aminotransferase (AST): a liver enzyme.

HIV–treatment naïve: an individual who has never taken anti-HIV medications is referred to as treatment naïve.

Hypogammaglobulinemia: an immune deficiency in which the body's production of antibodies and, in some cases, other immune responses are insufficient.

Log: Because viral loads can range from <5 copies/mL to tens of millions of copies, researchers sometimes use logarithmic—rather than linear—metrics to describe viral load levels. For example, a one-log decrease—or a 90% decrease—in viral load would be a reduction from 1 million to 100,000. A two-log decrease would be a reduction to 10,000. A one-log increase in viral load from 1 million would be a rise to 10 million.

Phylogenetic analysis: testing performed on a virus to identify genetic "family resemblance" among viral strains.

IV. Diagnostics

Summary

Since the discovery of the hepatitis C virus in 1988, the field of HCV diagnostics has advanced rapidly. Key tests for diagnosing chronic HCV infection, assessing prognosis, predicting and monitoring the effects of treatment, and measuring other complications of HCV infection include: HCV-antibody and RNA-PCR (viral load) tests; liver function tests (LFTs); HCV-genotypic tests; and body scanning technologies such as liver scan, computerized tomography (CT) scans, magnetic resonance imaging (MRI) scans, and ultrasound surveillance for hepatocellular carcinoma (HCC). Liver biopsy is still considered the "gold standard" for assessing HCV-induced liver damage and determining the need for HCV treatment, though researchers are working intensively to discover accurate but less invasive techniques.

Diagnosis of Acute Hepatitis C Infection. Most individuals will develop antibodies to hepatitis C virus (HCV) six weeks to six months after they have been infected, although in some instances antibodies to HCV will not appear for years after infection (M. J. Alter 1992; Beld 1999). The EIA-3 (the latest version of the HCV antibody test) has a sensitivity of 97%; however, it has a positive predictive value of only 25% in low-risk individuals (Gretch 1997). Therefore, any positive EIA-3 results, especially in individuals at low risk or individuals with rheumatoid factor or high immunoglobulin levels, need verification with confirmatory testing using either a recombinant immunoblot assay (RIBA, another test for antibodies to hepatitis C) or HCV-RNA testing (which identifies the presence of viral RNA and hence the actual virus). Because people who have achieved spontaneous viral clearance of HCV may remain HCV-antibody-positive, confirmatory testing for the presence of HCV RNA is needed to distinguish resolved infections from active, current infections. HCV-infected individuals may be intermittently viremic (Beld 1999; Villano 1999). In order to confirm or rule out current HCV infection, it is recommended that people with undetectable HCV-RNA levels have a follow-up HCV-RNA test six months later.

Individuals with acute HCV infection may receive a false negative antibody test result prior to seroconversion (when blood tests change from antibody-negative to antibody-positive). Some people may not be able to produce antibodies to hepatitis C. Transplant recipients, active injection drug users, and HIV-positive individuals—especially those with CD4 counts below 200—may require confirmatory HCV-RNA testing (Beggren 2001; Beld 1999; Busch 2001; H. H. Lin 2002; Thomas 1995).

Distinguishing Acute from Chronic Infection. Acute hepatitis C virus infections do not inevitably develop into chronic infections. Proper diagnosis of acute HCV infection with appropriate diagnostic follow-up can distinguish acute infections from resolved or chronic infections. Acute HCV infection may be diagnosed using information from several tests that rule out acute infection with other hepatitis viruses, measure alanine aminotransferase (ALT) levels, and look for the presence of HCV antibodies and HCV RNA. Chronic hepatitis C infection may be diagnosed by confirming positive EIA-3 results with RIBA or HCV-RNA testing.

HCV-RNA Testing. Hepatitis C RNA is detectable between one and two weeks after exposure (H.J. Alter 1991). The amount of hepatitis C virus in a person's blood varies among individuals,

but mode of transmission, length of infection, coinfection with hepatitis B, age, amount of alcohol consumption, and HIV status may have an effect on HCV-RNA levels (Oshita 1994; Pessione 1998; Sawada 1993; Thomas 2000). HIV-positive individuals who are coinfected with HCV have higher levels of HCV RNA than individuals with HCV alone (Cribier 1995; Di Martino 2001; Eyster 1994; Sherman 1993; Sulkowski 2002; Thomas 2001; Zylberberg 1996). Although HCV RNA is not predictive of disease progression or indicative of liver injury, individuals with HCV-RNA levels below 2,000,000 copies/mL or 800,000 international units (IU)/mL have better responses to interferon treatment (Fried 2002; Lau 1993; McHutchison 1998; Poynard 1998; Rumi 1997).

Two different types of RNA tests, quantitative and quantitative, are available. Qualitative HCV-RNA tests measure the presence or absence of HCV RNA, giving a positive or negative result, while quantitative tests measure the amount of HCV RNA per milliliter (mL) of blood. Qualitative testing is usually used to diagnose HCV infection, and may be used to measure response to HCV treatment (when quantitative test results are below the threshold of detection). Quantitative testing is used to establish the baseline amount of HCV RNA in a person's blood or tissue, to assess response to treatment and, sometimes, to diagnose HCV.

Liver Panel. The liver panel comprises a number of different blood tests and is used to provide diagnostic information for acute and chronic HCV infection as well as to assess the liver's capacity to metabolize drugs. Liver panel testing can identify possible liver damage and provide some information about response to HCV treatment (see A Guide to the Liver Panel at the end of this chapter). Frequent monitoring of liver enzyme levels is especially important for HIV-coinfected individuals in order to serially evaluate for evidence of HAART-associated hepatotoxicity.

Alanine Aminotransferase (ALT; a liver enzyme). Alanine aminotransferase (ALT) levels may remain normal, or become transiently or persistently elevated during chronic HCV infection. More than two-thirds of HCV-infected individuals have abnormally elevated ALT levels (Dufour 2000); However, ALT elevations do not predict disease progression (Herve 2001; Jamal 1999; Persico 2000). Some researchers have found less liver damage in individuals with persistently normal ALT levels, while others have observed liver damage despite persistently normal ALT levels (Herve 2001; Jamal 1999; Persico 2000; Puoti 1997).

Genotypic Tests. There are at least six different genotypes (viral strains) of HCV, and many subtypes. The genotype is the most important prognostic indicator of response to interferon treatment. Most HCV infections in the United States are genotype 1. Genotypes 1, 4, and 5 do not respond as well to interferon as do genotypes 2 and 3 (Fried 2002; Germer 2001; Mondelli 1999; Neumann 2000; Nousbaum 1998; Poynard 1998; Rosenberg 2001; Zein 1996b). A 48-week course of treatment is recommended for individuals with genotypes 1, 4, and 5; only 24 weeks of treatment are recommended for individuals with genotypes 2 and 3 (McHutchinson 2002; Poynard 2000; Soriano 2002). It is possible to become re-infected with HCV, or to have a mixed infection with more than one genotype (Accapezzato 2002; De Socio 1996; García- Samaniego 1997; Jarvis 1994; Tuveri 1997), although it is not clear if mixed infection can accelerate HCV progression.

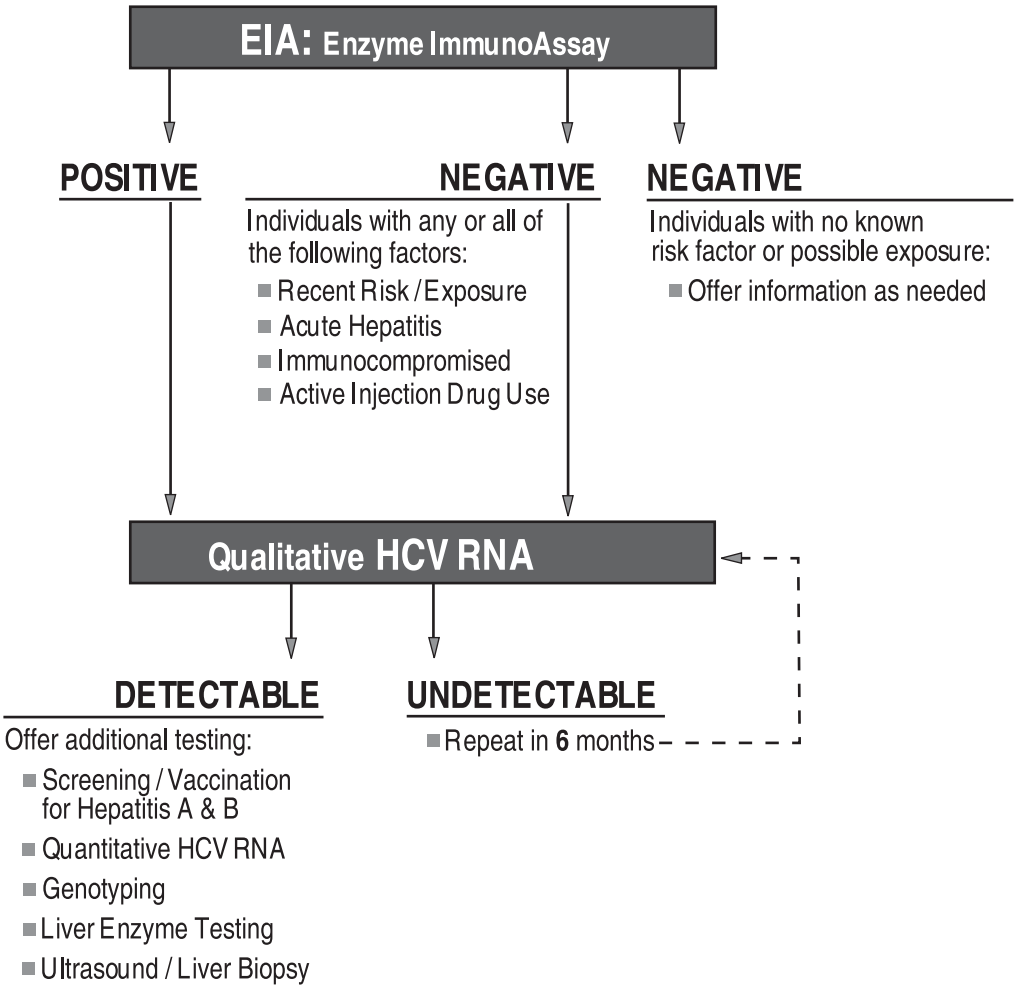
Scans. Additional information about the condition of the liver can be obtained from tests such as liver scans, CT scans, MRI, and ultrasonography, but these tests cannot replace biopsy. A combination

of blood tests has shown promising results as an alternative to biopsy in some situations, but more research is needed to identify and validate additional alternatives to biopsy (Boeker 2002; Forns 2002; Guechot 1994; Imbert-Bismut 2001; Leroy 2004; Mehta 2004; Myers 2002; Patel 2003; Sud 2004; Wai 2003).

Liver Biopsy. Liver biopsy is the only way to assess the condition of the tissue in an individual's liver. Information from liver biopsy is used to grade disease activity (assess the amount of inflammation and cell death) and stage the amount of damage to the liver; to identify any other causes of liver injury; and to make treatment decisions. Liver biopsy is usually performed in a hospital. A thin needle is quickly inserted between the ribs to remove a tiny sample of liver tissue. Liver biopsy can be painful and, occasionally, complications such as hemorrhage or puncture of adjoining organs may occur. Fatalities from biopsy are very rare (0.01–0.1%). Having a biopsy performed by an experienced physician using ultrasound guidance may reduce the risk of pain, complications, and sampling errors (Cadranel 2000; Pokorny 2002).

Ultrasound. Ultrasound surveillance for HCC in cirrhotic individuals (who are at higher risk for HCC than other HCV-infected individuals) increases early detection, but has not been proven to increase survival (Larcos 1998; Solmi 1996).

Figure 1. HCV Diagnostic Algorithm



Hepatitis C Antibody Testing

Antibodies to hepatitis C usually develop six weeks to six months after infection, although some individuals will not seroconvert (change from antibody-negative to antibody-positive) until 94 months after infection (Alter 1992; Beld 1999). Some individuals never develop antibodies to HCV, even when HCV RNA is detected in their blood (Durand 2000). Others may lose their antibodies to HCV years after infection, although they have detectable HCV RNA (M. J. Alter 1992; Beld 1999).

The sensitivity of the EIA-3 is 97%, but in low-prevalence populations, it has a positive predictive value of only 25% (Thomas 1997). False negative antibody test results may occur among people who are acutely infected with HCV and have not yet seroconverted. Immunocompromised individuals, such as transplant recipients and HIV-positive persons, may receive false negative results due to an inability to produce antibodies to hepatitis C. In some cases, injection drug users may not have antibody responses to HCV, although they have detectable HCV RNA (Beld 1999; Thomas 1995). Confirmatory HCV-RNA testing may be used to diagnose HCV infection in current injection drug users.

Because individuals who have had a recent exposure to HCV, as well as immunocompromised individuals who have been at risk for HCV infection in the past, may receive a false negative antibody test result, confirmatory testing for HCV RNA (testing that looks for the actual virus) is recommended. False positive results to HCV antibody testing may occur, especially among people with rheumatoid factor or high immunoglobulin levels. In order to properly diagnose hepatitis C infection, a positive antibody test result needs to be confirmed by recombinant immunosorbent assay (RIBA) or by testing for HCV RNA.

The RIBA has been used as a supplemental test, but the EIA-2 and the EIA-3 are more sensitive than the RIBA-2 and the RIBA-3 (Vrielink 1997). The RIBA test is useful for ruling out false positive antibody test results in low-risk groups. In individuals with a recent exposure to HCV, a negative RIBA does not rule out current HCV infection; these individuals need HCV-RNA testing to confirm or rule out current HCV infection.

The serum ELISA assay for HCV antibodies has been modified for oral fluid testing. The oral fluid ELISA was tested in 109 individuals who were HCV-antibody-positive by serum ELISA and 107 individuals who were HCV-antibody-negative by serum ELISA. The sensitivity of oral ELISA fluid testing for HCV antibodies was 98.2% and the specificity was 99.1% (Sherman 1994). This is comparable to the sensitivity (99%) and specificity (99.8%) of the serum EIA-3 (Abdel-Hamid 2002). Despite the accuracy and convenience of oral fluid testing for HCV antibodies, the oral fluid test has not been marketed.

Many current and former injection drug users have poor venous access. In addition, they may not seek HCV testing in clinical settings. An oral fluid test will facilitate individual testing in non-traditional venues such as syringe exchange programs and facilitate epidemiological surveys.

Diagnosis of Acute HCV Infection

Individuals with acute HCV do not invariably develop chronic HCV infection. Proper diagnosis of acute HCV with appropriate diagnostic follow-up is necessary to distinguish between resolved or chronic infections. Some preliminary research has indicated that treatment of acute HCV may reduce the likelihood of chronic infection (see Chapter V, Hepatitis C Treatment).

Acute hepatitis C infection may be identified by a combination of tests, beginning with screening to rule out acute infection with hepatitis A (HAV) and hepatitis B (HBV). If HAV and HBV test results are negative, acute HCV may be diagnosed by the presence of alanine aminotransferase levels (ALT) >7 times the upper limits of normal, combined with HCV antibody testing and confirmatory HCV-RNA (viral load) testing if the HCV antibody test result is positive. Identification of the seroconversion from hepatitis C antibody-negative to antibody-positive is the only reliable method to classify acute HCV infection.

Diagnosis of Chronic HCV Infection

Chronic hepatitis C infection is diagnosed by the presence of HCV antibodies, with either detectable HCV RNA on more than one occasion over a six-month interval or the presence of abnormal liver enzyme levels for at least six months.

Liver Enzymes

Measurements of enzymes, proteins, and bilirubin from a blood sample constitute a liver panel. Information from a liver panel can be used to help diagnose hepatitis C infection, identify possible liver damage, assess the liver's capacity to metabolize drugs, and partially evaluate the response to HCV treatment. A liver panel is usually comprised of measurements of the levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin, albumin, and total protein. Other tests are often included, such as measurements of prothrombin time (PT), gamma-glutamyl transferase (GGT), and lactic acid dehydrogenase (LDH). For more information, see A Guide to the Liver Panel at the end of this chapter.

Liver enzyme levels in people with chronic HCV infection can be normal, periodically elevated, or persistently elevated (Herve 2001; Jamal 1999; Persico 2000). Liver enzyme levels are only rough indicators of liver disease and are not predictive of disease progression. In chronic HCV disease, ALT levels are more frequently elevated than AST levels (71% vs. 33%) (Dufour 2000).

Some studies have found less HCV-related liver damage in people with persistently normal alanine aminotransferase (ALT) levels (Jamal 1999; Persico 2000). In a two-year case-control study, Jamal and colleagues followed 75 people with normal ALT levels (defined as four consecutive normal ALT values within 12 months) and 200 people who had abnormal ALT levels. Those with persistently normal ALT levels had less severe liver disease; just 6% had cirrhosis compared to 19% of the group with elevated ALT levels ($P=0.007$). Those with persistently normal ALT levels had less fibrosis ($P<0.05$) and a slower rate of fibrosis progression ($P<0.001$), as well as significantly lower HCV-RNA levels ($P=0.02$) (Jamal 1999). In a study in chronically HCV-infected individuals comparing 80 with persistently normal ALT levels and 455 with elevated liver enzymes, Herve and

colleagues found an association with less severe liver disease, slower fibrosis progression, and persistently normal ALT levels (Herve 2001).

However, mild-to-serious liver damage has been identified in HCV-infected persons with persistently normal ALT. In a retrospective study of 864 HCV-infected persons, some degree of fibrosis, usually mild, was observed in a significant number of those with persistently normal ALT levels (Pradat 2002). Puoti and colleagues found no difference in grading and staging of liver disease between those with persistently normal levels (N=46) and those with ALT elevations (n=52) (Puoti 1997). A study of HCV-infected Japanese hemodialysis patients reported that although most patients had persistently normal ALT levels, ultrasonography revealed liver damage among 72% (Furusyo 2000).

Elevated aspartate aminotransferase (AST) levels may help to identify damaged liver tissue, although their predictive value for the development of cirrhosis is controversial (Assy 2000; T. F. Imperiale 2000; Michielsen 1997; Park 2000; Sheth 1998). Higher AST:ALT ratios have been associated with increasing grades of fibrosis (Park 2000; Sheth 1998). However, when AST:ALT ratios were examined as potential predictors of cirrhosis, the findings were inconsistent. Sheth and colleagues found an AST:ALT ratio of ≥ 1 in 25 of 47 cirrhotic individuals and in none of 92 non-cirrhotic individuals. An AST:ALT ratio of ≥ 1 distinguished 100% of cirrhotic individuals from non-cirrhotic individuals in this study (positive predictive value of 100%). The AST:ALT ratio accurately ruled out cirrhosis in 80.7% of non-cirrhotics (negative predictive value of 80.7%) (Sheth 1998). Park and colleagues found that only 5 of 123 non-cirrhotic individuals had an AST:ALT ratio of ≥ 1, while 14 of 30 cirrhotics had an AST:ALT ratio of ≥ 1. The AST:ALT ratio correctly identified cirrhosis in 73.7% and accurately ruled out cirrhosis in 88.1%. Imperiale and colleagues attempted to validate the AST:ALT ratio as a clinical decision aid. The test incorrectly identified 13 of 36 individuals (36%) as cirrhotic (T. F. Imperiale 2000).

HCV RNA

Hepatitis C RNA levels are detectable between one and two weeks after infection (H. J. Alter 1991). Those with chronic HCV infection may be intermittently viremic (Beld 1999; Villano 1999). Therefore, it is recommended that individuals who are HCV-antibody-positive but have undetectable HCV-RNA levels undergo retesting for HCV RNA in six months to confirm or rule out ongoing HCV infection.

Individual hepatitis C viral loads vary, although hepatitis C replicates rapidly; an estimated ten trillion virions (individual virus particles) are produced per day (Neumann 1998). HCV-RNA levels may be influenced by mode of transmission, duration of infection, concurrent infection with hepatitis B, age, consumption of alcohol, and HIV status. Individuals who acquired HCV from blood transfusion have higher viral loads than those infected from injection drug use or needlestick injury (Lau 1993). However, all the transfusion recipients were over 60 years of age, and age may have been a surrogate for duration of infection or infection at an older age. In a cohort of injection drug users, Thomas and colleagues found a range of serum HCV-RNA levels from <200,000 to >120 million eq/mL. Higher HCV-RNA levels were found in older participants. In this group, age was strongly correlated with duration of drug use and HCV infection, suggesting that HCV viral load levels may increase with longer duration of infection. Lower HCV-RNA levels were associated with ongoing hepatitis B infection and not sharing needles (Thomas 2000b).

Alcohol consumption increases HCV RNA (Oshita 1994; Pessione 1998; Sawada 1993). Pessione and colleagues found a strong correlation with HCV-RNA levels and alcohol consumption (P= 0.0001). They detected a dose-response relationship between HCV RNA and alcohol even in individuals with moderate intake (30 grams/day). HCV-RNA levels in HIV-coinfected individuals are higher than those found in individuals HCV monoinfection (Cribier 1995; Di Martino 2001; Eyster 1994; Sherman 1993; Sulkowski 2002; Thomas 2000b; Zylberberg 1996).

Quantitative viral load testing, which measures the amount of hepatitis C virus from a blood or liver tissue sample, provides important information for making a treatment decision and measuring the response to treatment. Although hepatitis C viral load is not a reliable surrogate marker for disease progression, it is a prognostic indicator for response to treatment. Viral loads of less than two million copies have been associated with better responses to interferon-based treatment (Fried 2002; Lau 1993; McHutchison 1998; Poynard 1998; Rumi 1997). Two different methods are used for quantitative testing of HCV RNA: bDNA and RT-PCR.

Table 1. Commonly Used Quantitative HCV-RNA Assays

Assay	Method	Range (Without sample dilution)	As International Units
Roche COBAS Amplicor HCV Monitor 2.0	RT-PCR	100 to 5 x 10^6 HCV RNA copies/mL	~40 to 2.0 x 10^6 HCV IU/mL
National Genetics Institute HCV SuperQuant	RT-PCR		600 to 8.5 x 10^5 HCV IU/mL
Bayer Quantiplex HCV RNA 2.0	bDNA	0.2 to 120 MEq/mL	~3.2 x 10^4 to 1.9 x 10^4 HCV IU/mL
Bayer Versant HCV RNA 3.0*	bDNA	2500 to 4.0 x 10^7 HCV RNA copies/mL	521 to 8.3 x 10^6 HCV IU/mL
Abbott Laboratories Lcx HCV RNA	RT-PCR		23 IU/mL to 2.3 X 10^6 IU/mL

Adapted from Germer and Zein, 2001; Leckie 2004

*The Versant 3.0 has received FDA premarket approval (an approved Premarket Approval Application (PMA) is, in effect, a private license granted to the applicant for marketing a particular medical device).

Although bDNA and RT-PCR testing are both used to measure the amount of hepatitis C virus, their results are not interchangeable, even when converted into international units (IU), the World Health Organization's standard for measuring HCV RNA. Despite strong within-test correlation between RNA copies/ml and IU, there was a wide variation between RT-PCR and bDNA results, especially in high-titer, HIV-coinfected patients (Sherman 2002c). Therefore, to ensure an accurate assessment of treatment response, it is recommended that clinicians use the same RNA test assay before and during HCV treatment, especially for those with high viral loads. After the course of treatment, more sensitive assays may be needed to detect low levels of HCV RNA.

Qualitative testing has a much lower threshold of detection than quantitative testing and has been used to identify individuals with acute HCV infection, to confirm or rule out chronic HCV infection, and to assess virologic response to HCV treatment.

Table 2. Qualitative Assays for Detection of HCV RNA

Assay	Threshold of Detection	As International Units
Bayer TMA (Transcription-Mediated Amplification)	Can detect 50 copies HCV RNA/mL	~ 5 HCV IU/mL
Roche COBAS AmpliScreen™ HCV Test		Can detect 60 HCV IU/mL

The sensitivity and specificity of testing for HCV RNA may differ, due to assay variability and a lack of standardization among laboratories. Consistent use of the same quantitative assay throughout the course of HCV treatment will increase the comparability of the results. Although consistent use of one assay is preferable, if necessary, results from different quantitative assays can be converted into IU.

Table 3. Conversion Chart for HCV RNA Assays: Copies per Milliliter (copies/mL) to International Units (IU)

Assay	Conversion Factor
Amplicor HCV Monitor v 2.0 (manual procedure)	1 IU/mL = 0.9 copies
Cobas Amplicor HCV Monitor v 2.0 (semi-automated procedure)	1 IU/mL = 2.7 copies
Versant HCV RNA 3.0 Quantitative Assay	1 IU/mL = 5.2 copies
Lcx HCV RNA Quantitative Assay	1 IU/mL = 3.8 copies
SuperQuant	1 IU/mL = 3.4 copies

Hepatitis C Support Project 2003

Hepatitis C Genotyping

There are at least six known HCV genotypes (different hepatitis C viral strains, identified by their specific genetic makeup). Different viral isolates from around the world may vary genetically by as much as one third (Davis 1999; Okamoto 1992). Before 1994, there was no consistent classification system to distinguish different HCV genotypes, making it very difficult to evaluate the role of HCV genotype in different studies. In 1994, Simmonds and colleagues suggested a system for nomenclature of hepatitis C viral genotypes, classifying them on the basis of their nucleotide sequence similarities into major genetic groups designated as genotypes. Each genotype was assigned a number, based on the order of its discovery. The most closely related strains within genotypes were designated as subtypes named in alphabetical order. All genetic variants within a single isolate of the virus are designated as quasispecies. Quasispecies are a result of viral mutations in the host; since HCV makes up to 10 trillion copies of itself on a daily basis, and replication is error-prone, quasispecies diversity can be significant.

Liver Scan, Computed Axial Tomography (CAT) Scan, Magnetic Resonance Imaging (MRI), and Ultrasound Testing

The liver scan is used to determine liver shape and function. It starts with an injection of a radioactive isotope. After injection, the person lies on a table under a scanner, which detects the pattern of the radioisotope's distribution and sends images of the area to a computer. The amount of radiation used in a liver scan is less than that of an X-ray. A liver scan is an efffective and non-invasive method used to identify portal hypertension (high blood pressure in liver blood vessels), cirrhosis, hepatocellular carcinoma, and other liver disease.

Computed axial tomography (CT or CAT) scans provide X-ray images of the body from many angles. Beams from an X-ray device that quickly rotates around the body are sent to a computer, creating a cross-sectional image. Sometimes, as part of this procedure, a person will receive an injection of dye to enhance the contrast between organs. CT scans are not painful, but the injection of dye may be painful or dangerous to individuals with iodine allergies. CT scans can detect tumors and other irregularities of liver architecture.

Magnetic resonance imaging (MRI) uses magnets and radio waves to produce images of the body. During an MRI, a person lies on a narrow slab which is slid into a large tube. Inside the tube, radio waves are broadcast towards hydrogen atoms in the body alinged with the magnetic field from the MRI scanner. These atoms reflect a signal back to the machine. MRI scans can distinguish different different body organs and tissues. Sometimes, if a sharper image is needed, small devices called body coils, which transmit and receive the radio waves, may be used. Sometimes an intravenous agent is given to increase the contrast. An MRI scan is used to detect cancers, obstructions in the flow of blood in the liver's portal vein, and liver enlargement. The procedure is not painful, but some peope find it claustrophobic.

Sonographic or ultrasound screening can identify irregularities in the shape of the liver, or screen individuals at high risk for hepatocellular carcinoma. Ultrasound testing uses very high frequency sound waves which bounce off of the body to create an image of an organ. It has the advantage of being non-invasive and painless, although it does not provide information about the condition of liver tissue and the grade and stage of liver disease.

Hepatocellular Carcinoma: Screening and Surveillance

A key feature in the clinical management of people with cirrhosis is prompt identification of hepatocellular carcinoma. HCC can be identified by measuring alpha-fetoprotein (AFP) levels and ultrasound imaging, but the value of these tests for early detection of hepatocellular carcinoma in cirrhotic individuals has not been sufficiently demonstrated. However, it is common practice for cirrhotic individuals to undergo hepatic ultrasound and alpha-fetoprotein (AFP) surveillance at six-month intervals in the hope of early detection of HCC. The sensitivity and specificity of AFP levels in the detection of HCC has varied considerably (from 39% to 64%, or 76% to 91%, respectively) in different studies (Collier 1997). Intermittent and persistent elevations in AFP levels have been observed in individuals without HCC. Some research has shown that ultrasound surveillance increases early detection of HCC without reducing mortality (Larcos 1998; Solmi 1996).

Tong and colleagues performed a seven-year prospective assessment of the predictive value of ultrasound and AFP for detecting HCC. Five hundred and twenty-six of 602 participants were biopsied, 173 (33%) of them cirrhotic. Ultrasound and AFP results were used to guide follow-up CT scans and biopsies to confirm HCC diagnosis. During surveillance, HCC was detected in 5% (31/602). All of those with HCC were cirrhotic. The mean AFP concentration was significantly higher among individuals diagnosed with HCC than those with no HCC (426.06 ± 67.23 ng/mL vs. 8.91 ± 11.34 ng/mL; $P<0.001$), although seven individuals who were diagnosed with HCC had normal AFP levels. By themselves, AFP levels alone are not sufficiently sensitive or specific for detection of HCC. Ultrasound identified nine lesions that were not HCC; these were described as false positives. HCC was identified by ultrasound in 78% of cases (positive predictive value of 78%). Despite early HCC detection, 77% (24/31) individuals died within a mean of 16.70 ± 19.40 months after diagnosis of HCC (Tong 2001).

A scoring system may help to differentiate cirrhotics at low risk from those at high risk for progressing to HCC. Demographic, clinical, and biochemical data collected from 463 cirrhotics followed for a range of 1–96 months were analyzed to determine the predictive value of each variable. In cirrhotics aged 55 or over, the presence of HCV antibodies, prothrombin activity of <75%, or a platelet count below 75,000/mm^3 were identified by multivariate analysis as independent predictors of HCC. Using a scoring range of 0 to 4.71 points, a threshold to distinguish high vs. low risk was identified, with 2.33 as the cutoff. Among 270 low-risk individuals with a score of 2.33 or less, cumulative four-year HCC incidence was 2.3% (4/270), while in the high-risk group, cumulative HCC incidence was 30.1% (34/193) ($P=0.0001$) (Velázquez 2003). This system may help direct HCC surveillance efforts towards those who are at greatest risk.

Liver Biopsy

Liver biopsy is the best way to assess the grade and stage of HCV disease. Biopsy can identify or rule out other causes of liver disease, and is the only test that provides specific information about the condition of a person's liver tissue. Biopsy results are used to inform treatment decisions. Those with no liver damage or mild damage may choose to delay treatment, while those with more serious damage may need to initiate treatment.

During liver biopsy, a thin needle is quickly inserted between the ribs, where it collects a very small sample of liver tissue. Although it is an outpatient procedure, biopsy is usually performed in the hospital. Patients usually are kept under observation for three to six hours after the biopsy to watch for complications (hemorrhage, severe abdominal pain, punctured gall bladder), which usually become clinically apparent during the observation period (Janes 1993; Piccinino 1986). The risk of death from biopsy is low, ranging from 0.01% to 0.1%. The risk of complications ranges from 125 to 278 cases per 100,000 (Piccinino 1986). McGill and colleagues collected data from 9,212 biopsies performed over 21 years. During that period there were ten fatal and nine non-fatal hemorrhages (0.11% and 0.24%, or a total incidence of 0.35%) (McGill 1990). Liver biopsy can be painful. Thirty percent of biopsy recipients report mild pain, 3% report moderate pain, and 1.5% report severe pain (Gilmore 1995; Perrault 1978; Pokorny 2002).

New technology can reduce the risk of pain and complications from liver biopsy. The use of ultrasound to guide biopsy decreases the likelihood of puncturing the gallbladder, colon, bile

ducts, or large blood vessels, while increasing the likelihood of collecting adequate specimens in one pass (Soyer 1993). Cadranel and colleagues evaluated factors which increased or decreased complications from 2,084 liver biopsies. They observed reduced incidence of complications when biopsies were performed by more experienced physicians (>150 biopsies performed), when sedation or medication was provided prior to biopsy, and when ultrasound guidance was used (Cadranel 2000). Pokorny and colleagues biopsied 251 individuals, using ultrasound or CT scans to guide the procedures. Pain at the site of the biopsy or in the right shoulder was reported as severe in 1.2% (3/ 251), moderate in 2.4% (6/ 251), and mild in 21.5% (54/ 251) of biopsy recipients. Only one complication was reported—bleeding into the liver tissue (Pokorny 2002).

To reduce the risk of pain, complications, and sampling errors, liver biopsy should be performed by experienced physicians guided by ultrasound, and pain management should be provided to those undergoing biopsy.

About Liver Architecture

The liver is composed of many small, grape-like structures called lobules. Lobules are made up of plates of hepatocytes (liver cells) radiating outward from a central vein. The lobules are squeezed together in clusters. The portal zones are found between the lobules of the liver. Liver damage from hepatitis C can cause small areas of cell death (focal necrosis). As this necrosis worsens, dense, fibrous scar tissue may develop in portal zones. As the fibrosis worsens, it may extend from one portal zone to adjoining portal zones; this is called bridging fibrosis. Bridging fibrosis is the stage before cirrhosis. Cirrhosis is characterized by serious scarring that has damaged the liver's structure and ability to function.

Evaluating Biopsy

Different systems have been used to evaluate the grade and stage of liver disease. Grading measures the amount of disease activity in a sample of liver tissue, while staging identifies the degree of liver damage from the onset of disease to the development of cirrhosis. While liver disease grading may improve or worsen, staging is often stable or progressive.

The first system for scoring biopsy samples was developed by Knodell and colleagues in 1981; it has four components. Three components grade disease activity by area and extent; they are added up with the fourth component to make up the histological activity index (HAI). The fourth component stages disease, scoring the degree of fibrosis from zero to four. The HAI system is used more frequently in research settings than in clinical practice due to its complexity. In 1994 Desmet urged that that the grading score be separated from the staging, and that periportal necrosis and bridging necrosis be evaluated separately from each other, because of differences in prognostic implications (Desmet 1994).

Table 4. The Histological Activity Index

Periportal ± Bridging Necrosis	Score	Intralobular Degeneration and Focal Necrosis	Score	Portal Inflammation	Score	Fibrosis	Score
None	0	None	0	No Portal Inflammation	0	No Fibrosis	0
Mild piecemeal necrosis	1	Mild (acidophilic bodies, ballooning degeneration and/or scattered foci of hepatocellular necrosis in 1/3 of lobules or nodules)	1	Mild (sprinkling of inflammatory cells in <1/3 of portal tracts)	1	Fibrous portal expansion	1
Moderate piecemeal necrosis (involves <50% of the circumference of most portal tracts)	3	Moderate (involvement of 1/3-2/3 of lobules or nodules)	3	Moderate (increased inflammatory cells in 1/3-2/3 of portal tracts)	3	Bridging Fibrosis (portal-portal or portal-central linkage)	3
Marked piecemeal necrosis (involves >50% of the circumference of most portal tracts)	4	Marked (involvement of >2/3 of lobules or nodules)	4	Marked (dense packing of inflammatory cells in >2/3 of portal tracts)	4	Cirrhosis	4
Moderate piecemeal necrosis plus bridging necrosis	5						
Marked piecemeal necrosis plus bridging necrosis	6						
Multilobular necrosis	10						

Knodell 1981

A modification of the HAI described by Ishak and colleagues has supplanted the traditional HAI in many studies. It adds finer detail to the fibrosis subscore to help delineate more subtle changes.

Table 5. The Ishak Modification of the Knodell HAI: Architectural Changes, Fibrosis and Cirrhosis

Change	Score
No fibrosis	0
Fibrous expansion of some portal areas, with or without short fibrous septa	1
Fibrous expansion of most portal areas, with or without short fibrous septa	2
Fibrous expansion of most portal areas, with occasional portal to portal bridging	3
Fibrous expansion of portal areas with marked bridging as well as portal-central	4
Marked bridging (portal to portal and/or portal-central) with occasional nodules (incomplete cirrhosis)	5
Cirrhosis, probable or definite	6
Maximum score	**6**

Ishak 1995

Table 6. The Ishak Modification of the Knodell HAI: Grading

Feature	Score
A. Periportal or periseptal interface hepatitis (piecemeal necrosis)	
Absent	0
Mild (focal, few portal areas)	1
Mild/moderate (focal, most portal areas)	2
Moderate (continuous around <50% of tracts or septa)	3
Severe (continuous around >50% of tracts or septa)	4
B. Confluent necrosis	
Absent	0
Focal confluent necrosis	1
Zone 3 necrosis in some areas	2
Zone 3 necrosis in most areas	3
Zone 3 necrosis + occasional portal-central bridging	4
Zone 3 necrosis + multiple portal-central bridging	5
Panacinar or multiacinar necrosis	6
C. Focal (spotty) lytic necrosis, apoptosis and focal inflammation	
Absent	0
One focus or less per 10 X objective	1
Two to four foci per 10 X objective	2
Five to ten foci per 10 X objective	3
More than ten foci per 10 X objective	4
D. Portal inflammation	
None	0
Mild, some or all portal areas	1
Moderate, some or all portal areas	2
Moderate/marked, all portal areas	3
Marked, all portal areas	4
Maximum score	**18**

Ishak 1995

A newer, simpler system, the METAVIR—devised by the French research group of the same name—is more commonly used in clinical practice. Simpler grading and precise definition of pathological features in the METAVIR system may decrease observer variation. The METAVIR system evaluates liver tissue specimens for fibrosis stage and two major components of disease activity: inflammation and necrosis (cell death). The grading of disease activity is based on the severity of periportal inflammation or injury to the portal zones between the lobules of the liver—when severe, it overlaps across portal zones—and the degree of focal parenchymal necrosis (the death of small areas of liver tissue). Specimens are graded on a scale from A0 (no activity) to A3 (severe activity). The degree of fibrosis is staged from F0 (for none) to F4 (for cirrhosis).

Table 7. The METAVIR System

Histological Activity: Lobular Necrosis and Piecemeal Necrosis	Fibrosis Stage
no activity = A0	F0 = no fibrosis
mild activity = A1	F1 = portal fibrosis without septa
moderate activity = A2	F2 = portal fibrosis with rare septa
severe activity = A3	F3 = numerous septa without cirrhosis
	F4 = cirrhosis

Bedossa 1996

Other systems used to grade and stage liver disease include the Scheuer, the Sciot and Desmet (unpublished), and the Ludwig Batts with the Tsui modification.

Evaluating Cirrhosis: Child-Pugh and MELD Systems

Once an individual has been diagnosed with cirrhosis, a scoring system known as the Child-Pugh can assess its severity. The Child-Pugh system combines information from biochemical testing (bilirubin, albumin, and prothrombin time) with evaluations of hepatic encephalopathy and ascites. Another scoring system called MELD (Model of End-Stage Liver Disease) has replaced Child-Pugh scores for grading of severity during work-up and prioritization for liver transplantation. The MELD score is based on results from serum bilirubin, serum creatinine, and prothrombin time (calculated as the International Normalized Ratio).

Table 8. The Child-Pugh Score

Score	Bilirubin	Albumin	Prothrombin Time	Hepatic Encephalopathy	Ascites
1	< 2 mg/dl	> 3.5 gm/dl	1-4 seconds	None	None
2	2-3 mg/dl	2.8-3.5 gm/dl	4-6 seconds	grade 1-2	Mild (detectable)
3	> 3 mg/dl	< 2.8 gm/dl	> 6 seconds	grade 3-4	Severe (tense)

Pugh 1973

Problems with Biopsy and Alternatives

The risk of complications and possible pain involved with liver biopsy has made it an unpopular procedure with patients. Sampling errors and variation between observers also occur. Bejarano and colleagues assessed diagnostic inconsistencies from 125 biopsy specimens by comparing reports from community-based pathologists to reports from a hepatopathologist and a hepatologist. Results were classified as: major discrepancy (a diagnosis which might have resulted in inappropriate management and/or treatment decisions, misdiagnosis, and inappropriate assessments), minor discrepancy (considered unlikely to have an effect on future procedures or treatments, omissions and incomplete descriptions were included as well), and full concordant agreement. Twenty-eight percent (35/125) were categorized as major discrepancies. Failure to properly identify hepatocellular activity was identified in 28% (9/35) within this group. Eleven of these cases involved discordant

assessments of cirrhosis: six of the eleven initial reports failed to diagnose established cirrhosis, and five initial reports diagnosed cirrhosis where none had developed. Minor discrepancies were identified in 38% (47/125) of the reports (Bejarano 2001). To ensure proper diagnosis of HCV-related liver damage, biopsies should be read by pathologists who are skilled in reviewing liver biopsies.

The average biopsy specimen ranges from a few millimeters to several centimeters long, representing from one-hundred-thousandth to one thirty-thousandth of the liver (Scheuer 2003). Since liver damage is not evenly distributed throughout the liver, it is difficult to accurately assess the grade and stage of liver disease from a small biopsy sample (Bedossa 2003; Colloredo 2003). Colloredo and colleagues examined 161 biopsy samples from people with chronic hepatitis B and C. When they reduced the size of individual samples, their grading and staging scores decreased significantly; samples that were 1 mm in width were underscored for both the grade and stage of liver disease, no matter their length (Colloredo 2003). Bedossa and colleagues studied 17 large liver tissue samples from individuals with chronic hepatitis C. They used the same liver tissue to create virtual samples of differing lengths. The virtual samples were compared with the original samples to determine the minimum size for accurate liver biopsy specimens. The minimum length needed for accurate METAVIR system staging was 25 mm (Bedossa 2003).

Within an individual, the grade and stage of liver disease may vary according to the area of the liver from which the sample is obtained. Regev and colleagues compared 124 liver biopsy samples simultaneously obtained from the right and left liver lobes of 62 people with chronic hepatitis C. Samples were coded, and randomly divided between two hepatopathologists; after assessment by the Scheuer system, they were uncoded and samples from the left and right lobes of the same liver were compared to one another. The differences between right and left lobes were significant; one third of samples differed by at least one stage, and one quarter differed by at least one grade. In 14.5% (18/124) of samples from the same liver, cirrhosis was present in one of the lobes, while stage 3 fibrosis was present in the other lobe. Fifty samples were de-identified and re-examined by the hepatopathologists to assess intraobserver bias. Grading and staging of the 50 re-examined samples did not differ significantly from the first examination. Most of the differences in grading and staging were attributed to sampling (Regev 2002).

Reliable, reproducible and non-invasive biopsy alternatives are highly desirable. In a prospective study, Imbert-Bismut and colleagues assessed the predictive value of a combination of serum biochemical markers to diagnose fibrosis in people with chronic hepatitis C. They analyzed the concordance between 339 biopsy samples evaluated by the METAVIR scoring system and 11 serum biochemical markers. They selected six serum biochemical markers (α_2 macroglobulin, haptoglobulin, GGT, γglobulin, total bilirubin, and apolipoprotein-A1), and added ALT to increase diagnostic accuracy. These markers were validated in a group of 134 individuals by comparing them with liver biopsy samples. A serum biomarker index score of <0.10 predicted no fibrosis or mild fibrosis (F0 or F1) in more than 90%, while a score ≥ 0.60 accurately predicted moderate to severe fibrosis and cirrhosis (F2, F3, or F4) in more than 90%. However, more than half of the scores fell in the range between 0.10 and 0.60; these scores did not have a high enough predictive value to eliminate the need for a biopsy (Imbert-Bismut 2001).

A panel of five serum biochemical markers (α_2 macroglobulin, apolipoproteinA1, haptoglobulin, GGT, and bilirubin)— called Fibrotest— has been marketed for diagnosing fibrosis. Myers and colleagues compared Fibrotest scores from 534 people to their METAVIR biopsy scores. They

reported that a Fibrotest score below 0.50 ruled out cirrhosis in 329 of 337 individuals (98% negative predictive value). A score >0.50 predicted cirrhosis in only 55/197 (positive predictive value of 28%) (Myers 2002). Fibrotest is not as accurate for identifying mild-to-moderate liver damage.

Researchers continue to investigate non-invasive alternatives to liver biopsy (Boeker 2002; Forns 2002; Guechot 1994; Leroy 2004; Patel 2003; Sud 2004; Wai 2003). Sud and colleagues identified five independent predictors of fibrosis in people with chronic hepatitis C: age, AST, total cholesterol level, insulin resistance and past alcohol intake. These five markers accurately predicted significant fibrosis in 87% of cases among 176 individuals (Sud 2004). Wai and colleagues reported that using an AST to platelet ratio index (APRI) predicted liver damage in individuals with chronic hepatitis C. The APRI accurately predicted significant fibrosis in 51%, and cirrhosis in 81% (Wai 2003).

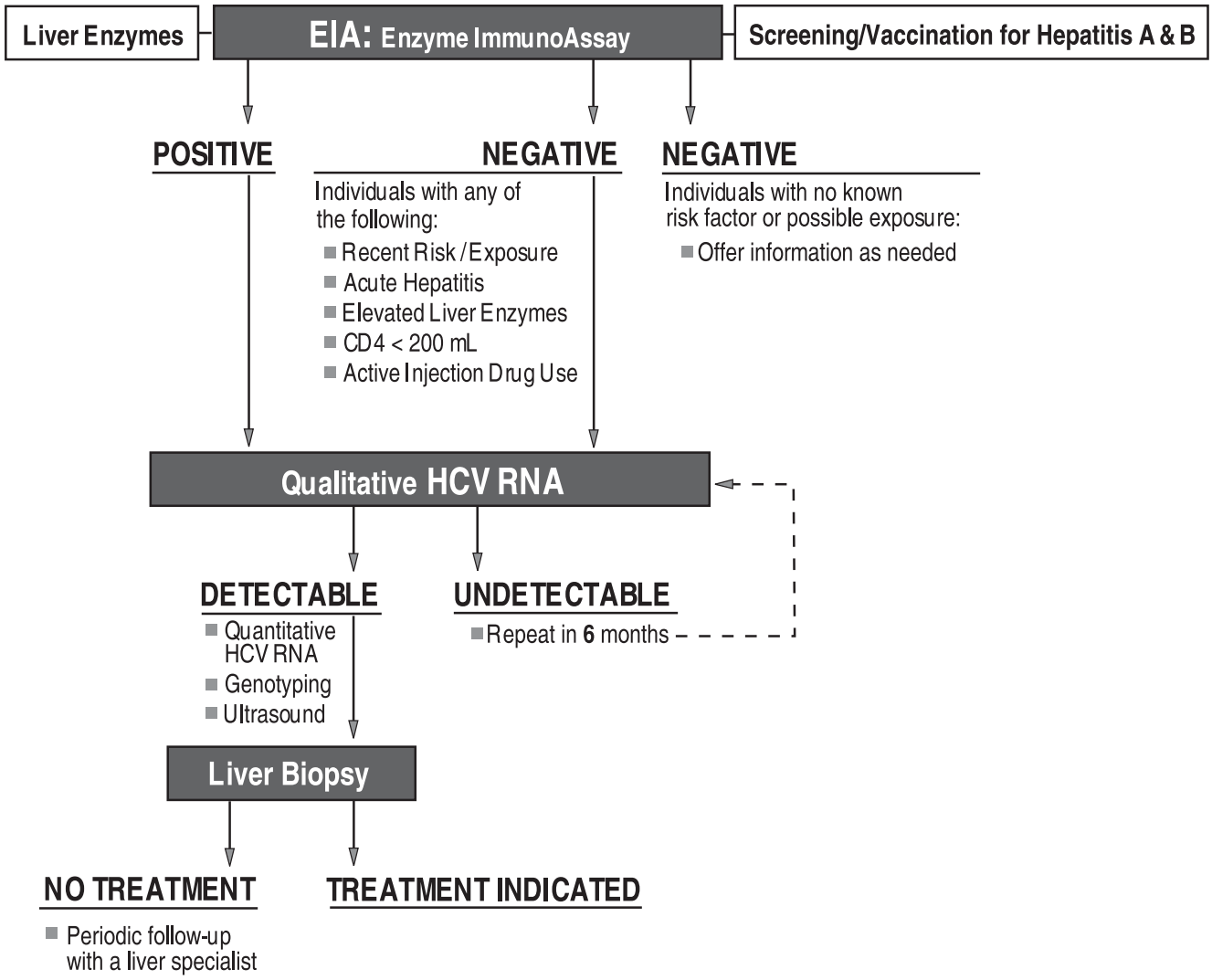
While these serum biomarkers cannot provide data on liver histology equivalent to that from a liver biopsy, these tests may be a viable first-line alternative when biopsy is contraindicated or refused. Further research is needed to determine which tests are most appropriate for diagnosing and staging fibrosis, monitoring fibrosis progression over time in untreated persons, and assessing response to HCV treatment, as well as how frequently these tests should be used.

HIV Coinfection: Diagnostic Considerations

Because of the overlap in modes of transmission, the CDC has recommended that all HIV-positive individuals undergo screening for hepatitis C infection. All positive results from HCV antibody testing should be verified with qualitative HCV-RNA testing. In addition, confirmatory testing may also be used in certain situations when antibody test results are negative but HCV infection is suspected. For example, immunodeficient individuals may not be producing HCV antibodies. Qualitative HCV-RNA testing may be needed to diagnose HCV infection in HIV-positive individuals, especially those with CD4 cell counts below 200/mm^3 (Berggren 2001; Busch 2001; H. H. Lin 2002). CD4 cell counts <100/mm^3 have been significantly associated with false-negative HCV ab (OR=49; P<0.01) (Berggren 2001). George and colleagues found a startling incidence of occult HCV infection among 131 HIV-positive individuals. Although only 31 of the HIV-positive individuals (23.7%) had antibodies to HCV, 19 of the remaining 100 (19% of the HCV-antibody-negative individuals, or 14.5% of the entire sample) had detectable HCV RNA despite being HCV-antibody-negative (George 2002).

HCV-RNA levels in coinfected individuals are higher than in those with HCV alone (Cribier 1995; Di Martino 2001; Eyster 1994; Sulkowski 2002; Thomas 2001; Zylberberg 1996). Sánchez-Quijano and colleagues found HCV-RNA levels close to ten times higher in coinfected persons. Higher HCV-RNA levels have been found in the liver and the blood of coinfected individuals (Bonacini 1999). Some studies have found HCV-RNA levels inversely correlated with CD4 cell counts (Di Martino 2001; Eyster 1994; Ghany 1996; Thomas 2001). Although no association with HCV-RNA levels and HCV disease progression has been found, HCV viral loads under 2,000,000 copies/ml (equivalent to 800,000 IU/mL) are associated with better responses to interferon-based HCV treatment.

Figure 2. Alternative HCV Diagnostic Algorithm for HIV+ Individuals



Liver Enzymes in Coinfected Individuals

Highly active antiretroviral therapy (HAART), OI prophylaxis and treatment, and medications used to manage treatment-related complications and comorbidities can adversely affect the liver. In the United States, 90% of those with an AIDS diagnosis use at least one hepatotoxic drug (Orenstein 2002). Therefore, monitoring of liver enzymes at one month after initiation of therapy and then every three months is recommended for coinfected individuals receiving HAART. More frequent monitoring is indicated for specific antiretroviral regimens, or for individuals with advanced liver disease (see Chapter VI, HIV Treatment in HIV/HCV Coinfection). HAART-mediated immune reconstitution may cause flares in ALT levels, possibly related to the restoration of HCV-specific immune responses. Severe elevations in ALT and AST levels may necessitate modification of the HIV treatment regimen in certain circumstances.

Elevations in AST have been associated with poorer survival in HIV-positive individuals (Justice 2002; Rancinan 2002). Justice and colleagues examined survival in two cohorts of HIV-positive individuals: the Collaboration in HIV Outcomes Research-US (CHORUS; n=5,985; 87% white

male MSM) and the Veterans Aging 3-Site Cohort (VACS 3; n=881; 99% male and 55% African-American, 53% infected with HIV from IDU or heterosexual exposure). There were 400 deaths in the CHORUS cohort from 1997 until 2002, and 71 deaths in the VACS 3 cohort from 1999 until 2000. In a multivariable analysis of survival, the hazard ratio of elevated AST for mortality was 6.40 (P<0.001) in the CHORUS cohort, and 2.62 (P=0.09) in the VACS 3 cohort. Although coinfection with hepatitis C was associated with elevated AST in both cohorts, (HR, 15.5; P<0.001 and HR, 6.1; P<0.001 respectively), HCV coinfection was not an independent predictor of survival (CHORUS HR, 1.53; P=0.1; VACS 3 HR, 0.24; P=0.8).

In an examination of survival in the Aquitaine cohort, (995 HIV-positive individuals; 576 of them HCV coinfected) Rancinan and colleagues found a significant association between AST elevations and poorer survival (HR for elevations >200 IU/l of 2.30; 95% CI, 1.32-4.03; P=0.004), although they did not find an association between HCV seropositivity itself and poorer survival. However, more coinfected individuals had elevated AST (15%, vs. 7% of those with HIV alone).

Liver damage may be present in coinfected individuals, regardless of persistently normal alanine aminotransferase (ALT) levels. Mendes-Corrêa and colleagues reviewed clinical information and liver biopsy samples from 195 coinfected persons, 28 with normal ALT. They found moderate to severe liver damage in biopsy samples from 32% (9/28) of coinfected individuals with normal ALT (Mendes-Corrêa 2003). Uberti-Foppa and colleagues investigated the extent of liver damage among coinfected people with persistently normal ALT (PNALT) in a retrospective examination of liver biopsies from 354 coinfected people, 26 with PNALT (three consecutive normal ALT levels over 12 months). The CD4 cell count, HIV RNA, antiretroviral regimens and HCV RNA did not differ significantly by ALT level, although HCV genotype 1 was significantly more common among those with PNALT (52% [14/26] vs. 31% [102/328]; P= 0.01). Those with PNALT had significantly lower scores for grading and staging of liver disease (4.12 ± 1.64 vs. 7.35 ± 2.7; P=0.0007 for grading; 1 ± 1.69 vs. 2.24 ± 1.79; P=0.0147 for staging).

Table 9. Fibrosis Scores in Coinfected People with Normal and Abnormal ALT

Ishak Fibrosis Score	PNALT (N=26)	Abnormal ALT (N=328)
0-1 (absent to mild)	75%	42.9%
2-3 (moderate to serious)	12.5%	33.8%
4-6 (bridging fibrosis to cirrhosis)	12.5%	23.2%

Uberti-Foppa 2004

Information on the duration of HCV and HIV infection, age, nadir CD4 cell count and duration of antiretroviral therapy was not included; any prospective study of coinfected individuals with PNALT should incorporate this information.

Although it is tempting to regard ALT measurements as a surrogate for biopsy, liver histology cannot be assessed without a liver biopsy. These data underscore the need for biopsy to ensure that liver disease is assessed accurately. Biopsy may be especially important for assessment of liver disease in coinfected persons, because it is frequently assumed that they have severe liver damage when this is not invariably the case. Merchante and colleagues performed a cross-sectional study

of liver histology in coinfected people. They examined biopsy samples collected from 152 coinfected people between November of 1989 and March of 2003. Fibrosis was absent, or mild (F0 or F1) in 37.5% (57/152) (Merchante 2003).

Genotype

García-Samaniego and colleagues investigated the influence of hepatitis C genotype on the liver histology of coinfected individuals. In a cohort of 59 HCV-infected individuals, 48 (82%) coinfected with HIV, they found significantly higher histological activity scores (a measurement of disease activity by the amount of inflammation and damage in liver tissue) and more fibrosis among individuals with HCV genotype 1b or a mixed infection including genotype 1b. The odds ratio for an association between genotype 1b infection and higher histological activity scoring was 3.5 (95% CI, 1.1–11.3; P=0.036). Genotype 1b was significantly associated with fibrosis (OR, 20.9; 95% CI, 2.8-–157.2; P=0.003). Infection with genotype 1b was significantly associated with piecemeal necrosis (liver cell death) and portal inflammation. HIV infection was also significantly associated with fibrosis, with an odds ratio of 17.9 (95% CI, 2.5–129.0; P=0.004) (García-Samaniego 1997). Because coinfection with HIV is known to accelerate HCV disease progression, and this study was finished before the HAART era, it is not possible to generalize the results to other circumstances and populations. Further study would be necessary to evaluate the influence of genotype 1b on the severity of HCV disease in coinfected individuals during the HAART era.

Alternatives to Liver Biopsy: Serum Biochemical Markers in HIV/HCV Coinfection

A panel of six serum biochemical markers—total bilirubin, γ-glutamyltranspeptidase (GGT), α_2 macroglobulin, apolipoprotein-A1 and haptoglobulin—has been evaluated for use as an alternative to liver biopsy for HIV/HCV coinfected individuals. The score from an index comprised of age, sex, and biomarker test results was compared to liver biopsy samples from 130 coinfected persons. If a score of 50 was used as a cutoff, the absence of cirrhosis would be accurately predicted in 100% of cases, and the presence of cirrhosis would be accurately predicted in 65% of cases (Myers 2003). Although this index is not equivalent to a biopsy, and does not accurately predict mild to moderate liver damage, it may be a useful screening tool for fibrosis, especially in situations where biopsy is contraindicated or refused.

Mehta and colleagues compared serum marker testing with liver biopsy results from 96 coinfected people. The threshold for each serum marker was as follows: alanine aminotranferase <93 IU/L; aspartate aminotransferase <61 IU/L; albumin >3.6 g/dL; total bilirubin <1.2 mg/dL; and hyaluronic acid <42. They found that hyaluronic acid (HA) levels were higher in coinfected persons with moderate to severe fibrosis and cirrhosis than in those with no fibrosis. A fibrosis score of >2 on the Ishak system was 12 times more likely among individuals with HA levels > 85 ng/mL (95% CI, 3.46–43.35) and almost three times more likely with HA levels from 42 to 85 (95% CI, 0.87–9.20). Lower levels of HA, albumin and AST predicted milder liver damage. Thirty-five individuals had HA, albumin and AST levels below the threshold; all had fibrosis scores of ≤ 2 (Mehta 2004).

These results merit prospective investigation of serum biochemical markers as a biopsy alternative for HIV/HCV coinfected persons. Evaluation of serum biomarkers could be incorporated into large observational studies and treatment trials.

Recommendations

Develop and market oral fluid test kits for HCV antibody testing.

Oral fluid HCV antibody testing will provide opportunities to perform initial HCV screening in individuals with poor venous access, and in venues frequented by high-risk individuals, including syringe exchange programs, methadone clinics, drug treatment facilities, shelters and correctional facilities. Oral fluid collection kits for HCV antibody testing would also increase the capacity to collect epidemiological data. The National Institutes of Health and Industry should support surveillance initiatives for oral fluid testing of hard-to-reach populations.

Identify and validate prognostic markers and effective screening methods for early diagnosis of hepatocellular carcinoma (HCC).

Hepatocellular carcinoma is a known complication of hepatitis C. In the United States, the incidence of hepatocellular carcinoma (HCC) in the general population has increased from a rate of 1.4 cases per 100,000 between 1976 and 1980, to 2.4 cases per 100,000 during the period between 1991 and 1995 (El-Serag 1999). This rise may reflect the consequences of an epidemic of increased HCV transmission that occurred decades earlier. The annual incidence of HCC in hepatitis C-infected cirrhotics ranges from 1% to 4% (Di Bisceglie 1997; Lauer 2001).

HCC can be identified by measuring alpha-fetoprotein (AFP) levels and by ultrasound imaging, but the value of these tests for early detection of HCC in cirrhotic individuals has not been sufficiently demonstrated. The sensitivity and specificity of AFP levels for detection of HCC varies considerably (from 39% to 64% and from 76% to 91%, respectively) in different studies (Collier 1997). Some research has shown that ultrasound surveillance increases early detection of HCC, but it may not reduce mortality (Larcos 1998; Solmi 1996). Mortality from hepatocellular carcinoma is extremely high, with five-year survival rates of less than 5% (El-Serag 1999). Better interventions to facilitate the early diagnosis of HCC and reduce the high fatality rate are urgently needed. The National Institutes of Health (NIH) must fund this research

Promote use of a standardized system for evaluation of liver biopsy.

A standardized system should be selected for evaluating the results of liver biopsy in research to enable cross-study analysis.

Continue research on non-invasive testing methods to replace or reduce the need for liver biopsy.

Liver biopsy is still the only way to assess the condition of liver tissue. Information from liver biopsy is used to assess the degree of inflammation, gauge hepatic cell death and damage, identify other causes of liver injury, and guide treatment decisions. Although fatalities from biopsy are extremely rare (0.01% to 0.1%), liver biopsy can be painful, and occasional complications such as hemorrhage or puncture of adjoining organs may occur. The risk of complications and the potential pain of the procedure have made liver biopsy unpopular with many patients.

Alternatives to liver biopsy using panels of serum biochemical markers are under investigation, and some have been marketed. Although these panels may serve as substitutes in cases where a biopsy is contraindicated or refused, they yield far less precise information. The identification, development, and validation of a non-invasive, cost-effective replacement for liver biopsy would be an important breakthrough and merits research from the National Institutes of Health (NIH).

Educate primary care providers about diagnosis of acute and chronic hepatitis C infection.

Acute hepatitis C infection is clinically silent for most infected people, with only 15% to 20% of individuals developing symptoms (Koretz 1993). Symptoms, when they occur—low-grade fever, fatigue, appetite loss, abdominal pain, nausea, and vomiting—are typical of many common viral infections. Chronic hepatitis C infection is also often asymptomatic, and both acute and chronic hepatitis C infections may go undiagnosed by physicians who fail to ask about risk factors (Shehab 2001; Shehab 2002; Villano 1999).

Many physicians are unaware of the proper procedures for diagnosing hepatitis C (Shehab 1997). HIV-positive individuals (especially those with fewer than 200 CD4 cells), injection drug users, and transplant recipients may harbor occult hepatitis C infection (Beggren 2001; Beld 1999; Busch 2001; H. H. Lin 2002; Thomas 1995). Routine HCV-RNA confirmatory testing following a negative HCV-antibody test result should be considered in populations who may harbor occult infection, including HIV-positive individuals and injection drug users. Provider education initiatives must be available from the Centers for Disease Control, the American Medical Association, and the AIDS Education and Training Centers.

A Guide to the Liver Panel

Alanine aminotransferase (ALT; formerly known as serum glutamic pyruvic transaminase or SGPT) is an enzyme produced in the liver that metabolizes amino acids. During acute HCV infection, ALT levels in the blood may rise to twenty times above normal. Certain medications, including some antiretroviral agents, may cause ALT level increases. ALT levels usually decrease again when these drugs are discontinued. Excess ALT seeps into the bloodstream when liver cells are injured or dying. However, if damage to the liver is widespread and the liver is incapable of producing sufficient ALT, enzyme levels in the blood may remain in a normal range. Blood ALT levels cannot reliably diagnose or predict the state or progression of HCV disease. However, a reduction in ALT levels during HCV treatment—a biochemical response—is regarded as a positive development.

The normal reference range for ALT levels usually falls between 1 and 45 U/L, although this varies by laboratory. ALT levels typically reach a peak during the afternoon and are at their lowest levels at night. Within an individual, ALT levels can vary by 10-30% from day to day. Body weight also affects ALT levels. People with a high body mass index (BMI) tend to have ALT levels 40–50% higher than thinner people. Strenuous exercise may decrease ALT levels by 20%. ALT levels vary with age; standard reference ranges may not apply to children or to people over 60. Men typically have higher ALT levels than women. Liver injury in women may be underreported if judged by reference ranges derived primarily from males.

Aspartate aminotransferase (AST; formerly known as serum glutamic oxalocetic transaminase or SGOT) is an enzyme found in the heart, kidney, pancreas, spleen, liver, lungs, skeletal muscle, brain tissue, and red blood cells. When tissues in these organs are injured, AST is released into the bloodstream. AST is neither as sensitive nor as specific to liver injury as ALT. However, AST levels may rise to twenty times above normal during acute HCV infection, and may remain elevated during chronic HCV infection. Anti-HIV medications may produce elevations in AST as the liver works to metabolize these drugs, but AST levels usually decrease when these drugs are discontinued. AST levels do not predict HCV disease progression or severity, although a decrease in AST levels during HCV treatment is considered to be a good biochemical response.

The normal reference range for blood levels of AST falls between 1-36 U/L, though this varies by laboratory. Individual AST levels can vary by 5-10% from day to day. Normal AST levels among African American males are typically 15% above the reference normal. Body weight also affects AST levels. Individuals with a high body mass index (BMI) may have AST levels 40-50% above those of thinner people. Cardiac and skeletal muscle injuries may produce significant elevations in AST. Strenuous exercise before a blood test can produce a three-fold elevation in AST.

Alkaline phosphatase (ALP) is an enzyme primarily found in the intestines, bones, and liver; some is produced and found in cells lining the bile duct and in the placenta. Obstruction in the bile duct due to liver disease can cause an accumulation of bile acids in the liver, which in turn can stimulate the production of ALP. Therefore, elevated ALP may indicate an underlying cholestatic (obstructive) liver disease. Drugs that reduce bile transport from liver cells to the bile duct can also stimulate increased production of ALP. In HCV-infected individuals, ALP elevations are associated with a type of serious liver injury called post-hepatitic cholestasis.

The normal reference range for blood ALP levels falls between 35-150 U/L for adults, 100-500 U/L for adolescents and 100-350 U/L for children. These values may vary by laboratory. Individual ALP levels can vary from 5-10% from day to day. Someone with a full stomach may have ALP increases of up to 30 U/L. Normal ALP levels are usually 15% higher than the reference range in African-American males and 10% higher in African-American females. ALP levels tend to increase in post-menopausal women. Body weight affects ALP; levels increase by 25% with higher BMI (body mass index). Smoking increases ALP by 10%. Oral contraceptives decrease ALP levels by 20%. ALP levels are increased between two-and three-fold during the third trimester of pregnancy.

Gamma-glutamyl transferase (GGT) is an enzyme produced by the bile ducts. GGT levels may be elevated in individuals with any type of liver disease, and especially in individuals with bile duct diseases. As many as 80 to 95% of individuals with acute hepatitis have elevated GGT levels. GGT levels increase with cirrhosis and hepatocellular carcinoma. Alcohol and certain medications elevate blood levels of GGT, while other medications lower GGT.

The normal reference range for GGT falls between 0-51 IU/L. These values may vary by laboratory. Within each individual, GGT levels vary by 10-15% from day to day. Among African-Americans, normal GGT levels may be two times above the reference range. Men under 50 have GGT levels 25-40% higher than those in women. Among those with moderately high body mass index (BMI), GGT levels may increase by 25%. When BMI is over 30, GGT levels may increase by 50%. Pregnancy reduces GGT levels by 25%. Smoking twenty cigarettes per day increases GGT levels by 10%; levels double with heavier smoking. GGT levels may remain elevated for weeks after heavy drinkers stop drinking.

Bilirubin is a by-product of the breakdown of red blood cells. In the spleen, hemoglobin from red blood cells is broken down and the released heme is processed into bilirubin and transported to the liver. In the liver, bilirubin undergoes further processing to become water-soluble and is excreted into the gut as part of bile, which helps to break down fats. Non-water-soluble bilirubin is called unconjugated or indirect bilirubin. Water-soluble bilirubin that has been metabolized by the liver is called conjugated or direct bilirubin. Two tests measure bilirubin levels. Total bilirubin includes both direct and indirect bilirubin levels, while direct bilirubin testing measures the amount of bilirubin processed in the liver. When both bilirubin levels are normal, it reflects a balance between bilirubin production from red blood cell breakdown and bilirubin elimination by the liver. Liver cell injury is indicated when the total bilirubin level is high while direct bilirubin is low. Hepatitis C infection can slow the processing of bilirubin in the liver, and bilirubin levels can become elevated, causing jaundice (yellowing of the skin and eyes). Some medications, including certain HIV protease inhibitors, can increase the level of total bilirubin by affecting the rate of conversion of indirect to direct bilirubin.

The normal reference range for blood levels of total bilirubin falls between 0.3-1.1 mg/dl (5.1-19.0 mmol/L in international units). The normal reference range for direct bilirubin falls between 0.1-0.4 mg/dl (1.7-6.8 mmol/L in international units). Values may vary by laboratory. Within an individual, bilirubin levels may vary by 15-30% from day to day. Overnight fasting typically increases bilirubin levels by 20-25%. Normal bilirubin levels are 33% below the reference range in African-American males, and 15% lower in African-American females. Exercise can increase bilirubin levels by 30% in males. Oral contraceptives reduce bilirubin levels by 15%. Hemolytic

anemia, a potential side effect of ribavirin, causes an increase in indirect bilirubin.

Albumin is a protein made by liver cells. It helps maintains the pressure that prevents fluids from seeping out of the bloodstream and into tissues. Albumin also carries drugs, hormones and waste products through the bloodstream. A seriously damaged liver is unable to produce sufficient albumin. Albumin levels usually remain normal during acute hepatitis. In chronic hepatitis, albumin levels may decrease gradually as progression to cirrhosis occurs. Abnormally low levels of albumin can be a prognostic marker for liver decompensation and hepatocellular carcinoma.

The normal reference range for albumin blood levels is between 3.5-5.4 gm/dl, though values may vary from lab to lab. Dehydration temporarily lowers albumin levels.

Total protein measures two major blood proteins, albumin and globulin. Approximately 60% of total protein is albumin. There are three types of globulins: alpha-, beta-, and gammaglobulins. Alpha globulins are made in the lungs and the liver. Betaglobulins, also known as low-density lipoproteins (LDLs), transport fat throughout the body. The gammaglobulins are antibodies. Total protein and globulin levels may increase with cirrhosis. When a cirrhotic liver is unable to produce sufficient albumin, the body produces extra globulin in an attempt to maintain sufficient total levels of protein.

The normal reference ranges for these tests are 6.4-8.3 g/dl for total protein, 3.5-5.4 g/dl for albumin and 2.3-3.4 g/dl for globulin. Values may vary by laboratory.

Alpha-fetoprotein (AFP) is produced in fetal liver tissue. After birth, blood AFP decreases to very low levels. AFP levels may increase in cases of acute hepatitis, chronic hepatitis, cirrhosis, and liver cancer.

AFP levels over 50 ng/ml are considered abnormal, while levels between 11-100 ng/ml may indicate liver cell regeneration. AFP levels over 100 ng/ml may indicate hepatocellular carcinoma (HCC); levels over 1,000 usually indicate HCC.

Prothrombin Time (PT) measures the amount of time needed for blood to clot. Clotting factors are made in the liver. PT elevations may occur during acute hepatitis. In chronic hepatitis, PT usually remains normal, though abnormal PT prolongation usually accompanies progression to cirrhosis. PT is elevated in cirrhotic individuals, because a significantly damaged liver may not be able to produce enough clotting factors. Some medications increase PT. A PT in excess of three seconds above the normal range is associated with increased risk of bleeding.

The normal prothrombin time is about 11-15 seconds, though values may vary from lab to lab. Some laboratories report the international normalized ratio (INR), a standardized ratio for sample clotting time versus a control value; 1.0 represents normal.

List of Terms Used in This Chapter

Branched-chain DNA assay (bDNA): a test used to measure the amount of a virus in blood plasma.

Eq: is an abbreviation for virus equivalent. Eq is used to indicate that HCV genetic material has been measured by weight in picograms. To convert picograms, multiply the result by 1 million.

Reverse-transcriptase polymerase chain reaction (PCR) assay: a very sensitive test used to detect and measure RNA or DNA of organisms and viruses in blood plasma or tissue.

V. Hepatitis C Treatment

Summary

The current standard of treatment for hepatitis C virus (HCV) is a combination of two drugs: pegylated interferon and ribavirin. The virological response rate, treatment duration, and ribavirin dose vary according to several prognostic factors: genotype, baseline HCV RNA (viral load), race, body weight, age, and liver histology. In one study, 48 weeks of pegylated interferon plus ribavirin led to treatment responses ranging from 47% to 82% (Hadziyannis 2004). Overall, approximately 50% of those treated for hepatitis C will achieve a sustained virological response (SVR), meaning that no virus is detected in the bloodstream six months after completing treatment. Whether or not an SVR is equivalent to a "cure" is a controversial matter. Treatment may be beneficial for individuals who do not achieve an SVR; some have an improvement in liver condition or a stabilization of disease progression, although the durability and clinical benefits of these improvements are unknown at present.

The decision to treat hepatitis C is a complex one. The current guidelines recommend treatment for individuals with the greatest risk of developing cirrhosis (NIH 2002). The rationale for treatment is less clear-cut for members of understudied populations. Pivotal treatment trials excluded children; the elderly; individuals with renal disease; individuals with mild or advanced liver disease; liver transplant recipients; hemophiliacs; individuals with psychiatric co-morbidities; and active drug and alcohol users. Therefore, few data exist about safety and efficacy of treatment in these populations. Studies have shown that treatment is less effective for African Americans, although the reasons for diminished efficacy are not clear. The potential benefits of treatment must be carefully weighed against the side effects, which range from uncomfortable to debilitating, and in rare instances are life-threatening. Interventions are available to minimize side effects, but more research is needed to improve the tolerability of HCV treatment. Questions about dosing and duration of therapy remain as well.

Other key concerns related to HCV therapy include access to treatment and quality of care. Treating HCV is costly; a year of therapy (not including medications to ameliorate side effects) may cost up to $40,000. Treatment is rarely available to incarcerated persons, despite a shockingly high prevalence of hepatitis C among prisoners. Hepatitis C is also prevalent among people with significant co-morbidities, including drug and alcohol addiction and mental illness; these individuals require multidisciplinary care. Primary care providers often lack adequate information about the diagnosis, care, and treatment of hepatitis C. Finally, alternative and complementary therapies, although widely used, have not been adequately researched.

Although results from three large trials of pegylated interferon plus ribavirin are available, people weighing the potential benefits of HCV treatment against considerable side effects are still without a simple answer to the key question: "Will this work for me?" An algorithm that considers individual prognostic factors (genotype and baseline HCV viral load, liver histology, baseline liver enzyme levels, age, sex, and race) does shed some light on the likelihood of achieving a sustained virological response, yet people with hepatitis C, clinicians, researchers, and advocates continue to seek information on optimal treatment and side effect management strategies while awaiting better therapies.

For information about treatment of HCV in HIV-coinfected individuals, see Chapter VII, HCV Treatment in HIV/HCV Coinfection.

Who Needs Treatment?

If the natural history of hepatitis C infection followed an identical and predictable course in each infected individual, and HCV treatment were universally efficacious, had minimal side effects and were not exceedingly costly, the question of whom to treat would become moot. Active drug users, liver transplant recipients, people with decompensated cirrhosis, HIV and/or HBV coinfection, mental illness, and other significant co-morbidities have been excluded from these trials. HCV treatment may indeed be less effective and less tolerable for those who need it most. Despite improved treatment efficacy, the side effects remain problematic. For some individuals, they may be insurmountable. The cost of combination treatment—up to $40,000 per year, not including other agents often used for side effects management—creates an additional barrier to treatment for many who need it.

The National Institute of Health's 2002 *Consensus Statement on the Management of Hepatitis C* (NIH 2002) recommends that hepatitis C treatment be offered to:

> Patients with an increased risk of developing cirrhosis. These patients are characterized by detectable HCV RNA levels higher than 50 IU/mL, a liver biopsy with portal or bridging fibrosis, and at least moderate inflammation and necrosis. The majority also have persistently elevated ALT values. In some patient populations, the risks and benefits of therapy are less clear and should be determined on an individual basis or in the context of clinical trials.

The decision to treat chronic hepatitis C is more complex for people with normal or only slightly elevated ALT values (less than two times the upper limit of normal) and symptomatic mild liver disease; individuals with advanced liver disease; those with kidney disease; the elderly; and children. More research is necessary to guide treatment decisions in these populations.

Hepatitis C Treatment: Pegylated Interferon and Ribavirin

The standard of care for treatment of HCV is a combination of two drugs: pegylated interferon alfa (taken as injections) and ribavirin (taken in pill or capsule form; a liquid form of ribavirin for pediatric use has been approved by FDA). The course of treatment may be 24 or 48 weeks, depending on the HCV genotype.

Pegylated Interferon

Interferons are cytokines (chemical messengers) that are naturally produced by white blood cells to help fight infections and inhibit abnormal tissue growth in the body. Interferon (IFN) has antiviral and immunomodulatory effects. Different types of recombinant interferon—alfa, beta, and consensus—have been used to treat hepatitis C. Interferon alfa-2a and interferon alfa-2b have been used to treat hepatitis C since 1989 (Davis 1989; Di Bisceglie 1989). The only difference between these two interferons is the amino acid at position 23, which is lysine in alfa-2a and arginine in alfa-2b.

Pegylation—the attachment of a nontoxic molecule called polyethylene glycol—keeps interferon in the bloodstream longer and at more constant levels, thus increasing the efficacy of interferon treatment while reducing the frequency of injections (Perry 2002; Reddy 2001; Zeuzem 2000). Two forms of pegylated interferon have been approved by FDA for treatment of chronic HCV: pegylated interferon alfa-2a (Pegasys®), which uses a large (40kd) branched molecule of polyethylene glycol, and pegylated interferon alfa-2b (Peg-Intron®), which uses a smaller (12kd) linear molecule of polyethylene glycol. Attachment of the PEG molecule extends the half-life of Peg-Intron® to approximately 40 hours (compared to 3.6 hours for the parent molecule); the mean half-life of Pegasys® is 80 hours, with a range from 50 to 140 hours (compared to a mean of 5.1 hours for the parent molecule). Pegylated interferon alfa-2a is given at a fixed dose and is premixed; pegylated interferon alfa-2b is dosed according to weight and must be reconstituted with sterile water before administration (the manufacturer has developed a pre-filled dosing pen to simplify the process). The most commonly reported side effects of interferon are fatigue and flulike symptoms. Other side effects include hematologic toxicities and depression. Side effects may range from uncomfortable to debilitating; in rare instances, they may be life-threatening.

Ribavirin

Ribavirin (RBV) belongs to the family of nucleoside analogs (a class of drugs also used to treat HIV, although ribavirin has no effect against HIV). By itself, ribavirin is ineffective against hepatitis C, but when it is used in combination with interferon, the combination is more effective than interferon monotherapy (Di Bisceglie 1995; McHutchison 1998; Poynard 1998). It has been speculated that ribavirin may force hepatitis C virus into "error catastrophe" by increasing mutation of hepatitis C until it can no longer replicate (Cameron 2001; Crotty 2002; Graci 2002). Ribavirin is available under the name of Copegus® (Roche), Rebetol® (Schering), and from compounding pharmacies. It is also available as a generic. Ribavirin may be given at a fixed dose based on efficacy by genotype, or dosing may be weight-based. The most frequently reported side effect of ribavirin is hemolytic anemia, which is usually reversible.

The combination of pegylated interferon and ribavirin is the most effective hepatitis C treatment to date. Approximately half of those treated will achieve a sustained virological response (DiBisceglie 2002; Fried 2002a; Glue 2000; Manns 2001).

Assessing Responses to Hepatitis C Treatment: EVR, ETR, and SVR

Treatment for HCV can be evaluated by virological, histological, and biochemical responses, and at different time points: early, at the end of treatment, and six months after completion of treatment.

- Virological response is defined as either undetectable or significantly decreased HCV RNA.

- Histological response refers to an improvement in the condition of liver tissue, assessed by a better-than-baseline histological grade (amount of disease activity) in a post-treatment biopsy.

- A biochemical response reflects liver enzyme (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) levels that have decreased to within the normal range at the end of treatment, or for at least six months after completion of treatment.

- An early virological response (EVR) is defined as either a 2 $\log_{10}$ drop (a decrease in viral load by an order of 100; for example, decreasing from 2,000,000 to 20,000 copies) in HCV RNA, or undetectable HCV RNA twelve weeks after initiating treatment.

- An end-of-treatment response (ETR) means that an individual has no detectable HCV RNA in the bloodstream upon completion of treatment; many studies report ETR. While providing useful data, ETR should not be confused with the more robust SVR, as some individuals relapse during the six-month period between ETR and SVR reports. The rate of ETR is always higher than the rate of SVR.

- A sustained virological response (SVR) occurs when an individual has undetectable HCV RNA six months after completing HCV treatment. Many consider an SVR to be a cure or at least an indication of long-term remission, though this is controversial.

- One study found no detectable HCV RNA in the serum of five individuals who had achieved SVR ten years earlier (Lau 1998), while two studies reported relapse rates among 9% to 12% of sustained virological responders after five years of follow-up (Pradat 2003; Veldt 2003).

- A non-responder is someone who does not have a significant reduction in HCV RNA levels (<2 $\log_{10}$) after a specified interval of treatment (usually 24 weeks), or who has a significant decrease in HCV RNA, but never becomes HCV-RNA-undetectable during treatment. Some non-responders may have improved liver histology after treatment (Shiffman 1997).

- Not all individuals with an end-of-treatment response will maintain a sustained virological response; individuals with an ETR, but not an SVR are referred to as relapsers.

Long-Term Follow-Up of Sustained Virological Responders

Reports of the durability of sustained virological responses to HCV treatment vary. Early reports may have overestimated the proportion of sustained virological responders who remained virus-free years later. Older, less sensitive assays may have failed to detect low levels of viremia, thus some may have initially been misclassified as sustained virological responders. Others may have been reinfected. Very low levels of replication-competent hepatitis C have been discovered in blood from 11 individuals up to five years after they achieved SVR (Pham 2004). Corresponding liver biopsy samples were not available; the impact of low-level, replicating HCV on the liver histology of these sustained virological responders is unknown. Long-term follow-up of sustained virological responders treated with standard and pegylated interferon-based regimens is needed.

Table 1. Number and Percent of Sustained Virological Responders 3.5–10 Years Later

Author & date	N achieved SVR	Duration of follow-up	Undetectable HCV RNA at follow-up
Lau 1998	5	10 years	100% (5/5)
Sim 1998	5	Median: 48.2 months (range: 23–66)	100% (5/5)
Reichard 1999	26	3.5–8.8 years	92% (24/26)
Veldt 2003	286	59 months (range: 12–20)	91% (25/286)
Collier 2000	16	Mean: 38 months (range: 6–92)	88% (14/16)
Pradat 2003	59	5–7 years	88% (7/59)

Care for Hepatitis C

> *When I went back for the [HCV test] results,… she offered almost no information at all about the virus, explaining that she "just doesn't see it" in her surgery, and handed me a brochure produced in 1991, which said that there was little in the way of treatment, that the prognosis was not good…. All of this was simply untrue in 2000, but I did not know that then.*
>
> —Lisa Waller
> *Medical Journal of Australia*

Provider Education

Not all primary care physicians are well educated about hepatitis C. Consequently, their patients receive suboptimal care and inaccurate information despite recent medical advances in hepatitis C diagnostics and treatment. In 1999, Shehab and colleagues released their landmark survey of practice patterns of primary care physicians in the management of hepatitis C. The survey included an assessment of general knowledge of hepatitis C and clinical vignettes. The surveys were completed and returned by 33% (404/1,233) of physicians in a large HMO in Michigan. Birth from a mother with hepatitis C was ranked as a significant risk factor for hepatitis C infection by 80%; 20% thought that a blood transfusion in the United States after 1994 presented a significant risk;

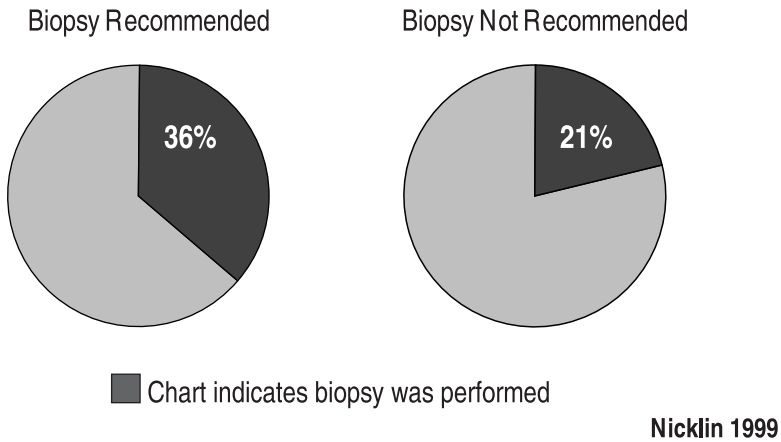
and 8% thought that casual household contact with an HCV-infected person presented a significant risk for acquiring hepatitis C. Only 2% had read the Consensus Development Conference Statement. Most respondents agreed to liver biopsy and interferon treatment if recommended by a gastroenterologist, but 72% either overestimated the response rate to interferon therapy or did not know what the response rate was (Shehab 1999).

Shehab and colleagues published results from a national survey in 2001 that assessed provider knowledge of risk factors for hepatitis C, attitudes about HCV testing, and actual management of patients with HCV. Completed surveys were received from 39% (1,412/4,000) of primary care providers across the United States. Although more than 90% were aware of the most common risk factors for hepatitis C, only 59% asked all of their patients about their risk factors for hepatitis C. Only 70% tested patients who had disclosed being at risk for hepatitis C infection, and a quarter of survey respondents did not know which treatment to recommend for hepatitis C (Shehab 2001).

Another group of researchers assessed the care received by patients with hepatitis C from an integrated medical delivery system in Philadelphia in which approximately 855 physicians provide medical care at 108 sites to about 500,000 patients. Surveys were sent to 222 physicians; 172 responses were analyzed. In addition, the medical charts of 186 individuals with hepatitis C were reviewed. Although ALT testing was frequently ordered as a part of routine medical care, 34% of physicians reported that they did not order HCV antibody testing for patients with elevated ALT levels. HCV antibody testing was ordered by only 21% of physicians with patients who disclosed parenteral (injecting) risk factors. Seventy-nine percent did not routinely test these patients for HCV. Screening for HCV antibodies was more frequently offered by physicians with practices in affluent, mostly white suburban areas; this is concerning because HCV is prevalent among African Americans and individuals with low socioeconomic status (M. J. Alter 1992).

There were substantial gaps between physicians' survey responses and the information from medical charts of 186 hepatitis C patients. Although 79% (147/186) of the HCV patients had elevated ALT levels, only 55% (102/186) had been seen by a gastroenterologist. According to their survey responses, physicians indicated that they referred 75% of HCV patients to specialty care. Only three individuals (2%) had been vaccinated against hepatitis A; only six (3%) had been vaccinated against hepatitis B (Nicklin 1999).

Figure 1. Physician-Reported Biopsy Recommendation vs. Documentation of Biopsy on Chart



Nicklin 1999

In 2003, Shehab and colleagues published another survey of the diagnosis and management of hepatitis C among patients of primary care clinics. They reviewed medical records from three groups of 229 individuals. Members of group one were HCV-antibody-positive, members of group two were HCV-antibody-negative, and members of group three had never been tested. Hepatitis C testing was initiated by the physician for just 16% (37/229) of group one and for just 10% (22/229) of group two. In group three, only 1% (2/229) had documented evidence of having had a discussion about hepatitis C with their physician. Although a majority (77%) of group one patients with detectable HCV RNA and elevated liver enzymes were referred to specialty care, almost half (40%; 24/59) of those biposied were diagnosed with bridging fibrosis or cirrhosis (Shehab 2003). These data underscore the importance of timely provider-initiated discussion of, and screening for, hepatitis C.

Guidelines for the management of persons with hepatitis C are extremely valuable. They must, however, be accompanied by initiatives for provider education to ensure the identification of and optimal care for individuals with hepatitis C.

Prognostic Factors

Coinfection with HIV

See Chapter VII, HCV Treatment in HIV/HCV Coinfection.

Genotype

Genotype is the strongest predictor of response to treatment. Genotypes 1 and 4 do not respond to treatment as well as genotypes 2 and 3, regardless of the type of interferon used (Berg 2003; Fried 2002a; S. Lee 2002; McHutchison 1998; Poynard 1998). In their meta-analysis of data from three trials of pegylated interferon alfa-2a, Lee and colleagues found a non-1 genotype to be the strongest independent predictor of SVR (OR, 4.11; 95% CI, 2.90–5.86; P=0.0001) (S. Lee 2002). A 24-week course of treatment for individuals with genotypes 2 and 3 appears to be sufficient; a 48-week course of treatment is recommended for individuals with genotypes 1 and 4 (Di Bisceglie 2002; Hadziyannis 2004).

Genotype 3 does not appear to be as sensitive to treatment as genotype 2 (Mangia 2004: Zeuzem 2003). Zeuzem and colleagues treated 224 individuals with HCV-2 or HCV-3 with 1.5 μg/kg of pegylated interferon alfa-2b plus weight-based rivavirin (800 to 14,000 mg/day) for 24 weeks. Overall, 81% achieved sustained virological response, but SVR rates were lower in those with genotype 3 (79% [143/182]) than in genotype 2 (93% [39/42]). Relapse rates were higher in genotype 3 (14% vs. 7% in genotype 2). The difference in response rates may be attributed in part to steatosis and high baseline viral load. Steatosis was significantly more prevalent in genotype 3 (P=0.003), and it was associated with a high baseline viral load (P=0.001). Steatosis of <5% was significantly associated with SVR (P=0.015) (Zeuzem 2003).

Genotype 4 may be more responsive to treatment than genotype 1. Sustained virological response rates from two studies that used 48 weeks of pegylated interferon plus ribavirin have ranged from 40% to 61% (Esmat 2003; Hassan 2003).

The viral kinetics of hepatitis C during early treatment differ depending on the genotype of HCV (A. U. Neumann 2000; Pawlotsky 2002; Zeuzem 2001). Frequent blood sampling from 12 individuals with HCV genotypes 1a, 1b, 2a, and 2b over the first 14 days of high-dose interferon revealed significant differences. Individuals with genotypes 2a and 2b had larger and more rapid decreases in HCV RNA after 48 hours than those with genotypes 1a and 1b (2.95 log copies/mL vs. 1.65 log copies/mL; $t^1/_2 = 2.0 \pm 0.5$ hours vs. $t^1/_2 = 3.0 \pm 1.0$ hours). At the end of 14 days, a significantly larger proportion of individuals with genotypes 2a and 2b had undetectable HCV RNA ($P=0.03$) (A. U. Neumann 2000). An examination of first and second-phase viral kinetics by genotype and mode of treatment (standard or pegylated interferon monotherapy) revealed more rapid first-phase and second-phase viral decay slopes for non-1 genotypes treated with pegylated interferon (Zeuzem 2001). Pawlotsky and colleagues observed less marked decreases in second-phase viral decay in genotypes 1 and 4 vs. genotype 3. At four weeks of treatment, individuals with genotypes 1 and 4 were less likely to be classified as rapid responders (individuals with decreases ≥ 0.3 log per week) (Pawlotsky 2002).

Baseline HCV RNA

A low baseline HCV-RNA level (≤ 2 million copies or ≤ 800,000 International Units) is a significant predictor of response to treatment. Numerous trials of both standard and pegylated interferon have confirmed that those with low baseline HCV RNA levels have a greater likelihood of achieving SVR (Fried 2002a; Hadziyannis 2002; Manns 2001; Poynard 1998).

The Role of Race/Genetics

In the United States, HCV is most prevalent among African Americans (see Chapter I, Epidemiology of Hepatitis C), who are more likely to be infected with genotype 1 than Whites, Hispanics, or Asian Pacific Islanders ($P<0.001$) (Blatt 2000; Jacobson 2002; Wiley 2002). It has been observed in several studies that African-Americans have impaired responses to interferon (De Maria 2002; Jeffers 2002; Kinzie 2001; McHutchison 2000; Reddy 1999; Theodore 2003). Results from a study of 472 individuals treated with either consensus interferon or interferon alfa-2b thrice weekly for 24 weeks found markedly poorer responses among African Americans than among Whites, Hispanics, and Asian Americans both during and after therapy. HCV-RNA levels were measured at baseline and weeks 0, 2, 4, 6, 8, 12, 20, 24, 36, 44, and 48. During treatment, HCV-RNA levels during treatment decreased by approximately 2.3 log in Whites vs. decreases of approximately 0.3 log in African Americans. Only 1 African-American participant (2%) achieved an SVR, while 46 white participants (12%), 4 Hispanic participants (10%), and 3 of the Asian-American participants (30%) achieved SVR (Reddy 1999).

Two efforts to increase virological response among African Americans by using high-dose interferon were unsuccessful. In one study, after 24 weeks of therapy, 26% of African Americans had undetectable HCV RNA vs. 60% of Whites ($P<0.01$). After 48 weeks, response rates among African Americans diminished to 10%, vs. 53% for Whites ($P<0.0001$) (De Maria 2002). A retrospective analysis of a treatment trial using two doses of interferon alfa-2b (3 MIU thrice weekly or 5 MIU daily) in African-American and white individuals with genotype 1 infections found similar initial responses among those treated with 3 MIU of interferon; however, when HCV-RNA levels on the high, daily-dose regimen were compared, African Americans had slower reductions in HCV

RNA than Whites (Theodore 2003).

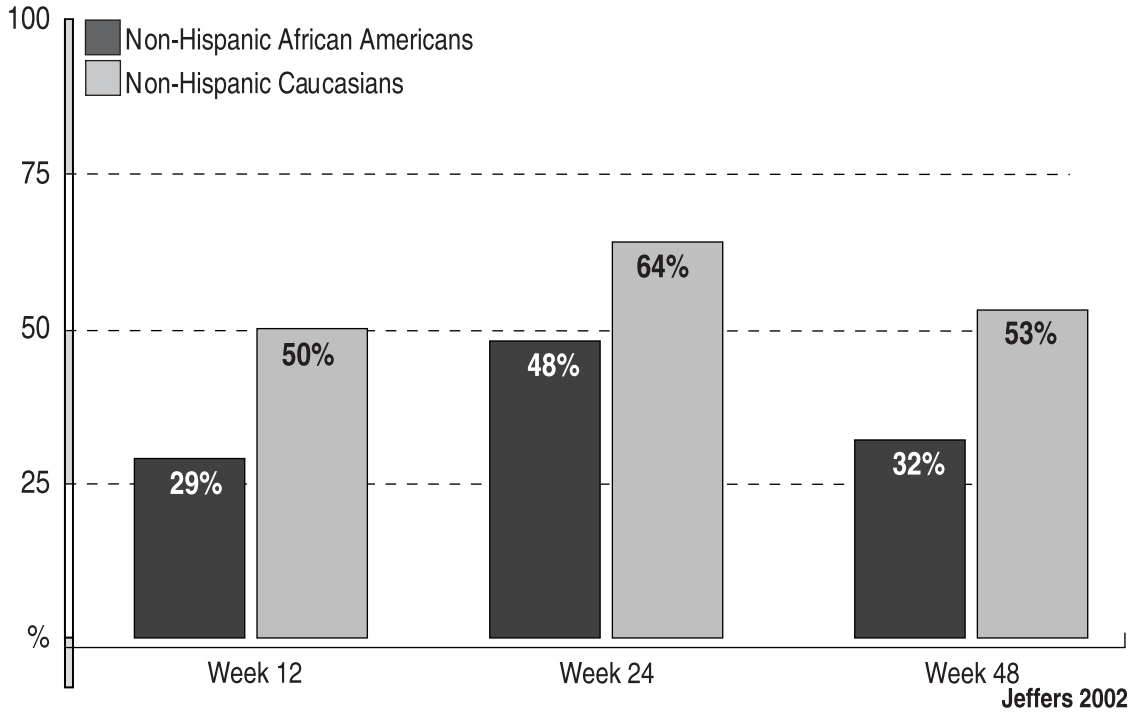
Data from two large clinical trials suggest that the addition of ribavirin increases virological response rates among African Americans. McHutchison and colleagues examined response rates from two large randomized trials utilizing four different treatment regimens. After 48 weeks of interferon monotherapy, no African-American participants achieved SVR. Adding ribavirin to interferon increased the percentage of African Americans achieving SVR from 0% to 23% (vs. 42% of Whites). Fewer than 4% (53/1744) of all participants were African Americans. Trials are rarely designed to ensure that the demographics of HCV infection in the United States are accurately reflected.

A majority of HCV-infected African Americans have HCV genotype 1 (Blatt 2000). Although genotype 1 infections do not respond to treatment as well as infections with genotypes 2 and 3, the differential response rates among African Americans cannot be attributed solely to genotype. Three treatment trials using standard interferon have reported impaired responses in African Americans with HCV genotype 1 (Kinzie 2001; Reddy 1999; Theodore 2003). Reddy and colleagues found fewer biochemical and virological response rates among African Americans vs. Whites with genotype 1 (6% vs. 34% for biochemical response rates; $P=0.001$; 6% vs. 22% for virological response rates during therapy; $P=0.038$). Kinzie and colleagues compared end-of-treatment responses of genotype 1-infected African Americans and Whites, finding that 2% (1/45) of African Americans achieved an ETR vs. 15% (5/33) of Whites ($P<0.05$) (Kinzie 2001). Although Theodore and colleagues saw similar response rates between African Americans and Whites with genotype 1 infections treated with 3 MIU of interferon thrice weekly, when the dose was increased to 5 MIU daily, Whites were most likely to have an initial response ($P<0.001$). Conversely, McHutchison and colleagues observed similar response rates among Blacks and Whites with genotype 1 infections (23% and 22%, respectively) in two large clinical trials (McHutchison 2000).

The pivotal studies of pegylated interferon had few African-American participants. Although data from subgroup analyses of these trials are available, the number of non-white participants has been too small to allow confident conclusions.

The efficacy of pegylated interferon alfa-2a plus ribavirin (1,000 mg–1,200 mg/day) has been assessed in 78 non-Hispanic African Americans and 28 non-Hispanic Whites, all with genotype 1 infections. Although sustained virological response rates among African Americans occurred more frequently in this trial than in previous studies, response rates remained greater among non-Hispanic Whites (Jeffers 2003).

Figure 2. Virological Response to Treatment in Non-Hispanic African Americans and Non-Hispanic Whites*



*Treatment was completed by 63/78 non-Hispanic African Americans and 22/28 non-Hispanic white participants.

Recent evidence suggests that some African Americans who do not achieve SVR may attain histological benefit from HCV treatment, although the durability of the improvement is currently unknown. Cassidy and colleagues evaluated histological responses from paired biopsies of 53/78 African Americans treated with pegylated interferon alfa-2a and ribavirin (1,000–1,200 mg/day) for 48 weeks. SVR was achieved by 32% (17/53) with paired biopsies. Improvement in fibrosis (≥ 1 point decrease in Knodell fibrosis score) occurred in 29% (5/17) of those who achieved SVR as well as 22% (8/36) of virological non-responders (Cassidy 2003).

There is an urgent need to investigate the contribution of additional genetic, environmental, and other factors to these differential responses, so that interventions to improve virological and histological treatment outcomes among African Americans may be developed.

A retrospective analysis of data from a multicenter HCV treatment trial and a compassionate access program in Australia and New Zealand reported that response to HCV treatment may be different in Southeast Asians. Dev and colleagues analyzed data from 70 Southeast Asian and 50 white individuals with standard interferon alfa-2, using induction/maintenance or regular dosing with 1,000–1,200 mg/day of ribavirin. Those with HCV genotypes 2 and 3 were treated for 24 weeks; all others were treated for 48 weeks. HCV genotypes 7, 8, and 9—regarded as new genotypes rather than subtypes of genotype 6 as previously thought (Tokita 1994)—were present in 33 Southeast Asians and emerged as independent predictors of an SVR (OR, 16.56; 95% CI, 4.16–18.04). SVR was achieved by 79% (26/33) of those with genotypes 7, 8, and 9. Unfortunately, because the sample size was small in this study (33 Southeast Asians with genotype 7, 8, or 9; 7

Southeast Asians and 44 Whites with genotype 1b), it is difficult to tease out the role of genotype vs. that of race. Southeast Asians with genotype 1b were five times as likely as Whites to achieve an SVR (OR, 4.63; 95% CI, 1.9–18.04), and there were no significant differences in treatment response by genotype or regimen among Southeast Asians (Dev 2002).

The duration of treatment may contribute to response rates. In San Jose, California, Nguyen and colleagues analyzed data from 38 Southeast Asians with HCV genotypes 6, 7, 8, and 9 who were treated for 24 weeks with either standard or pegylated interferon plus ribavirin. SVR was achieved by 54% (21/38), a lower rate than that reported in those with genotypes 6, 7, 8, and 9 after 48 weeks of treatment (Dev 2002; Hui 2003). There were no significant differences in response rate by treatment regimen, and the sample size was too small for analysis by genotype (M. H. Nguyen 2003).

It is not clear whether these differences reflect race, geographic diversity among Southeast Asians, genotype, a combination of these factors, or these and other additional factors. Identification of the factor(s) involved with differential responses to treatment may lead to improved treatment outcomes.

Weight

Lower body weight (≤ 85 kg /187 lbs; Fried and colleagues identified a slightly lower predictive threshold of ≤ 75 kg/165 lbs) is a known predictor of virological response to HCV treatment, whether standard or pegylated interferon is used (Fried 2002a; S. Lee 2002; Manns 2001).

Body mass index—the ratio of body weight in kilograms to the square of its height in meters—has been associated with virological and histological response to HCV treatment. Bressler and colleagues retrospectively reviewed data from 253 individuals treated with standard interferon with or without ribavirin. After controlling for age, sex, history of heavy alcohol consumption and cirrhosis at baseline, they found that a body mass index >30 kg/mg^2 was an independent predictor of virological non-response to HCV treatment (Bressler 2003). Greater body mass index also has a negative effect on histological response to treatment. In a meta-analysis of data from three HCV treatment trials, Cammá and colleagues reported that obese and overweight individuals were less likely to experience improvement of fibrosis than those with a body mass index ≤ 30 kg/mg^2 (OR, 0.56; 95% CI, 0.35–0.9) (Cammá 2004).

Weight is unique among prognostic factors, since it is the only one that may be modified by the individual. Pegylated interferon alfa-2b is dosed by body weight, as is ribavirin. It is clear that the dose of ribavirin has an impact on treatment outcomes for individuals with HCV genotype 1. The impact of ribavirin dosing on treatment outcomes has been difficult to analyze, because the dose of ribavirin has often been used as a surrogate for body weight. For individuals with HCV genotype 2 or 3, low body surface area and low body weight were the only variables significantly associated with achieving SVR (P=0.005 for low body surface areas; P=0.04 for low body weight) (Berg 2003).

Age and Sex

The likelihood of achieving a sustained virological response is greater in persons under 40 years old, and it continues to decrease with aging (Foster 2003; S. Lee 2002; Manns 2001; Poynard 1998; Poynard 2000). Females are more likely to achieve SVR than males (Manns 2001; Poynard 1998; Poynard 2000). Female sex and body weight are favorable prognostic indicators. Since females are usually smaller than men, a portion of this effect may be attributable to sex. The confluence of youth and sex appears to favor to premenopausal females, although the effect of hormones on response to treatment has not been characterized. If the favorable prognosis for treatment of young women does indeed have a hormonal component, perhaps hormones may be manipulated to increase treatment efficacy in other groups as well.

Cirrhosis

Treatment is contraindicated for individuals with decompensated cirrhosis, due to the risk of hepatic decompensation and death. Individuals with bridging fibrosis or compensated cirrhosis who have an urgent need for HCV treatment do respond to treatment, albeit less frequently than those with less advanced liver disease.

In a prospective, randomized study of standard interferon alfa-2a vs. three doses of pegylated interferon alfa-2a given to 271 individuals with bridging fibrosis or cirrhosis, the greatest virological response rate—30% SVR—was achieved with the highest dose of pegylated interferon (180 μg), although response rates for participants with poor prognostic factors (such as HCV genotype 1 and a high baseline HCV RNA level) receiving the same dose dwindled to 10%. Histological improvements occurred most frequently among those with a virological response (88% vs. 35% for non-responders) (Heathcote 2000).

Unfortunately, most data on the efficacy of pegylated interferon plus ribavirin in cirrhotics come from subgroup analyses from trials that have included only a small number of individuals with bridging fibrosis/compensated cirrhosis.

Steatosis

Hepatic steatosis—deterioration of liver tissue marked by fat deposits in liver cells—has been associated with hepatitis C infection, particularly genotype 3a, and linked with fibrosis progression (Castéra 2003; Gochee 2003; Hu 2003; Romero-Gomez 2003; Westin 2002). The presence of steatosis may decrease the probability of achieving SVR (Poynard 2003; Zeuzem 2003).

ALT and GGT Levels

High baseline alanine aminotransferase (ALT) levels and low-to-normal baseline levels of gamma-glutamyl transferase (GGT) are predictors of sustained virological response to HCV treatment (see Chapter IV, Diagnostics) (Berg 2003; S. Lee 2002; Pawlotsky 1996). Lee and colleagues, looking for baseline factors associated with achievement of an SVR, analyzed data from 814 participants in three randomized trials of pegylated interferon and found that pre-treatment ALT >3 times the upper limit of normal (ULN) was independently associated with SVR (OR=2.34; P<0.0001) (S.

Lee 2002). In an analysis of clinical, biochemical, histological, and virological characteristics of 260 participants in HCV treatment trials (of pegylated and standard interferons), a low baseline GGT level ($P<0.0001$), and a high baseline ALT level ($P=0.002$) were identified as predictors of SVR in individuals with HCV genotype 1 (Berg 2003).

Liver Histology Index

The Knodell Histological Activity Index (HAI; see Chapter IV, Diagnostics) at baseline has been identified as an independent predictor of SVR by an analysis of pooled data from three large, randomized clinical trials of pegylated interferon alfa-2a. A pre-treatment HAI score of >10 was significantly associated with SVR in cirrhotics as well as non-cirrhotics (overall, $P=0.0410$; for non-cirrhotics, $P=0.0268$) (S. Lee 2002).

Viral Kinetics

Hepatitis C viral kinetics are steady state; the continuous release of virions is balanced by a constant removal of virions from the bloodstream. The number of newly infected hepatocytes is counterbalanced by the apoptosis of infected hepatocytes. The estimated serum half-life of an HCV virion is between two and three hours (A. U. Neumann 1998). HCV-infected cells have a half-life of 1–70 days (Herrmann 2000).

Initial- and Second-Phase Viral Decay

Neumann and colleagues observed a biphasic decline in HCV by looking at blood samples from 23 HCV-infected individuals at initiation of treatment with interferon. HCV RNA levels remained at baseline for 8.7 ± 2.3 hours; then, an initial-phase decline occurred as interferon began to inhibit the production and release of new virions into the bloodstream. The amount of viral decay ranged from 0.5 to 2.0 log, depending on the dose of interferon. This decline stabilized after 24 to 48 hours of treatment. A less rapid, second-phase decline occurred between day two and day fourteen. During this second phase, interferon continued to block production of HCV, and virions were cleared from the bloodstream. The second-phase decrease in HCV-RNA is not dose-dependent (A. U. Neumann 2000).

Both the initial-phase rapid decline and the slower, second-phase decline in HCV RNA levels may be predictors of response to treatment. Although the second-phase decline has been regarded as the best predictor of SVR, the initial phase decline (at 24 hours after initiation of treatment) may be an important predictor of second-phase decline and, therefore, an early predictor of response to treatment (Carlsson 2002; Jessner 2001; Layden 2002a; Layden 2002b). A retrospective analysis of two studies by Layden and colleagues found strong correlations with lower viral loads at the end of the 24-hour initial-phase decline and more rapid second-phase declines ($P<0.001$). Individuals with HCV RNA <250,000 copies/mL after the first phase of viral decay were the only ones who achieved sustained virological responses (Layden 2002a). In another study of the predictive value of HCV RNA levels 24 hours after initiation of treatment, Jessner and colleagues observed that individuals with viral load decreases of less than 70% of baseline were likely to be non-responders after 24 weeks of treatment. This would mean that an individual with a baseline viral load of 2,000,000 copies/mL would most likely be a non-responder if his or her viral load remained above

600,000 copies/mL 24 hours after initiating treatment. This approach identified non-responders with a specificity of 100%, a sensitivity of 83%, a positive predictive value of 100%, and a negative predictive value of 77% (Jessner 2001).

More evidence to support the predictive value of 24-hour viral loads comes from Ferenci and colleagues, who observed that those with 12-week EVRs experienced sharper declines in 24-hour viral loads than non-responders. In week-12 responders, 24-hour viral load declines were 1.19 log ± 0.43 (SD), while non-responders had 24-hour viral load declines of 0.55 log ± 0.36 (SD) (Ferenci 2002).

Hopefully, research will identify individuals who are likely to have virological, biochemical, and histological responses early in the course of treatment. Until more information about predictors of biochemical and histological responses in the absence of virological responses is available, treatment decisions based on a 24-hour viral load must be considered premature. Some individuals may see much-needed improvements in liver histology even in the absence of virological response; discontinuing treatment would prevent an opportunity for histological benefit.

Early Stopping Rules

The likelihood of achieving sustained virological response to treatment may be predicted by early virological response after 12 weeks of treatment. Individuals who do not have either an undetectable HCV RNA or a 2-log decrease in HCV RNA are unlikely to have an SVR (Davis 2003b; Castro 2002; Civeira 1999; Fried 2002a; S. Lee 2002; A. U. Neumann 1998; Rosen 2002). Fried and colleagues found that 65% (253/390) of those treated with pegylated interferon alfa-2a plus ribavirin who achieved EVR also achieved SVR. Only 3% (2/63) of the individuals without an EVR had an SVR (Fried 2002a).

In a meta-analysis of data from trials of pegylated interferon alfa-2a, Lee and colleagues found a negative predictive value (NPV) of EVR of 91% at week 4; it increased slightly to 95% at week 8, and rose to 98% at week 12 (a high NPV is used to determine when therapy can be discontinued, because achieving an SVR after completing the full course of treatment is extremely unlikely). The positive predictive value (PPV) of an EVR, according to this meta-analysis, was not as useful for guiding treatment decisions (the higher the PPV, the more likely that an individual may achieve an SVR; a high PPV may encourage people to continue treatment). At week 4, the positive predictive value of EVR was 54%; it decreased to 49% at week 8 and to 46% at week 12 (S. Lee 2002).

Data from an earlier study by McHutchison and colleagues, which used standard interferon (either with or without ribavirin), revealed that detectable HCV RNA at 12 weeks of therapy predicted non-response in 89% of individuals; waiting until 24 weeks to identify non-responders (by detectable HCV RNA) increased this to 99% (McHutchison 2001). It is possible, however, that the regimen of standard interferon used in this study may have influenced the length of time necessary for identifying non-responders. Using a 24 week cutoff for non-response derived from a standard interferon treatment trial may not be applicable to persons treated with pegylated interferon.

In an effort to develop an algorithm for early discontinuation of HCV treatment applicable to both standard and pegylated interferon-based regimens, Berg and colleagues analyzed data from 209 individuals enrolled in five different HCV treatment protocols. Pre-treatment virological, histological,

biochemical, and clinical parameters were examined for their importance in predicting SVR. Participants received 24 or 48 weeks of treatment. Treatment included ribavirin (dose of 800 –1,200 mg/day), with the exception of 19 individuals who received pegylated interferon alfa-2a monotherapy. Regimens included two different induction/maintenance strategies with standard interferon alfa-2a, thrice weekly standard interferon (alfa-2a and alfa-2b), and two pegylated interferon-based regimens—alfa-2b (Peg-Intron®) and alfa-2a (Pegasys®). HCV RNA testing was performed on stored serum samples at baseline and after 4 and 12 weeks of treatment.

The predictive thresholds for baseline HCV RNA, baseline ALT and baseline GGT levels, and HCV RNA levels at week 4 and week 12 were identified; week 12 cutoff values were used for the algorithm. At week 12, the NPV of HCV RNA ≤ 30,000 IU/mL was 100%; positive predictive value was 64.8%. There were no significant differences in the applicability of these thresholds by treatment regimen.

The algorithm proposed by Berg and colleagues recommends discontinuation of treatment at week 12 if HCV RNA is >30,000 IU/mL, or if there has been less than a 2-log (99%) decrease in HCV RNA from baseline. If HCV RNA is between 30,000 and 35,000 IU/mL, repeat testing is recommended. For those with HCV RNA below the threshold of discontinuation, a qualitative HCV RNA test should be performed at 24 weeks; if HCV RNA is detectable at that time, the algorithm recommends discontinuation of treatment (Berg 2003).

Additional research using viral kinetics to determine early stopping rules is underway. Using data from 127 participants treated with 180 μg/week of pegylated interferon alfa-2a plus 1,000–1,200 mg/day of ribavirin for 48 weeks, Neumann and colleagues worked to identify the earliest reliable time point and decrease in HCV RNA level for predicting sustained virological response. No one achieved an SVR unless their HCV RNA was <5.5 log on treatment day four, or they had a decrease of >0.5 log (approximately a threefold drop) on treatment day seven. These parameters had a negative predictive value of 100% (A. U. Neumann 2003). Prospective studies are needed to validate these and other early stopping rules.

Duration of Treatment

Extending HCV treatment for an additional 24 weeks has been suggested as a strategy to improve treatment outcomes in genotype 1. Drusano and colleagues developed a model to predict SVR after treatment with pegylated interferon alfa-2b, using data from participants in the Manns trial. The model predicted that individuals with genotype 1 would need to have continuously undetectable HCV RNA for at least 32 weeks to achieve a sustained virological response. Since the model found that, on average, it took 30.2 weeks for HCV RNA to become undetectable, the authors suggested that 48 weeks of treatment might be insufficient for genotype 1 (Drusano 2004). Although this model has limits, a prospective investigation could help to identify individuals who might benefit from extended therapy.

However, extending duration of therapy may increase treatment discontinuations rather than sustained virological response rates. Sanchez-Tapias randomized 326 individuals who had detectable HCV RNA after 4 weeks of treatment to either 44 or 68 additional weeks of pegylated interferon alfa-2a plus 800 mg/day of ribavirin, for a total of 48 or 72 weeks of treatment. Although they did not observe an increase in neutropenia or thrombocytopenia with longer

treatment, they reported a difference in withdrawal rates by treatment duration (17% vs. 36% in the extended duration group). Sustained virological response rates did not differ significantly by treatment arm (30% for 48 weeks vs. 36% for 72 weeks) (Sanchez-Tapias 2004). The rate of sustained virological responses was not broken out by genotype in this study, so it is difficult to assess the effect of extended treatment on virological responses in genotype 1.

Conversely, a subset of individuals with genotype 2 or genotype 3 may achieve sustained virological responses after less than 24 weeks of treatment. In a randomized, prospective study of 280 individuals with genotype 2 and 3, Mangia and colleagues used HCV RNA level after 4 weeks of treatment to determine duration of treatment for 210/280 individuals; the remaining 70 were treated for 48 weeks. When HCV RNA was undetectable at 4 weeks, treatment was discontinued at 12 weeks. Those with detectable HCV RNA at week 4 were treated for a total of 24 weeks. SVR was achieved more frequently among those treated for 12 weeks than 24 or 48 weeks (89.9%, 78.7% and 81.4%, respectively). Relapse rates were lowest after 48 weeks of treatment; they increased from 0-2.5% after 24 weeks of treatment and 10% after 12 weeks of treatment (Mangia). Response rates were greater among those with genotype 2 (82%) than those with genotype 3 (64%), regardless of duration of treatment. This study used a lower dose of pegylated interferon alfa-2b (1.0 μg/kg per week) and a higher dose of ribavirin (1,000–1,200 mg/day) than has been recommended for treatment of genotype 2 and genotype 3 (P-IFN 1.5 μg/kg per week; RBV 800 mg/day). Prospective study of early virological responses with different doses of pegylated interferon and ribavirin will help to clarify optimal regimen and duration of therapy for persons with genotype 2 and genotype 3.

The Difficulty of Comparison

Although study results from trials of each drug have been compared (often by one company or the other, to indicate its product's advantage), there has not been a head-to-head comparison of the safety, efficacy, and tolerability of the two pegylated interferons. Efficacy, safety, and tolerability of each product appear similar, but without a direct comparison we must rely on the experience of clinicians who have used both products. While it is tempting to compare the two, a true comparison is not possible; participant characteristics and treatment regimens differ across studies. The only comparison to date is a small study of hepatitis C viral kinetics during treatment with Pegasys® or Peg-Intron® and ribavirin. A suboptimal dose of Peg-Intron® was used in this study (1.0 μg/kg, the recommended dose for Peg-Intron® monotherapy; 1.5 μg/kg is the recommended dose for combination therapy). The study compared mean week-12 viral loads, finding that those who received Pegasys® had significantly lower viral loads (2.82 $\log_{10}$ vs. 3.87 $\log_{10}$; $P<0.01$) (Bruno 2002). This information raises questions about the recommended dose for Peg-Intron® monotherapy.

Key Studies of Combination Therapy: Pegylated Interferon Plus Ribavirin

Three pivotal large, randomized clinical trials of pegylated interferon plus ribavirin (with similar inclusion/exclusion criteria) have shown that the combination of pegylated interferon plus ribavirin is the most effective treatment for chronic hepatitis C. Overall sustained virological response rates are often presented as evidence of treatment efficacy in all individuals with chronic hepatitis C although individuals with one or more poor prognostic factors were excluded from these trials.

The Manns Data

Manns and colleagues conducted a large (1,530 person), three-arm study, comparing the safety and efficacy of:

- Standard interferon alfa-2b (3 MIU, thrice weekly), plus ribavirin dosed at 1,000–1,200 mg/day for 48 weeks;
- Pegylated interferon alfa-2b (1.5 μg/kg, once weekly), plus ribavirin dosed at 800 mg/day for 48 weeks; and
- Pegylated interferon alfa-2b (1.5 μg/kg, once weekly for four weeks, then reduced to 0.5 μg/kg), plus ribavirin dosed at 1,000–1,200 mg/day for 48 weeks.

Comparisons across treatment arms are problematic in the Manns study. It is possible that the induction/maintenance arm did not offer its participants a sufficient dose of pegylated interferon, while those in the higher-dose pegylated interferon arm may not have received a sufficient dose of ribavirin. It's as if someone tried to bake three cakes: one with a proper proportion of known ingredients (but using inferior flour), one with not quite enough baking powder, another with not quite enough flour—and then looked to see if the cakes rose nonetheless.

Figure 3. Sustained Virological Responses by Treatment Regimen

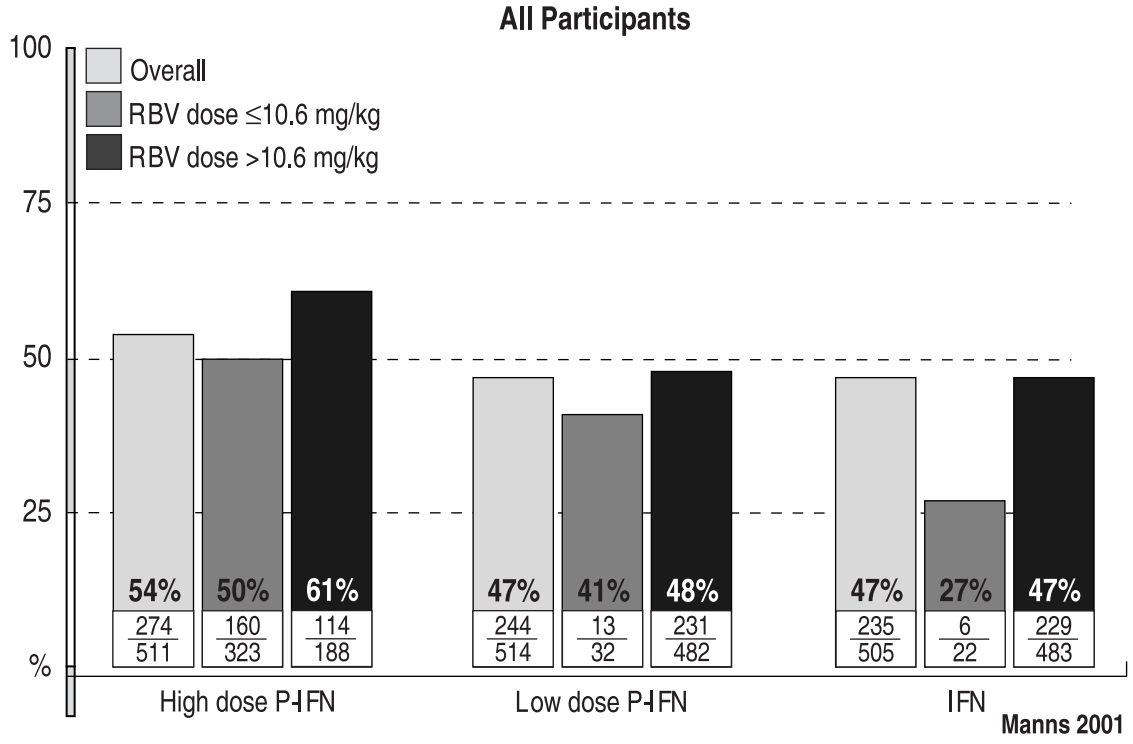
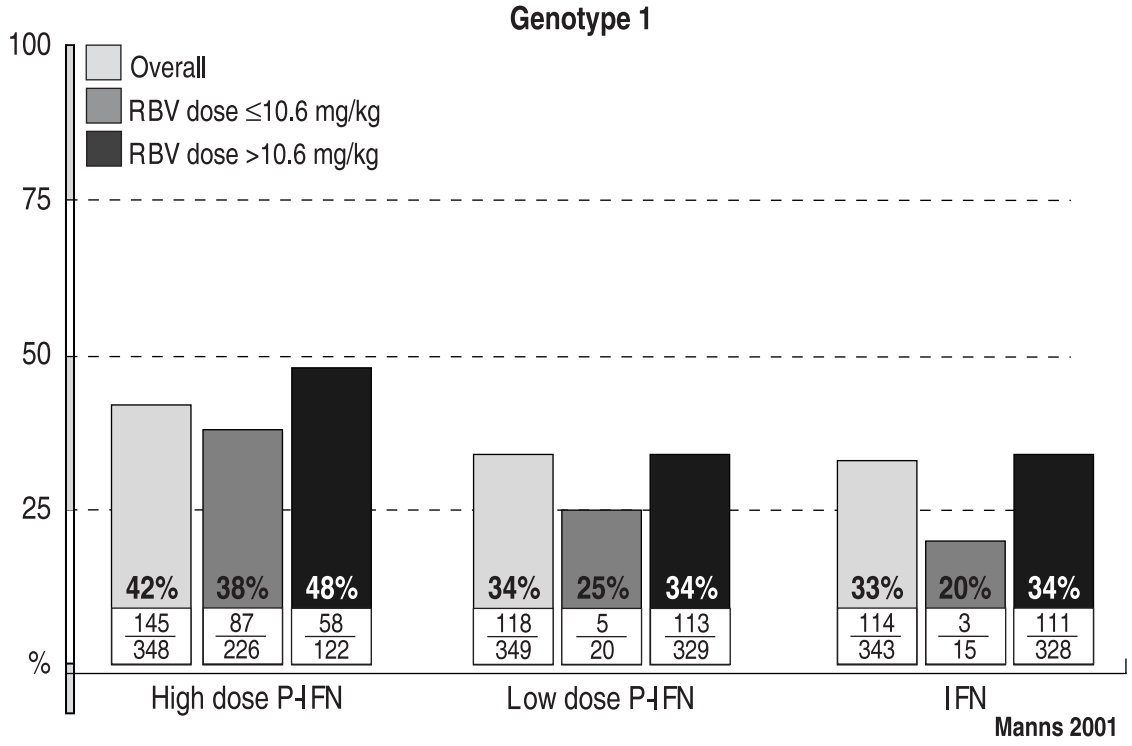


Figure 4. Sustained Virological Response by Genotype



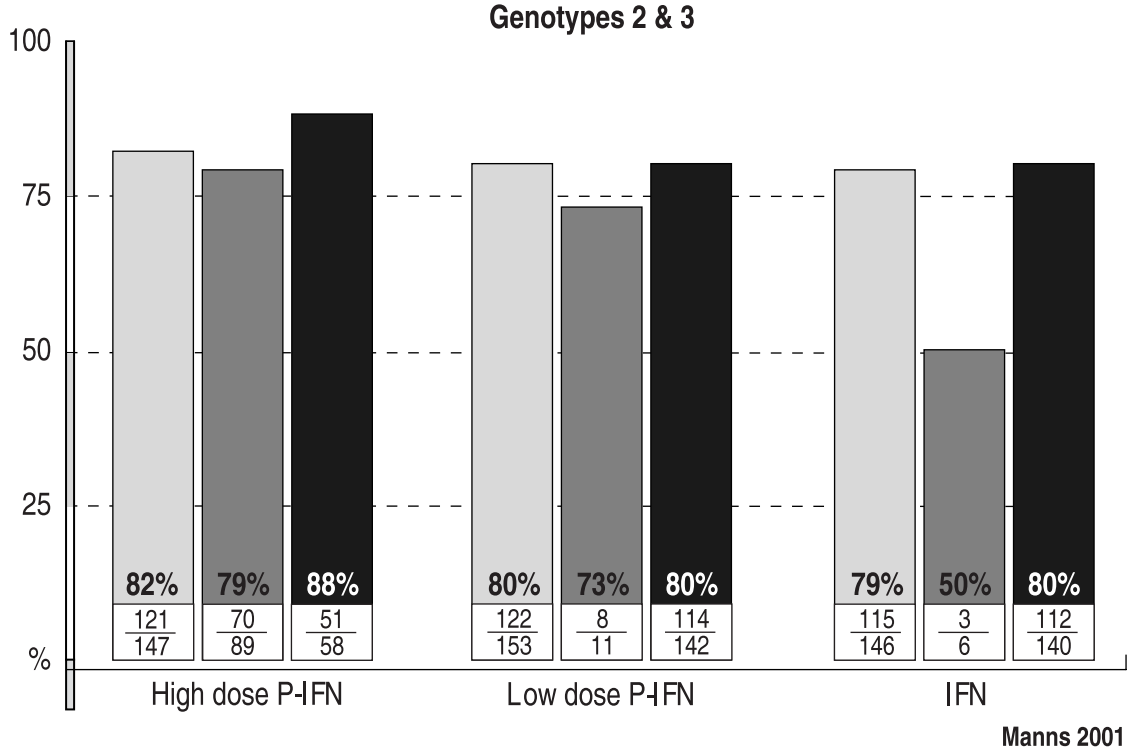
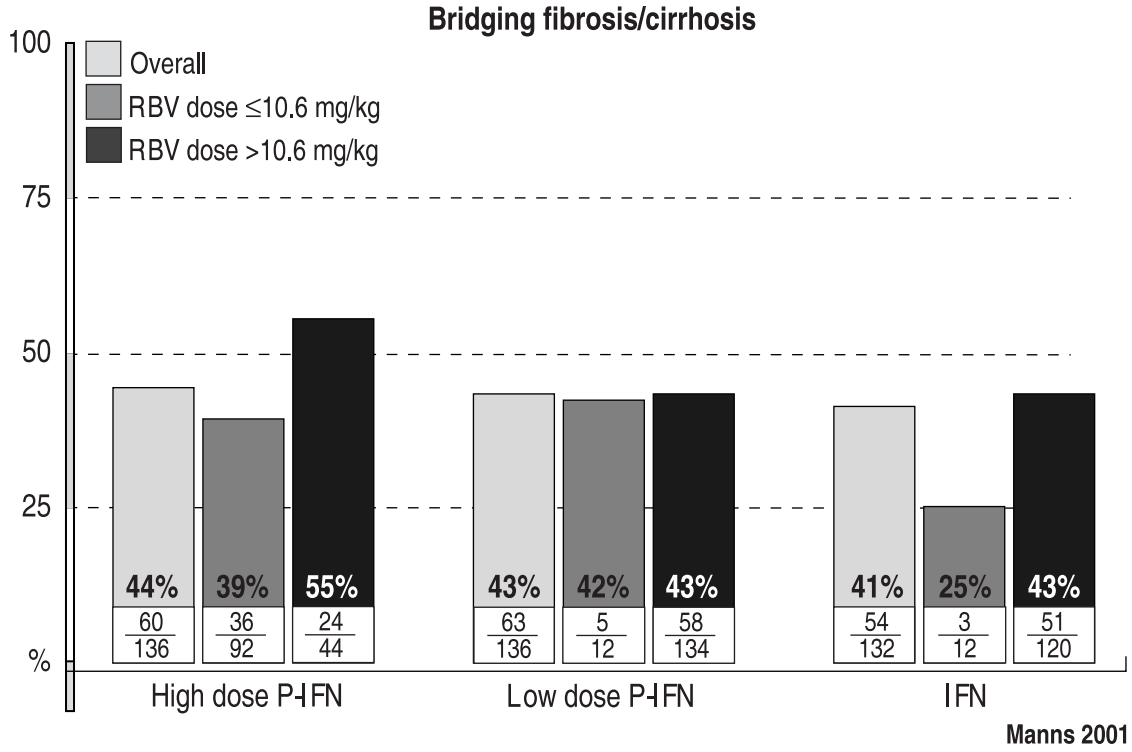
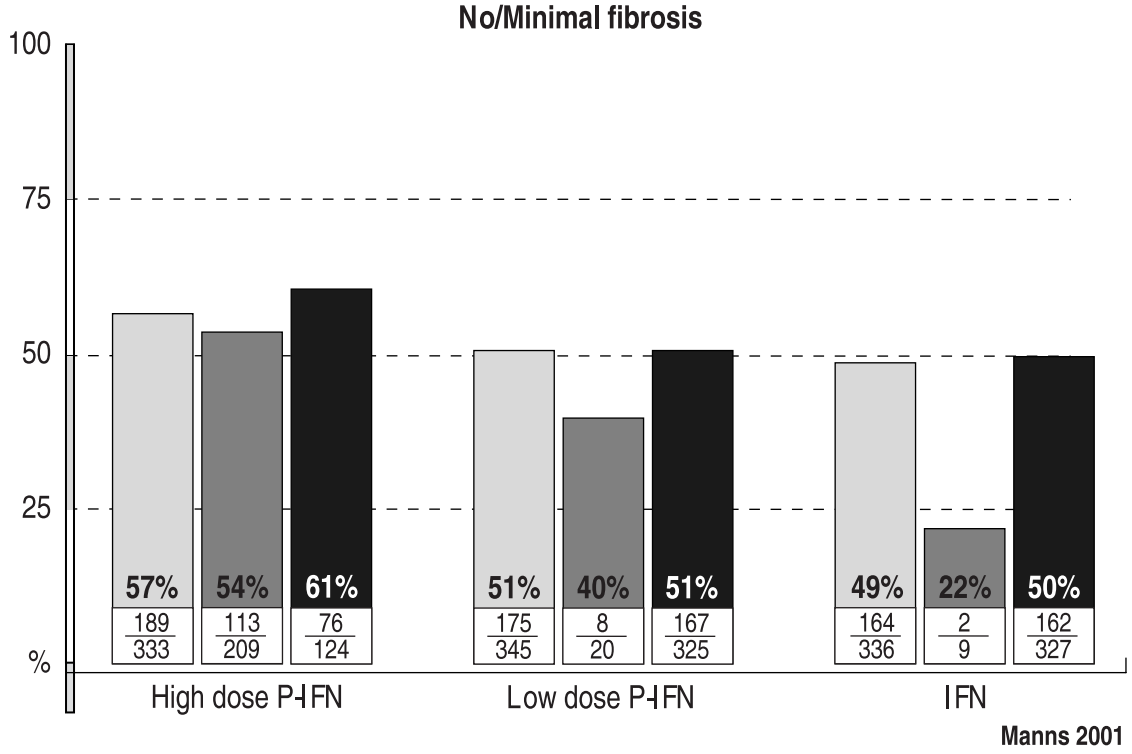


Figure 5. Sustained Virological Response by Liver Histology



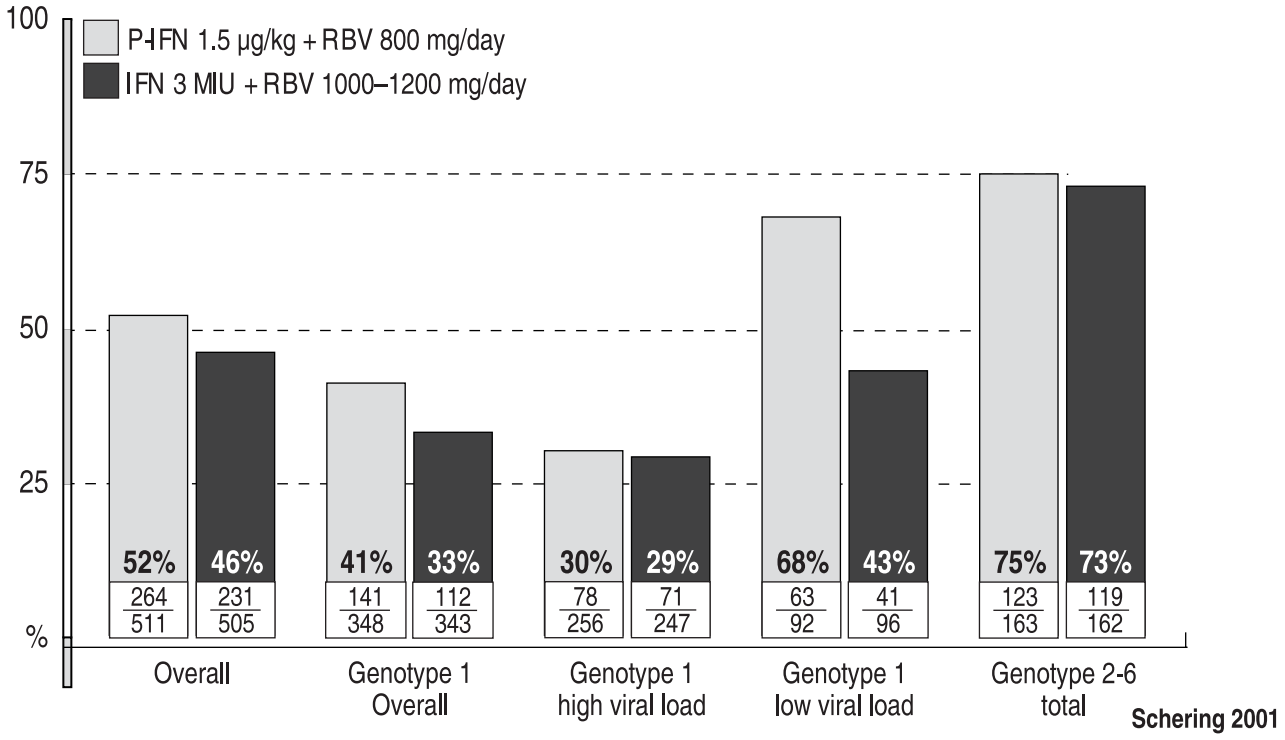


The probability of achieving an SVR increased with the higher doses of ribavirin and pegylated interferon (OR, 1.7; P=0.002). When weight-based dosing of ribavirin was taken into account, the estimated effect of high-dose (vs. lower dose) pegylated interferon was larger (OR, 1.7); however, post hoc analysis used ribavirin dose as a proxy for, rather than a reflection of, body weight. (Weight-based dosing of ribavirin has been correlated with greater response rates in many studies of RBV plus standard or pegylated interferon.) Unfortunately, these data led to the approval of Peg-Intron® with a recommended daily dose of 800 mg of ribavirin, which may be suboptimal for some individuals. Higher doses of ribavirin are recommended in the European Union (1,000 mg daily for individuals who weigh less than 75 kg (165 lbs) and 1,200 mg daily for individuals who weigh 75 kg or more), but statistically significant prospective data on the efficacy of weight-based ribavirin are not yet available.

Overall, SVR occurred most frequently with the higher dose of pegylated interferon (54% vs. 47% for the other two dosing arms). In the same higher dose arm, 42% of those with genotype 1 and 42% of those with a high baseline viral load (>2 million) achieved SVR.

Possessing genotype 1 and a high baseline viral load substantially influenced the response to treatment. When response rates are broken out by baseline viral load and genotype, significant differences by baseline viral load among those with genotype 1 emerge. Only 30% of those with a high baseline viral load achieved SVR after treatment with pegylated interferon 1.5 μg/kg plus 800 mg of RBV, while 68% with low baseline viral loads achieved SVR. Among those with genotype 1/high baseline viral load, there was virtually no difference in response by regimen (30% for P-IFN vs. 29% for standard IFN). While the package insert includes this data, the study did not include this analysis, which is relevant for the majority of people in the United States contemplating HCV treatment.

Figure 6. Response by Genotype, Baseline HCV RNA Level, and Treatment Regimen



The Manns study did not address questions about the optimal duration of therapy for each HCV genotype. All participants received 48 weeks of therapy, which may have been longer than necessary for those with genotype 2 or genotype 3. A year before the Manns study was published, Poynard and colleagues, based on their analysis of data from 1,744 treatment-naïve persons in two large trials, suggested discontinuation of treatment (standard interferon plus ribavirin) for individuals with HCV genotype 2 or genotype 3 who had undetectable HCV RNA after completing 24 weeks of therapy. They found that 82% of those with HCV genotype 2 or genotype 3 who were HCV-RNA-undetectable after 24 weeks of therapy achieved SVR. If therapy was continued for an additional 24 weeks, the rate of SVR rose by only 2% (Poynard 2000). A look at the Manns data broken out by genotypes 2 and 3 shows very little difference by treatment regimen. It is difficult to tell whether the similarities in response between the lower-dose pegylated interferon arm and the standard interferon arm are a consequence of an insufficient dose of pegylated interferon, differing baseline HCV RNA levels within genotypes, or other prognostic factors.

Figure 7. Sustained Virological Response Rate by Treatment Regimen and Genotype

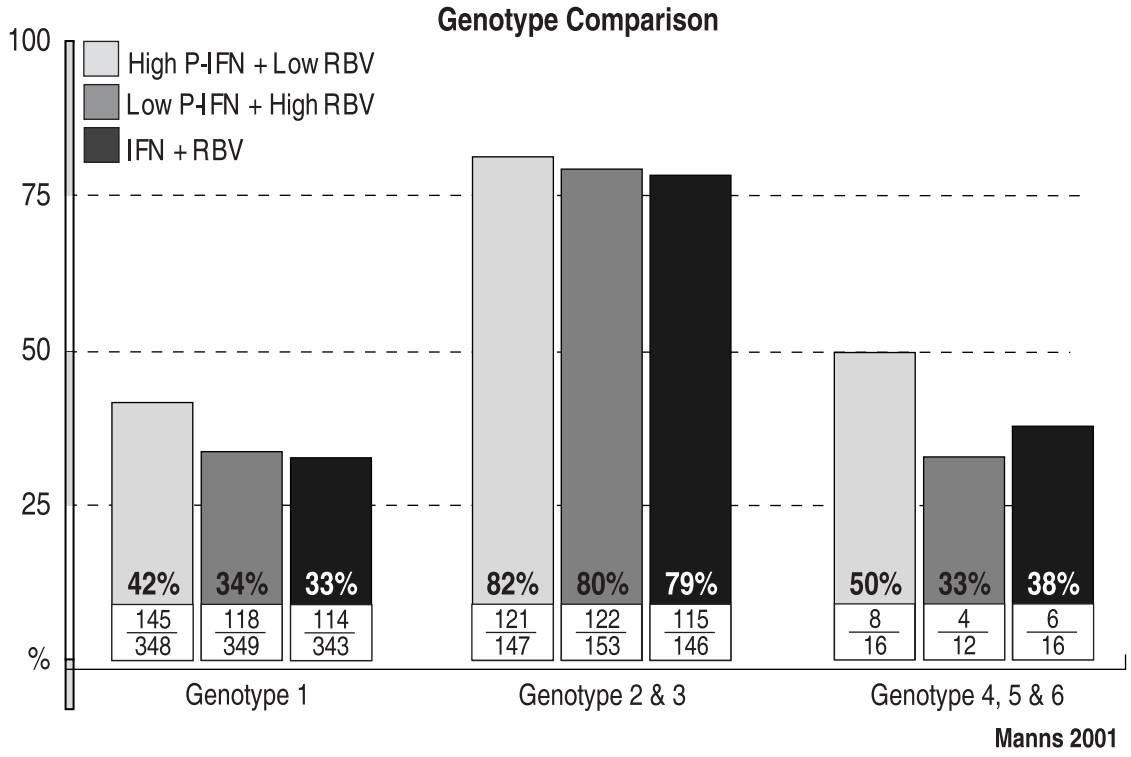
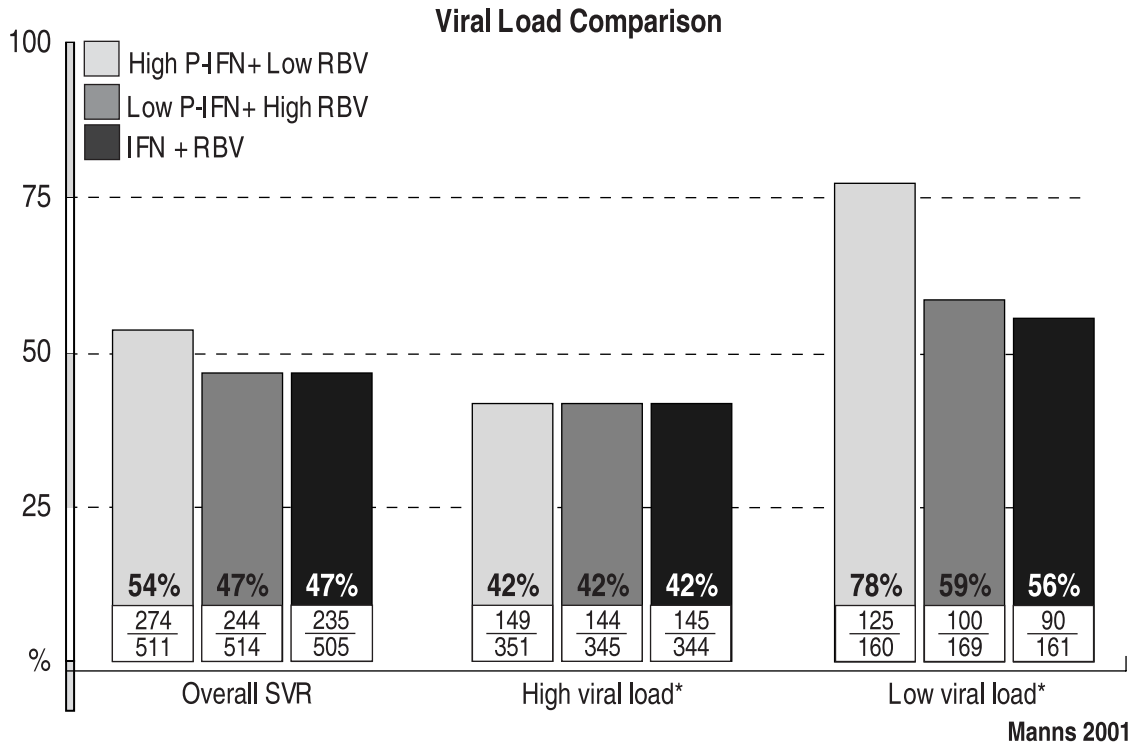
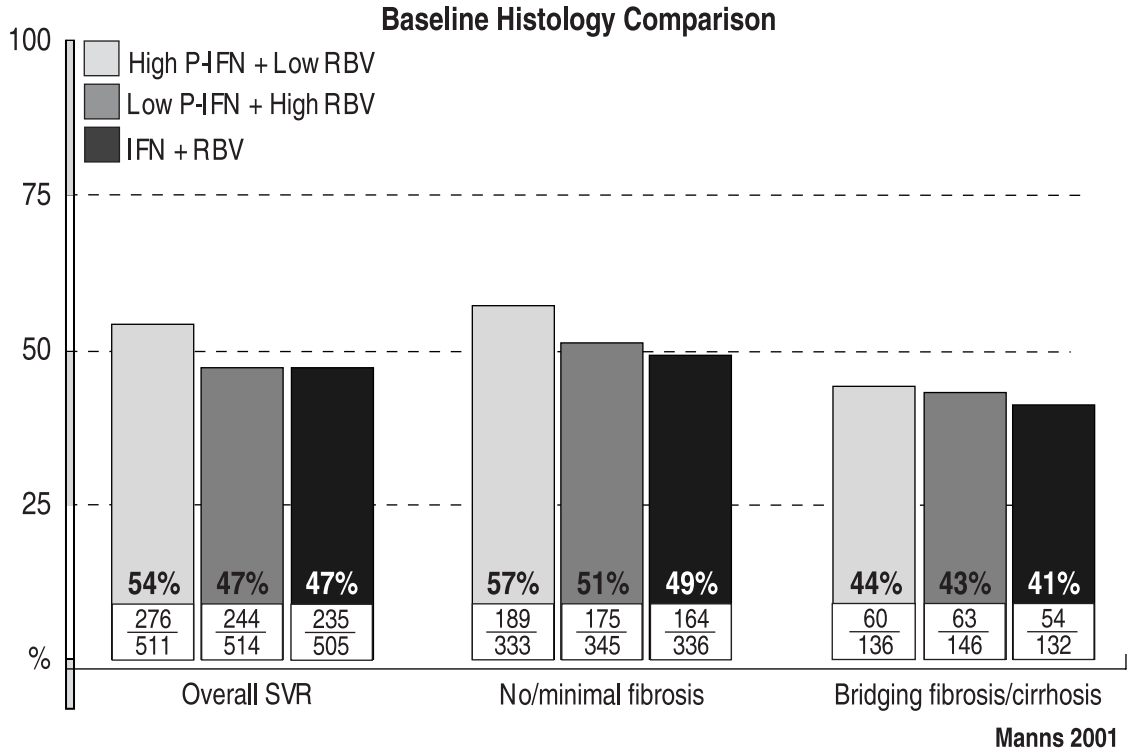


Figure 8. Sustained Virological Response Rate by Treatment Regimen and Baseline Viral Load



* High viral titer, >2 million; low viral titer, ≤ 2 million

Figure 9. Sustained Virological Response Rate by Treatment Regimen and Liver Histology



Although many did not achieve a sustained virological response, histological improvement was observed in all treatment groups. Histological improvement occurred most frequently with SVR; 90% of those who achieved an SVR also had improvement in liver histology. Some degree of histological improvement (ranging from -0.8 to -1.3) was seen in 44% of non-responders. About 20% of each treatment group had improvement of fibrosis (defined as a decrease of at least one in the Knodell HAI score). However, without long-term follow up, it is not possible to assess the durability and clinical impact of such improvements in relapsers and non-responders.

Dose reductions due to neutropenia occurred in 18% of those on high-dose pegylated interferon vs. 8% on standard interferon. Only 1% discontinued treatment because of neutropenia. Dose modifications were more frequent with pegylated interferon/high-dose ribavirin than with standard interferon/high-dose ribavirin arm (49% vs. 34%), mostly due to neutropenia.

Table 2. Treatment Discontinuations and Dose Reductions for Adverse Events

Adverse Events	High dose P-IFN/ Low dose RBV	Low dose P-IFN/ High dose RBV	IFN/ High dose RBV
Asthenia (weakness)	18 % (92/511)	16% (82/514)	18% (91/505)
Fatigue	64% (327/511)	62% (318/514)	60% (303/505)
Fever	46% (235/511)	44% (226/514)	33% (167/505)
Headache	62% (316/511)	58% (298/514)	58% (293/505)
Rigors (stiffness and/or chills)	48% (245/511)	45% (231/514)	41% (207/505)
Weight decrease	29% (148/511)	17% (87/514)	20% (101/505)
Dizziness	21% (107/511)	21% (108/514)	17% (86/505)
Arthralgia (joint pain)	34% (174/511)	34% (175/514)	28% (141/505)
Musculoskeletal pain	21% (107/511)	17% (87/514)	19% (96/505)
Myalgia (muscle pain)	56% (286/511)	48% (247/514)	50% (252/505)
Anorexia	32% (163/511)	29% (149/514)	27% (136/505)
Diarrhea	22% (112/511)	16% (82/514)	17% ((86/505)
Nausea	43% (220/511)	36% (185/514)	33% (167/505)
Vomiting	14% (71/511)	14% (72/514)	12% (61/505)
Impaired concentration	17% (87/511)	16% (82/514)	21% (106/505)
Depression	31% (158/511)	29% (149/514)	34% (172/505)
Insomnia	40% (204/511)	40% (206/514)	41% (207/505)
Irritability	35% (179/511)	34% (175/514)	34% (172/505)
Coughing	17% (87/511)	15% (77/514)	13% (66/505)
Dyspnea (difficulty breathing)	26% (133/511)	23% (118/514)	24% (121/505)
Alopecia (hair thinning or loss)	36% (184/511)	29% (149/514)	32% (162/505)
Pruritus (itching)	29% (149/511)	26% (134/514)	28% (141/505)
Rash	24% (123/511)	22% (113/514)	23% (116/505)
Dry skin	24% (123/511)	18% (93/514)	23% (116/505)
Injection site inflammation	25% (128/511)	27% (139/514)	18% (91/505)
Injection site reaction	58% (296/511)	59% (303/514)	36% (182/505)

Manns 2001

Adverse events were clustered in five groups: flulike symptoms, gastrointestinal symptoms, psychiatric symptoms, respiratory symptoms, and dermatological symptoms. The adverse events reported in this chart occurred in at least 10% of study participants. The number of individuals experiencing more than one adverse event was not provided.

Table 3. Adverse Events By Treatment Regimen

Adverse Events	High dose P-IFN/ Low dose RBV	Low dose P-IFN/ High dose RBV	IFN/ High dose RBV
Dose discontinuation for any adverse event	14% (71/511)	13% (67/514)	13% (65/503)
Dose reduction for any adverse event	42% (214/511)	36% (185/514)	34% (171/503)
for anemia	9% (46/511)	12% (62/514)	13% (65/503)
for neutropenia	18% (92/511)	10% (51/514)	8% (40/503)

Manns 2001

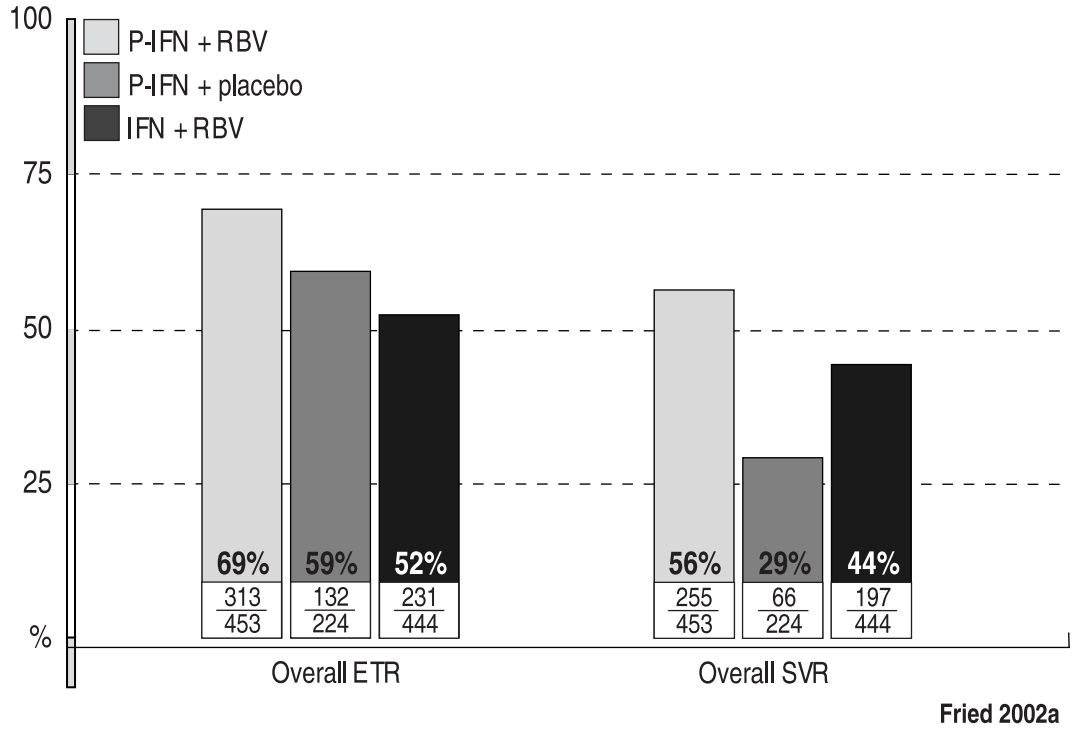
Discontinuation rates were similar across arms (13% from the pegylated interferon arm and 14% from the standard interferon arm).

The Fried Data

Fried and colleagues conducted a large, international trial, in which 1,121 individuals with chronic HCV were randomized into one of three treatment arms:

- Pegylated interferon alfa-2a ,180 μg once weekly, plus ribavirin 1,000–1,200 mg/day for 48 weeks;
- Pegylated interferon alfa-2a, 180 μg once weekly, plus placebo for 48 weeks; or
- Standard interferon alfa-2b, 3 MIU thrice weekly, plus ribavirin 1,000–1,200 mg/day for 48 weeks.

Figure 10. End-of-Treatment and Sustained Virological Responses by Regimen



The label of Copegus® (Roche's ribavirin) breaks out SVR data by genotype.

Figure 11. Sustained Virological Responses by Regimen and Genotype

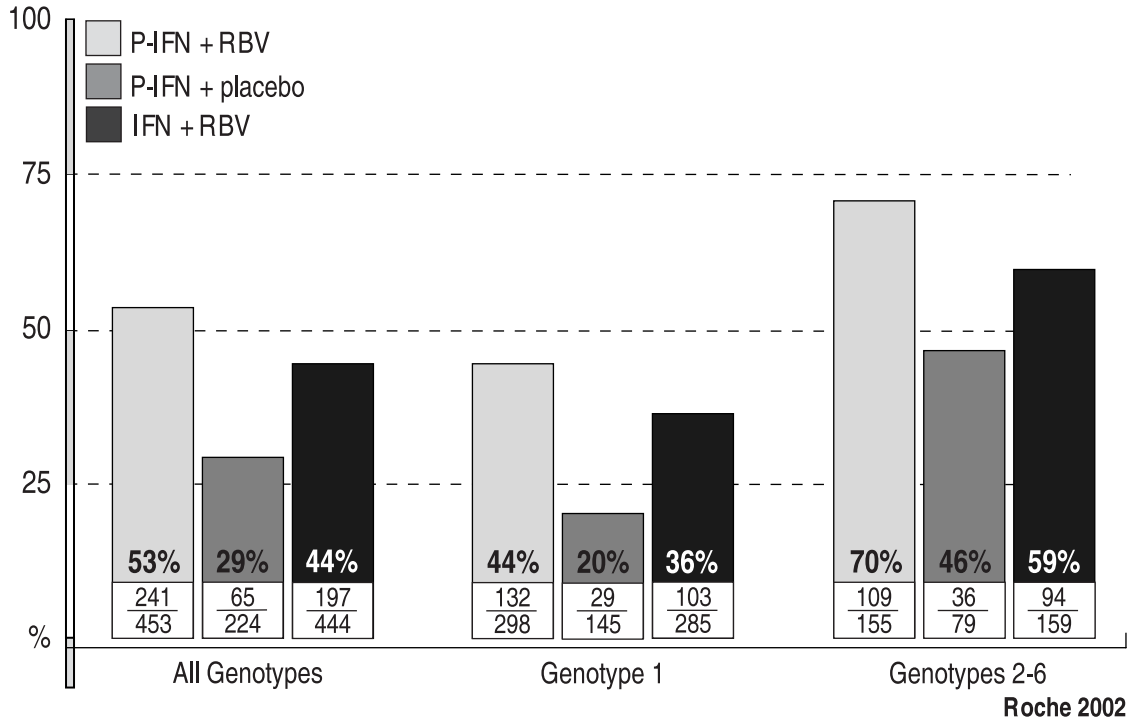


Figure 12. Sustained Virological Response Rate by Regimen and Genotype: High Baseline HCV RNA

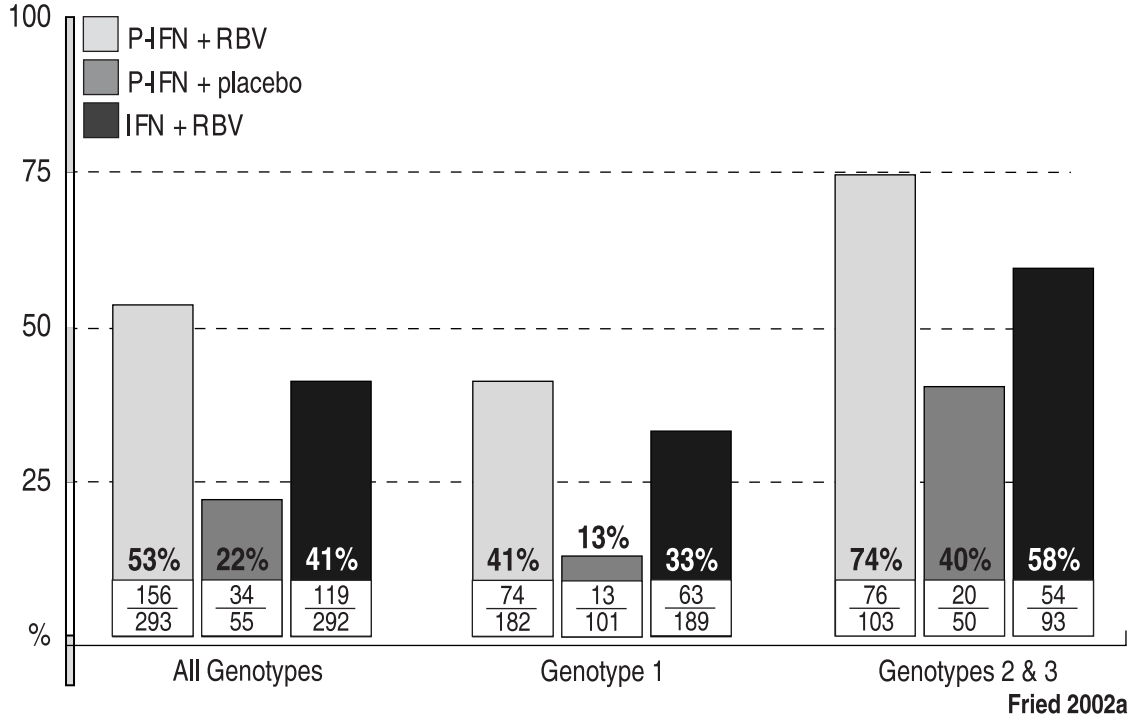


Figure 13. Sustained Virological Response Rate by Regimen and Genotype: Low Baseline HCV RNA

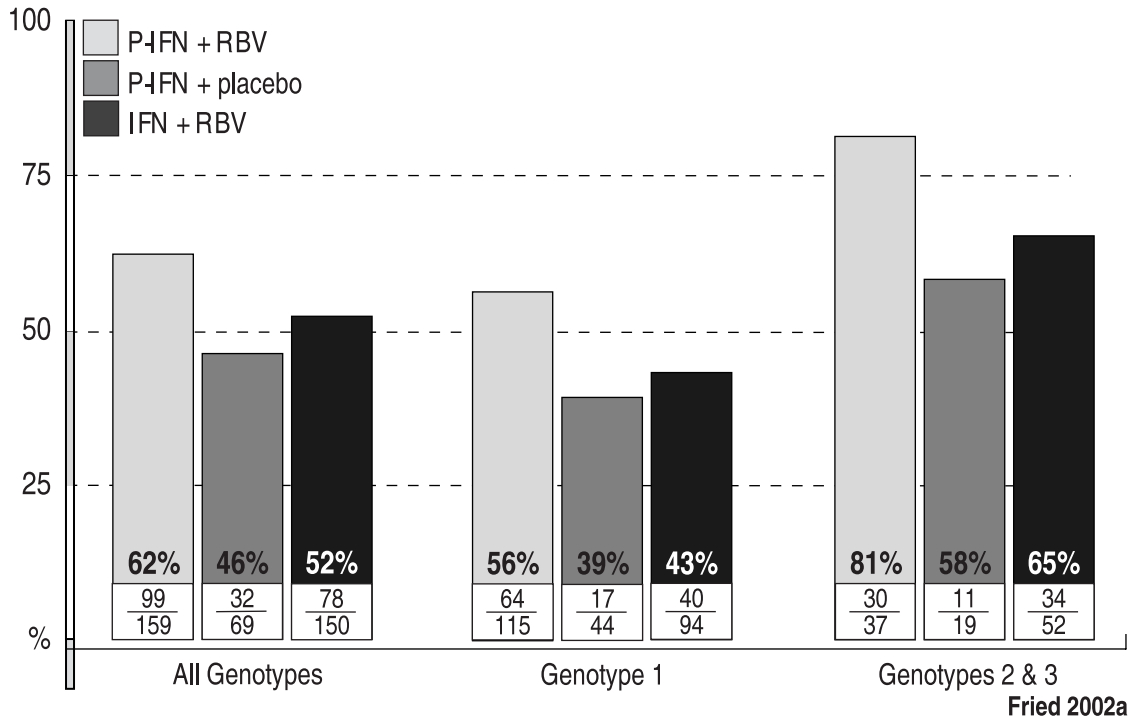
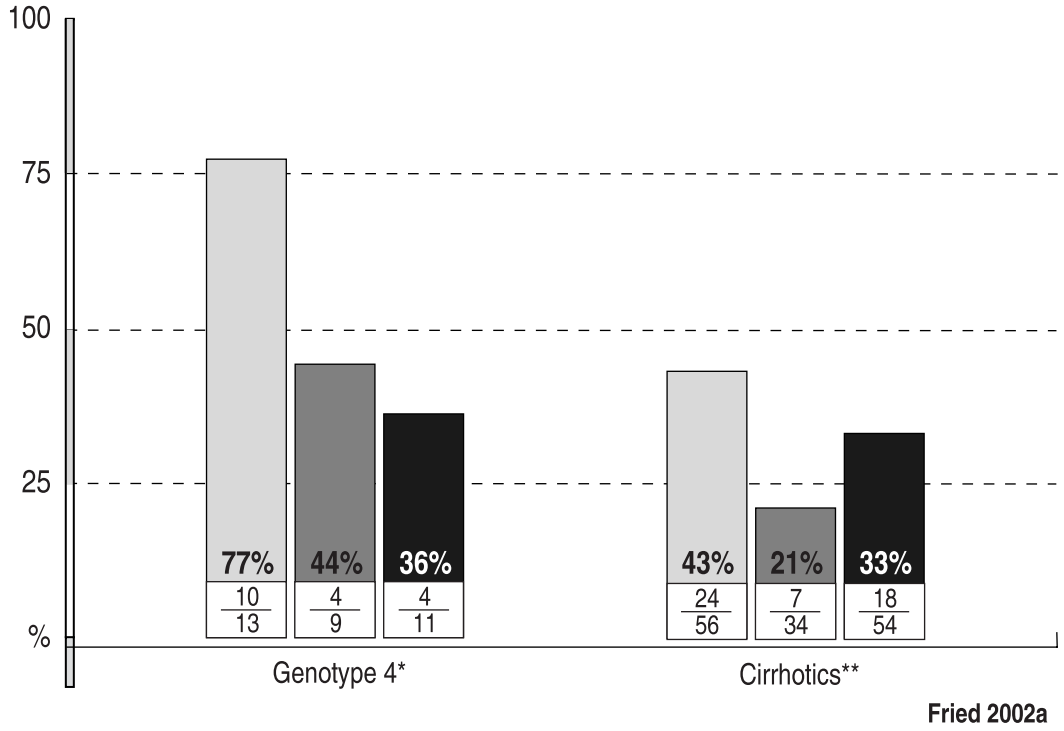


Figure 14. Sustained Virological Response Rate: Genotype 4 and Cirrhosis



* Data not broken out by baseline viral load.
** Data not broken out by baseline viral load or genotype.

Duration of therapy by genotype was not addressed in this study, nor were questions about optimal dosing of pegylated interferon and ribavirin, although interesting information emerged about SVR and dose modifications. The rate of SVR among individuals with early virological responses in the P-IFN/RBV arm was 75%. When the authors looked at early virological responders from the same arm that had significant dose reductions (of at least 20% in doses of both drugs), the rate of SVR only dropped to 67%. Further exploration of the safety and efficacy of lower doses of pegylated interferon alfa-2a (150 μg or 135 μg) is needed.

Aside from a greater frequency of neutropenia and thrombocytopenia, adverse events from the pegylated interferon arm were similar to those in the standard interferon arm. People on pegylated interferon had fewer flulike symptoms and less frequent depression than those on standard interferon.

Table 4. Dose Modifications Due to Laboratory Abnormalities*

Lab Abnormalities	P-IFN + RBV		IFN + RBV		P-IFN + Placebo	
Anemia	1% (4/453)	22% (99/453)	3% (13/444)	19% (83/444)	0% (0/224)	4% (8-224)
Neutropenia	20% (91/453)	1% (6/453)	5% (24/444)	<1% (1/444)	17% (38/224)	0% (0/224)
Thrombocytopenia	4% (18/453)	<1% (2/453)	<1% (1/444)	0% (0/453)	6% (14/224)	<1% (1/224)

Fried 2002a

*Laboratory abnormalities also included elevations of alanine aminotransferase levels (not shown).

Table 5. Discontinuations for Laboratory Abnormalities and Adverse Events

Discontinuations	P-IFN + RBV	IFN + RBV	P-IFN + Placebo
Overall	22% (100/453)	32% (140/444)	32% (72/224)
For lab abnormalities	3% (12/453)	1% (4/444)	1% (2/224)
For adverse events	7% (32/453)	10% (44/444)	6% (13/224)

Fried 2002a

There were three deaths in this trial (due to hypertensive heart disease, drowning, and liver cancer), none considered to be related to treatment.

The Haziyannis Data

Hadziyannis and colleagues took a closer look at ribavirin dosing and duration of therapy by genotype in a multinational, randomized, four-arm study of 1,284 individuals with chronic hepatitis C. Participants received a fixed dose of pegylated interferon alfa-2a (180 μg once weekly) for either 24 or 48 weeks, with ribavirin doses of either 800mg/day or 1,000–1,200 mg/day. The four arms of the study were:

- Arm one: pegylated interferon alfa-2a, 180 μg once weekly, plus 800 mg/day of ribavirin for 24 weeks;
- Arm two: pegylated interferon alfa-2a, 180 μg once weekly, plus 1,000–1,200 mg/day of ribavirin for 24 weeks;
- Arm three: pegylated interferon alfa-2a, 180 μg once weekly, plus 800 mg/day of ribavirin for 48 weeks; and
- Arm four: pegylated interferon alfa-2a, 180 μg once weekly, plus 1,000–1,200 mg/day of ribavirin for 48 weeks.

Randomization was stratified by genotype and viral titer (low vs. high: below or at least 2 million copies). Individuals with genotype 1/high viral load were randomized 1 to 1 to 4 to 4 (10%, 10%, 40%, 40%). Individuals with a non-1 genotype and a low viral titer were randomized 1 to 1.5 to 1 to 1.5 (20%, 30%, 20%, 30%). Due to this randomization scheme, the results of this trial are comparable only within a particular genotype and viral load stratum; the overall virological response across arms does not reflect a purely random distribution of baseline characteristics.

Table 6. Randomization and Allocation

	24-week		48-week	
Genotype & Viral Load	P-IFN + RBV 800	P-IFN + RBV 1000–1200	P-IFN + RBV 800	P-IFN + RBV 1000–1200
Genotype 1 & high VL	1 (10%)	1 (10%)	4 (40%)	4 (40%)
Genotype 1 & low VL	1 (20%)	1.5 (30%)	1 (20%)	1.5 (30%)
Genotype non-1 & high VL	1 (20%)	1.5 (30%)	1 (20%)	1.5 (30%)
Genotype non-1 & low VL	1 (20%)	1.5 (30%)	1 (20%)	1.5 (30%)

FDA 2002

Table 7. Baseline Characteristics

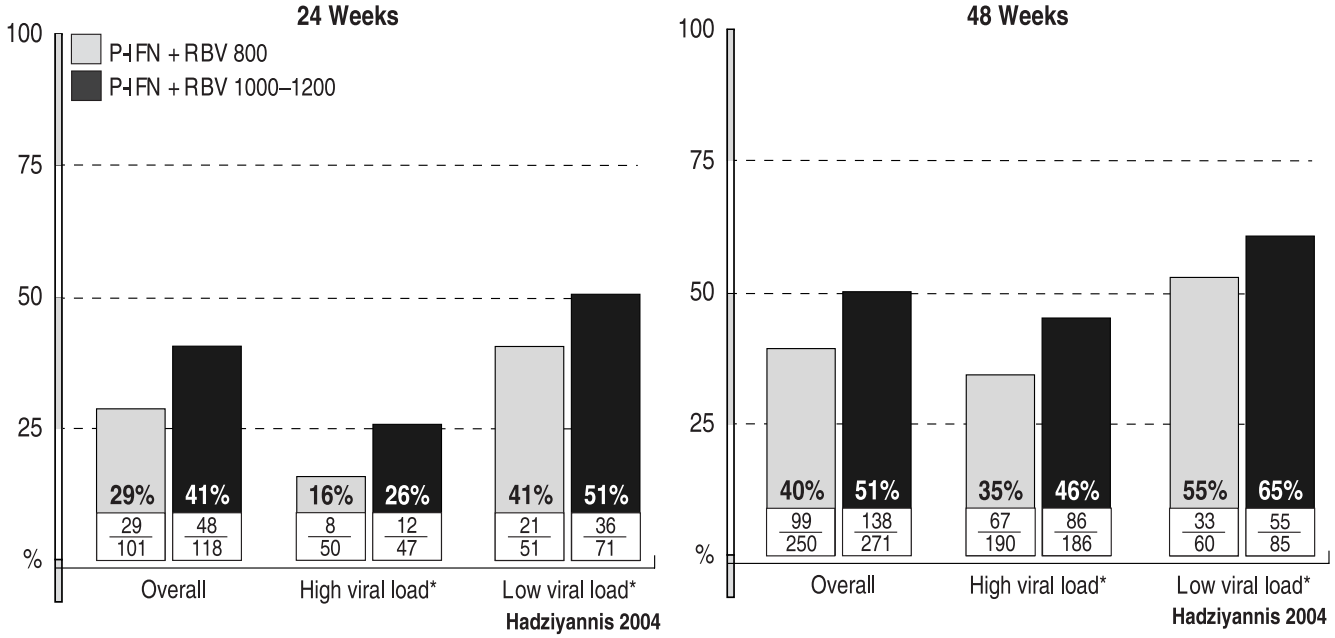
Characteristics	Total (N=1284)	U.S. (N=441)	Non-U.S. (N=843)
High viral load	64 % (819)	65% (288)	63 % (531)
Genotype 1	58% (740)	61% (270)	56% (843)
Cirrhosis	25% (321)	29% (127)	23% (194)

FDA 2002

Data were analyzed by modified intention-to-treat, including everyone who received at least one dose of medication (1284/1311), rather than everyone who was enrolled. Individuals who did not achieve a week-24 virological response were offered the option of treatment discontinuation.

Most participants were white males (65% male; 89% white). Roche is sponsoring another trial (the National Institutes of Health's Study of Viral Resistance to Anti-Viral Therapy [Virahep-C]) that will evaluate response to treatment among African Americans. Unfortunately, the number of African Americans with genotype non-1 infections enrolled in this trial was too small to draw any conclusions about dosing, duration, and the likelihood of achieving SVR in this group.

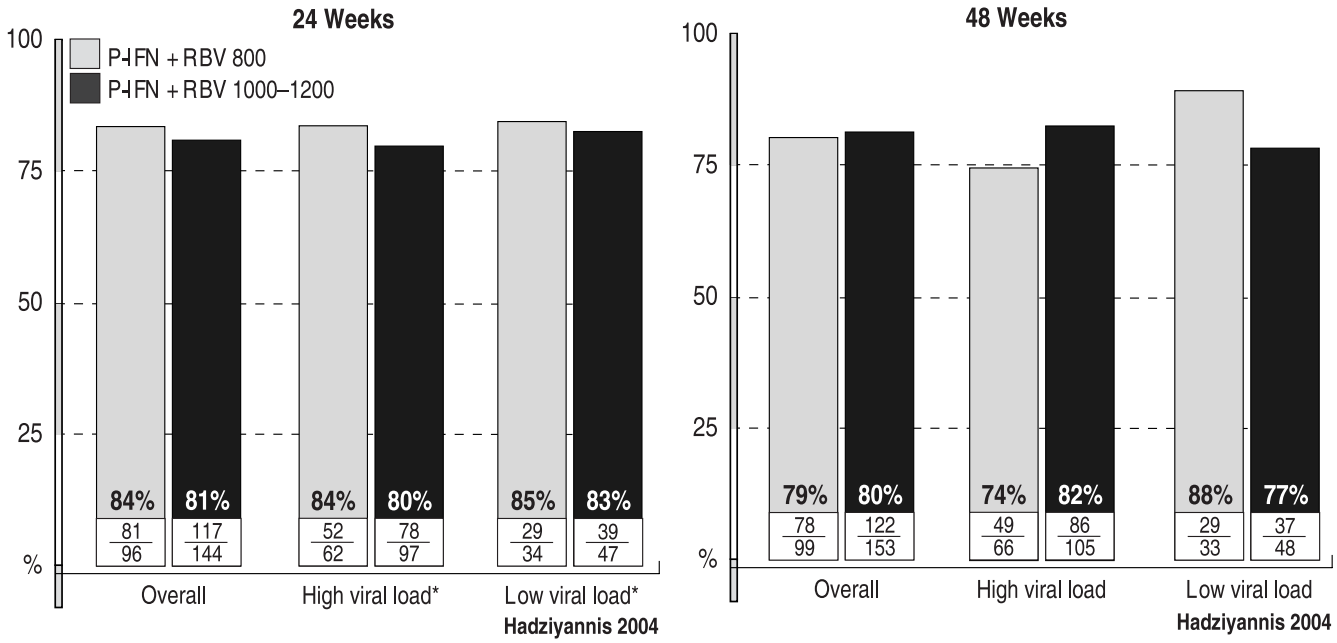
Figure 15. Sustained Virological Response Rate in Genotype 1 by Regimen, Treatment Duration, and Baseline Viral Load



* High viral titer is >2 million copies; low viral titer is ≤ 2 million copies.

The Hadziyannis data is especially relevant to most people in the United States, where genotype 1 is predominant. Forty-eight weeks of treatment with weight-based dosing of ribavirin yielded the highest rate of sustained virological responses for individuals with genotype 1, regardless of baseline viral load.

Figure 16. Sustained Virological Response Rate in Genotypes 2 and 3 by Regimen, Treatment Duration, and Baseline Viral Load



Response rates for HCV genotypes 2 and 3 did not differ significantly by regimen or duration of treatment . For people with genotype-2 and genotype-3 infections, 24 weeks of treatment and a lower ribavirin dose appear to be as effective as a 48-week course of treatment and a higher ribavirin dose. There were fewer severe adverse events and discontinuations among those who received 24 weeks of therapy with the lower dose of ribavirin. This is good news for people with HCV genotypes 2 and 3.

Figure 17. Sustained Virological Response Rate in Genotype 1 by Regimen, Treatment Duration, and Baseline Liver Histology

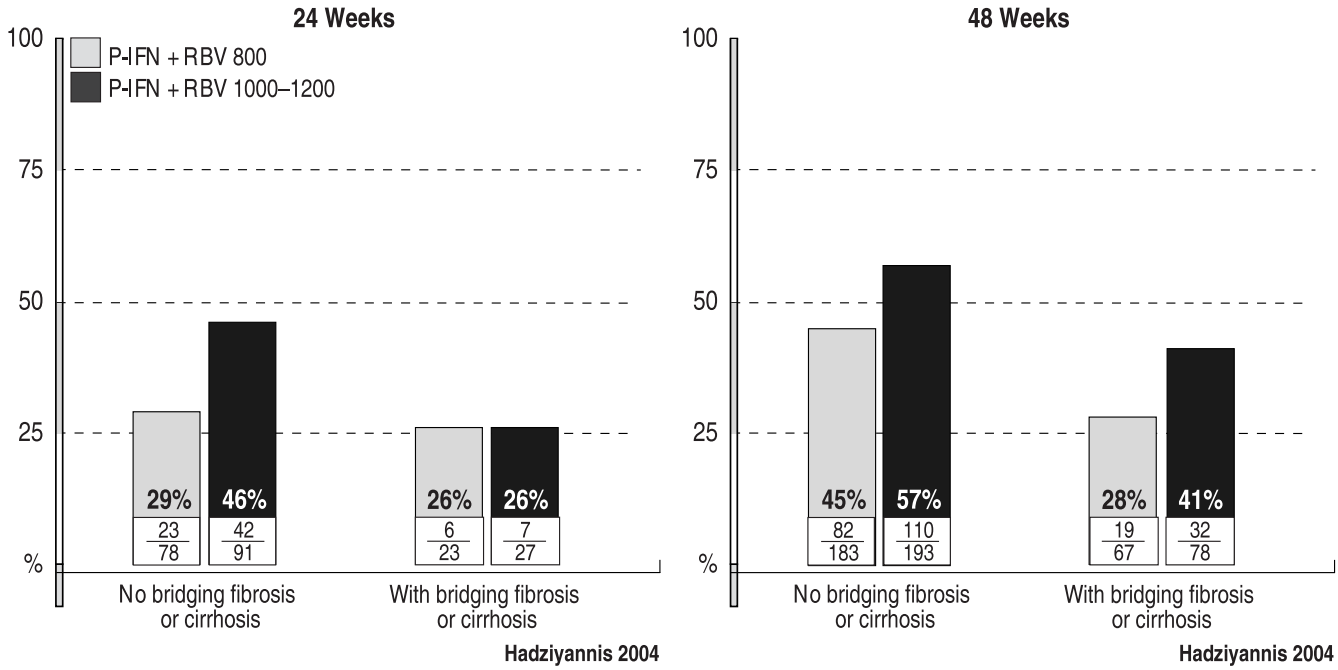
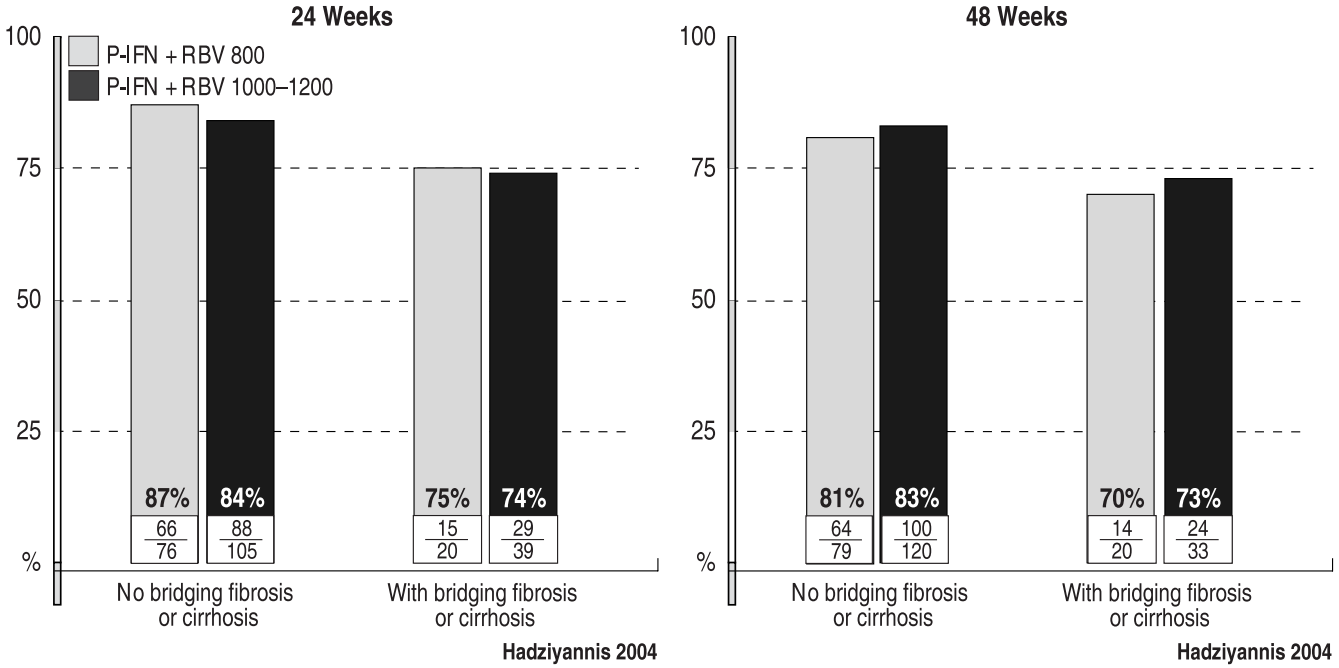


Figure 18. Sustained Virological Response Rate in Genotype 2 and 3 by Regimen, Treatment Duration, and Baseline Liver Histology



Here, HCV genotype remains a significant prognostic factor among individuals with bridging fibrosis or cirrhosis, although the sample size was small. Among those with genotype 2 or 3 and serious liver damage, neither duration of treatment nor dose of ribavirin had a significant impact on SVR. In genotype 1, the highest rate of SVR was achieved with both the greater duration of treatment and the higher of ribavirin. Unfortunately, no data on changes in liver histology were available.

Other interesting information emerged about treatment response and toxicities based on geographic region. There were 441 participants in the U.S. and 843 non-U.S. participants (from Australia, Belgium, Brazil, Canada, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Mexico, the Netherlands, New Zealand, Norway, Portugal, Spain, Sweden, Taiwan, and the UK). Participants in the U.S. experienced more treatment-related toxicities and achieved SVR less frequently than did non-U.S. participants (FDA 2002).

Table 8. Prognostic Factors in U.S. and Non-U.S. Participants

Characteristics	U.S. Participants	Non-U.S.
HCV genotype 1	61%	56%
> 44 years of age	52%	33%
Body weight > 85 kg	47%	22%
Cirrhosis	29%	23%

FDA 2002

The difference in response rates may be attributed in part to a greater proportion of poor prognostic factors among U.S. participants. However, HCV genotype 1 in the U. S. may be a different entity than non-U.S. genotype 1, since there can be substantial genetic diversity among strains within a genotype (see Chapter IV, Diagnostics). It is possible that genotype 1 infections in the U.S. may

respond differently to treatment than genotype 1 infections in other parts of the world.

This study did not resolve questions about optimal dosing of pegylated interferon. Initially, a fixed dose of pegylated interferon was given to all participants. Treatment-related hematologic abnormalities were managed by dose modification rather than through administration of hematopoietic growth factors. Overall, more than 30% of those receiving 48 weeks of therapy modified or omitted at least one dose of pegylated interferon.

Table 9. Pegylated Interferon and Ribavirin Dose Modifications by Study Arm

	24-week		48-week	
Dose Modification	**P-IFN + RBV $_{800}$**	**P-IFN + RBV $_{1000–1200}$**	**P-IFN + RBV $_{800}$**	**P-IFN + RBV $_{1000–1200}$**
RBV	19% (39/207)	27% (76/280)	28% (101/361)	38% (166/436)
P-IFN	30% (63/207)	26% (73/280)	33% (120/361)	36% (159/436)

Hadziyannis 2004

Adverse events reported by 20% of those who received at least one dose of study medications, from most to least common, included flulike symptoms, insomnia, irritability, hair loss, itching, depression and skin inflammation.

Table 10. Severe and Serious Adverse Events (SAEs)

	24-week		48-week	
Adverse Events	**P-IFN + RBV $_{800}$**	**P-IFN + RBV $_{1000–1200}$**	**P-IFN + RBV $_{800}$**	**P-IFN + RBV $_{1000–1200}$**
Overall severe events	22% (46/207)	23% (63/280)	32% (116/361)	32% (141/436)
Overall serious events	3% (7/207)	7% (19/280)	9% (33/361)	10% (44/436)
Treatment-related serious events	1% (3/307)	3% (8/280)	4% (15/361)	3% (14/436)
Deaths*	0	1 (<1%)	1 (<1%)	2 (<1%)

Hadziyannis 2004

* Two deaths were unrelated to therapy (both drug overdoses). Of the two therapy-related deaths, one was caused by septicemia associated with neutropenia, and one by suicide.

Table 11. Treatment Withdrawals By Study Arm

	24-week		48-week	
Treatment Withdrawl	**P-IFN + RBV $_{800}$**	**P-IFN + RBV $_{1000–1200}$**	**P-IFN + RBV $_{800}$**	**P-IFN + RBV $_{1000–1200}$**
For any reason	7% (14/207)	8% (22/280)	32% (117/361)	27% (117/436)
For AE & lab abnormality	5% (10/207)	5% (13/280)	16% (59/361)	15% (67/436)
For insufficient response	0 (<1%)	0 (<1%)	9% (31/361)	6% (24/436)

Hadziyannis 2004

Pegylated Interferon Monotherapy

Although combination therapy with ribavirin is considered to be the current standard of care for the treatment of chronic hepatitis C, ribavirin may be contraindicated for some individuals who still need to treat their HCV. Ribavirin has caused birth defects and death in exposed animal fetuses at doses as low as one-twentieth of those recommended for human beings. Consequently, it is contraindicated for men and women who are planning a pregnancy; pregnant women and their sexual partners; and breast-feeding women.

For those engaging in procreative sex, the use of two forms of contraception during treatment and for six months after completion of treatment is recommended. Ribavirin is contraindicated for persons with severe renal impairment (creatinine clearance of <50 mL/min); individuals with a history of significant or unstable cardiac disease; individuals with hemoglobinopathies (e.g., sickle-cell anemia, thalassemia major); autoimmune hepatitis; advanced hepatic decompensation (before or during treatment); and anyone allergic to ribavirin.

Little is known about the safety and efficacy of ribavirin in transplant recipients and individuals with HIV/HBV/HCV infection. More data are needed on safety and efficacy of ribavirin in persons under 18 years of age. Roche's pediatric safety and efficacy study of Pegasys® with and without Copegus® is expected to open in mid-2004; final data will not be available for several years. Schering has not performed pharmacokinetic evaluations of ribavirin in this population. Little is known about the response to ribavirin in geriatric individuals, as there have been so few study participants over 65 years of age, nor have there been pharmacokinetic evaluations of ribavirin in the elderly. Clearly, data concerning safety, efficacy and pharmacokinetics are needed in these populations.

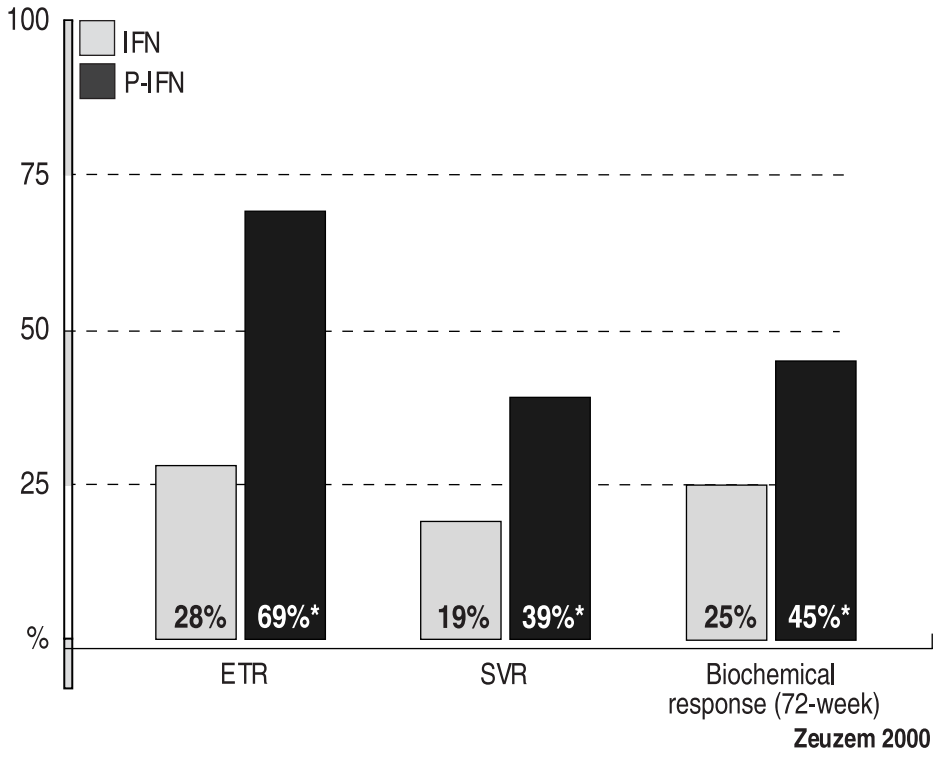
Results from two large randomized, controlled trials of treatment-naïve individuals established the efficacy of pegylated interferon monotherapy is superior to that of standard interferon monotherapy (Lindsay 2001; Zeuzem 2000). These studies provide a wealth of data on the dosing, efficacy, side effects and adverse events of pegylated interferon therapy.

The Zeuzem Data

Zeuzem and colleagues compared pegylated interferon monotherapy to standard interferon monotherapy in a study of 531 individuals randomized to receive either:

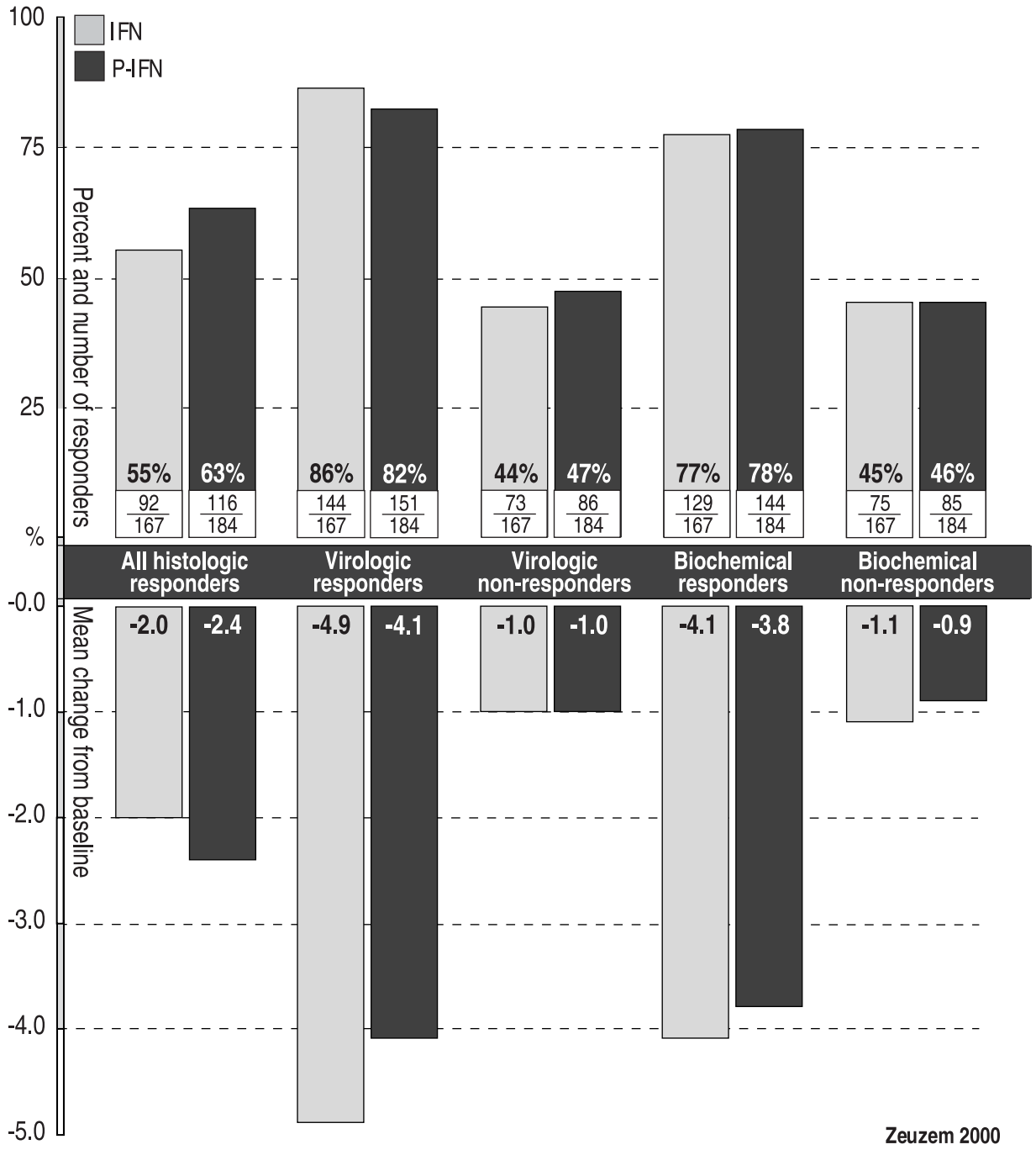
- Six million units of standard interferon alfa-2a thrice weekly for 12 weeks, continuing on a lower dose of three million units thrice weekly for 36 weeks (total course of therapy: 48 weeks); or
- Pegylated interferon alfa-2a (Pegasys ®) 180 μg once weekly for 48 weeks.

Figure 19. End-of-Treatment Response, Sustained Virological Response, and Biochemical Response by Treatment Regime



*P=0.001 for comparison of pegylated interferon vs. standard at 48 and 72 weeks, as well as for sustained biochemical response.

Figure 20. Histological Response at Week 72 from Paired Biopsy Specimens* by HAI.



*A subgroup of 351 (66%) participants had paired baseline and post-treatment liver biopsies (184 received pegylated interferon and 167 received standard interferon). Biopsies were evaluated with the Knodell Histological Activity Index (HAI).

Adverse events, dose reductions, and discontinuations were similar across arms. The adverse events reported in Table 12 occurred in at least 10% of study participants.

Table 12. Adverse Events by Treatment Regimen

Adverse Events	IFN	P-IFN
Headache	66% (173/261)	60% (166/265)
Fatigue	65% (170/261)	60% (160/265)
Pyrexia (fever)	52% (135/261)	37% (99/265)
Myalgia	43% (111/261)	42% (110/265)
Rigors	43% (112/261)	27% (72/265)
Alopecia	37% (96/261)	27% (72/265)
Nausea	35% (91/261)	21% (55/265)
Insomnia	24% (62/261)	18% (48/265)
Depression	23% (59/261)	16% (43/265)
Decreased appetite	21% (55/261)	20% (53/265)
Diarrhea	20% (53/261)	19% (51/265)
Dizziness	16% (42/261)	23% (60/265)
Upper abdominal pain	14% (37/261)	13% (35/265)
Vomiting	12% (32/261)	6% (16/265)
Pruritus	12% (32/261)	18% (49/265)
Impaired concentration	11% (29/261)	5% (14/265)
Cough	10% (25/261)	9% (25/265)
Nasopharyngitis (inflammation of nasal passages and pharynx)	8% (22/261)	11% (28/265)
Inflammation at injection site	7% (17/261)	10% (27/265)

Zeuzem 2000

Table 13. Dose Modifications and Discontinuations

	IFN	P-IFN
Discontinuations	10% (27/261)	7% (19/265)
Dose modifications*	18% (47/261)	19% (51/265)
Due to adverse events	11% (30/261)	8% (21/265)
Due to laboratory abnormalities	9% (24/261)	14% (37/265)

Zeuzem 2000

* Some individuals had adverse events and laboratory abnormalities.

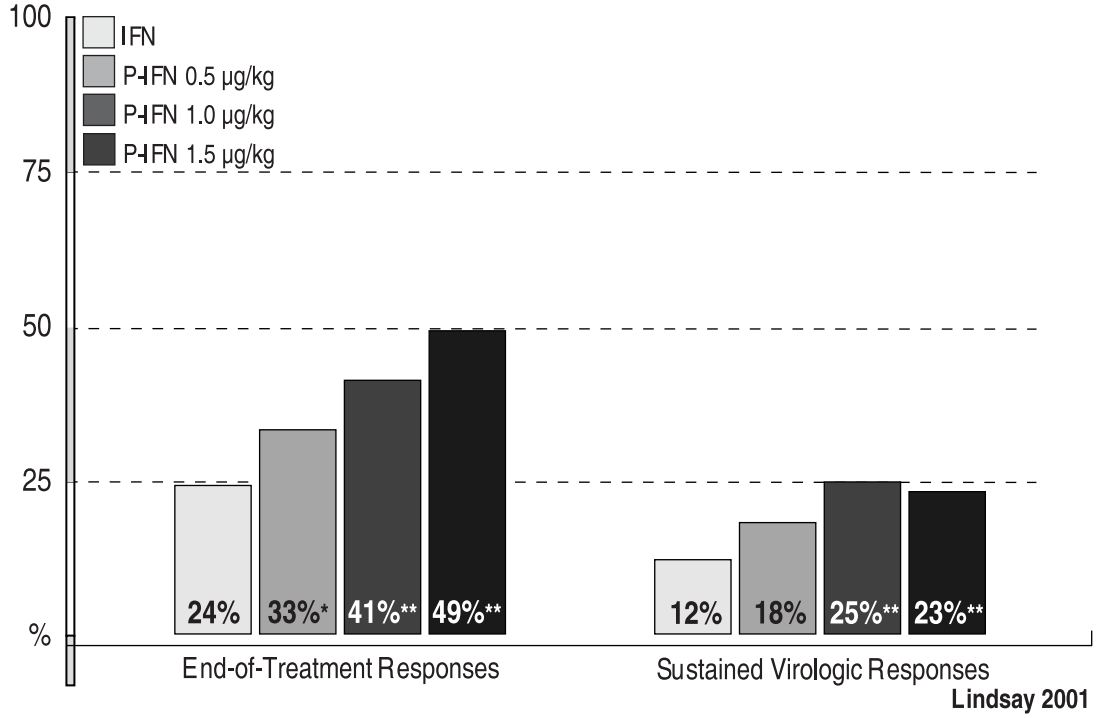
The Lindsay Data

In a study of 159 individuals with chronic hepatitis C, Lindsay and colleagues compared three different doses of pegylated interferon alfa-2b (Peg-Intron®) to a fixed dose of standard interferon. Participants were randomized to one of four arms:

- Pegylated interferon alfa-2b, 0.5 μg/kg once weekly;
- Pegylated interferon alfa-2b, 1.0 μg/kg once weekly;
- Pegylated interferon alfa-2b, 1.5 μg/kg once weekly; or
- Standard interferon alfa-2b, 3 MIU thrice weekly.

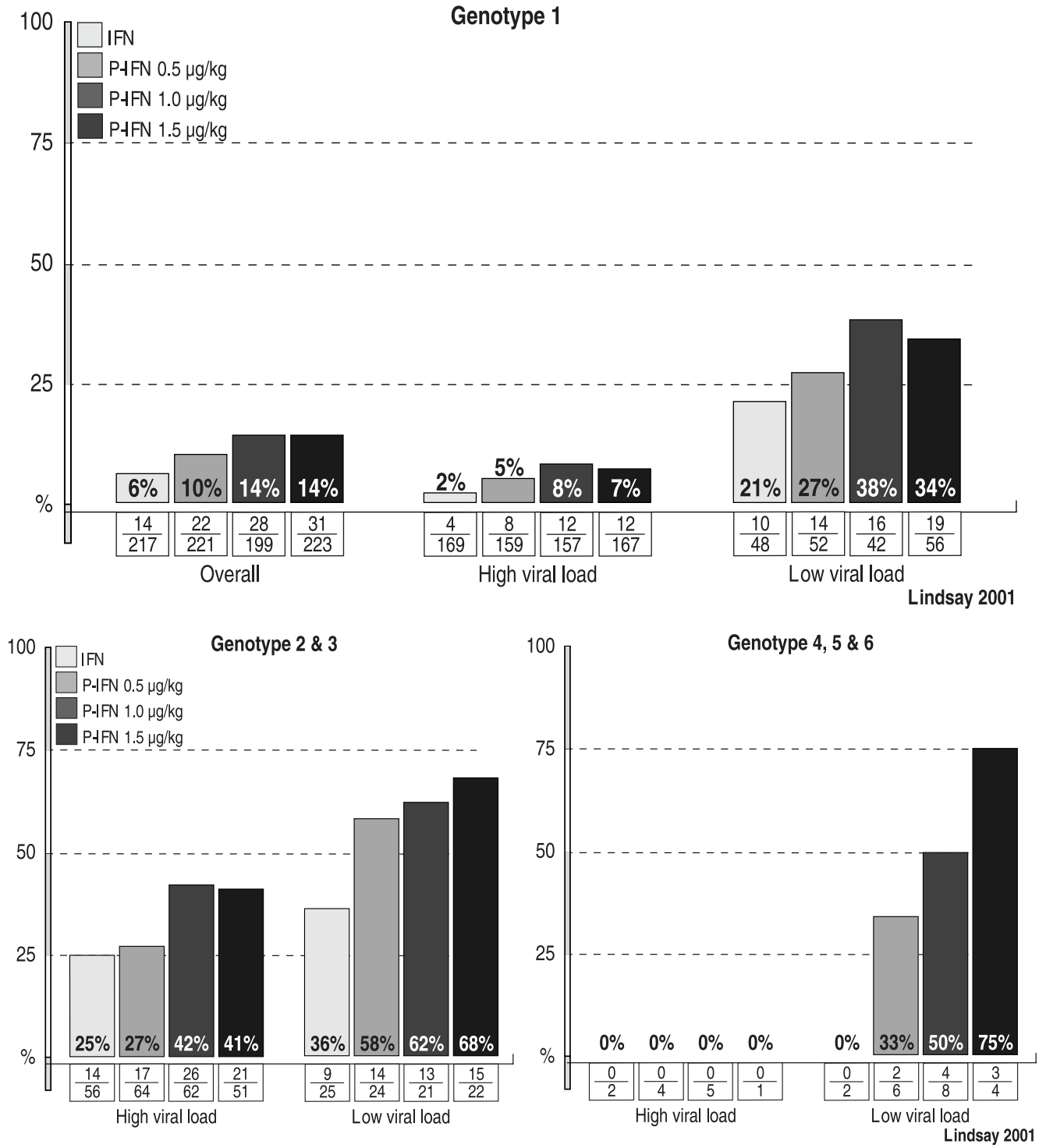
Virological responses at the end of treatment were significantly greater in those who received the two higher doses of pegylated interferon (1.0 and 1.5 μg/kg). Responses through week 48 were dose-dependent; by week 72, the discrepancy in response rates between the two higher doses disappeared, due to high rates of post-treatment relapse. The authors attributed this discrepancy to two factors: higher relapse rates for those with genotype 1 who were treated with 1.5 μg/kg vs.1.0 μg/kg (66% (57/87) and 46% (23/50) respectively; P=0.025); and a greater proportion of participants with genotype 1 in the 1.5 μg/kg arm (73% vs. 67% for the other two arms; P=0.09). Relapse rates among participants with genotypes 2 and 3 were similar (36% in the 1.0 μg/kg arm and 38% in the 1.5 μg/kg arm).

Figure 21. End-of-Treatment Response Rate and Sustained Virological Response Rate



*P=0.01 for P-IFN 0.5.
**P≤ 0.001 for P-IFN 1.0 and P-IFN 1.5 at ETR and for SVR.

Figure 22. Sustained Virological Response by Dose and Formulation of Interferon, Baseline HCV RNA and Genotype



Paired liver biopsy specimens were available from 61% (744/1219) of study participants. The proportion of individuals with a histological response to treatment, as well as the degree of improvement, was similar across all arms (47% to 50%; mean decreases of -1.2 to -1.8 by the Knodell Histologic Activity Index). Although histological improvement was more frequent among those with an SVR (77% to 90%), 33% to 46% of relapsers and 31% to 41% of non-responders achieved histological improvement.

Table 14. Histological Response by Knodell HAI Scoring System: Mean Changes from Baseline

Treatment Outcome	Histologic Responders	HAI (mean changes from baseline)
SVR	77% to 90%	-4.0 to -5.0
Relapsers	33% to 45%	-0.2 to -0.9
Non-responders	31% to 41 %	-0.3 to -0.7

Lindsay 2001

It is encouraging that a percentage of virological and biochemical relapsers and non-responders were able to achieve some degree of improvement in liver histology from pegylated interferon monotherapy, although longer follow-up is needed to assess the durability and clinical value of such improvements.

Although the virological response rates seen with pegylated interferons are an improvement over standard interferon, the side effects are still considerable.

Table 15. Adverse Events by Treatment Regimen

Adverse Events	IFN	P-IFN 0.5 µg/kg	P-IFN 1.0 µg/kg	P-IFN 1.5 µg/kg
Headache	19% (58/303)	19% (61/315)	21% (64/297)	21% (64/304)
Fatigue	14% (43/303)	16% (51/315)	15% (45/297)	16% (50/304)
Chills	11% (33/303)	11% (34/315)	13% (40/297)	14% (44/304)
Fever	10% (30/303)	11% (31/315)	15% (45/297)	14% (44/304)
Myalgia	17% (53/303)	15% (48/315)	18% (54/297)	20% (61/304)
Musculoskeletal pain	7% (22/303)	6% (19/315)	9% (28/297)	6% (20/304)
Nausea	6% (20/303)	7% (21/315)	9% (26/297)	8% (25/304)
Anorexia	5% (17/303)	3% (10/315)	7% (20/297)	8% (25/304)
Irritability	8% (24/303)	6% (19/315)	6% (18/297)	5% (17/304)
Insomnia	7% (23/303)	5% (17/315)	7% (23/297)	6% (20/304)
Alopecia	7% (22/303)	6% (20/315)	7% (22/297)	11% (34/304)
Injection site inflammation	5% (16/303)	14% (44/315)	14% (42/297)	13% (40/304)

Lindsay 2001

Leukocyte, neutrophil, and platelet counts decreased in all arms initially, stabilized after the first few weeks of treatment, and returned to baseline after treatment. Dose reductions for neutropenia occurred more frequently in the 1.5 µg/kg arm (5% vs. 2–3%).

Dose reductions for thrombocytopenia occurred more frequently in the pegylated interferon arms (2–3% vs. 0.3% for standard interferon). Dose reductions occurred most frequently with the two higher doses of pegylated interferon. Discontinuations were similar across the pegylated interferon arms (9–11%), and higher than those in the standard interferon arm (6%) (Lindsay 2001).

Table 16. Discontinuations and Dose Reductions by Dose and Formulation of IFN

	IFN	P-IFN 0.5 µg/kg	P-IFN 1.0 µg/kg	P-IFN 1.5 µg/kg
Discountinuations	1.9% (6/303)	2.8% (9/315)	3.7% (11/297)	2.9% (9/304)
Dose Reductions	1.9% (6/303)	2.8% (9/315)	4.7% (14/297)	6.2% (19/304)

Lindsay 2001

Unresolved Dosing Issues: The Formann Data

The rationale for recommending a different dose of pegylated interferon alfa-2b (Peg-Intron®) for monotherapy (1.0 µg/kg) than that for use in combination with ribavirin (1.5 µg/kg) is unclear. Formann and colleagues randomized 20 individuals to receive 1.0 µg/kg of Peg-Intron® either once or twice weekly for four weeks. Blood levels of Peg-Intron® were below the level of detection by day seven in all but one of the once-weekly dosing group. Those who were randomized to receive twice-weekly dosing had constantly detectable levels of drug. Throughout the four-week induction period, members of the once-weekly group had higher levels of HCV RNA. Viral loads appeared to increase as drug levels decreased. At day 28, 5/10 of the twice-weekly dosing group had undetectable HCV RNA vs. 3/10 of the once-weekly dosing group (Formann 2002). This has raised concern about the recommended dose of 1.0 µg/kg Peg-Intron® for monotherapy; it may be suboptimal.

The Reddy Data

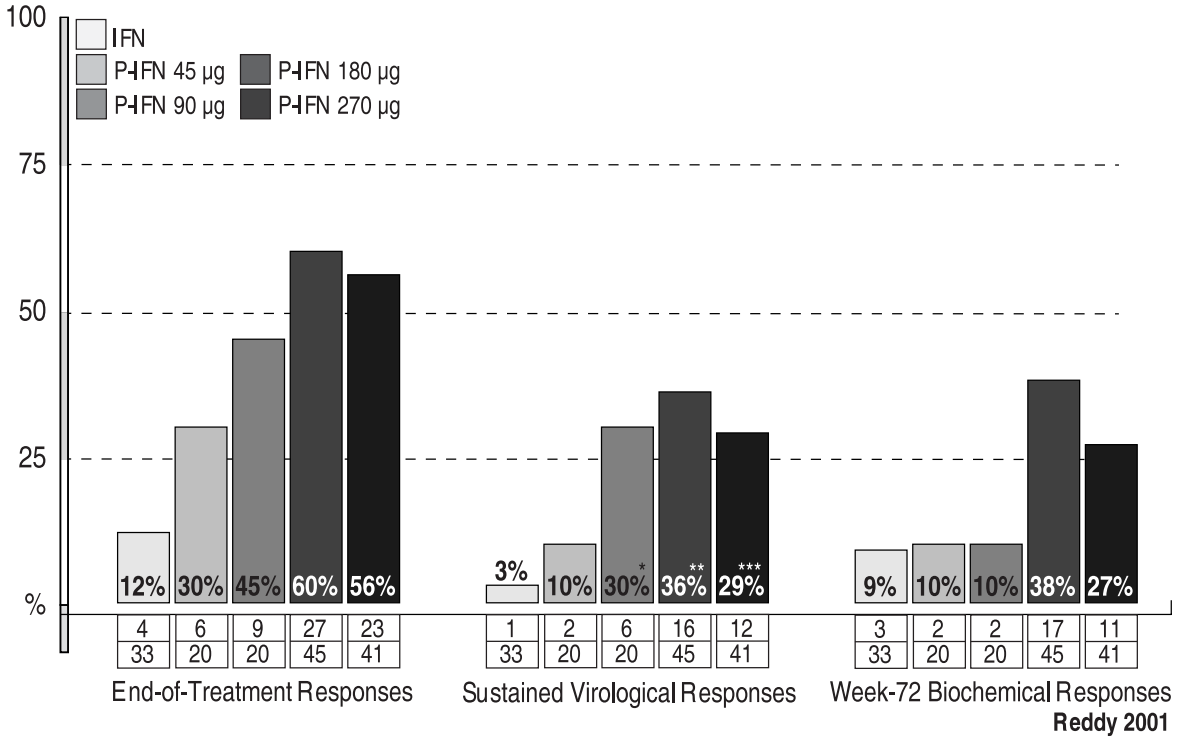
Pegylated interferon alfa-2a (Pegasys®) is not dosed by weight. Dosing for subsequent clinical trials of pegylated interferon alfa-2a was determined by Reddy and colleagues in a dose-ranging study completed by 122 of 159 original participants. The high discontinuation rates in the 180 and 270 µg arms (22% and 20%, respectively) may be attributable to the original protocol design, which initially did not allow dose modifications. Dose modification guidelines were not instituted until several months after the study opened (Reddy 2001).

Participants were randomized to receive either:

- Pegylated interferon alfa-2a, 45 µg once weekly, for 48 weeks;
- Pegylated interferon alfa-2a, 90 µg once weekly, for 48 weeks;
- Pegylated interferon alfa-2a, 180 µg once weekly, for 48 weeks;
- Pegylated interferon alfa-2a, 270 µg once weekly, for 48 weeks; or
- Standard interferon alfa-2a, 3 MIU thrice weekly, for 48 weeks.

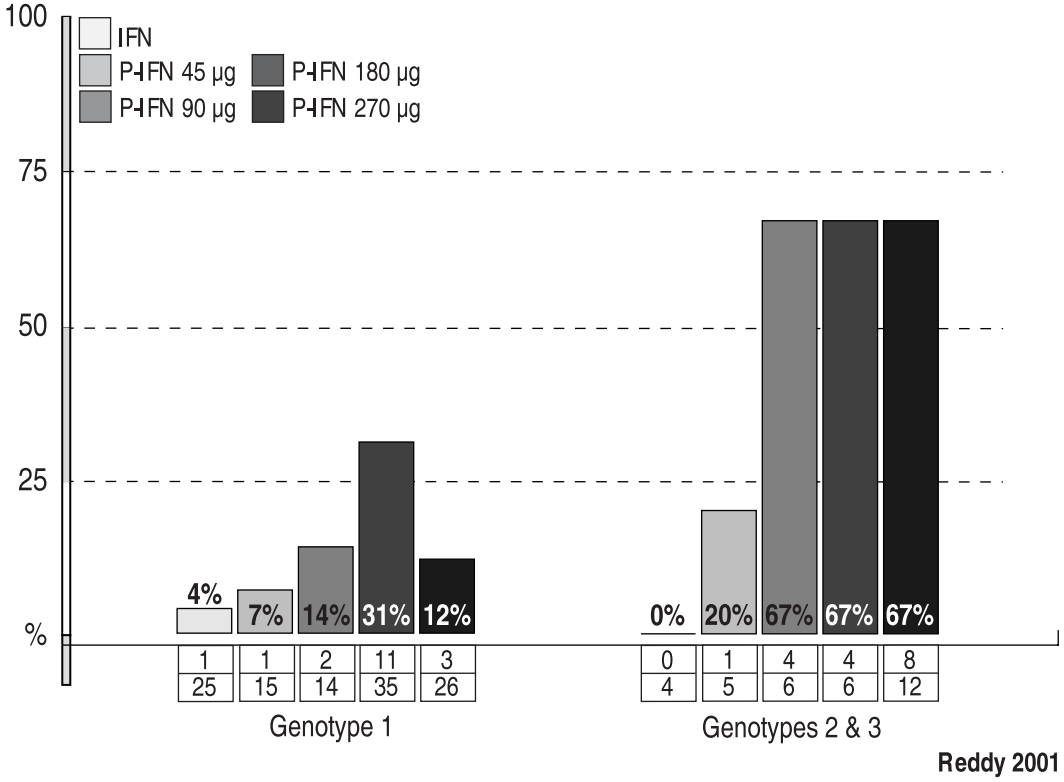
End-of-treatment and week-72 virological response rates were significantly greater in the pegylated interferon dosing arms of 90, 180, and 270 µg.

Figure 23. End of Treatment Response, Sustained Virological Response, and Week-72 Biochemical Response by Dose and Formulation of IFN



All P values are vs. IFN; *P=0.009 for P-IFN 90 µg vs. IFN; **P=0.006 P-IFN 180 µg vs. IFN; ***P=0.004 P-IFN 270 µg vs. IFN.

Figure 24. Sustained Virological Response by HCV Genotype and Dose and Formulation of IFN



The standard interferon arm included more individuals with HCV genotype-1 infections, as well as a higher mean HCV RNA and a slightly larger proportion of participants with bridging fibrosis, which may have contributed to its poorer response rate. In addition, the difference in SVR between the 90 μg arm and the 180 μg arm was relatively narrow. It is unclear whether this may be attributed to a larger proportion of individuals with favorable prognostic factors in the 90 μg arm (genotype non-1 infections, lower baseline HCV RNA levels, and fewer individuals with bridging fibrosis) or because the efficacy of the 90 μg and the 180 μg doses may be roughly equivalent. No further increase in efficacy was observed with the highest dose of pegylated interferon.

Overall, improvements in liver histology (based on paired biopsy samples from a proportion of participants) were similar across groups, but median improvements were greatest in the arm receiving pegylated interferon 180 μg (-3.0). Histological response rates among virological non-responders ranged from 42% to 60% in the pegylated interferon arms vs. 55% in the standard interferon arm.

Table 17. Adverse Events by Dose and Formulation of IFN*

Adverse Events	IFN	P-IFN 45 μg	P-IFN 90 μg	P-IFN 180 μg	P-IFN 270 μg
Fatigue	70% (21/30)	70% (14/20)	85% (17/20)	67% (30/45)	70% (28/40)
Headache	60% (18/30)	40% (8/20)	35% (7/20)	58% (26/45)	48% (19/40)
Myalgia	63% (19/30)	40% (8/20)	65% (13/20)	31% (14/45)	48% (19/40)
Rigors	47% (14/30)	5% (1/20)	20% (4/20)	47% (21/45)	50% (20/40)
Nausea	47% (14/30)	45% (9/20)	15% (3/20)	44% (20/45)	30% (12/40)
Depression	10% (3/30)	30% (6/20)	35% (7/20)	27% (12/45)	38% (15/40)
Diarrhea	20% (6/30)	25% (5/20)	25% (5/20)	31% (14/45)	33% (13/40)
Irritability	13% (4/30)	35% (7/20)	20% (4/20)	29% (13/45)	33% (13/40)
Injection site inflammation	20% (6/30)	35% (7/20)	30% (6/20)	24% (11/45)	25% (10/40)
Insomnia	23% (7/30)	25% (5/20)	5% (1/20)	33% (15/45)	30% (12/40)
Arthralgi	23% (7/30)	20% (4/20)	40% (8/20)	18% (8/45)	30% (12/40)
Pyrexia	30% (9/30)	15% (3/20)	10% (2/20)	24% (11/45)	28% (11/40)
Alopecia	20% (6/30)	5% (1/20)	30% (6/20)	22% (10/45)	25% (10/40)
Upper abdominal pain	17% (5/30)	30% (6/20)	10% (2/20)	18% (8/45)	28% (11/40)
Dizziness	23% (7/30)	10% (2/20)	20% (4/20)	13% (6/45)	18% (7/40)
Impaired concentration	7% (2/30)	10% (2/20)	20% (4/20)	7% (3/45)	30% (12/40)
Dermatitis (skin inflammation)	7% (2/30)	15% (3/20)	0% (0/20)	13% (6/45)	28% (11/40)
Pain	13% (4/30)	20% (4/20)	0% (0/20)	20% (9/45)	13% (5/40)
Decreased appetite	7% (2/30)	15% (3/20)	20% (4/20)	16% (7/45)	13% (5/40)
Back pain	17% (5/30)	0% (0/20)	15% (3/20)	16% (7/45)	15% (6/40)
Pain in limb	13% (4/30)	15% (3/20)	25% (5/20)	9% (4/45)	8% (3/40)
Vomiting	17% (5/30)	20% (4/20)	0% (0/20)	16% (7/45)	3% (1/40)
Pruritus	3% (1/30)	10% (2/20)	15% (3/20)	11% (5/45)	13% (5/40)

Reddy 2001

* The adverse events listed were seen in at least 10% of study participants.

Table 18. Discontinuations by Dose of and Formulation of IFN

	IFN	P-IFN 45 µg	P-IFN 90 µg	P-IFN 180 µg	P-IFN 180 µg
Discountinuations	9%	10%	0%	22%	22%

Reddy 2001

The highest-dose (270 µg) arm had a greater incidence of dose modification because of laboratory abnormalities or adverse events than did the 180 µg group (53% vs. 31%). It is unfortunate that the investigators did not consider using a dose of 135 µg. Offering this dose may have resulted in fewer adverse events, dose modifications, and treatment discontinuations while offering efficacy similar to that of the 180 µg dose. Data from a dose-ranging study comparing the efficacy of pegylated interferon alfa-2a 180 µg to 135 µg (using standard interferon as a comparator) found that both doses yielded an SVR of 28% (vs. 11% for standard interferon), although histological improvement occurred more frequently in the 180 µg arm (58% vs. 48%) (Pockros 2001).

Long-Term Benefits of Hepatitis C Treatment

Interferon can slow or halt HCV disease progression in some individuals, thus treatment may decrease liver-related mortality among people with hepatitis C. Imazeki and colleagues retrospectively analyzed data from 459 people with hepatitis C, 104 untreated, over an eight-year interval. They found an overall reduction in the risk of liver-related death among treated persons. Although the decrease was greater in sustained virological responders (RR, 0.030; 95% CI, 0.003–0.267; P=0.0017), it also decreased among virological non-responders (RR, 0.257; 95% CI, 0.108–0.609; P=0.020) (Imazeki 2003).

Although the duration of follow-up and baseline participant characteristics differ widely across studies of the long-term effects of interferon, the data suggest that among individuals who achieve an SVR or a biochemical response to treatment, fibrosis progression is slowed or arrested and, in some cases, pre-treatment liver damage can be reversed (Cammá 2004; Lau 1998; Marcellin 1997; Schvarcz 1999; Shiatori 2000; Shindo 2001; Yabuuchi 2000; Yoshida 2002). Lau and colleagues followed ten individuals for six to thirteen years after completion of HCV treatment (with varying regimens of interferon alfa-2b). Half of the group achieved SVR; the other five were non-responders. Liver biopsies were performed five to eleven years after therapy. All five of the sustained responders had no detectable HCV RNA in their serum or their liver at final follow-up. Biopsy samples from all five responders reflected improvements over baseline and end-of-treatment scores for fibrosis and inflammation; one individual had normal liver tissue and the other four had non-specific, mild inflammation without significant fibrosis. All five of the non-responders had detectable HCV RNA at final follow-up, and two had increased fibrosis scores. One non-responder developed hepatocellular carcinoma (HCC) five years after treatment and had a liver transplant, while another progressed to decompensated liver disease and died from an intracerebral bleed while awaiting transplantation (Lau 1998).

Marcellin and colleagues evaluated the long-term benefit of interferon among 80 individuals who achieved sustained virological and biochemical responses to interferon treatment. Follow-up ranged from 1.0 to 7.6 years. HCV RNA remained undetectable in 96%, and 93% maintained persistently normal liver enzyme levels. Before treatment, 60% experienced fatigue; after treatment,

none reported fatigue. Baseline liver biopsy samples were available from all 80 participants, and at least one post-treatment biopsy was performed on 69 individuals between one and six years after completion of treatment. Normal—or nearly normal—liver histology was observed after treatment in 62%, while 94% had an improvement in liver histology. No new cases of cirrhosis were diagnosed after treatment. Of the five individuals with pre-treatment cirrhosis, four had post-treatment biopsies. An improvement was seen in two; disease progression without liver decompensation or hepatocellular carcinoma was found in the other two (increases of one and two points on the Knodell HAI, respectively) (Marcellin 1997).

Although achieving a sustained virological response increases the likelihood of histological benefit, a sustained biochemical response to treatment appears to reduce the risk of HCV disease progression. Shindo and colleagues studied the pre- and post-treatment liver histology of 250 individuals treated with standard interferon and a control group of 89 untreated individuals. Follow-up ranged from 8 to 11 years post-treatment. The treated cohort was categorized by response to therapy as: complete responders (defined as sustained virological and sustained biochemical response), biochemical responders, relapsers, and non-responders. The annual incidence of cirrhosis was significantly lower in complete responders, biochemical responders, and relapsers than in an untreated control group (P=0.0001).

Table 19. Annual Incidence of Cirrhosis and HCC among Responders, Relapsers, Non-Responders, and Untreated Controls

Treatment Response	% of total	Annual Incidence of	
		Liver Cirrhosis	Hepatocellular Carcinoma
Complete responders	27% (67/250)	0%	0.37%
Biochemical responders	10% (26/250)	0%	0.50%
Relapsers	28% (70/250)	1%	0.80%
Non-responders	35% (87/250)	14.9%	5.5%*
Untreated controls	89	6.4%	1.2%

Shindo 2001

*P=0.0001 for complete responders, biochemical responders, relapsers, and controls vs. non-responders.

Complete responders had improvements in the grade (amount of disease activity) and stage (structural progression of disease) of liver histology at the end of treatment. Grading scores continued to decrease at one and two years after treatment, while staging scores decreased at one year after treatment and then stabilized. Biochemical responders had decreases in grading and staging scores by the end of treatment, but their scores did not change subsequently (Shindo 2001).

Fibrosis does not invariably improve after hepatitis C treatment. Shiratori and colleagues performed a retrospective cohort study, assessing post-treatment changes in fibrosis and inflammatory activity in biopsy samples from 487 interferon-treated individuals and 106 untreated controls. Liver biopsy was performed within six months of treatment initiation and 1–10 years (median, 3.7) after completion of treatment. Regression of fibrosis and improvement in histological activity occurred most frequently among those who achieved sustained virological responses, although some relapsers experienced histological improvement as well (Shiratori 2000).

Table 20. Post-Treatment Changes in Fibrosis among Sustained Responders and Relapsers, Plus Untreated Controls

Treatment Response	Fibrosis Activity		
	Regression	No Change	Progression
Sustained responders	59% (108/183)	40% (73/183)	1% (2/183)
Relapsers	19% (57/304)	57% (173/304)	24% (74/304)
Untreated controls*	5% (5/106)	57% (61/106)	38% (40/106)

Shiratori 2000

*Lower baseline ALT level, milder histologic activity and fibrosis than treated cohort

Table 21. Post-Treatment Changes in Disease Activity among Sustained Responders and Relapsers, Plus Untreated Controls

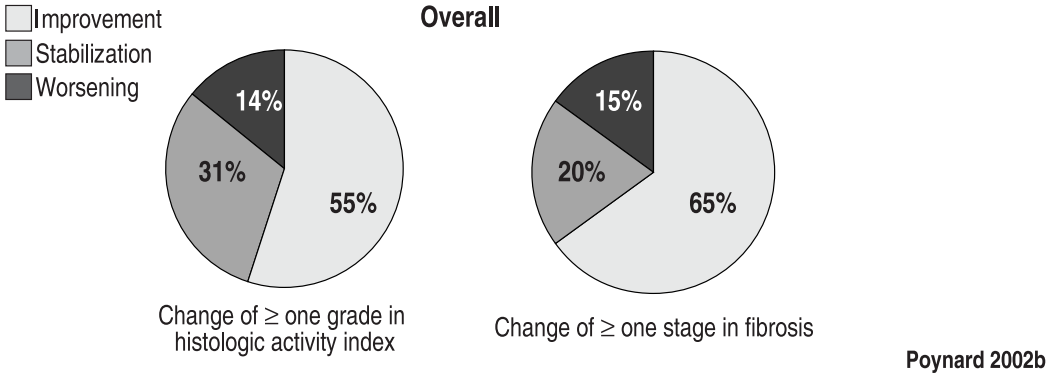
Treatment Response	Inflammatory Disease Activity		
	Decreased	Stable	Increased
Sustained responders	89% (162/183)	10% (19/183)	1% (2/183)
Relapsers	32% (98/304)	49% (148/304)	19% (58/304)
Untreated controls*	20% (21/106)	58% (62/106)	22% (23/106)

Shiratori 2000

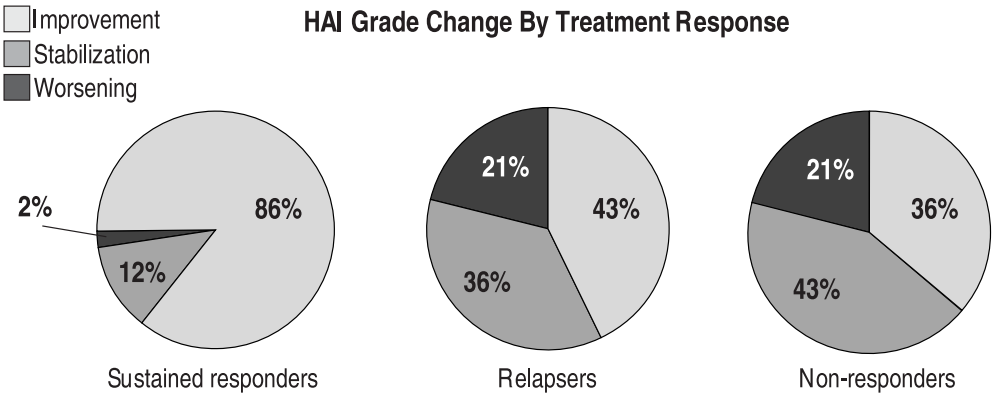
* Lower baseline ALT level, milder histological activity and fibrosis than treated cohort

Poynard and colleagues used a modeled estimate of fibrosis progression (see Chapter II, Natural History of Hepatitis C) to compare baseline and post-treatment changes in fibrosis progression and disease activity by treatment regimen. Data were pooled from four randomized treatment trials with 3,010 participants. They examined rates of pre- and post-treatment fibrosis progression, liver histology after therapy, and histological response by regimen. The mean duration between baseline and post-treatment biopsies was 20 months.

Figure 25. Changes in Grade of Histological Activity and Fibrosis Stage



Poynard 2002b



Poynard 2002b

Improvements in the histological activity grade occurred most frequently among those who received pegylated interferon alfa-2b 1.5 μg/kg plus high-dose ribavirin (73%), and least frequently (39%) among those who received 24 weeks of standard interferon alfa-2b. Worsening of fibrosis occurred least frequently in those who received pegylated interferon alfa-2b 1.5 μg/kg with high-dose ribavirin (8%), and occurred most frequently among those treated with 24 weeks of standard interferon monotherapy (23%).

Among cirrhotics, 49% (75/153) had improvement in fibrosis after treatment. All of them received 48 weeks of standard interferon alfa-2b, with or without ribavirin. Improvement from stage 4 to stage 3 was observed in 23 individuals; from stage 4 to stage 2 in 26; and from stage 4 to stage 1 in 23. Three individuals improved by four stages (to stage 0; no remaining fibrosis) (Poynard 2002b). Although this is a promising report, it is preliminary, and may be limited by the use of estimated fibrosis progression rates per year, since fibrosis progression is not always linear.

Cammà and colleagues performed a meta-analysis of data from three HCV treatment trials, to examine the effect of pegylated interferon alfa-2a on liver histology, using baseline and post-treatment biopsy samples from 1,013 people. All were treated for 48 weeks with pegylated interferon alfa-2a or standard interferon. Overall, 280 achieved SVR (215 from pegylated interferon vs. 65 from standard interferon; P=0.001). Reduction in fibrosis was most likely among those treated with pegylated interferon (P=0.04) and sustained virological responders (P<0.001). Sustained virological response was significantly associated with reductions in disease activity (P<0.00001). Relapse was associated with improvement in fibrosis (P=0.0007) and disease activity (P=0.004), while no significant changes were observed among virological non-responders (Cammà 2004).

Table 22. Changes in Fibrosis and Disease Activity After Treatment with Standard or Pegylated Interferon

Treatment Response	Inflammatory Disease Activity		
	Improved	Stabilized	Worsened
Fibrosis (Staging)	25.7% (261/1013)	63.6% (645/1013)	10.5% (107/1013)
Disease Activity (Grading)	48.4% (491/1013)	34.5% (350/1013)	16.9% (172/1013)

Cammà 2004

Longer-term follow-up will reveal the durability and clinical benefits of improvements in grading and staging of liver disease.

Treatment of Acute Hepatitis C Infection

It is difficult to identify cases of acute hepatitis C infection for many reasons. The incidence of new infections—especially from transfusions and blood products—has decreased and the majority of acute HCV infections are asymptomatic. Fewer than 25% of individuals with acute HCV infection seek medical attention. Clinicians may overlook HCV infection if it is not suspected (Villano 1999). In addition, there is not a specific diagnostic test to identify acute HCV infection (see Chapter IV, Diagnostics). Despite these obstacles, several studies have examined different doses and durations of interferon therapy in individuals with acute HCV. A meta-analysis of data from four randomized, controlled trials of acute, post-transfusion hepatitis C, all treated with interferon alfa-2b revealed a 29% increase in SVR among treated vs. untreated individuals (P=0.00007) (Alberti 2002). In three of these studies, the interferon dose was identical: three MIU of interferon alfa-2b, thrice weekly, for twelve weeks (Hwang 1994; Lampertico 1994; Viladomiu 1992). Two treatment regimens were used in the remaining study. Eight participants received 3 MIU daily for one week, which was followed with 3 MIU every other day for eleven weeks; another group of eight participants were given 3 MIU every other day for twelve weeks (Li 1993). The response rates from these studies are not much different from the response rates in chronic hepatitis, with almost two-thirds of the treated individuals developing chronic hepatitis.

More promising results came from two uncontrolled studies of interferon therapy during acute-phase hepatitis C in which 90% and 98% of participants achieved sustained virological responses. Vogel and colleagues treated 24 individuals with acute hepatitis C with 10 MIU of subcutaneous (SQ) interferon alfa-2b per day until liver enzyme levels reached normal levels (18–43 days). Pre-treatment ALT levels ranged from 531 to 1,940 IU/liter, with a mean of 1055; normal ALT was considered to be <22 IU/liter. Twenty-two of 24 participants completed treatment; 18 (90%) of these individuals remained virus-free during a follow-up interval of 18.65 ± 9.7 months (Vogel 1996). Jaeckel and colleagues treated 44 individuals with subcutaneous injections of 5 MIU of interferon alfa-2b per day for four weeks, followed with thrice weekly dosing for 20 more weeks. Treatment was initiated at an average of 89 days (with a range of 30–112) after infection. All but one individual completed treatment. One individual was re-treated (with interferon alfa-2b and ribavirin) 89 days after finishing study treatment. After six months of follow-up, 98% (43/44) had an undetectable HCV-RNA level (including the individual who discontinued treatment at week 12 and the individual undergoing re-treatment) (Jaeckel 2001).

It is important to note that both studies were uncontrolled. Without a randomized, untreated control group, it is not possible to determine how many study participants would have achieved spontaneous viral clearance without undergoing treatment. Other factors may have had contributed to the high rates of a viral clearance: homogeneity of study participants—many of whom were symptomatic and jaundiced (both of which have been associated with higher rates of spontaneous viral clearance)—and the mode of acquisition. In these two studies, all of the participants acquired their infections from occupational or nosocomial exposures, injection drug use, sexual contact, or sporadic (unknown) means. HCV infections acquired from transfusions appear to have a higher rate of chronicity than those acquired by other means (Alberti 2002). In addition, the follow-up periods may have been too brief, or RNA testing too infrequent to detect intermittent viremia. HCV RNA may have been present in levels below the threshold of detection; Jaeckel and colleagues used an assay with a lower limit of detection of 600 copies, the most sensitive

available at the time (Jaeckel 2001).

Gerlach and colleagues identified 60 individuals with acute hepatitis C over a seven-year interval. Each was offered HCV treatment at diagnosis; ten individuals either declined or were ineligible (due to active injection drug use or other medical conditions) and 24 others achieved spontaneous viral clearance before HCV treatment was initiated. Of the 26 who were treated, 21 (81%) achieved SVR. Risk factor and interval between diagnosis and initiation of treatment differed among individuals, as did the regimen and duration of therapy (Gerlach 2003).

Table 23. Acute HCV: Risk Factor, Interval from Diagnosis to Treatment, HCV Genotype, Regimen and Response

Risk Factor	Genotype	Months from Diagnosis	TX Regimen	TX Duration
Sustained Virologic Responders (N=21)				
IV drug use	1b	4.2	IFN 3 MU 3 x week + RBV	50 weeks
IV drug use	1b	26.2	IFN 5 MU/3 MU, 3 x week	52 weeks
IV drug use	1b	3.4	P-IFN 80µg 1 x week + RBV	14 weeks
IV drug use	2a	6.6	P-IFN 80µg 1 x week + RBV	23 weeks
IV drug use	3	7.6	IFN 5 MU 3 x week	34 weeks
IV drug use	3	6.5	IFN 5 MU 3 x week + RBV	50 weeks
IV drug use	Unknown	1.0	IFN 3 MU 3 x week + RBV	52 weeks
Sexual	1a	5.5	IFN 5 MU 3 x week + RBV	26 weeks
Sexual	1b	2.4	IFN 3 MU 3 x week	29 weeks
Medical procedure	1b	9.7	IFN 5 MU/3MU 3 x week	38 weeks
Medical procedure	3	12.9	P-IFN 100µg 1 x Week	46 weeks
Surgery	3b	1.9	P-IFN 80µg 1 x week + RBV	25 weeks
Dental surgery	1a	3.7	IFN 5 MU 3 x week + RBV	26 weeks
Unknown	1b	10	IFN 5 MU 3 x week + RBV	61 weeks
Unknown	1b	0.9	IFN 5 MU 3 x week	17 weeks
Unknown	2a	7.1	IFN 3 MU 3 x week + RBV	52 weeks
Unknown	3	1	IFN 3 MU 3 x week	35 weeks
Unknown	3	12.6	IFN 5 MU 3 x week	50 weeks
Needlestick	1a	5.9	IFN 5 MU 3 x week + RBV	53 weeks
Needlestick	1b	0	IFN 5 MU 3 x week + RBV	26 weeks
Needlestick	1b	0.3	IFN 5 MU 1 x day + RBV P-IFN 100µg 1 x week +RBV	51 weeks
Relapsers (N=2)				
Sexual	1a	8.6	IFN 3 MU 3 x week	37 weeks
Medical procedure	1a	0.4	P-IFN 80µg 1 x week	36 weeks
Non-responders (N=3)				
Surgery	1b	20.5	IFN 3 MU 3 x week	31 weeks
Surgery & transfusion	4	3.3	IFN 3 x 6 MU	26 weeks
Unknown	1a	11.6	IFN 3 MU 3 x week + RBV	23 weeks

Gerlach 2003

Amid the compelling evidence that treating acute hepatitis C infection is beneficial, questions remain about the dosing and duration of treatment, choice of therapeutic agent(s), and determination of the need for treatment. Optimal dose and duration of standard interferon therapy have yet to be identified, and pegylated interferons have not been adequately explored as treatments for acute HCV. The higher response rates to pegylated interferon in chronic hepatitis C suggest that they will be more effective against acute hepatitis C. Information about dosing from studies of standard interferon may not be applicable to pegylated interferon, because it is difficult to translate the dosage of standard interferon (in millions of international units) into doses of pegylated interferon (in micrograms). Preliminary information suggests that 1.0 μg/kg may be a suboptimal dose of pegylated interferon alfa-2b for treatment of acute HCV (Wiegand 2003). Data on interferon and ribavirin during acute HCV infection are scant.

Determining when to initiate treatment for acute hepatitis C is an important issue. Treatment may not be necessary for those who will achieve spontaneous viral clearance. Identifying these individuals before initiating treatment will spare them the side effects and expense of unnecessary treatment. Findings from a study of twelve individuals with acute HCV indicated a high rate of spontaneous viral clearance relatively soon after exposure and onset of symptoms. Eight individuals achieved spontaneous viral clearance by 74 ± 25.3 days after exposure and 34.7 ± 22.1 days after the onset of symptoms. HCV-RNA levels decreased rapidly in the individuals with viral clearance. In the remaining four individuals HCV-RNA levels stayed high or increased (Hofer 2003). Larghi and colleagues noted longer intervals of detectable HCV RNA among seven acutely infected individuals. Before achieving spontaneous viral clearance, these seven individuals had detectable HCV RNA for between four and thirteen months after infection (Larghi 2002). Larger studies are needed to determine when treatment of acute HCV should be initiated.

Treatment for Relapsers and Non-Responders

As HCV treatments become more effective, options for re-treatment of relapsers and non-responders have increased. The likelihood of achieving an SVR after re-treatment of HCV hinges in part upon the difference in efficacy of the first regimen and any subsequent regimen. Using the identical treatment regimen usually does not improve treatment outcomes; the re-treatment regimen should have superior efficacy to the initial regimen.

Prognostic factors—genotype, baseline HCV RNA—and ability to tolerate HCV treatment influence the likelihood of successful re-treatment. The type of response to the initial course of treatment may also contribute to the success of re-treatment. Relapsers (who become HCV RNA undetectable but do not remain aviremic after completion of treatment) are more likely to achieve an SVR after re-treatment than non-responders (Shiffman 2002a). There are two patterns of non-response to HCV therapy. A partial response indicates a decrease in HCV RNA of >2.0 log and persistently detectable HCV RNA during treatment; a flat response is characterized by a decrease of <2.0 log in HCV RNA while on treatment, which may be an indication of interferon resistance.

Strategies for effective re-treatment have included higher-dose interferon monotherapy, different types of interferon, a longer duration of therapy and re-treatment with a combination of interferon plus ribavirin or pegylated interferon plus ribavirin. Data from two meta-analyses of re-treatment for non-responders to interferon monotherapy report low overall SVR rates (ranging from 13% to

20%). Individuals were re-treated with inteferon plus ribavirin. Reponse rates depended on duration of re-treatment therapy and individual prognostic factors (Cheng 2001; Cummings 2001).

Pegylated Interferon in Relapsers and Non-Responders

End-of-treatment results are available from a study examining responses to re-treatment with 48 weeks of pegylated interferon alfa-2a plus ribavirin in 64 individuals who relapsed after 24 weeks of treatment with the same regimen. The maximum allowable dose for pegylated interferon was 180 μg once weekly; the maximum dose for ribavirin was 1,000–1,200 mg/day. Some individuals received lower doses of one or both drugs based on their experience with each drug during the previous regimen. End-of-treatment results are available from 59 participants who completed 48 weeks of treatment (Goncales 2002). Since all participants achieved undetectable HCV RNA after their initial course of treatment (with the exception of one person, who was withdrawn from this study) the efficacy of re-treatment with the same regimen for a longer interval can be determined only at week 72.

Table 24. HCV-RNA Levels at Week 48 of Re-treatment

	Undetectable	Detectable	Not Tested
Overall (N=59)	90% (53)	5% (3)	5% (3)
Genotype 1 (N=41)	88% (36)	7% (3)	5% (2)
Genotype 2 & 3 (N=14)	93% (13)	0%	7% (1)

Goncales 2002

HALT-C: Treatment in Non-Responders with Advanced Liver Disease

The Hepatitis C Antiviral Long-Term Treatment Trial Against Cirrhosis (HALT-C) is assessing tolerability and rate of SVR in individuals with advanced fibrosis or cirrhosis (Ishak score 3 by liver biopsy; see Chapter IV, Diagnostics) who were non-responders to prior therapy with standard interferon, with or without ribavirin. During the lead-in phase of HALT-C, all participants received 24 weeks of pegylated interferon alfa-2a 180 μg once weekly plus ribavirin (1,000–1,200 mg/day, based on weight). Individuals with detectable HCV RNA after 20 weeks of treatment were rolled into HALT-C. Those with undetectable HCV RNA at week 20 were treated for an additional 28 weeks, then followed for 24 more weeks.

So far, SVR data is available from 604/863 who have completed treatment and follow-up. Overall, 18% (109/604) achieved SVR. Prior interferon monotherapy, genotype 2 or 3, a lower AST:ALT ratio and no cirrhosis were associated with achievment of a sustained virological response. SVR was more likely among those who received ≥ 60% of the ribavirin dose (21% vs. 11%; P=0.05) (Shiffman 2004).

In a sub-group of 212 HALT-C participants who completed therapy by late 2002, the likelihood of SVR was significantly greater in non–African Americans, non-1 genotypes, those with a 2.0 log decrease in HCV RNA at week 12, and persons less than 50 years old (P<0.005 for all). More than half of these the participants had dose reductions of pegylated interferon and/or ribavirin.

Week 24 discontinuations for fatigue, depression, or hematologic abnormalities were reported in 5% (Shiffman 2002b).

Treatment for Compensated Cirrhotics

Cirrhotics may remain stable for several years. During this window, initiation of HCV treatment may delay progression to hepatic decompensation or hepatocellular carcinoma. In a retrospective follow-up of 384 compensated cirrhotics with hepatitis C, the five-year survival probability was 91%, decreasing to 79% at ten years, suggesting that this interval presents a valuable opportunity for HCV treatment (Fattovich 1997).

A retrospective analysis of data from 637 cirrhotics, treated and untreated, found that treatment with interferon—regardless of the outcome—seems to affect the oncogenic mechanisms of HCV. Interferon alfa is active against a number of cancers, including AIDS-associated Kaposi's sarcoma (KS). The International Interferon-a Hepatocellular Carcinoma Study Group identified predictors of progression from compensated cirrhosis to hepatocellular carcinoma (male sex, older age, and signs of portal hypertension) and time from diagnosis of cirrhosis to development of hepatocellular carcinoma. The study compared outcomes of two matched groups, one of 356 untreated cirrhotics and one of 281 cirrhotics treated with interferon. The median duration of therapy was 7 months (range: 3–30 months). Participants were followed for at least three years. The overall risk of progression to HCC was 1.99 for untreated individuals (95% CI, 1.09–3.6; P=0.027), with 66 untreated individuals and 29 treated individuals developing HCC during an interval of 36–250 months. Among cirrhotics with HCV infection, the relative risk of progression to hepatocellular carcinoma among untreated individuals was 3.14 times that of those treated with interferon (95% CI, 1.46–6.80; P=0.004). In a subgroup of cirrhotics who were HCV-antibody-positive and anti-HBV-negative, the risk of progression to hepatocellular carcinoma for untreated individuals was 6.28 times greater (95% CI, 1.65–23.97; P<0.007) (The International Interferon-α Hepatocellular Carcinoma Study Group 1998).

The effect of interferon on the clinical outcomes of 189 cirrhotics was retrospectively assessed by Benvegnù and colleagues during a mean follow-up of 71.5 ± 23.6 months; 7.9% of those who received treatment (88/189) and 21.8% of untreated individuals (101/189) had progressive liver disease (by Child's staging; see Chapter IV, Diagnostics). Hepatocellular carcinoma developed in 5.6% of treated persons vs. 26% of untreated individuals (P<0.001) (Benvegnù 1998).

Imazeki and colleagues retrospectively analyzed the effect of interferon on survival rates of people with hepatitis C. Of the 459 individuals in this study, 104 were untreated. Among cirrhotics, those who achieved SVR had a reduced rate of mortality during the eight-year follow-up. Hepatocellular carcinoma accounted for 25 deaths overall; only one was a sustained virological responder (Imazeki 2003).

There are particular safety concerns for cirrhotics; many are more vulnerable to side effects and adverse events, especially the hematologic toxicities of pegylated interferons. As a result, dose reductions may be more frequent, and the efficacy of treatment may be diminished. For example, there were dose reductions among 83% (44/53) of those participating in an ongoing study of the viral kinetics of pegylated interferon alfa-2a plus ribavirin in cirrhotics (Gane 2002).

Interim data from an HCV treatment trial in people with advanced liver disease (bridging fibrosis or cirrhosis) suggests that full-dose pegylated interferon and weight-based dosing of ribavirin, especially in non-1 genotypes, may increase the likelihood of sustained virological responses. Participants were randomized to receive 48 weeks of treatment with either full-dose (1.5 μg/kg once weekly) or half-dose (0.75 μg/kg once weekly) pegylated interferon alfa-2b, plus 800 mg/day of ribavirin. Sustained virological response data are available from 165 of 210 participants who have completed follow-up (Abergel 2003). No information on adverse events, dose reductions, or discontinuations was provided.

Table 25. Sustained Virological Response by Regimen and Genotype

	P-IFN 0.75 μg/kg		P-IFN 1.5 μg/kg	
	+ RBV > 10.6 mg/kg	+ RBV < 10.6 mg/kg	+ RBV > 10.6 mg/kg	+ RBV < 10.6 mg/kg
Genotypes 1, 4 & 5	17% (6/35)	15% (3/20)	29% (9/31)	26% (5/19)
Genotypes 2 & 3	85% (11/13)	57% (8/14)	91% (21/23)	60% (6/10)

Abergel 2003

A subset of individuals with bridging fibrosis and cirrhosis have participated in large HCV treatment trials. The treatment regimens and study populations differ, so it is difficult to draw conclusions from pooled data.

Table 26. Sustained Virological Response Rate Among Persons With Bridging Fibrosis and Cirrhosis: Subgroup Data From Four Trials

Study	Best SVR Rate	Regimen
Manns 2001	44% (60/136)	P-IFN alfa-2b 1.5mg/kg + RBV 800mg for 48 weeks
Fried 2002a	43% (25/56)	P-IFN alfa-2a 180μg + RBV 1000–1200mg for 48 weeks
Marcellin 2003	49% (38% for genotype 1;72% for non-1)	P-IFN alfa-2a 180μg + RBV 1000–1200mg for 48 weeks
Hadziyannis 2004	41% for genotype 1	P-IFN alfa-2a 180μg + RBV 1000–1200mg for 48 weeks
Hadziyannis 2004	75% for genotype 2 or 3	P-IFN alfa-2a 180μg + RBV 800mg for 24 weeks

The goals of therapy may be different for those with advanced liver disease. Averting liver transplantation, slowing disease progression, and improvement in liver histology may be relevant outcomes in the absence of achieving SVR, although histological response often correlates with virological response. Without long-term follow-up, it is impossible to know if histological improvement and/or viral eradication translate into increased quality of life and survival. Long-term studies of interferon maintenance therapy for non-responders with advanced liver disease are underway.

Treatment for Decompensated Cirrhotics

Individuals with decompensated cirrhosis need treatment urgently, as their five-year survival is 50% (Fattovich 1997). The safety of interferon and ribavirin in decompensated cirrhotics is a significant concern. Individuals with decompensated cirrhosis are at greater risk of life-threatening complications during therapy, such as deteriorating liver function, bone marrow suppression, and infections. Because of these concerns, individuals with decompensated cirrhosis have been excluded from pivotal clinical trials. Data on treatment of decompensated cirrhotics are very limited; there have been no randomized, controlled treatment trials in this population.

Everson and colleagues studied safety, efficacy, and tolerability of a gradually accelerated dosing regimen. This strategy resulted in SVR among 22% (20/91), with 40% (8/20) of those who achieved SVR remaining HCV-RNA-undetectable after liver transplantation. The regimen started with low doses of interferon (1.5 MIU thrice weekly), plus ribavirin (600 mg/day). Doses of each drug were gradually increased every two weeks, as tolerated. Growth factors were used to maintain blood cell counts when needed (Everson 2000). Information on changes in hepatic function, Child-Pugh scoring after treatment (see Chapter IV, Diagnostics), and serious adverse events was not available.

Crippin and colleagues conducted a pilot study of the safety, tolerability and efficacy of interferon with or without ribavirin in individuals with decompensated cirrhosis. Fifteen participants awaiting liver transplantation were randomized to:

- Interferon alfa-2b, 1 MIU/day;
- Interferon alfa-2b, 3 MIU/thrice weekly; or
- Interferon alfa-2b, 1 MIU/day, plus ribavirin 800 mg/day.

At the end of treatment, 33% had undetectable HCV RNA, and 55% had reduced viral loads. During the study, two individuals had liver transplants; both had recurrent hepatitis C. Adverse events were frequent and serious; 20 of the 23 adverse events were serious (severe thrombocytopenia and neutropenia, hepatic encephalopathy, and serious infections). One person died from infectious complications (Crippin 2003). The study was ended because of the frequency of severe adverse events.

Garcia-Retortillo and colleagues treated 30 individuals (13 cirrhotics and 17 with hepatocellular carcinoma) awaiting liver transplantation. Treatment was initiated when the anticipated interval before transplantation was less than five months and continued until transplantation. At the time of transplantation, 9/30 had undetectable HCV-RNA levels and 6/9 remained undetectable after transplantation (median follow-up of 26 weeks; range: 5–60 weeks).

The original regimen was standard interferon alfa-2b (3 MIU daily) plus ribavirin (400 mg every 12 hours). The dose of interferon was reduced in 60% (18/30); the ribavirin dose was reduced in 20% (6/30). Growth factors were given when necessary (G-CSF to 10/30; Epoetin-alfa to 8/30). Treatment was discontinued permanently by four individuals and temporarily by two. There were three serious adverse events: two cases of sepsis and one case of hepatitis. Leukopenia was reported in 18/30, thrombocytopenia in 13/30, and anemia in 5/30 (Garcia-Retortillo 2002b).

Treatment in Liver Transplant Recipients

In the United States, end-stage liver disease resulting from chronic hepatitis C infection is the leading indication for liver transplantation. As of June 30, 2003, 17,001 people were waiting for a liver (http://www.ustransplant.org/facts.html, accessed on 8 April 2004). After liver transplantation, hepatitis C infection of the graft occurs almost universally (Gretch 1995; Terrault 1995; Wright 1992; Zekry 2003). Viral replication begins within hours of liver transplantation (Garcia-Retortillo 2002a).

The overall survival rate at one year after liver transplantation is 85%; at three years, 75.9%; and at ten years, it decreases to 59% (C. M. Smith 2000; United Network for Organ Sharing, 2000). For transplant recipients with hepatitis C, survival rates at one year, three years, and five years are 86.4%, 77.8%, and 69.9%, respectively (Forman 2002). Progression of post-transplant hepatitis C disease varies (see Chapter III, Natural History of HCV in HIV Coinfection; HCV and Immunosuppression). Cirrhosis develops in 10–25% of transplant recipients with recurrent HCV within five years (Everson 2002). Individuals with early recurrence of HCV (less than six months after transplantation) are at greater risk of progression to bridging fibrosis or cirrhosis (Shuhart 1997; Testa 2000).

There are three strategies for treating recurrent hepatitis C: preemptive treatment prior to transplantation, initiating treatment as soon as possible after transplantation, or delaying treatment until post-transplantation hepatitis has recurred. The goals of preemptive treatment are to stabilize or improve hepatic function and reduce the likelihood of recurrent hepatitis C infection.

Data on preemptive treatment are scarce. A retrospective analysis of outcomes of 26 cirrhotic transplant candidates treated with interferon, with or without ribavirin, reported no recurrent HCV among 6/6 individuals who achieved SVR prior to transplantation, although adverse events were frequent and severe (Alvarez 2003). Preemptive treatment carries significant risks such as serious adverse events and potential acceleration of liver deterioration (see Treatment for Decompensated Cirrhosis section in this chapter), but some individuals may benefit. More research is needed before this approach becomes the standard of care.

The goal of early post-transplant therapy is to avert histological damage from recurrent HCV. Treatment is more effective in individuals with low viral loads. HCV-RNA levels are usually at their lowest immediately after transplantation, before rising to levels up to 20-fold higher than before transplantation (Feray 1994). Singh and colleagues found that early treatment with six months of interferon did delay recurrence of HCV. Recurrence occurred at a median of 408 days after transplantation in the treated group vs. a median of 193 days after transplantation in the untreated controls; P=0.05). Otherwise, no significant differences were observed, either in the frequency of recurrence or the severity of recurrent HCV disease (Singh 1998). Sheiner and colleagues randomized 86 transplant recipients to a regimen of interferon alfa-2b, 3 MIU thrice weekly, or to a control arm who did not receive interferon. Recurrent hepatitis C occurred less frequently in the interferon arm (8 vs. 22; P=0.017). HCV-RNA levels were categorized as low, moderate, or high. In the treated group, high HCV-RNA levels at one and three months were significantly associated with risk of recurrence (risk was 3.1 times greater at month one; P=0.01; at month three, risk was 3.9 times greater; P=0.006). There was no significant difference in actuarial survival between groups at one and two years (Sheiner 1998).

Early treatment with standard interferon plus ribavirin has shown more promising results. Beginning three weeks after transplantation, 36 individuals were given combination therapy for one year. At 36 months after completion of therapy, 33% (12/36) achieved sustained virological and biochemical responses. Progression to severe hepatitis occurred in 11% (4/30) of non-responders (Mazzaferro 2001). Dose reductions due to hemolytic anemia occurred frequently. Terrault and colleagues have treated 25/49 eligible transplant recipients for 48 weeks; 23 have completed treatment and follow-up. Treatment was initiated 1.7–9.3 weeks (median: 5.1 weeks) after transplantation. Participants were randomized to receive an induction/maintenance regimen of standard or pegylated interferon, with or without a gradually escalating dose of ribavirin (400 mg/day to 1.0–1.2 g/day, by body weight). Only 23% received full-dose ribavirin; 84% were able to tolerate full-dose interferon. Overall, only 3/23 achieved SVR; those with undetectable HCV RNA prior to treatment were more likely to achieve SVR (P=0.0009). After completion of treatment, most had mild liver disease; 78% had stage 0 fibrosis, and 72% had ≤ grade-1 disease activity, suggesting histological benefit in the absence of virological response. The discontinuation rate was high: five individuals left before starting treatment, and 19 discontinued due to adverse events. Five deaths occurred during the study, none treatment-related (Terrault 2003). Larger, randomized studies of safety, efficacy, and tolerability of combination therapy for this indication are needed.

The outcome of treatment for recurrent hepatitis C varies, depending on pre-transplant HCV-RNA levels, genotype, regimen, duration of treatment, and an individual's capacity for tolerating treatment. Adverse events requiring dose modifications are common, especially hemolytic anemia due to ribavirin (De Vera 2001; Kornberg 2001; Lavezzo 2002; Narayanan 2002; Samuel 2003). Ribavirin is eliminated by the kidneys. Levels of ribavirin tend to build up when renal function is impaired, and renal impairment is common in liver transplant recipients. Jain and colleagues examined the incidence of hemolysis and renal impairment among transplant recipients on combination therapy. Serum creatinine levels were higher (median of 1.3 mg/dL vs. 1.0 mg/dL), and clearance of creatinine was significantly lower (median 66.47 vs. 96; P=0.018), among those who experienced hemolysis (Jain 2002a).

Table 27. Treatment of Recurrent Hepatitis C: Outcomes/Dose Reductions/Discontinuations

Author	Regimen	Duration	N Participants	% SVR	Histological Outcomes	Dose Reduction	Discont-inuations
Samuel 2003a	IFN 3 MIU 3 x week + RBV 1,000–1,200 mg/day or control group	12 months	N=52 treated: 28 controls: 24	21% (6/28)	No significant improvement in liver histology	Not available	43%
Bizollon 2003	IFN + RBV for 24 weeks, then one year of RBV maintenance therapy	18 months	N=54	24% (13/54) 3 years post TX	For virological responders: improved in 12/14*; 5/14 had normal or nearly normal liver histology	Not available	Not available
Firpi 2002	IFN 3 MIU 3 x week + RBV 800–1,000 mg/day	12 months	N=54	30% (16/54)	For virological responders, no significant progression of liver fibrosis	72%	Not available
Lavezzo 2002	IFN 3 MIU 3 x week + RBV 800 mg/day	6 or 12 months	N=57 6 months: 27 12 months: 30	22% (6/27) 17% (5/30) 1 year post TX	In end-of-treatment responders, decreases of >2 points on the HAI score	51%	Not available
Narayanan 2002	IFN 3 MIU 3 x week + RBV 800–1,000 mg/day	12 months (or more)	N=26	23% (6/26) > 6 months post TX	Decreases of >2 points on the HAI score achieved by 75% of virological responders and 67% of non-responders	66%	50% after 1 year
Nelson 2002	IFN 3 MIU 3 x week + RBV 800–1,000 mg/day	12 months	N=54	30% (16/54) 6 months post TX	In those with SVR, no significant fibrosis progression within 6 months post TX	72%	Not available
Ahmad 2001 (Arm A)	IFN 3 MIU 3 x week for 1 month, then IFN 5 MIU 3 x week for 5 months	6 months	N=20	2.5% (1/20)	No improvement in inflammatory scoring; worsening fibrosis score	Not available	50% (10/20)
Ahmad 2001 (Arm B)	IFN 3 MIU 3 x week + RBV 1,200 mg/day for 1 month, then IFN 5 MIU 3 x week + RBV 1,200 mg/day for 5 months	6 months	N=20	20% (4/20)	No improvement in inflammatory scoring; worsening fibrosis score	Not available	40% (5/20)

* At the end of treatment, 14 of 54 participants (26%) had undetectable HCV RNA; all 14 were followed for a mean interval of three years after completion of treatment.

End-of-treatment results are available from a study of safety, efficacy, and tolerability of pegylated interferon monotherapy in transplant recipients. Vogel and colleagues randomized 65 transplant recipients with recurrent HCV to either 48 weeks of treatment with 180 μg/week of pegylated interferon alfa-2a (33) or no treatment (32). Participants were stratified by high (>1,000,000) or low (<1,000,000) HCV-RNA levels. Week 48 results were available from 49 participants (23 treated and 26 contols). A total of 16 individuals discontinued participation in this study; 10 from the treatment arm and 6 from the control arm.

Table 28. Hepatitis C RNA Levels During Treatment

HCV RNA Level	Week 4	Week 12	Week 24	Week 48
Undetectable	12% (4/33)	33% (11/33)	31% (10/32)	35% (8/23)
2.0-log drop	36% (12/33)	45% (15/33)	50% (16/32)	48% (11/23)

Vogel 2002

During the study, four rejection episodes occurred in the treatment arm; two of these individuals completed the trial. In the treatment arm, 45% (15/33) had at least one serious adverse event. In the control arm, 25% (8/32) had at least one serious adverse event. There were two deaths in the treatment arm (one from hepatic and renal failure and another from pulmonary metatases); neither were considered to be related to treatment (Vogel 2002).

Preliminary data from several ongoing studies of pegylated interferon alfa-2b plus ribavirin are available.

Table 29. Treatment of Recurrent HCV with Pegylated Interferon and Ribavirin

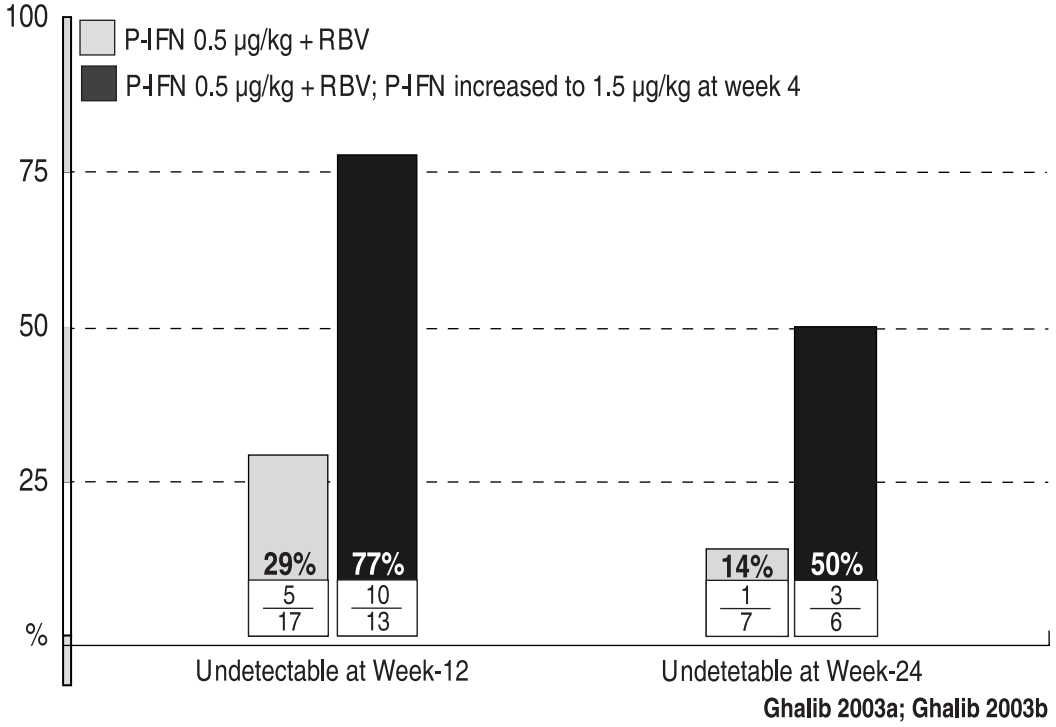
Author	Regimen	Duration	N Participants	% SVR or ETR	Histological Outcomes	Dose Reduction	Discontinuations
Lorenzini 2004	P-IFN alfa-2b 80 µg/week + RBV 600 mg/day	24 weeks; if HCV RNA undetectable, 24 additional weeks	N=18	SVR: 39%	Data not provided	RBV reduced in 10 & discontinued in 2 G-CSF used; no P-IFN dose modifications	3 cirrhotics had to discontinue due to hepatic decompensation
Dumortier 2003	P-IFN alfa-2b, escalated from 0.5 µg/kg to 1.0 µg/kg/week + RBV, escalated from 400 mg to 1,000–1,200 mg/day	12 months	N=20 Genotype 1=16	SVR: 45% (9/20)	Mean decrease of METAVIR score post TX: A 1.8 to A 0.3 F 2.2 to F 1.6	P-IFN: 37% (6/16) RBV: 81% (13/16)	20% (4/20)
Mukherjee 2003	P-IFN alfa-2b 1.5 µg/kg/week + RBV 800 mg/day + folic acid 1 mg/day	Genotype 1 & 4: 12 months Genotype 2 & 3: 6 months	N=39 Genotypes 1&4=34; Genotypes 2&3=5	SVR: 30% (12/39); data pending from 3 people	Fibrosis at ETR: improvement: 33% (6/18) stable: 56% (10/18) worsening: 11% (2/18)	Not Available	44% (17/39)
Lavezzo 2003	P-IFN alfa-2b 1.0 mg/kg/week + RBV 800 mg/day	6 or 12 months	N=16 (treatment-naive)	Not available	Not available; 3 with normal ALT levels at ETR	88% (14/16)	44% (7/16)
Neff 2003a	P-IFN alfa-2b 1.5 mg/kg/week + RBV 400–600 mg/day	Not available	N=32 (non-responders to IFN + RBV)	ETR:18% (6/32)	Not available; biochemical improvement seen in 50% (16/32)	P-IFN: 60% (19/32) RBV: 28% (9/32)	28% (9/32)
Neff 2003b	P-IFN alfa-2b 1.5 mg/kg/week + RBV 400–600 mg/day	At least 24 weeks	N=30 (treatment-naive)	ETR: 27% (8/30)	Not available	P-IFN: 40% (12/30) RBV: 43% (13/30)	None
U. P. Neumann 2003	P-IFN alfa-2b 1–1.5 µg/kg/week + RBV 400–800 mg/day	48 weeks	N=25	SVR: 36% (9/25)	No change in fibrosis or inflammation from baseline to week 72	Doses reduced for side effects neutropenia: 60% (15/25) fever, chills, headache & vertigo: 48% (12/25) anemia: 20% (5/25) psychiatric: 8% (2/25)	None
Samuel 2003b	P-IFN alfa-2b 1 µg/kg/week (mean dose) + RBV 7.5 mg/kg/day (mean dose)	Genotype 1: 12 months Genotype non-1: 6 months	N=22 Genotype 1=17 Genotype non-1=5 treatment-naïve=15 non-responders=6 relapser=1	SVR Overall: 18% (4/22) genotype 1: 13% (2/17) non-1: 40% (2/5) TX-naïve: 26% (4/17) prior TX: 0%	Normal ALT 50% (11/22) 6 months after completion of treatment	P-IFN: 27% (6/22) RBV: 45% (10/22)	63% (14/22) for intolerability: 27% (6/22)* for non-response: 36% (8/22)

* One death was reported; its cause and relationship to treatment were not described.

Serious adverse events occurred frequently across these studies, including severe depression, neutropenia, thrombocytopenia, anemia, acute pancreatitis (N=1; relationship to study treatment not described), organ rejection (N=3; relationship to study treatment not described), jaundice, and severe flulike symptoms. Neff and colleagues reported using multiple therapeutic interventions in one study, where 10% were given blood transfusions, 20% received erythropoietin, 43% were given neupogen, and 43% received treatment for clinical depression (Neff 2003b). Each investigator concluded that efficacy and tolerability were poorer in transplant recipients. Initiation of treatment with lower doses, and gradual dose escalation are current strategies for increasing the efficacy and tolerability of treatment for recurrent hepatitis C in transplant recipients.

Higher doses of pegylated interferon appear to be more effective, based on interim reports of week-12 and week-24 virological responses from 30 transplant recipients treated with two different doses of pegylated interferon alfa-2b (0.5 μg/kg or 1.5 μg/kg) plus 600 mg/day of ribavirin, increased to 800 mg/day at week 4. Growth factors were used to decrease dose reductions, although 18% (3/17) in the high-dose arm and 21% (4/19) in the low-dose arm had reductions of their pegylated interferon doses. Both arms had reductions in ribavirin doses (41% and 37%). No differences in toxicity by pegylated interferon dose have been reported (Ghalib 2003a; Ghalib 2003b).

Figure 26. Week-12 and Week-24 Virological Response by Treatment Arm



Ghalib 2003a; Ghalib 2003b

Hepatitis C Treatment in Kidney Transplant Recipients

Hepatitis C infection is common among individuals with end-stage renal disease (ESRD) on hemodialysis;. Estimates of prevalence in the United States range from 6% to 38% (Zacks 2001). Overall, survival in HCV-positive individuals with ESRD improves with kidney transplantation vs. maintenance with hemodialysis (Fabrizi 2002; Knoll 1997; Siren 2002). Although rapidly progressive hepatitis C appears less frequently in kidney transplantation, long-term follow-up indicates that HCV infection does have an adverse effect on survival, with the risk of graft rejection increasing at five years after transplantation (Fabrizi 2002; Siren 2002). Mathurin and colleagues compared survival at ten years after transplantation between three groups (HBV-infected, HCV-infected and matched, uninfected controls), finding that HCV infection significantly decreased survival (65 ± 5% vs. 80 ± 3% for controls; $P<0.001$). Graft survival at ten years after transplantation was also significantly lower in those with HCV infection (49 ± 5% vs. 63 ± 3% for controls; $P<0.0001$) (Mathurin 1999).

Interferon treatment for HCV in kidney transplant recipients has resulted in episodes of graft rejection (Kakimoto 1994; Rostaing 1996; Takahara 1995). Due to the risk of acute renal failure during treatment, and the frequency of relapse after completion of treatment for HCV, treatment of hepatitis C after kidney transplantation is contraindicated (Pol 2002).

Interferon Monotherapy: Efficacy in Dialysis Recipients before and after Kidney Transplantation

Although promising data on safety and efficacy of interferon monotherapy in dialysis recipients have emerged, tolerability remains a significant consideration. Degos and colleagues planned a multicenter, prospective trial to assess the tolerance and efficacy of interferon in 120 dialysis recipients with HCV. The initial dose of interferon was 3 MIU thrice weekly, with reduction to 1.5 MIU thrice weekly in case of side effects; planned duration of treatment was 48 weeks. By the time 37 individuals had been enrolled in the study, it was prematurely terminated due to the frequency of severe adverse events and discontinuations. Treatment was stopped in 19/37 and life-threatening side effects were recorded in 12 individuals. Dose reductions were necessary in 21 individuals by week 24; only 18 reached the 48th week of treatment. Of these 18, 38% (7/18) achieved SVR (Degos 2001).

Izopet and colleagues treated 23 dialysis recipients with 3 MIU of interferon thrice weekly for either 6 (N=12) or 12 months (N=11). Sustained viral clearance was achieved by 42% (5/12) of those treated for 6 months and 64% (7/11) of those who received 12 months of treatment (Izopet 1997).

Another prospective, controlled study evaluated the outcome of kidney transplantation in 30 individuals with HCV infection awaiting transplantation. A year of interferon monotherapy (3 MIU thrice weekly) was given to 15 individuals; another 15 were untreated. Kidney transplantation was performed in 11/15 who received HCV treatment, and in 10/15 controls. A year after transplantation, HCV RNA was undetectable in 4/11 treated individuals, and liver biopsy was performed on all transplant recipients. Those who had received HCV treatment had significantly lower mean HAI scores than untreated controls (1.82 ± 0.6 vs. 5.5 ± 1.35; $P<0.0001$) (Huraib 2001). In another study of efficacy and tolerance of interferon monotherapy in 19 HCV-infected individuals with renal impairment, treatment was discontinued in 9/19. Of those who completed treatment, 7/10 achieved SVR. Renal transplantation was performed in 10 individuals; 3 of them had undetectable HCV RNA at the time of transplantation, and 2 of the 3 remained virus-free 24 months after transplantation (Campistol 1999).

Combination Therapy

Safety, efficacy, and tolerability of combination therapy for HCV in individuals with kidney dysfunction are significant concerns. Clearance of interferon and ribavirin decreases with renal impairment (Bruchfeld 2002; Pol 2000; Rostaing 1998). Results from a pilot study of combination therapy using lower doses of ribavirin (200–400 mg/day), careful monitoring of hemoglobin levels, and the use of growth factors for anemia as needed, indicate that HCV infection in dialysis recipients may be treated with combination therapy. Further study is necessary (Bruchfeld 2001).

Pegylated Interferon

The safety and efficacy of pegylated interferon in individuals with renal impairment is being assessed in ongoing studies (Fabrizi 2002).

Management of Side Effects and Adverse Events

The side effects of interferon (whether standard or pegylated) and ribavirin are considerable (see adverse events tables from Fried 2002; Lindsay 2001; Reddy, 2001; Zeuzem 2001). The most common side effects of interferon are flulike: fever, headache, chills, muscle aches, and fatigue. Scheduling interferon injection before bedtime (or on Friday nights for once-weekly pegylated interferon) may help to decrease side effects. Muscle aches, headaches, and fever can be treated with acetaminophen or other nonsteroidal anti-inflammatory drugs prior to injection of interferon. Weight loss is common; eating several small light meals daily or larger meals when possible may help. Nausea and anorexia can be managed with antiemetics or marinol. Adequate hydration and light exercise for 30 minutes, at least three times per week, may alleviate fatigue and headaches. Insomnia can be treated with medication if necessary. A thorough knowledge of potential side effects by clinicians and those undergoing treatment is crucial to preparing for treatment of hepatitis C infection.

Some treatment-related adverse events may be life-threatening. A range of severe adverse events have been recorded and are categorized below, with current strategies for their management.

Neuropsychiatric Side Effects

Neuropsychiatric adverse events, such as depression, anxiety, irritability, and insomnia, have been associated with interferon. Depression, irritability, and insomnia were reported by 30–40% of participants in the Peg-Intron®/Rebetol® trial; overall, psychiatric adverse events occurred among 77% of trial participants (Schering package insert, 2001). In studies of Pegasys®, 33–38% of participants reported anxiety, nervousness, or irritability (Roche package insert, 2002). These adverse events are a significant concern, because depression can be a symptom of untreated hepatitis C (see Chapter II, Natural History of Hepatitis C), and many of the high-prevalence populations (such as injection drug users, HIV-positive individuals, and veterans) have a high prevalence of depression and other psychiatric disorders. Careful monitoring during treatment is important.

The most serious neuropsychiatric adverse events are severe depression, suicidal ideation, and suicide attempts. Suicidal behavior (attempts and suicide) has occurred in <1% (Roche) to 2% (Schering) of trial participants (Roche package insert, 2002; Schering package insert, 2001). Treatment with interferon must be discontinued if an episode of severe depression with suicidal ideation occurs. Suicides and attempted suicide have been reported during interferon therapy in individuals with no prior history of mental illness (Fattovich; Janssen 1994; Schering package insert, 2001). One case report of a 50-year-old woman with no significant psychiatric history provides a harrowing illustration of interferon-induced depression. During treatment with interferon, she developed irritability, anxiety, insomnia, and depression; she poured lamp oil on herself and set herself on fire. Fortunately, she survived (Fukunishi 1998). Severe interferon-related depression

may not always disappear after treatment discontinuation. In one study, prevalence of suicide attempts among 306 individuals during and after interferon therapy increased from 0% (during therapy) to 1.3% in the six months after therapy (Rifflet 1998).

Clinical trials have used different instruments to assess depression, or have relied upon self-reporting of depression, which may not reflect the true incidence of depression among those on treatment (estimated at 20–30%) (Fried 2002b). It may be difficult for people on HCV treatment and their clinicians to distinguish clinical depression from other common side effects from interferon (such as insomnia and fatigue). Although there is no specifically validated instrument to assess interferon-related depression, different instruments have been used to measure interferon-induced depression. The Montgomery-Asberg Depression Rating Scale (MADRS) was used in a small (N=33) prospective evaluation of the incidence of, and predictive factors for, depression prior to starting interferon. Participants with a high baseline MADRS (≥ 3) had more intense depressive symptoms than those with low baseline scores (<3) (Castéra 2002). Another group used the Minnesota Multiphasic Personality Inventory (MMPI) at baseline and three months after initiation of interferon to identify individuals at risk for depression. Three months after initiation of interferon, 64% (9/14) of those with a baseline score of ≥ 60/100 developed a depressive mood, and 11% (5/44) with baseline scores <60/100 showed medium-level depression after three months on treatment (Scalori 2000).

Sanchez and colleagues used the Beck Depression Inventory (BDI) to predict and identify HCV-treatment-related depression in a study of 76 individuals from three HCV treatment trials. Depression increased significantly during HCV treatment, regardless of the regimen used (P≤ 0.001). The severity of treatment-induced depression correlated with the baseline BDI score (P≤ 0.001). Individuals with severe depression had a greater incidence of early withdrawal from treatment trials than those with no, or mild-to-moderate depression (34% vs. 11% and 15% respectively). Those with severe depression who continued treatment had lower rates of week-12 viral response than did those with mild-to-moderate depression (34% vs. 62%) (N. Sanchez 2002).

When mild-to-moderate interferon-induced depression is identified, it can often be managed, thus improving quality of life and, possibly, treatment adherence and outcomes. Because they appear to be safe and easily tolerated in individuals with liver disease, selective serotonin reuptake inhibitor (SSRI) antidepressants are often used to treat interferon-induced depression (Gleason 2002; Hauser 2002: Krauss 2002; Schramm 2000).

Some individuals and their clinicians may choose to start preemptive treatment for depression before the initiation of interferon. Schafer and colleagues evaluated the effect of pre-treatment with citalopram (an SSRI) among 25 methadone recipients with psychiatric histories. Episodes of major depression were significantly less frequent during four months of HCV treatment in the citalopram group than in those who were not pre-treated (14% vs. 64%; P=0.028) (Schafer 2003b).

Careful assessment of baseline depression and an ongoing screening process during therapy should be a routine part of HCV treatment. Exploration of the pathophysiology of interferon-induced depression is needed, so that better interventions may be developed.

Although uncommon, other severe, interferon-induced neuropsychiatric adverse events have been reported, including acute psychosis, confusion and coma, memory loss, neuropathy, panic attacks

and personality changes, and seizures in persons with and without a history of such disorders (Ahmed 2003a; Anton 2000; Fried 2002b; Hosoda 2000; Kanno 1999; Schafer 2000; Shakil 1996).

Sensory Adverse Events

Interferon-related adverse events may affect hearing and vision. Tinnitus (ringing or roaring noises) and hearing loss are rare, usually reversible side effects of interferon. Ocular pathologies, such as blocked blood supply to the retina, retinal hemorrhage, and cotton wool spots are known side effects of standard interferon (Fried 2002b; Kadayifclar 1999; Norcia 1999). The effect appears to be dose-dependent, with reported incidences across different studies ranging from 18% to 85% (Hayasaka 1998). In some instances, interferon may need to be discontinued to prevent permanent damage.

Pegylated interferon-based therapy has been linked with serious ophthalmologic side effects. Ahmed and colleagues reported serious ophthalmic adverse events in 20/4800 people who received at least one dose of pegylated interferon alfa-2b and fixed or weight-based dosing of ribavirin. Ophthalmic damage was diagnosed in 16; 1 needed surgery for a detached retina. Treatment was discontinued in 17/20; 3 had persistent symptoms after treatment discontinuation (Ahmed 2003b).

Assessment of ocular problems at baseline, and regular monitoring, including color vision testing, are recommended.

Autoimmune Disorders and Adverse Events

Extrahepatic manifestations of untreated hepatitis C may appear as immunologic disorders (see Chapter II, Natural History of Hepatitis C). Autoimmune disorders may be induced or worsened during interferon therapy. Interferon therapy may be contraindicated for individuals with preexisting autoimmune hepatitis or thyroid disease, depending in part on the severity of the condition, because it can exacerbate these disorders (Dumoulin 1999; Heller 1996). Interferon has been associated with rare instances of celiac disease (damage to the intestinal mucosa caused by an immune response), inflammatory bowel disease, autoimmune hepatitis, induction of autoantibodies (antibodies that attack parts of the tissues in a person's own body), psoriasis, sarcoidosis (chronically inflamed tissue; formation of nodules in the lymph nodes, bones and skin), myasthenia gravis (progressive muscle weakness), type 1 diabetes mellitus, thrombocytopenia purpura (platelet destruction), and lupus-like syndrome (Bell 1999; Fattovich 1996; Fried 2002b; Leveque 2001; Nawras 2002; Neglia 2001; Papo 2002; Tada 1996; C. Taylor 2000; Wolfer 1996; Zuffa 1996).

Cardiac Adverse Events

A range of rare cardiac adverse events from interferon and ribavirin has been reported, from arrhythmias to acute congestive heart failure (Fried 2002b). Interferon and ribavirin are contraindicated for individuals with a history of significant or unstable cardiac disease. During treatment with interferon and ribavirin, close monitoring of individuals with a history of cardiovascular disease (heart attack, arrhythmia) is recommended.

Hematologic Toxicities

Interferons are known bone marrow suppressants, causing significant decreases in white blood cell counts, hemoglobin, and platelet counts (Peck-Radosavljevic 2002; Wong 1996). The adverse events induced by pegylated interferons are similar to those from standard interferons, with the exception of an increased frequency of hematologic toxicities (neutropenia, a decrease in white blood cells called neutrophils, which resolves after discontinuation or completion of therapy; and thrombocytopenia, a decrease in platelets). Ribavirin is associated with reversible hemolytic anemia and it may exacerbate interferon-induced neutropenia (Bodenheimer 1997; De Franceschi 2000; Dusheiko 1996; Schering package insert, 2001). In rare instances, interferon induces aplastic anemia (Schering package insert, 2001; Roche package insert, 2002).

Data from chemotherapy recipients have been used to evaluate the risk of infection from interferon-induced neutropenia. Low neutrophil counts have been a criterion for exclusion in many HCV treatment trials, and used as triggers for dose reductions of interferon, both in clinical trials and clinical practice. Individuals with chronic hepatitis C, however, may not be at the same risk for infections as immunosuppressed chemotherapy recipients, with the possible exceptions of cirrhotics and coinfected persons. A retrospective analysis of data from 119 persons treated for HCV with interferon and ribavirin found that no bacterial infections occurred in neutropenic individuals during treatment (Soza 2002). Additionally, Blacks have significantly lower neutrophil counts than Whites (Freedman 1997; Reed 1991; Zezulka 1987). Soza and colleagues have estimated that there may be 76,000 black Americans with HCV and constitutional neutropenia. Using a universal neutrophil cutoff for hepatitis C treatment trials may result in the exclusion of many black volunteers. Research to identify an appropriate neutrophil threshold for Blacks, and a safe threshold for triggering dose reductions is needed.

Dose reduction is the standard of care for interferon-induced neutropenia, yet suboptimal dosing of interferon may impair treatment outcomes. Maintaining doses of at least 80% of both drugs, for at least 80% of the course of therapy, increases the likelihood of achieving an SVR (McHutchison 2002). Hematopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF) can be used to boost neutrophil counts. Clinical trials of G-CSF as a therapy for hepatitis C, either alone or with interferon, have demonstrated safety, but not antiviral efficacy (Carreno 1996; Carreno 2000; Schiffman 1998). It is not current clinical practice to use G-CSF for neutrophil rescue therapy during hepatitis C treatment. More information is needed to determine if G-CSF rescue therapy is a viable option.

Thrombocytopenia (low platelets) may be a manifestation of untreated hepatitis C itself, especially among cirrhotics and those with advanced liver damage, or may be induced by interferon therapy (Giannini 2002; Pockros 2002; Ramos-Casals 2003). Interferon has been used as a treatment for the thrombocytopenia caused by HCV, and platelet counts have increased after therapy (Benci 2003; Rajan 2001). If platelet counts drop markedly during therapy with pegylated interferon, dosing may need to be modified or treatment discontinued, as there is growing concern among clinicians about spontaneous intracranial bleeding in patients with fewer than 50,000 platelets/mm^3.

Hemolytic anemia (destruction of red blood cells) is a common, usually reversible side effect of

ribavirin. Dose reduction is one strategy used for management of ribavirin-induced anemia, but suboptimal dosing may result. Epoetin-alfa, a genetically engineered version of erythropoietin (EPO), a human protein that stimulates production of red blood cells, has been used to maintain or restore full doses of ribavirin, resulting in significantly higher hemoglobin levels (Dieterich 2001; Dieterich 2002; Gergely 2002; Senkbeil 2003; Wasserman 2000; Weisz 1998). When ribavirin is used with pegylated interferon, the recovery period from treatment-induced anemia may be longer than that observed with ribavirin and standard interferon; use of epoetin-alfa may contribute to recovery (Azzam 2003).

Menstrual Irregularities

Menstrual irregularities—amenorrhea or prolonged menstruation—have been reported in female cynomolgus monkeys given pegylated interferon alfa-2a every other day for one month, at a dose approximately 180 times that recommended for a 60-kg person. Menstrual irregularities were accompanied by a decrease and a delay in the peak levels of two hormones, 17ß estradiol and progresterone. When the dose was lowered to approximately 30 times the weekly recommended dose, no effect on the duration of menstruation or on estradiol and progesterone levels was observed. After treatment was discontinued, normal menstrual rhythm returned. According to the label, pegylated interferon alfa-2a may impair fertility (Roche package insert 2002).

Care During Treatment

Preliminary data from a randomized, controlled study suggest that a multidisciplinary approach to providing care during hepatitis C treatment decreases the number of discontinuations and increases quality of life during the first three months of therapy. One of two models, standard of care (involving routine supportive care from a gastroenterologist or hepatologist) or an active intervention (involving patient education, regular, scheduled telephone consultations with experienced nurses, aggressive side effects management, and behavioral therapy) were provided to 67 individuals from 9 different medical practices during the first 12 weeks of HCV treatment. Both groups were monitored for frequency of adverse events, and a week-12 assessment of quality of life was performed. At week 12, 4/39 (10%) of the standard of care group had discontinued treatment vs. 1/38 (3%) of the active intervention group. Members of the active intervention group reported higher quality of life scores in all domains, with the exception of bodily pain and mental health (Flamm 2002).

Hepatitis C Treatment in Other Understudied Populations

People with Hemophilia

People with hemophilia have been excluded from pivotal treatment trials, mainly because of the risk of biopsy on people with coagulation disorders. Data on interferon with and without ribavirin in hemophiliacs come from small studies. Information about safety and efficacy of pegylated interferon, with and without ribavirin, is not yet available in this population.

Table 30. HCV Treatment Outcomes for Hemophiliacs

Author	Regimen	Duration	N Participants	% SVR	Biochemical Improvement	Discontinuations
Hanley 1996	IFN alfa-2a 3 MIU, 3 x week	24 weeks	N=31	24% (7/31)	28% (8/31) sustained normal ALT	Not available
Rumi 1997	IFN alfa-2b 3MIU, 3 x week	12 months*	N=101 Treated: 50 Controls: 51	13% (6/45) SVR + sustained normal ALT	13% (6/45) SVR + sustained normal ALT	Treated: 5/50 Controls: 1/51
Shields 2000	IFN alfa-2b 3MIU, 3 x week + RBV 1–1.2 g/day	48 weeks	N=28	71% (20/28) 16-month mediam follow-up	Not available	11/28 (3 of 4 who discontinued TX early achieved SVR)
Sauleda 2000	IFN + RBV	12 months	N=20	35% (7/20)	Not available	0%
Fried 2002c	3 Arms (see below)	48 weeks	Total N=113 Adolescents: 37 Adults: 76	Adolescents: 59% Adults: 15%	Not available	14% (16/113)
	IFN alfa-2b 3 MIU, 3 x week + RBV 1,000 mg/day	48 weeks	N=56	29% (16/56)		
	IFN monotherapy	48 weeks	N=14	7% (4/57)		
	IFN monotherapy for first 12 weeks, then IFN + RBV for 36 weeks	48 weeks	N=43			
Franchini 2002	IFN alfa-2b 5 MIU, 3 x week + RBV 1–1.2 g/day	12 months	N=33 (IFN non-responders)	33% (11/33)	42% (14/33) normal ALT levels at the end of TX	12% (4/31)

*Biochemical non-responders or those with persistently detectable HCV RNA discontinued treatment at week 24.

Children

In the United States, between 68,000 and 102,000 children are chronically infected with hepatitis C (Jonas 2002). Hepatitis C appears to run a more benign course in children than it does in adults (Guido 1998; Kage 1997; Vogt 1999); however, hepatitis C is not invariably less severe in children. Badizadegan and colleagues examined liver biopsy specimens from 40 children (age range: 2–18.6), finding significant fibrosis in 58% (23/40) and cirrhosis in 8% (3/40). In this study, the average duration of infection was 6.8 years (Badizadegan 1998). Fibrosis progression may be a function of duration of infection and aging, as the severity of liver damage increases with adolescence and young adulthood (Jara 2003); therefore, hepatitis C treatment during childhood may present an opportunity to avert progressive disease.

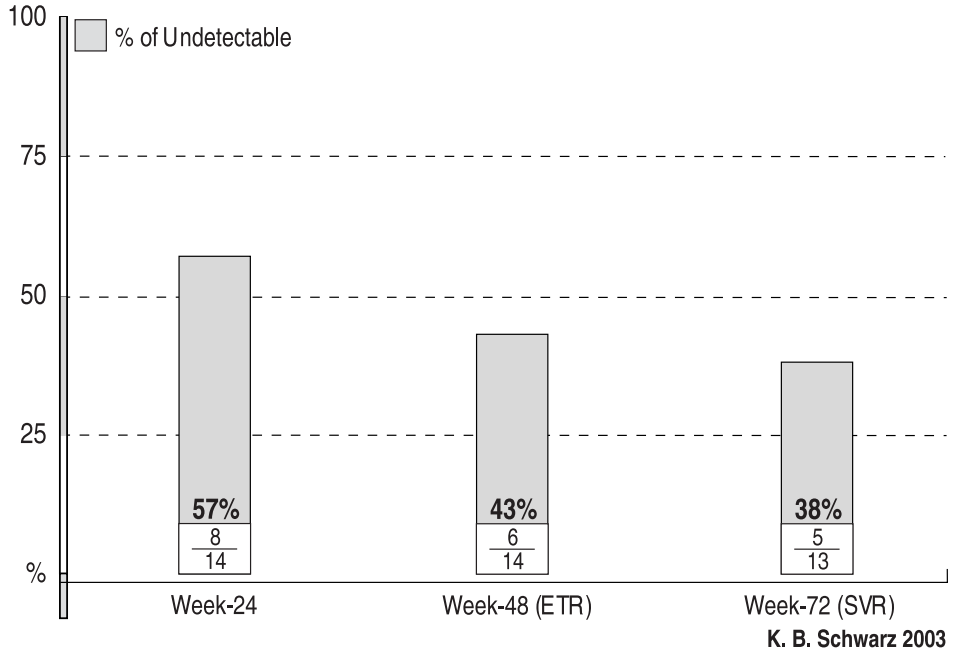
There have not been any randomized, controlled trials of the safety, efficacy, and tolerability of HCV treatment in children, although data from several small studies of interferon monotherapy

have yielded SVR rates ranging from 33% to 50% (Bortolotti 1995; Jonas 1998; Marcellini 1997; Nakashima 2003; A. Sawada 1998; Yuce 2000). Wirth and colleagues conducted an uncontrolled pilot study of efficacy and tolerability of 3 or 5 MIU of interferon thrice weekly plus ribavirin (15 mg/kg per day) in 41 children. Those with detectable HCV RNA after six months of treatment discontinued. When HCV RNA was undetectable at week 24, treatment continued for another 24 weeks. One child discontinued treatment because of severe anemia, and 12 discontinued due to week-24 non-response. Of the 25 children who completed treatment without virological breakthrough, all achieved SVR; the overall SVR rate was 61% (25/41). The rate of SVR in children with genotype 1 was 53% (18/34); all children with non-1 genotypes achieved SVR (Wirth 2002). Two other studies of interferon plus ribavirin in children have yielded sustained virological response rates of 41–45% (Kelly 2001; Süoglu 2002).

Wirth and colleagues reported that children were better able to tolerate combination therapy than adults, although all of them experienced flulike symptoms during the first weeks of treatment. Thyroid autoantibodies developed in 21% (6/28) after six months of treatment, and 11% (3/28) had markedly increased levels of thyroid-stimulating hormone. Dry skin and hair loss developed in three children. All side effects resolved after treatment was ended (Wirth 2002).

Encouraging data on safety and efficacy of pegylated interferon alfa-2a monotherapy in children have been reported from a multicenter, open-label study. The 14 participants were given 180 μg/kg once weekly for 48 weeks.

Figure 27. Virological Response to Pegylated Interferon Monotherapy at Weeks 24, 48, and 72



No serious adverse events were reported; the adverse events most frequently reported—fever, headache, vomiting, appetite loss, and abdominal pain—were described as "mild in intensity." There were four withdrawals from this study: one for week-24 virological non-response, two from elevations in liver enzymes, and one from an exacerbation of preexisting hypertriglyceridemia (K. B. Schwarz 2003).

Wirth and colleagues have reported interim results from an open-label pilot study of a 12-month course of pegylated interferon alfa-2b (1.5 μg/kg) plus ribavirin (15 mg/kg per day) in 52 children and adolescents (mean age: 11.3 years; range: 2–17). Treatment was discontinued at week 24 if HCV RNA was detectable. Of the 46 participants with six month follow-up data, 61% (28/46) achieved SVR. All participants with non-1 genotypes achieved SVR; 52.6% (20/38) with genotype 1 achieved SVR. The remaining 39% (18/46) were primary non-responders, relapsers, and one treatment discontinuation for side effects. No serious adverse events were reported; treatment was characterized as "well tolerated" (Wirth 2003).

More study of pegylated interferon, with and without ribavirin, is needed in pediatric populations.

HCV Treatment and Care for Individuals with Psychiatric Disorders, Active Drug and/or Alcohol Users, and the Dually Diagnosed

> *Treatment of HCV infections should not be withheld from patient populations with complicated social problems.*
>
> —G. Robaeys
> *Acta Gastroenterology*

Revised Indications for Active Drug Use

Until 2002, active drug use was regarded as a contraindication for treatment with interferon. Thankfully, the NIH Consensus Panel revised its guidance in the 2002 Statement, which reads:

> Many patients with chronic hepatitis C have been ineligible for trials because of injection drug use, significant alcohol use, age, and a number of co-morbid medical and neuropsychiatric conditions. Efforts should be made to increase the availability of the best current treatments to these patients. Recent, albeit limited, experience has demonstrated the feasibility and effectiveness of treating chronic hepatitis C in people who use illicit injection drugs, known as injection drug users (IDUs). This is potentially important because injection drug use is the most common risk factor for new HCV infections in the United States, and successful treatment may reduce transmission. Management of HCV-infected IDUs is enhanced by linking these patients to drug treatment programs. Treatment for drug and alcohol abuse should be made available to all patients who want and need it. Access to methadone treatment programs should be fostered for HCV-infected IDUs whether or not they are receiving treatment for HCV. Methadone treatment has been shown to reduce risky behaviors that can spread HCV infection, and it is not a contraindication to HCV treatment. Efforts should be made to promote collaboration between experts in HCV and healthcare providers specializing in substance-abuse treatment. HCV therapy has been successful even when the patients have not abstained from continued drug or alcohol use or are on daily methadone. However, few data are available on HCV treatment in active IDUs who are not in drug treatment programs. Thus, it is recommended that treatment of active injection drug use be considered on a case-by-case basis, and that active injection drug use in and of itself not be used to exclude such patients from antiviral therapy.

Hepatitis C Treatment in Psychiatric Risk Groups and Active and Recovering Drug and Alcohol Users: The Value of Multidisciplinary Care

Clinicians approach these patients from a perspective reflecting their respective training and background. Medical clinicians typically address the toxic effects (such as seizures or alcoholic cirrhosis) of a particular substance or the health consequences of a high-risk lifestyle (such as infectious hepatitis or HIV infection). Psychiatrists and other mental health clinicians focus on the mental health issues prevalent among substance-dependent patients. Chemical dependency counselors typically focus on the individual's destructive preoccupation with obtaining and consuming a psychoactive chemical substance and the negative consequences thereof. For the patient, the issues from all of these perspectives are pressing, often inseparable problems, yet health care providers operate in separate systems of care. The shortcoming of these parallel approaches is that the patient's problems are interrelated and require input from all systems for optimal treatment.

—J. H. Samet
Archives of Internal Medicine

Providing opportunities for hepatitis C care and treatment in populations for which it was formerly contraindicated is complicated. Encouraging results have emerged from different studies of active drug users and individuals with dual diagnoses (mental illness and addiction), as well as those on methadone maintenance. All speak to the need for patient-centered, multidisciplinary care.

Schafer and colleagues found no rationale for continued contraindications to interferon therapy if psychiatric evaluation and multidisciplinary care are provided before and during interferon therapy. They prospectively assessed efficacy, psychiatric side effects, and adherence to hepatitis C treatment (standard interferon 3 MIU, thrice weekly, plus ribavirin 1,000–1,200 mg/day; duration according to genotype and week-24 response) in four groups: individuals with a history of psychiatric disorders; individuals receiving methadone maintenance; former injection drug users; and controls (no past or present psychiatric disorders or drug use). Preexisting and interferon-induced depression did not have a significant impact on the dropout rate or treatment outcomes.

All participants were seen by a psychiatrist twice weekly during their first eight weeks on interferon, and on a monthly basis thereafter. Although five individuals were admitted to the psychiatric ward, not one of these admissions could be directly linked with interferon. Suicidal thoughts were reported in 4–6% of participants, and two individuals dropped out for this reason. In every instance, suicidal thoughts vanished during psychiatric care. Overall, 16% (13/81) developed new depression during treatment and were treated with antidepressants. Six individuals (four with a history of addiction and two from the methadone group) were treated for alcohol abuse during the study; only one of these individuals dropped out of the study (Schafer 2003a).

Table 31. SVR, Adherence, Discontinuation, Depression, and Suicidal Thoughts

	All (N=81)	Control (N=23)	Psychiatric (N=16)	Methadone (N=21)	Former Addiction (N=21)
SVR	37% (30/81)	35% (8/23)	38% (6/16)	48% (10/21)	28% (6/21)
Nonadherence	13% (10/81)	9% (2/23)	6% (1/16)	14% (3/21)	13% (3/21)
Discontinuation	22% (18/81)	13% (3/23)	18% (3/16)	14% (3/21)	43% (9/21)
Mild depression	15% (11/81)	4% (1/23)	25% (4/16)	14% (3/21)	14% (3/21)
Moderate depression	7% (6/81)	4% (1/23)	6% (1/16)	10% (2/21)	10% (2/21)
Severe depression	5% (4/81)	4% (1/23)	13% (2/16)	0%	5% (1/21)
Antidepressants given	16% (13/81)	4% (1/23)	62% (10/16)	24% (5/21)	10% (2/21)
Suicidal thoughts	5% (4/81)	4% (1/23)	6% (1/16)	5% (1/21)	5% (1/21)

Schafer 2003a

Veterans with HCV

El-Serag and colleagues examined the occurrence of psychiatric disorders, and drug and/or alcohol addiction among veterans with hepatitis C who received inpatient care at a VA facility between 1992 and 1999. Of the 33,824 veterans with hepatitis C, 86% had at least one prior or current psychiatric, drug, or alcohol disorder. After controlling for age, sex, and ethnicity, drug and alcohol use, depression, post-traumatic stress disorder, and anxiety were significantly associated with hepatitis C infection (El-Serag 2002).

Despite the significant prevalence of these co-morbidities among veterans with hepatitis C, treatment for hepatitis C using a multidisciplinary model has produced encouraging results. A team of providers, including a hepatologist, psychiatrist, pharmacologist, and nurses, assessed prevalence of co-morbid behavioral emotional disorders (BED) in veterans with hepatitis C at the Cincinnati VA Medical Center. Over 95% had experienced or been diagnosed with a BED (67% had one or more disorders, and 89% had been diagnosed with drug or alcohol addiction). At the time of publication, 90% (83/92) had completed six months of treatment; 50% (47/92) had completed the entire course of therapy and 28% (26/92) remained on treatment. Withdrawals and dropouts (29%) were attributed to personal problems (9%), psychiatric adverse events (7%), disappearance due to suspected drug/alcohol relapse (6%), medical adverse events (5%), and known drug/alcohol relapse (2%). Overall, 20% achieved sustained virological responses, with the highest rate of SVR seen in white males (42%) (Goldsmith 2002).

Nguyen and colleagues looked at the medical records of 206 veterans with hepatitis C who received care at a multidisciplinary medical and psychiatric chronic hepatitis clinic. Psychiatric disorders and/or drug/alcohol addiction were prevalent; 89% had been diagnosed with one or both. Treatment was not given to individuals with minimal liver fibrosis or persons with worsening medical, psychiatric, or drug/alcohol problems. Of the 206, 145 (71%) were treated for hepatitis C with interferon or interferon plus ribavirin. Sustained virological responses were within expected parameters: 16% of those on interferon monotherapy, and 28% of those on interferon plus ribavirin (H. A. Nguyen 2002).

HCV Treatment in Active Injection Drug Users

In the United States, an estimated 3,372,000 individuals have injected drugs (National Household Survey on Drug Abuse, 2000 and 2001). Hepatitis C is highly prevalent among injection drug users; an estimated 70–90% have been infected (M. J. Alter 1998; Donahue 1991; Garfein 1996; Thomas 1995a). Although injection drug users comprise the majority of those infected with hepatitis C, little is known about the safety, efficacy, and tolerability of hepatitis C treatment in this population; until recently, hepatitis C treatment was withheld from active users until they had completed a six-month period of abstinence from drugs and alcohol.

Backmund and colleagues studied the feasibility of initiating hepatitis C treatment during inpatient detoxification treatment. HCV treatment was not withheld or discontinued if relapse to injection drug use occurred during the study. Fifty individuals enrolled in the study. Hepatitis C treatment was initiated two weeks before discharge. After discharge, participants either attended a weekly outpatient program or were sent to an inpatient clinic. Relapse to active drug use occurred in 80% of study participants; 30% began replacement therapy with methadone or dihydrocodeine.

After 12 weeks of treatment, 54% discontinued (10% due to side effects, 10% because of non-adherence and 34% because of virological non-response). Week-12 responders to interferon monotherapy continued treatment for another 36 weeks. The duration of combination therapy was assigned according to genotype: 24 weeks for genotypes 2 and 3, and 48 weeks for genotype 1. Overall, 36% achieved a sustained virological response to treatment (Backmund 2001). This rate of response is within the range from two pivotal clinical trials of interferon monotherapy and combination therapy in non–drug users, although baseline characteristics were different in these trials.

Table 32. SVR After Treatment with Combination Therapy or Interferon: Data from Two Large Clinical Trials

Author	Regimen	Duration	% SVR, Overall
McHutchison 1998	IFN alfa-2b monotherapy	48 weeks	13% (29/255)
McHutchison 1998	IFN alfa-2b + RBV 1,000–1,200 mg/day	24 weeks	31% (70/228)
McHutchison 1998	IFN alfa-2b + RBV 1,000–1,200 mg/day	48 weeks	38% (87/228)
Poynard 1998	IFN alfa-2b + placebo	48 weeks	19% (53/278)
Poynard 1998	IFN alfa-2b + RBV 1,000–1,200 mg/day	24 weeks	35% (96/277)
Poynard 1998	IFN alfa-2b + RBV 1,000–1,200 mg/day	48 weeks	43% (118/277)

Schafer 2003a

Table 33. SVR in Current/Former IDUs by Regimen, Setting, Appointment Attendance, and Baseline Characteristics

Variable	% SVR
Regimen:	
IFN monotherapy	35% (12/34)
IFN + RBV*	38% (6/16)
Setting:	
Inpatient program	20% (1/5)
Home	60% (3/5)
Post-relapse substitution program	53% (8/15)
Relapse to heroin injection	24% (6/25)
Appointment attendance:	
Less than 2/3	6% (1/12)
More than 2/3	45% (17/38)
Baseline Characteristics:	
Genotype 1	26% (7/27)
Genotype 2 or 3	48% (11/23)
HCV RNA <300,000 copies	38% (9/24)
HCV RNA >300,000 copies	35% (9/26)
<29 years old	37% (10/27)
>29 years old	35% (8/23)
Female	41% (7/17)
Male	33% (11/33)

Backmund 2001

*After October 1998, all participants received combination therapy.

Reinfection with HCV

Reinfection with hepatitis C may occur in injection drug users after spontaneous viral clearance of acute infection or sustained virological response to treatment (Dalgard 2002; Proust 2000). The frequency of re-infection is unknown. Backmund and colleagues assessed HCV RNA at 12 and 24 weeks after completion of treatment. Although 56% (10/18) of the cohort had injected heroin (for a range of 4–140 days), none became reinfected during this period. The investigators planned to continue follow-up for one or two more years (Backmund 2001). Dalgard and colleagues followed a group of 27 injection drug users and 18 non-injecting controls who had been successfully treated for hepatitis C five years earlier. Every participant was tested for HCV RNA and underwent a risk assessment. Although 33% (9/27) had injected drugs since completion of therapy, only one individual had detectable RNA at follow-up. Genotypic testing revealed a new infection with genotype 1a (rather than the previous genotype 1b infection).

HCV Treatment in Individuals on Methadone Maintenance Therapy

Hepatitis C infection is common among methadone maintenance recipients. Prevalence estimates range from 67% to 87% (Piccolo 2002; Stein 2001). Psychiatric co-morbidities are prevalent as well, with estimates ranging from 47% to 76% (Brooner 1997; Callaly 2001). Methadone maintenance programs have been successful venues for directly observed therapy with highly active antiretroviral therapy (HAART) and isoniazid (Batki 2002; Clarke 2002; McCance-Katz 2002). Three studies examined safety, tolerability, and efficacy of HCV treatment for individuals on methadone maintenance.

Interim data from the Organization to Achieve Solutions in Substance Abuse (OASIS) reported that 54% achieved an ETR after a six or twelve month course of interferon and ribavirin. A subset of 59/105 achieved SVR (Sylvestre 2002a).

Table 34. Sustained Virological Response Rates from 59/105 Study Participants*

Participant Characteristics	% SVR
Overall	28%
Treatment discontinuation	24%
Pre-treatment psychiatric diagnosis	22%
No prior psychiatric diagnosis	37%
Antidepressant use before therapy	50%
Antidepressant use during therapy	88%
Alcohol consumption during therapy (21% overall)	25%
No alcohol consumption during therapy	29%
>6 months pre-treatment sobriety	37%
<6 months pre-treatment sobriety	30%
No pre-treatment sobriety	17%
Active drug use during treatment (35% overall)	20%
Infrequent drug use during treatment	20–29%
Frequent drug use during treatment	0%

Sylvestre 2002b

*Data on the remaining participants will be available at completion of study.

HCV treatment is feasible for recent drug users, and methadone maintenance may support adherence to treatment. Van Thiel and colleagues treated 120 recent drug users, 52 on methadone maintenance. Their baseline characteristics, preclinical parameters and treatment outcomes were compared to those of 120 non-drug using controls. Discontinuation rates were astonishingly low, despite a grueling regimen of 5 MIU of interferon daily for a minimum of one year (virological responders were continued on therapy until their HCV RNA was undetectable for fifteen consecutive months). Only 15% (18/120) of drug users discontinued (vs. 7% [112/120]) of the control group). Sustained virological response rates did not differ significantly between drug and methadone users (33%) and controls (37%). Access to methadone during this study may have

increased adherence to treatment, because some participants initiated methadone maintenance as a side effects management strategy. Methadone dosing remained stable or increased by 10–15% (Van Thiel 2003).

Two other studies demonstrated the safety, feasibility, and efficacy of providing HCV treatment with methadone maintenance. Buggisch and colleagues retrospectively analyzed data from 39 individuals on methadone maintenance who were treated for HCV with standard interferon plus ribavirin. Participants had to be drug-free (with the exception of methadone) for six months before enrolling. The SVR rate was 46% (18/39)—comparable to that seen in non-methadone-using populations (Fried 2002a; McHutchison 1998; Poynard 1998). Many participants had genotype 2 or 3 (31% or 12/39), and SVR occurred more frequently among these individuals (75% vs. 33% for genotype 1) contributing to the overall SVR rate. There were four discontinuations, two each for side effects and relapse to active drug use (Buggisch 2002). Mauss and colleagues compared HCV treatment outcomes between 50 individuals using methadone maintenance and 50 matched controls. Participants received pegylated interferon plus ribavirin according to genotype. The end-of-treatment response rate in the control arm was 74% (37/50) vs. 50% (25/50) in the methadone arm. In the control group, 54% (27/50) achieved SVR vs. 39% (19/49) in the methadone group. Discontinuation rates for side effects or lack of adherence were 18% (8/45) in the control arm vs. 42% (18/43) in the methadone arm. Most of the discontinuations in the methadone arm occurred before week eight. There was no significant difference in the response rates for those who remained on treatment after week eight; 50% (19/38) of methadone users achieved SVR, as did 56% (27/48) of the control arm. No serious psychiatric side effects were reported in either arm. No information was provided about care and ancillary psychiatric services available to study participants (Mauss 2003a; Mauss 2003b).

The pharmacokinetics of methadone and pegylated interferon alfa-2a (180 μg/week) were evaluated in 24 individuals receiving concomitant methadone maintenance. Baseline levels of methadone were compared with serum samples after single (week 1) and multiple doses (week 4) of pegylated interferon, and pegylated interferon levels were compared with historical data from non-methadone users. The levels of pegylated interferon at week 1 and week 4 were similar to levels in non-methadone users, and methadone levels were similar at baseline and at week 4. No signs of opioid withdrawal were observed. The most frequently reported adverse events—headache, myalgia, fever, fatigue, and appetite loss—were mild or moderate (Sulkowski 2003a).

HCV Treatment and Alcohol Use

Alcohol consumption of over 50 g/day (equivalent to four or five glasses of wine) during HCV treatment decreases the efficacy of antiviral therapy (Ohnishi 1996; Okazaki 1994; Peters 2002). Several factors may contribute to the poorer response to treatment seen in alcohol users. Heavy alcohol intake (>70 g/day) increases HCV quasispecies complexity, which may make HCV less responsive to interferon (Sherman 1999). Alcohol may increase HCV replication; some studies have found higher levels of HCV RNA in alcohol users while others have not observed significant differences between drinkers and nondrinkers (Cromie 1996; Khan 2000; Oshita 1994; Pessione 1998; M. Sawada 1993; Wiley 1998).

A case-controlled study evaluated the effect of different amounts of alcohol on treatment efficacy

in 65 individuals. Alcohol use per day was categorized into four groups: none, ≤ 40 g/day, 41–80 g/day, and >80 g/day. HCV-RNA levels were significantly higher in drinkers, with the heaviest drinkers having the highest titers of HCV RNA. Response to treatment decreased with heavier alcohol intake. Fewer than 5% of those reporting alcohol use of any amount achieved SVR, and non-response to treatment occurred at a significantly greater rate among drinkers (63.1% vs. 10.7%; P<0.001) (Loguercio 2000).

Specific information about the effect of light-to-moderate alcohol intake (<20 g/day) on HCV treatment efficacy is unavailable. Decreasing or eliminating alcohol intake during treatment is recommended.

Treatment Issues for Recovering Addicts

For those in recovery from alcohol or drug use, relapse is a significant concern. Interferon's side effects have been compared to those of opioid withdrawal, which may trigger drug cravings. Interferon is given by injection, which may also be an issue for some recovering injection drug users.

HCV Treatment in Correctional Institutions

> *Correctional facilities are critical settings for the efficient delivery of prevention and treatment interventions for infectious diseases. Such interventions stand to benefit not only inmates, their families, and partners, but also the public health of the communities to which inmates return.*
>
> —T. M. Hammett
> *American Journal of Public Health*

Estimates of hepatitis C prevalence among the almost 2 million inmates of state and federal correctional facilities range from 255,000 to 500,000 (Allen 2003; CDC 2003). A serosurvey of 3,914 Maryland inmates reported that 29.7% had antibodies to hepatitis C (Goldstein 2003). Screening for and treatment of hepatitis C in correctional facilities is extremely inconsistent. According to results from a national survey from Spaulding and colleagues, only one state (Colorado) routinely screens for hepatitis C. California was the only state to perform a seroprevalence survey. A standard protocol for HCV treatment was followed by four states, while 73% of respondents "sometimes consider" treatment with interferon (Spaulding 1999). Data on treatment outcomes are available from Rhode Island; their Department of Corrections treated 90 inmates with interferon plus ribavirin; of the 41 who completed treatment, 26 (62%) achieved sustained virological response (Allen 2003).

Inmates have had to resort to litigation to obtain treatment for hepatitis C in Montana, Oregon and Pennsylvania (Gustavson 2003; J. Lin 2002; McKee 2002). Withholding necessary treatment from prisoners is unacceptable. A valuable tool for advocates comes from the Centers for Disease Control (CDC) in the form of guidelines for *Prevention and Control of Infections with Hepatitis Viruses in Correctional Settings*.

Alternative and Complementary Therapies

As practitioners educating and treating patients with liver disease, we are obliged to be informed about popular alternative therapies, understanding of our patients' need to be partners in their care, and open-minded to the possibility that some benefit may come from therapies currently regarded as alternative.

—N. M. Bass
Current Gastroenterology Report

Complementary and alternative treatments for liver disease come from many cultures. They may be useful as alternatives to standard HCV treatment, as adjunctive therapies, or to minimize the side effects of interferon and ribavirin.

At present, these therapies have not been adequately researched. The lack of standardization of these preparations, and the inconsistent manufacturing practices involved with their production, make it difficult to perform pharmacokinetic evaluations, safety and efficacy studies, and investigations of potential interactions.

In 1999, 809 individuals receiving care at six different liver clinics completed a questionnaire on their use of complementary and alternative medicine (CAM). Overall, 74% of respondents indicated CAM use, although 26% did not inform their physician. Silymarin (milk thistle) was used by 12% as a treatment for liver disease (Seeff 2001; Strader 2002). Silymarin has been used to treat liver disorders for at least 2,000 years, and has been reported to work as an antioxidant and have anti-inflammatory and regenerative properties. Silymarin may increase hepatocyte protein synthesis, decrease activity of tumor promoters, and protect against liver injury by blocking various toxins from entering liver cells (Flora 1998; Giese 2001; Luper 1998; Wellington 2001).

Conflicting results have emerged from two trials evaluating silymarin's effect on cirrhosis. Ferenci and colleagues randomized 170 individuals with varying degrees of cirrhosis (alcohol- and non-alcohol-related) to receive either 140 mg of silymarin three times daily, or a placebo for two years. After a mean observation period of 41 months, the survival rate in the silymarin group was 58 ± 9% vs. 39 ± 9% for the placebo group (P=0.036). A subgroup analysis revealed that silymarin appeared to be most beneficial for individuals who had alcoholic cirrhosis (P=0.03). No side effects were reported (Ferenci 1989). Pares and colleagues evaluated the effects of silymarin in 200 individuals with alcoholic cirrhosis, who were randomized to receive either 450 mg of silymarin thrice daily or placebo for two years. Survival was similar in treated and placebo groups, and no significant effect on the clinical course of liver disease was observed (Pares 1998). Two other studies have noted significantly reduced levels of ALT and AST in individuals with liver disease (Buzzelli 1993; Salmi 1982). So far, one study from the National Center for Complementary and Alternative Medicine (NCAM) examined the effect of silymarin in chronic hepatitis C. The estimated completion date for this study was June 2002. Results are not yet available.

Recommendations

Increase knowledge of treatment and care for hepatitis C patients among primary care providers.

Surveys of primary care providers have revealed significant gaps in the care and treatment provided to patients with hepatitis C. A national survey of primary care providers found that a quarter of the 1,412 physicians who responded did not know what treatment to recommend for hepatitis C (Shehab 2001). Provider education initiatives, such as continuing medical education credits (CMEs) are urgently needed.

Identify optimal dosing strategies for pegylated interferon and ribavirin.

Although there are approved doses for both brands of pegylated interferon (Pegasys® [pegylated interferon alfa-2a] and Peg-Intron® [pegylated interferon alfa-2b]), there are unresolved dosing issues with each product. The FDA has required that Roche and Schering conduct studies examining 1) the potential safety and efficacy of higher doses of Pegasys and/or ribavirin in people with genotype 1, high viral load, and weight >85 kg (Roche); 2) the safety and efficacy of fixed-dose (800 mg) vs. weight-based (800–1,400 mg) ribavirin in combination with Peg-Intron (Schering's WIN-R); and 3) the safety and efficacy of low-dose (1.0 ug/kg) vs. high-dose (1.5 ug/kg) Peg-Intron in combination with ribavirin for people with genotype 1 (Schering's IDEAL).

Dose reductions have occurred frequently during pivotal HCV treatment trials, yet data on efficacy and tolerability of lower doses of pegylated interferon alfa-2a are scarce. A 135 μg dose of pegylated interferon alfa-2a may be equally efficacious as, and more tolerable than a 180 μg dose (Pockros 2001).

As for pegylated interferon alfa-2b, pharmacokinetics data suggests that the once-weekly 1.0 μg/kg dose recommended for monotherapy may be suboptimal. The upper limit for weight-based dosing of pegylated interferon alfa-2b has not been adequately defined. Obese individuals typically have lower response rates, but it is unclear whether this is due to inadequate dosing of pegylated interferon and/or ribavirin or to other poor prognostic factors, viral resistance, or a combination of these elements.

Schering and Roche must support research to answer these questions.

Increase research on treatment safety and efficacy in understudied populations.

Most studies of HCV treatment efficacy and safety have focused on populations with favorable prognostic factors. Individuals with medical and psychiatric co-morbidities have been excluded from the pivotal studies of pegylated interferon and ribavirin, and results from these trials may not be applicable to a majority of individuals with HCV infection. More research is urgently needed on the safety, efficacy, and optimal dosing and duration of HCV treatment in African Americans, cirrhotics, active drug users, individuals on methadone maintenance, the mentally ill, transplant recipients, individuals with renal disease, individuals with autoimmune disorders, the elderly, young children, adolescents, and non-responders and relapsers after prior HCV treatment. This

research should be funded by NIH.

African Americans have been underrepresented in clinical trials. This may be attributed in part to the exclusion criteria for neutropenia, as African Americans are constitutionally neutropenic (Freedman 1997; Reed 1991; Zezulka 1987). Investigation of a safe threshold for neutropenia for African Americans, and modification of the standard exclusion criteria for neutropenia will help to minimize underrepresentation of African Americans in clinical trials.

Treatment should not be withheld from active drug users; decisions should be made on an individualized basis.

Treatment has traditionally been withheld from active drug users. A survey of 306 former IDUs in a methadone maintenance program revealed that 53% were interested in treating their HCV after hearing about the risks and benefits of interferon therapy (Stein 2001). Three studies have assessed feasibility, safety, and efficacy of HCV treatment in groups of individuals who were undergoing detoxification and/or receiving methadone maintenance, and a subset who were using drugs and/or alcohol during HCV treatment (Backmund 2001; Sylvestre 2002; Van Thiel 2003). Response rates from one trial were within the expected range from clinical trials of non-drug-using individuals (Backmund 2001; McHutchison 1998; Poynard 1998). Another study demonstrated that response rates to treatment were increased in individuals who had been drug-free for six months prior to treatment, yet a proportion of those who used drugs infrequently during HCV treatment still achieved SVR (Sylvestre 2002b).

The risk of reinfection is often used as a reason not to offer treatment to active injection drug users. So far, there has been scant documentation of reinfection in IDUs who achieved SVR after HCV treatment, although this may reflect the paucity of studies rather than the infrequency of reinfection. At any rate, provider concern should be focused on ensuring that injectors have access to sterile syringes by referral to syringe exchange programs (when possible) or pharmacy sale; other strategies include referral to methadone maintenance programs, prescription of buprenorphine and drug treatment upon request.

Develop integrated, multidisciplinary systems of care for individuals with multiple co-morbidities (HCV, mental illness, addiction).

Individuals with hepatitis C are often grappling with additional issues: the stress involved with illicit drug use; maintaining recovery from addiction; severe, debilitating fatigue; poverty; homelessness; or incarceration. HCV is more prevalent among the mentally ill, and individuals with HCV have a greater prevalence of depression (Zdilar 2000).

Our health care system is not prepared to accommodate the needs of active users or dually and triply diagnosed individuals. Multidisciplinary systems of care have been proven successful in treating active users, individuals with addiction and psychiatric co-morbidities, and individuals in a methadone maintenance program (Backmund 2001; Samet 2001; Samet 2003; Schwartzapfel 2002; Sylvestre 2002; Van Thiel 2003). Cross-disciplinary care must become an integral part of the care and treatment of people living with HCV.

Provide full access to hepatitis C care and treatment for all those in need.

Current treatments for HCV can cost up to $40,000 per year. The uninsured, underinsured, and those ineligible for patient assistance and entitlement programs go untreated, even when treatment is urgently needed. Advocacy efforts to increase access to HCV treatment must continue. Entitlement programs and private insurers should cover the costs of HCV treatment, including laboratory monitoring and medications to manage treatment side effects. Medicaid programs must receive the necessary funding from Congress to cover HCV treatment. Strategies must be developed to provide coverage for HCV therapy among the uninsured who do not qualify for entitlements or patient assistance programs.

Provide full access to hepatitis C care and treatment for incarcerated individuals.

In the United States, close to 2 million individuals are incarcerated. HCV infection is endemic among prisoners. A 1994 study of HCV prevalence among 4,513 inmates (87% male; 13% female) in the California correctional system reported that 39.4% of the males and 53.5% of the females were HCV-antibody-positive (Ruiz 1999). The need for HCV treatment remains largely unmet in correctional systems. Policies about HCV treatment in prison differ in every state, and incarcerated individuals do not have uniform access to treatment for HCV. Some inmates have had to resort to legal action to obtain treatment. This is an unacceptable situation. State and national advocacy efforts must be coordinated to demand access to HCV treatment for inmates. A valuable tool for advocates comes from the Centers for Disease Control (CDC) in *Prevention and Control of Infections with Hepatitis Viruses in Correctional Settings* (http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5201a1.htm, accessed on 10 April 2004).

Increase research on strategies to manage side effects from HCV treatment.

The side effects of treatment for hepatitis C range from the uncomfortable to life-threatening. In a recent 1,100-person phase III trial of pegylated interferon alfa-2a (with placebo or ribavirin) and interferon alfa-2b, the rate of treatment discontinuation due to adverse events and/or laboratory abnormalities was 10% in the pegylated interferon/ribavirin arm and 11% in the standard interferon arm. Dose reductions were necessary for 32% of the individuals in the pegylated interferon/ribavirin arm (Fried 2002a). Significant dose reductions may have an impact on the response to treatment (Fried 2002a; McHutchison 2002).

A comparison of adverse events from a recent trial comparing peg-interferon alfa-2b and ribavirin to interferon alfa-2b and ribavirin found that more than 20% of participants in each arm experienced fatigue, headache, fever, muscle aches and stiffness, insomnia, nausea, hair loss, irritability, joint pain, loss of appetite and weight loss, depression, and injection site reactions (Manns 2001). The list of serious adverse events associated with interferon treatment, although occurring in less than 1% of individuals studied so far, is daunting and includes severe neuropsychiatric complications and suicidal ideation, as well as skin, kidney, blood, liver, heart, and autoimmune diseases, and sensory organ disorders (Fried 2002b).

Research on the safety and efficacy of interventions to increase the tolerability of, and adherence to, HCV treatment is a priority. More research is needed to identify the proper threshold to initiate

the use of growth factors to treat anemia and neutropenia, and to study their impact on HCV treatment efficacy. Interventions to decrease neuropyschiatric side effects are a priority: the instruments used to screen for depression have not been validated for this purpose. More exploration of the instruments used to diagnose depression and evaluate the efficacy, side effects, and indications of SSRIs, other antidepressants, and anti-anxiety agents is needed to optimize individual side effect management strategies.

The manufacturers of interferon, ribavirin, and ancillary mediations used as treatment for HCV treatment-induced side effects should provide the drugs, and their sponsorship to government-funded research networks so that additional strategies for side effects management may be developed.

Establish prospective, long-term follow-up studies to assess the durability and clinical benefit of histological responses in responders, relapsers, and non-responders.

Achieving a sustained virological response after HCV treatment increases the probability of histological improvement, but decreases in both grade and stage of liver disease have occurred in the absence of an SVR (Cammà 2004; Poynard 2002b; Shindo 2001). The risk of liver-related mortality from HCV appears to be lower among individuals who have been treated with interferon (Imazeki 2003). Longer follow-up of participants in pivotal HCV treatment trials will provide more information on the potential histological benefits of therapy, regardless of the response to treatment. Improvements in the grade of histological activity appear to occur most frequently among individuals treated with interferon plus high-dose ribavirin (Poynard 2002b). At present, there are not enough data to determine whether this post-treatment stabilization of HCV-related liver disease—especially in relapsers and non-responders—will confer clinical benefit. NIH should fund long-term research on the effect of HCV treatment on liver histology.

Evaluate durability and clinical benefits of sustained virological response.

Although many regard a sustained virological response as a "cure" or sign of remission, more data on the long-term outcomes of sustained virological responders are needed, especially in light of improved treatment efficacy and increased sensitivity of HCV-RNA assays.

Late relapse rates of up to 12% have been reported among sustained virological responders (Collier 2000; Pradat 2003). Low levels of HCV RNA have been detected in blood from 11 sustained virological responders up to five years after HCV treatment (Pham 2004). Industry-sponsored trials are ideal venues for establishing systems to collect data on long-term virological, histological, and clinical outcomes. NIH should support this research.

Identify when treatment for acute hepatitis C should be initiated, and what the optimal regimen and duration of therapy should be.

Treatment of acute HCV infection presents an opportunity for viral eradication; the rate of SVR in two recent studies ranged from 90% to 98% (Jaeckel 2001; Vogel 1996). These promising results require further study. Randomized, controlled trials of treatment of those with acute HCV are needed to define the interval during which spontaneous viral clearance is likely to occur, so that treatment may be initiated in the absence of spontaneous viral clearance. Optimal regimens and

duration of treatment should be identified. Roche and Schering should support this research.

Create an "opt-out" system for organ donation in the United States and include discussion of organ donation as part of school health education programs and regular medical care.

Between 1988 and 1999, the number of liver transplants in the United States increased from 1,713 to 4,689, and the number of centers performing liver transplantation rose from 59 to 117 (C. M. Smith 2000). As of June 30th, 2003, 17,001 Americans were awaiting liver transplantation. In the period between July 1, 2003 and June 30, 2003, only 5,486 had a liver transplant; 1,772 others died while waiting for a liver (Scientific Registry of Transplant Recipients, 2004). Many of these individuals would still be alive today if the supply of donor organs was adequate, as the one-year survival rate for HCV-related liver transplantation is 86.4% (Scientific Registry of Transplant Recipients, 2002; C. M. Smith 2000; United Network for Organ Sharing, 2000). It is estimated that, if untreated, the proportion of persons with HCV who will develop cirrhosis by 2020 will increase from 16% to 32%. Complications of cirrhosis, such as hepatic decompensation, hepatocellular carcinoma, and liver-related deaths will increase by 106%, 81%, and 180%, respectively (Davis 2003a). Increasing the supply and accessibility of available donor organs is an urgent priority. In the United States, potential organ donors may opt-in. Switching to a system that assumes organs will be donated unless an individual specifically opts-out will save lives.

A discussion of organ donation should be incorporated into school health education initiatives and primary care visits, so that it becomes normalized and premeditated, instead of being associated with shock, grief, and loss.

Research safety and efficacy of alternative therapies for HCV infection.

A national survey assessing the use of complementary and alternative therapies found that 42% of Americans reported use of complementary and alternative medicine (CAM); and an estimated 41% of individuals receiving care at six liver disease clinics reported use of CAM (Seeff 2001). Despite this widespread usage, we do not have data on the safety and efficacy of these therapies. The National Center for Complementary and Alternative Medicine (NCCAM) has performed a few exploratory studies of silymarin and mixed herbs for treatment of hepatitis C; we need larger, more rigorous investigation of pharmacokinetics, potential interactions, and safety and efficacy of complementary and alternative therapies in the treatment of hepatitis C. NIH should support this research.

List of Terms Used in This Chapter

Apoptosis: programmed cell death.

Estradiol: a female steroid sex hormone, is the most potent form of estrogen. It has many important functions, including growth of the uterus, fallopian tubes and vagina.

Half-life: the time needed for half of something to be eliminated from the bloodstream.

Hepatocytes: liver cells.

Intent-to-treat: an analysis of clinical trial results that includes all data from trial participants in the groups that they were randomized to, even if they never received the treatment or completed the trial.

MIU: million International Units. An International Unit is a measurement of the amount of the biologically active substance in the standard amount of the preparation producing this activity—such as a vitamin—that is agreed upon as an international standard, especially for comparison with other biologicals containing the substance. Internal Units are also used in Hepatitis C viral load testing; the results are usually reported as International Units per milliliter (IU/mL).

MU: million units.

Progesterone: Progesterone is a female steroid sex hormone that prepares the uterus for pregnancy, primes the breasts for making milk and protects the developing fetus.

Virion: an individual virus particle.

VI. HIV Treatment in HIV/HCV Coinfection

Summary

There are many unresolved questions regarding treatment of HIV in people coinfected with HIV and HCV. There are no United States treatment guidelines created specifically for HIV/HCV coinfection. This creates confusion among coinfected people and their clinicians, and variations in patient care. The optimal sequencing of treatment for HIV and HCV is unclear. Treating HIV first may prevent HCV disease progression by preserving the immune system; however, some studies have reported a blunted immune response to antiretroviral therapy in coinfected persons (Greub 2000; Law 2002; Torriani 2001; Zala 2004).

Antiretroviral-induced hepatotoxicity—abnormal elevations in liver enzyme levels—is a significant concern among coinfected persons. HCV coinfection increases the risk for hepatotoxicity. Protease inhibitors, non-nucleoside reverse transcriptase inhibitors, and nucleoside analog reverse transcriptase inhibitors are all associated with hepatotoxicity. The mechanism of liver toxicity differs by class of drug, as well as by agents within a class. Several factors may contribute to hepatotoxicity, such as flares of preexisting hepatitis due to immune restoration; genetic factors that influence drug metabolization; pharmacokinetic (drug-drug) interactions; heavy alcohol consumption; and perhaps even hepatitis C genotype. Treating HCV first may decrease the risk of hepatotoxicity by improving the condition of the liver (Uberti-Foppa 2003).

Coinfected persons may be more vulnerable to certain antiretroviral-associated side effects and toxicities such as lipoatrophy and diabetes. Antiretroviral agents should be selected carefully by coinfected patients and their clinicians. In some instances, antiretroviral agents may need to be changed, or treatment discontinued. HCV-related liver damage may compromise hepatic metabolization of antiretrovirals, thus increasing the potential for side effects, toxicities, and interactions with other drugs commonly used by people with HIV. Data on antiretroviral drug levels in people with hepatic impairment are scant. More information is needed.

Care, Treatment, and Research Issues

Treatment Guidelines and Provider Education

Currently, there are no United States guidelines for the care and treatment of HIV/HCV-coinfected persons. Coinfection-specific guidelines are needed to ensure that coinfected individuals receive optimal care for HIV and HCV. Coinfected individuals are not always referred to a liver specialist. If specialty care is available, coinfected persons may need to coordinate their care between infectious disease doctors and gastroenterologists. Some infectious disease physicians routinely offer referral to a gastroenterologist or hepatologist, but others do not. Infectious disease doctors have said that some gastroenterologists and hepatologists prefer not to treat coinfected persons, while some liver specialists report that some infectious disease doctors are not providing adequate care to those with liver disease.

The American Association for the Study of Liver Disease (AASLD) and the Infectious Diseases Society of America (IDSA) have collaborated on *Practice Guidelines for Diagnosis, Management and*

Treatment of Hepatitis C (Strader 2004). The *Practice Guidelines* include less than two pages on the diagnosis, natural history, and treatment of hepatitis C in coinfected persons. These guidelines are a first step towards establishing consistent standard of care for hepatitis C in HIV-positive persons.

Current resources include the *Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents* (DHHS 2003), the *Guidelines for the Prevention of Opportunistic Infections in Persons Infected with Human Immunodeficiency Virus* (USPHS/IDSA 2001), the *Consensus Statement on the Management of Hepatitis C: 2002* (NIH 2002), and the AASLD/IDSA's *Practice Guidelines for Diagnosis, Management and Treatment of Hepatitis C* (Strader 2004). These resources need to be integrated into guidelines for the care and treatment of HIV/HCV coinfection. In the meantime, other resources available to coinfected individuals, clinicians, educators, and advocates include:

- The British HIV Association (BHIVA) *Guidelines for Treatment and Management of HIV and Hepatitis C Coinfection* (BHIVA Coinfection Guidelines Committee 2003);
- The HIV-HCV International Panel's *Care of Patients with Chronic Hepatitis C and HIV Co-infection* (Soriano 2004);
- The Australasian Society for HIV Medicine (ASHM) *Coinfection HIV & Viral Hepatitis. A Guide for Clinical Management* (2003);
- *Coinfection by HIV and Hepatitis A, B and C Virus in Adult Patients* (GESIDA/PNS 2003).

Initiatives for provider education must accompany treatment guidelines. The need for provider education is underscored by surveys of clinician adherence to both the United States *Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents* (DHHS 2004) and the *Treatment Recommendations for Patients with Chronic Hepatitis C: 2002 Version 3.0* from the Department of Veteran's Affairs (VA). The survey of clinician adherence to the DHHS Guidelines reported that only 6% (116/1,933) of coinfected persons had been vaccinated against the hepatitis A virus (HAV), although the Guidelines recommend HAV vaccination for all susceptible coinfected individuals (Teshale 2002).

The VA's *Treatment Recommendations for Patients with Chronic Hepatitis C: 2002 Version 3.0* states that,

> All patients with HIV should be tested for hepatitis C . . . Patients infected with both HIV and hepatitis C appear to be at higher risk of liver disease progression than those with HCV infection alone. Therefore, they should be seriously considered for HCV therapy.

A recent review of patient and provider surveys, and of laboratory, pharmacy, and administrative medical records from the VACS-3 cohort (881 HIV-positive veterans) revealed that 79.5% (700/881) had been tested for antibodies to HCV, yet only 21.7% (65/300) of anti-HCV-positive individuals had HCV-RNA testing performed. Sixty-five individuals had no contraindications for HCV treatment, yet only 18% received a liver biopsy, and only 3% were prescribed HCV treatment. Overall, the investigators found that of the approximately 5,510 coinfected veterans in the VA system,

only 2.5% (138) had been prescribed HCV treatment (Fultz 2003b).

Until there are U.S. coinfection treatment guidelines and tools—such as an algorithm to help determine which coinfected individuals should be offered HCV treatment—as well as widespread initiatives to educate clinicians on the care and treatment of coinfected individuals, many HIV/HCV-coinfected people will continue to receive substandard care.

HIV Treatment in Coinfected Persons

When to Start Antiretroviral Therapy

The United States DHHS *Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents* recommend initiation of antiretroviral treatment in all symptomatic HIV infected persons and in those with CD4 cell counts below 200/mm^3. For those with CD4 cell counts of 200–350/mm^3 "treatment should be offered, although [it is] controversial" and for those with CD4 cell counts over 350/mm^3 "some" clinicians would initiate therapy in those with HIV RNA levels over 55,000 copies/mL, while "most" experienced clinicians would recommend deferring therapy for those with HIV RNA levels below that level (DHHS 2004).

The optimal time for initiating antiretroviral therapy in coinfected persons is not established. It may be beneficial to initiate antiretroviral therapy in coinfected individuals at a higher CD4 count than that which is currently recommended by the *Guidelines*. Some evidence suggests that coinfected persons have a blunted immune response to HAART, although this remains controversial (Greub 2000; Law 2002; Law 2004; Pineda 2002; Torriani 2001; Zala 2004). Starting HIV treatment at higher CD4 cell counts may help coinfected individuals preserve and maintain high CD4 cell counts.

HAART may delay HCV disease progression by increasing CD4 cell counts, because the risk of cirrhosis increases with CD4 counts below 200/mm^3 (Allory 2000; Goedert 2002; Lesens 1999; Ragni 2001). A retrospective analysis of the effect of HAART on survival of HIV/HCV-coinfected persons reported significant reductions in overall and liver-related mortality ($P<0.001$ and $P=0.018$, respectively) with triple-combination therapy vs. nucleoside analog reverse transcriptase inhibitors alone or no HIV treatment (Qurishi 2003). Maintaining a CD4 cell count >200/mm^3 increases the likelihood of achieving a sustained virological response to HCV treatment with standard interferon (Mauss 1998; J. F. Sanchez 1994; Soriano 1996). Although CD4 cell percentages often increase during interferon therapy, absolute CD4 cell counts decrease (Ballesteros 2002; Chung 2002b; Hoffman-Terry 2002; Soriano 1995; Torriani 2004). Concomitant or pre-emptive use of HAART may be necessary to prevent CD4 cell counts from dropping below 200/mm^3 during HCV therapy. Absolute CD4 cell count and CD4 percentage usually return to baseline after completion of HCV therapy (Chung 2004; Torriani 2004).

On the other hand, HAART-mediated immune reconstitution may exacerbate HCV infection, at least in the initial weeks of HAART. Some studies have found that antiretroviral therapy may increase HCV RNA levels, although others have not (Chung 2002a; Matsiota-Bernard 2001; Qurishi 2003; Torriani 2002; Torre 2001; Rutschmann 1998; Zylberberg 1998b). After initiation of HAART, Chung and colleagues found greater increases in HCV RNA among individuals with

lower baseline CD4 cell counts. In 10 individuals with baseline CD4 cell counts <350/mm^3, HCV RNA increased by 0.44 log at 16 weeks and 0.59 log at 48 weeks. HCV RNA increasesd by 0.26 log at week 16 and 0.1 log at week 48 in the 30 individuals with a baseline CD4 cell count >350 (Chung 2002c). The clinical implications of these increases in HCV RNA are unknown.

The decision to initiate antiretroviral therapy depends on a number of non-clinical factors as well, but in the absence of large, prospective, long-term clinical trials, coinfected persons and clinicians lack the information they need in order to make key HIV treatment decisions.

Sequencing of Treatment for HIV and HCV

For coinfected individuals with CD4 counts in the range of 300–400/mm^3, there are compelling arguments for treating either virus first, but there is no completed research addressing this question. In those with high CD4 counts, the presence of advanced liver disease makes HCV treatment the priority. Offering liver biopsy to assess liver damage will help to inform the decision making process, as biochemical and virological tests are not reliable surrogate markers for fibrosis or cirrhosis and the need for HCV treatment cannot be definitively assessed without a liver biopsy (Mendes-Corréa 2003; Merchante 2003; Sterling 2003). Individuals with CD4 counts ≤ 200/mm^3 need HIV treatment first. Those with high CD4 cell counts and mild liver disease may not need to treat either virus immediately.

Immune activation is associated with HIV disease progression in untreated individuals, and has been identified as a key element in HIV pathogenesis (Giorgi 1999; Grossman 2002; Liu 1998). During treatment with antiretroviral therapy, persistent immune activation has been associated with decreased CD4 cell gains (Anthony 2003). Coinfection with HCV has been associated with increased immune activation (Hunt 2003; Valdez 2000). Treating hepatitis C first may decrease or eliminate its contribution to immune activation if a sustained virological response is achieved. On the other hand, early initiation of antiretroviral therapy may decrease immune activation. Higher levels of immune activation have been observed in individuals who initiate HAART at low CD4 cell counts (Lange 2002). However, immune activation appears to decrease as the duration of viral suppression increases (Hunt 2003).

- **Treating HIV First**

The arguments for treating HIV first include the well-recognized risk of progression to AIDS in untreated HIV disease and the known efficacy of antiretrovirals against HIV. Virological suppression of HIV may slow fibrosis progression (Braü 2004b). Maintaining a high CD4 cell count may reduce the risk of HCV disease progression. Furthermore, preserving the immune system may slow HCV disease progression, allowing coinfected individuals to defer HCV therapy until more effective and less toxic HCV therapies reach the market. Finally, maintaining a high CD4 cell count with HAART may contribute to the success of future HCV treatment, as standard interferon-based regimens are more effective in those with CD4 cell counts >500/mm^3 (Mauss 1998; J. F. Sanchez 1994; Soriano 1996).

- **Treating HCV First**

The rationale for treating HCV first is informed by data on the natural history of HCV in HIV-positive individuals, as HCV infection is known to progress more rapidly in coinfected persons. A proportion of coinfected persons treated for HCV may achieve a sustained virological response. Although treating hepatitis C does not invariably result in eradication of hepatitis C, some virological non-responders may have improved liver histology. HCV monoinfection studies (see Chapter V, Hepatitis C Treatment) have reported that HCV disease progression may stabilize or that there may be a reversal of liver damage after treatment, even in the absence of an SVR. This may apply in coinfection as well, although the long-term histological and clinical benefits of pegylated interferon-based regimens in mono- or coinfected persons are currently unknown.

Even in the absence of an SVR, many clinicians and researchers have speculated that treating HCV first may lower the risk of antiretroviral hepatotoxicity by improving overall liver health. Uberti-Foppa and colleagues studied the effect of pretreatment for HCV on the risk for hepatotoxicity after initiation of HAART in an unrandomized, prospective study of 105 coinfected participants. HIV and HCV parameters of all participants were similar at baseline. Sixty-six chose to be pretreated for HCV (with standard interferon monotherapy or standard interferon plus ribavirin) prior to initiating antiretroviral therapy. The risk for discontinuation of anti-HIV therapy was significantly greater among individuals who were not pretreated for HCV (RR=10.4; 95% CI, 1.6–66; P=0.0127) and those with elevated liver enzyme levels before initiation of HAART. The discontinuation risk increased with ALT elevations (for increases of 10 U, 50 U and 100 U, the risk ratios were 1.14, 1.96 and 3.86, or 1.014 per unit of ALT; P=0.005). The probability of remaining on HAART for 24 months was 95% ± 4.5% in the pretreated group versus 85% ± 15.4% in the non-pretreated group, suggesting that pretreatment of hepatitis C may increase tolerability of antiretroviral therapy. All of those who achieved an SVR were able to tolerate HAART (Uberti-Foppa 2003). Although a baseline assessment of liver histology was available, post-treatment liver biopsies were not performed in this study.

Assessment of baseline and post-treatment liver histology must be included in future studies.

- **Treating HIV and HCV Simultaneously**

Both viruses may be treated at the same time, although side effects may be unendurable for some individuals. In the absence of prospective, randomized studies, treatment decisions should be made on an individualized basis.

HIV Treatment Issues in HIV/HCV Coinfection

Hepatotoxicity

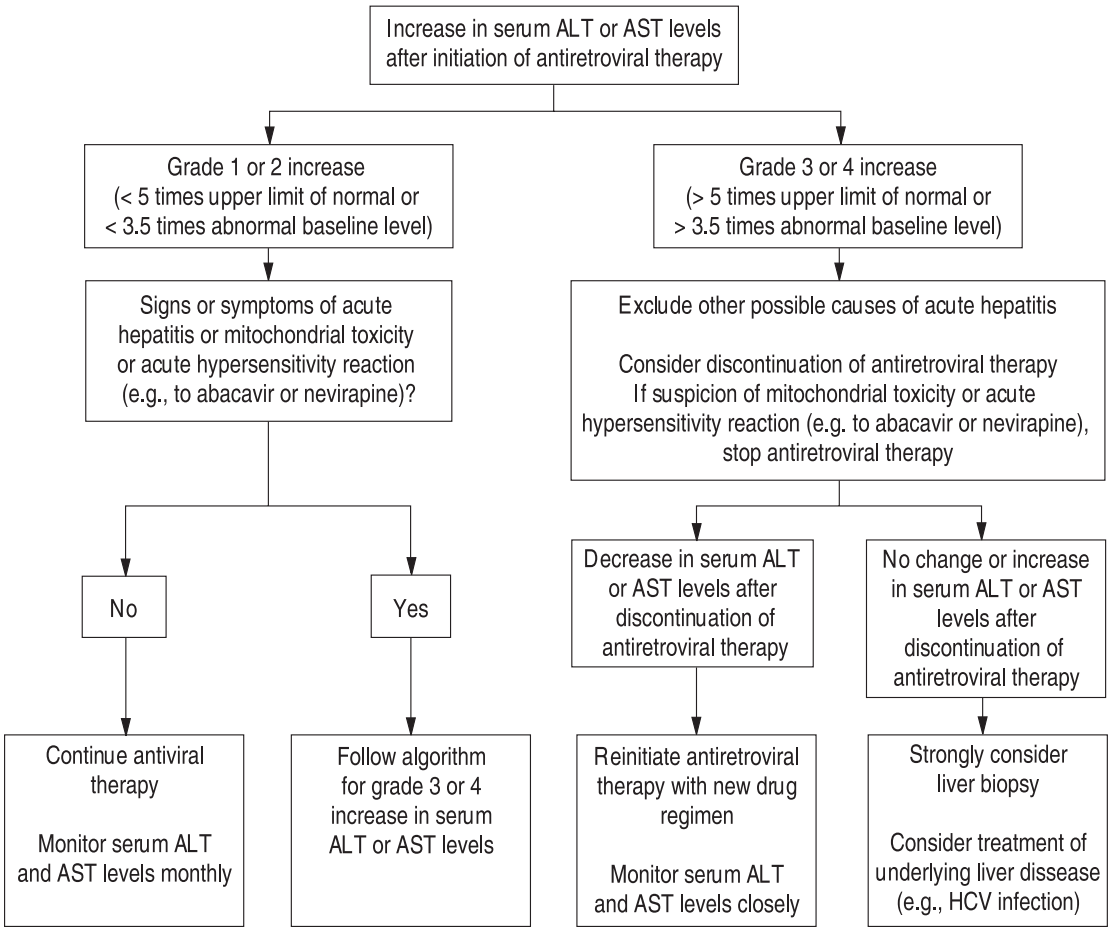
One of the most vexing areas in care and treatment for coinfected individuals is the potential for hepatotoxicity from antiretrovirals or other ancillary medications commonly used to treat HIV disease, opportunistic infections, complications and co-morbidities. Antiretroviral-related hepatotoxicity is characterized by abnormal elevations in liver enzyme levels and, in some instances, flares of symptomatic hepatitis after initiation of HAART. In some cases, antiretroviral

agents may need to be switched or discontinued. Although people who are not HCV-coinfected also experience HAART-related hepatotoxicity, the risk is greater for coinfected people (Aceti 2002; Bonnet 2002; Bonfanti 2001; den Brinker 2000; S. M. Imperiale 2002; Law 2003; Martinez 2001; M. Núñez 2001; Reisler 2002; Savès 1999; Sulkowski 2000a; Sulkowski 2000b; Torriani 2000; Zylberberg 1998a).

Several mechanisms may cause or contribute to hepatotoxicity, such as direct toxicity of antiretrovirals, pharmacokinetic interactions, drug-induced mitochondrial damage in liver cells, and enhanced immune responses to hepatitis C after HAART-mediated immune restoration. Host factors are involved as well; hereditary differences in the genes encoding drug-metabolizing enzymes may alter the risk for hepatotoxicity. Heavy alcohol use has been associated with an increased risk for hepatotoxicity (Aceti 2002; Lana 2001; M. Núñez 2002). The stage of HIV disease at initiation of HAART, and the immunologic response to therapy may contribute as well.

Much remains unknown about hepatotoxicity. The relationship between the degree of liver damage present at initiation of HAART and the incidence of hepatotoxicity has not been thoroughly examined. Research must include baseline assessments of liver histology at initiation of HAART in order to determine when hepatotoxicity is likely to occur, since ALT levels are not an adequate surrogate for HCV disease progression (Kouvatsos 2002). Antiretroviral regimens for coinfected persons must be carefully selected, and regular monitoring of liver enzyme and bilirubin levels is required.

Table 1. Clinical Management of Antiviral-Associated Hepatotoxicity in HIV/HCV Coinfection



Sulkowski 2003d

There is no universal definition for hepatotoxicity. Different studies have used different parameters to define hepatotoxicity, ranging from AST and ALT levels that are 5–10 times the upper limits of normal to levels >10 times the upper limits of normal. When ALT and AST levels are elevated at baseline, definitions have ranged from >200 above baseline to 3.5 times the baseline amount. Adopting or developing a uniform characterization of hepatotoxicity, such as the AIDS Clinical Trials Group parameters (see Table 2) will benefit research and clinical care. Definitions of, and parameters for, liver toxicity must be included in coinfection treatment guidelines.

Table 2. AIDS Clinical Trials Group Parameters for Liver Transaminase Elevations*

	Grade 1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially life-threatening
AST (SGOT)	1.25–2.5 x ULN**	>2.5–5.0 x ULN	>5.0–10.0 x ULN	>10.0 x ULN
ALT (SGOT)	1.25–2.5 x ULN	>2.5–5.0 x ULN	>5.0–10.0 x ULN	>10.0 x ULN
GGT	1.25–2.5 x ULN	>2.5–5.0 x ULN	>5.0–10.0 x ULN	>10.0 x ULN
ALK PHOS	1.25–2.5 x ULN	>2.5–5.0 x ULN	>5.0–10.0 x ULN	>10.0 x ULN

AIDS Clinical Trials Group 1992

*For more information on liver transaminases, see Chapter IV, Diagnostics.
**ULN = upper limit of normal.

Antiretrovirals and Hepatotoxicity

Three classes of antiretroviral agents—protease inhibitors (PIs), nucleoside analog reverse transcriptase inhibitors (NRTIs), and non-nucleoside reverse transcriptase inhibitors (NNRTIs)—may cause hepatotoxicity, although their causal mechanisms may vary by class and by individual agent (Bonnet 2002; John 1998; Lana 2001; Martín-Carbonero 2002; M. Núñez 2001; Rutschmann 1998; Savès 1999; Sulkowski 2000a; Sulkowski 2000b).

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

All of the NNRTIs have been associated with elevations in liver enzymes. They may interfere with liver function and affect metabolism of drugs by inhibiting or inducing the liver's cytochrome P450 system. Drugs may build up to hepatotoxic levels, or be excreted too rapidly, leading to drug failure and the emergence of drug-resistant virus. The NNRTI nevirapine (Viramune®) may induce fulminant hepatitis, which can be fatal.

- Nevirapine

With nevirapine (Viramune®), potentially life-threatening clinical hepatitis may develop, with two-thirds of cases developing within six to twelve weeks after starting the drug. Symptoms may include fatigue, appetite loss, nausea, jaundice, a swollen or tender liver, rash, and fever, with or without elevated liver enzyme levels. In February of 2004, nevirapine's manufacturer, Boehringer Ingelheim, added to the boxed warning on the drug's label: "Severe, life threatening, and in some cases fatal hepatotoxicity, including fulminant and cholestatic hepatitis, hepatic necrosis and

hepatic failure, has been reported in patients treated with Viramune®. In some cases, patients presented with non-specific prodromal signs or symptoms of hepatitis and progressed to hepatic failure. These events are often associated with rash. Women, and patients with higher CD4 counts, are at increased risk of these hepatic events. Women with CD4 counts >250 cells/mm^3, including pregnant women receiving chronic treatment for HIV infection, are at considerably higher risk of these events. Patients with signs or symptoms of hepatitis must discontinue Viramune® and seek medical evaluation immediately." The manufacturer states, "Intensive clinical and laboratory monitoring, including liver function tests, is essential at baseline and during the first 18 weeks of treatment. The greatest risk of severe rash or hepatic events (often associated with rash) occurs in the first six weeks of therapy. However, the risk of any hepatic event, with or without rash, continues past this period and monitoring should continue at frequent intervals." (Boehringer Ingelheim. Dear Health Care Provider Letter. 2004a).

An analysis of data from several clinical trials revealed that the incidence of hepatic events was 12 times greater among women with CD4 cell counts >250 cells/mm 3 than in women with CD4 cell counts <250/mm^3 (11% vs. 0.9%). For men, the incidence of hepatic events was greatest in those with CD4 cell counts >400/mm^3 (6.3% vs. 2.3% among men with CD4 cell counts <400/mm^3). Some cases of hepatic adverse events were asymptomatic. Nevirapine discontinuation did not always stop progression of liver injury (Boehringer Ingelheim 2004a).
In a 468-person treatment trial that compared lamivudine (3TC; Epivir®) to emtricitabine (FTC; Emtriva™) in combination with stavudine (d4T; Zerit®) grade 4 liver enzyme elevations occurred among 9.4% (36/385) of those who received nevirapine vs. 0% of those who received efavirenz (Sustiva™). Grade 4 elevations occurred among 12% of females vs. 6% of males (P=0.05). Two participants died from liver failure (Bartlett 2001).

The risk for nevirapine-related hepatic adverse events is greater among individuals who have elevated liver enzymes at initiation of therapy, as well as in those who are coinfected with hepatitis C (Martínez 2001). Sulkowski and colleagues examined the incidence of nevirapine-related hepatotoxicity among 568 individuals, 43% (244/563) of whom were coinfected. They reported that only 32% of cases of nevirapine-related hepatotoxicity occurred during the first 12 weeks of therapy. Overall, nevirapine-related hepatotoxicity was observed in 19.3% (23/119) of coinfected individuals vs. 12.4% (17/137) of those with HIV alone (Sulkowski 2000a). In coinfected individuals, nevirapine plasma concentrations over 6 mcg/ml have been associated with a 92% risk for hepatotoxicity (Gonzalez 2002).

- Efavirenz

With efavirenz, Sulkowski and colleagues observed severe hepatotoxicity among 15.3% (19/124) of coinfected recipients vs. 3.2% (6/188) of those with HIV alone (RR 4.8; 95% CI, 2.0–11.8). Half of the episodes of efavirenz-associated hepatotoxicity occurred during the first twelve weeks of therapy. Adding a protease inhibitor to an efavirenz-containing regimen increased the likelihood of severe hepatotoxicity among coinfected persons (Sulkowski 2000a).

Table 3. ALT and AST Levels Before, During, and After Treatment by HCV Status and Use of Nevirapine or Efavirenz

	Nevirapine HCV +	Nevirapine HCV –	P value	Efavirenz HCV +	Efavirenz HCV –	P value
Baseline ALT	37 (24–61)	24 (14–41)	<0.0001	36 (25–52)	26 (19–41)	<0.0001
Maximum ALT	88 (47–134)	47 (28–99)	<0.0001	56 (32–98)	35 (21–55)	<0.0001
Change in ALT	37 (10–82)	16 (1–60)	<0.01	17 (-2–49)	6 (-6–24)	<0.01
Baseline AST	47 (35–70)	28 (20–44)	<0.0001	48 (36–73)	29 (22–40)	<0.0001
Maximum AST	84 (57–144)	45 (28–80)	<0.0001	84 (39–148)	37 (26–56)	<0.0001
Change in AST	32 (6–87)	10 (0–36)	<0.001	19 (-3–79)	6 (-5–21)	<0.001

Sulkowski 2002a

Protease Inhibitors (PIs)

All protease inhibitors (PIs) are metabolized in the liver. PIs may cause elevated liver enzyme levels at any time during treatment. Protease inhibitors affect hepatic metabolism by inhibiting or inducing the liver's cytochrome P450 system, so drugs may build up to hepatotoxic levels or be cleared too rapidly, leading to therapeutic failure and the emergence of drug resistance. Of the PIs, full-dose ritonavir (Norvir®) has most often been associated with elevated liver enzymes (Aceti 2002; Bonfanti 2001).

- **Full and Boosting Doses of Ritonavir**

Ritonavir is an extremely powerful cytochrome P450 CYP3A4 metabolic inhibitor, often used to boost levels of other protease inhibitors such as amprenavir (Agenerase ®), atazanavir (Reyataz™), fosamprenavir (Lexiva™), indinavir (Crixivan®), and saquinavir (Fortovase®). Ritonavir is coformulated with lopinavir in Kaletra®. It is rarely administered at the full dose of 600 mg twice daily due to poor tolerability and the availability of other treatment options.

Sulkowski and colleagues evaluated the incidence of severe hepatotoxicity from full-dose ritonavir among a cohort of 294 HIV-positive persons, 158 of whom were coinfected (53.7%). The overall risk for hepatotoxicity was fivefold greater from full-dose ritonavir than saquinavir, indinavir, and nelfinavir (Viracept®), regardless of HCV status. Severe hepatotoxicity occurred in 30% (6/30) of coinfected individuals receiving full-dose ritonavir, and among 30% (9/30) with HIV alone (Sulkowski 2000b).

Aceti and colleagues retrospectively analyzed hepatotoxicity among 1325 individuals, 731 HCV-coinfected, who received protease inhibitors as part of their HIV treatment regimen. HCV coinfection significantly increased the risk for hepatotoxicity after six months of HAART (OR 6.79; 95% CI, 3.66–9.16; P<0.0001). Liver toxicity (of any grade) occurred more frequently among coinfected persons receiving full-dose ritonavir than in those with HIV alone (P=0.006), and more often with full-dose ritonavir than other protease inhibitors (26.1% (17/65) for ritonavir vs. 17.3% (9/52) for nelfinavir, 16.5% (41/248) for saquinavir, and 11.7% (44/375) for indinavir). Severe liver toxicity

occurred more frequently in coinfected persons taking full-dose ritonavir (20% (13/65)) than any other protease inhibitor (5% (12/248) for saquinavir; 1.86% (7/375) for indinavir; and 0% (0/52) for nelfinavir) (Aceti 2002).

Low-dose ritonavir has also been associated with an increased risk for hepatotoxicity in coinfected persons. In an analysis of the incidence of liver toxicity among 120 individuals (52 coinfected) after initiation of a regimen containing lopinavir/ritonavir (Kaletra®), Soriano and colleagues reported a 4% overall cumulative incidence of grade 3 and grade 4 hepatotoxicity at 12 months. The cumulative incidence of hepatotoxicity rose to 8% among coinfected individuals, although there was no significant difference in lopinavir/ritonavir levels by HCV status (Soriano 2003).

Sulkowski and colleagues reported hepatotoxicity data from 1,061 HIV-positive persons, 488 (46%) coinfected. Although 405 (83%) coinfected persons did not experience hepatotoxicity, their risk for developing hepatotoxicity was twice as high as that of the HIV-positive, HCV-negative group. The risk was greatest for those receiving combinations including higher doses of ritonavir (Sulkowski 2003c).

Table 4. Incidence of Hepatotoxicity (Grade 3/4) by HCV Status and Antiretroviral Agent

	Nelfinavir N=605	Lopinavir + 200 mg RTV* N=89	Indinavir + 200-400 mg RTV** N=92	Saquinavir + 400 mg RTV*** N=273
All	11.1%	9%	12.8%	17.2%
HCV/HIV coinfected	15.8%	12.8%	14.8%	26.2%
HIV monoinfection	6.5%	6%	10%	11.4%

Sulkowski 2003c

*Lopinavir is coformulated with 100 mg of ritonavir and is taken twice daily.
**Indinavir can be combined with different doses of ritonavir; in this study, ritonavir doses ranged from 200 to 400 mg/day.
***Saquinavir has been combined with different doses of ritonavir; in this study, participants received 400 mg of each per day.

Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

The NRTIs are not metabolized through the cytochrome P450 system. These drugs can cause hepatotoxicity by damaging mitochondria, the small, rod- or oval-shaped bodies inside of cells that produce energy. NRTI-induced mitochondrial dysfunction may cause hyperlactatemia (mild to serious elevations in serum lactate levels), sometimes accompanied by liver enzyme elevations and hepatic steatosis (liver tissue degeneration marked by fat globules in the cells) (Lonergan 2000). Lactic acidosis—a rare but life-threatening consequence of mitochondrial toxicity—has been linked with the use of all NRTIs, and particularly with zalcitabine (ddC; Hivid®), didanosine (ddI; Videx®), stavudine, and zidovudine (AZT; Retrovir®) (Boubaker 2001; Brinkman 2000; Falcó 2002; Fortgang 1995; John 2001; Marceau 2003; K. D. Miller 2000). The symptoms of lactic acidosis may include weakness, abdominal pain and distention, nausea, vomiting, diarrhea, muscle aches, ascending neuromuscular weakness, elevations in liver enzyme levels, and shortness

of breath. Severe NRTI-induced lactic acidosis is often accompanied by hepatic steatosis, hepatomegaly (swollen liver), and hepatic failure (Bienvenu 2001; Fortgang 1995; K. D. Miller 2000; Mokrzycki 2000; Olano 1995).

Mitochondrial abnormalities have been detected in liver tissue from individuals using NRTIs (Batisse 2002; Van Huyen 2003). In addition, hepatitis C has been associated with mitochondrial abnormalities in liver tissue (Barbaro 1999). NRTIs may exacerbate HCV-induced mitochondrial toxicity. Verruci and colleagues examined liver tissue samples from 39 coinfected individuals, 34 of whom had been using antiretroviral therapy (including NRTIs) for a median duration of 8.7 years; the other 5 had never been treated for HIV. All 34 had mitochondrial abnormalities in their liver tissue, as did 3/5 in the untreated group. Although some abnormalities (polymorphism, crystalline inclusions, and hyperplasia) were found in all 39, certain abnormalities (glycogen inclusions, reduction or loss of cristae, and reduction of matrix density) were present only among those who had received antiretroviral therapy (Verucchi 2004). These data are limited by small sample size of the treatment-naïve group and the absence of a comparator group with HCV monoinfection. A larger study is warranted.

- **"D" Drugs: Didanosine (ddI), Stavudine (d4T), and Zalcitabine (ddC)**

Walker and colleagues examined serum lactate levels and liver tissue samples from 94 people with hepatitis C, 80 coinfected. Participants were grouped as coinfected, no antiretroviral therapy at biopsy (N=11); coinfected, uninterrupted antiretroviral therapy (N=69); and HCV-monoinfected (N=14). Thirty-four coinfected individuals were using a "d" drug (didanosine (ddI), stavudine (d4T), zalcitabine (ddC)) as part of their antiretroviral therapy. Their hepatic mitochondrial DNA (mtDNA) was significantly decreased ($P<0.0001$ for "d" drugs vs. non-"d" drugs). Dual "d" drug therapy further reduced hepatic mtDNA by 53% ($P<0.0001$ for stavudine plus didanosine vs. stavudine alone). There was no significant difference in hepatic mtDNA levels by HIV status or use of antiretroviral therapy without "d" drugs, and the difference in mtDNA could not be attributed to any other variables. Only three individuals had lactate levels equal to, or above, the upper limits of normal. All were using stavudine, and two were also using didanosine; their hepatic mtDNA levels were significantly lower than all untreated participants ($P=0.003$) as well as the other 31 individuals using "d" drugs ($P=0.017$). Serum lactate levels at liver biopsy were higher among those using antiretroviral therapy than among untreated persons; among treated persons, there were no significant differences in lactate levels by antiretroviral agent (Walker 2004).

Drug Interactions

Inhibiting or inducing hepatic drug metabolism via the cytochrome P450 system leads to drug interactions between antiretroviral agents and other drugs. Ritonavir, in particular, is a potent inhibitor of the hepatic metabolism of other drugs, which "…may create the potential for serious and/or life threatening reactions such as cardiac arrhythmias, prolonged or increased sedation, and respiratory depression" (Abbott 2003).

Alterations in the capacity for hepatic metabolization of antiretrovirals may result in accumulation of drugs to toxic levels, decreases to suboptimal levels or increased potential for interactions, side effects, and toxicities. There is abundant potential for interactions among antiretroviral agents;

drugs used as prophylaxis for opportunistic infections; ancillary medications for the complications of HIV disease and side effects of antiretroviral therapy; hormonal contraceptives; and treatments for other conditions including methadone, anti-anxiety drugs, vitamins, supplements, and herbal preparations.

For more information on specific drug-drug interactions among medications used for treatment of hepatitis C, antiretroviral agents, and immunosuppressants, see Chapter VII, HCV Treatment in HIV/HCV Coinfection.

Pharmacokinetics of Antiretroviral Agents

The liver's capacity to metabolize drugs may be compromised by damage from hepatitis C. George and colleagues compared 50 cirrhotic livers (which were removed during transplantation) and 21 normal livers. They discovered a significant reduction in cytochrome P450 in the cirrhotic livers (George 1995). Although the livers studied were damaged enough to warrant transplantation, and HCV was not the only cause of cirrhosis, this research sheds some light on metabolic impairment resulting from liver damage. The impact of liver damage on antiretroviral drug levels has not been adequately explored; only a small body of data is available. More research is required.

Table 5. Dosing Recommendations for Persons with Hepatic Dysfunction

Nucleoside Reverse Transcriptase Inhibitors	Daily Dose	Dosing in hepatice impairment
Abacavir (*Ziagen)*	300 mg bid	No dosage recommendation
Didanosine (*Videx)*	Weight >60 kg: 400 mg qd or 200 mg bid Weight <60 kg: 250 mg qd or 125 mg bid	No dosage recommendation
Emtricitabine (*Emtriva)*	200 mg qd	No dosage recommendation
Lamivudine (*Epivir)*	300 mg qd or 150 mg bid	No dosage recommendation
Stavudine (*Zerit)*	Weight >60 kg: 40 mg bid Weight <60 kg: 30 mg bid	No dosage recommendation
Tenofovir (*Viread)*	300 mg qd	No dosage recommendation
Zalcitabine (*Hivid)*	0.75 mg tid	No dosage recommendation
Zidovudine (*Retrovir)*	300 mg bid	No dosage recommendation

Non-Nucleoside Reverse Transcriptase Inhibitors	Daily Dose	Dosing in hepatice impairment
Delavirdine (*Rescriptor)*	400 mg tid	No dosage recommendation; use with caution in patients with hepatic impairment
Efavirenz (*Sustiva)*	600 mg qd	No dosage recommendation; use with caution in patients with hepatic impairment
Nevirapine (*Viramune)*	200 mg bid	No data available; avoid use in patients with moderate to severe hepatic impairment

Protease Inhibitors	Daily Dose	Dosing in hepatice impairment	
Amprenavir (*Agenerase)*	1200 mg bid	Child-Pugh Score	Dose
		5–8	450 mg bid
		9–12	300 mg bid
Atazanavir (*Reyataz)*	400 mg qd	Child-Pugh Class	Dose
		Class B	300 mg qd
		Class C	not recommended
Fosamprenavir(*Lexiva)*	1,400 mg bid	Child-Pugh Score	Dose
		5–8	700 mg bid
		9–12	not recommended
Indinavir (*Crixivan)*	800 mg q8h	For mild-to-moderate hepatic insufficiency due to cirrhosis: 600 mg q8h	
Lopinavir/Ritonavir (*Kaletra*)	400/100 mg bid (coformulated)	No dosage recommendation; use with caution in patients with hepatic impairment	
Nelfinavir (*Viracept*)	1250 mg bid	No dosage recommendation; use with caution in patients with hepatic impairment	
Ritonavir (*Norvir*)	600 mg bid	No dosage adjustment in mild hepatic impairment; no data for moderate to severe impairment, use with caution	
Saquinavir (*Fortovase*) soft gel cap	1200 mg tid	No dosage recommendation; use with caution in patients with hepatic impairment	

Fusion Inhibitor	Daily Dose	Dosing in hepatice impairment
Enfuvirtide (*Fuzeon)*	90 mg SQ q 12h	No dosage recommendation

Department of Health & Human Services 2004

- **Nucleoside Reverse Transcriptase Inhibitors**

  - **o Abacavir**

There are no data on the pharmacokinetics of abacavir (Ziagen®) in persons with hepatic impairment.

  - **o Didanosine**

There are no data on the pharmacokinetics of didanosine in persons with hepatic impairment.

  - **o Emtricitabine**

The pharmacokinetics of emtricitabine have not been studied in persons with hepatic impairment (Gilead package insert 2003).

o Lamivudine

The pharmacokinetics of lamivudine did not differ in individuals with hepatic impairment; safety and efficacy for individuals with hepatic decompensation have not been evaluated (Glaxo Smith Klein package insert 2002).

o Stavudine

The pharmacokinetics of a single 40 mg dose of stavudine have been evaluated in five HIV-negative individuals with cirrhosis (Child-Pugh Class B and C); the pharmacokinetics of stavudine were unaltered in these individuals (Bristol Myers Squibb package insert 2002).

o Tenofovir

The pharmacokinetics of a single 300 mg dose of tenofovir have been evaluated in 24 HIV-negative persons; 7 had moderate hepatic impairment, 8 had severe hepatic impairment, and the other 8 were unimpaired controls. The pharmacokinetics of tenofovir were not substantially altered in persons with moderate or severe hepatic impairment (Kearney 2004).

o Zalcitabine

No data are available on the pharmacokinetics of zalcitabine in persons with hepatic impairment.

o Zidovudine

Data on the pharmacokinetics of zidovudine in persons with hepatic impairment are limited; because it is metabolized in the liver, dose reduction may be necessary (Glaxo Wellcome package insert 2001).

- **Non-Nucleoside Reverse Transcriptase Inhibitors**

o Delavirdine

Delavirdine (Rescriptor ®) has not been studied in persons with hepatic impairment.

o Efavirenz

A study monitoring levels of efavirenz reported that 50% (6/12) of coinfected participants had minimum concentrations (C_{min}) that were above the upper limit of the therapeutic range. The dosing in this group was decreased from 600 mg qd to 400 mg qd; after a mean interval of six months, all had maintained undetectable HIV RNA (Jeantils 2003).

o Nevirapine

The pharmacokinetics of a single 200 mg dose of nevirapine have been studied in 10 HIV-negative individuals: 6 had mild hepatic impairment, and 4 had moderate hepatic impairment.

One individual with moderate hepatic impairment had a significant increase in the area under the curve (AUC; a measurement of the total amount of a drug in blood) of nevirapine. Nevirapine is not recommended for persons with severe hepatic impairment (Boehringer Ingelheim package insert 2004b). Vigilant monitoring for signs and symptoms of clinical hepatitis and elevated liver enzyme levels, especially during the first 18 weeks of treatment, is crucial. Nevirapine can cause severe and life-threatening hepatic events.

- **Protease Inhibitors**

    - **o Amprenavir and Fosamprenavir**

Amprenavir has been evaluated in persons with hepatic impairment. Veronese and colleagues compared drug levels in three groups of ten: a control group (no cirrhosis), a moderate cirrhosis group, and a severe cirrhosis group. A linear relationship between the AUC of amprenavir and the severity of liver disease was identified; with AUC increased 2.5-fold in the group with moderate cirrhosis, and by 4.5-fold in the group with severe cirrhosis (Veronese 2000); see Table 5 for dosing recommendations.

Fosamprenavir, a prodrug of amprenavir, has not been evaluated in persons with hepatic impairment, nor are there any data on ritonavir-boosted fosamprenavir.

o Atazanavir

The concentration of atazanavir is increased in people with hepatic impairment. After a single 400 mg dose of atazanavir, the AUC was 42% greater in 16 individuals with moderate to severe hepatic impairment than that of study volunteers without hepatic impairment. Hepatic impairment decreased clearance of atazanavir; its mean half-life was 12.1 hours vs. 6.4 hours in persons without hepatic impairment. Individuals with "…underlying hepatitis B or C viral infections or marked elevations in liver transaminases prior to treatment may be at risk for developing further transaminase elevations or hepatic decompensation" (Bristol Myers Squibb package insert 2003). There are no data on ritonavir-boosted atazanavir in persons with hepatic impairment.

o Indinavir

Indinavir has been studied in 12 people with mild-to-moderate hepatic impairment and evidence of clinical cirrhosis. After one 400 mg dose, the AUC was approximately 60% greater and the half-life of indinavir was extended (2.8 $\pm$ 0.5 hours vs. 1.8 $\pm$ 0.4 hours with no hepatic impairment) (Merck package insert 2001). There are no data on ritonavir-boosted indinavir in persons with hepatic impairment.

o Lopinavir/Ritonavir

Levels of lopinavir/ritonavir (LPV/r) are higher in persons with HCV-related liver disease. Arribas and colleagues conducted a pharmacokinetic evaluation of drug levels among 24 individuals (12 HIV-positive controls; 6 HCV-coinfected with mild hepatic impairment and 6 HCV-coinfected with moderate hepatic impairment). They reported that the AUC of lopinavir was 0.98–1.96 times

greater in mild hepatic impairment and 0.89–1.76 times greater with moderate hepatic impairment. The AUC of ritonavir was 41% greater with mild hepatic impairment and 185% higher with moderate hepatic impairment (Arribas 2003).

o Nelfinavir

Regazzi and colleagues studied the clinical pharmacokinetics of nelfinavir. They looked at plasma concentrations of the drug in 42 HIV-positive individuals, and two groups of HIV/HCV-coinfected individuals (24 without cirrhosis and 14 with cirrhosis). Nelfinavir levels were significantly higher in both groups of coinfected persons. The AUC increased by 58% in coinfected non-cirrhotics and by 155% in coinfected cirrhotics (Regazzi 2003). The implications for dosing are unclear; a reduced dose of nelfinavir could result in suboptimal drug levels. There are no data on ritonavir-boosted nelfinavir in persons with hepatic impairment.

o Saquinavir

The pharmacokinetics of saquinavir and ritonavir-boosted saquinavir have not been studied in people with hepatic impairment.

- **Fusion Inhibitors**

o Enfuvirtide

The pharmacokinetics of enfuvirtide (T-20; Fuzeon®) in persons with hepatic impairment have not been studied.

Immune Reconstitution and Hepatotoxicity

Transient flares of hepatitis after initiation of HAART—characterized by significant increases in liver enzyme levels and in some cases, symptomatic hepatitis—may be the result of HAART-mediated immune reconstitution. John and colleagues reported two cases of symptomatic hepatitis after initiation of HAART. One had been taking dual nucleoside analog reverse transcriptase inhibitors; adding a protease inhibitor increased the CD4 cell count from 266/mL to 416/mL. The other had a history of multiple opportunistic infections; therapy with two nucleoside analog reverse transcriptase inhibitors and a protease inhibitor increased the CD4 cell count from 32/mL to 138/mL. Although both were anti-HCV-negative prior to initiation of HAART, a look at stored samples of their plasma revealed that each had detectable HCV RNA prior to initiating HAART. Both individuals developed antibodies to HCV after an immunological response to HAART (CD4 cell increase of >100 after four weeks of therapy) (John 1998).

The degree of virological and immunologic responses to HAART has been associated with hepatotoxicity in some studies, although this association remains controversial. Gavazzi and colleagues reported that increased liver enzyme levels occurred only in coinfected individuals with persistently undetectable HIV RNA after initiation of HAART. The achievement of undetectable HIV RNA levels correlated with an increase in CD8 cells. The authors speculate that these cytotoxic CD8 cells could be responsible for liver enzyme elevations and suggested further analysis of immunologic

and histological parameters (Gavazzi 2000).

Puoti and colleagues examined the incidence of severe hepatotoxicity (defined as ≥ 10 times the upper limit of normal or, if elevated at baseline, ≥ 5 times the amount of baseline) after initiation of antiretroviral therapy among a cohort of 755 HIV-positive individuals, 513 coinfected. Severe hepatotoxicity was experienced by 26 individuals, 25 of whom were coinfected. Overall, 5% (25/513) of coinfected persons experienced severe hepatotoxicity; 58% (15/26) with baseline CD4 cell counts of $<200/mm^3$. Seven of the individuals who experienced severe hepatotoxicity died from liver failure. A direct correlation between peak elevations in ALT levels and CD4 cell increase from baseline was observed ($P<0.001$). Paired pre- and post-treatment hepatotoxicity biopsy samples were available from 2 individuals; both showed worsening of HCV disease (Puoti 2003).

However, immune-mediated exacerbation of hepatitis C is not a universal phenomenon, and the contribution of immune reconstitution to hepatotoxicity remains controversial. Zylberberg and colleagues examined paired biopsy samples (pre-HAART and after 12 months of antiretroviral therapy) from 25 coinfected individuals, finding no significant relationship between immune reconstitution and histological progression of HCV disease (Zylberberg 2003). Martín-Carbonero and colleagues retrospectively studied liver injury among 42 coinfected persons. They did not identify an association between elevated liver enzymes and increases in HCV RNA, or virological and immunological responses to HAART. They measured liver enzyme levels, HIV- and HCV RNA levels and CD4 cell counts at baseline, and every three months for at least six months after initiation of antiretroviral therapy. Those who developed hepatotoxicity had their HIV and HCV RNA levels measured, and a CD4 cell count when their liver enzymes reached a peak. Baseline and peak liver enzyme measurements were compared with measurements in those who did not experience hepatotoxicity. Although immune reconstitution syndrome occurred more frequently in coinfected persons who initiated HAART with baseline CD4 cell counts of $<200/mm^3$, liver enzyme elevations did not (Martín-Carbonero 2002).

The extent of immunologic and virological responses to HAART may influence the likelihood of developing hepatotoxicity. After one year of HAART, Aceti and colleagues found that hepatotoxicity was more common in coinfected persons with no increase in CD4 cell counts than those with CD4 cell increases (20.9% vs. 10.2%; $P=0.017$). Additionally, liver toxicity was more common in coinfected individuals who had increases in HIV RNA after a year of HAART than in those who had stable or decreasing HIV RNA levels (34% vs. 13%; $P=0.18$) (Aceti 2002).

HAART may have beneficial or detrimental effects on ALT levels, according to individual characteristics. Torre and colleagues retrospectively studied CD4 cell counts, HIV and HCV RNA levels, and changes in ALT levels among 323 coinfected individuals over a follow-up period of at least two years. Individuals with normal baseline ALT had significant increases in ALT at 12, 18, 24, and 30 months, while those with elevations in ALT >4 times the upper limit of normal at initiation of HAART showed significant decreases in ALT at 12, 18, 24, 30, and 36 months. Participants with immune recovery—defined as an increase in CD4 cell count of ≤ 200– ≥ $400/mm^3$—had significant increases in ALT 6 months after initiation of therapy, but levels decreased for the 36 months of follow-up (Torre 2001).

Table 6. Duration of HAART, CD4 Cell Increases, and Changes in ALT

Months on HAART	# w/immune recovery*	Increase in DC4	Change in ALT
6	8	467.7 ± 288.1	+65.0 ± 51.3
12	12	450.0 ± 125.2	-21.0 ± 84.1
18	25	449.1 ± 154.7	-22.0 ± 76.6
24	25	493.4 ± 153.8	-31.8 ± 62.9
30	16	549.2 ± 203.5	-15.6 ± 59.0
36	6	537.8 ± 65.8	-57.7 ± 84.4

Torre 2001

HCV Genotype and Hepatotoxicity

Hepatitis C genotype may contribute to the risk for hepatotoxicity. Núñez and colleagues evaluated 70 coinfected individuals initiating HAART. They discovered that those with HCV genotype 3 were at greater risk for developing any grade of liver enzyme elevation than individuals with genotypes 1, 2, or 4. Severe liver enzyme elevations (defined as either ≥ 5 times above the upper limit of normal, or, when baseline ALT and AST levels were abnormal, elevations ≥ 3.5-fold above baseline) were more frequent among individuals with HCV genotype 3 (OR, 4.4; 95% CI, 1.2–16.1; P=0.02). The incidence of severe liver enzyme elevations by genotype was as follows: genotype 1, 13% (5/39); genotype 2, 0% (0/3); genotype 3, 33% (7/21); and genotype 4, 0% (0/7). In a multivariate analysis, the only significant factors associated with the development of severe hepatotoxicity were heavy alcohol intake and HCV genotype 3 (P=0.004 for heavy alcohol use; P=0.01 for HCV-3) (M. Núñez 2002). Another study, however, did not find a relationship between transaminase elevations and HCV genotype (Gavazzi 2000).

Metabolic Complications: HCV and HAART

Careful selection of antiretroviral regimens for coinfected persons is warranted, as risk for developing HAART-related metabolic complications (lipoatrophy, fat redistribution, pre-diabetic conditions such as insulin resistance, glucose intolerance, and hyperglycemia, as well as diabetes itself) appears to increase with HCV coinfection (Crane 2004; Duong 2001; Mehta 2003b; Patroni 2002; Rodriguez-Guardado 2003; Torti 2002; Zylberberg 2000). Conversely, coinfection with HCV may decrease total serum cholesterol, low-density lipoprotein cholesterol (LDL), and triglycerides.

Lipoatrophy and Fat Redistribution

Lipoatrophy may occur more frequently in coinfected individuals. Zylberberg and colleagues examined a cohort of 226 HIV-positive individuals (46 coinfected) for lipodystrophy, which they classified as pure lipoatrophy, pure truncal adiposity, and a mixed syndrome. Although prevalence of lipodystrophy did not differ by HCV status, pure lipoatrophy was significantly more prevalent among coinfected individuals (46.2% vs. 27.6% of those with HIV alone; P<0.03) (Zylberberg 2000). Duong and colleagues also found an association between hepatitis C, fat redistribution, and other metabolic abnormalities. They analyzed data from 226 individuals; 121 had HCV

monoinfection, and 105 were HIV-positive, using antiretroviral therapy. Of the 105, 29 were coinfected. Although the coinfected participants had a greater duration of HIV infection, there was no significant difference in duration of antiretroviral therapy between groups. Pure lipoatrophy was significantly more common among coinfected persons than in those with HIV alone (41% (12/29) vs. 14% (11/76); P=0.006) (Duong 2001).

Rodriguez-Guardado and colleagues retrospectively analyzed the development of lipodystrophy among 88 persons receiving antiretroviral therapy, 51 coinfected. In this study, lipodystrophy was classified as facial and/or limb lipoatrophy, with or without increased fat in the abdomen. Baseline characteristics—CD4 cell count, HIV RNA, sex, and age—did not differ significantly between groups, nor did use of specific nucleoside analog reverse transcriptase inhibitors. Lipodystrophy was more common among coinfected persons than in those with HIV alone (45% (23/51) vs. 30% (11/37); P=0.0031) (Rodriguez-Guardado 2003).

Although these results are preliminary, they merit further investigation in larger studies.

Insulin Resistance, Glucose Elevations, and Diabetes

HCV itself has been associated with diabetes; individuals with HCV monoinfection are 11 times more likely to develop diabetes than those who do not have HCV (Mehta 2003a). Diabetes is more prevalent among coinfected people than in those with HIV alone. Butt and colleagues assessed the prevalence of actual diabetes among a cohort of 41,262 HIV-positive male veterans, 17.9% (7,386) HCV-coinfected. Diabetes was significantly more prevalent in coinfected persons (19.7% vs. 13.7% in those with HIV alone; P<0.001) (Butt 2003). Crane and colleagues retrospectively studied new cases of diabetes among 699 HIV-positive patients of an urban clinic; from 1996 to 2003, 40 of those individuals developed diabetes. New-onset diabetes developed significantly more often in coinfected people than in those with HIV alone (43% vs. 23%; P<0.01), and coinfection increased the odds for developing diabetes 2.1-fold (95% CI, 1.1–4.2) (Crane 2004).

HCV coinfection and use of protease inhibitors have been associated with an increased risk for hyperglycemia (elevated glucose levels in the blood; a sign of diabetes). In a retrospective analysis of data from 1,230 individuals who initiated HAART between 1996 and 2002, hyperglycemia was more prevalent among coinfected individuals (5.9% vs. 3.3%; P=0.03) than in those with HIV alone. New-onset hyperglycemia also occurred more frequently among coinfected persons (5.8% vs. 2.8%; P=0.01) than in those with HIV alone. Both HCV coinfection and use of a protease inhibitor were independent risk factors for developing hyperglycemia (adjusted relative hazard (ARH), 5.02; 95% CI, 1.39–18.16 for protease inhibitor therapy and ARH 2.28; 95% CI, 1.23–4.22 for coinfection). Coinfected individuals who received a protease inhibitor had the greatest incidence of hyperglycemia (5.6 cases per 100 person-years) (Mehta 2003b).

HAART increases the risk for diabetes and it has been implicated in the development of two conditions that are precursors to diabetes: insulin resistance and glucose intolerance (Dube 2000; Hardy 2001; Metha 2000). HCV coinfection appears to contribute to the risk. Duong and colleagues identified a relationship between insulin resistance, antiretroviral therapy, and HCV in a study of 226. Of the 226, 105 were taking HAART and 29 were coinfected; 121 had HCV alone. Insulin resistance was significantly higher in individuals with HCV, both mono- and coinfected

(0.21 ± 0.34 for HCV monoinfection; 0.25 ± 0.28 for coinfection) than in those HIV alone (0.04 ± 37; P=0.01 and P=0.03, respectively). HCV coinfection—in the context of antiretroviral therapy— was linked significantly with the development of insulin resistance (OR, 8.9; 95% CI, 2.61–110.29; P=0.003).

Cholesterol and Triglycerides

HCV coinfection appears to exert an inhibitory effect on total serum cholesterol, low-density lipoprotein cholesterol (LDL), and triglycerides. This may occur because of hepatic metabolic dysfunction. A number of other factors may contribute as well, including diet, heredity, age, sex, race, and antiretroviral regimen. The hepatitis C virus itself may have an effect on cholesterol levels. A part of hepatitis C's envelope, called E2, binds with serum lipoproteins. Low-density lipoproteins carry cholesterol from the liver into the tissues via the bloodstream, and high-density lipoproteins (HDL) recycle cholesterol from the tissues to the liver via the bloodstream. When hepatitis C virions bind with lipoproteins, the uptake of LDL may increase, thereby lowering serum cholesterol levels (Wünschmann 2002).

Torti and colleagues retrospectively analyzed changes in cholesterol and triglyceride levels after initiation of HAART in a cohort of 205 HIV-positive individuals, 112 HCV-antibody positive. Median follow-up was 21.4 months. By multivariate analysis, the presence of antibodies to HCV was negatively associated with cholesterol (P<0.0001) (Torti 2002). Another retrospective study of elevations in cholesterol among 282 individuals on their first HAART regimen reported that the presence of antibodies to HCV was significantly associated with smaller increases in cholesterol (P<0.001), regardless of antiretroviral regimen (Patroni 2002). These findings are supported by data from the Veterans Aging Cohort 3 Site Study (VACS 3), which found an independent association with lower levels of both LDL and HDL cholesterol and HCV coinfection among HIV-positive individuals receiving antiretroviral therapy (P<0.001) (Stapleton 2002).

Recommendations

Develop guidelines for the care and treatment of HIV/HCV-coinfected individuals.

Despite the resources available to physicians who care for coinfected individuals in the United States, no one has yet integrated the recommendations from the Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents (DHHS 2003), the National Institutes of Health's 2002 Consensus Statement on the Management of Hepatitis C (NIH 2002) and the Practice Guidelines for Diagnosis, Management and Treatment of Hepatitis C (Strader 2004) into specific recommendations for care and treatment of persons with HIV/HCV coinfection. Care and treatment for HIV/HCV coinfection is not always consistent or well coordinated; some infectious disease doctors providing care for both HIV and HCV may be more focused on HIV disease. Referral to a gastroenterologist is not always feasible. When referral is possible, the gastroenterologist may not be well informed about the clinical management of HIV. Adapting, integrating, and updating existing recommendations under the aegis of the DHHS Guidelines Panel could address these concerns. Coinfection-specific care and treatment guidelines would provide an essential resource for coinfected individuals and their clinicians, as well as for treatment educators and advocates.

Investigate treatment strategies for HIV/HCV coinfection.

It is still unclear when antiretroviral therapy should be initiated in coinfected individuals. Some studies have found a blunted immune response to HAART in coinfected individuals (Greub 2000; Torriani 2001). Earlier initiation of HAART may help to preserve immune function and delay HCV disease progression, as end-stage liver disease (ESLD) occurs more frequently in individuals with low CD4 cell counts (Goedert 2002; Ragni 2001). Thus, for coinfected individuals, it is now critical to investigate whether earlier initiation of HAART—possibly at higher CD4 cell counts than today's recommended threshold of 200–350 CD4 cells/mm^3—will decrease the progression rate and incidence of ESLD among coinfected individuals. Alternatively, since it may be possible to eradicate HCV in people who experience a sustained virological response (SVR) to therapy, early initiation of HCV treatment in HIV-positive individuals should also be explored. Preemptive treatment of HCV, even if an SVR is not achieved, may improve tolerability of antiretroviral agents; this requires Congress and NIH to allocate sufficient resources to carry out long-term treatment strategy studies.

Establish a universal definition for hepatotoxicity and characterize its severity.

Coinfected individuals often have elevated liver enzyme levels, which may be due to both liver disease and hepatotoxicity of anti-HIV medications (Staples 1999). A universal definition of hepatotoxicity for research and clinical practice is needed to increase the consistency and interpretability of results from clinical trials, to guide antiretroviral treatment decisions, and to enable the collection of consistent adverse event data.

Hepatitis C coinfection significantly increases the risk of hepatotoxicity from HAART (Lana 2001). In some cases, individuals must discontinue HAART altogether because of liver toxicity; in other instances, only a particular drug must be switched. Hepatotoxicity has multiple causes, such as

underlying liver diseases unrelated to HCV, fatty liver, alcohol consumption, genetic variation, antiretrovirals, and other medications.

We need to be able to differentiate between transient elevations in liver enzymes and clinically significant indicators of progressive liver damage. Studies must include liver biopsy, so that the relationship between liver histology and hepatotoxicity may be more fully characterized. NIH and industry must support hepatotoxicity research.

Explore pharmacokinetics and drug levels in coinfected individuals.

Up to 90% of HIV-positive individuals receive at least one hepatotoxic drug (Orenstein 2002). The potential for drug interactions in HIV-positive individuals is abundant even without hepatitis C coinfection; often, people take hormonal contraceptives, lipid-lowering agents, methadone or buprenorphine, anti-anxiety medications, prophylaxis against opportunistic infections, vitamins, herbs, supplements, and antiretrovirals.

The liver metabolizes several important antiretrovirals; HCV-related liver damage may compromise the liver's ability to metabolize these drugs. HIV drug levels may be elevated in individuals with liver disease, increasing side effects, interactions, and toxicities. Alternatively, levels can also decrease, allowing loss of viral suppression. The incidence and severity of complications from antiretroviral therapy among coinfected individuals needs further investigation and documentation. The contribution of a specific class or agent to interactions, side effects, and complications also needs further exploration. Drug interactions between antiretrovirals and drugs used for opiate addiction treatment must be characterized. The FDA must mandate pharmacokinetic evaluation of antiretroviral agents in coinfected persons and individuals with hepatic impairment prior to approval.

List of Terms Used in This Chapter

Area under the curve (AUC): a measurement of the total amount of a drug in blood, defined by graphing the change in drug levels over a 24-hour period, then calculating the area under the curve.

BID: twice daily.

Cristae: distinctly structured folds projecting from the inner membrane into the matrix of mitochondria in liver cells.

Crystalline inclusions: an accumulation of crystals inside of a cell.

CYP3A4: one of six isoenzymes responsible for 90% of the oxidative metabolism of drugs in human beings. CYP designates the root, 3, the family, A, the subfamily and 4 designates the gene.

Cytochrome p450 (CYP): the CYP isoenzymes are involved with the metabolism of drugs and other substances. These are located mainly in liver cells where most CYP metabolism occurs, although some are present in the small intestine, kidney, lungs and brain.

Glycogen inclusions: an accumulation of glycogen in the liver.

Hepatic Steatosis: degeneration of liver tissue marked by accumulation of fat globules.

Hepatomegaly: swollen liver.

Hyperlactatemia: mild to serious elevations in serum lactate levels.

Hyperplasia: abnormal increase of cell growth in tissue and/or organs.

Lactic acidosis: a rare but life-threatening sequelae of mitochondrial toxicity, lactic acidosis occurs when lactate acid levels accumulate in the bloodstream.

Lipoatrophy: loss of fat under the skin.

Lipodystrophy: abnormal metabolism of fat, resulting in loss or accumulation of fat under the skin.

Matrix: the area between the inner and outer membrane of mitochondria in liver cells.

Polymorphism: occurring in many different forms.

Pro-drug: an inactive precursor to a pharmacologically active metabolite.

Q8H: every eight hours.

Q12H: every twelve hours.

QD: once daily.

SQ: subcutaneous; an injection under the skin.

TID: three times daily.

Truncal adiposity: fat in the main part of the body.

VII. Hepatitis C Treatment in HIV/HCV Coinfection

The burden of liver disease is too high to delay management of HIV/HCV coinfected persons while awaiting better data. Instead, the management of hepatitis C today must be based on data generated on persons without HIV and an understanding of both infections.

—David L. Thomas
Hepatology

Summary

Hepatitis C progresses more rapidly in coinfected persons (see Chapter III, Natural History of HIV/HCV Coinfection); consequently, the need for information about the safety and efficacy of HCV treatment in coinfection is urgent. Three key studies of HCV treatment in coinfected persons—the Adult AIDS Clinical Trials Group's A5071, Roche's Apricot and the ANRS HC02 (RIBAVIC)—have reported that pegylated interferon-based regimens are more effective than standard interferon-based regimens, although sustained virological response rates have been lower in these coinfection treatment trials than in HCV monoinfection treatment trials (Chung 2002b; Chung 2004; Perronne 2002; Perronne 2004; Torriani 2004).

Tolerability of and access to treatment for HCV are pressing issues for coinfected people, who tend to experience more severe side effects from HCV treatment. Significant interactions between ribavirin and didanosine (ddI; Videx®) have been reported, and ribavirin may exacerbate toxicities of other antiretrovirals. Discontinuations from clinical trials of pegylated interferon-based regimens have been a significant problem; dropout rates have been as high as 30–46% (Hopkins 2002; Perronne 2002; Rockstroh 2002). Access to HCV treatment is limited: currently, only 14 state AIDS Drug Assistance Programs (ADAPs) in the U.S. have been able to add pegylated interferon and ribavirin to their formularies. Twenty-three states have no plans to cover these drugs in the future, due to financial constraints (National ADAP Monitoring Project 2004). Insufficient federal and state funding of ADAPs may further limit access to HCV treatment. Expected cuts in Medicaid funding may also limit access to HCV treatment for many coinfected individuals in the U. S.

Up to 90% of people who acquired HIV from injection drug use are coinfected with hepatitis C (CDC 2002f). Although injection drug use is no longer a contraindication for HCV treatment, injection drug users still encounter significant barriers to obtaining treatment for HCV and HIV. HIV-positive and HIV/HCV coinfected injection drug users have generally received HAART later than non-users. (Bassetti 1999; Bogart 2000; Hare 2002; Maisels 2001; Mocroft 1999a; Murri 1999 Sulkowski 2002; Tedali 2003a; Tedali 2003b; Torriani 2001; Qurishi 2002a; Qurishi 2002b). These barriers may be attributable in part to discomfort reported by physicians who provide care to people with drug and/or alcohol problems, as well as inadequate training on assessment of and interventions for addiction (M. F. Fleming 1999; N. S. Miller 2001; Saitz 2002). Harm reduction strategies— referral to drug/alcohol treatment upon request, instruction on safe injection practices, referral to syringe exchange programs, methadone maintenance and prescription of buprenorphine—must be incorporated into the medical care of coinfected injection drug users.

The safety and efficacy of treatment for hepatitis C have not been adequately assessed in populations with high prevalence of HIV/HCV coinfection, such as hemophiliacs, individuals with psychiatric disorders and active drug users. There is limited information on treatment outcomes in coinfected cirrhotics. Successful outcomes have been reported from uncontrolled studies of treatment for acute HCV infection in HIV-positive persons, but the optimal regimen and duration of therapy are unknown.

Promising data has emerged from studies of coinfected liver transplant recipients, although access to transplantation is limited for HIV-positive candidates, and treatment for recurrent hepatitis C remains problematic.

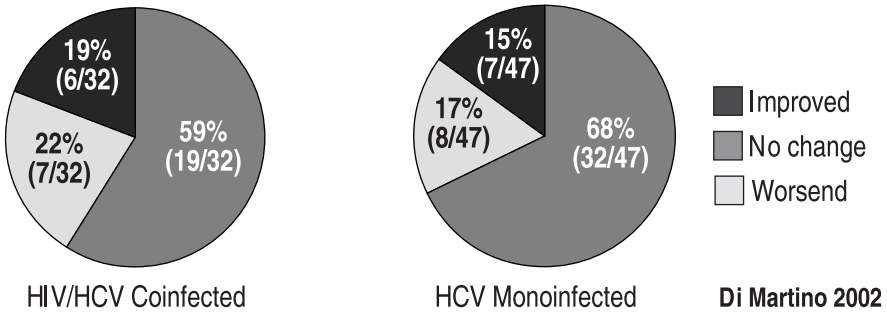
Interferon Monotherapy

Early studies of interferon monotherapy for treatment of HCV in HIV-positive individuals yielded sustained virological response (SVR) rates ranging from 22.5% to 30% (Mauss 1998; Soriano 1996).

- Histological Benefit of Interferon Monotherapy In Coinfection

A study from Di Martino and colleagues selected decrease in fibrosis score and reduction in disease activity after HCV treatment as the primary endpoints. A group of 79 injection drug users, 32 coinfected, received 3 MIU of interferon alfa-2b thrice weekly for 24 weeks. There was no significant difference in histological response or fibrosis score according to HIV status, nor was any relationship between CD4 cell count and histological response identified. Liver biopsies were performed at baseline and again at 23 ± 16 months after completion of treatment. Histological response was defined as a decrease in the Knodell score (see Chapter IV, Diagnostics) of >2 points; fibrosis improvement was a decrease in fibrosis score of >1. There were no significant differences in histological response by HIV status (40.6% [13/32] vs. 36.2% [17/47] for HCV alone). Predictors of histological response to treatment were: presence of a biochemical response to treatment (OR, 4.9; P=0.008), baseline HCV RNA <1,000,000 (OR, 4.1; P=0.023) and a high pretreatment Knodell score (P=0.0002).

Figure 1. Changes in Fibrosis Score After HCV Treatment



Predictors of improvement of fibrosis were biochemical response to treatment (OR, 4.6; P=0.039), non-1 HCV genotype (OR, 8.6; P=0.023), and a high pretreatment liver fibrosis score (P<0.0001) (Di Martino 2002). Long-term follow-up is needed to assess the durability of improvements in liver histology among coinfected persons.

Combination Therapy

Data from HCV monoinfection treatment trials indicated that adding ribavirin to interferon improved treatment efficacy (see Chapter V, HCV Treatment). Subsequent studies in coinfected individuals have investigated the safety and efficacy of combination therapy. While interferon-based regimens are no longer the standard of care for HCV treatment, these studies have yielded useful data about tolerability, drug interactions, adverse events and the relationship of CD4 cell count to treatment efficacy.

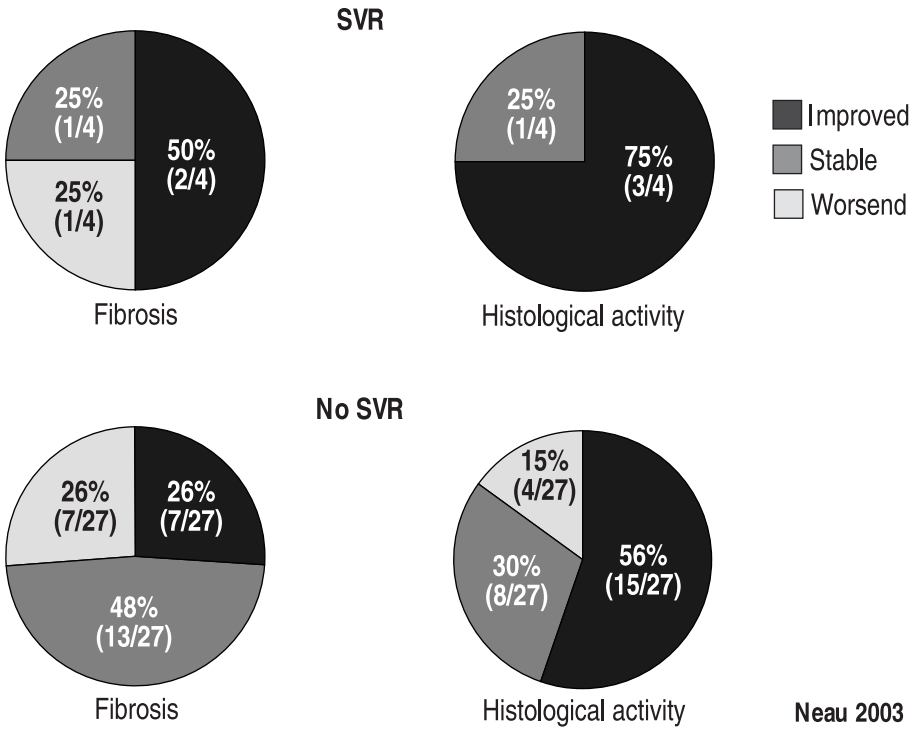
Table 1. Sustained Virological Response (SVR) Rates: Combination Therapy with Standard IFN + RBV

Author	Regimen & Duration	N Participants	CD4 Count	SVR	Discontinuation
Bochet 2001	IFN 3 MIU 3xweek + RBV 800–1200 mg/day For 6 months (10), 9 months (3), and 12 months (25)	Total N=56 treatment naïve: 54% (30/56) non-responders: 46% (20/56)	377 ± 27	Overall: 17.8% No difference by treatment experience	27% (15/56) For: anemia, depression, asthenia, anger, neuropathy
Nasti 2001	IFN alfa-2b 3 MIU 3xweek + RBV 1000–1200 mg/day For 24 weeks	N=17	Mean:445 SD: 144	19% (3/17)	6% (1/17) For: mild dizziness (interfered with ability to work)
Landau 2001	IFN alfa-2b 3 MIU 3xweek + RBV 1000–1200 mg/day For 12 months	N=51	412 ± 232	21% (11/51)	29% (15/51) For: adverse events (4) virological non-response (11)
Pérez-Olmeda 2002 Arm A	IFN 3 MIU 3xweek + RBV 800 mg/day For 6 months	Total N=111	>350	22% (24/111)	12% (13/111) For: adverse events (not specified)
Arm B	INF 6 MIU/day for 6 weeks, then cross over to Arm A regimen				
Santos 2002	IFN 3 MIU 3xweek + RBV 1000–1200 mg/day Genotypes 1&4: for 12 months Genotypes 2&3: for 6 months	Total N=26 Genotype 1: 13 Genotype 4: 2 Genotype 2: 1 Genotype 3: 10	Mean:632	27% (7/26) Genotypes 1&4: N=2 Genotype 3: N=5	15% (4/26) For: 'flu-like' syndrome (3) suicide (1)
Sanchez Montero 2002	IFN 3 MIU 3xweek + RBV 800 mg/day For 24 weeks	N=21	>350	43% (9/21)	None reported
Sulkowski 2003b Arm A	IFN 3 MIU 3xweek + RBV 800 mg/day For 48 weeks	Total N=180	Mean:533	4.3%	Virological non-response: 43% (39/90)
Arm B	IFN 3 MIU daily + RBV 800 mg/day For 48 weeks		Mean:551	9.3%	Virological non-response: 20% (18/90)
Braü 2004 Arm A	IFN 3 MIU 3xweek + RBV 800 mg/day For 48 weeks	N=53	Mean:458	11% (6/53)	Discontinued: 51% (55/107) for adverse events: depression, anxiety, anemia and fatigue: 21% (23/107) insufficient response: 11% (12/107) lost to follow-up: 12% (13/107) relapse to active drug use: 4% (4/107)
Arm B	IFN 3 MIU 3xweek for 16 weeks; then added RBV 800 mg/day for another 32 weeks	N=54	Mean: 520	6% (3/54)	

Histological Outcome of Combination Therapy in Coinfection

Although the virological response rate was disappointing—only 15% (11/68) achieved SVR—valuable histological data emerged from a small study comparing two different regimens of standard interferon (ribavirin was added at week 12 if HCV RNA was detectable). Paired biopsy specimens were available from 31 participants, four of whom achieved an SVR (Neau 2003).

Figure 2. Histological Responses at Week 72



Host determinants for achieving histological response—improvement in fibrosis and/or decreased necroinflammatory activity—have yet to be identified. Predicting the likelihood of histological response, especially in the absence of a virological response, will help guide decisions about continuation of HCV treatment.

Efficacy and Safety of Pegylated Interferon and Ribavirin in Coinfection

Early Viral Kinetics

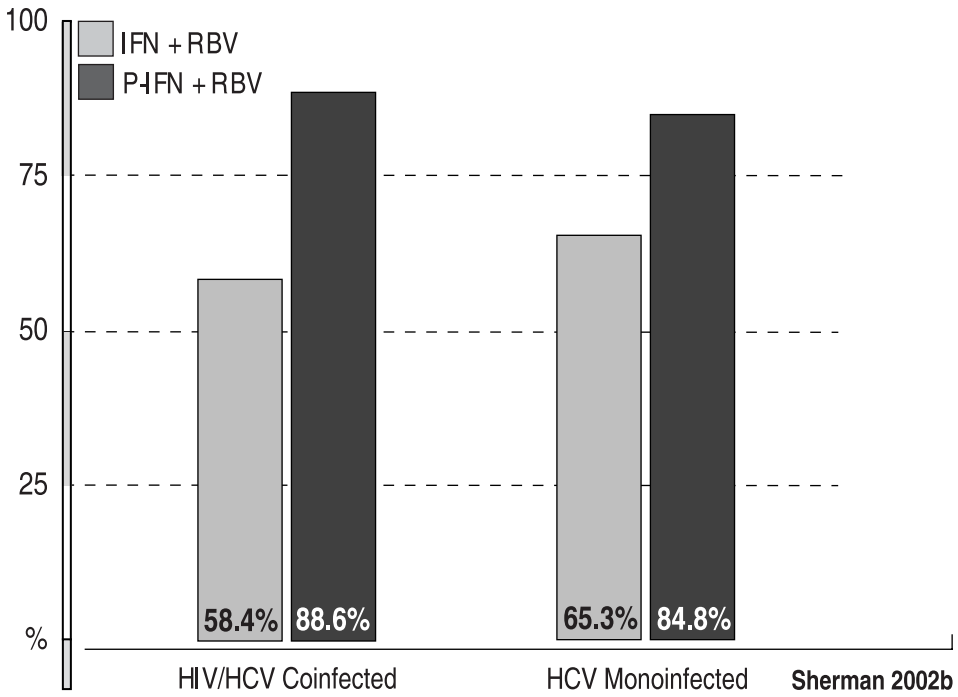
Viral clearance as early as 24 hours after initiating interferon-based treatment for hepatitis C may help to predict SVR in HCV monoinfection. Evaluating early responses to treatment in persons with HIV/HCV co-infection may offer information about the likelihood of HCV treatment efficacy, an opportunity for interventions to increase treatment efficacy, and spare those who are unlikely to benefit from treatment.

The decay rate of hepatitis C virions during early HCV treatment appears to be less rapid in coinfected individuals. In a viral kinetics substudy, three of ten coinfected persons (two of whom

received pegylated interferon) had a slow clearance of HCV. The interval before any reduction in HCV RNA was observed ranged from six days to more than twelve weeks (Torriani 2003). A look at hepatitis C viral decay during early HCV treatment in five coinfected individuals found a virion half-life of 7.0 ± 1.0 hours (vs. a virion half-life of two to three hours in HCV monoinfection) (Layden 2002b; Torriani 2002a).

Reports from two small studies of viral kinetics during the first 72 hours of HCV treatment reflect the superior efficacy of pegylated interferon despite the longer viral half-life of HCV observed in coinfected persons. Sherman and colleagues studied ten coinfected individuals; five received standard interferon alfa-2a and ribavirin, five pegylated interferon alfa-2a with ribavirin. Although the initial viral response was slightly delayed among individuals treated with pegylated interferon alfa-2a (9 hours vs. 7.7 hours for standard interferon), pegylated interferon increased the efficiency of initial-phase viral clearance from 65% (standard IFN) to 90% ($P<0.05$) (Sherman 2002b). In another study, Sherman and colleagues evaluated early viral clearance among 27 individuals (12 coinfected) treated with standard or pegylated interferon-based regimens. HCV RNA testing was performed at 0, 6, 12, 24, 48 and 72 hours. Again, pegylated interferon was more efficient than standard interferon, regardless of HIV status ($P<0.05$) (Sherman 2003).

Figure 3. Efficiency of Viral Clearance at 72 Hours by Regimen and HIV Status



Ballestros and colleagues studied early viral kinetics of hepatitis C in 28 coinfected persons treated with pegylated interferon alfa-2b (1.5 mg/kg once weekly) and ribavirin (800 mg/day). During the first 24 hours of treatment, viral kinetics differed significantly between responders and non-responders, and according to HCV genotype. Within the first 24 hours of treatment, the decrease in HCV RNA was significantly greater among virological responders than non-responders (-1.06 $\log_{10}$ vs. - 0.05 log 10; $P=0.002$). Sustained virological responders maintained lower levels of HCV RNA throughout treatment (24 weeks for genotype 3 and 48 weeks for genotypes 1 and 4). The

median decrease in HCV RNA during the first 24 hours of treatment differed significantly by HCV genotype (-1.20 log_{10} in genotype 3 vs. –0.06 log_{10} in genotype 1 and –0.37 log_{10} in genotype 4; P=0.022). SVR could be predicted by virological response at week 4; 100% of people who achieved either a two-log decrease or undetectable HCV achieved SVR, while 92.9% (13/14) of week 4 non-responders did not achieve SVR (Ballestros 2004). A greater number of sustained virological responders in this study had genotype 3 (60% [6/10] vs. 11.1% for both genotypes 1 and 4 [1/13 and 1/5, respectively]). The predictive value of week 4 virological response may be more applicable in genotype 3 than genotypes 1 and 4.

Coinfection Treatment Trials

The efficacy of pegylated interferon plus ribavirin in HCV monoinfection warranted investigation in coinfected individuals. Although a wealth of natural history data have established that HCV is more aggressive in people with HIV, studies of safety and efficacy of pegylated interferon-based regimens in coinfected persons have lagged years behind pivotal monoinfection treatment trials. Long-overdue data on the safety and efficacy of pegylated interferon plus ribavirin in coinfected persons from three randomized, controlled coinfection treatment trials (Roche's APRICOT, the ACTG's A5071 and ANRS HC02/RIBAVIC) were presented in February of 2004. All reported that pegylated interferon-based regimens are more effective for coinfected persons than standard interferon-based regimens, although less effective for coinfected persons than those with HCV alone (Chung 2004; Perronne 2004; Torriani 2004).

Table 2. Sustained Virological Response Rates From HCV Treatment Trials of Pegylated Interferon-Based Regimens by HIV Status and HCV Genotype

Author	Population	Regimen	Duration	N Participants	% SVR		
					Overall	Genotype 1	Genotypes 2&3
Fried 2003	HCV	P-IFN alfa-2a 180 µg 1xweek + 1,000–1200 mg RBV/day	48 weeks	N=453	56%	44%	70% (includes genotypes 4, 5 & 6)
Hadziyannis 2004	HCV	P-IFN alfa-2a 180 µg 1xweek + 1,000–1200 mg RBV/day	48 weeks	N=424	61%	51%	80%
Manns 2001	HCV	P-IFN alfa-2b 1.5 mg/kg 1xweek + 800 mg RBV/day	48 weeks	N=511	54%	42%	82%
Chung 2004	HIV/HCV	P-IFN alfa-2a 180 µg 1xweek + 600 up to 1,000 mg RBV/day*	48 weeks	N=66	27%	14%	73%
Perrone 2004	HIV/HCV	P-IFN alfa-2b 1.5 mg/kg 1xweek + 800 mg RBV/day	48 weeks	N=205	27%	15%	44%
Torriani 2004	HIV/HCV	P-IFN alfa-2a 180 µg 1xweek + 800 mg RBV/day	48 weeks	N=289	40%	29%	62%

*In A5071, ribavirin dose was gradually escalated by 200 mg every four weeks, as tolerated.

As in HCV monoinfection, genotype appears to be the most significant prognostic factor for a sustained virological response to treatment (Chung 2002b; Chung 2004; Fried 2002; Hadziyannis 2004; Hopkins 2003; Manns 2001; Pérez-Olmeda 2003b; Perronne 2002; Perronne 2004; Torriani 2004; Voigt 2003).

Since the treatment regimen, sample size and baseline characteristics of participants differed, direct and accurate comparisons of these trial results are not possible. Although ACTG A5071 and APRICOT used pegylated interferon alfa-2a, the initial dose of ribavirin was lower in A5071 than APRICOT (600 mg/day, gradually escalated by 200/mg day every four weeks vs. 800 mg/day). ANRS HC02 used pegylated interferon alfa-2b. The ribavirin dose in ANRS HC02 and APRICOT was the same (800 mg/day), but ribavirin dose reductions may have occurred more frequently in ANRS HC02, because it did not allow the use of growth factors to treat anemia, whereas the other two studies did.

Although all participants in APRICOT, ACTG A5071 and APRICOT were coinfected, some of their other characteristics varied; ANRS HC02 had a greater proportion of cirrhotics than the other two studies, while ACTG A5071 had a greater proportion of black participants (HCV treatment is less effective for cirrhotics than those with less advanced liver disease, and less effective for Blacks than non-Blacks). Well-controlled HIV disease and mild-to-moderate liver disease of participants were reflected by response rates in APRICOT.

APRICOT

Roche sponsored APRICOT (AIDS Pegasys Ribavirin International Coinfection Trial), an 868-person, multi-site, randomized, controlled study, comparing safety and efficacy of 48 weeks of treatment with:

- Pegylated interferon alfa-2a (180 μg once weekly) plus placebo;
- Pegylated interferon alfa-2a (180 μg once weekly) plus ribavirin (800 mg per day); and
- Interferon alfa-2a (3 MIU, thrice-weekly) plus ribavirin (800 mg per day).

Table 3. Baseline Characteristics in APRICOT by Treatment Arm

	IFN + RBV (N=285)	P-IFN + placebo (N=286)	P-IFN + RBV (N=289)
Male	81%	82%	80%
White	78%	79%	80%
Using ART	84%	85%	84%
CD4 cell count (mean)	542	530	520
CD4 <200μL	7%	5%	6%
HIV RNA (mean ±SD)	2.3 ± 1.0	2.4 ± 1.0	2.3 ± 1.0
HIV RNA <50 copies/mL	60%	60%	60%
HCV RNA (mean)	5.2	6.3	5.6
Genotype 1	60%	61%	61%
Genotypes 2 and 3	31%	32%	32%

Torriani 2004

Coinfected individuals who had stable HIV disease (with or without antiretroviral therapy) and a CD4 cell count of either >200/mL or 100–200/mL with an HIV RNA of <5,000 copies/mL were eligible for APRICOT. Participants were required to be HCV treatment naïve. A liver biopsy within 15 months of study entry was required. Cirrhotics with Child-Pugh Grade A (see Chapter IV, Diagnostics) were eligible. APRICOT stratified by genotype (1 vs. non-1), baseline CD4 count (≥ 100–200/mL vs. ≥ 200/mL), antiretroviral therapy vs. no antiretroviral therapy, presence or absence of cirrhosis, and geographic region.

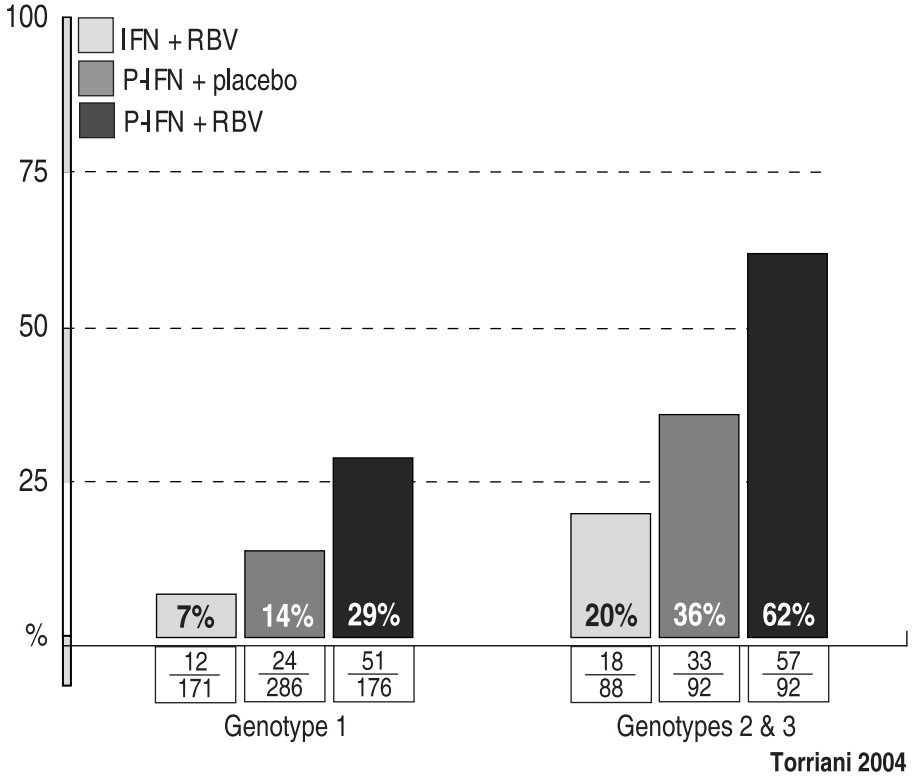
Although 24 weeks of therapy is the standard of care for HCV-monoinfected persons with genotype 2 or genotype 3, all of APRICOT's participants were treated for 48 weeks, regardless of their HCV genotype. The relapse rate (between the end of treatment and week 72) varied according to genotype and regimen. It was highest among participants with genotypes 2 and 3 who received pegylated interferon plus placebo, and lowest among those with genotypes 2 and 3 who received pegylated interferon plus ribavirin.

Table 4. APRICOT: End of Treatment (ETR) and Sustained Virological Response Rate (SVR) by Regimen and Genotype

	IFN + RBV		P-IFN + placebo		P-IFN + RBV	
	ETR	SVR	ETR	SVR	ETR	SVR
Genotype 1	8%	7%	21%	14%	38%	29%
Genotypes 2&3	27%	20%	57%	36%	64%	62%

Torriani 2004

Figure 4. APRICOT: Sustained Virological Response Rate (SVR) by Regimen and Genotype



Rodriguez-Torres and colleagues examined virological responses at week 12 and week 24 among 289 APRICOT participants randomized to pegylated interferon plus ribavirin. As in HCV mono-infection (Fried 2002), coinfected participants in APRICOT who did not achieve a ≥ 2 log decrease or undetectable HCV RNA by week 12 were extremely unlikely to achieve SVR (Rodriguez Torres 2004). Unfortunately, the positive predictive value of a virological response at week 12 or week 24 was less robust, especially among those with genotype 1 (Rodriguez Torres 2004).

Table 5. Predictive Value of Early Virological Response (EVR) at Week 12 and Week 24 (P-IFN + RBV only)

	EVR		SVR W 72	Negative Predictive Value		Positive Predictive Value	
	W 12	W 24		W 12	W 24	W 12	W 24
All	71% (204/289)	75% (216/289)	40% (116/289)	98%	99%	56%	53%
Genotype 1	63% (110/176)	68% (120/176)	29% (51/176)	98%	100%	45%	43%
Genotypes 2&3	84% (88/95)	85% (89/95)	62% (59/95)	100%	100%	70%	69%

Rodriguez Torres 2004

With regard to HIV, absolute CD4 cell counts decreased during treatment, but returned to baseline by week 72. The median decrease in CD4 cell counts at week 48 was greatest among those on pegylated interferon and ribavirin ([approximately] -140 vs. -120 for interferon plus ribavirin and pegylated interferon plus placebo). The CD4 percentage rose during treatment, peaked at week 36, and returned to baseline by week 72. HIV RNA decreased slightly during treatment in both of the pegylated interferon arms, and returned to baseline by week 72. It remained stable among those receiving interferon plus ribavirin.

The most common adverse events were fatigue and a constellation of flulike symptoms, insomnia, and depression. Serious adverse events possibly or probably related to HCV treatment were reported in 5% of the interferon arm, 10% of the pegylated interferon arm and 8% of those receiving pegylated interferon plus ribavirin. Two deaths (cardiac arrest and suicide during hospitalization for depression) occurred during APRICOT; both were considered possibly or probably related to study drugs (Torriani 2004).

Table 6. Treatment Discontinuation by Cause and Regimen

	IFN + RBV	P-IFN + placebo	P-IFN + RBV
Laboratory abnormality	0%	5%	3%
Adverse event	14%	12%	12%
Non-safety	24%	15%	10%

Torriani 2004

AACTG A5071

In the Adult AIDS Clinical Trials Group's A5071 study, 132 coinfected study volunteers were randomized to 24 weeks of treatment with:

- Pegylated interferon alfa-2a (180 μg once weekly) plus ribavirin (600 mg per day, increased by 200 mg every four weeks as tolerated, to a maximum of 1,000 mg/day); or
- Interferon alfa-2a (6 MIU, thrice-weekly for 12 weeks, then 3 MIU, thrice-weekly) plus ribavirin (600 mg per day, increased by 200 mg every four weeks as tolerated, to a maximum of 1,000 mg/day).

Table 7. Baseline Characteristics in 5071 by Treatment Arm

	IFN + RBV (N=67)	P-IFN + RBV (N=66)
Male	85%	79%
White	46%	50%
African American	34%	32%
Hispanic	12%	15%
Using ART	87%	85%
CD4 cell count (median)	444	482
HIV RNA >50 copies/mL	60%	61%
HCV RNA IU/mL	6.2 ± 0.3	6.2 ± 0.4
Genotype 1	78%	77%
Median fibrosis score	2.0 out of 6.0	2.0 out of 6.0
Cirrhosis	9%	11%

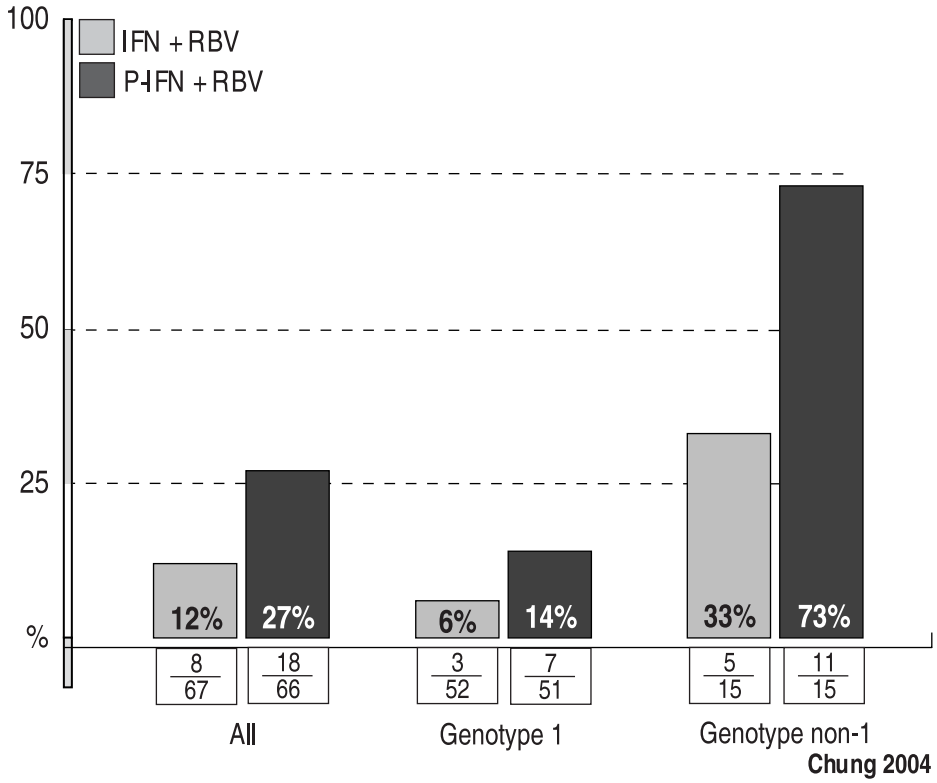
Chung 2004

Coinfected individuals who were on stable antiretroviral therapy for at least 12 weeks and had a CD4 cell count of >100 and an HCV RNA <10,000 were eligible for 5071. If the People with a CD4 cell count of >300 were eligible for A5071 if they had not received HIV treatment for 12 weeks before entering the study, and did not plan to start treatment for 24 weeks. Unless contraindicated, liver biopsy within a year of study entry was a prerequisite for participation. Compensated cirrhotics were eligible for 5071.

The primary endpoint of A5071 was the virological response at week 24. At week 24, participants with undetectable HCV RNA continued treatment for an additional 24 weeks, while people with detectable HCV RNA had a liver biopsy to assess histological response. Participants whose histological activity score decreased by at least two points from baseline continued treatment. Treatment was discontinued only for virological and histological non-responders.

Participants were stratified by HCV genotype (genotype 1 vs. non-1) and whether or not they were receiving antiretroviral therapy.

Figure 5. Sustained Virological Response by Genotype and Regimen: A5071

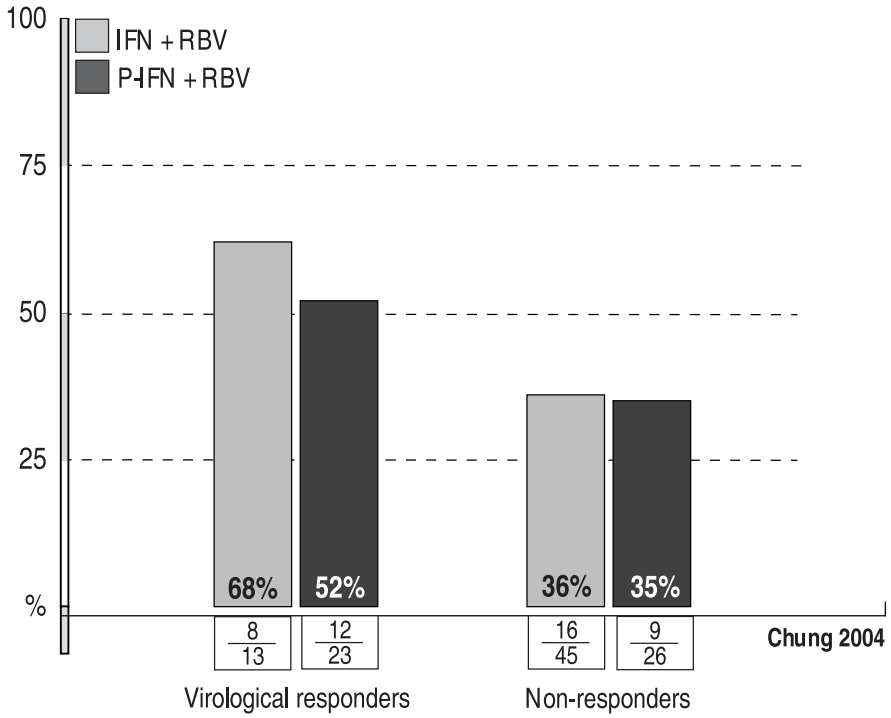


After controlling for other factors, independent predictors of a sustained virological response in A5071 were: a non-1 genotype (OR, 15.8; 95% CI, 4.94–50.5; P<0.001) treatment with pegylated interferon (OR, 4.76; 95% CI, 1.49–15.2; P=0.008), detectable HIV RNA at baseline (OR, 3.55; 95% CI, 1.19–10.6; P=0.023). Those with no history of injection drug use (IDU) were more likely to achieve SVR (OR, 0.48; 95% CI, 0.27–0.83; P=0.009) than current or former IDUs. Age, race, use of antiretroviral therapy with or without a protease inhibitor, CD4 cell count, baseline HIV RNA, liver histology and use of growth factors were not predictive of SVR (Chung 2004). This may be due to the small sample size and low SVR rate (26/133).

The relationship between previous injection drug use and treatment outcomes needs further exploration in larger studies. Depression may be a confounding factor, since it is a common side effect of interferon and is prevalent among IDUs with hepatitis C and HIV/HCV (Golub 2004, M. E. Johnson 1998; Garcia 2004). Interferon-induced or exacerbated depression may have had an effect on adherence to HCV treatment and/or study discontinuation. In A5071, treatment-related moderate-to-serious depression was reported among 18 study participants, some of whom may have had a history of IDU.

Although sustained virological response rates—particularly in genotype 1— were disappointing, a proportion of virological non-responders in each treatment arm derived histological benefit from treatment. Histological improvement was defined as a decrease in the HAI score of two or more points from the pre-treatment liver biopsy; see Chapter IV, Diagnostics. All histological responders had slight decreases in HCV RNA by week 24 (-0.61 with interferon; -1.01 with pegylated interferon), although these decreases did not differ significantly from those in histological non-responders (-0.58 with interferon; -0.71 with pegylated interferon).

Figure 6. Histological Improvement by Treatment Arm and Virological Response



During HCV treatment, absolute CD4 cell counts decreased, but they returned to slightly above baseline by week 72. The higher the CD4 cell count at study entry, the greater the decrease: people with CD4 cell counts above 700 at entry had decreases of 390 (pegylated interferon) vs. 149 (interferon), while individuals with CD4 cell counts under 700 at study entry had decreases of 111 (pegylated interferon) vs. 77 (interferon). As absolute CD4 cell counts dropped, the percentage of CD4 cells increased. After 24 weeks of treatment, the CD4 cell percentage rose by 2.5% in the standard interferon arm and 3.5% in the pegylated interferon arm. By week 72, CD4 cell percentages had returned to near-baseline levels. No opportunistic infections were reported during treatment or the follow-up period (Chung 2002b; Chung 2004).

Virological response at week 12 had a negative predictive value of 100%. In other words, the likelihood of achieving a sustained virological response without achieving either a two-log decrease or an undetectable HCV RNA by week 12 was zero percent. For week-12 virological responders, the likelihood of achieving SVR was 51%.

Moderate to severe flulike symptoms and depression were the most commonly reported side effects. Laboratory abnormalities occurred in both study arms. There were eight withdrawals (or 12%) from each study arm (Chung 2004).

Table 8. Laboratory Abnormalities in A5071 by Regimen and Severity*

	Grade 2: Moderate		Grade 3: Severe		Grade 4: Potentially life threatening	
	IFN	P-IFN	IFN	P-IFN	IFN	P-IFN
Anemia	1	0	0	0	0	3
Neutropenia	11	20	9	24	3	9
Thrombocytopenia	2	13	0	2	0	1
Glucose	18	17	3	7	0	5
ALT	13	23	7	4	1	0
Lipase	8	8	4	5	0	0
Lactate	0	0	1	0	0	0

Chung 2004

*from initiation of HCV treatment until week 72.

ANRS HC02 (RIBAVIC)

In ANRS HC02, 412 HIV/HCV-coinfected participants were randomized to 48 weeks of treatment with:

- Pegylated interferon alfa-2b 1.5μg/kg once weekly plus 800 mg/day of ribavirin; or
- Interferon alfa-2b 3MU three times per week plus 800 mg/day of ribavirin.

Table 9. Baseline Characteristics in ANRS HC02

Baseline Characteristics	
Male	74%
IDU (current or former not specified)	79%
Using ART	82%
CD4 cell count (mean)	541 ± 229/mL
HIV RNA <400 copies/mL	66%
Mean HIV RNA (if >400 copies/mL)	3.7 ± 0.7 log
With a previous AIDS-defining condition	17%
HCV RNA (mean)	5.9 ± 0.7 log
Genotype 1 or 4	58%
Genotype 3	34%
With bridging fibrosis or cirrhosis	39%

Perronne 2004

Coinfected persons with a CD4 cell count >200/mL and stable HIV disease (defined as no change in HIV RNA of ≥ 1 $\log_{10}$ within three months of study entry) were eligible, whether or not they were using antiretroviral therapy. Participants were required to be HCV treatment-naïve, and to have had a liver biopsy within 18 months of study entry.

The failure to achieve an SVR was most reliably predicted by the virological response (defined as either a two-log decrease in HCV RNA, or an undetectable HCV RNA) at week 12, while the virological response at week 4 was more likely to predict SVR. The negative predictive value of virological non-response at week 4 was 79%; it increased to 94% at week 12. The positive predictive value of an early virological response decreased from 92% at week 4 to 74% at week 12.

Figure 7. ANRS HC02: Sustained Virological Response Rate by Genotype and Regimen

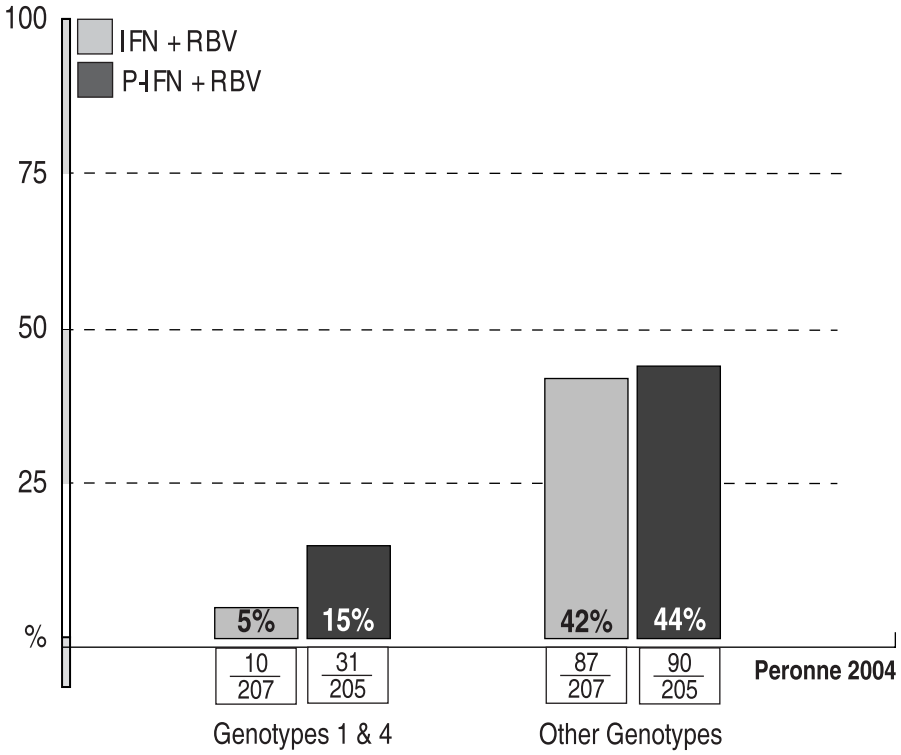


Table 10. ANRS HC02: Discontinuations and Sustained Virological Response Rate by Regimen, METAVIR Score, Baseline HCV RNA and Baseline CD4 Count

Variable	IFN + RBV (N=207)	P-IFN + RBV (N=205)
All	19% (39/207)	27% (55/205)
Completed treatment	28% (33/117)	36% (42/117)
% (N) who achieved SVR		
HCV RNA <1 X 10^6	23% (48/207)	28% (57/205)
HCV RNA >1 X 10^6	17% (35/207)	26% (53/205)
METAVIR score F0–F2	21% (44/207)	25% (51/205)
METAVIR score F3–F4	26% (54/207)	32% (66/205)
CD4 cell count >500	21% (43/207)	34% (70/205)
CD4 cell count <500	18% (37/207)	22% (45/205)

Peronne 2004

A preliminary analysis of histological responses indicated that virological responders had significant decreases in METAVIR scores (see Chapter IV, Diagnostics) for both fibrosis and disease activity (Perronne 2004).

The withdrawal rate in ANRS HC02 was shockingly high—43% of participants in each arm withdrew. Several factors may have contributed to the high discontinuation rate. A large proportion of ANRS HC02 participants had advanced liver disease (40%) and 17% had a history of an AIDS-defining condition; these people may have had difficulty tolerating simultaneous HAART and HCV treatment. Growth factors were not permitted for management of treatment-induced anemia or neutropenia, both of which may have led to withdrawals. Although it was unclear whether or not they were still active users, almost 80% of the study participants acquired HCV from injection drug use. No information is available regarding access to methadone or buprenorphine during this trial. Evaluating the contribution of current or former injection drug use to study discontinuations is not possible. Certainly, the importance of monitoring for, and managing side effects is underscored by discontinuations from ANRS HC02.

Common side effects in both treatment arms included flulike symptoms, weight loss, anxiety, insomnia, depression, hair loss and itching. At week 12, decreases in hemoglobin and platelets were significantly greater among those receiving pegylated interferon (-1.8 vs. -1.4 [P=0.002] for hemoglobin; -19,000 vs. -33,000 [P=0.031] for platelets), while decreases in neutrophil and absolute CD4 cell counts did not differ significantly by treatment arm. Severe adverse events were reported by 31% (127/ 410); 64 in the interferon arm and 63 in the pegylated interferon arm (Perrone 2004).

Table 11. Serious Adverse Events Reported in ANRS HC02 *

Adverse Events	
Psychiatric events	16
Sepsis	8
Pneumonia	7
Hepatic failure	4
Acute pancreatitis	5
Hyperlactatemia >5mM/l	6

Perronne 2002; Perronne 2004

*This is not a complete listing of serious adverse events from ANRS HC02.

An Uncontrolled Study of Pegylated Interferon Plus Ribavirin

Pérez-Olmeda and colleagues evaluated safety, efficacy and tolerability of HCV therapy in 68 coinfected individuals with CD4 cell counts of >300 and HIV RNA of <5000 (with or without antiretroviral therapy). Participants received a fixed dose of ribavirin (800 mg/day) with an induction dose of pegylated interferon alfa-2b. For the first 12 weeks of the study, they were given 150 μg per week. The dose of pegylated interferon was subsequently reduced to 100 μg per week until completion of therapy. For genotypes 1 and 4, the course of treatment was 48 weeks, for genotype 3, 24 weeks.

Table 12. Early Virological Response (EVR), End-of-Treatment Response and SVR According to Genotype

	EVR W 12	ETR W 24/48*	SVR W 48/72*
All	48.5% (33/68)	39.7% (27/68)	27.9% (19/68)
Genotypes 1&4	Not available	15% (10/68)	12% (8/68)
Genotype 3	Not available	81% (17/21)	52% (11/21)

Pérez-Olmeda 2003b

*ETR at 24 weeks for genotype 3. ETR at 48 weeks for genotypes 1 & 4

After controlling for other factors, the only significant predictor of achieving SVR was baseline HCV RNA <800,000 IU (OR, 5.5; 95% CI, 1.5–19.87; P=0.009).

Several factors contributed to low response rates. Doses of both drugs may have been suboptimal. For individuals weighing more than 75 kg, a higher dose of ribavirin is recommended if they can tolerate it (tolerability of ribavirin is a significant issue for HIV-positive persons, who often receive the 800 mg dose used in this trial). Based on the weight range of participants in this study, a quarter of the participants could have received a suboptimal dose of ribavirin, although the difference in response rates by ribavirin dose was not significant. Although pegylated interferon alfa-2b is dosed according to body weight, in this trial, it was given at two different fixed doses—150 μg per week for 12 weeks, then 100 μg per week until completion of treatment—regardless of body weight. The recommended dose for use in combination with ribavirin is 1.5 μg/ kg once weekly. The mean weight of participants in this study was 69 ± 11.2 kg. For the upper end of this weight range (80 kg), the recommended weight-based dose of pegylated interferon would be 120 μg; for the lower end of the weight range (58 kg), the recommended weight-based dose would be 87 μg. The post-induction dose (100 μg per week) may have been suboptimal for anyone weighing more than 66 kg. The rationale for this dosing scheme is unclear, and may have had a substantial impact on efficacy.

Although a 24-week course of treatment is recommended for genotype 2 or 3 in HCV moinfection, this may have been an insufficient duration of therapy for coinfected individuals with genotype 3. In this study, 30% of those with genotype 3 relapsed between the end of treatment and week 72. The authors suggested that the efficacy of treatment for coinfected people with genotype 3 may increase if treatment duration is extended to 48 weeks.

Fifteen percent of participants discontinued treatment due to adverse events. Some of the adverse events were unexpected, such as pancreatitis (N=1), asymptomatic hyperamylasemia (excess levels of amylase in the bloodstream) (N=4), and slightly elevated lactate levels (N=2). Both people with elevated lactate levels were taking stavudine (d4T; Zerit®). All of the individuals who experienced pancreatitis and asymptomatic hyperamylasemia were taking didanoisine (ddI; Videx®). These adverse events resolved after the discontinuation of therapy (Pérez-Olmeda 2003b). These and other data indicate that didanosine and stauvidine must be used cautiously, if at all, with ribavirin (see the section on drug interactions later in this chapter).

HCV Treatment Strategies For Coinfected People

Although the sustained virological response rates from APRICOT, ACTG A5071, ANRS HC02 and Pérez-Olmeda and colleagues were disappointing, these studies provide useful data and a path towards strategies for optimizing HCV treatment.

- **Identifying Prognostic Value of Baseline HCV RNA**

The prognostic value of baseline HCV RNA in coinfection is unknown. HCV viral loads are higher in coinfected people than those with HCV alone (Cribier 1995; Di Martino 2001; Eyster 1994; Sulkowski 2002; Thomas 2001; Zylberberg 1996). In HCV monoinfection, those with a low pre-treatment viral load (≤ 2,000,000 copies/mL or 800,000 IU) are more likely to achieve SVR. A different prognostic threshold may apply in coinfection. More information about likelihood of SVR according to baseline HCV RNA would be welcome.

- **Predicting SVR at Week 12**

Reports from APRICOT, ACTG A5071, ANRS and Pérez-Olmeda and colleagues concur: the virological response at week 12 has a negative predictive value of almost 100% in coinfected people, although the positive predictive value is not as robust (Chung 2004; Pérez-Olmeda 2003a; Perronne 2004; Torriani 2004). Other evidence supports the negative predictive value of the week 12 virological response in coinfected people. (Berenguer 2003b; Voigt 2003). Early, reliable prediction of treatment outcomes may support adherence among those who continue therapy, and spare non-responders from the side effects and expense of treatment.

- **Duration of Treatment for Genotype 2 or 3**

All participants in APRICOT, ACTG A5071, and ANRS HC02 were treated for 48 weeks, regardless of genotype. Extending treatment from 24 to 48 weeks for coinfected persons with genotype 2 or 3 decreased relapse rates in this group. Although there has not been a randomized, controlled trial comparing SVR rates in coinfected persons with genotype 2 or 3 by duration of treatment, the standard of care for this population may have already been redefined.

- **Extending Duration of Treatment in Genotype 1**

Perhaps using the same approach for improving treatment outcomes in genotypes 2 and 3—extending the duration of treatment—could increase efficacy in genotype 1. Some individuals with genotype 1 may be more likely to achieve SVR if duration of treatment is extended from 48 to 72 weeks. Further research is needed to identify those who are likely to achieve SVR by extending treatment.

- **Increasing Efficacy with Higher Doses of Ribavirin**

Using higher doses of ribavirin may increase treatment efficacy for coinfected people. Due to concerns about anemia in HIV-positive persons, the dose of ribavirin is usually 800 mg/day, regardless of weight. One HCV monoinfection trial reported more sustained virological responses

in individuals with HCV genotype 1 who received 1,000 to 1,200 mg/day of ribavirin than in those who received 800 mg/day (Hadziyannis 2004).

Hernández-Quero and colleagues are evaluating safety and efficacy of 48 weeks of pegylated interferon alfa-2a (180 mg/kg once weekly) with two doses of ribavirin (800 or 1,000 mg/day) among 149 coinfected people. So far, 108 have been treated for 24 weeks. Sixty-three percent (37/59) in the 1,000 mg arm had undetectable HCV RNA vs. 55% (27/49) in the 800 mg arm. With respect to genotype, 47.5% (28/59) with genotype 1, 84.4% (27/32) with genotypes 2 and 3, and 52.9% (9/17) with genotype 4 had undetectable HCV RNA at week 24. By week 24, 21 people had withdrawn from the trial; withdrawal rate did not differ by ribavirin dose. (Hernández-Quero2003). These results have not yet been broken out by both ribavirin dose and genotype. When the final data are available, the results must be evaluated specifically in genotype 1. Vigilant monitoring for, and prompt treatment of, anemia must accompany administration of high-dose ribavirin.

- **Decreasing Toxicity by Lowering the Dose of Pegylated Interferon**

Moreno and colleagues evaluated safety and efficacy of low-dose pegylated interferon plus ribavirin in 35 coinfected persons. All received 48 weeks of treatment with a fixed dose of 50 mg of pegylated interferon alfa-2b once weekly (the standard dose is 1.5 mg/kg once weekly) plus 800 mg/day of ribavirin. Overall, 31% (11/35) achieved SVR. The only significant predictor of SVR was genotype 2 or 3 (OR, 6.0; 95% CI, 1.1–31.7; $P<0.005$). SVR was achieved by 54% (7/13) with genotype 3, 21%(4/19) with genotype 1 and 0% (0/3) with genotype 4.

Although the response rates were comparable to those from ANRS HC02, decreasing the dose of pegylated interferon did not decrease side effects or adverse events. Ninety-one percent of the participants were receiving HAART during HCV treatment, which may have caused or exacerbated certain side effects of antiretroviral agents. Peripheral neuropathy developed in 9% (3/35), lactic acidosis in 6% (2/35) and weight loss of > 10kg (approximately 22 pounds) in 17% (6/35). An astounding 97% (35/35) reported flulike symptoms, while 40% (14/35) reported irritability and 9% (3/35) developed depression. Neutropenia developed in 60% (21/35) and anemia in 20% (7/35). Dermatological side effects (injection site reactions and dry and/or itchy skin) were common. Despite the adverse events, the discontinuation rate was fairly low (17%; 6/35) (Moreno 2004). Although data from this study do not warrant lowering the dose of pegylated interferon, it is encouraging that SVR rates—especially for genotype 1—did not differ significantly from those in ANRS HC02 or A5071.

- **Shifting the Treatment Paradigm**

For some coinfected people, the HCV treatment paradigm needs to shift from viral eradication to preventing additional liver damage. Long-term maintenance therapy with low-dose pegylated interferon may prevent HCV progression among HIV/HCV-coinfected people. This may be a viable option for those in whom SVR is unlikely, as well as for relapsers and virological non-responders. Maintenance therapy may stabilize liver disease until less toxic and more effective therapies are available. Two NIH-sponsored studies—one ongoing and another expected to open in late 2004—are evaluating safety and efficacy of maintenance therapy for coinfected virological non-responders.

Other Treatment Issues for Coinfected People

Drug Interactions

- **Ribavirin and Didanosine**

Cases of mitochondrial toxicity from the combination of ribavirin and nucleoside reverse transcriptase inhibitors (NRTIs) have been reported (Berenguer 2003a; Lafeuillade 2001; Pol 2003b; Salmon-Céron 2001; D. M. Smith 2002). A review of the Food and Drug Administration's Adverse Events Reporting System (AERS) revealed that combining didanosine with ribavirin was associated with a five-fold increase in the risk of mitochondrial toxicity-related events, such as pancreatitis, lactic acidosis with liver failure and/or hepatic steatosis. There were 65 unduplicated reports of adverse events in coinfected individuals receiving a combination of RBV and NRTI, and 26 of the 65 reported 53 events suggesting mitochondrial toxicity. Of these 26 individuals, 23 were taking didanosine and ribavirin. Three deaths occurred; two from liver failure, one from lactic acidosis and multi-organ failure (Fleischer 2003).

In September 2002, the FDA added a precaution about the co-administration of ribavirin and didanosine to the ddI label: "co-administration of ribavirin with VIDEX should be undertaken with caution, and patients should be monitored closely for didanosine-related toxicities. VIDEX should be suspended if signs or symptoms of pancreatitis, symptomatic hyperlactatemia, or lactic acidosis develops" (Bristol Myers Sqibb package insert 2003). In vitro, ribavirin increases levels of ddI's active metabolite, dideoxyadenosine 5'-triphosphate (ddATP), which may exacerbate didanosine-related toxicities, including pancreatitis, symptomatic hyperlactatemia, lactic acidosis, and peripheral neuropathy.

- **Ribavirin and Stavudine**

Although there is no data on interactions between ribavirin and stavudine, some adverse events reported during HCV treatment trials indicate that the side effects and toxicities from stavudine may be exacerbated by concomitant ribavirin use (Moreno 2004; Pérez-Olmeda 2003b; Perronne 2002). Moreno and colleagues reported two cases of lactic acidosis during their study of HCV treatment in 35 coinfected individuals. Both were taking stavudine and one was also taking didanosine. Three cases of moderate or severe peripheral neuropathy developed during this study; all among people using stavudine. Peripheral neuropathy is a known side effect of stavudine (Dragovic 2003; Reliquet 2001). Changing HIV treatment regimens led to significant improvements in symptoms of peripheral neuropathy in all three people (Moreno 2004).

During their study of HCV treatment in 68 coinfected individuals, Pérez-Olmeda and colleagues reported that significant weight loss—averaging 4.5 kg or approximately 10 pounds—occurred within six months of initiating HCV treatment. The authors speculated that since almost half of the study participants were taking stavudine, their weight loss may have been attributable in part to side effects from stavudine, potentiated by concomitant ribavirin use (2003b). Didanosine and stauvidine must be used cautiously, if at all, with ribavirin.

- **Ribavirin, Interferon and Zidovudine**

Interferon induces anemia through bone marrow suppression, which is the same mechanism associated with anemia induced by zidovudine (AZT; Retrovir®) (Dieterich 2003; Glaxo Wellcome 2000). Ribavirin causes anemia via hemolysis (Bodenheimer 1997; Dieterich 2002b; Dusheiko 1996). Combining the two drugs increases the risk for anemia, because myleosuppression makes compensation for the loss of red blood cells difficult. Braü and colleagues reported an association between ribavirin, zidovudine and anemia in a study of HCV treatment in coinfected people. When ribavirin and zidovudine were used together, hemoglobin nadir levels were significantly lower (10.1 g/dL vs. 13.0 g/dL for ribavirin without concomitant zidovudine; P=0.001) and decreases in hemoglobin were significantly greater (-3.64 g/dL vs. –2.08 g/dL; P=0.002). Dose reduction for anemia was significantly more common among those using ribavirin and zidovudine (60% vs. 16% for no AZT; P=0.001) (Braü 2004a). Moreno and colleagues reported a significant association between development and severity of anemia and zidovudine use during an HCV treatment trial in coinfected persons (71% vs. 28% of stavudine users; P=0.021). In this study, all of the participants who developed moderate or severe anemia were receiving zidovidine (Moreno 2004).

- **IFN and Nucleoside Analogs**

Although the focus of research on interactions between antiretroviral agents and hepatitis C therapy has been on ribavirin, interferon may be involved as well. Anderson and colleagues have speculated that toxicities from nucleoside and nucleotide analogs may be potentiated by interferon. Interferon may upregulate phosphorylation of NRTIs via cellular activation. Evidence to support this comes from studies of NRTI phosphorylation in people with advanced HIV disease, when cellular immune activation is common. Greater rates of NRTI phosphorylation and more toxicities have been reported in persons with advanced HIV disease receiving NRTIs (Barry 1994; Fischl 1997; Hoggard 2001: Wattanagoon 2000). Although the increased toxicity of didanosine reported in people being treated simultaneously for hepatitis C and HIV has been attributed solely to ribavirin, interferon may contribute as well (Anderson 2004). If increased mitochondrial toxicity resulting from hepatitis C treatment can be demonstrated in people receiving NRTIs, then further drug-drug interaction studies examining interferon's role in potentiating NRTI-induced mitochondrial toxicity are warranted.

Side Effects of Hepatitis C Treatment in HIV-Positive Persons

The side effects of interferon and ribavirin may occur more frequently, and may be more severe in coinfected persons. People who are HIV/HCV-coinfected are more vulnerable for a number of reasons: treating HCV may induce or exacerbate HIV-related complications such as anemia and neutropenia, the potential for drug interactions increases when interferon and ribavirin are added to antiretroviral therapy and additional drugs added to ameliorate side effects of HCV treatment may add new side effects or cause new interactions. Although treatment of HCV and HIV may alleviate symptoms of each virus, the side effects from their treatments may be worse.

For additional information on the side effects associated with standard and pegylated interferon and ribavirin, see Chapter V, Hepatitis C Treatment, Management of Side Effects and Adverse Events.

- **Depression**

Depression is common among HIV-positive individuals, occurring in 20–80% (Tate 2003; Valente 2003). Major depression is not always identified in people with HIV. Asch and colleagues used the Composite International Diagnostic Interview survey to assess depression among 1,140 HIV-positive people, all of whom were receiving medical care. The survey results indicated major depression among 37% (448/1,140). When they compared the survey results to data on each individual's medical record, they found that 45% (203/448) had no documentation of depression in their medical records (Asch 2003). It is crucial to diagnose and treat depression in HIV-positive people, because depression is associated with decreased quality of life and an increased risk for HIV disease progression and death (Farinpour 2003; Tate 2003).

Depression is a known side effect of interferon (Koskinas 2002; Kraus 2003). Interferon-induced depression ranges in severity; attempted suicides have been reported in <1–2% of HCV mono-infection treatment trial participants (Roche package insert 2002; Schering package insert 2001). Myers and colleagues reported that treatment-induced depression was responsible for 50% (3/6) of withdrawals due to neuropsychiatric side effects in a an HCV re-treatment in 32 coinfected persons; one required hospitalization (Myers 2004). Laguno and colleagues identified depressive symptoms among 37% (40/109) of coinfected individuals during treatment with interferon and ribavirin. Four were hospitalized for severe depression and had to permanently discontinue HCV treatment. Seventeen were treated for depression with citalopram (Celexa™; a selective serotonin reuptake inhibitor), and reported significant improvement in depressive symptoms (Laguno 2003). The authors suggested pre-emptive treatment of depression as an intervention to decrease treatment discontinuations.

Assessment for depression prior to initiation of HCV therapy, monitoring during treatment, and intervention when indicated should be key elements of HCV treatment. Furthermore, research on strategies for managing interferon-induced depression is needed.

- **Anemia**

Anemia is of great concern to HIV-positive people. It occurs among 30% of asymptomatic individuals and up to 75–80% of persons with an AIDS diagnosis (Levine 2001). If anemia is untreated or does not respond to treatment, it is associated with more rapid HIV disease progression and death (Diallo 2003; Lundgren 2003; Mocroft 1999b; Moore 1998; Semba 2002; Sullivan 2002). Anemia is prevalent among HIV-positive women; risk for developing anemia increases with African-American race, age, use of zidovudine, and CD4 cell counts <200/mL (Levine 2001; Semba 2002; Sullivan 1998; Volberding 2004). Recombinant human erythropoietin (EPO) has been used successfully for treatment of anemia in HIV-positive individuals, improving quality of life as well as increasing survival (Miles 1992; Moore 1999; Moore 2000; Revicki 1994; Sullivan 2002).

The risk for anemia is especially significant for coinfected persons who are considering or undergoing HCV treatment. Interferon may cause anemia through suppression of bone marrow, and hemolytic anemia is a common, usually reversible side effect of ribavirin (Bodenheimer 1997; Dieterich 2002b; Dieterich 2003 Dusheiko 1996). Ribavirin may cause anemia more often in coinfected persons than in those with HIV alone (Dieterich 1999). The risk for ribavirin-induced

anemia increases with higher doses (Chang 2002). Dose reduction may decrease treatment efficacy (Sulkowski 2003d), so an additional strategy—treatment with epoetin-alfa (EPO; a synthetic version of a human protein that stimulates production of red blood cells)—has been studied for management of ribavirin-induced anemia in coinfected individuals (Weisz 2000; Golie 2003).

Golie and colleagues randomized 20/21 coinfected people who were being treated for HCV to two different interventions for ribavirin-induced anemia. When hemoglobin levels decreased to ≤ 10 mg/dl during treatment with pegylated interferon alfa-2b and ribavirin (13 ± 2 mg/kg per day), participants were randomized to RBV dose reduction (N=13) or treatment with EPO (N=7). The ribavirin dose was reduced to 10 mg/kg per day. Two weeks after dose reduction, 7/13 had mean hemoglobin increases from 8.9 mg/dl to 9.7 mg/dl (P=0.03). In the EPO group, within two weeks, 4/7 had increases in mean hemoglobin from 9.9 mg/dl to 11.4 mg/dl (P=0.02) (Golie 2003). This study was too small to evaluate the impact of each strategy on treatment outcomes. Larger studies are needed.

The largest decreases in hemoglobin usually occur within the first four to eight weeks of treatment with ribavirin. A complete blood count should be obtained at baseline, at two and four weeks after initiation of ribavirin (or more frequently if clinically indicated) and periodically during treatment (Roche package insert 2002; Schering package insert 2001).

- **Neutropenia**

Neutropenia often occurs in advanced HIV disease, and significantly increases the risk for, and incidence of bacterial infections (Kuritzkes 2000). Several studies have reported that recombinant human granulocyte-colony stimulating factor (G-CSF; Neupogen®) is an effective treatment for neutropenia in HIV-positive people (Hermans 1996; Kuritzkes 1998, Kuritzkes 2000; Mitsuyasu 1999).

Interferon induces neutropenia. In HCV monoinfection treatment trials, neutropenia has been associated more frequently with pegylated interferon than standard interferon (Fried 2002a; Manns 2001). A retrospective chart review of the incidence of neutropenia among coinfected HCV treatment trial participants reported a higher incidence of neutropenia (42%) than that seen in HCV monoinfection treatment trials (18–20%) (Fried 2002a; Manns 2001; Slim 2003). Reducing the dose of interferon may compromise the response to HCV therapy, so Golie and colleagues randomized 10 coinfected participants who developed neutropenia during an HCV treatment trial to dose reduction of pegylated interferon (N=3) or G-CSF (N=7). The pegylated interferon dose was reduced from 1.5 mg/kg per week to 1.0 mg/kg per week in one group; within two weeks, absolute neutrophil count increased from 553/mm^3 to 2013/mm^3 . In the other group of seven, who received 5 mg/kg of G-CSF twice weekly, absolute neutrophil counts rose from 571/mm^3 to 1814/mm^3 within two weeks (Golie 2003). No data on the response to HCV treatment were provided. Although this study reported that both interventions were effective for management of neutropenia, it is not possible to determine which intervention is preferable without HCV treatment response data.

- **Thrombocytopenia**

Thrombocytopenia (low platelets) is a common complication of HIV, especially in advanced disease (Louache 1994; Moses 1998; Scaradavou 2002). Thrombocytopenia has been associated with HCV infection, and occurs more frequently in persons with advanced liver disease (Pockros 2002; Ramos-Casals 2003). Interferon has been used to treat HCV-related thrombocytopenia, although it can also induce thrombocytopenia. In HCV monoinfection treatment trials, thrombocytopenia was reported more frequently with pegylated interferon than standard interferon (Fried 2002a). Dose modification or treatment discontinuation may be necessary if platelet counts drop markedly during HCV treatment.

- **Hemoglobinuria**

Hemoglobinuria (the presence of unbound hemoglobin in the urine, causing black or very dark urine) is a rare side effect of ribavirin (Diamond 2004; Massoud 2003). Because hemoglobinuria occurs with pronounced hemolysis, hemoglobin levels should be checked if hemoglobinuria develops.

- **Weight Loss**

Weight loss continues to be a significant problem for HIV-positive individuals in the HAART era (Wanke 2000). A weight loss of 5–10% from baseline has been associated with increased risk of developing opportunistic infections and death (Wheeler 1998; Wheeler 1999; Williams 1999). Nausea, vomiting, loss of appetite and weight loss are common side effects of interferon (Fried 2002b; Lindsay 2001; Manns 2001; Zeuzem 2000). Significant weight loss has been reported during HCV treatment trials in coinfected persons (Moreno 2004; Pérez-Olmeda 2003b; Pomova 2003).

Careful monitoring of weight loss during HCV therapy is recommended for coinfected persons. Possible strategies for management of weight loss include eating several small light meals throughout the day, and/or prescribing antiemetics or dronabinol (Marinol®).

- **Ocular Side Effects of Pegylated Interferon in Coinfected Persons**

A rare side effect of pegylated interferon is optic neuropathy, which may cause color blindness or complete loss of vision. In a study of 18 coinfected persons, one case of optic neuropathy was identified, two individuals developed cataracts and seven others developed cotton wool spots. Overall, ocular pathologies were observed in 39% (7/18) (Farel 2003). In many cases, the damage is reversible, but in some instances treatment may need to be temporarily or permanently discontinued to prevent partial or total loss of vision. Baseline assessment and vigilant monitoring for ocular problems, including color vision testing, are recommended.

HCV Treatment for Coinfected People with Hemophilia

A majority of the hemophiliacs who acquired HIV from clotting factors prior to 1985 (when viral inactivation procedures were instituted) also contracted hepatitis C. Coinfection with HIV is known to accelerate HCV progression in hemophiliacs and to increase their risk for developing end-stage liver disease (Goedert 2002; Lesens 1999; Ragni 2001).

The extent and severity of liver disease among coinfected hemophiliacs is difficult to assess unless clinical indications of advanced liver disease are present. Due to the risk for bleeding, liver biopsy is not routinely performed on hemophiliacs. Duration of HCV infection and liver enzyme levels before, during, and after HCV treatment are insufficient surrogate markers for assessing the severity of, and measuring improvements in HCV disease.

Data on safety and efficacy of standard interferon—with or without ribavirin—are available from few studies.

Table 13. HCV Treatment Efficacy In Coinfected Hemophiliacs: Interferon With or Without Ribavirin

Author	Regimen & Duration	N Participants	% SVR	Biological improvement	CD4 (mean)	Discont-inuations
Hanabusa 2002 Arm A: HIV/HCV Arm B: HCV	9 MIU of IFNa-2a once daily for 2 weeks, then 3 x week for 22 weeks 24 weeks total	Total N=30 Arm A: N=15 Arm B: N=15	Overall: 33.3% (10/30) Arm A: 33% (4/12) Arm B: 40% (6/15)	At end of treatment: Arm A: 50% (6/12) Arm B: 47% (7/15) At 2-year follow-up: Arm A: 58% (7/12) Arm B: 50% (7/14) At 4-year follow-up: Arm A: 100% (8/8) Arm B: 43% (6/14)	363 ± 29	Arm A: 20% (3/15) Arm B: 0% (0/15)
Sauleda 2001	3 MU IFNα-2b 3xweek; + 800 mg/day ribavirin after one month IFN monotherapy 24 weeks if HCV RNA was detectable; otherwise, 48 weeks	N=20	Overall: 40% (8/20) Genotypes 2&3: 80% (4/5) Genotypes 1&4: 27% (4/15)	Virological responders: 75% (6/8) had normalization of ALT during TX, and maintained normal ALT after TX. Virological non-responders: none had normalized ALT, but ALT levels decreased during and after TX.	490 ± 76	For toxicity: 0% For week 24 non-response: 60% (12/20)

Hayashi and colleagues studied the same interferon regimen used by Hanabusa and colleagues (9 MIU of IFNα-2a once daily for 2 weeks, then thrice-weekly for 22 weeks). They reported that 0/7 achieved a sustained virological response. A majority of participants in the Hayashi study had high levels of HCV RNA and/or low CD4 cell count, and most were receiving dual nucleoside therapy rahter than HAART (Hayashi 2000). These factors may have contributed to the disappointing response rate.

In March of 2002, a study sponsored by the National Institutes of Health (NIH) opened to evaluate the efficacy of pegylated interferon alfa-2a plus ribavirin in hemophiliacs with HCV and HIV/HCV coinfection, *Treatment of Hepatitis C in Hemophilic Patients with HIV*. The study is expected to conclude in mid-2005.

Treatment of Acute HCV in HIV/HCV Coinfection

Nelson and colleagues diagnosed acute hepatitis C infections in 49 HIV-positive men who have sex with men (MSM), and studied the outcome of both treated and untreated acute HCV infections. Due to the possibility of spontaneous viral clearance, it was suggested that treatment be delayed for 12 weeks (12/22 achieved spontaneous viral clearance during this period). Twenty-six men chose to be treated for HCV. All but four received pegylated interferon plus ribavirin. Three were treated with pegylated interferon, and one with interferon plus ribavirin. So far, 18 have completed treatment; ten have maintained undetectable HCV RNA during follow-up ranging from one to eight months. The only factor significantly associated with a virological response to HCV treatment was a higher peak ALT (482 vs. 177; P=0.021), although CD4 cell count, HIV and HCV viral load, HCV genotype, treatment regimen and the interval between diagnosis and initiation of treatment were also considered. The adverse events reported during treatment included depression, neutropenia, and flulike symptoms; one individual required a transfusion for anemia (Nelson 2003).

Vogel and colleagues treated eight HIV-positive individuals during acute hepatitis C infection. A majority had HCV genotype 1 (5/8). Five received pegylated interferon plus ribavirin, two received pegylated interferon and one received standard interferon. Of the eight, six (75%) achieved an SVR (Vogel 2003).

Different regimens and durations of treatment for acute HCV infection in HIV-positive people merit further investigation.

Retreatment of HCV in HIV/HCV Coinfected Individuals

Rodriguez Torres and colleagues studied the efficacy of HCV re-treatment in a group of 76 coinfected non-responders to interferon with or without ribavirin. Participants were randomized to receive pegylated interferon alfa-2a with or without 800 mg/day of ribavirin for 24 weeks. At week 24, individuals who did not have a decrease of ≥ 2 $\log_{10}$ in HCV RNA discontinued treatment. Virological responders who received pegylated interferon monotherapy added 800 mg/day of ribavirin at week 24. Sustained virological responses were achieved by 5.7% (2/35) of those initially randomized to pegylated interferon monotherapy vs. 19.5% (8/41) of those randomized to pegylated interferon plus ribavirin. SVR rates were not broken out by baseline HCV RNA or HCV genotype.

More than a quarter (20/76) discontinued treatment. There were more withdrawals from the pegylated interferon monotherapy arm than the combination therapy arm (15 and 5, respectively). Discontinuations were attributed to lost-to-follow-up (9/20), adverse events (7/20) and laboratory abnormalities (4/20). Overall, laboratory abnormalities occurred more frequently among those on combination therapy (26/41) than pegylated interferon monotherapy (13/35) (Rodriguez-Torres 2003).

Myers and colleagues re-treated 32 coinfected people who relapsed (N=6) or did not respond (N=26) to previous HCV therapy (interferon with or without ribavirin). Participants received 24 weeks of pegylated interferon alfa-2b (median dose 1.0 μg/kg once weekly; range: 0.5–1.5 μg/kg) and ribavirin (median dose of 1,000 mg/day; range: 600–1,200 mg/day). Those with detectable

HCV RNA discontinued treatment at 24 weeks (34% or 11/32), while those with undetectable HCV RNA continued treatment for an additional 24 weeks.

SVR was achieved by 16% (5/32). The small sample size—and consequently, the smaller number of sustained virological responders—make it difficult to identify any significant predictor of a SVR, although there were non-significant trends associated with achievement of an SVR (non-1 genotype, lower baseline HCV RNA [5.32 ± 0.93 vs. 6.16 ± 0.79 $\log_{10}$], higher CD4 cell count [580 ± 172 vs. 460 ± 211/mL] and higher ALT [139 ± 37 vs. 96 ± 68 IU/l).

Most participants (94%) were receiving antiretroviral therapy during HCV treatment. Six people changed antiretroviral regimens during HCV treatment due to increasing HIV RNA or decreasing CD4 cell counts (N=4), anemia (N=1) and diarrhea (N=1). Although 78% (N=25) experienced decreases in absolute CD4 cell count (median –135/ml; range: -441 to + 16//ml), their CD4 cell counts returned to baseline by week 36. Both CD4 cell percentage and HIV RNA remained stable during treatment.

The withdrawal rate in this study was quite high (47%), and can be attributed to both virological non-response and side effects. Fourteen participants cited side effects rather than virological non-response as their rationale for treatment discontinuation (depression, anxiety, agitation and delirium [N=6]; fatigue, insomnia and weight loss [N=6]; neutropenia and thrombocytopenia]N=2]), while one person withdrew because of hepatocellular carcinoma (Myers 2004).

Strategies to optimize re-treatment of coinfected relapsers and non-responders are needed.

HCV Treatment in Coinfected Cirrhotics

A small study evaluated the safety and efficacy of 24 weeks of standard interferon alfa-2b plus ribavirin in six coinfected cirrhotics. All six participants had been on stable antiretroviral therapy for at least two months before they started HCV treatment. Four of the six received 6 MU of interferon thrice weekly; the other two participants received 3 MU thrice weekly. All received 1000–1200 mg/day of ribavirin. Although 5/6 had an end-of-treatment response, only 2/6 had a sustained virological response. No hepatic decompensations or other severe adverse events were reported (De Bona 2002).

In ANRS HC02, 39% of participants had advanced fibrosis or cirrhosis. When sustained virological response rates were broken out by liver histology, the response rates did not differ significantly according to the degree of liver damage. In the pegylated interferon arm, 25% (51/205) of those with a METAVIR score of F0–F2 achieved SVR, while 32% (66/205) of those with F3–F4 achieved SVR (Perronne 2004).

Liver Transplantation in HIV-Positive People

The United Network for Organ Sharing (UNOS) policy on transplantation for HIV-positive transplant candidates states,

> A potential candidate for organ transplantation whose test for HIV-Ab is positive but who is in an asymptomatic state should not necessarily be excluded from candidacy for organ transplantation, but should be advised that he or she may be at an increased risk of morbidity and mortality because of immunosuppressive therapy.

The UNOS policy was last revised on June 24th, 1992—prior to the era of highly-active antiretroviral therapy. UNOS does not govern the policy on transplantation in HIV-positive candidates at individual transplant centers, but an updated policy would certainly contribute to greatly needed efforts towards broader access to transplantation for HIV-positive people. A survey of 148 directors of transplantation at renal transplant centers was released in 1998. Eighty-eight percent of survey respondents would not transplant a kidney from a cadaveric donor, and 91% would not transplant a kidney from a living donor into a person with asymptomatic HIV infection who was otherwise a good candidate for transplantation. A majority of centers reported a fear of transplantation in HIV-positive individuals; some believed that transplantation into HIV-positive persons was "a waste of precious organs" (Spital 1998). The reluctance to perform organ transplantation in HIV-positive people may persist, despite substantial data on the clinical and immunologic benefits of antiretroviral therapy (Palella 1998; Hogg 1999; Sendi 1999). Clearly, research on patient and graft survival in HIV-positive transplant recipients is needed.

Post Transplantation Survival Before the HAART Era

Before antibody screening for HIV was developed, some organ recipients contracted HIV via organs or blood transfusions from infected donors, while others who were infected prior to transplantation were not diagnosed until after transplantation. Before HAART, HIV treatment was not potent enough to control HIV disease progression for long periods of time in many individuals. Lack of effective treatment for HIV reduced post-transplant survival of some HIV-positive organ recipients. Bouscarat and colleagues followed eleven liver transplant recipients who were infected from 1985 to 1987, during or after transplantation. The seven-year post-transplantation survival rate was 36% for HIV-positive organ recipients vs. 70% for HIV-negative transplant recipients (Bouscarat 1994). A study of transplantation in 15 HIV-positive adults followed between 1981–1988 reported that AIDS was the leading cause of death; 5/15 died during a follow-up period ranging from 0.7–6.6 years (Tzakis 1990).

Erice and colleagues studied 88 HIV-positive organ recipients. The mean time for progression to AIDS after transplantation was 27.5 months. Individuals who were HIV-negative before transplantation did not progress to AIDS as quickly as those infected prior to transplantation, despite the use of immunosuppressive agents (32 months vs. 17 months) (Erice 1991).

There has been much concern about the effects of immunosuppressive therapy in HIV-positive persons, yet encouraging data on one immunosuppressive agent—cyclosporine—has emerged from the pre-HAART era and from more recent research on a potential role for cyclosporine as an

adjunct to HAART in primary HIV infection (Rizzardi 2002). Dummer and colleagues performed a retrospective analysis of data from 1,043 transplant recipients, 18 HIV-positive. After a mean interval of 43 months, 50% (9/18) of the HIV-positive organ recipients were still alive, suggesting that immunosuppressive therapy with cyclosporine did not exacerbate HIV disease (Dummer 1989). Schwarz and colleagues evaluated the medical records of 53 renal transplant recipients, who contracted HIV from infected organ donors. The five-year cumulative incidence of AIDS was significantly lower in the 40 individuals who had received an immunosuppressive regimen containing cyclosporine than that of 13 individuals who did not receive cyclosporine (31% vs. 90%; P=0.001) (A. Schwarz 1993).

Transplantation in the HAART Era

As HAART has brought significant clinical benefits for people with HIV disease, HCV- related end-stage liver disease has become a leading cause of death among people with HIV (Bica 2001; Martín-Carbonero 2001; Monga 2001; Quintana 2002). Thus, an increasing number of HIV-positive transplant candidates with end-stage liver disease have an urgent need for a transplant. Promising reports of post-transplant survival in HIV/HCV coinfected liver recipients have come from transplantation centers in Miami, Pittsburgh, San Francisco and Paris (Neff 2003c; Ragni 2003a; Ragni 2003b; Roland 2003; Samuel 2003).

Table 14. Survival Rates at One Year After Liver Transplantation

Source	Population	Survival Rate
Organ Procurement & Transplantation Network 2002	HIV-negative, from living donor	85.2%
	HIV-negative, from deceased donor	86.3%
Roland 2003	HIV/HCV or HBV coinfected from UCSF	79% (16/19)
Neff 2003c	HIV/HCV or HBV coinfected from UM	100% (6/6)
	HIV/HCV or HBV coinfected from UPMC	90% (9/10)
	Overall from UM and UPMC	94% (15/16)
	HIV/HCV coinfected liver recipients from UM and UPMC	90.0%(10/11)
Samuel 2002	HIV/HCV coinfected from Hôpital Paul Brousse, France	85.7% (6/7)

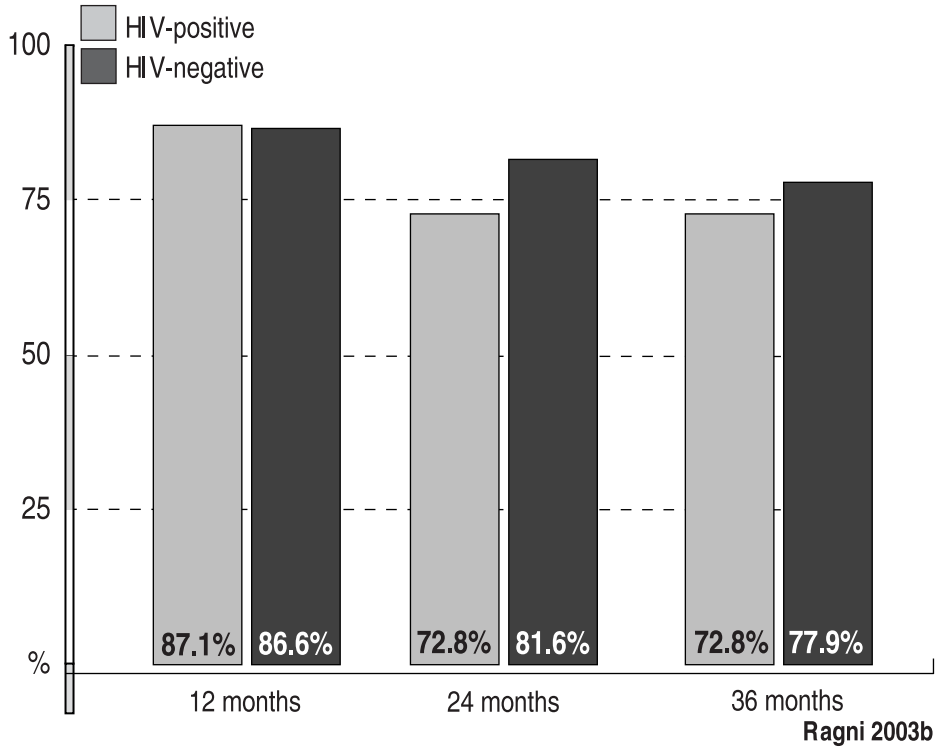
UM = University of Miami; UPMC = University of Pittsburgh Medical Center

A retrospective analysis of the outcomes of liver transplantation in the HAART era reported on CD4 cell counts, HIV RNA and graft survival among 19 HIV-positive liver recipients. During a follow-up interval ranging from 3–1696 days (median: 314 days), four deaths occurred. They were caused by recurrent HCV (N=1), rejection resulting from drug interaction (N=1), post-operative pancreatitis (N=1) and sinus thrombosis 4.5 years after transplantation (N=1). CD4 cell counts were stable, and most people had undetectable HCV RNA. The rate of graft rejection during follow-up was 21%, which is similar to the UNOS rejection rate in HIV-negative individuals (Roland 2003).

Ragni and colleagues reported survival data at one, two and three years after liver transplantation among 24 HIV-positive transplant recipients, 15 HCV coinfected. Post-transplant antiretroviral

intolerance developed in 40% (6/15) of the coinfected liver recipients, four of whom died. All four developed recurrent HCV, and were being treated with interferon and ribavirin. The remaining two resumed antiretroviral therapy.

Figure 8. Survival Rate at 12, 24 and 36 Months After Transplantation by HIV Status



Survival was significantly associated with the post-transplant CD4 cell count and HIV RNA. Survival after transplantation was significantly poorer among those with a post-transplantation CD4 cell count of <200 cells/μL and an HIV RNA >400/mL (P=0.005 and P=0.016, respectively). Thus, the authors suggest that survival may be predicted more accurately by the virological and immunological responses to antiretroviral therapy before end-stage liver disease (ESLD). ESLD may make it impossible for some candidates to tolerate HAART, so using pre-transplant CD4 cell count and HIV RNA as the sole eligibility criteria may unnecessarily disqualify good candidates.

Survival was significantly poorer among the 15 HCV-coinfected liver recipients (P=0.023) than those with HBV/HIV-coinfection (N=7) and HIV-positive persons transplanted because of fulminant liver failure (N=3). However, post-transplantation survival of coinfected transplant recipients did not differ significantly from that of persons with HCV monoinfection (Ragni 2003b). The sample size was small; data from larger groups of coinfected transplant recipients are needed.

A review of data from eleven HIV/HCV coinfected patients who had liver transplants at the University of Miami (UM) and the University of Pittsburgh (UP) between September of 1997 and December of 2001 reported that ten of the eleven transplant recipients maintained undetectable HIV RNA levels during follow up. Prior to transplantation, all had CD4 cell counts of <200; these have increased to >200 after transplantation (Neff 2003c).

Nowak and colleagues studied outcomes of four coinfected liver transplant recipients. They reported that HIV RNA was controlled, and CD4 cell counts increased when HAART was reinitiated after transplantation. One of the four had a primary HIV infection after transplantation; in this individual, HIV RNA decreased during the second week after infection, despite immunosuppressive therapy. By the third week, HIV RNA began to increase, and antiretroviral therapy was started. All four individuals maintained undetectable HIV RNA. One individual died at three months, while the other three were alive at three months, nine months and three years after transplantation (Nowak 2003).

Rufi and colleagues are conducting a prospective cohort study evaluating safety and efficacy of liver transplantation in HIV-positive persons. So far, six sites have performed liver transplants in 21 HIV-positive people, 19 HCV-coinfected. All received antiretroviral therapy before transplantation, when the median CD4 cell count was 247/mL (range: 110–589) and almost all (95%) had undetectable HIV RNA. All initiated antiretroviral therapy within a median of 5 days (range: 3–30) after transplantation. Only one person experienced immunological progression of HIV disease (CD4 count <100/mL) after transplantation.

Table 15. CD4 Cell Count and HIV RNA After Liver Transplantation

Timepoint and number	CD4 cell count/ mL median (range)	HIV RNA <200 copies/mL
Prior to transplantation (N=19)	247 (110–589)	95%
1 month after transplantation (N=19)	228 (119–564)	88%
3 months after transplantation (N=16)	250 (130–462)	86%
6 months after transplantation (N=12)	216 (154–480)	100%
12 months after transplantation (N=5)	253 (165–440)	100%

Rufi 2004

Although there were eight cases of acute graft rejection, none required re-transplantation. Recurrent HCV was cited as the major concern; it developed among 75% (15/19), appearing between 1–12 months after transplantation. Almost half (47%) are being treated with pegylated interferon plus ribavirin. Two transplant recipients have died: one at three months from sepsis and one from cirrhosis resulting from recurrent HCV at 14 months (Rufi 2004).

Treatment of Recurrent HCV

The need for effective HCV treatment after transplantation was underscored by a report from King's College Hospital in London. All four HIV/HCV coinfected liver recipients survived transplantation, although HCV recurred. Two of the four were treated with interferon and ribavirin, yet all four died from HCV-related complications between 3–25 months after transplantation (Boyd 2001).

Reports of treatment outcomes from Miami and Pittsburgh have been more encouraging. In Miami, two of three coinfected liver recipients developed recurrent HCV. Both were treated with pegylated interferon alfa-2b plus ribavirin. Although HCV treatment did not result in SVR for either individual, one had a reduction in inflammation and the other had reductions in both inflammation

and HCV RNA levels. Five of six HIV/HCV coinfected liver recipients in Pittsburgh developed recurrent HCV. One individual experienced graft rejection after the physician discontinued treatment with a protease inhibitor without making a corresponding adjustment to the dose of his immunosuppressive therapy; then, the treatment for graft rejection was complicated by recurrent HCV. The patient developed renal failure and died 19 months after transplantation. One of the remaining four was not been treated for HCV, because a biopsy showed no evidence of hepatitis. The other three individuals have been treated with interferon and ribavirin. One achieved SVR and the other two have had normalization of liver function tests while undergoing HCV treatment (Neff 2003c).

Aside from the pressing clinical need for better data and more access to transplantation for coinfected individuals, transplantation also presents an opportunity for immunologic and pharmacokinetic research. The effects of two immunosuppressive agents—cyclosporine and mycophenolate mofetil—on HIV disease, and the potential for synergistic as well as dangerous interactions with these drugs and certain antiretrovirals merit further investigation. The case histories of coinfected transplant recipients generate numerous clinical management issues: prevention and reversal of graft rejection, selection of antiretrovirals, proper dosage of immunosuppressive agents, the incidence and management of opportunistic infections in the milieu of immunosuppression, especially those for which there is no prophylaxis (Kaposi's sarcoma and human papilloma virus). The efficacy and tolerability of treatment for recurrent hepatitis C is a crucial issue. The National Institutes of Health has funded the *Kidney and Liver Transplantation in People with HIV Study*, a prospective, multi-center cohort study of 275 HIV-positive kidney and liver recipients, who will be followed for two to five years. It opened in late 2003.

Drug Interactions: Protease Inhibitors and Immunosuppressants

Drug-drug interactions are a significant concern for coinfected liver transplant recipients and their medical providers. There are significant interactions between protease inhibitors and the immunosuppressive agents used after transplantation to prevent graft rejection. A two-year evaluation of interactions between antiretrovirals and cyclosporine in 22 HIV-positive transplant recipients reported that cyclosporine increased or decreased the area under the curve (AUC) of protease inhibitors, while the AUC of NNRTIs remained close to levels seen in published studies. Pharmacokinetic studies were performed prior to transplantation, during weeks 1, 2, 4, 8, 12, 28, 52, 104, and if antiretroviral therapy was changed. The dose of cyclosporine needed progressive reduction in individuals taking protease inhibitors with or without NNRTIs, due to the increasing intestinal bioavailability of cyclosporine that occurs over time (Frassetto 2003).

Significant interactions between tacrolimus (another immunosuppressant), and protease inhibitors — especially lopinavir/ritonavir (Kaletra®) and nelfinavir (Viracept®)— have been reported (Jain 2002b; Jain 2003; Schvarcz 2000; Sheikh 1999). Jain and colleagues reported that lopinavir/ritonavir significantly increased tacrolimus levels in three coinfected liver transplant recipients. In one individual, tacrolimus dosing was adjusted from 5mg twice daily to 0.5 once weekly after introduction of lopinavir/ritonavir. In another, the area under the curve (AUC; a measurement of the amount of a drug that reaches the bloodstream during a specific period of time) for tacrolimus increased from 31 ng/mL/h to 301 ng/mL/h after lopinavir/ritonavir was introduced. They recommended using "great caution" when adjusting tacrolimus dosing at initiation and

discontinuation of lopivavir/ritonavir (Jain 2003). The interaction between tacrolimus and nelfinavir was crudely demonstrated when a transplant recipient discontinued nelfinavir without a concomitant adjustment in tacrolimus dosing; this led to graft rejection, renal failure and eventual death (Neff 2003c). Jain and colleagues studied interactions with protease inhibitors and tacrolimus in six liver transplant recipients (one of whom received indinavir; the other five, nelfinavir-based regimens). They compared tacrolimus dosing in the six HIV-positive liver recipients on HAART to that of HIV-negative transplant recipients. The proper dose of tacrolimus in nelfinavir-using liver transplant recipients was determined to be 38 times lower than the regular dose (Jain 2002b). A case report of the interaction between tacrolimus and protease inhibitors (saqinavir [Fortovase®], ritonavir [Norvir®], and nelfinavir) in one individual found that tacrolimus dosing had to be decreased by >95% when administered with nelfinavir (Sheikh 1999). An interaction between sirolimus (a newer immunosuppressant) and nelfinavir has been identified in a case report, where sirolimus levels were five to nine times higher in an individual who was also taking nelfinavir (Jain 2002c).

MELD (Model for End-Stage Liver Disease) Scoring: Impact on HIV-Positive Transplant Candidates

The Model for End-Stage Liver Disease (MELD) system is used to evaluate transplant candidates; MELD replaced the Child-Pugh Score in February of 2002 (see Chapter IV, Diagnostics). The MELD system is regarded as the most accurate and objective method of identifying those with the most urgent need for liver transplantation within a three month period and prioritizing them on the waiting list, thus decreasing the mortality rate among transplant candidates (Kamath 2003).

The MELD allocation system may extend the wait for organs among HIV-positive transplant candidates. More data is needed before the effect of the MELD system on HIV-positive transplant candidates can be evaluated. The need for transplantation may be more urgent among coinfected candidates with hepatic decompensation. A retrospective chart review analyzed survival among 41 coinfected individuals with decompensated cirrhosis, reporting that the median survival time after development of ascites was 123 days; the probability of survival at six months was 38% (Von Wichmann 2003b). In HCV monoinfection, the survival rate at five years after hepatic decompensation is 50% (Fattovich 1997).

Access to transplantation does not depend solely on an individual's MELD score. Organs are allocated regionally. The MELD score at which a candidate receives a transplant may vary, depending on the availability of organs and the number of other candidates in a particular region. Candidates with high MELD scores and the most urgent need for a transplant may be less likely to survive transplantation than those with less immediate need. This is mainly due to a chronic shortage of donor organs. Because of this shortage, the MELD system may have a negative impact on overall survival of transplant candidates, regardless of their HIV status.

A retrospective comparison of the MELD scores of transplant recipients with hepatitis C prior to and after the implementation of the MELD system did not find any significant differences in allocation for transplantation. Before the MELD system was adopted, the mean MELD score in transplant recipients was 19 (range: 11–35). After implementation of the MELD system, the mean MELD score in transplant recipients was 17.2 (range: 7–31). There were no differences in

allocation by gender, or allocation by MELD score over a period of one, two and three months before transplantation. The investigators did identify a trend towards increased transplantation of individuals with HCV-related hepatocellular carcinoma after the implementation of the MELD system (Meyer 2003).

The impact of the MELD allocation system on overall post-transplant survival rates—particularly on survival of HIV-positive liver recipients—needs evaluation.

Access to Transplantation

Limited supply of organs, a fragmented transplantation system, and financial coverage for transplants are all critical for HIV/HCV coinfected individuals. Many insurers consider it to be an experimental procedure and have declined coverage. An experimental designation should not apply to an established procedure simply because a candidate is HIV-positive.

Other Factors Affecting Treatment and Survival of Coinfected Persons

HCV antibody status may be serving as a marker for poorer access to care and competing problems with addiction that lead to delays in care or failure to implement the standard of care…if we are to improve the health status of patients with HIV-HCV coinfection, perhaps we should focus on these issues as well as the presence of the 2 viruses.

—C. S. Graham
Clinical Infectious Diseases

Presenting With Advanced HIV (and HCV) Disease and Limited Access to Care

The number of people diagnosed with AIDS immediately after their HIV diagnosis reflects limited access to care among people who are at high risk for HCV coinfection. HIV and AIDS surveillance data collected from 25 states during the period from 1994–2000 found that 26% (33,144/128,813) of those who were diagnosed with HIV already had AIDS. Twenty-four percent of this group (7,955/33,144) acquired HIV from injection drug use (CDC 2002c). Since HCV is widely prevalent among IDUs, it is safe to assume that a majority of these 33,144 individuals are coinfected.

Coinfected individuals may receive HAART significantly later, or at lower baseline CD4 cell counts than those with HIV monoinfection, despite evidence of the clinical benefits of HAART in people who are coinfected (Hare 2002; Sulkowski 2002; Tedali 2003a; Tedali 2003b; Torriani 2001; Qurishi 2002a; Qurishi 2002b). The delay in initiation of HAART may be attributed to several factors that also have an effect on survival: limited access to care, injection drug use and poverty. For example, individuals from lower socioeconomic strata are less likely to be prescribed triple-combination therapy regardless of clinical indications (Mc Farland 2003; Wood 2002).

Ineligibility for HCV Treatment

Until recently, active drug use has been a contraindication for HCV treatment. Although interferon has not been expressly contraindicated in people with psychiatric co-morbidities, many have traditionally been considered ineligible for HCV treatment. HIV and HCV are common among individuals with mental illness and/or drug addiction (Bolumar 1996; Marsh 2002; Meyer 2003; Rohrig 1990; Schmitt 1994). Rosenberg and colleagues screened 931 individuals receiving treatment for severe mental illness in Connecticut, Maryland, New Hampshire and North Carolina for HIV and HCV. They reported that HIV prevalence in this group was approximately eight times greater than that of the general population (3.1% vs. 0.335%) and HCV prevalence was approximately eleven times greater (19.6% vs. 1.4%) than that of the general population (Rosenberg 2001).

In a clinic at Chicago's Cook County Hospital, Soto and colleagues screened 241 HIV-positive adults with and without psychiatric co-morbidities (including addiction to drugs/alcohol) for HCV antibodies. Participants were grouped by the presence or absence of psychiatric co-morbidities and addiction. The overall prevalence of HCV was 28%. HCV was far more prevalent among individuals with psychiatric co-morbidities and addiction (40% vs.14.6% for individuals without a psychiatric or addiction diagnosis) (Soto 2002).

These significant co-morbidities have resulted in reduced eligibility for HCV treatment among a significant proportion of coinfected individuals (Bini 2004; C. A. Fleming 2003; Jarousse 2002; Restrepo 2003; Schwartzapfel 2002; L. E. Taylor 2002).

Bini and colleagues assessed eligibility for HCV treatment in a cohort of 280 coinfected people. Eligibility for treatment was established by combining well-known inclusion and exclusion criteria with the treating clinician's assessment. Coinfected people were considered significantly less likely to be eligible candidates for HCV treatment by both parameters than those with HCV monoinfection (P=0.01 according to exclusion criteria; P=0.02 according to clinician opinion). Predictors of treatment ineligibility according to clinician opinion were: ongoing or recent substance abuse (OR, 26.0; 95% CI, 5.2–128.8), co-morbid medical conditions (OR, 19.4; 95% CI, 6.4–58.9), albumin <3.2 g/dl (OR, 15.2; 95% CI, 1.5–157.2), psychiatric co-morbidity (OR, 5.7; 95% CI, 1.2–26.6), and annual income of <$10,000 per year (OR, 2.6; 95% CI, 1.0–6.4) (Bini 2004).

Fleming and colleagues evaluated eligibility for HCV treatment in a cohort of 149 coinfected persons. Only 29% (44/149) met the criteria for treatment eligibility.

Table 16. Reasons for HCV Treatment Ineligibility

Cause of Treatment Ineligibility	% (N=105)
Non-adherence to medical visits	23% (24/105)
Drug/alcohol use during previous six months	23% (24/105)
Active psychiatric disease	21% (22/105)
Advanced HIV disease	13% (14/105)
Decompensated liver disease	12% (13/105)
Medical comorbidities	8% (8/105)

C. A. Fleming 2003

Of those eligible for HCV therapy, 64% (28/44) chose not to undergo treatment (C. A. Fleming 2003).

Table 17. Reasons for HCV Treatment Refusal

Cause of Treatment Refusal	% (N=28)
Concern about potential side effects of HCV treatment	31% (9/28)
Did not return for treatment	21% (6/28)
Relocated	11% (3/28)
Concern about ability to work during treatment	11% (3/28)
Unstable social circumstances	11% (3/28)
Concern about relapse to active injection drug use	7% (2/28)
Pregnancy of partner	3.5% (1/28)
Death by unrelated cause	3.5% (1/28)

C. A. Fleming 2003

Taylor and colleagues investigated low enrollment of coinfection clinic patients in an HCV treatment trial. They reported that a majority of patients were ineligible due to medical contra-indications (58%), psychiatric illness (26%), active drug use (20%), and previous HCV treatment (6.5%); the remaining 26% did not choose to participate in the study (reasons not specified) (L. E. Taylor 2002). Another evaluation of 231 coinfected patients found that only 24% (56/231) were eligible for HCV treatment. Reasons for HCV treatment ineligibility included alcohol consumption of more than 30 grams per day (43/231), severe mental illness (28/231), and active injection drug use (6/31) (Von Wichmann 2003a).

These considerable barriers to care will only be surmounted by outreach initiatives to underserved and at-risk populations. Outreach initiatives must be linked to medical and mental health providers, drug and alcohol treatment programs and methadone maintenance facilities.

Multidisciplinary Care

Encouraging results have emerged from a pilot program for treating HCV in HIV-positive individuals with co-morbid mental illness and/or addiction. In a Rhode Island clinic, Schwartzapfel and colleagues evaluated 38 coinfected individuals, 95% of whom had a history of addiction and 84% of whom had a history of mental illness. Liver biopsy was performed in 23, revealing that eight had stage 3 fibrosis and six were cirrhotic; three developed decompensated liver disease during the evaluation process. Based on the urgent need for treatment, individualized care plans were developed with a team including an HIV specialist, a gastroenterologist, a psychiatrist, a nurse and an outreach worker who made frequent contact with those undergoing HCV treatment. The first two individuals who received HCV treatment and care from this multidisciplinary team have had virological responses to treatment without serious adverse events (Schwartzapfel 2002).

Hepatitis C Treatment in Coinfected Injection Drug Users

Little is known about HCV treatment outcomes or side effects in coinfected injection drug users, as they have often been excluded from clinical trials, or so few have participated that it is impossible to determine this information. Clearly, research on treatment of coinfected active injection (and non-injection) drug users is a priority.

Impact of Repeated Exposure to HCV From Injection Drug Use on Efficacy of HCV Treatment

Since injection drug users may be repeatedly exposed to HCV, it has been suggested that the low rates of virological response to HCV treatment among coinfected people might be partially attributable to infection with more than one genotype of HCV. Hypothetically, since HCV-genotypes 2 and 3 are more sensitive to treatment than genotypes 1 and 4, an initial viral clearance of an HCV genotype 2 or genotype 3 infection might allow a shift to a masked infection with a more treatment-resistant genotype. Soriano and colleagues examined this possibility in a group of 30 coinfected former IDUs. Ten of these individuals had HCV genotype 3; although they initially cleared HCV, while still on treatment, their virus became detectable. A comparison of baseline and post-treatment genotypic results revealed no difference in HCV subtypes (Soriano 2003).

Issues Concerning Medical Care and Treatment of Injection Drug Users

> *As someone who was closely involved in the original (illegal) efforts to establish needle exchange in New York City and having worked in one position or another as an advocate for the health care needs of illicit drug users for the past ten years, I was intimately aware of the incredible stigma, discrimination, and outright hostility and disgust injection drug users routinely face when attempting to seek health care services of any kind. Suddenly, I was my own client, and all those years I'd spent advocating for other drug users, while giving me insight into some of the systems I would now have to negotiate for myself, did not prepare me for the treatment I would also receive as a heroin injector with AIDS.*
>
> —I. Thaca
> *Harm Reduction Communication*

> *I felt my GP's diagnosis was not that I had a serious liver disease, but an untreatable moral malady.*
>
> —Lisa Waller
> *Medical Journal of Australia*

Injection drug use was identified as the mode of transmission by 21% (21,469/101,881) of people diagnosed with HIV from 1999 through 2002 (Glynn 2004). According to the Centers for Disease Control, injection drug use directly and indirectly accounts for 36% of AIDS cases in the United States (CDC 2002e). Up to 90% of people who acquired HIV from injection drug use with unsterilized equipment are coinfected with hepatitis C (CDC 2002f). The needs of coinfected

active and former injection drug users must be addressed by clinicians and incorporated into medical care and treatment.

Even in the HAART era, injection drug use remains linked with an increased risk for progression to an AIDS-defining condition or death in HIV-positive and HIV/HCV coinfected persons (Egger 2002: Voirin 2003; Schlanger 2004). An analysis of data from a cohort of 3,547 HIV-positive individuals collected during 1990–1995 and 1996–2000 revealed that injection drug users derived less disease-free survival time from HAART than non-IDUs. HAART increased disease-free survival time for non-IDUs with <200 CD4 cells by 135% vs. 34% for IDUs with <200 CD4 cells (Poundstone 2001). From 1996 until 2002, Voirin and colleagues found that the risk of death among 1,470 HIV-positive people did not differ significantly by HCV status (HR 0.76; 95% CI, 0.28–2.08; P=0.59). Injection drug use increased the risk of death for coinfected persons. Coinfected IDUs had a significantly greater risk than that of HIV-positive or HIV/HCV coinfected non-IDUs (HR 2.92; 95% CI, 1.63–5.23; P<0.001) (Voirin 2003). In New York City, HCV-related death rates increased among coinfected IDUs from 0.3% in 1993 to almost 4% in 1999. HCV-related liver disease was the leading cause of death among coinfected IDUs (79.2% vs. 50.2% for other causes of death; P<0.0001) (Schlanger 2004).

The institutional norms and stigma associated with injection drug use have created considerable barriers to care and treatment for people with HIV and hepatitis C. Until 2002, active injection drug use was a contraindication for treatment of hepatitis C, and many physicians still consider it to be one. Barriers to treatment for injection drug users are reflected in several reports of HIV-positive injection drug users who have not received HAART, or have initiated HAART later than non-drug users (Bassetti 1999; Bogart 2000; Maisels 2001; Mocroft 1999a; Murri 1999).

Physicians have perceived drug use as an indicator of poor adherence to antiretroviral therapy (Escaffre 2000). However, predictions by medical providers of which patients are likely to be adherent to HAART are often inaccurate (Bangsberg 2001; Escaffre 2000; Gross 2002; L. G. Miller 2002; Paterson 2000). While some studies have linked active drug and/or alcohol use with poor adherence (Carrieri 2003; Chesney 2000; Haubrich 1999; Lucas 2001), others have not found significant differences in adherence between drug users and former or non-drug users (Broers 1994; Gebo 2001; Roca 1999). The frequency of drug and/or alcohol use and the substance(s) being used vary, making generalizations about the impact of drug and/or alcohol use on adherence difficult.

For patients who are seeking treatment for drug and/or alcohol problems, combining primary care with addiction treatment may increase adherence to antiretroviral therapy (Lucas 2001; O'Connor 1992). However, treatment for drug and/or alcohol problems should not be a prerequisite for medical care for HIV and HCV. Barriers to adherence should be identified and addressed by several interventions, including screening for, and (when indicated) treating depression. Patients should receive education and counseling from HIV and HCV treatment education programs as well as their clinicians. Antiretroviral regimens should be selected carefully to reflect patient concerns regarding side effects, dosing frequency, pill burden and dietary restrictions (Murphy 2003; Proctor 1999; Starace 2002; Tucker 2003; Turner 2003). Interventions to support adherence, such as aggressive monitoring and management of side effects, ongoing counseling and provision of reminder devices and pill boxes should be incorporated into individually tailored adherence strategies (Haynes 2002; Stone 2001; Tuldra 2002).

Education About Addiction

Education on drug and/or alcohol addiction and treatment must be available to medical providers, some of whom have cited difficulties in providing care to people with drug and/or alcohol problems. A survey of 144 physicians reported that they experienced a lower satisfaction rate in caring for patients with drug and/or alcohol problems than those with other illnesses. Greater satisfaction in caring for patients with drug and/or alcohol problems was associated with a positive attitude towards treatment of addictions (adjusted OR 4.60, 95% CI, 1.59–13.29), physician confidence in assessment and intervention (adjusted OR, 2.49; 95% CI, 1.09–5.69) and perceived responsibility for addressing problems with substance use (adjusted OR 5.59, 95% CI, 2.07–15.12) (Saitz 2002).

These low satisfaction rates may reflect a lack of training on the identification and treatment of addiction. An astonishing 41% (28/66) of accredited medical schools surveyed in 1996 did not include curricula on addiction in lecture or discussion hours (N. S. Miller 2001). A national survey of 769 faculty members who teach residents about drug and alcohol problems reported that less than 10% of the instructors had actually done clinical work in alcohol or drug treatment programs, and only 19% were certified in addiction treatment (M. F. Fleming 1999).

Providing training on assessment of, and interventions for addiction increases physician confidence and comfort with providing care for people with drug and alcohol problems. Karam-Hage and colleagues surveyed attitudes and beliefs about addiction among 52 general psychiatry residents before and after a one-day educational conference on addiction. After the conference, participants were more likely to believe they could motivate patients to seek treatment for drug and/or alcohol problems, and had an increased interest in more training on addiction (Karam-Hage 2001). The University of Massachusetts Medical School has provided an intensive one or two-day interclerkship on substance abuse for third-year medical students and performed an evaluation on its impact on their knowledge and attitudes concerning substance abuse. After the interclerkship, participants had significant improvements in their ability to assess for drug and/or alcohol problems ($P=0.005$) and to provide appropriate intervention ($P<0.05$) (Matthews 2002). Curricula on assessment and interventions for addiction must be included in medical education, and CME programs.

Harm Reduction as Part of Medical Care

Harm reduction—a set of practical strategies that reduce negative consequences of drug use by addressing the conditions of drug use along with drug use itself—must be incorporated into the care and treatment of coinfected injection drug users. Instruction on safe injection practices and referral to syringe exchange programs or prescription of syringes will reduce the risk of HCV reinfection, bacterial infections and infection with other bloodborne pathogens. Resources are available to physicians to support integration of harm reduction into medical care; there are more than 200 syringe exchange programs in the United States (Edlin 2002). Prescription of syringes is illegal in only three jurisdictions (Burris 2002). A survey of 39 infectious disease and addiction medicine physicians in Rhode Island reported that 95% of respondents felt that there was a legitimate medical reason for injection drug users to obtain sterile syringes (Rich 2001).

Other interventions include referral to methadone maintenance programs or prescribing buprenorphine (a semi-synthetic opiate approved by the FDA in 2002 for maintenance and

detoxification treatment of opiate addiction). Although methadone is a safe and effective treatment for opioid addiction, its availability is limited; in the United States; only 20% of the estimated 810,000 heroin addicts receive methadone maintenance treatment (Office of National Drug Control Policy 2000). Buprenorphine is subject to fewer restrictions—doctors may apply for a waiver to prescribe or dispense it— and it has been associated with increased adherence to antiretroviral therapy (Moatti 2000). High-dose burpenorphine for treatment of opiate addiction has been available by prescription in France since 1996. A prospective study of retention in care among recipients of prescription high-dose buprenorphine found that 56.9% (508/909) were still with the same physician two years later. Their self-reported heroin use had decreased significantly ($P<0.001$), while their social situations (housing and work) improved significantly ($P<0.001$). The rates of seroconversion for HCV and HIV were low (4.1% and 0.8%, respectively) (Fhima 2001).

Recommendations

Provide full access to hepatitis C care and treatment for all HIV-positive persons in need.

Current treatments for HCV can cost as much as $40,000 per year. Cash-strapped State AIDS Drug Assistance Programs (ADAPs) are unable to offer HCV treatment; few have the resources available to provide pegylated interferon and ribavirin. ADAPs must receive the necessary funding from Congress to cover HCV treatment. Strategies must be developed to provide coverage for HCV therapy among the uninsured who do not qualify for entitlements or patient assistance programs.

Include HIV/HCV coinfected individuals in early-phase HCV treatment trials.

Hepatitis C treatment with pegylated interferon plus ribavirin is less effective in coinfected persons (Chung 2004; Pérez-Olmeda 2003b; Perronne 2002, Perrone 2004; Torriani 2004). Because HCV is more aggressive in HIV-positive individuals, the need for new, more effective treatments is particularly urgent. Research on the safety and efficacy of HCV treatment in coinfected individuals has lagged; usually, coinfected individuals and clinicians must wait for several years before these data are available to them. This is unacceptable. Coinfected individuals must be offered the opportunity to participate in clinical trials of new agents as soon as it is safe to do so. A good benchmark here would be to ensure enrollment of coinfected individuals as soon as a safe and active dose is defined. Trial sponsors could stratify such studies by HIV status.

Explore Strategies to Optimize HCV Treatment for HIV/HCV-Coinfected Persons.

In the absence of new drugs, research on strategies for optimizing current HCV treatment for HIV/HCV-coinfected persons is needed. Sustained virological response rates from three large, randomized HCV treatment trials in HIV/HCV-coinfected persons have been disappointing, especially for people with genotype 1. Extending treatment from 48 weeks to 72 weeks may increase sustained virological response rates among this population. Although induction therapy with high-dose interferon has not been a successful intervention for people with HCV monoinfection, it may improve treatment outcomes for coinfected people. NIH should sponsor this research.

Side effects of HCV therapy may have severe consequences for coinfected people. For example, anemia, weight loss and depression are common side effects of HCV therapy. These are significant concerns for HIV-positive people, as all three conditions, when untreated, have been associated with more rapid HIV disease progression and poorer survival (Diallo 2003; Fairnpour 2003; Lundgren 2003; Mocroft 1999b; Moore 1998; Semba 2002; Sullivan 2002; Tate 2003; Wheeler 1998; Wheeler 1999; Williams 1999).

Ribavirin and both formulations of interferon may potentiate side effects and toxicities from antiretroviral agents (Anderson 2004; Berenguer 2003a; Braü 2004; Fleischer 2003; Lafeuillade 2001; Moreno 2004; Pérez-Olmeda 2003b; Perronne 2002; Pol 2003b; Salmon-Céron 2001; D. M. Smith 2002). Continued research on side effect management strategies for coinfected people during HCV treatment is crucial. Drug-drug interactions between agents used for HCV and HIV therapies need further study. Manufacturers of HCV and HIV therapies should offer their sponsorship for such research.

Interventions such as pre-emptive treatment for anemia and depression prior to, or upon initiation of HCV therapy may increase tolerability and adherence and thus, the likelihood of achieving SVR. These and other strategies for managing side effects and improving HCV treatment outcomes merit prospective, randomized trials. Manufacturers of HIV and HCV therapies, growth factors and anti-depressants should contribute their support towards this research.

Increase research of HCV treatment safety and efficacy in understudied coinfected populations.

HCV treatment trials in coinfected individuals have excluded people with medical and psychiatric co-morbidities; active drug users have been virtually excluded as well. Since HIV/HCV-coinfection is prevalent among injection drug users and individuals with severe mental illness, HCV treatment trials must be designed to include them. This will ensure that results will be applicable to these high-prevalence populations.

Coinfected individuals with compensated cirrhosis have an urgent need for treatment, yet little is known about the safety and efficacy of pegylated interferon–based regimens in this group. More research is needed.

The NIH must support research on the safety and efficacy of HCV treatment in these understudied coinfected populations.

Develop a treatment protocol for acute HCV infection in HIV-positive people.

As in HCV monoinfection, the optimum regimen and duration of treatment for acute hepatitis C infection are unknown. The NIH should support research on treatment of acute HCV infection in HIV-positive people.

Strengthen linkages between substance abuse treatment programs, methadone maintenance programs, medical and mental health providers, and HIV/HCV prevention and service programs.

Between 1994–2000, 7,955 current and former injection drug users who had already developed AIDS were tested for HIV (CDC 2002c). Since HCV is highly prevalent among IDUs, it is safe to assume that a majority of these individuals are HCV-coinfected. HAART has significantly decreased overall and liver-related mortality (Qurishi 2003), but when people present with advanced HIV disease and/or advanced liver disease, they may be unable to benefit fully from treatment for one or both infections (Wood 2003). Efforts to reach underserved and at-risk populations and provide them with medical care must increase.

Public and private systems of care must address multiple needs: mental health, addiction, medical care and treatment. Linkages between prevention programs for HIV and HCV and medical care delivery systems must be strengthened.

Increase capacity to provide individualized medical care and treatment to coinfected active drug users.

Harm reduction must be incorporated into the medical care of coinfected injection drug users. Interventions must range from education on safer drug use to supporting abstinence and recovery.

Adequate education on assessment of and treatment for drug and alcohol addiction is an important component of providing care to active and recovering drug and/or alcohol users. Cross-disciplinary collaboration between injection drug users, medical providers, experts in harm reduction and substance abuse treatment are necessary to develop best practices for medical care for coinfected injection drug users. The National Institutes of Health and its National Institute on Drug Abuse (NIDA) should support the development of best practices for treatment of coinfected injection drug users.

Support access to and research on organ transplantation for HIV-positive individuals

Although HAART has significantly increased the survival of HIV-positive individuals, the risk for end-stage organ disease in this population remains significant. In the HAART era, HIV-positive individuals may have post-transplantation outcomes equivalent to HIV-negative individuals (Gow 2001; Kuo 2001; Neff 2003c, Prachalias 2001; Ragni 1999; Ragni 2003a; Ragni 2003b; Roland 2002; Roland 2003; Roland 2004; Rufi 2004).

Although The United Network for Organ Sharing (UNOS) does not consider HIV infection to be a contraindication for organ transplantation, its policy on transplantation has not been updated for more than a decade. Consequently, the UNOS policy does not reflect virological, immunological and survival benefits from the use of highly active antiretroviral therapy. A revised policy may help dispel the unwillingness to perform transplantation in HIV-positive candidates at individual transplant centers, where the decision to perform transplantation in HIV-positive candidates is made.

Despite the emerging reports of favorable outcomes in HIV-positive individuals, insurers have sometimes denied reimbursement for transplants when HIV is involved, deeming it “experimental.” Expanding an indication to include people with HIV does not transform an established procedure into an “experiment.” Transplantation must be reimbursable for HIV-positive individuals.

The National Institutes of Health has funded a prospective, multi-center cohort study evaluating the safety and efficacy of solid organ (kidney and liver) transplantation in people with HIV. An evaluation of the effect of MELD on the survival of HIV-positive transplant candidates and organ recipients will be included.

This and other prospective studies of transplantation in HIV-positive individuals will provide vitally important information about the specific risks for those undergoing transplantation, as well as help to identify the optimal clinical management strategies for improved and extended survival of HIV-positive organ recipients. This important research—and transplantation in HIV-positive candidates outside of research settings— will provide crucial data that may be used to broaden indications and secure reimbursement for transplantation in HIV-positive candidates.

List of Terms Used in This Chapter

Absolute CD4 cell count: the number of CD4 lymphocytes in one cubic millimeter (mm^3) of blood.

Anitemetic: a drug used to control nausea and vomiting.

Buprenorphine: a semi-synthetic opiate. Buprenorphine was approved by the FDA in 2002 for maintenance and detoxification treatment of opiate addiction.

CD4 cell percentage: the percentage of total lymphocytes made up by CD4 CELLS. Hemolysis: destruction of red blood cells. When the membrane of a red blood cell is ruptured, hemoglobin is released from the cell.

Hemoglobinuria: an abnormal condition marked by the presence of hemoglobin in urine.

Hyperamylasemia: abnormally high levels of amalyse in the blood or urine. Amalyse is a digestive enzyme produced by the pancreas and salivary glands.

Myleosuppression: a decrease in the ability of the bone marrow cells to produce blood cells, including red blood cells, white blood cells and platelets.

Negative predictive value (NPV): The accuracy of predictions that the target outcome is not present. In this case, a sustained virological response to hepatitis C treatment was not present, based on virological response to hepatitis C treatment at a specific timepoint during treatment (such as week 4 or week 12). For example, an NPV of 99% means that a 99/100 people without a virological response to hepatitis C treatment at week 12 did not achieve a sustained virological response.

Optic neuropathy: damage to the optic nerve, which may result in impairment or loss of vision.

Pancreatitis: inflammation of the pancreas. Pancreatitis is a potentially life-threatening condition. Symptoms include: severe abdominal pain, nausea, vomiting, constipation, and slow pulse. The onset of pancreatitis can be predicted by rises in blood levels of the pancreatic enzyme amylase.

Peripheral neuropathy: nerve damage characterized by sensory loss, pain, muscle weakness and wasting of muscle in the hands or legs and feet. It may start with burning or tingling sensations or numbness in the toes and fingers.

Phosphorylation: the addition of a phosphate group to an organic molecule.

Positive predictive value (PPV): the proportion of all people who were identified by a measurement or screening test as apparently having a target outcome who actually do have the target outcome. In this case, PPV refers to the proportion of people who have a virological response to hepatitis C treatment at a specific timepoint during treatment (such as week 4 or week 12) who will actually achieve a sustained virological response. For example, a PPV of 50% means that 50/100 people who had a virological response to hepatitis C treatment at week 12 achieved a sustained virological response.

Sepsis: an infection in the bloodstream or tissues

Symptomatic Hyperlactatemia: mild to serious elevations in serum lactate levels. accompanied by symptoms including fatigue, anorexia, nausea, abdominal pain, weight loss.

Treatment naïve: a person who has never received any treatment for a specific condition. Upregulation: an increase in the rate at which something occurs.

Volume II: Basic Science

by Daniel Raymond

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Introduction to Volume II: Basic Research and Drug Development

This is an exciting time for basic research and drug development in hepatitis C. Since 2003, a number of important papers and presentations have substantially added to our body of knowledge about the hepatitis C virus (HCV) and offered hope for the advent of new therapies. Key findings include:

- Proof-of-concept from human studies of an HCV NS3 protease inhibitor (BILN 2061), with other candidates moving into clinical trials;
- Initial clinical data on a new HCV polymerase inhibitor (NM283);
- A range of other HCV drugs entering phase II and III studies;
- Encouraging research on the prospects for developing a vaccine to prevent HCV, based on increased understanding of the correlates of protective immunity in chimpanzees and humans;
- New insights into the role of HCV proteins and the dynamics of viral replication;
- New data on the role of the HCV NS3 protease in regulating the interferon response within cells, offering clues to viral persistence;
- Advances in HCV replicon models used to study viral replication *in vitro* and test the activity of new drugs;
- A promising new *in vitro* model for studying HCV entry into target cells;
- Establishment of an HCV genetic database at the Los Alamos National Laboratory, with a database of T cell epitopes to follow.

These developments represent the culmination of 15 years of research since the discovery of HCV. But despite remarkable progress, much work remains. Critical aspects of HCV pathogenesis and the viral replication cycle are not fully understood, and further refinement of cell culture systems and animal models is an urgent priority. While the current HCV drug pipeline contains an ever-expanding roster of promising compounds, few have moved into advanced clinical trials.

These antiviral agents could bring about a paradigm shift in HCV treatment over the next decade, potentially supplanting current combination therapy with pegylated interferon and ribavirin. Yet dramatic improvements in the efficacy and tolerability of HCV treatment are unlikely to reach the market for several years. Furthermore, most observers anticipate that the development of an effective preventative vaccine for HCV will require decades of research.

HCV prevention, screening, care, and treatment have attracted a growing constituency of advocates, including people with hepatitis C and activists working on HIV, harm reduction and substance abuse, and prisoner health. This volume of the report aims to broaden the advocacy agenda to include advocacy for basic research and drug development. The following chapters provide a foundation for understanding current issues and research priorities in HCV virology, immunology, and drug and vaccine development.

Current changes in how research is conceived and conducted provide a range of opportunities for advocacy:

- The National Institutes of Health (NIH) unveiled a new initiative, the NIH Roadmap, intended to accelerate biomedical and translational research through new forms of collaboration. Yet NIH has entered a phase of relatively flat funding, following a five-year period during which Congress doubled the NIH budget. Funding for HCV research in fiscal year 2005 is estimated at $130 million. Flat funding will limit the pace of new advances and discoveries in HCV research.

- Within NIH, the National Institute of Diabetes and Digestive and Kidney Diseases created a Liver Disease Research Branch that encompasses research on HCV within the institute. The Liver Disease Research Branch is developing an Action Plan for Liver Disease Research that attempts to coordinate and prioritize liver disease research across NIH. The Action Plan has been soliciting comments from the research community and the public.

- The Food and Drugs Administration (FDA) has launched a 'Critical Path' initiative to accelerate drug development by stimulating research on scientific barriers to assessing the efficacy and safety of new drugs. FDA intends to identify and prioritize a list of opportunities for targeted research and collaboration between government and industry. FDA is soliciting comments on potential areas of focus.

- As new classes of HCV drugs—particularly protease and polymerase inhibitors—begin moving into clinical trials, FDA can also take a leadership role in providing guidance to industry on trial design, study populations, and conditions for accelerated approval. Advocates can work with FDA and industry to speed the development, testing, and approval of safe and effective new HCV drugs.

- New international research initiatives are stimulating novel research into liver disease and therapies. The Human Liver Proteome Project, spearheaded by China and coordinated by the Human Proteome Organization, aims to map the interactions of proteins in healthy and diseased livers. The Gates Foundation's 'Grand Challenges in Global Health' initiative will fund innovative research projects in areas including preventative and therapeutic vaccines, with HCV listed as a priority area. On a smaller scale, the Canadian Network for Vaccines and Immunotherapeutics (CANVAC) coordinates HCV vaccine research, and the European Commission funds the HepCVax collaborative project to research and develop preventative and therapeutic vaccines for HCV. The European Union has also funded the European Network for Hepatitis C Virus Envelope Glycoprotein Research consortium (ENHCV) to study the structure and function of HCV envelope proteins for the development of new HCV drugs. Finally, HIV vaccine research offers provocative models for effective collaboration and coordination of HCV vaccine research efforts, including the incipient Global HIV Vaccine Enterprise and the Neutralizing Antibody Consortium, led by the International AIDS Vaccine Initiative.

Future advances in HCV basic research and drug and vaccine development will require creative partnerships between government, industry, academia, and community. Basic and clinical research will mutually benefit from further integration, as occurs within the NIH-funded Hepatitis C Cooperative Research Centers. The science points to a new era of innovation and discovery; this vision can only be realized through increased leadership, resources, and advocacy.

Recommendations

- **Support and intensify research into the molecular biology of HCV.**
- **Support and intensify research into immune response, persistence and pathogenesis.**
- **Increase funding and coordination of research.**
- **Support refinement of in vitro and animal models.**
- **Promote drug development efforts that study safety and efficacy in real-world populations.**
- **Initiate partnerships between industry, government, academia, and community.**

VIII. The Molecular Virology of Hepatitis C

Introduction and Summary

The identification of HCV in the late 1980s was the culmination of a decade of work by several research groups and nearly half a century of investigation into the causes of post-transfusion hepatitis (Feitelson 2002). Every conventional method used to isolate viruses had failed until a group of researchers at the Chiron Corporation successfully identified HCV using the polymerase chain reaction (PCR), a new research tool developed in the mid-1980s that had not been previously applied to the discovery of a virus (Q. L. Choo 1989). Notably, the discovery of HCV was aided by the work of government researchers at the Centers for Disease Control and the National Institutes of Health.

These factors—the creative application of new technologies when conventional methods had failed, and the contribution of multiple researchers and groups, both public and private—have also characterized the best of subsequent research into HCV. The ongoing struggle to develop an adequate cell culture system and a small-animal model for studying hepatitis C continues to tax the resourcefulness of HCV researchers, although these efforts are finally beginning to bear fruit. Similarly, investments by government and industry have generated synergy between the respective domains of basic science and drug development. There are two other recurring themes in the field that should be noted: one is the strength of international contributions, ranging from U.S. based to East Asian and European research groups working independently and in collaboration. A second theme is the crerative utilization of technological innovations to advance the study of HCV.

The initial discovery of HCV in 1987–88 was rapidly followed by reports from the United States and Japan characterizing the entire HCV genome (Q. L. Choo 1991; N. Kato 1990a; Kuo 1989; Takamizawa 1991). Subsequent work by several research groups in Europe, Japan, and the United States successfully determined the viral proteins produced by HCV, the rudiments of viral replication, and the extent of viral diversity in different regions of the world. Just as the discovery of HCV might not have been possible before PCR technology was available, research into the dynamics of its replication has benefited from additions to the molecular biology toolbox. Similarly, over the last 15 years, the field of virology has embarked on new avenues of inquiry into viral protein synthesis, viral diversity, and viral–host cell interactions. The general insights yielded by this work have paved the way for understanding aspects of hepatitis C virology. Key findings from this substantial, and growing, body of research are highlighted in this chapter.

HCV is a small, enveloped RNA virus, consisting of nothing more than the viral genome (a single strand of RNA, or ribonucleic acid) enclosed in a capsid shell and surrounded by a viral envelope, or membrane. HCV's genome encodes at least ten viral proteins, including two envelope proteins, as well as protease, helicase, and polymerase enzymes (Bartenschlager 2000). HCV has been classified as a member of the Flaviviridae family of viruses based on these characteristics and similarities to other members of the Flaviviridae family (Büchen-Osmond 2003; Francki 1991). Most of the fundamental stages of HCV replication (entry, viral protein synthesis, cleavage, genome replication, and viral assembly and release) have been documented and analyzed; however, gaps in knowledge persist in important areas, particularly in the very earliest and latest stages of the viral replication cycle (how HCV gets inside cells, and how newly produced viral particles are

assembled and released). Two key related questions require further elucidation: how does the virus interact with cellular components at each point in the replication cycle? and What factors, viral and cellular, regulate the activities of each stage?

Despite substantial progress in addressing these questions, complete answers to the problems in the study of HCV replication may not be soon forthcoming. Research has been stymied by the lack of an efficient cell culture system, a way to study viral replication in laboratory-grown cells. For reasons not well understood, HCV does not replicate reliably or reproducibly in cell cultures, which has frustrated attempts to construct thorough maps of the dynamics of viral replication (Bartenschlager 2001; N. Kato 2000a). In the absence of cell cultures supporting HCV replication, synthetic genetic constructs called replicons have recently been developed as a model for the hepatitis C virus (Blight 2000; Lohmann 1999). These replicons contain some or all of the genes of HCV and can replicate autonomously in cell cultures (Blight 2002; M. Ikeda 2002; Pietschmann 2002).

The development of a replicon model was a watershed in HCV research, dramatically expanding the possibilities for studying viral replication, as well as for screening potential anti-HCV drugs for activity against viral enzymes (Bartenschlager 2002; Grakoui 2001; Lanford 2002; Randall 2001; V. Wu 2001). Replicons, however, cannot assemble into new viral particles capable of infecting other cells (Molenkamp 2003; Pietschmann 2002). Other limitations in the replicon model suggest that additional refinements will be necessary for the full simulation of HCV replication (Bartenschlager 2002; Blight 2002; Blight 2003; Bukh 2002; Lohmann 2003; Murray 2003; Pietschmann 2003).

The only other established model for hepatitis C is the chimpanzee, virtually the only species besides humans susceptible to HCV infection (Lanford 2001a). Chimpanzee research is prohibitively expensive; thus, there is an urgent need for a more broadly available and convenient small-animal model for HCV infection. The recent development of transgenic mice transplanted with cells from human livers may be a major step towards this goal (Dandri 2001; Ilan 2002; Mercer 2001). Currently, however, these transgenic mice are more an initial proof-of-concept than the final realization of a small-animal model for HCV (Brass 2002; Fausto 2001; Pietschmann 2003).

The immediate value of a cell culture system or small-animal model lies in its potential to illuminate the basic science and pathogenesis of hepatitis C, bringing into focus the full workings of viral replication and HCV's interactions with cells. Ideally, such research would begin to shed light on three urgent issues in the study of HCV:

- Persistence—How does HCV establish chronic infection and elude cellular and immune system defenses?
- Pathogenesis—How does HCV cause disease, and why does the course of disease vary in different people?
- Prospects for new therapies—How can drugs successfully target viral replication, offset liver damage, or prevent hepatitis C infection?

This chapter, focusing on viral replication, lays the foundation for beginning to answer these questions, which are more fully explored in subsequent chapters (see Chapter IX, Immune Response, Persistence, and Pathogenesis, and Chapter X, The Future of HCV Therapy).

Discovery of the Hepatitis Viruses

Hepatitis has a long history in humans. Jaundice, a common symptom of hepatitis, was first described by Hippocrates in the fifth century B.C., and documentation of hepatitis outbreaks extends back for centuries. Despite speculation in the early twentieth century that hepatitis might result from an infectious agent, it took several decades and the development of more sophisticated research methods and technology before scientists identified the hepatitis A, B, and C viruses. These discoveries were facilitated by the recognition that many blood transfusion recipients developed hepatitis. Post-transfusion hepatitis (PTH) (a syndrome associated with abnormal laboratory values on liver enzyme tests and symptoms which could include nausea, fever, and jaundice) was recognized as early as the 1940s, nearly 150 years after the first human blood transfusion was performed (Beeson 1943). Different clinical patterns suggested the existence of more than one form of hepatitis, originally described as "infectious" and "serum" hepatitis; the terms hepatitis A and hepatitis B were introduced in 1947 to distinguish between the two hypothesized forms of PTH (MacCallum 1947).

The concept of separate forms of hepatitis, with different epidemiological patterns and clinical presentations, had been firmly established by the 1960s (Krugman 1967). During this decade, a protein found in the blood of an Australian aborigine (thus designated Australia antigen) was linked to type B hepatitis (Blumberg 1967; Okochi 1968; Prince 1968). Despite initial controversy, the Australia antigen (later identified as hepatitis B surface antigen) formed the basis of the first test to screen blood for type B hepatitis, introduced in United States blood banks in 1971. A virus-like particle containing Australia antigen, initially called the Dane particle and later identified as the hepatitis B virus (HBV), was subsequently discovered in the early 1970s, spurring further research. A vaccine protecting against HBV was introduced in 1981 (CDC 1982; Dane 1970; Francis 1982; Szmuness 1980; Tobler 1997).

The viral nature of the infectious agent causing type A hepatitis was demonstrated in the late 1960s, through transmission by human serum to marmoset monkeys (Deinhardt 1967). A virus-like particle associated with type A hepatitis was also identified in the early 1970s, followed by a serological test for antibodies to the hepatitis A virus (HAV); by the end of the decade, researchers had developed methods to study viral replication in cells through an *in vitro* culture (Feinstone 1973; Miller 1975; Provost 1979).

The hepatitis C virus was more elusive and would not be identified until 1987–88 (Q. L. Choo 1989). The availability by the mid-1970s of serum tests and blood screening for hepatitis A and hepatitis B indicated, by process of exclusion, that at least one other unidentified infectious agent was responsible for a significant number of cases of post-transfusion hepatitis, designated non-A, non-B hepatitis, or NANBH (Alter 1975; Feinstone 1975; Prince 1974). A number of researchers would pursue the identification of NANBH's causative agent for nearly a decade, with various hypotheses—a retrovirus, a picornavirus, a relative of HBV—tentatively proposed and ultimately ruled out, though the agents ultimately named the hepatitis D (for hepatitis delta) and hepatitis E viruses were identified during this period (Balayan 1983; Bonino 1984; Rizzetto 1977). Unlike HAV and HBV, the hepatitis C virus proved refractory to conventional methods of serological testing for antibodies and direct visualization through electron microscopy, and was, therefore, equally refractory to identification. However, chimpanzees were susceptible to NANBH when

infused with serum from patients diagnosed with non-A, non-B hepatitis, establishing an animal model for infection. Further work suggested that NANBH was caused by a fairly small virus, surrounded by an envelope, and carrying its genetic information in the form of RNA (ribonucleic acid; see Viral Classification section below), though the search for viral particles, antigens, and antibodies was hindered by the relative paucity of circulating virus in NANBH patients and infected chimpanzees (Feitelson 2002).

To evaluate candidate pathogens, Harvey Alter of the National Institutes of Health had gathered blood samples from individuals with clinical and epidemiological profiles indicative of NANBH, and from chimpanzees infected with sera from NANBH patients. Additional blood samples were taken from control groups of healthy individuals and persons with other liver diseases. The Alter panel, as this sample collection was dubbed, provided a valuable screen to assess whether candidate pathogens were indeed associated with NANBH. If a proposed agent were truly the cause of NANBH, it would react only to antibodies in Alter panel samples that were from NANBH patients and infected chimpanzees, and exclude those samples from healthy or uninfected controls.

The identification of the hepatitis C virus in 1987–88, a landmark in the history of virology, ultimately resulted from a public-private collaboration, initiated in 1982, between the Chiron Corporation and the Centers for Disease Control (CDC). Dr. Daniel Bradley of CDC provided chimpanzee plasma containing a highly infectious, high-titer form of the NANBH agent produced through serial passage of infectious sera through chimpanzees. Michael Houghton, George Kuo, Qui-Lim Q. L. Choo, and colleagues at Chiron extracted plasma genetic material (RNA and DNA, or deoxyribonucleic acid) from an infectious chimpanzee. In theory, much of this genetic material should derive from chimpanzee genes, but a portion of the genetic material would be viral in origin; therefore, any genetic material not from chimpanzee genes could be from the NANBH agent. The Chiron team used a new molecular cloning technique, reverse transcriptase-polymerase chain reaction (RT-PCR), to search for this agent.

The researchers cloned from the chimpanzee plasma a genetic fragment that met the criteria for the NANBH agent. First, antibodies from NANBH patients recognized a section of a protein encoded by the genetic fragment. This suggested that the fragment was from an infectious agent that evoked an immune response. Also, the fragment did not correspond to chimpanzee (or human) DNA, further indicating that it was derived from or produced by a foreign agent, such as a virus. In addition, the fragment corresponded to RNA found in NANBH-infected (but not uninfected) chimpanzee blood and livers, suggesting an RNA virus. Finally, the relationship between the genetic fragment and NANBH was validated through the Alter panel, where the putative agent could successfully distinguish between blood from NANBH-infected patients and chimpanzees, and samples from uninfected controls. This research, published in 1989, provided the foundation for christening the agent hepatitis C, the causative agent of a majority of NANBH cases (Q. L. Choo 1989). The cloning of HCV represented the first time that a virus had been detected without first being visualized through electron microscopy or identified through serological techniques. The first HCV antibody test was quickly developed based on this work, and several researchers in the U.S. and Japan subsequently succeeded in cloning full-length HCV (Q. L. Choo 1991; N. Kato 1990a; Kuo 1989; Takamizawa 1991). The work of Michael Houghton and Harvey Alter in identifying HCV, developing sensitive screening methods, and virtually eliminating the risk of transfusion-associated hepatitis was recognized in 2000 when they received the prestigious

Albert Lasker Award for Clinical Medical Research.

The actual isolation of HCV particles from sera would not occur until the mid-1990s, which finally allowed viral particles to be studied under a microscope. Electron microscopy of HCV virions in the mid-1990s enabled rough descriptions of the size and structure of individual viral particles (de Vos 2002; Kaito 1994; X. Li 1995; Y. K. Shimizu 1996). The hepatitis C virus is a spherical virus with three major components: an external envelope, an internal shell, and a strand of RNA. The HCV genome is encoded by an RNA strand approximately 9,600 nucleotides in length (nucleotides being the basic units, or building blocks, of RNA). The single strand of genomic HCV RNA is surrounded by a shell, or protein coat, called the capsid. The capsid is structured as an icosahedron, a form consisting of 20 identical and symmetrical equilateral triangles that are made up of viral proteins. The combination of genomic RNA and capsid is referred to as the nucleocapsid. An envelope encompasses the HCV nucleocapsid and is composed of viral proteins and cellular membrane. The HCV envelope is a membrane composed of a lipid bilayer, derived from host cells, studded with viral envelope proteins. The diameter of an HCV virion is estimated at approximately 50–65 nanometers (nm), larger than hepatitis A (17–19 nm) and hepatitis B (42 nm) (Kaito 1994; Y. K. Shimizu 1996). By contrast, the diameter of HIV virions is at least twice as large, with estimates ranging from about 110 nm to 145 nm for mature HIV virions (Briggs 2003; Gentile 1994).

Viral Classification: HCV as an RNA Virus

The identification through molecular cloning of HCV was only the first step in understanding the virus. At first, virtually nothing was known about the genetic organization of the virus, the viral proteins that could be synthesized from the HCV genome, and the details of how HCV replicated. Such information would be crucial in order to understand how HCV caused liver disease, and how to develop drugs to treat HCV. Researchers in the early 1990s drew upon the knowledge of other viruses to construct tentative models for HCV's genetic structure and protein function; however, HCV bore little resemblance to the hepatitis A virus or the hepatitis B virus, even though all three viruses targeted the liver. Therefore, scientists turned to other viruses that had greater genetic similarities, using them to form testable hypotheses about HCV. These functional and structural comparisons were enabled by established systems of viral classification, called taxonomies. Current hepatitis C research continues to draw upon these systems in developing surrogate models for HCV from related viruses.

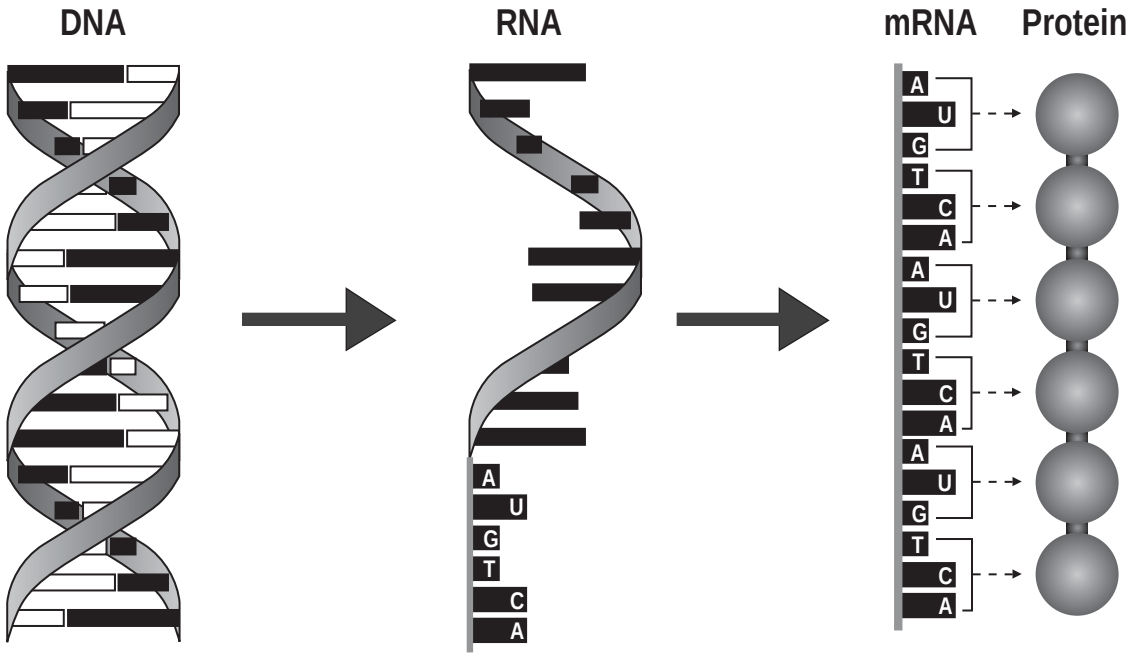
A major basis for distinction among viruses is how they encode their genetic information. Living organisms, including humans and other animals, bacteria, fungi, and plants, use DNA to store their genetic code. Some viruses also encode their genetic information as DNA, while others use RNA. Viruses are therefore classified as DNA viruses or RNA viruses. HCV is an RNA virus, and each viral particle contains a single RNA strand. HCV RNA has two functions:

1. Genomic RNA: The single RNA strand serves as HCV's genome, meaning that it contains all of HCV's genetic information. When a virus replicates, all new viral particles must contain a copy of the viral genome; therefore all viruses produce new copies of their genome during the replication process.

2. Messenger RNA (mRNA): All proteins (cellular and viral) are synthesized from an RNA template called messenger RNA. Messenger RNA is a single RNA strand that contains the genetic code or "message" describing the composition of the desired protein. The composition of mRNA provides the instruction set for protein synthesis. HCV uses the proteins encoded in its mRNA to replicate itself.

An RNA strand that can function as messenger RNA is also referred to as positive-sense RNA. Therefore, HCV is described as a positive-sense, single-stranded RNA virus. During replication, HCV synthesizes a negative-sense strand of RNA, a mirror-image copy of genomic RNA. This negative-sense RNA strand becomes the template for producing additional copies of positive-sense RNA. On this basis, HCV has been grouped with other viruses that have positive-sense RNA for genomes and have similar replication processes.

Figure 1. DNA, RNA, and Proteins



Genetic information in cells and many viruses is stored as DNA. DNA is transcribed into RNA. RNA, in the form of messenger RNA (mRNA), forms the template for synthesizing proteins. The HCV genome consists of a strand of RNA containing all of the virus' genetic information. The HCV genome also functions as mRNA, the template for synthesizing all HCV proteins.

Viruses are classified phylogenetically, that is, by presumed evolutionary history or genetic "family tree." This classification system considers several factors: similarities between viral genomes and proteins, as well as size and shape, physical and chemical properties, and type of host (i.e., whether viruses infect and replicate in plants, insects, or animals, etc.). In this system, viruses have been grouped hierarchically by:

1. Species (the most specific category—A species is a particular virus);
2. Genus (an intermediate category—A group of species make up a genus, the Latin word for "kind" or "class"); and
3. Family (the broadest category—There are 30 recognized families of viruses that infect vertebrates).

The hepatitis C virus is a species within the hepacivirus genus (Büchen-Osmond 2003). The hepacivirus genus belongs to the Flaviviridae family of viruses, which also includes flaviviruses and pestiviruses (Francki 1991). All Flaviviridae have positive-sense, single-stranded RNA genomes. The flavivirus genus includes viruses such as Dengue fever and yellow fever, as well as the Japanese and tick-borne encephalitis viruses. Pestiviruses are animal pathogens such as bovine viral diarrhea virus and classical swine fever virus. Research on these and other related viruses continues to inform the understanding of how HCV replicates and interacts with infected cells.

HCV was classified as a member of the Flaviviridae family based on its similarity to flaviviruses and pestiviruses, but categorized as the first member of a new genus designated hepacivirus. Because of significant genetic differences, other major hepatitis viruses are members of different viruses families, and are not directly related to HCV. The hepatitis A virus, like HCV, has a single-stranded, positive-sense genome, but is classified within the picornaviridae family. The hepatitis B virus (HBV) is a double-stranded DNA virus classified among the hepadnaviridae family. Three other viruses—GB viruses A, B, and C (the last previously misidentified as hepatitis G virus)—have been proposed for membership in the Flaviviridae family, but the GB viruses have not been assigned to a specific genus (see below for more on GB viruses).

Viral Diversity and the Origins of HCV

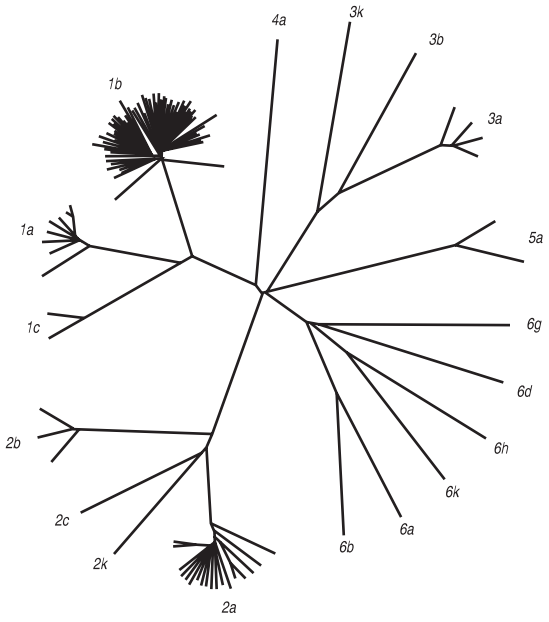
Hepatitis C isolates (individual samples of the virus) from around the world show significant genetic variability, as is common in RNA viruses. This variability results from the error-prone nature of HCV replication. RNA strands are composed of molecules called nucleotides. These nucleotides form the building blocks of RNA; the specific sequence of these nucleotides in the RNA strand determines the nature of the proteins encoded by the RNA. Each RNA nucleotide contains one of only four possible chemical bases—adenine, cytosine, guanine, and uracil. The synthesis of new strands of HCV RNA entails the assembly, in proper sequence, of a string of nearly 10,000 nucleotides. The synthesis of new strands of genomic RNA is an imperfect process, with no mechanism for "proof-reading" and correcting errors.

Mistakes during strand synthesis get incorporated into HCV RNA as mutations. When a new strand of genomic RNA is synthesized, these mutations are introduced more or less randomly; at any given position on the genome, there is a possibility that a "wrong" nucleotide may be added to the new RNA strand in place of the "right" one that would correspond to the original template. Mutations introduced into a prior round of replication will carry over every time the new RNA strand undergoes another round of replication. Over time, these and other mutations can accumulate with every new cycle of viral replication; therefore, a person chronically infected with hepatitis C will harbor a viral population that consists of a cloud or swarm of minor genetic variants. This viral ensemble is called a quasispecies, the term for a dynamic population of closely related but distinct genetic sequences.

The quasispecies nature of HCV was recognized during early research comparing sequences of various strains of the HCV genome (N. Kato 1990b). The extent of HCV replication provides ample opportunity for the introduction of mutations into the viral population within an infected individual. Viral production has been estimated at 10^{12} (one trillion) new HCV virions per day (Neumann 1998). In comparison with other RNA viruses, HCV has a relatively high mutation rate. The mutation rate of RNA viruses generally ranges between 10^3 and 10^5 nucleotide substitutions per genomic site per year. The mutation rate of HCV has been calculated at 0.816–1.92 x 10^3 (just under one or two per 1,000) substitutions per genomic site per year, based on studies of chronically infected humans and chimpanzees (W. Lu 2001; Ogata 1991; Okamoto 1992; Rispeter 2000). This relatively high rate would translate into the accumulation of between 8 and 18 mutations in genomic HCV RNA for each year of infection.

The extent of global HCV heterogeneity suggests that HCV has a complex evolutionary history with distinctive epidemiological patterns (Bukh 1995; Q. L. Choo 1991; Davidson 1995; Dusheiko 1994; Mellor 1995; D. B. Smith 1997). HCV variants have been classified into six major genotypes, or groupings, based on genetic similarities. Clusters of closely related variants within a genotype are categorized as subtypes (Robertson 1998; Simmonds 1994a; Simmonds 1994b). Genotypes are numbered, in order of their identification, while a letter denotes subtypes (for example, 1b refers to genotype 1, subtype b). The genetic sequences of HCV genomes differ by about one-third between genotypes; comparison of subtypes within a single genotype shows a lesser but still significant degree of variation. Some differences in pathogenicity between genotypes have been reported, though the nature and extent of these differences remain controversial (see Chapter II, Natural History of Hepatitis C); however, HCV genotype strongly influences the likelihood of responding to treatment (see Chapter V, Hepatitis C Treatment). The distribution of HCV genotypes and subtypes may shed light on the origins of hepatitis C.

Figure 2. HCV Phylogenetic Tree



A map of the genetic distances between HCV genotypes and subtypes (adapted from Los Alamos National Laboratory HCV sequence database, http://hcv.lanl.gov).

In theory, knowledge of the origins of a virus could help in understanding how it spreads, and how to develop strategies for prevention and disease control. This information might also shed light on how HCV causes disease and provide clues for designing effective drugs and vaccines. Unfortunately, little is known about the origin and history of the hepatitis C virus. Attempts to trace the origins of HCV have used the methods of molecular epidemiology. This approach combines epidemiology (the study of the global distribution of HCV genotypes and subtypes) with phylogenetic analysis (examining different viral strains for genetic similarities in order to construct a family tree of genotypes and subtypes). In the case of HCV, this form of analysis is highly speculative, depending on multiple unverifiable assumptions about transmission patterns and viral evolution (Simmonds 2001). A common obstacle in phylogenetic investigations of viruses, including HCV, is the absence of a historical archive of viral isolates suitable for study. Reconstructions of trends in viral evolution over time cannot be based on the kinds of evidence (such as fossils) available for studies of other forms of life. As a result, phylogenetic studies of HCV have relied on mathematical models, using recent estimates of the evolutionary rate of HCV.

Presumably, all HCV subtypes and genotypes have descended from a common ancestor, with genetic divergence occurring over time. The greatest diversity among variants is found in sub-Saharan Africa and Southeast Asia, suggesting that human populations in these regions have harbored HCV for longer periods. By analyzing calculated rates of sequence divergence in particular regions of the HCV genome and the geographical distribution of genotypes, initial estimates suggest that HCV genotypes diverged at least 500–2,000 years ago, while subtypes diverged over 300 years ago (D. B. Smith 1997). According to one model, subtypes 1a and 1b first emerged in the early twentieth century, while genotypes 4 and 6 date back hundreds of years (Pybus 2001). These models assume that viral heterogeneity (the extent of the differences between HCV isolates) has increased progressively over time. The evolutionary rate of HCV can be used to calculate when HCV subtypes and genotypes may have diverged from each other. This approach relies on a concept in evolutionary biology called the molecular clock, which assumes that genetic variation and divergence occurs at constant, ascertainable rates over time (Bromham 2003). Those rates vary by species, but known rates of variation can help to reconstruct evolutionary patterns and genetic divergence. The molecular clock has been applied to species evolution in general—the evolution of primates into various families and species, for example.

Some researchers, however, have noted that these calculations are at best conjectural and difficult to interpret due to uncertainties about the applicability of the molecular clock model to viral evolution (Holmes 2003; Simmonds 2001). In contrast to the evolution of animal species, viral evolution may not always occur at a steady rate, especially over the course of centuries. On the one hand, viruses mutate much more rapidly than other organisms; on the other hand, viral genetic constraints and trasmission bottlenecks may result in relative stability over long periods of time. Host immune pressure and genetic factors can also shape the evolutionary dynamics of viruses at a population level (Grenfell 2004; Moore 2002).

Furthermore, the historical record offers virtually no information about how HCV was transmitted during the periods when viral genotypes and subtypes supposedly diverged (Simmonds 2001). HCV transmission patterns in prior centuries must have differed substantially from those seen in recent decades. Most current HCV infections have occurred primarily through parenteral routes, such as drug injection and blood transfusions, factors which were non-existent until relatively

recently. Medical developments, and especially the invention of the hypodermic syringe in the mid-1800s, ushered in an era of widespread potential for exposure to pathogens through injection drug use, shots and vaccinations with unsterile syringes, and blood transfusions. The appearance of these new transmission risks coincides with the relatively recent global emergence of a hepatitis C pandemic during the middle decades of the twentieth century (Holmes 1995). The transmission patterns prior to this era, and the mechanisms for the apparent presence and persistence of HCV in human populations over several centuries, are unclear. HCV transmission is associated with direct blood-to-blood contact; sexual and perinatal transmission of HCV is fairly inefficient (see Chapter I, Epidemiology of Hepatitis C).

HCV most likely originated in non-human primates, but no clear viral antecedent or primate counterpart for HCV has been established. One candidate, GB virus B (GBV-B), bears the closest genetic relationship to HCV. GBV-B is infectious in tamarins, a species of small monkeys found in Central and South America. GBV-B was identified in 1995 from a tamarin infected with plasma derived from the blood of a surgeon (whose initials were G. B.) who had been diagnosed with acute hepatitis of unknown etiology in 1964 (Muerhoff 1995; Simons 1995a; Simons 1995b). Evidence suggests that GBV-B may be native to tamarins, though its prevalence in tamarins or other primates is unknown and warrants further investigation (Simons 1995b; N. D. Wolfe 1998). The value of such evolutionary studies can be seen in HIV research, where the search for viral ancestors in other primates has yielded important insights into the origins of HIV. These investigations have dated cross-species transmission events for HIV to the early decades of the twentieth century (B. H. Hahn 2000). Similar inquiries into HCV's origins may be more difficult. Phylogenetic analysis would indicate that cross-species transmission of HCV occurred well before the twentieth century. Subsequent primate population changes may have resulted in the extinction of a reservoir of antecedent virus. The ultimate origins of HCV, therefore, may never be known, but much can be learned from the virus in its current-day form.

Overview of HCV Research Methods

The innovation, ingenuity, and persistence required in the hunt for the hepatitis C virus in the 1980s also characterize subsequent research efforts aimed at unraveling the viral replication cycle. Such research is vital in order to understand the major challenges and questions posed by HCV infection:

- Persistence—How does HCV establish chronic infection and elude cellular and immune system defenses?
- Pathogenesis—How does HCV cause disease, and why does the course of disease vary in different people?
- Prospects for new therapies—How can drugs successfully target viral replication, offset liver damage, or prevent hepatitis C infection?

These issues require knowledge of the genetic organization of the virus, the structure and function of viral proteins, and how the virus interacts with the cell. Traditionally, researchers study the replication and pathogenesis of viruses through model systems—cell cultures (cells grown in laboratory conditions) and small animals such as mice—that are susceptible to infection and support viral replication. Unfortunately, for reasons that are not well understood, HCV does not

replicate efficiently in cell cultures or mouse livers (Bartenschlager 2001; Grakoui 2001; N. Kato 2000a). For most of the short history of HCV research, the only animal model for infection has been the chimpanzee, which shares 95–98% of its DNA with humans (Britten 2002; Bukh 2001a; Lanford 2001a). These constraints have inspired the creative use of both conventional and novel tools of molecular biology to study the hepatitis C virus.

Defining the genetic code of HCV RNA (the sequencing of the HCV genome) followed rapidly on the heels of the initial identification of HCV (Q. L. Choo 1991; N. Kato 1990a; Takamizawa 1991). The next major task was to identify and characterize the viral proteins encoded by HCV RNA. Researchers developed tools for protein expression (the production of individual HCV proteins) by using cloning vectors (DNA molecules that smuggle HCV genetic material inside a cell that would then synthesize viral proteins). This work led to determinations of the structure and function of the viral proteins, as well as to explorations of their location within infected cells and their interactions with cellular components and other HCV proteins (reviewed in Bartenschlager 2000; N. Kato 2001; Penin 2004). Viral protein research has been critical in developing strategies for designing drugs that can block HCV protein functions and interrupt viral replication (see Chapter X, The Future of HCV Therapy); however, approaches for studying individual viral proteins cannot fully substitute for systems capable of modeling the replication cycle of the entire hepatitis C virus.

Model systems for studying HCV replication

Studies of how viruses replicate inside hosts draw upon three main types of model systems:

Cell cultures use laboratory-grown cell lines derived from human or animal cells to study viral replication at the cellular level. This enables the examination of viral replication dynamics and viral-host cell interactions. Established cell lines, generated from the progeny of a single ancestor, are particularly useful because every cell in the line is genetically identical. This increases the consistency, reproducibility, and reliability of experimental results. The cells can be experimentally modified, and the virus mutated, to pinpoint the role of specific components in the replication process. Cell cultures may also use primary cells, derived directly from virus-infected individuals or animals. HCV primarily infects liver cells, called hepatocytes. Hepatocyte cell lines are thus the most relevant for HCV research. Recent technical advances have yielded hepatocyte cultures sustainable for several months, which will ultimately aid the study of HCV (Lázaro 2003).

Tissue cultures use actual tissue from human or animal organs (for example, liver tissues containing hepatocytes and surrounding structures). They can thus provide information on how viruses affect infected cells and the nearby environment, including other cell types. A tissue culture for HCV would enable better understanding of the mechanisms of fibrosis and liver injury. Currently no adequate tissue culture system is available that can model HCV pathogenesis in the liver (Lanford 2002; Pietschmann 2003).

Animal models examine viral infection in a living animal, studying the effects of a virus on the entire organism. Such research may be ethically unsound and logistically unfeasible in humans. Animal models allow researchers to look at overall viral-host dynamics beyond the cell and tissue level (for instance, the effects of the immune response on viral infection, and the distribution of the virus throughout the body). Primate models, such as chimpanzees, can provide particularly valuable information because of their genetic closeness to humans. However chimpanzees, an endangered species, are expensive and limited in availability for research. Small animals (particularly mice) offer several advantages in virology research, including their relative convenience and availability. The substantial genetic differences between mice and humans can be compensated for by introducing foreign genes into mice, producing transgenic mice. Human organ tissue, such as liver tissue, cannot be grown in mice, and must be grafted or transplanted into mice. However, engrafted human liver cells can divide after being transplanted into mice.

Success with cell and tissue cultures for HCV research has been limited until recently. Attempts to culture the hepatitis C virus itself in laboratory cell lines or primary cells have suffered from poor reproducibility and very low levels of viral replication (Bartenschlager 2001; N. Kato 2000a). For unknown reasons, introducing HCV into cell lines (even ones derived from hepatocytes, which should be an optimal environment for the virus) does not reliably result in sustained viral replication at detectable levels.

Subsequent efforts to develop cultures have attempted to use HCV genes, combined with the genetic material of other viruses or bacteria, as a surrogate for the virus itself. Initial progress in developing a cell culture system supporting HCV replication came with the construction of molecular clones based on full-length genomic HCV RNA (Kolykhalov 1997; Yanagi 1997). These clones have enabled studies of viral infectiousness in chimpanzees. Other researchers introduced specific HCV genes into other viruses, producing hybrid viral forms referred to as pseudotyped or chimeric viruses. Genetic material from HCV has been incorporated into several viruses for which cell culture systems exist, including bovine viral diarrhea virus, poliovirus, vesicular stomatitis virus, and yellow fever virus (Blanchard 2003a; Ezelle 2002; Macejak 2000; Matsuura 2001; Molenkamp 2003; Nam 2001; W. D. Zhao 2000; W. D. Zhao 2001). These chimeric viruses are used to study the role of various HCV proteins in viral replication and viral–host cell interactions, and to screen for potential anti-HCV drug candidates against select HCV proteins.

The most important development in HCV cell culture systems has been the advent of HCV replicons. Replicons are genetic material capable of autonomous replication and protein synthesis (Blight 2000; Lohmann 1999). HCV replicons are generated from synthetic DNA molecules that contain genetic material corresponding to some or all of the HCV genome. In cells, these molecules yield RNA transcripts containing HCV genes, along with other RNA sequences, that allow viral replication to occur and be measured. In lieu of a cell culture system for the virus itself, HCV replicons presented a major breakthrough for examining the molecular biology of HCV and developing screening methods for drug discovery (Bartenschlager 2002; Grakoui 2001; Lanford 2002; Randall 2001; V. Wu 2001). The first HCV replicons were described as subgenomic, since they only contain parts of the HCV genome (Blight 2000; Lohmann 1999). Recent efforts have led to the development of genomic replicons incorporating the full HCV genome (Blight 2002; M.

Ikeda 2002; Pietschmann 2002). A cell-free replication system derived from HCV replicon-bearing cells has also been described (N. Ali 2002); however, the assembly and release of infectious virions or HCV-like particles following replication still have not been observed in cell culture systems (Molenkamp 2003; Pietschmann 2002). Therefore even full-length genomic HCV replicons lack the capacity of actual hepatitis C virions to infect other cells, and cannot help elucidate the process of viral entry. Despite these limitations, replicon systems have opened up dramatic new possibilities for the study of viral replication, and will be particularly valuable in the preclinical assessment of potential antiviral drugs targeting HCV replication.

HCV replicons have other limitations:

- Replicons thus far only replicate efficiently in a particular cell line derived from human hepatocytes, designated Huh7. Replication of most HCV replicons has not been observed in other cell lines derived from human hepatocytes, though new research documents replication of the JFH-1 replicon in HepG2 and IMY-N9 hepatocyte-derived cell lines, albeit at lower efficiencies than those seen in Huh7 cells (Date 2004). Recent reports have also documented replication in a human embryonic kidney cell line, a human non-hepatic epithelial cell line, and a mouse hepatoma cell line (S. Ali 2004; Bartenschlager 2002; Q. Zhu 2003).
- Even among Huh7 cell lines, not all cells support replication; selection of specific lines of permissive "daughter" cells from the "parental" Huh7 cell line can increase replication efficiency 100-fold. Presumably, permissive cells either contain factors that support, or lack factors that inhibit, HCV replication, but these factors have not been identified (Blight 2002; Lohmann 2003; Murray 2003).
- Replicons have generally required adaptive HCV gene mutations to replicate at adequate levels within Huh7 cells; however, some of these adaptations are infrequently seen in naturally occurring HCV genomes, and may reduce the infectiousness of HCV. In genotype 1 replicons, the original HCV strains that replicate best *in vivo* seem to require more mutations to function efficiently as replicons (Bartenschlager 2002; Blight 2000; Bukh 2002; Evans 2003; Krieger 2001; Lohmann 2001; Lohmann 2003).
- Replication-competent replicons have been constructed from only certain strains of HCV, almost exclusively genotype 1 HCV RNA. Prior to recent reports describing the establishment of replicons derived from genotypes 1a and 2a, only genotype 1b replicons had succeeded in replicating in cell cultures; replication efficiency also varies among replicon strains (Bartenschlager 2002; Blight 2003; Date 2004; Evans 2003; Gu 2003a; T. Kato 2003b; Pietschmann 2003)

These challenges in refining HCV replicon models may derive from the characteristics of specific cell lines, which vary in levels of cellular gene expression and response to viral proteins. Even subtle variations in host factors between cell lines may produce divergent experimental outcomes (Borman 1997; Collier 1998; Koev 2002; Lagging 1998; Meng Soo 2002; Podevin 2001). Further refinements in HCV replicon systems will require more detailed knowledge of viral–host cell interactions. Recent major advances in extending host cell range and developing viable genotype

1a and 2a replicons provide crucial opportunities to study the role of host factors in HCV replication.

Aside from humans and chimpanzees, only one other species has been observed to have susceptibility to HCV infection. Two studies have reported that Tupaia belangeri, a primate-like species of Asian tree shrews, can be infected with HCV (Lanford 2001a; Lanford 2002; Xie 1998; X. Zhao 2002). Chimpanzees have had tremendous significance to hepatitis C research, beginning with the discovery of HCV and continuing with research into infectivity, viral clearance, immune responses, and gene expression; however, a cheaper and more widely available primate model for HCV infection would be extremely valuable for the testing of potential anti-HCV drugs and vaccines before they enter clinical trials in humans.

GB virus B, the virus perhaps most closely related to HCV, does infect tamarins, though HCV itself does not infect this family of small new world monkeys (Bukh 2001b). GBV-B may provide a surrogate animal model for certain studies exploring the dynamics of HCV infection. Chronic GBV-B infection has recently been observed in a tamarin, increasing the relevance of this model to HCV, with some close parallels to hepatitis C pathogenesis (A. Martin 2003). A tissue culture system using tamarin hepatocytes has been developed (Beames 2000; Beames 2001). Ideally, infectious molecular clones containing genomic elements of both GBV-B and HCV could be used in tamarins instead of chimpanzees (Bukh 1999; De Tomassi 2002; Sbardellati 2001). Marmosets, a related small New World primate, are also susceptible to GBV-B infection, and have been used to study the activity of antiviral drugs targeting HCV (Bright 2004); however, differences between the viruses can affect their relative susceptibility to inhibition by antiviral compounds (Ranjith-Kumar 2003).

Developing a mouse model for HCV infection is a high priority for drug discovery as well as for research on pathogenesis. Mice are not naturally susceptible to HCV infection, though transgenic mice can be genetically engineered to constitutively express HCV proteins (Feitelson 2001). These transgenic mice have been used to study the viral mechanisms underlying complications of HCV infection such as hepatocellular carcinoma (Ishikawa 2003; Koike 2002a; Koike 2002b; Moriya 1997; Moriya 1998; Moriya 2001a; Moriya 2001b). Other groups have developed more sophisticated approaches, transplanting human hepatocytes or HCV-infected human liver tissue into immune-deficient mice (Dandri 2001; Ilan 2002; Mercer 2001). Viral replication has been observed in these mice, suggesting that these models could be useful to screen potential anti-HCV drugs (Ilan 2002).

Despite their promise, these methods need refining. The transplantation process is difficult and time-consuming. About one third of these transgenic mice die shortly after birth. The limited availability of fresh human hepatocytes poses further difficulties, and techniques for the cryopreservation, or freezing, of hepatocytes, which would reduce the need for fresh cells, require further work (Brass 2002; Pietschmann 2003). These factors limit the utility and availability of a small-animal model. One commentator noted that, despite the importance of these models, he "suspect[s] that not many virologists will jump into hepatitis C research upon reading this [research]" (Fausto 2001). Furthermore, studies of viral pathogenesis and viral-host interactions would require that these mice be engineered to simulate the human immune system (Brass 2002; Pietschmann 2003). Nonetheless, this work constitutes a major advancement in HCV research and encourages the development of more refined mouse models of HCV infection and replication.

While the last five years have ushered in dramatic advances in HCV model systems (including replicons and small-animal models) further advances will require a sustained effort, ultimately bringing science closer to the holy grails of HCV research: a reliable, efficient cell culture system, a tissue culture system supporting viral infection and replication, and a small-animal model mimicking HCV pathogenesis in humans.

The Hepatitis C Viral Replication Cycle

Background: Viral Replication

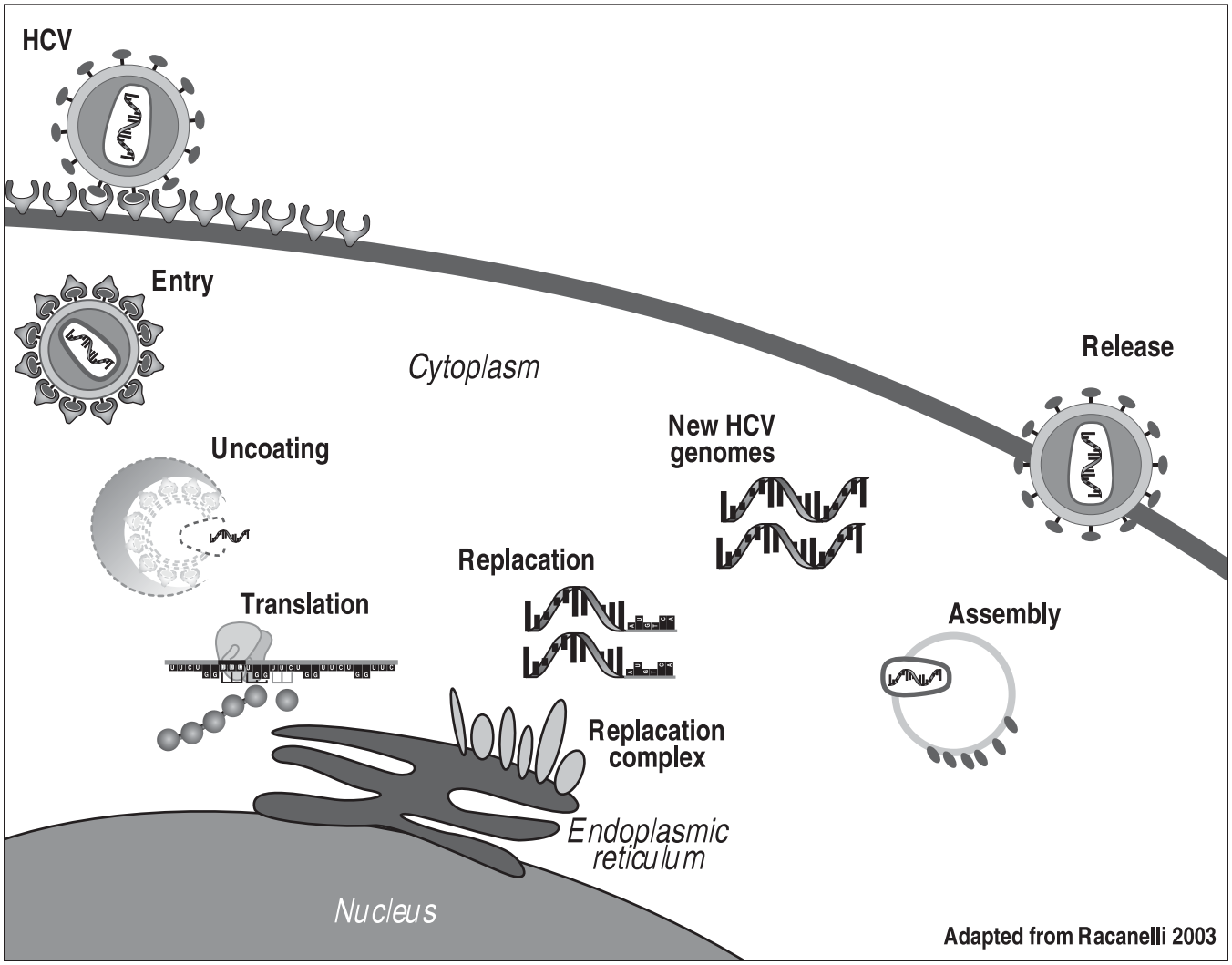
The primary goal of all viruses, including hepatitis C, is to replicate, to make new copies of itself. Unlike other organisms, a virus cannot replicate on its own; it simply doesn't possess all the tools necessary for the task. To replicate, HCV and other viruses must infect other cells and hijack the cellular apparatus, including enzymes and other proteins. An infected, or target, cell is called a host cell, because it "hosts" the virus. The term "host" is also used to describe the species susceptible to infection by a given virus. The hepatitis C virus primarily targets hepatocytes in the liver. Because HCV preferentially infects liver cells, the virus is considered hepatotropic (tropos is Greek for turning; hence, HCV "turns" toward the liver).

Once HCV enters the host cell, all subsequent events in the replication cycle occur in the cell's cytoplasm, the main area of the cell inside its membrane but outside of its nucleus. Both cellular proteins and viral proteins facilitate the progression of HCV through its replication cycle. The HCV genome encodes at least ten different viral proteins, including structural proteins (the envelope and core proteins) and nonstructural proteins. The structural proteins are incorporated into the capsid and envelope of new virions, while the nonstructural proteins are involved in the viral replication process. Research over the last decade has elucidated the functions of many of these proteins, but some proteins' roles in viral replication remain unclear, and many aspects of HCV replication and viral–host cell protein interactions are poorly understood (Ahlquist 2003).

HCV replicates through the following cycle (See Figure 3):

1. Host cell attachment, entry, and uncoating;
2. Translation of the HCV genome into viral proteins;
3. Cleavage and processing of viral proteins;
4. Replication of HCV genome; and
5. Assembly of new virions and release from host cell.

Figure 3. HCV Replication Cycle



The following sections will summarize current knowledge about the viral replication cycle and provide a foundation for discussion in subsequent chapters about HCV pathogenesis and drug development.

Stage 1: Host Cell Attachment, Entry, and Uncoating

The virus must enter a target cell to replicate. The events surrounding HCV's entry into cells are not known in any detail and have been difficult to research. In general, viruses in the Flaviviridae family enter cells in three stages:

1. Attachment. The viral envelope attaches to molecules on the surface of the target cell.
2. Entry. The virus passes through the cell's outer membrane (the plasma membrane), entering the target cell.
3. Uncoating. The virus sheds its envelope and releases the viral genome from the inner capsid shell into the cell's cytoplasm.

The study of viral entry attempts to address three broad questions:

- Which cells does HCV target?
- How does HCV attach to target cells?
- How does HCV enter cells and release its genome into the cell?

Cells targeted for infection by HCV

HCV, like other viruses, can infect and replicate only inside of certain cell types. Cell types that are susceptible to HCV infection are referred to as permissive cells. The range of cell types that a virus can enter is referred to as cell tropism (Baranowski 2001; Schneider-Schaulies 2000). HCV primarily infects hepatocytes, the main cell type in liver tissue; however, HCV has also been found in a range of other cells outside of the liver. These cells include white blood cells, components of the immune system. In particular, HCV has been found in certain peripheral blood mononuclear cells (PBMCs), specifically monocytes and macrophages, dendritic cells, T cells, and B cells (Caussin-Schwemling 2001; Goutagny 2003; Rodríguez-Iñigo 2000). HCV infection has also been observed in bone marrow cells (pluripotent hematopoietic CD34+ cells), the progenitors of these white blood cells (Radkowski 2000; Sansonno 1998). Many if not most viral particles circulating outside of cells are found in complexes bound to immunoglobulins (antibodies), lipoproteins, and platelets (André 2002; S. H. Choo 1995; Hamaia 2001; Hijikata 1993a; Kono 2003; Thomssen 1992; Thomssen 1993)

HCV replication in hepatocytes is well established. What is less clear is the extent of extrahepatic viral replication (whether HCV is capable of replicating outside of the liver) in PBMCs or bone marrow cells. In theory, HCV may be able to infect some cell types that do not themselves support viral replication. The ability of a particular cell type to support viral replication may depend on the requirements of later stages of the viral replication process, such as protein synthesis (Yanagiya 2003). Claims for replication in these other cell types have been controversial, though recent refinements in research methods may help clarify this issue (Laskus 1997; L. Lin 2002; Meier 2001).

The implications of HCV infection of PBMCs and bone marrow cells are also uncertain. These cells may constitute a viral reservoir (a pool of virus outside of the liver). In theory, successful treatment could clear HCV from the liver, while leaving viral reservoirs untouched. This could allow HCV to reestablish itself in the liver, making viral eradication through treatment more difficult. This scenario has not been documented in chronic HCV infection; however, such reservoirs may be partly responsible for HCV resurgence among liver transplant recipients (see Chapter V, Hepatitis C Treatment).

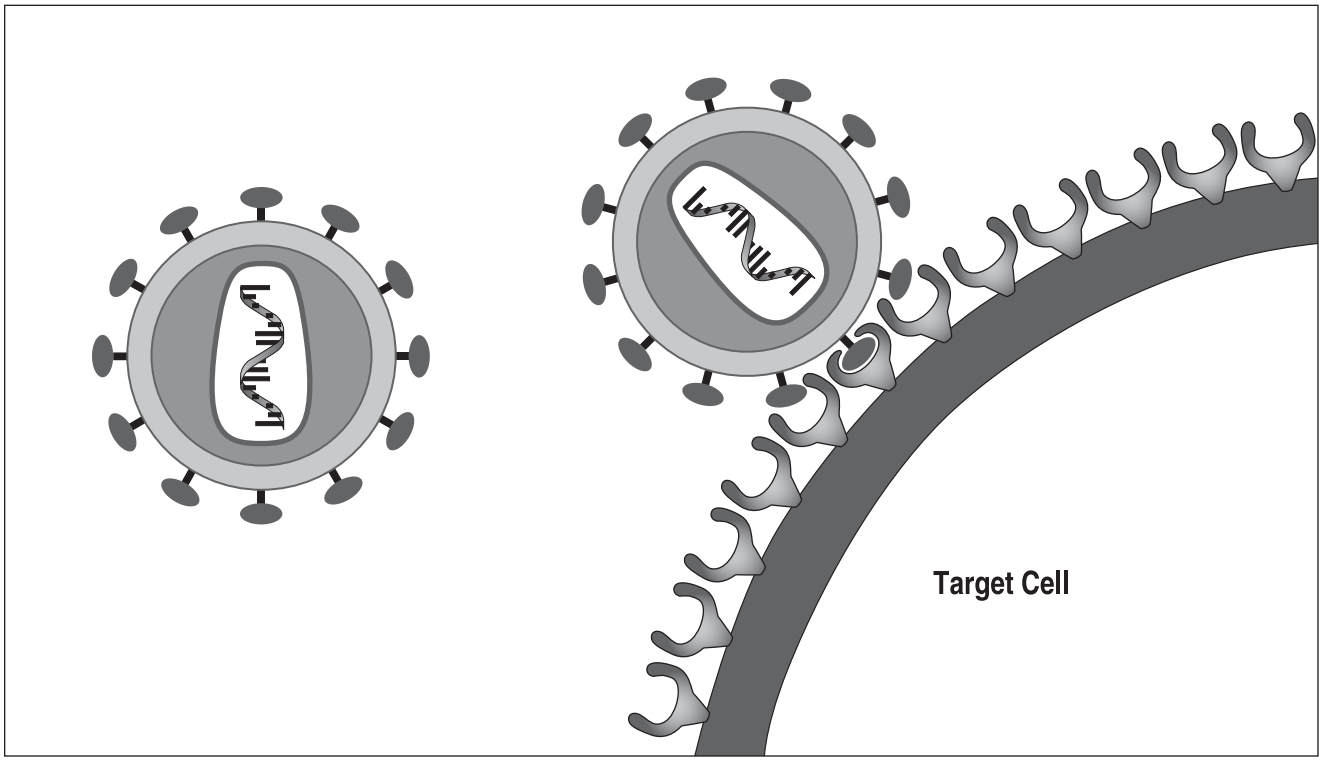
The extent of HCV infection of PBMCs may differ among HCV-infected individuals; it appears more common among people with a history of injection drug use (Resti 2002). Some evidence suggests that HCV replication in PBMCs is more likely to occur in people coinfected with HIV (Laskus 1998b; Laskus 2000; Laskus 2004). Even among HIV-negative persons, HCV infection of PBMCs and bone marrow cells may help impair immune responses to HCV (see Chapter IX, Immune Response, Persistence, and Pathogenesis). Infected PBMCs may also shuttle the virus to other parts of the body, and perhaps cause other extrahepatic manifestations of HCV disease (see

Chapter II, Natural History of Hepatitis C). For instance, HCV has been found in autopsied brain tissue, presumably carried by macrophages, perhaps contributing to the neurological complications sometimes associated with HCV (Radkowski 2002). Finally, HCV-infected PBMCs may facilitate viral transmission. A study of mother-to-child transmission found a strong association between detection of HCV in PBMCs and the likelihood of vertical transmission (Azzari 2000; see also Chapter I, Epidemiology of Hepatitis C). Further research on HCV cell tropism may shed light on viral pathogenesis, HIV coinfection, and HCV transmission.

Attachment of HCV to target cells

Attachment is believed (by analogy to other viruses) to occur through interactions between HCV's envelope and molecules on the surface of potential host cells. The HCV envelope includes two envelope proteins, E1 and E2, produced by the virus. E1 and E2 are bound together on the envelope, forming heterodimers (complexes formed by two different proteins) (Op de Beeck 2001). HCV, like other viruses, uses one or both of these envelope proteins to attach to molecules on target cells, whose outer membranes are studded with various molecules called cell surface receptors. These receptors are proteins that play a role in communication between cells. There are many kinds of cell surface receptors, each binding to one or more specific molecules or proteins. Receptor-binding proteins such as hormones and cytokines use the receptor to send signals to the cell's interior. The molecule that binds to a particular receptor is referred to as its ligand. While some receptors are ubiquitous, appearing on the surfaces of virtually all cell types, other receptors are specific to a particular type of cell. The receptors HCV can bind to, therefore, determine which kinds of cells the virus can enter.

Figure 4. Attachment of HCV to Target Cells



While several candidate HCV receptors have been proposed, their roles in viral entry have not been definitively established. HCV may require more than one type of receptor for attachment, for instance a primary receptor and a co-receptor, as with HIV infection (Schneider-Schaulies 2000). The search for cell surface receptors capable of binding to HCV virions has yielded three primary candidates: the CD81 receptor, the low-density lipoprotein (LDL) receptor, and the human scavenger receptor class B type I (SR-BI). HCV also binds to the molecules DC-SIGN (dendritic cell–specific intercellular adhesion molecule 3-grabbing non-integrin) and L-SIGN (liver/lymph node–specific intercellular adhesion molecule-3-grabbing integrin). Researchers have used a number of criteria to identify potential HCV receptors:

- Is the receptor expressed on the types of cells (hepatocytes, etc.) that HCV infects?
- Does the receptor bind to HCV particles, and can the binding site(s) for E1 or E2 be identified?
- Does blocking the receptor's binding site(s) prevent viral entry?
- Is the presence of the receptor sufficient for viral attachment and entry, or are co-receptors required?

CD81: CD81 is nearly ubiquitous on human cells, including hepatocytes and B cells (Maecker 1997). CD81 is involved in many cellular processes, including activation, adhesion, and differentiation, as well as the production of antibodies by B cells (S. Levy 1998; Maecker 1998). CD81's ability to bind to a region of the HCV E2 envelope protein is well documented, but CD81 appears to be insufficient for HCV entry into hepatocytes or binding to other cells (Hamaia 2001; M. Hsu 2003; Masciopinto 2002; Meola 2000; Roccasecca 2003; Sasaki 2003; Takikawa 2000; X. Zhao 2002). Some *in vitro* studies show that HCV-like particles containing E1 and E2 envelope proteins can still bind to cell lines lacking CD81 (Hamaia 2001; Roccasecca 2003; Sasaki 2003; Wellnitz 2002). Other studies have cast further doubt on CD81's specific role in viral entry, finding that viral binding to target cells is only partially inhibited by blocking the CD81 receptor. CD81 also appears to be relatively inefficient at internalizing E2 after binding (Germi 2002; Hamaia 2001; Petracca 2000; Roccasecca 2003; Triyatni 2002).

Some research, however, suggests that CD81 may function as a co-receptor aiding HCV in binding to another receptor (M. Hsu 2003; Pileri 1998). In particular, CD81 may facilitate the initial attachment of HCV E2 to the target cell as part of a receptor complex, with one or more other receptors responsible for binding and entry (Bartosch 2003b). Some studies that questioned CD81's role in viral entry used a truncated form of E2, which may not adequately model actual *in vivo* interactions. A recent report found that E1-E2 heterodimeric complexes bind to CD81 much more efficiently than truncated E2 alone (Cocquerel 2003b). Another recent study found that CD81 expression on target cells is necessary but not sufficient for viral entry (J. Zhang 2004).

LDL receptor (LDLR): Lipoproteins are proteins bound to fat molecules (cholesterol and triglycerides). Low-density lipoprotein receptors (LDLRs) help to regulate cellular cholesterol levels. LDLRs shuttle cholesterol-bearing lipoproteins into cells through receptor-mediated endocytosis. While all cells use cholesterol, the liver is a primary site for cholesterol metabolism. The HCV E1 and E2 envelope proteins both bind to low-density, very-low density, and high-density lipoproteins (Kono 2003; Monazahian 2000; Prince 1996; Thomssen 1992; Thomssen 1993). Indeed, many

circulating viral particles in HCV-infected people are bound in complexes with lipoproteins (Kono 2003; Thomssen 1992; Thomssen 1993). This suggests another potential mechanism of viral attachment, perhaps independent of the E1 and E2 envelope proteins, through binding of HCV-associated lipoproteins to cell surface receptors (Agnello 1999; André 2002; Germi 2002; Monazahian 1999). However, research on HCV attachment and entry has not yielded convincing evidence establishing a role for the LDL receptor (M. Hsu 2003; Wellnitz 2002; Wünschmann 2000).

SR-BI: SR-BI, like the LDL receptor, is a lipoprotein receptor involved in lipid metabolism. While the LDLR binds to low-density lipoproteins and is expressed on a wide range of cell types, SR-BI binds to high-density lipoproteins and is found in high concentrations primarily on hepatocytes. SR-BI was recently shown to bind to the HCV envelope protein E2 (Op de Beeck 2003; Scarselli 2002). A subsequent *in vitro* study found that SR-BI expression alone was not sufficient for infection of target cells (M. Hsu 2003). Most recently, another group found that on hepatic cells expressing CD81, SR-BI expression was essential for viral entry, which could be blocked by anti–SR-BI antibodies that prevented E2 binding (Bartosch 2003b). The latter study, however, indicated that one or more cellular factors in addition to SR-BI and CD81 were likely involved in viral entry.

DC-SIGN and L-SIGN: DC-SIGN and L-SIGN are closely related members of a class of carbohydrate-binding molecules called C-type lectins. DC-SIGN is predominantly expressed on dendritic cells, components of the immune system that patrol the body for foreign particles such as viruses and bacteria. Dendritic cells capture and process these particles, bringing them to lymph nodes (small tissue sites scattered throughout the body that serve as central meeting grounds for immune system cells). Dendritic cells then present the processed particles to CD4 T cells, which launch an immune response (see Chapter IX, Immune Response, Persistence, and Pathogenesis). L-SIGN is expressed on endothelial cells, a type of cell that lines blood vessels and separates blood from surrounding organ tissue. L-SIGN is primarily found on a type of endothelial cell found in the liver called liver sinusoidal endothelial cells (LSECs), as well as on the endothelial cells in lymph nodes. According to a recent report, LSECs can also express DC-SIGN (W. K. Lai 2004).

Both DC-SIGN and L-SIGN can bind to E2, though it is not clear whether this results in viral entry. HCV infection has been observed in dendritic cells, but no reports have yet documented the presence of HCV within endothelial cells (Goutagny 2003; Laporte 2003; Navas 2002). L-SIGN and DC-SIGN may act not primarily as viral entry receptors themselves, but rather as facilitators of HCV entry into hepatocytes and perhaps PBMCs (M. Hsu 2003). This form of cellular hijacking would mirror the role of DC-SIGN (and L-SIGN) in HIV infection, where these molecules bind to HIV and bring the virus into contact with its main target, CD4 T cells (Bashirova 2001; Geijtenbeek 2003; Pöhlmann 2001; Soilleux 2002; Soilleux 2003). For HCV, capture by L-SIGN could serve as a Trojan horse strategy, with liver sinusoidal endothelial cells inadvertently passing HCV on to hepatocytes (Feng 2004). Alternatively, viral binding to dendritic cells via DC-SIGN may function to disrupt immune responses, independent of any role in viral entry (see Chapter IX, Immune Response, Persistence, and Pathogenesis).

Other candidates: Viral entry may also be facilitated by other cell surface molecules that do not themselves function as receptors. Some researchers have suggested a role in HCV attachment and entry for glycosaminoglycans (GAGs), a group of polysaccharides such as heparin and heparan sulfate present on cell surfaces. In this model, GAGs would facilitate HCV's initial attachment to target cells, and support or strengthen the association between HCV and its receptor, as has been seen with other viral infections (Germi 2001; Germi 2002; Takikawa 2000). One group recently suggested that the E2 protein requires heparan sulfate proteoglycans, a type of GAG, for binding to target cells (Barth 2003). A section of E2 called the hypervariable region 1 (HVR1) can bind to cell surface GAGs, though this interaction is neither sufficient nor perhaps necessary for viral entry (Basu 2004).

Despite the range of candidates, there is good reason to believe that at least one other, as yet unidentified, HCV receptor is necessary for viral entry (M. Hsu 2003; Op de Beeck 2003; Pandya 2002; J. Zhang 2004). However, the ability of a particular viral envelope protein to bind to a given receptor does not constitute proof of that receptor's involvement in the infection of target cells. The binding properties of E2 may provide valuable clues for understanding HCV pathogenesis and immune response, but viral entry appears to require the presence of both E1 and E2 in their heterodimeric form (Bartosch 2003a; M. Hsu 2003; Takikawa 2000; Triyatni 2002). Similarly, the ability of an entire viral particle to bind to a particular receptor (such as L-SIGN) does not guarantee that HCV uses this receptor for cell entry.

Many of the challenges in studying binding and attachment stem from the lack of an efficient cell culture system that supports both viral replication and infection of new cells. Currently available HCV replicon models do not produce viral particles that are infectious to other cells in culture. Recent encouraging advances in the methods for researching viral entry will likely aid in identifying the actual receptors that HCV uses to infect cells (Bartosch 2003a; Cocquerel 2003b; M. Hsu 2003; Lambot 2002; Op de Beeck 2003). HCV pseudotype particles have envelopes that bear HCV E1 and E2 proteins on their surfaces; this envelope surrounds a core particle from a different type of virus, such as HIV. Theses infectious pseudotype particles have already begun to clarify HCV entry requirements (Bartosch 2003b; Castet 2003; Dumonceaux 2003; M. Hsu 2003; Op de Beeck 2004; J. Zhang 2004). The arrangement of HCV envelope proteins on retrovirus core particles appears to resemble the natural conformation and properties found on HCV virions, suggesting that HCV pseudotype particles are a valid model for studying viral entry (Op de Beeck 2004). Future research should elucidate the mechanisms of viral attachment leading to entry, and may provide clues for developing drugs that inhibit HCV attachment and entry (see Chapter X, The Future of HCV Therapy).

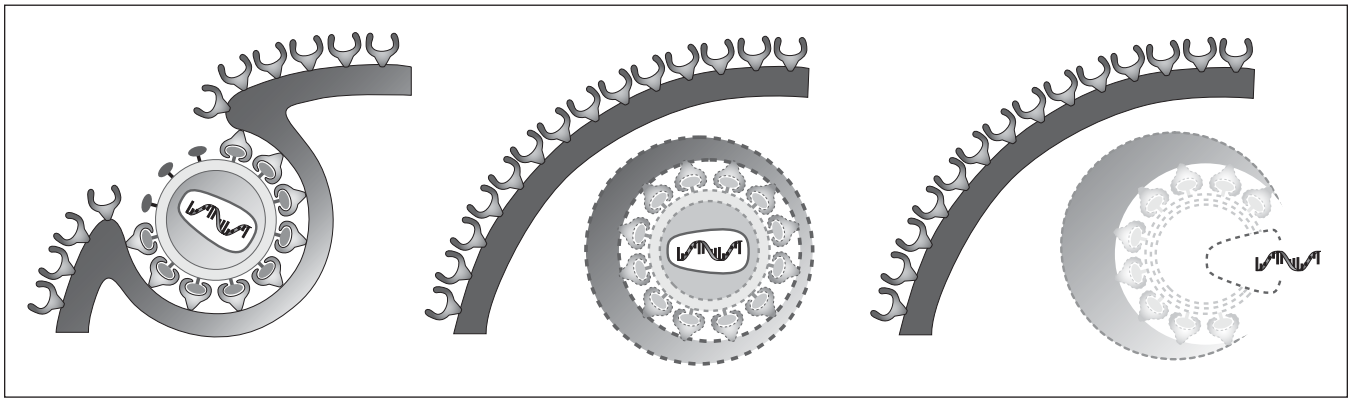
HCV entry into target cells and uncoating

The entry of HCV into target cells most likely occurs through receptor-mediated endocytosis, in which a cell internalizes a ligand-bound surface receptor. The receptor-ligand complex enters the cell encapsulated within a vesicle, a small pocket of fluid surrounded by a thin membrane. Cells use endocytosis to internalize particles involved in signaling and cell growth such as hormones, proteins, growth factors, and cholesterol. Receptor-mediated endocytosis is also used by flaviviruses closely related to HCV, though this mode of cell entry has not been conclusively demonstrated for HCV (Op de Beeck 2003). Other modes of viral entry, such as direct fusion with the cell

membrane (as with HIV's viral fusion protein, gp41) cannot be ruled out (Flint 2001; Hernandez 1996). Recent descriptions of the structure and mechanism of the envelope fusion proteins for the flaviviruses dengue virus and tick-borne encephalitis virus have spurred speculation that HCV may also enter host cells through fusion (Bressanelli 2004; Drummer 2004; Modis 2004). Recent evidence, however, indicates that hepatitis C viral entry is dependent on pH levels (the degree of acidity), a characteristic of receptor-mediated endocytosis but not of direct fusion (Bartosch 2003b; M. Hsu 2003).

Uncoating is the process that releases the viral genome into the cell's cytoplasm. Following entry, HCV must release its genome from its capsid shell and surrounding viral envelope in order to begin replication. Though the specific uncoating process for HCV has not been characterized, it likely follows a sequence of events common to other enveloped viruses entering through receptor-mediated endocytosis. In this model, the HCV envelope would fuse to the vesicle membrane once inside the cell. Fusion may depend on a change in pH levels within the vesicle. Envelope–vesicle membrane fusion results in the degradation of the viral capsid surrounding the viral genome, releasing the viral RNA into the cell's cytoplasm.

Figure 5. HCV Entry and Uncoating



The events surrounding HCV's entry into the cell are some of the least well-characterized aspects of the viral replication cycle. Improvements in research methods and model systems will clarify viral entry and uncoating, and could lead to therapies targeting these events in the viral life cycle.

Summary of HCV entry:

- HCV is an enveloped virus; the viral envelope contains two HCV proteins, E1 and E2, joined as heterodimers.
- HCV entry is thought, by analogy with other flaviviruses, to enter cells through receptor-mediated endocystosis.
- Candidate receptors include CD81, LDLR, and SR-BI.
- HCV entry may require more than one receptor, and the receptor(s) may differ according to cell type.
- Binding and entry may be mediated by cellular or endogenous proteins such as lipoproteins and glycosaminoglycans, as well as by HCV envelope proteins E1 and E2.
- There may be other HCV receptors that have not yet been identified.

Stage 2: Translation of the HCV Genome into Viral Proteins

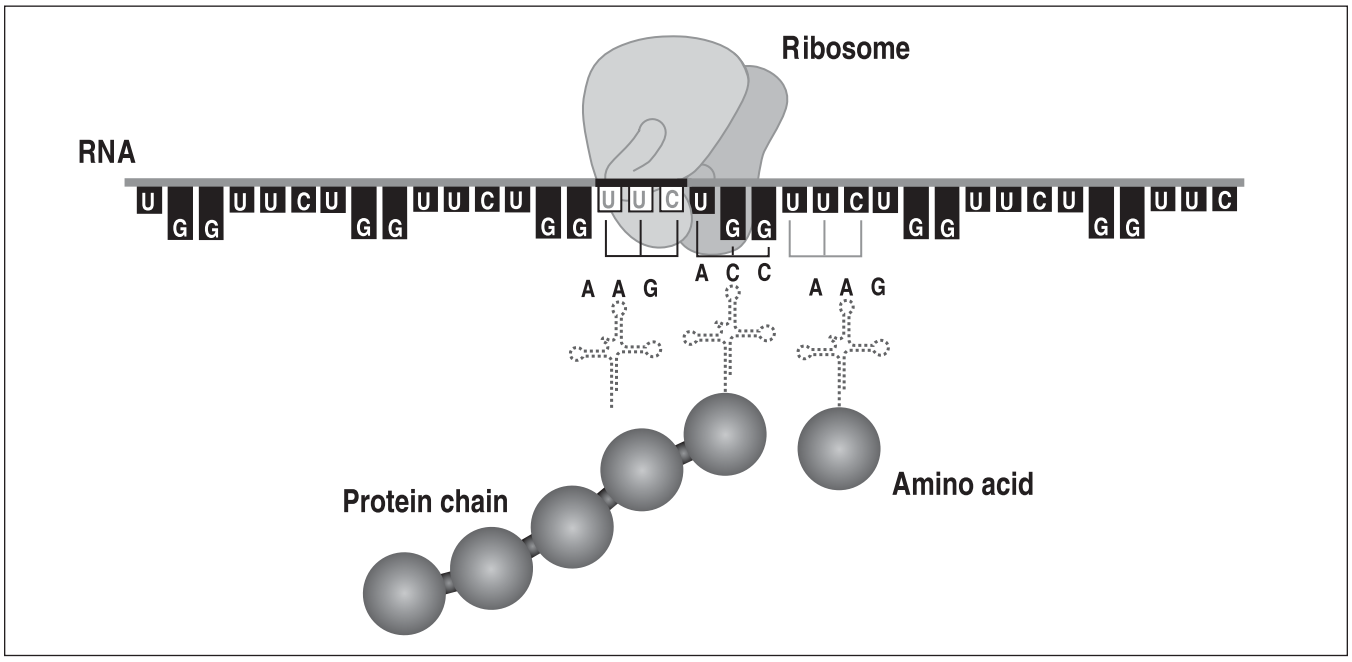
Once released inside the cell, HCV RNA is used as a blueprint for the production of viral proteins. The process of protein synthesis from RNA is called translation and uses cellular components (in particular, ribosomes) that are also employed in translating the cell's own mRNA into proteins. The primary product of HCV translation is a single polyprotein (a long protein chain) consisting of over 3,000 amino acids. The polyprotein contains all ten HCV proteins required for HCV replication.

Research into HCV translation falls into three overlapping areas:

- How does HCV initiate protein synthesis?
- How does the regulation of viral protein synthesis differ from that of cellular protein synthesis?
- What viral and cellular factors regulate this process?

All proteins, both viral and cellular, are assembled from molecules called amino acids. The structure and function of a protein is determined by its amino acid sequence and composition. Messenger RNA (mRNA) provides the genetic blueprint for protein assembly and determines the amino acid sequence of a given protein. In the translation process, large cellular complexes called ribosomes "read" this mRNA blueprint, translating the genetic code into instructions for protein synthesis. The ribosome works with other cellular components to assemble a chain of amino acids corresponding to the mRNA genetic sequence. These amino acids, or peptide chains, will form a new protein when translation is complete.

Figure 6. Translation



Translation is remarkably complex and exacting, requiring the choreography of multiple cellular components. Their availability and interactions with mRNA regulate translation, which is more

efficient when these components are more abundant. Cellular conditions determine the relative abundance of components required for translation, upregulating or downregulating protein translation depending on the cell's needs.

Protein synthesis is key to HCV replication, since new virions cannot be produced until key viral proteins have been synthesized. HCV translation requires some, but not all, of the translation components used by cells. This may allow HCV to initiate efficient translation even when cellular conditions do not favor protein synthesis. Indeed, conditions that limit the translation of cellular proteins may actually promote the translation of hepatitis C proteins.

Initiation of HCV protein synthesis

HCV RNA contains a complex structure called an internal ribosomal entry site (IRES) at the beginning of its genome (Tsukiyama-Kohara 1992; C. Wang 1993). The ribosome binds directly to the HCV IRES, which directs the ribosome to the mRNA site where translation is initiated. Virtually the entire HCV genome is translated, except for regions at either end of HCV RNA. These regions are therefore called the untranslated regions (UTRs), and designated the 5′ UTR (5-prime untranslated region) and the 3′ UTR (3-prime untranslated region). Translation of mRNA is directional, beginning at the RNA site immediately following the 5′ UTR and proceeding until reaching the 3′ UTR. The long region of RNA between the 5′ UTR and the 3′ UTR contains the genetic sequence encoding all HCV proteins. This region is called the open reading frame, because it frames the genetic sequences read by the ribosome.

HCV also appears to use an alternate form of translation, called frameshifting, in which the ribosome initiates translation from a slightly different site on HCV RNA, through which HCV synthesizes at least one other viral protein, ARFP (alternative reading frame protein) or F (frameshift) protein (Boulant 2003; Choi 2003; Roussel 2003; Varaklioti 2002; Vassilaki 2003; Xu 2001). The function of the F protein, and the conditions governing its expression, are currently unknown. Some evidence suggests that F protein expression may vary by genotype (Boulant 2003).

The IRES, comprising almost the entire 5′ UTR of HCV RNA, is folded into a three-dimensional scaffolding which contains various structural elements such as stem and hairpin loops, helices, and a pseudoknot (Beales 2001; Brown 1992; Fukushi 1994; M. Honda 1996; Kieft 1999; Rijnbrand 1995; C. Wang 1994; C. Wang 1995). These elements allow the IRES to bind to and assemble the ribosome and other cellular proteins required to initiate translation. Some of these proteins are called eukaryotic initiation factors (eIFs), cellular proteins that facilitate translation by coordinating ribosome assembly and its proper positioning at the mRNA site where translation begins. HCV uses two of these initiation factors, designated eIF2 and eIF3, to initiate translation.

Differences between HCV protein synthesis and cellular protein synthesis

Cellular mRNA, like HCV, uses ribosomes to assemble amino acids into proteins; however, internal ribosomal entry sites, though not uncommon in viral mRNA, are extremely rare in cellular mRNA, which therefore lacks a structure that can directly bind to the ribosome. Instead of an IRES, cellular mRNA has a chemical group at its 5′ end, called a methylated cap (because it "caps" the mRNA), which binds to another eukaryotic initiation factor, the eIF4 complex. This complex guides the

mRNA to the ribosome to initiate translation. This form of translation is called cap-dependent, because it relies on the interaction between the methylated cap and the eIF4 complex. HCV does not have a methylated cap, instead using its IRES to bind directly to the ribosome, a mechanism of translation initiation called cap-independent (synonymous with IRES-directed). Thus HCV does not require the eIF4 complex to begin translation. Another difference between cellular and HCV translation hinges on how the ribosome is positioned at the proper mRNA initiation site. HCV positions the ribosome directly at the initiation site, but the ribosome must scan cellular mRNA until it finds the initiation site. By replacing eukaryotic initiation factors and omitting key steps such as scanning required by cap-dependent translation initiation, HCV expedites viral protein synthesis (Kieft 2001; Kolupaeva 2000; Pestova 1998; Pestova 2001; Spahn 2001).

Some research has linked the regulation of HCV translation to the cell cycle, the series of events that a cell undergoes when it divides into two cells. HCV IRES activity seems to be relatively low in resting, nondividing cells, but viral protein synthesis increases in actively dividing cells (M. Honda 2000; Shimazaki 2002). This pattern contrasts with that observed in cap-dependent translation of most cellular mRNA, which dramatically decreases during cell division (Pyronnet 2001a; Pyronnet 2001b; Sachs 2000). Cell division disrupts the eIF4 complex that is essential for cap-dependent translation (Pyronnet 2001b). The relatively few cellular proteins synthesized through cap-independent translation tend to function during periods of cellular stress, such as cell death, cell division, and oxygen shortage (Lang 2002; Morrish 2002; Pyronnet 2000; Stein 1998; Stoneley 2000). Cells may use IRES-directed protein synthesis in emergency conditions when cap-dependent translation initiation is decreased or inhibited (Fernandez 2001).

These differences in translation efficiency suggest that the IRES benefits HCV RNA translation in actively dividing cells. By allowing HCV to bypass eIF4 to initiate translation, IRES permits HCV translation to occur during cell division, when most other protein synthesis shuts down. Conversely, HCV proteins would not be efficiently synthesized in resting cells—the state of most hepatocytes at any given time. Rather, resting cells would favor the synthesis of cellular proteins. These observations may prove to have important implications for developing new approaches to anti-HCV therapy that exploit differences in cellular and viral translation.

The cell cycle model of translation regulation also suggests a complex trade-off between factors promoting cellular and HCV protein synthesis. Viruses, including HCV, must ensure efficient synthesis of their proteins in order to replicate. This may entail competition with cellular mRNA for the host cell factors (including eIFs and ribosomes) required for translation (Sarnow 2003). Notably, both the 5′ UTR and the 3′ UTR of HCV RNA can bind to proteins within the ribosome, perhaps helping HCV to compete with cellular mRNA for ribosomes (Fukushi 2001b; Mauro 2002; Odreman-Macchioli 2001; Otto 2002; Pestova 1998; Wood 2001). Viewing the dynamics of protein synthesis in terms of competition for cellular resources may shed light on other factors involved in regulating HCV translation initiation.

Viral and cellular factors regulating HCV translation

The regulation of HCV translation can be understood as a balance between several requirements:

1. Efficient viral replication demands an adequate supply of viral proteins, and thus a minimum level of protein synthesis.
2. Viral replication also requires the preservation of the host cell environment, at least until new virions have been produced. Overproduction of viral proteins might damage or kill the host cell before the HCV replication cycle is complete.
3. RNA (both viral and cellular) does not persist indefinitely in cells; cellular enzymes target RNA for degradation or decay. HCV must either protect its RNA from degradation or complete protein synthesis before its RNA can be degraded.
4. Cellular defenses against viruses and other invaders can guard against replication by foreign (i.e., viral) RNA in part by depriving such RNA of cellular factors required for translation. HCV must block or overcome the triggering of host cell defenses in order to guarantee the synthesis of its proteins.
5. The same HCV RNA used for protein synthesis (translation) will also be used to make new copies of the HCV genome (transcription), but the RNA can be used for only one of these processes at a time. Therefore, HCV must devise a way to manage "traffic" (for example, ribosome movement) while ensuring that RNA is available for the execution of both translation and transcription.

Because of these multiple requirements, some factors stimulate HCV translation, while other factors repress it, depending on such conditions as cell cycle phase and viral replication stage.

The overall regulation of HCV protein synthesis remains poorly understood. Many factors are likely to regulate translation by increasing or decreasing its efficiency. Some cellular proteins may regulate translation by binding to various regions of HCV RNA, particularly the 5′ UTR and the 3′ UTR. These untranslated regions fold into complex structures that need to balance stability with flexibility. The IRES structure within the 5′ UTR contains several loops that bind components needed to start translation, such as the ribosome and eIF3. Other cellular proteins not directly involved in translation initiation can also bind to the HCV IRES. Some of these proteins may stabilize the IRES structure, increasing translation efficiency, while others may make the IRES structure too rigid, preventing it from functioning.

Among these cellular proteins, the most important regulatory factor may be the La antigen, which binds to the HCV IRES (N. Ali 1997; N. Ali 2000; Izumi 2004; Pudi 2003; Pudi 2004). The La antigen increases the efficiency of protein synthesis and may actually be required for translation initiation (N. Ali 2000; S. Das 1998a; Isoyama 1999; Pudi 2003; Shimazaki 2002). The La antigen also appears to bind to a region within the 3′ UTR, possibly protecting HCV RNA from degradation by cellular enzymes (McClaren 1997; Pannone 1998; Spångberg 2001). Some research suggests that the polypyrimidine tract binding protein (PTB) may also stimulate, or even be required for, HCV translation initiation (N. Ali 1995; Anwar 2000; Gosert 2000). Several other

cellular, RNA-binding proteins also bind to the HCV IRES, including polycytosine-binding proteins 1 and 2 (PCBP-1 and -2) and heterogeneous nuclear ribonucleoprotein L (hnRNP L), though their role in translation has not been fully established (Fukushi 2001a; Hahm 1998; Spångberg 1999). Similarly, some proteins bind to the 3′ UTR (including PTB, hnRNP C, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and ribosomal protein L22) giving them a potential role in modifying translation (Blight 1997; Gontarek 1999; Y. Inoue 1998; Ito 1997; Ito 1998; Luo 1999; Petrik 1999; Tsuchihara 1997; Wood 2001); however, significant controversy remains about whether and how 3′ UTR is involved in regulating translation (Fang 2000; Friebe 2002; Imbert 2003; Kong 2002; Murakami 2001; Qi 2003; Wiklund 2002).

Determining which factors regulate HCV translation, and how, has been difficult in the absence of an efficient cell culture system, particularly in view of the potential interactions of multiple factors. In addition to cellular proteins, viral proteins synthesized during prior rounds of translation may also influence the efficiency of translation. Studies of the HCV core protein and its coding RNA region have reported conflicting results on its role in regulating translation (Fan 1999; M Honda 1999; Ito 1999; D. Li 2003; H. H. Lu 1996; Reynolds 1995; Shimoike 1999; Tanaka 2000; T. H. Wang 2000; J. Zhang 2002). Other reports suggest that HCV nonstructural proteins NS4A and NS4B may also regulate translation (Florese 2002; Y. He 2003; J. Kato 2002a). Part of the difficulty in defining the roles of these factors is that their observed effects in experimental systems may not reflect their role *in vivo*.

For example, some studies suggest that the HCV core protein inhibits HCV translation (D. Li 2003; Shimoike 1999); however, the core protein may take on different roles at other stages of the HCV replication cycle, such as regulating the switch between translation and transcription (J. Zhang 2002). Alternately, interactions between the core protein and the 5′ UTR may occur only during virion assembly rather than during translation regulation (Fan 1999; Tanaka 2000).

Other viral proteins (particularly NS5A) may indirectly promote viral translation by blocking cellular defenses. Cells defend against viral infection through an enzyme called PKR (double-stranded RNA-dependent protein kinase R), an important component of the antiviral effects of interferon alpha (see Chapter IX, Immune Response, Persistence, and Pathogenesis). PKR inactivates eIF2, a eukaryotic initiation factor required for protein synthesis. The HCV NS5A protein binds to PKR, preventing it from inactivating eIF2 and shutting down protein synthesis (Y. He 2001; Y. He 2003; C. Wang 2003); however, the degree to which eIF2 inactivation regulates HCV translation remains unclear (J. Kato 2002b; Koev 2002).

Directions for future research on translation regulation

The last five years have been enormously productive for the study of HCV protein synthesis and of internal ribosomal entry sites in general. While the broad outlines of HCV IRES–directed translation have been established, significant questions remain regarding the mechanisms of translation regulation. Researchers have identified a broad range of viral and cellular factors that may affect the efficiency of translation, though whether they are essential and how exactly they work have not been fully established. HCV translation regulation is a dynamic system involving the interaction of multiple factors operating synergistically, antagonistically, or redundantly, and may vary in significance depending on cellular conditions. Current knowledge of HCV protein

synthesis cannot yet provide a definitive model for translational control, but still permits the initial identification and prioritization of targets for anti-HCV drug development.

Summary of HCV translation:

- HCV initiates protein synthesis from its genome through an internal ribosomal entry site (IRES) contained within the 5′ UTR.
- HCV translation operates under a tightly regulated regime that results in sufficient protein synthesis but may also limit the production of viral proteins. This degree of regulation may be necessary in order to ensure successful viral replication while protecting the host cell from premature death.
- Several cellular proteins may play a role in regulating of HCV translation, including the La antigen, PTB, and hnRNP L, though it is unclear whether all of these proteins are essential or how they function.
- Several viral elements have also been implicated in regulating HCV translation, including the core protein and/or its RNA coding sequence. The NS5A protein may block cellular defenses that would otherwise shut down protein synthesis during viral infection.
- HCV translation may be regulated by the cell cycle, operating most efficiently in actively dividing cells.
- HCV may have a competitive advantage in ensuring its translation over cellular mRNA during conditions of cellular stress such as cell division.

Stage 3: Cleavage and processing of viral proteins

The product of HCV translation is a single, long polyprotein containing all viral structural and nonstructural proteins. The polyprotein must first be cleaved, or sliced up, into several individual proteins, in order for the viral proteins to function properly. Some viral proteins must then undergo further modifications. Cleavage of the HCV polyprotein uses both viral and cellular enzymes. These enzymes are called proteases; they catalyze reactions that separate the individual proteins. This stage of the viral replication cycle is an important target for HCV drug development; if cleavage or processing is blocked, viral proteins cannot produce new hepatitis C virions.

Studies of HCV cleavage and processing have addressed three key issues:

- Which viral proteins are produced as a result of cleavage and processing?
- Which cellular and viral enzymes are involved in the cleavage of HCV proteins?
- What further modifications following cleavage are required for viral protein function?

HCV viral proteins

The HCV polyprotein yields ten main viral proteins. These proteins are classified as either structural proteins, which are incorporated into new virions, or nonstructural proteins. The ten proteins are listed below, along with their functions in viral replication (some of which are described later in this chapter; see Stage 4 and Stage 5):

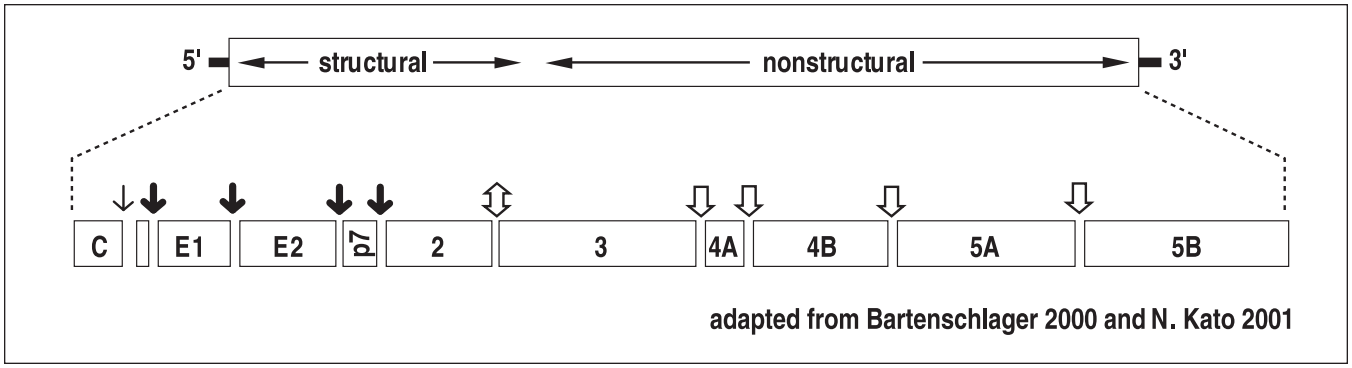
Structural proteins:

- Core protein—forms the capsid shell surrounding genomic HCV RNA
- E1 (envelope 1)—an envelope protein; probably involved in host cell attachment
- E2 (envelope 2)—an envelope protein; also involved in attachment to host cells
- p7—unknown function; may be involved in viral assembly and release; technically, not a known structural element of HCV virions, but generally grouped with the core, E1, and E2 structural proteins

Nonstructural proteins:

- NS2 (nonstructural protein 2)—part of the NS2-NS3 protease; possibly involved in the viral replication complex
- NS3 (nonstructural protein 3)—part of the NS2-NS3 protease; also contains the NS3 serine protease and a helicase/NTPase enzyme involved in HCV replication; probably involved in the viral replication complex
- NS4A (nonstructural protein 4A)—a cofactor for the NS3 serine protease, facilitating cleavage; probably involved in the viral replication complex
- NS4B (nonstructural protein 4B)—unknown function; probably involved in the viral replication complex
- NS5A (nonstructural protein 5A)—unknown function; probably involved in the viral replication complex
- NS5B (nonstructural protein 5B)—contains the RNA-dependent RNA polymerase enzyme, which copies the HCV genome via the viral replication complex

Figure 7. HCV Proteins and Cleavage Sites



Cellular and viral enzymes involved in cleavage of the HCV polyprotein

Cleavage of the HCV polyprotein occurs along the cell's endoplasmic reticulum (ER), a large internal network of membranes involved in protein synthesis, folding, and transport (Bartenschlager 2000; Reed 2000; J. Z. Wu 2001). Some cleavage occurs during translation (co-translational cleavage), but most cleavage occurs after the entire polyprotein has been translated (post-translational cleavage). HCV uses cellular proteases called signal peptidases to cleave its structural proteins. Two viral proteases, the NS2-NS3 protease and the NS3 serine protease, cleave HCV's nonstructural proteins:

- Signal peptidases cleave the core, E1, E2, and p7 proteins (Grakoui 1993a; Harada 1991; Hijikata 1991; C. Lin 1994a; Santolini 1994; Selby 1993). A full-length form of the core protein is further trimmed by signal peptidases to yield its mature, truncated form (Hedge 2002; Hussy 1996; T. Kato 2003a; Lemberg 2002; McLauchlan 2002; Weihofen 2002; M. S. Wolfe 2002).
- The cleavage of NS2 and NS3 is accomplished through the action of a protease comprised of the NS2 and NS3 proteins themselves, a process described as auto cleavage (Grakoui 1993c; Hijikata 1993b; Hirowatari 1993; Reed 1995). The NS2-NS3 protease has generally been described as a zinc protease, but some studies also suggest that it may belong to the cysteine protease family* (Pallaoro 2001; Pieroni 1997; Thibeault 2001).
- A separate NS3 protease enzyme, the NS3 serine protease, cleaves the remaining nonstructural proteins (Bartenschlager 1993; Bartenschlager 1999; Eckart 1993; Grakoui 1993b; Manabe 1994; Tomei 1993). The activity of the NS3 serine protease involves the HCV protein NS4A serving as a cofactor to facilitate cleavage and stabilize NS3 (Bartenschlager 1994; Failla 1994; Failla 1995; C. Lin 1994b; Tanji 1995).

Post-cleavage modifications to viral proteins

The HCV envelope proteins E1 and E2 must undergo further modifications before their assembly into the envelope of new HCV virions (Op de Beeck 2001):

- Localization and membrane anchoring—During translation and cleavage, E1 and E2 enter the lumen, or interior, of the endoplasmic reticulum. Both envelope proteins subsequently remain anchored to the ER membrane.
- Glycosylation—Oligosaccharide chains, or sugar molecules, are added to up to 6 sites on E1 and 11 sites on E2 inside the ER.
- Heterodimerization—Following glycosylation, E1 and E2 can be folded into their proper three-dimensional forms and joined into heterodimers, with the help of cellular "chaperone" proteins that assist in protein folding.

These steps are crucial for viral infectivity. Without heterodimerization of E1 and E2, new HCV virions cannot attach to and infect other cells (see Stage 1: Host cell attachment, entry, and uncoating). Similarly, without glycosylation, E1 and E2 cannot be properly folded into heterodimers. Indeed, clumps of improperly folded E1 and E2 proteins (called misfolded protein aggregates) have been found in the lumen of the ER, suggesting that proper folding does not always occur automatically (Choukhi 1999; Deleersnyder 1997; Dubuisson 1994; Dubuisson 1996; Duvet 1998; Grakoui 1993a; Michalak 1997). In theory, the accumulation of misfolded

* All proteases are classified into one of five categories, depending on their mechanism of action and the type of amino acids involved in catalyzing cleavage: aspartyl, cysteine, serine, threonine, and zinc proteases (also called metalloproteases). A diverse range of proteases falls within each category; human genes encode hundreds of different serine proteases. This classification encompasses viral as well as cellular proteases; HIV, for example, encodes an aspartyl protease that has been successfully targeted by HIV protease inhibitors.

E1-E2 protein aggregates may trigger an ER stress response, or unfolded protein response. These cellular defenses could potentially shut down translation initiation and conceivably lead to cell death, as has been seen *in vitro* with the related flaviviruses bovine viral diarrhea virus (BVDV) and Japanese encephalitis virus (JEV) (Jordan 2002; H. L. Su 2002). While some research supports a role for ER stress in HCV pathogenesis, available evidence is ambiguous, and other viral proteins, such as NS5A, may be involved (Gong 2001; Pavio 2003a; Tardif 2002; Tardif 2003; Tardif 2004; Waris 2003). Advances in mouse models of ER stress may clarify these questions (Iwawaki 2004).

Other viral proteins are also modified following cleavage. For example, the HCV protein NS5A undergoes a chemical modification called hyperphosphorylation that adds multiple phosphate groups to the protein (Reyes 2002). The role of NS5A hyperphosphorylation in viral replication is unclear, though it may be necessary for the assembly of the viral replication complex (see Stage 4: Replication of HCV genome) or for NS5A's role in pathogenesis (see Chapter IX, Immune Response, Persistence, and Pathogenesis). A set of cellular enzymes called protein kinases catalyze phosphorylation; over 500 kinases have been identified in the human genome (Manning 2002). Screening of yeast kinases has provided clues into which human kinases may be responsible for NS5A hyperphosphorylation, and NS5A phosphorylation by the human kinase c-Raf1 (an NS5A binding partner) has been demonstrated *in vitro* (Buerckstuemmer 2004; Coito 2004). Other HCV nonstructural proteins such as NS3, NS4A, and NS4B probably mediate the hyperphosphorylation of NS5A (Kaneko 1994; Koch 1999; Neddermann 1999).

Directions for future research on HCV cleavage and processing

While several facets of viral protein cleavage and post-translational modifications require further investigation, many fundamentals of polyprotein processing are now well established. In particular, a significant body of research has examined the function and structure of the NS3 serine protease and its NS4A cofactor. The NS3 serine protease—arguably the most extensively researched HCV viral enzyme—makes an attractive target for drug development. The success of HIV protease inhibitors no doubt contributes to the prominence of the NS3 serine protease in HCV research, despite little structural similarity between the proteases (Bartenschlager 1999; Bianchi 2002; Lahm 2002; Narjes 2003). Moreover, several drug companies have directed significant resources to the study of NS3, increasing our understanding of its molecular biology and their ability to develop NS3 protease inhibitors*. Indeed, the productive convergence of industry and academic research activities around the NS3 serine protease is unparalleled in the field of HCV, distantly echoing the public-private collaboration leading to the discovery of HCV. This impressive body of research provides hope that many lingering mysteries surrounding HCV could finally be resolved if similar resources and talents are brought to bear upon other aspects of viral replication.

*Drug companies include Agouron (now Pfizer), Boehringer Ingelheim, Bristol-Myers Squibb, Hoffmann-LaRoche, Merck (working in part through the Italian Istituto di Ricerche di Biologia Molecolare P. Angeletti), Schering-Plough, Vertex, and Wyeth.

Summary of HCV cleavage and processing

- The HCV polyprotein contains ten viral proteins—four structural proteins and six nonstructural proteins.
- Polyprotein processing occurs at the ER during and after translation.
- The structural proteins, core, E1, E2, and p7, are cleaved by cellular signal peptidases.
- The NS2/NS3 juncture is cleaved by the NS2-NS3 protease, thought to be a zinc protease but also sharing properties with cysteine proteases.
- The remaining nonstructural proteins are cleaved by the NS3 serine protease, using NS4A as a cofactor.
- The envelope proteins E1 and E2 undergo further post-translational modifications in the ER lumen, where they are glycosylated and folded with the help of chaperone proteins into their proper heterodimeric form.
- Other post-translational modifications, including the hyperphosphorylation of NS5A, also occur following cleavage.

Stage 4: Replication of the HCV Genome

Replication of the HCV genome, pivotal event in the viral life cycle, follows translation and viral protein processing. Each new virion requires its own viral genome.

These new HCV genomes are RNA strands that have been copied, or transcribed, from the original HCV RNA; however, the original RNA cannot simply be duplicated; it must first go through an intermediary stage. Replication, therefore, has two major steps: 1) the original viral genome is used as a template for the synthesis of the negative-sense strand of viral RNA, and 2) the negative-sense RNA then serves as the template for the production of new, genomic, positive-sense HCV RNA. A type of enzyme called a polymerase carries out strand synthesis; another enzyme, called a helicase, keeps the RNA strands separated during synthesis. Both polymerase and helicase are viral enzymes but other viral and cellular proteins are also involved in the replication process. Indeed, replication involves virtually all of the nonstructural proteins, which assemble to form the viral replication complex, the site where RNA strand synthesis occurs. Genome replication, like HCV translation, is a tightly orchestrated process regulated by viral and host factors.

The study of the replication of the HCV genome has perhaps benefited the most from the development of HCV replicon systems. As with prior stages in HCV replication, the synthesis of new genomic RNA presents several opportunities for therapeutic intervention. In particular, the viral polymerase and helicase enzymes make attractive targets for drug development.

Research on HCV RNA replication has explored three major areas:

- How does HCV replicate its genome?
- Which viral and cellular proteins are involved in replication?
- Where does replication occur?

Synthesis of HCV RNA

HCV polymerase enzyme, located within the HCV NS5B protein, synthesizes new RNA strands. This enzyme is called the RNA-dependent RNA polymerase (RdRp; reviewed in Lesburg 2000; Lohmann 2000). The RdRp operates through mechanisms similar to those used by cellular polymerase enzymes in the production of RNA. RNA is composed of nucleotides, molecules supplied by cells and containing one of four possible chemical bases—adenine, cytosine, guanine, and uracil. Each base has a complementary base to which it binds. Adenine binds to uracil, forming a base pair, and cytosine base pairs with guanine.

The NS5B RdRp "reads" the nucleotides on the genomic, positive-sense strand of HCV RNA and begins to synthesize a complementary negative-sense RNA chain, matching each original nucleotide with its complementary base. If the polymerase reads an A, it adds a complementary U to the new negative strand; when it reads a C on the original strand, it adds a G on the growing RNA chain, and so on. The NS5B RdRp reads the original RNA strand from the 3′ end to the 5′ end, reversing the direction that the ribosome uses for translation. In this way, the RdRp synthesizes a new RNA strand that is fully complementary, but not identical, to the original genomic RNA. The negative-sense RNA strand is therefore a molecular mirror image of the viral RNA.

Because the positive-sense and negative-sense strands are complementary, each can serve as a template for synthesizing the other. After the RdRp synthesizes negative-sense RNA, it repeats the process, this time using the negative-sense strand as a template for synthesizing a new positive-sense strand. This new positive-sense HCV RNA will form the genome of a new hepatitis C virion. The HCV RdRp has no proofreading mechanism to correct errors during strand synthesis, so mistakes made by the HCV RdRp get incorporated into new HCV RNA as mutations (Behrens 1996). This propensity for error during genomic replication results in a quasispecies population of closely related but genetically distinct HCV variants.

Complementary RNA strands can bind to each other, forming double-stranded RNA. Double-stranded RNA (dsRNA) rarely appears in cells. Cells tend to interpret the presence of dsRNA as a sign of viral infection, triggering a set of cellular defenses (including interferon) that aim to block viral replication and destroy viral RNA (see also the discussion of interferon mechanisms of action in Chapter X, The Future of HCV Therapy). Furthermore, the HCV polymerase cannot "read" RNA strands bound into dsRNA form; strand synthesis requires a template of single-stranded RNA. Therefore, HCV must keep positive-sense and negative-sense RNA strands separated in order to avoid cellular defenses and maintain the replication process. The helicase enzyme, contained within the HCV NS3 protein, accomplishes this task by unwinding or separating RNA strands during genome replication. The helicase acts as a wedge, binding to an RNA strand and moving from the 3′ end to the 5′ end, disrupting dsRNA formation, and possibly dislodging cellular RNA-binding proteins that would otherwise interfere with HCV polymerase activity. The HCV helicase operates with another NS3 enzyme, called NTPase (nucleotide triphosphatase), which catalyzes chemical reactions that support the movements and RNA binding of the helicase (J. L. Kim 1998; Levin 2003; Paolini 2000). Multiple HCV helicase enzymes may be required for efficient RNA strand synthesis (Levin 2004).

Strand synthesis occurs within a viral replication complex (or replicase) formed by the HCV proteins NS3, NS4A, NS4B, NS5A, and NS5B (and possibly NS2) on the membrane of the endoplasmic reticulum (Dimitrova 2003; El-Hage 2003; Fipaldini 1999; Moradpour 1998; Mottola 2002; Pietschmann 2001; Tardif 2002; Waris 2004). This replication complex sequesters the process of strand synthesis, protecting viral RNA strands from host cell defenses and degradation. The replicase probably also provides the scaffolding for replication and helps to retain the negative strand for further rounds of synthesis. The viral replication complex has not been well characterized and likely involves cellular and viral proteins. One candidate cellular protein, alpha-actinin, interacts with HCV NS5B and appears to be required for HCV replication (S. Lan 2003). NS5B and NS5A can each bind to the cellular protein hVAP-33 (human vesicle–associated membrane protein–associated protein of 33 kDa), which facilitates the formation of the viral replication complex (Tu 1999). Interference with hVAP-33 expression dramatically reduces intracellular HCV RNA and viral protein levels *in vitro* (L. Gao 2004).

The viral protein structure formed on the endoplasmic reticulum during replication has been described as a "membranous web" (Egger 2002; Gosert 2003; Miyanari 2003). The HCV protein NS4B appears to play a major role in forming these membranous webs, presumed to function as the viral replication complex. NS4B also possesses a domain capable of binding nucleotides, and replicon studies show that mutations to that domain impair or abolish viral replication (Einav 2003a). However, other researchers have suggested that viral replication may actually occur not on the ER membrane, but on lipid rafts (cholesterol-rich bits of membrane) (Shi 2003).

HCV RNA strand synthesis is regulated by a variety of viral and cellular factors. Evidence from chimpanzees suggests that some mutations to HCV NS5B may increase the rate of viral RNA strand synthesis (Lou 2003). Conversely, some highly conserved elements in HCV RNA, common across genotypes and individual viral isolates, may also regulate viral replication. The NS5B coding sequence includes a conserved RNA structural element that folds into a stem loop and functions as a *cis*-acting replication element (CRE) (S. You 2004). *Cis*-acting replication elements function as conserved structures embedded in viral RNA that can mediate translation, RNA strand synthesis, or other aspects of the viral replication cycle. CREs have also been found in the HCV 5′ UTR, 3′ UTR, and core protein coding sequence; key RNA structures in the 3′ UTR are required for HCV strand synthesis (Friebe 2002; Penin 2004; Schuster 2002; Yi 2003a; Yi 2003b). The NS5B CRE is also essential for replication, presumably by interacting with one or more viral and/or cellular proteins (S. You 2004).

Interactions between NS5A and NS5B appear particularly important for effective HCV replication (Shimakami 2004). Viral proteins (including NS3, NS4B, and NS5A) may directly or indirectly contribute to the efficiency of HCV genome replication, inhibiting or stimulating HCV RdRp activity (Piccininni 2002; Shirota 2002). This may partly account for differing rates of synthesis for positive-sense and negative-sense RNA strands. Generally the amount of positive-sense HCV RNA exceeds that of negative-sense RNA, suggesting a regulatory mechanism at work (M. Chang 2003; Komurian-Pradel 2004).

Some cellular proteins may also regulate viral RNA strand synthesis, though their roles are less clear. Morphine can increase HCV RNA replication *in vitro*, possibly by stimulating intracellular signaling pathways rather than via direct interaction with viral proteins (Y. Li 2003). Evidence

suggests that NS5B interacts *in vitro* with nucleolin, an RNA-binding molecule, and hPLIC1 (human homolog 1 of protein linking intergrin-associated protein and cytoskeleton), a cellular protein that reduce replication by targeting NS5B for degradation via ubiquitination (L. Gao 2003; Hirano 2003). NS5A interacts with the cellular protein amphiphysin II, though the function of this interaction is unclear (Zech 2003). In vitro models suggest that the NS5A protein and the viral replication process itself trigger the ER stress response, with possible consequences for HCV RNA strand synthesis (Gong 2001; Tardif 2002; Waris 2002).

Cellular proteins such as PTB and hnRNP C that bind to the 3′ UTR, where negative-sense strand synthesis initiates, may modulate the efficiency of HCV genome replication (Ito 1997; Luo 1999). Further research may elucidate the role of these factors and identify additional cellular proteins that can regulate RdRp activity. Inhibition of geranylgeranylation (the transfer of chemical groups involved in protein prenylation, which directs cellular proteins to cell membranes) disrupts the HCV replication complex, suggesting a role for an unidentified geranylgeranylated host protein in viral replication and a possible avenue for therapeutic inhibition (J. Ye 2003). Cellular factors almost certainly influence HCV replication, given that viral replication may be most active during cell growth and division, as with protein synthesis (M. Honda 2000; Pietschmann 2001; Scholle 2004). The availability of pools of nucleotides for incorporation in new HCV RNA strands, and perhaps cytosine and uracil in particular, would also determine the rate of replication and may provide a strategy for inhibition (Stuyver 2003b).

Summary of HCV genome replication

- HCV genome replication entails synthesis of a negative-sense RNA strand complementary to the original viral RNA, which then serves as a template for synthesis of positive-sense, genomic viral RNA.
- Negative-sense and positive-sense strand synthesis is performed by the HCV NS5B protein, an RNA-dependent RNA polymerase, which assembles a chain of nucleotides complementary to the original strand.
- During RNA replication, the NS3 helicase/NTPase, with NS4A acting as a cofactor, moves along the template RNA strand to keep the original and newly synthesized RNA strands separate.
- RNA replication begins with the assembly of a viral replication complex consisting of all HCV nonstructural proteins and possibly one or more as yet unidentified cellular proteins.
- The viral replication complex is associated with the ER membrane, at least initially, though viral replication may occur on a lipid raft detached from the ER in association with the replication complex.
- The highest levels of viral genome replication are observed during cell growth and division, suggesting the importance of cellular factors in modulating replication efficiency.

Stage 5: Assembly of new virions and release from host cell

New HCV virions consist of three main components: single-stranded genomic HCV RNA, a capsid shell, and the viral envelope. HCV assembles new virions from components produced through translation (the core and envelope proteins) and strand synthesis (genomic HCV RNA). The genomic RNA is packaged within the capsid, which is formed from core proteins. The combination of HCV RNA with the capsid shell is known as the nucleocapsid, which is then enveloped within a section of cellular membrane, presumably derived from the endoplasmic reticulum. This envelope incorporates the viral envelope proteins, completing new virion assembly. These new viruses are then transported to the plasma membrane and released to infect new cells. It is unclear whether viral release results in the death of the host cell (see Chapter IX, Immune Response, Persistence, and Pathogenesis). The dynamics of viral assembly and release are not well understood, largely due to the limitations of experimental models; however, these late events in the viral replication cycle are crucial for maintaining HCV infection, and may also offer additional targets for antiviral intervention.

Research on the late stages of HCV replication poses the following questions:

- How are new HCV virions assembled?
- Where does assembly occur?
- How are new virions released from the cell?

Viral assembly can be roughly divided into two phases: the formation of the nucleocapsid and the formation of the viral envelope. The capsid is composed of at least 420 core protein subunits assembled into an icosahedral form (Kunkel 2001; Matsumoto 1996). Interactions between core proteins and the 5′ UTR of HCV RNA trigger the assembly of the capsid, and enclose genomic RNA within the capsid shell (Fan 1999; Kunkel 2001; Kunkel 2002; Santolini 1994; Shimoike 1999; Tanaka 2000). How this process is regulated remains an open question, though cellular factors may be involved. In particular, the cellular enzyme tissue transglutaminase promotes the formation of core protein dimers (two core proteins joined together), impairing the ability of the core protein to bind to RNA and thus possibly impeding nucleocapsid formation (W. Lu 2001).

How the HCV nucleocapsid acquires its envelope is not yet clear. The assembly of other members of Flaviviridae viruses is generally thought to involve a process called "budding" into vesicles, wherein the viral capsid encircles itself in a cellular membrane, which becomes the viral envelope (Garoff 1998; Lindenbach 2001). This membrane is studded with viral envelope proteins (E1 and E2, in the case of HCV). E1-E2 heterodimers are largely retained within the ER prior to virion assembly, providing indirect evidence that budding of new HCV virions likely occurs at the ER membrane, though functional E1-E2 heterodimers have also been detected on cell surface membranes in some *in vitro* systems (Charloteaux 2002; Cocquerel 1998; Cocquerel 1999; Drummer 2003; Dumonceaux 2003; Duvet 1998). The HCV core protein appears to play a key role in directing the budding process (Blanchard 2003b).

Not all viral particles are released in enveloped form. Nonenveloped viral particles that appear to consist solely of a nucleocapsid shell, presumably surrounding HCV RNA, have been found in the blood of people chronically infected with HCV (Maillard 2001). The role of these particles in viral replication and pathogenesis is unclear; however, HCV nucleocapsids can bind to certain types of

antibodies (IgG, or immunoglobulin G), which may interfere with immune responses or otherwise contribute to HCV pathogenesis (Maillard 2004; Sansonno 2003; see also Chapter IX, Immune Response, Persistence, and Pathogenesis).

The actual release from the cell of new hepatitis C virions has not been observed, so speculation on this process again relies on analogy to other members of the Flaviviridae family. In this model, newly enveloped virions would be transported in vesicles to the plasma cell membrane. When the vesicles fuse to the plasma membrane, new virions would be released from the cell in a process called exocytosis, the reverse of endocytosis (Lindenbach 2001; Serafino 2003). This form of release follows the cell's own secretory pathway (the route taken by proteins secreted by the cell) moving from the ER to the Golgi apparatus to the plasma membrane.

Alternately, viral release may involve the HCV p7 protein. Recent research has shown that p7 can form pores or protein-lined tunnels on the plasma cell membrane called ion channels, which allow ions such as calcium, sodium, and potassium to enter cells (Carrère-Kremer 2002; Griffin 2003; Pavlović 2003). These p7 ion channels may effectively remder the cell's plasma membrane permeable to substances that would otherwise be blocked. Increased permeability of the cell membrane might also facilitate the release of hepatitis C virions. This hypothesis remains speculative, but gains credibility from the observation that HIV produces a similar protein, Vpu, which forms ion channels that enhance the release of new HIV virions (C. Ma 2002). Despite uncertainty about its function, p7 appears essential to viral replication. Chimpanzee studies demonstrate that viral HCV RNA transcripts without p7 or with p7 mutations are not infectious, and that p7 function may be genotype-specific (Sakai 2003). The p7 protein may present a viable target for anti-HCV drug development Pavlović 2003).

HCV assembly and release are presumably not automatic or passive events, and the viral and cellular factors that mobilize and direct these factors require elucidation. Evidence from several other viruses, including HIV, indicates that cellular proteins can play a significant role in viral assembly and release (Erturk 2003; Freed 2002). The existence and nature of such cellular factors has not yet been determined for HCV (Pietschmann 2002). Despite progress in HCV replicons and surrogate viral replication models, the assembly and release of infectious virions or HCV-like particles following replication have not been observed in cell culture systems (Molenkamp 2003; Pietschmann 2002). Analogies to the maturation and budding processes seen in other viruses may aid in forming hypotheses, but a definitive model for HCV assembly and release will require further research. Better experimental models for HCV assembly and release are badly needed.

Summary of HCV assembly and release

- The capsid self-assembles from multiple subunits of the core protein.
- Capsid formation requires interactions between the core protein and the HCV 5′ UTR.
- The viral envelope is formed from cellular membranes by a process called budding.
- HCV is thought to acquire its envelope through budding into the lumen of the endoplasmic reticulum.
- In this model, the assembled virion would be released through the secretory pathway.
- The viral protein p7 may also be involved in viral release by forming ion channels, potentially increasing the permeability of the cell's plasma membrane.

Research Recommendations

Support and intensify research into the molecular biology of HCV.

The initial identification of hepatitis C virus (Q. L. Choo 1989) ushered in a highly productive era in virology research, as scientists began investigating the genetic structure of the virus and the role of viral proteins in HCV replication. Despite enormous advances, numerous challenges remain. Key aspects of the HCV replication cycle are not fully understood, and further work on cell culture systems and animal models is an urgent priority. Continued elucidation of the molecular biology of HCV will be critical in understanding viral pathogenesis and developing new therapies.

Efforts must be made to increase the utility of the mouse model and enhance the efficiency and reproducibility of *in vitro* cell culture systems. The chimeric mouse model incorporating human hepatocytes shows promise, though further work is necessary to better mimic human immune responses (Brass 2002; Pietschmann 2003). The National Institute of Allergy and Infectious Diseases' Division of Microbiology and Infectious Diseases' (DMID) currently supports small-animal model HCV research. Increased NIAID funding and additional resources from other funders of biomedical research would help to accelerate this work. Further exploration of the potential for chimeric GB virus B in tamarins and marmosets as a surrogate model for HCV infection should also be supported (Beames 2000; Beames 2001; Bright 2004; Bukh 1999; De Tomassi 2002; Sbardellati 2001).

Finally, recent advances in HCV replicon systems offer hope for the eventual development of a viable cell culture model for HCV (Bartenschlager 2002; Pietschmann 2003). Further refinements in replicon models will require sustained support from public and private funders. Prominent examples of such support include the recent five-year, unrestricted grant from Bristol-Myers Squibb to Ralf Bartenschlager's laboratory at the University of Heidelberg in Germany, and the support from government grants and the Greenberg Medical Research Institute for the work of Charles Rice and colleagues at Rockefeller University's Center for the Study of Hepatitis C. The National Institutes of Health and other governmental and private funders should coordinate and extend their efforts to ensure that the work of Bartenschlager, Rice, and other groups focusing on replicons continues. DMID must also update its "Framework for Progress on Hepatitis C" (NIAID 1997) and receive additional funding to increase its commitment to intramural and extramural basic research on HCV infection.

List of Terms Used in this Chapter

3′ UTR (3-prime untranslated region): non-coding region of HCV RNA; site of initiation of negative-sense strand synthesis.
5′ UTR (5-prime untranslated region): non-coding region of HCV RNA; contains the internal ribosomal entry site (IRES); site of initiation of translation.
Adenine: a nucleoside base; one of the four building blocks of RNA.
Amino acids: the building blocks of proteins; the sequence and composition of amino acids determines a protein's structure and function.
ARFP (alternate reading frame protein): an HCV protein encoded within an alternate reading frame of the core protein coding sequence; also called F protein or frameshift protein.
Cap-dependent translation: the method of translation (protein synthesis) predominantly used for cellular proteins.
Cap-independent translation: translation (protein synthesis) via an internal ribosomal entry site (IRES); the method used by HCV.
Capsid: the shell surrounding the HCV genome, formed by the HCV core protein.
CD81: a cell surface receptor believed to be involved with HCV entry into cells.
Cell culture: growing cells in a laboratory; an *in vitro* research tool.
Cell line: a group of cells maintained in cell culture that continue to survive and divide; an *in vitro* research tool.
Chimeric virus: an engineered hybrid of two different viruses (e.g., the core particles of a retrovirus bearing HCV envelope proteins on the surface).
***Cis*-acting replication element (CRE):** conserved structures embedded in viral RNA that can mediate translation, RNA strand synthesis, or other aspects of the viral replication cycle.
Cloning vectors: DNA molecules (i.e., plasmids) or viruses that smuggle HCV genetic material inside a cell to synthesize viral proteins.
Core: an HCV protein; forms the capsid.
Cryopreservation: freezing (e.g., of cells or tissue for later study).
C-type lectins: carbohydrate-binding molecules; includes DC-SIGN and L-SIGN.
Cytokine: secreted proteins that function as chemical messengers between cells by binding to cell surface receptors.
Cytoplasm: the main area of the cell inside its membrane but outside of its nucleus.
Cytosine: a nucleoside base; one of the four building blocks of RNA.
DC-SIGN (dendritic cell–specific intercellular adhesion molecule 3-grabbing non-integrin): a cell surface receptor; possibly involved with HCV entry into cells.
E1: one of two HCV envelope proteins.
E2: one of two HCV envelope proteins.
Endoplasmic reticulum (ER): a membrane within cells; the site of HCV translation (protein synthesis) and strand synthesis.
Envelope: the outer layer of HCV; a membrane containing HCV envelope proteins E1 and E2 that surrounds the nucleocapsid.

Eukaryotic initiation factors: cellular proteins involved in translation (protein synthesis).
Extrahepatic: cells or tissue outside the liver.
F protein (frameshift protein): an HCV protein encoded within an alternate reading frame of the core protein coding sequence; also called the alternate reading frame protein (ARFP).
Flaviviridae: the family of viruses that HCV belongs to.
Flavivirus: a genus within the Flaviviridae family; closely related to HCV.
Frameshift: the translation of 2 or more proteins from overlapping sections of RNA. HCV contains at least one frameshift protein (the F protein or ARFP).
Geranylgeranylation: the transfer of chemical groups involved in protein prenylation.
GAPDH (glyceraldehyde-3-phosphate dehydrogenase): a cellular protein that binds to the HCV 3′ UTR.
Genome: the total genetic information of an organism; the HCV genome is a single strand of positive-sense RNA.
Glycosaminoglycans: a group of polysaccharides (e.g., heparin and heparan sulfate) found on cell surfaces and possibly involved in HCV attachment to target cells.
Glycosylation: a chemical modification that adds sugar molecules to proteins. HCV envelope proteins E1 and E2 undergo glycosylation in the endoplasmic reticulum.
Guanine: a nucleoside base; one of the four building blocks of RNA.
Helicase: an HCV enzyme, contained within NS3, which unwinds and separates RNA strands during strand synthesis.
Hepacivirus: the genus of viruses that HCV belongs to.
Hepadnaviridae: the family of viruses that hepatitis B belongs to.
Heparan sulfate: a glycosaminoglycan found on cell surfaces and possibly involved in HCV attachment to target cells.
Heparin: a glycosaminoglycan found on cell surfaces and possibly involved in HCV attachment to target cells.
Hepatocyte: a liver cell; the main cell type that HCV infects and uses for replication.
Hepatoma cell line: a cell line derived from cancerous hepatocytes (liver cells); an *in vitro* research tool.
Hepatotropic: targeting the liver; HCV is a hepatotropic virus (as are hepatitis A and hepatitis B).
Heterodimer: complexes formed by two different proteins; the HCV envelope proteins E1 and E2 join to form heterodimers.
hnRNP (heterogeneous nuclear ribonucleoprotein): a type of cellular protein. HnRNP L binds to the HCV internal ribosomal entry site (IRES). HnRNP C binds to the HCV 3′ UTR.
Host cell: a cell infected by a virus.
hPLIC1 (human homolog 1 of protein linking integrin-associated protein and cytoskeleton): a cellular protein that reduces replication by targeting HCV NS5B for degradation via ubiquitination.
Hyperphosphorylation: a type of chemical modification performed by cellular enzymes; specifically, the addition of multiple phosphate groups to a molecule. HCV NS5A undergoes hyperphosphorylation.

In vitro: research performed outside of a living organism (e.g., not in humans or animals); test tube or laboratory research.
In vivo: research performed inside of a living organism (e.g., humans and animals).
Ion channels: a gateway, composed of one or more proteins, that allows ions (charged atoms or molecules such as calcium) to pass through cell membranes.
IRES (internal ribosomal entry site): a structure with the HCV RNA 5′ UTR that binds directly to the ribosome to initiate translation.
La antigen: a cellular protein that binds to the HCV internal ribosomal entry site (IRES) and increases the efficiency of translation (protein synthesis). The La antigen also binds to the HCV 3′ UTR.
LDLR (low-density lipoprotein receptor): a cell surface receptor; possibly involved with HCV entry into cells.
Ligand: molecules that bind to a particular receptor.
LSECs (liver sinusoidal endothelial cells): endothelial cells that line blood vessels in the liver and separate blood from surrounding organ tissue; express L-SIGN receptors.
L-SIGN (liver/lymph node–specific intercellular adhesion molecule-3-grabbing integrin): a cell surface receptor; possibly involved with HCV entry into cells.
Membraneous web: the structure formed by HCV replication complex.
Molecular cloning: duplication of genetic material (e.g., RNA) through PCR techniques.
mRNA (messenger RNA): positive-sense RNA that encode proteins; the HCV genome functions as mRNA.
Negative-sense RNA: the mirror image of positive-sense RNA; serves as a template for synthesizing positive-sense RNA; cannot function as messenger RNA (mRNA).
NS2 (non-structural protein 2): an HCV protein; part of the NS2-NS3 protease.
NS3 (non-structural protein 3): an HCV protein; contains part of the NS2-NS3 protease; the NS3 serine protease; and the NS3 helicase/NTPase
NS4A (non-structural protein 4A): an HCV protein; a co-factor for the NS3 serine protease and part of the HCV replication complex.
NS4B (non-structural protein 4B): an HCV protein; part of the HCV replication complex.
NS5A (non-structural protein 5A): an HCV protein; part of the HCV replication complex.
NS5B (non-structural protein 5B): an HCV protein; contains the RNA-dependent RNA polymerase (RdRp).
NTPase (nucleotide triphosphatase): an HCV enzyme, contained within NS3, which catalyzes chemical reactions that support the movements and RNA binding of the HCV NS3 helicase.
Nucleocapsid: the HCV genome, surround by the capsid shell.
Nucleolin: an RNA-binding molecule that interacts with HCV NS5B.
Nucleoside: a base (adenine, cytosine, guanine, or uracil) attached to a sugar molecule.
Nucleotide: the phosphorylated form of a nucleoside; the building block of RNA.
Open reading frame: the section of HCV RNA that encodes viral proteins; does not include the 5′ UTR and the 3′ UTR.
p7: an HCV protein of unknown function; may create ion channels.

PBMCs (peripheral blood mononuclear cells): white blood cells, including monocytes and lympocytes (e.g., T cells); HCV can infect PBMCs, and may replicate within them.
PCR (polymerase chain reaction; also RT-PCR for reverse transcriptase polymerase chain reaction): a technique to amplify and clone genetic material (DNA and RNA); PCR technology enabled the original identification of HCV.
Permissive cells: cells susceptible to HCV infection and capable of supporting HCV replication (e.g., hepatocytes).
Pestivirus: a genus within the Flaviviridae family; closely related to HCV.
Phosphorylation: a type of chemical modification performed by cellular enzymes; specifically, the addition of a phosphate group to a molecule.
Phylogenetic: referring to HCV's evolutionary history; i.e., HCV's "family tree."
Picornaviridae: the family of viruses that hepatitis A belongs to.
PKR (Double-stranded RNA-dependent protein kinase R): a cellular defense against viral infection that shuts down protein synthesis.
PCBP (polycytosine-binding protein): a type of cellular protein. PCBP 1 and 2 bind to the HCV internal ribosomal entry site (IRES).
Polymerase: an enzyme that synthesizes new DNA or RNA strands. HCV NS5B contains a polymerase enzyme, the RNA-dependent RNA polymerase, which synthesizes new HCV RNA.
Polyprotein: a long protein chain; HCV RNA is translated into a polyprotein, which is then cleaved (split or separated) into individual HCV proteins by viral and cellular protease enzymes.
Polysaccharides: carbohydrate molecules composed of sugars; glycosaminoglycans are a type of polysaccharide.
Positive-sense RNA: RNA strands that can function as messenger RNA (mRNA) and be translated into proteins; the HCV genome is a single positive-sense RNA molecule.
Prenylation: a type of chemical modification that directs cellular proteins to cell membranes.
Protease: an enzyme that breaks down proteins. HCV contains two viral protease enzymes: the NS2-NS3 protease, and the NS3 serine protease.
Protein: large molecules composed of amino acids. The genetic template or blueprint for proteins is stored (encoded) in RNA and DNA. HCV RNA encodes at least ten viral proteins. HCV structural proteins (core, E1, E2) become components of new virions; HCV non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) play a variety of roles in viral replication.
Protein expression: the production of individual HCV proteins; an *in vitro* research tool.
Pseudotyped virus: an engineered hybrid of two different viruses (e.g., the core particles of a retrovirus bearing HCV envelope proteins on the surface).
PTB (polypyrimidine tract binding protein): a cellular protein that binds to the HCV 3' UTR.
Quasispecies: a dynamic population of closely related but distinct genetic sequences; the population of HCV within an individual, including strains containing mutations.
Receptors: molecules on cell surfaces that other molecules bind to, for signaling and cell entry. HCV uses one or more receptors to enter cells.

Receptor-mediated endocytosis: the mechanism through which HCV is believed to enter cells; HCV envelope proteins would bind to one or more cell surface receptors, triggering the cell to internalize the virus, encapsulating it into a vesicle.
Replicase: the HCV replication complex, the structure within which HCV RNA strand synthesis occurs. Composed of non-structural (and probably cellular) proteins.
Replication: the process of creating new HCV virions; occurs within cells (primarily hepatocytes).
Replication complex: the structure within which HCV RNA strand synthesis occurs. Composed of non-structural (and probably cellular) proteins. Also called the replicase or membraneous web.
Replicon: genetic material capable of autonomous replication and protein synthesis; HCV replicons are the main *in vitro* model for research on viral replication.
Retrovirus: a type of RNA virus; HIV (but not hepatitis C) is a retrovirus.
Ribosome: cellular machinery responsible for translation (protein synthesis); the ribosome "reads" mRNA.
Ribosomal protein L22: a component of the ribosome that binds to the HCV 3′ UTR.
RNA: a molecule (composed of nucleotides) containing genetic information that can be translated into proteins; the HCV genome is a single strand of positive-sense RNA.
RNA-dependent RNA polymerase (RdRp): the HCV polymerase enzyme, contained within HCV NS5B and responsible for synthesizing new positive-sense and negative-sense HCV RNA.
Serial passage: taking sera (the clear fluid portion of blood) containing HCV and r epeatedly passing it through (i.e., infecting) animals, from one animal to the next; this can increase the virulence (infectiousness) of the virus.
Signal peptidases: a type of cellular protease enzymes; signal peptidases cleave (split or separate) part of the HCV polyprotein.
SR-BI (human scavenger receptor class B type I): a cell surface receptor; possibly involved with HCV entry into cells.
Strand synthesis: the creation of new RNA strands by a polymerase enzyme; HCV uses the NS5B RNA-dependent RNA polymerase to synthesize new negative-sense HCV RNA from a positive-sense HCV RNA strand, and new positive-sense HCV RNA from a negative-sense HCV RNA strand. Strand synthesis is vital to HCV replication.
Subgenomic: containing only part of the genome; subgenomic HCV replicons typically contain only the RNA sequences that encode the HCV non-structural proteins.
Taxonomies: systems of classification.
Tissue culture: organ tissue (e.g., from the liver) grown and maintained in a laboratory; an *in vitro* research tool.
Translation: protein synthesis; the ribosome translates HCV RNA into viral proteins. Translation is vital to HCV replication.
Ubiquitination: a cellular process that targets proteins for destruction.
Uracil: a nucleoside base; one of the four building blocks of RNA.
UTR (untranslated region): regions of HCV RNA that do not encode viral proteins (the 5′ UTR and the 3′ UTR).

Vesicle: a small pocket of fluid within cells surrounded by a thin membrane.
Viral heterogeneity: genetic diversity among viral isolates.
Viral isolates: an individual strain of virus.
Virion: an individual virus particle.

IX. Immune Response, Persistence, and Pathogenesis

Introduction

Research into the natural history of hepatitis C infection is shaped by two questions: Why do some people clear HCV during acute infection, while most people proceed to chronic infection? And why do the rates and degrees of liver damage associated with chronic HCV infection vary so widely among individuals? These questions have stimulated a vast body of research into the mechanisms of viral persistence (how HCV establishes and maintains chronic infection) and viral pathogenesis (how HCV causes disease). Explanations of viral persistence and pathogenesis have implicated both viral and host factors. Proposed viral factors include the high mutation rate of HCV and the deleterious effects of particular viral proteins both on the proper functioning of infected cells and on key components of the immune system responsible for defense against viruses. Host factors range from individual genetic differences to the vigor and quality of the immune response to HCV.

Chronic HCV infection can lead to fibrosis (scarring of the liver), cirrhosis (severe and widespread scarring that compromises liver function and interferes with blood flow), steatosis (build-up of fats in the liver), and hepatocellular carcinoma (liver cancer). These complications represent the consequences of injuries to the liver that damage or kill hepatocytes. These clinical manifestations are not unique to chronic HCV infection; they typify liver disease that may result from chronic hepatitis of various origins, including hepatitis B infection, heavy drinking, and hereditary disorders. Nevertheless, in HCV infection, the virus itself (or particular viral proteins) may directly contribute to or exacerbate these conditions, especially steatosis and hepatocellular carcinoma.

The nature of the human immune response to HCV is considered pivotal to both persistence and pathogenesis; indeed, the immune response, rather than the direct effects of the virus, is generally assumed to cause most if not all liver damage associated with HCV infection. Though not discussed in this chapter, immunologic factors drive a range of extrahepatic manifestations of diseases associated with chronic HCV infection, including autoimmune and lymphoproliferative disorders such as mixed cryoglobulinemia, Sjögren's syndrome, and B-cell non-Hodgkin's lymphoma (Agnello 2004; von Herrath 2003; see Chapter II, Natural History of Hepatitis C). The central role of the immune system in HCV pathogenesis also has particular consequences for people coinfected with HIV. HIV coinfection, characterized by progressive immune deficiency and dysfunction, results in the acceleration of HCV-related liver damage (Einav 2002). Yet much remains to be learned about the precise workings of viral persistence and HCV-related liver disease. Several themes recur in research on the immune response to HCV and viral pathogenesis: the immunological environment of the liver; functional impairments in the immune response to HCV; host-virus interactions; and host and viral genetic diversity.

The immunology of the liver

The liver constitutes a distinctive immunologic environment. The immune system guards the body against harmful foreign agents such as viruses and bacteria, including those that pass through the gut and into the liver. At the same time, the liver plays a primary role in metabolizing foods—substances technically foreign to the body but harmless and a source of essential nutrients. While

the immune system generally attempts to block, neutralize, and eliminate foreign substances, the functions of the liver require the immune system to tolerate the presence of such substances when they are benign. The liver therefore tends to induce a state of immunologic tolerance, which may contribute to viral persistence by dampening the intrahepatic immune response to HCV. Other aspects of the hepatic immunologic milieu may actually augment HCV-related liver damage (Crispe 2003).

Immune dysfunction

Defects in the immune response to HCV may account for viral persistence as well as aspects of pathogenesis. These defects may be exacerbated by HIV coinfection, but can be observed even in individuals with no signs of immune deficiency. The immune response to HCV exhibits several signs of functional impairment, from the earliest events during acute infection and throughout the course of chronic infection. These impairments may contribute to liver damage. The immune response to HCV in the liver results in inflammation; hepatitis literally means "inflammation of the liver." The inflammatory response, an attempt to control the virus and destroy infected cells, may eventually produce scarring, or fibrosis. A dysfunctional immune response in chronic HCV infection may promote a persistent state of inflammation, in which the immune system consistently fails to suppress the virus and inadvertently increases liver damage.

Host-virus interactions

In vitro research has documented an ever-expanding array of mechanisms by which HCV usurps or interferes with cellular processes. Some of these mechanisms foster a cellular environment that permits or enhances HCV replication, while others may directly influence viral persistence and pathogenesis by disabling cellular defenses. Interactions with components of the immune system may contribute to dysfunction in the immune response to HCV. Hepatitis C viral proteins subvert or hijack key intracellular signaling pathways that normally regulate major cell processes by stimulating or inhibiting the expression of genes. The effects of HCV proteins on cell activation, proliferation, and death are of particular interest in understanding the development of hepatocellular carcinoma.

Host and viral genetic diversity

Some of the differences in the outcomes of acute and chronic HCV infection may be attributable to variations in individual genes, particularly those that influence immune responses. Genetic differences between individuals and groups may influence the likelihood of clearing acute HCV infection, the rate of fibrosis progression, and the risk of hepatocellular carcinoma. The virus also exhibits genetic diversity; viral replication is error-prone, so mutations are constantly being introduced into new virions. Some of these mutations may enable viral evasion of the immune response to HCV. The presence of these mutations correlates with failure to resolve acute infection.

Research on immune response, persistence, and pathogenesis has been hindered by the lack of suitable cell and tissue culture systems and small-animal models for HCV infection. A better understanding of the mechanisms of HCV persistence and pathogenesis can provide important insights for the treatment and prevention of HCV infection. Therapeutic approaches that could mitigate fibrosis and prevent hepatocellular carcinoma would provide crucial options for

individuals who do not respond to, or are unable to tolerate, current anti-HCV therapy. The parameters of immune response and pathogenesis may also inform the development of diagnostic tools that can guide decisions on when and whether to initiate treatment.

Vaccine development also requires an understanding of the dynamics of the immune response to acute infection, and of the immune correlates of viral clearance. Dysfunctional immune responses to HCV offer intriguing clues and highlight serious challenges to developing potential vaccine strategies. Further research may suggest possible therapeutic approaches for augmenting the capacity of the immune system to control chronic HCV infection. Host and viral genetic variation also have important implications for anti-HCV vaccines and immune-based therapies.

Immune Response and Viral Persistence

Early events in the immune response to HCV infection determine whether acute HCV infection will become chronic. In some people, the immune system succeeds in clearing the virus within a few months (during the acute phase of infection) by eliminating all viral particles in circulation and destroying all infected cells. In the majority of people, however, the immune system is unable to eradicate hepatitis C, and HCV infection persists (see Chapter II, Natural History of Hepatitis C). Much of the current knowledge of immune responses to HCV has come from studies investigating the determinants of viral clearance in order to understand the correlates of successful immune responses to HCV. Ideally, this line of inquiry could help to identify strategies for augmenting less successful immune responses. These strategies could then be applied to the development of vaccines that prevent HCV infection, and therapies or vaccines that improve the response to HCV treatment in chronically infected people.

Determinants of Viral Clearance

Studies of clearance of acute HCV infection generally point to the importance of a robust, HCV-specific cell-mediated immune response (Pavio 2003b; Racanelli 2003; see box below). A number of studies in humans have documented an association between the resolution of acute infection and the breadth and magnitude of CD4 and CD8 T cell responses directed against HCV (K. M. Chang 2001; Cramp 1999; Cucchiarini 2000; Day 2002; Day 2003; Diepolder 1995; Grüner 2000; Lancaster 2002; Lechmann 1996; Lechner 2000; Missale 1996; Pape 1999; Rosen 2002; K. Sugimoto 2003a; K. Sugimoto 2003b; Thimme 2001; Wertheimer 2003; Woollard 2003). One report underscores the importance of maintaining a strong CD4 T cell response directed against HCV during acute infection. Six acutely-infected patients who initially mounted a strong HCV-specific CD4 T cell response appeared to clear HCV, only to experience a recurrence of viremia when their CD4 T cell responses to HCV were lost (Gerlach 1999; see also Thimme 2001).

Studies in transplant recipients provide further support for the importance of HCV-specific T cell responses in the outcome of acute infection. A case study of an individual infected with HCV during a kidney transplant found that viral clearance only occurred when immunosuppressive drugs (used to prevent transplant rejection) were discontinued, which restored cell-mediated immune responses (Somsouk 2003). Differences in immune status and the quality of the immune

response to HCV can influence viral persistence. Various host factors that govern the nature and degree of particular immune responses to HCV may largely determine the outcome of acute infection, and general declines in immune function corresponding to age may contribute to the decreased rate of viral clearance associated with older age at time of infection (Isaguliants 2003; Pawelec 2002).

Adaptive immunity

Adaptive immune responses play a major role in the defense against pathogens (foreign invaders such as viruses and bacteria that can cause disease). The adaptive immune response, in which the immune system adapts its responses to a particular threat (in this case, the hepatitis C virus), involves T cells and B cells (white blood cells, also called lymphocytes). These white blood cells can specifically recognize and target a particular pathogen. The adaptive immune response has two arms: cell-mediated immunity and humoral immunity.

Cell-mediated immunity targets intracellular pathogens (i.e., virus that has already entered and infected cells). In cell-mediated immune responses, T cells (specifically CD4 and CD8 T cells) collaborate to kill virus-infected cells. A subset of T cells can recognize components of HCV and mount an immune response specific to the virus; these cells are thus called HCV-specific T cells. HCV-specific CD4 T cells coordinate the cell-mediated immune response and signal to CD8 T cells. HCV-specific CD8 T cells then recognize cells that have been infected with HCV and kill them, preventing further viral replication. The subset of CD8 T cells responsible for destroying infected cells is called cytotoxic T lymphocytes (CTLs).

Humoral immunity guards against extracellular pathogens, such as viral particles circulating in the blood. Humoral immunity involves antibodies produced by B cells. B cells generate antibodies specific to a particular pathogen. Antibodies against HCV bind to sections of the virus, particularly exposed sections on the HCV envelope. Antibody binding aims to neutralize HCV and target virions for destruction. HCV-specific CD4 T cells also direct humoral immunity and promote antibody production from B cells.

Depending on the nature of the threat (circulating pathogens vs. infected cells) the CD4 T cells can stimulate either B cells or CD8 T cells. Humoral immune responses deploying B cells and antibodies are called T_H2 responses; cell-mediated immune responses focused on CD8 T cells are called T_H1 responses.

T cells and B cells contain receptors on their surfaces capable of binding to various pathogen components (for instance, an HCV protein) called antigens. Antigens are foreign substances, generally proteins, that trigger an immune response. The T cell and B cell receptors recognize specific segments of antigens. These segments are called epitopes. The breadth of an immune response depends on the number of different epitopes recognized by the immune system. Broad responses are more likely to be successful than more narrow responses.

Key Terms

Antibodies: small proteins produced by B cells that can target and neutralize circulating virus.
B cells: antibody-producing cells.
CD4 T cells: helper T cells that coordinate the immune response.
CD8 T cells: T cells that recognize and kill infected cells.
CTLs (cytotoxic T lymphocytes): the subset of CD8 T cells responsible for killing infected cells.
Epitopes: peptides or short sections of a pathogen (for instance, a small slice of a viral protein) recognized by a T cell receptor or antibody.
T_H1 response: synonym for cell-mediated immunity, involving CD8 T cells.
T_H2 response: synonym for humoral immunity, involving B cells and antibodies.

Studies of acute HCV infection in chimpanzees have also linked the viral cleareance to vigorous T cell responses, though one recent report found no clear correlation between viral clearance and cell-mediated immunity in these animals (Cooper 1999; Shata 2002; Thimme 2002; Thomson 2003). Humoral responses, involving the production of antibody to HCV, may also facilitate viral clearance, though studies have largely failed to correlate anti-HCV antibody responses with the outcomes of acute infection (Bassett 1999; Baumert 2000; M. Chen 1999; Cooper 1999; Cramp 1999; Ishii 1998; Lagging 2002; Wodarz 2003; Zein 1999; Zibert 1997). Some researchers have proposed that viral clearance may depend on sustained T_H1 responses to HCV, and that dominant T_H2 responses are associated with viral persistence (Kamal 2001; Sarih 2000; Tsai 1997). While antibody responses may play a secondary role to cell-mediated immunity in the clearance of HCV, the relationship or even interdependence between T_H1 and T_H2 responses may have important functions in the HCV-specific immune response (Antonaci 2001; Bertoletti 2003; Christensen 2003). Of note, some individuals with primary antibody deficiencies (such as hypogammaglobulinemia) have been able to clear acute infection in the absence of a strong antibody response. Nevertheless, chronic HCV infection takes a more aggressive course in this group, suggesting that anti-HCV antibodies may have some role in long-term viral control (Chapel 2001; Razvi 1997).

The association between cell-mediated immunity and viral clearance raises the question of why the immune system fails to control acute HCV infection in most people but succeeds in others. Specifically, what factors determine the effectiveness of the HCV-specific immune response during acute infection? A number of demographic characteristics, including age, sex, and race, have been associated with differences in viral clearance rates, suggesting the importance of host factors in determining the outcome of HCV infection (see Chapter II, Natural History of Hepatitis C). The exact mechanisms through which these differences operate have not been established.

Explanations for disparate outcomes of acute HCV infection fall broadly into three categories:

- Immunogenetics—the genetic variations among individuals that influence how the immune system recognizes and responds to HCV;
- Immune escape—the appearance of viral mutations that allow HCV to elude immune control; and
- Functional impairments—defects in the quality of the immune response to HCV, particularly those caused by viral interference with or subversion of components of the immune system.

Immunogenetics

Variations in genes associated with the immune system may provide a partial answer to differences in the outcomes of HCV infection. Genetic differences influence a range of immunologic parameters, including the levels of expression of various proteins involved in immune responses as well as the breadth and specificity of responses to specific pathogens (Trowsdale 2004). Studies of genetic factors in HCV persistence look for associations between particular genetic variations and viral clearance. If a genetic variation is found more frequently among people who have cleared HCV infection than in people with chronic infection, then that variation may in theory influence immune responses that protect against chronic HCV infection. For example, a recent report compared 100 people with cleared HCV infection to 198 people with chronic HCV infection, and found different patterns of genes involved in humoral immunity (immunoglobulin GM and KM) associated with viral clearance and persistence (Pandey 2004).

Some research has linked susceptibility to HCV infection with a mutation in the gene encoding the CCR5 receptor (Ahlenstiel 2004). CCR5 is a receptor expressed on activated T cells that binds to certain chemokines (a set of proteins involved in cell migration and trafficking). People with the CCR5 Δ32/Δ32 genotype (Δ32 refers to a 32-base pair deletion in the CCR5 gene) have a version of CCR5 that cannot function as a receptor. This genotype has been associated with protection from HIV infection, since HIV uses CCR5 to enter cells (Dean 1996; Huang 1996). Direct interactions between CCR5 and HCV have not been established, and the role of the Δ32/Δ32 genotype in HCV infection is more controversial.

An initial report compared frequency of CCR5 genotypes between a cohort of white hemophiliacs and healthy blood donors. This study found a higher frequency of the CCR5Δ32/Δ32 genotype in people with chronic HCV infection than in those who had never been infected with HCV (Woitas 2002), yet several subsequent studies failed to demonstrate an association between the CCR5Δ32/Δ32 genotype and susceptibility to HCV infection (Glas 2003; Hellier 2003; Promrat 2003a; Promrat 2003b; Wasmuth 2004). Attempts to reconcile these disparate results have noted the high exposure to HIV- and HCV-infected blood among hemophiliacs in the 1980s, resulting in a high rate of HIV-related mortality in this group. Several observers have suggested that the frequency of the Δ32 mutation in the original study may have reflected a degree of protection against HIV among surviving hemophiliacs, rather than increased susceptibility to HCV infection. The debate around CCR5 genotypes illustrates the complexities of studying the contribution of immunogenetics to HCV infection, in particular the challenges of selecting appropriate cohorts for this research (Wasmuth 2004).

A significant source of genetic variation in the immune response among individuals comes from human leukocyte antigen (HLA) genes. HLA genes encode two classes of major histocompatibility complex (MHC) molecules. MHC molecules can bind to viral peptides (fragments of HCV proteins, for example) and present the peptides to T cells. The two classes of MHC molecules, MHC class I and MHC class II, present antigens to CD8 and CD4 T cells, respectively. The T cell receptor of CD4 T cells can recognize only a peptide bound to MHC class II molecules. CD8 T cells recognize peptides bound to MHC class I molecules.

Genetic inheritance determines the particular forms of MHC molecules found in a person. A single individual will be able to express no more than six different class I molecules and up to a dozen distinct class II molecules, depending on the HLA complex he or she inherited. Possible variations of these genes are referred to as HLA alleles. The set of HLA alleles present within an individual defines their HLA type. Because HLA type is a genetic trait based on heredity, the distribution of HLA alleles varies according to racial and ethnic group.

Each MHC molecule can bind to a broad but finite variety of peptides; therefore, the number of HCV epitopes (viral peptides, or segments of virus) presented to T cells—and hence, the breadth of the immune response—depends in part on an individual's specific HLA type. Not all epitopes are equally immunogenic—that is, different epitopes elicit different degrees of immune response. The epitopes that stimulate the strongest immune responses are called immunodominant. Thus, HLA type determines which epitopes are presented to T cells, and influences the hierarchy of immunodominance. Favorable HLA types would present either a wider variety of HCV epitopes (thus generating a broader immune response) or more immunogenic epitopes (eliciting a stronger immune response) (Messaoudi 2002).

A number of studies have explored the association between HLA alleles and the outcome of HCV infection. Several HLA alleles, encoding both MHC class I and class II molecules, have been associated with clearance of HCV, suggesting that they facilitate stronger or broader HCV-specific CD4 and CD8 T cell responses (Isaguliants 2003). Conversely, other HLA alleles are more prevalent in individuals who progress to chronic infection (McKiernan 2000; Thio 2001; Thio 2002; Thursz 1999; Vejbaesya 2000). Certain alleles may be associated with viral clearance in some, but not all, racial and ethnic groups (Thio 2001). Some research has suggested that certain alleles may protect against initial HCV infection, while others may confer susceptibility (Isaguliants 2003).

Favorable HLA types presumably confer protection by enabling stronger and/or broader immune responses to HCV. Yet the array of HLA associations reported in these studies, and conflicting results in some research, make it difficult to assess the contribution to viral clearance of genetic variation in HLA alleles. Ideally, hypotheses about the relative value of a specific HLA type would incorporate knowledge of the HCV epitope(s) that can bind to the corresponding MHC molecule (Day 2003; Ward 2002). Mapping viral epitopes to HLA types would allow analyses of genetic variables to consider the relative role of key epitopes in the hierarchy of immune responses. New techniques may ultimately facilitate epitope-MHC molecule mapping projects (Purcell 2004). A number of studies indicate that CD4 T cell epitopes within the HCV core, NS3, NS4, and NS5 viral proteins may be particularly important in the immune response to HCV (Day 2002; Diepolder 1995; Diepolder 1997; Ferrari 1994; Hoffmann 1995; Lamonaca 1999; Lohr 1996; Penna 2002; Rosen 2002; Wertheimer 2003; Woollard 2003).

The advantages associated with some HLA types may reflect a greater ability to present these CD4 T cell epitopes and stimulate HCV-specific immune responses, though this has not been clearly demonstrated.

The involvement of HLA types in determining the breadth and magnitude of HCV-specific CD8 T cell responses is more difficult to investigate. The wide range of possible CTL epitopes can complicate attempts to identify immunodominant epitopes (Anthony 2002; Cerny 1995; Himoudi 2002; Lauer 2002b; Urbani 2001; Wertheimer 2003; Wong 1998; Wong 2001). Discerning patterns of immunodominance is complicated by the fact that some virus-specific T cells may cross-react to other viruses (Cerny 2002). For example, HCV and influenza A share an epitope recognized by the same T cells (Wedemeyer 2001). Cross-reactivity can therefore shape hierarchies of immunodominant epitopes, potentially influencing the efficacy of the HCV-specific immune response (Brehm 2002; S. K. Kim 2002; Selin 1999). Ultimately, no single viral epitope or HLA type assures viral clearance. While genetic variation in HLA may have a major influence on the likelihood of clearance vs. persistence, it cannot fully account for different outcomes of infection. Other potential explanations must be considered.

Immune escape

HCV, like other viruses including HIV, can evade virus-specific T cell and antibody responses. The quasispecies nature of HCV has profound implications for the adaptive immune response. Viral genetic variation may also be involved in determining the success of the immune response during acute HCV infection. Adaptive immune responses depend on the immune system's recognition of specific HCV epitopes, and the composition of these epitopes reflects genetic sequences in HCV RNA. Viral replication introduces mutations into the HCV genome that can change the respective epitope (i.e., by altering the amino acid sequence in an HCV protein; see Chapter VIII, The Molecular Virology of Hepatitis C).

If an HCV epitope changes too much, it may no longer be recognized by the immune system, in effect escaping immune control. Virions containing such mutations are therefore called escape mutants. These escape mutants are effectively invisible to adaptive immune responses (T cells and antibodies) which target the original epitope. In theory, mutations to regions that border HCV epitopes (called flanking regions) could also generate escape mutants, as has recently been shown in HIV infection (Draenert 2004). However, the immune response to HCV targets multiple epitopes, so that a virus containing an escape mutation would still be susceptible to immune responses targeting other epitopes on different parts of the virus (Pavio 2003b).

Ongoing viral replication virtually guarantees the emergence of escape mutations. Broad immune responses targeting multiple epitopes can offset the impact of individual escape mutations, while strong immune responses will control viral replication and minimize the generation of new escape mutations. Less than optimal immune responses (those that are weaker and narrower) favor the emergence of escape mutations. Without optimal immune responses, certain escape mutants will ultimately have a survival advantage—they can evade at least part of the adaptive immune response. Over time, such mutant strains will come to dominate the viral population, as other strains lacking escape mutations succumb to the immune response. This process is called positive selection, a form of pressure that the immune response exerts on HCV. Not all mutations lead to

immune escape, and some mutations cripple the virus by rendering HCV proteins nonfunctional or reducing the efficiency of viral replication. These deleterious mutations tend not to accumulate, since they confer no survival advantage to HCV. This process is called negative selection, and effectively purges the viral population of weaker strains. The dynamics of positive and negative selection shape the composition of HCV quasispecies (Grenfell 2004).

The evolution of HCV quasispecies is more rapid during the acute phase of infection directly following transmission than in the subsequent chronic phase (Cantaloube 2003). This suggests that immune escape may be a mechanism for viral persistence in HCV infection (Pavio 2003b; Racanelli 2003). Selective pressure favoring escape mutations could, in theory, account for the failure of the majority of people infected with HCV to clear the virus during acute infection. Unfortunately, the hypothesis (that quasispecies evolution is responsible for viral persistence) has been difficult to confirm, in part because of inadequate data. Research on acute HCV infection in humans has been limited, since relatively few cases are diagnosed during that phase, and so the numbers of patients studied are typically small (see Chapter II, Natural History of Hepatitis C); resource constraints also limit the availability of chimpanzees for this research. Nevertheless, substantial evidence links escape mutations to chronic infection, though the causal relationship between the two remains controversial. Mutations arising within the HCV NS3 and E2 proteins have been the focus of particular interest exploring the connection between viral escape and persistence.

A number of studies have explored NS3 epitopes for their immunogenicity (the ability to stimulate an immune response) and their association with viral clearance during acute infection (Brinster 2001; Chiang 1998; Diepolder 1995; Diepolder 1997; Pan 2002; Pape 1999; Shata 2002; Wertheimer 2003; F. Zhu 2002). Since CD4 and CTL responses to NS3 have been detected in significant proportions of individuals with both cleared and chronic HCV infection, these epitopes present an opportunity to characterize the role of escape mutations in HCV immunopathogenesis. Some investigators have studied viral isolates sampled from chronically infected individuals, identifying epitope variants with mutations in the NS3 helicase region suggestive of immune escape (Eckels 1999; H. Wang 1999; H. Wang 2002). These variant epitopes were, at best, only weakly immunogenic, implying that the immune responses in these individuals lost the ability to recognize this region of the HCV NS3 protein. A study of HCV infection in a chimpanzee offers further support for a potential immune escape mechanism. The chimpanzee developed a strong and durable CTL response to an NS3 epitope, but mutant variants of the epitope that emerged four months after infection were unable to elicit an immune response (Weiner 1995).

Antibody-mediated immune responses to HCV have also been studied in the context of immune escape. Because the viral envelope is the most exposed area of circulating virions, epitopes on the envelope proteins capable of inducing antibodies that neutralize circulating virus or prevent the virus from binding to target cells are of particular interest. Much of this research has been directed at the highly variable region 1 (HVR1) of the HCV envelope protein E2. As its name implies, this region shows a very high level of sequence diversity compared to the rest of the HCV genome (Hijikata 1991; N. Kato 1992a; N. Kato 1992b; Ogata 1991; Weiner 1991). The HVR1 region has been shown to contain a number of epitopes, including ones targeted by potentially neutralizing antibody responses.

If HVR1 is subject to positive selection pressure by the humoral immune response, then variants containing HVR1 mutations would be expected to emerge shortly after the development of an antibody response during acute infection. Indeed, some work has indicated that HVR1 sequence heterogeneity evolves most rapidly during acute infection, at which stage positive selective pressure is highest (L. Lu 2001; Yamaguchi 1994). One group studied twelve patients infected from transfusions in the 1980s. This study used stored serum samples taken at different time points during acute infection (including before and after detectable antibody responses to HCV). These researchers found that viral evolution (reflected in HVR1 mutations) occurred at roughly the same time as the emergence of detectable antibody responses. The temporal association suggested that antibody responses drove HVR1 mutations through positive selection. They also noted an association between increased genetic diversity in the HVR1 region and failure to resolve HCV infection (Farci 2000). Similarly, a large retrospective analysis of HVR1 sequence evolution in HCV-infected blood donors and transfusion recipients found an initial period of genetic stability immediately following infection; however, a rapid evolution in HVR1 ensued during the phase of acute infection, coinciding with the induction of an adaptive immune response (H. J. Lin 2001).

Other groups have also produced data supporting a correlation between antibody response and HVR1 sequence heterogeneity. Mondelli and colleagues noted that strong, cross-reactive antibody responses were accompanied by increased diversification of HVR1 sequences over time (Mondelli 1999). Researchers in Sweden followed five individuals infected from a common source for up to three years after they were infected with HCV. The researchers observed that the pattern of mutations in HVR1 sequences compared against changes in antibody reactivity appeared consistent with positive selection pressure leading to immune escape, though other interpretations based on potential impairment of HVR1-specific humoral immune responses could not be ruled out (Hjalmarsson 2001). These findings were echoed in a study of acutely infected chimpanzees, which found that HVR1 sequence evolution corresponds to the development of humoral immune responses (van Doorn 1995); however, a second report by the same group found no obvious relationship between changes in HVR1 and humoral response in three chimpanzees followed during chronic infection (van Doorn 1997).

Indeed, research does not provide unequivocal support for a correlation between HVR1 sequence evolution and humoral immune pressure. If present, this dynamic may not follow a single, predictable course. A study of three acutely infected patients, followed for up to one year, found varying degrees of evidence for selective pressure and no obvious common pattern of viral evolution (Manzin 1998). A recent phylogenetic analysis of two data sets from acute infection studies found that the number of sites in the HCV E1 and E2 envelope proteins under selection pressure corresponded to disease outcomes (Sheridan 2004). Rapid progressors who develop chronic infection showed the greatest number of sites under selective pressure, followed by slow progressors; those who cleared acute infection had a yet lower number of sites, demonstrating the effects of immune selection. Yet no correlation between immune selection and disease outcome could be established when restricting the analysis to the HVR1 sequence alone.

A chimpanzee study tracked the evolution of HVR1 sequences in two animals that were inoculated intrahepatically with infectious HCV clones and followed for five years. Researchers found relatively stable HVR1 sequences despite the presence of antibody responses targeting this region, and did not find evidence that viral persistence depended on HVR1 escape mutations

(Major 1999). A subsequent study of six chimpanzees that cleared HCV infection, two of which had received prior immunization with HVR1 peptides, found no evidence of viral evolution or selection pressure on HVR1 sequences, regardless of the degree of antibody response (Y. H. Zhou 2002b). Other researchers have questioned the extent to which HCV-specific humoral responses in chimpanzees mimic those seen in human HCV infection (Bassett 1999; S. C. Ray 2000).

Substantial evidence ultimately supports an association between escape mutations and viral persistence (K. M. Chang 1997; Erickson 2001; Farci 2000; Weiner 1995). Yet it remains unclear whether this association is the cause or effect of disparate outcomes in acute infection. Does immune escape cause viral persistence, or is immune escape a result of an underlying failure of the immune system to control HCV? This question seems to hinge on the degree of pressure exerted by the immune system on HCV during acute infection. Viral clearance is associated both with a broad T cell response and with relative stability of HCV RNA sequences, as mutations do not become established in the HCV quasispecies during a successful immune response to acute infection. Random mutations inevitably occur during viral replication, but do not accumulate against a background of robust immune responses. This would suggest that escape mutations are a consequence of inadequate immune responses (Grenfell 2004).

HCV quasispecies evolution in immunocompromised individuals provides some context for untangling the relationship between immune response and escape mutations. The effect of HIV coinfection on HCV quasispecies populations is somewhat ambiguous. A majority of studies report less quasispecies complexity and diversity in HCV/HIV-coinfected individuals, particularly those with lower CD4 T cell counts, but diversity tends to increase in people on antiretroviral therapy (Babik 2003; Blackard 2004; Neau 2003; Roque-Alfonso 2002). Other studies have looked at HCV infection in individuals with agammaglobulinemia and hypogammaglobulinemia, conditions that eliminate or profoundly impair the ability to mount antibody responses. In these groups, humoral selective pressure would thus be low or non-existent. In fact, little or no evolution of HVR1 sequences or evidence of positive selection has been detected in HCV-infected agammaglobulinemic and hypogammaglobulinemic individuals (Booth 1998; U. Kumar 1994; Odeberg 1997). These patterns in immunocompromised individuals mirror those at the other end of the immunological spectrum: robust immune responses during acute infection suppress quasispecies evolution, while profoundly weak or impaired immune responses apply no selective pressure and result in relatively static viral populations.

The establishment of escape mutations in a quasispecies population can be viewed as a function of partial but inadequate immune control—just enough immune pressure to provide an advantage to escape mutants, but not enough to fully control the virus. Immune escape surely plays a role in viral persistence, and has important implications for the development of an HCV vaccine (see Chapter X, The Future of HCV Therapy). The direction of HCV quasispecies evolution may also have some correspondence with HLA types, as they both influence epitope recognition. In this regard, certain escape mutations would be associated with particular HLA alleles. This association has not been explored in HCV infection and would require a relatively large study to identify potentially subtle effects, but recent HIV research supports an HLA-dependent model of the generation of escape mutations (Brander 2003; Grenfell 2004; Moore 2002). Nevertheless, escape mutations cannot explain the well-documented functional deficiencies in the HCV-specific immune response (Racanelli 2003). For example, a recent study examining the relationship

between mutations to a common NS3 epitope and immune control in acute infection found a lower CD8 response to that epitope in individuals who became chronically infected than in those who had cleared HCV. The diminished response could not, however, be attributed to escape mutations, suggesting that other factors must influence the degree of the epitope-specific CD8 response (Kantzanou 2003).

Dramatic impairments in the function of HCV-specific CTLs have been observed in newly infected individuals who progress to chronic disease (Gruener 2001; Sobao 2001; Urbani 2002; Wedemeyer 2002). Some evidence suggests that individuals who ultimately clear acute HCV infection also experience these functional defects, but only temporarily; the HCV-specific CTLs in this group somehow recover, restoring their capability to control HCV (Lechner 2000; Urbani 2002). In chronic HCV infection, functional impairments would most likely precede, and perhaps even foster, the emergence of escape mutations. The antibody response to HCV may also drive quasispecies evolution, since it fails to eliminate circulating virus (Hunziker 2003). Since some HCV-antibody epitopes overlap with viral T cell epitopes, antibody-driven escape mutations may also facilitate viral escape from T cell responses directed against these shared epitopes (Frasca 1999; Hwang 1996; Khudyakov 1995; Mondelli 1994; Ou-Yang 1999; Sallberg 1996; Shirai 1999 (see also correction in Shirai 2001); Wodarz 2003; Z. X. Zhang 2000).

There may be other links between quasispecies evolution and HCV-specific immune dysfunction. Some epitope mutations affect immune responses not by escaping detection but by actively inhibiting epitope-specific immune responses. These mutated epitopes can occupy T cell receptors without stimulating a response (Ruppert 1993). Such variants, called T cell receptor (TCR) antagonists, have been identified in HVR1 and NS3 sequences from chronically infected individuals (K. M. Chang 1997; Frasca 1999; Tsai 1998). While these variants block epitope-specific CD4 and CD8 T cell responses *in vitro*, their influence on HCV persistence has not been established; however, TCR antagonism could in theory account for some of the associations between HLA alleles and the outcome of acute HCV infection (Vukmanovi ć 2003).

The question then turns to dynamics of the HCV-specific T cell response: what determines the quality and magnitude of the T cell response to HCV, and how does the immune system overcome functional impairment to ensure viral clearance?

Functional Impairments

HCV-specific CD8 T cells control viral infection by various mechanisms. CTLs can secrete certain proteins called cytokines, which are involved in intracellular signaling and regulation of immune responses. CTLs secrete interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), cytokines that induce an antiviral state in cells. This antiviral state increases cellular resistance to viruses and inhibits viral replication. CTLs are also directly cytotoxic (that is, they kill infected cells) through mechanisms that often involve the release of perforin, a protein that can punch holes in the membranes of targeted cells. CD8 T cells also proliferate during an antiviral response, undergoing several rounds of cell division to expand the population capable of responding to a particular pathogen. All of the following activities are impaired in the context of HCV infection:

- Reduced levels of synthesis of antiviral cytokines IFN-γ and TNF-α (Gruener 2001; Lechner 2000; Thimme 2001; Urbani 2002; Wedemeyer 2002);
- Reduced levels of perforin (Urbani 2002);
- Reduced cytotoxicity (Urbani 2002; Wedemeyer 2002); and
- Reduced proliferative capacity (Urbani 2002; Wedemeyer 2002).

Reduced cytotoxicity would clearly impair the ability of CTLs to control HCV infection. Reductions in IFN-γ synthesis may be equally damaging, given recent evidence that IFN-γ plays a significant role in mechanisms of HCV control independent of cell-killing (Cheney 2002; Frese 2002; C. Liu 2003; A. I. Su 2002; Thimme 2001; Thimme 2002). These deficits are not uncommon in other persistent viral infections, and resemble functional impairment of virus-specific CD8 responses in HIV infection.

In chronic HCV infection, the problems with CD8 T cells reflect a specific rather than global immune dysfunction, since CD8 responses to other viruses do not show comparable impairments in people with HCV (Gruener 2002). Moreover, parameters of the HCV-specific immune response may vary with degree of liver damage (Anthony 2001). Individuals with higher fibrosis scores show decreased levels of IFN-γ production in response to HCV core antigen, though responses to another virus (cytomegalovirus) are unaffected (Watson 2003).

These impairments may be associated with a skewed maturation process (Racanelli 2003; Wherry 2004). Typically, a virus-specific CD8 T cell population begins in a naïve state before its first encounter with virus. When these naïve cells are activated by engagement with a virus, they begin to proliferate, undergoing several rounds of cell division. The new virus-specific CD8 T cells are no longer naïve; some have differentiated into effector cells, the CTLs capable of killing infected cells. Others become memory cells, which are CD8 T cells capable of mounting a stronger, more rapid response to the virus than the original naïve cells. HCV-specific memory CD8 T cells are absolutely essential in resolving HCV infection (Shoukry 2003). These different CD8 T cell subsets can be distinguished by certain cell surface markers.

One study that explored HCV-specific CD8 T cell subset function in acute infection found that the majority of these cells were memory-effector cells, a phenotype sometimes referred to as "pre-terminally differentiated" (Urbani 2002). This subset represents an intermediary stage between memory cells and terminally differentiated effector cells, the CTLs capable of generating the most potent virus-specific response. By analogy with HIV infection, an abnormally high memory-effector subset may reflect an inability to fully mature and a consequent impairment in function (Champagne 2001). The majority of HCV-specific CD8 T cells in chronic infection have also been described as pre-terminally differentiated (Knuchel 2003). However, other studies have found somewhat differing distributions of HCV-specific CD8 T cell subsets during acute and chronic infection, and in some cases have used different cell surface markers to determine phenotype (Appay 2002; Francavilla 2004; X. S. He 1999; M. Murata 2002; Sobao 2001; Wedemeyer 2002). Some evidence also suggests that the HCV core protein may interfere with proper CD8 T cell differentiation (Accapezzato 2004a).

Furthermore, the relationship between CD8 T cell subsets (assigned through surface marker expression) and function (measured by levels of perforin and cytokine secretion and proliferative

capacity) may not always be straightforward (Urbani 2002). Distinguishing relevant subsets of CD8 T cells can be difficult, as surface markers and cell populations display substantial overlap. A subset of "double-positive" T cells that bear both CD4 and CD8 markers also appears to have effector functions and concentrate in HCV-infected livers; in a chimpanzee infected with HCV, decreases in viral load correlated with increases in activated double-positive T cells (Nascimbeni 2004).

To further complicate matters, a recent study found that people with chronic HCV infection had a population of CD8 T cells specific for cytomegalovirus (CMV), a common viral infection, that were skewed towards an "early memory" phenotype. People with chronic HCV infection had lower proportions of mature CMV-specific memory cells, though this distribution did not alter the *in vitro* proliferative capacity of these cells (Lucas 2004). Altogether, these findings suggest that some alteration in HCV-specific CD8 T cell maturation may occur in chronic HCV infection, and may have more generalized effects on CD8 T cell responses.

The study of the nature and function of HCV-specific CD8 T cells is made difficult by their low levels in the blood, potential T cell population differences by compartment (blood vs. liver), and technical issues in CD8 T cell measurement that may distort results (discussed in K. M. Chang 2003; see also Welsh 2001). However, a number of possible explanations may account for CD8 dysfunction and viral persistence. Epitope-specific CD8 T cell populations (or clonal populations—clones of the original naïve CD8 T cells that all share the same epitope specificity) may be exhausted or overwhelmed by high viral loads early in infection through two related scenarios:

- Clonal deletion—the virus-specific CD8 T cell population rapidly expands during acute infection, but fails to control the infection due to high viral loads. The expanded population is composed of short-lived, activated cells that ultimately die out, thus "deleting" the population of epitope-specific cells (Moskophidis 1993).
- Clonal anergy—the virus-specific CD8 T cell population rapidly expands during acute infection, but results in a large population of activated cells that are functionally deficient. This state of anergy, or non-responsiveness to stimulation, is typically associated with inadequate CD4 T cell help (Zajac 1998).

In both scenarios, sustained T cell exhaustion effectively results in a state of immunologic tolerance—the immune system "tolerates" the presence of a pathogen by failing to mount a significant T cell response.

The potential role of clonal deletion in HCV persistence is unclear. Studies of HCV-specific CTLs during acute infection have shown no clear evidence of clonal deletion (Wedemeyer 2002). However, this mechanism cannot be excluded, particularly since its detection may be especially challenging in acute HCV infection for several reasons:

- HCV-specific CTLs concentrate in the liver, the primary site of infection (Grabowska 2001; X. S. He 1999; Thimme 2002).
- The intrahepatic CTL population may differ from that detectable in the blood, containing broader epitope specificities and possibly altered functional patterns and subset distributions (Grabowska 2001; Koziel 1993; Thimme 2002).

- Other immune cells in the liver may contribute to CTL deletion. Experiments in mice show that Kupffer cells (liver-resident macrophages, or scavengers that ingest dead cells and present antigen) contribute to the death of CD8 T celsl entering the liver (Kuniyasu 2004).
- The liver, even in the absence of viral infection, is a site of widespread death of activated CD8 T cells (Crispe 2003).

The effects of clonal deletion occurring in the liver during acute infection may thus be obscured in studies of immune responses in humans that rely exclusively on blood samples. However, clonal deletion cannot fully account for failures in the HCV-specific immune response, since CD8 T cells responding to various epitopes remain detectable throughout the course of infection.

The relative contributions of deletion and anergy to HCV-specific CD8 T cell impairment are unclear, but some insights from other models of viral infection may be relevant. The mechanism of CD8 T cell exhaustion—clonal deletion or clonal anergy—may vary depending on characteristics of particular viral epitopes. The two mechanisms are not mutually exclusive and may even reflect different points on a spectrum of CD8 T cell dysfunction (Fuller 2004; Reignat 2002; Wherry 2003; Wherry 2004; Zajac 1998). CD8 T cells that bind with a high affinity to viral epitopes or encounter particularly large amounts of antigen containing their respective epitopes will be more susceptible to clonal deletion. In turn, the functional capacities of the remaining virus-specific CD8 T cells may be attenuated to various degrees by clonal anergy. The consequent downregulation of CD8 T cell responses through exhaustion results in viral persistence.

This scenario, drawn from studies of other persistent viral infections, resembles the observed dynamics of immune response to HCV in individuals who remain chronically infected. Alternately, another model suggests that slower viral replication rates and the delayed appearance of higher antigen titers could lead to persistent HCV infection. The slow onset of intense viral replication may result in weaker immune responses, allowing HCV to “sneak through” and avoid triggering an effective immune response (Bocharov 2004). While intriguing, this hypothesis has not been confirmed in experimental models or studies of acute infection.

Even functional HCV-specific CD8 T cells may not effectively target virus-infected cells. Some research using HCV replicons has found that levels of expression of MHC class I molecules, which display antigen on HCV-infected cells, is reduced in cells harboring replicons (Tardif 2003). Viral interference with MHC class I expression, perhaps as a consequence of the endoplasmic reticulum stress response triggered by HCV replication, could allow virus-infected cells to evade detection and destruction by HCV-specific CD8 T cells. However, other *in vitro* data has suggested that HCV proteins do not repress MHC class I expression, or alternately that the HCV core protein actually increases MHC class I expression, indicating a need for further research (Herzer 2003; Moradpour 2001).

CD4 T cells and immune dysfunction

Several lines of evidence suggest a role for CD4-mediated clonal anergy in explaining the functional impairment of HCV-specific CD8 T cells. The vital importance of CD4 T cell help for effective CD8 responses was recently demonstrated in a study of two chimpanzees that had

cleared previous HCV infections. After experimental depletion of the chimpanzees' CD4 T cells, the animals were reinfected with HCV. Both chimpanzees failed to resolve HCV infection despite the presence of HCV-specific CD8 T cells, and both developed escape mutations (Grakoui 2003). This study confirms the critical role of HCV-specific memory CD4 T cells in supporting the essential function of HCV-specific memory CD8 T cells (Grakoui 2003; Shoukry 2003).

Weak HCV-specific CD4 T cell responses have been associated with functional impairments in HCV-specific CD8 T cells, while stronger CD4 responses accompany stronger CD8 responses (K. M. Chang 2001; Wedemeyer 2002). More generally, weak HCV-specific CD4 T cell responses are a hallmark of chronic infection, while robust, durable responses have been linked to viral clearance (K. M. Chang 2001; Day 2003; Diepolder 1995; Godkin 2001; Missale 1996; Pape 1999; K. Sugimoto 2003b; Thimme 2002). HIV coinfection is also associated with HCV persistence, further emphasizing the significance of the strength and quality of the CD4 T cell response (S. H. Mehta 2002; Thomas 2000).

Evidence of functional impairments in HCV-specific CD4 T cells is limited; methodological issues make the detection of non-functional populations of virus-specific CD4 T cells difficult. However, defects in proliferative capacity and/or IFN-γ secretion among HCV-specific CD4 T cells, associated with viral persistence, have recently been reported (K. Sugimoto 2003a; Ulsenheimer 2003).

Effective development of virus-specific memory CD8 T cells appears to depend on a vigorous CD4 T cell response (Bourgeois 2002; Janssen 2003; Shedlock 2003; Sun 2003). CD4 T cells can provide help to CD8 T cells in two ways: by secreting particular cytokines, and by stimulating antigen-presenting cells (see 'Dendritic Cells' in the next section). Skewed maturation of HCV-specific CD8 T cells may be a byproduct of deficient IL-2 production from CD4 T cells (Francavilla 2004).

The particular cytokines secreted by CD4 T cells depend on the type of immune response—cell-mediated (T_H1) or humoral (T_H2). The T_H1 subset of CD4 T cells secretes IL-2 (interleukin-2) and IFN-γ, which promote the activation of CD8 T cells. By analogy with findings from HIV research, IL-2 production by CD4 T cells may be especially relevant to functional virus-specific immune responses. IL-2-producing HIV-specific CD4 T cells are necessary for the establishment of long-term HIV-specific memory CD4 T cells, and associated with effective viral control, while CD4 T cells that only secrete IFN-γ are typically short-lived and correspond to high HIV viral loads (Boaz 2002; Harari 2004; Palmer 2004; Younes 2003). In these models, persistently high levels of HIV skew the immune response away from IL-2-secreting CD4 T cells, thus impairing the establishment of an HIV-specific memory CD4 T cell population that can support functional HIV-specific CD8 T cell responses. These findings suggest new directions for understanding effective HCV-specific CD4 T cell responses based on cytokine secretion profiles.

The T_H2 subset secretes IL-4 (interleukin-4) and IL-10 (interleukin-10), cytokines that can indirectly inhibit the proliferation of CD8 T cells. Some research has suggested that a T_H1/T_H2 imbalance may be involved in viral persistence, though most studies indicate that even in chronic infection, the CD4 T cell response is overwhelmingly T_H1 (Bergamini 2001a; Bertoletti 1997; Kawakami 2000; Penna 2002; Rico 2002; Tsai 1997). However, different epitopes may stimulate

different types of helper responses (Eckels 1999; Woitas 1997).

A different subset of CD4 T cells, described as regulatory T cells, may also be involved in the downregulation of HCV-specific cell-mediated immune responses. One subset—T_R1 CD4 T cells—secretes both IFN-γ and IL-10, but not IL-4. HCV core protein-specific CD4 T cells with a T_R1 profile were found in blood samples from individuals chronically infected with HCV (A. J. MacDonald 2002). Furthermore, IL-10 levels circulating in blood were higher in samples from individuals with chronic infection than in those who had cleared HCV during acute infection.

Another study of 24 individuals infected with HCV, half of whom resolved infection, found that those with chronic infection had a higher frequency of regulatory T cells (identified as cells expressing both CD4 and CD25 markers), which were capable of suppressing HCV-specific CD8 T cell responses *in vitro* (K. Sugimoto 2003b). Some evidence consistent with the presence of regulatory T cells as well as T_H0 cells (secreting IL-4 and IFN-γ) has also been noted in other studies (Godkin 2001; Penna 2002; Ulsenheimer 2003; Woitas 1997). A recent report also described a population of IL-10-secreting regulatory HCV-specific CD8 T cells isolated from the livers of people with chronic HCV; these regulatory CD8 T cells were capable of suppressing CTL function *in vitro* (Accapezzato 2004b).

The significance of these findings is unclear, but may relate to immunological features of the liver that tend to promote tolerance in T cells rather than inducing effective cell-mediated responses (Crispe 2003). The liver regulates tolerance induction by several methods, including IL-10 secretion and incomplete activation of T cells through antigen presentation by the liver sinusoidal endothelial cells (LSECs) that form a loose boundary between hepatocytes and blood circulating through the liver. Thus T cells activated in the liver may be more prone to tolerance than T cells primed in other parts of the body. Again, the relevance of this phenomenon to HCV infection is unclear. However, one study examining cells from people chronically infected with HCV found that the HCV NS4 protein stimulated IL-10 secretion, inhibiting dendritic cell maturation and suppressing T_H1-type responses (Brady 2003).

Explaining the mechanisms of T cell dysfunction in HCV disease may ultimately require an exploration of the events preceding the priming and activation of HCV-specific T cells. Acute HCV (and hepatitis B virus) infection is distinguished by a delay in the onset of detectable adaptive immune responses; HCV-specific T cells do not appear until several weeks after infection, and antibodies to HCV become detectable shortly thereafter (Thimme 2001; Thimme 2002). In the case of HCV, this delayed appearance of cell-mediated immune responses coincides with signs of liver damage, as measured by alanine aminotransferase levels (ALTs), suggestive of the killing of infected cells by HCV-specific CTLs (see Chapter IV, Diagnostics). Yet the induction of an HCV-specific T cell response lags behind an early and substantial rise in the levels of HCV RNA detectable in the blood beginning shortly after infection. This suggests that HCV manages to subvert the innate immune response—the first line of defense against pathogens.

Innate immune response to HCV

Innate immune responses include cellular defenses that are automatically activated when a cell is infected, as well as special cells that respond to certain motifs common to many pathogens. Since

innate responses do not rely on specific recognition of a pathogen, they appear almost immediately after infection. Innate responses typically do not clear viral infections on their own, but attempt to contain them until the adaptive immune response kicks in. These early responses also establish conditions that influence the course of the subsequent adaptive immune responses. The overall immune response can be viewed as a chain reaction: a series of linked and interdependent processes initiated by innate responses and culminating in adaptive responses. Disrupting early events in this response—through viral interference with components of innate immunity, for example—can have severe repercussions on the efficacy of later events in the immune response and the outcome of acute infection.

Type I Interferons

In the case of HCV, the innate response begins with the synthesis by infected cells of type I interferons—the antiviral cytokines IFN-α (interferon alpha) and IFN-β (interferon beta). Signs of type I interferon synthesis were seen as early as two days into acute HCV infection in an experimentally infected chimpanzee (Bigger 2001). IFN-α and IFN-β synthesis is triggered by cellular signaling pathways that respond to the presence of double-stranded RNA (dsRNA). The formation of double-stranded RNA typically occurs during the replication of RNA viruses (see Chapter VIII, The Molecular Virology of Hepatitis C). An infected cell therefore recognizes the presence of dsRNA as a sign of viral replication. Cells containing dsRNA immediately begin to express the antiviral cytokines IFN-α (interferon alpha) and IFN-β (interferon beta). These type I interferons induce an antiviral state in adjacent cells; if these neighboring cells become infected, they stimulate a broad range of genes that encode various proteins and enzymes that inhibit viral replication. Defects in the ability to respond to type I interferons during an acute viral infection have been associated with functional defects in CD8 T cell responses (Ou 2001).

Several interferon-stimulated gene products have been studied in the context of cellular inhibition of HCV replication:

- PKR (double-stranded RNA-dependent protein kinase R) inactivates eukaryotic initiation factor 2 (eIF2, involved in viral and cellular translation initiation), shutting down protein synthesis in the cell in order to disrupt the viral replication cycle.
- (2′,5′)-oligoadenylate synthetase (2′,5′-OAS) activates RNase L, which degrades viral RNA.
- MxA is a guanine triphosphatase (GTPase) protein that can inhibit viral replication.
- p56 is a protein that binds to eukaryotic initiation factor 3 (eIF3, involved in viral and cellular translation initiation) and inhibits protein synthesis.

In addition to their antiviral effects, IFN-α and IFN-β also increase the expression of MHC class I proteins on the cell surface, increasing the likelihood that infected cells will be detected and killed by CTLs. Type I interferons also influence the activity of other cells involved in the innate immune response. Individual genetic variations in PKR, MxA, and 2′,5′-OAS have been associated with greater likelihood of clearing acute HCV infection (Knapp 2003).

HCV appears capable of inhibiting the antiviral effects of type I interferons through several mechanisms observed through *in vitro* studies. The HCV NS3 serine protease can block the activation of interferon regulatory factor-3 (IRF-3), a protein critical to the synthesis of type I interferons and interferon-stimulated genes (Foy 2003). HCV proteins may also directly or indirectly disrupt the cellular signaling pathway used by IFN-α to stimulate a cascade of antiviral gene expression (Blindenbacher 2003; Duong 2004; Heim 1999). The HCV core protein may be involved in this disruption, though the effects of the core protein may depend on the cellular environment and require clarification (Basu 2001; Bode 2003; Hosui 2003). HCV envelope proteins E1 and E2 may also impede IFN-α activity, at least *in vitro* (Keskinen 2002). HCV NS5A has also been implicated in the inhibition of type I interferon responses through various proposed mechanisms (Aizaki 2000; Geiss 2003; Reyes 2002; J. Song 1999). The NS5A protein appears to induce the synthesis of interleukin-8, a cytokine reported to inhibit the antiviral effects of IFN-α (Girard 2002; Polyak 2001a; Polyak 2001b). However, studies on whether chronic HCV infection decreases IFN-α-mediated gene expression have yielded conflicting results (Abbate 2003; Castelruiz 1999; MacQuillan 2002; MacQuillan 2003; Yu 2000).

The viral proteins E2 and NS5A have each been proposed to inhibit the activation of PKR, preventing it from blocking protein synthesis (Gale 1998; Gale 1999; Y. He 2001; Taylor 1999; C. Wang 2003). The actual involvement of E2 and/or NS5A in regulating PKR activation in HCV infection—and the significance of PKR inhibition for viral replication—remains controversial. NS5A in particular may disrupt the antiviral effects of IFN-α through other pathways independent of PKR itself or of its effects on protein synthesis (François 2000; Geiss 2003; Koev 2002; Pflugheber 2002; Podevin 2001; Tan 2001; Taylor 2001b). It has recently been suggested that the HCV internal ribosomal entry site (IRES) binds to and inhibits PKR, while the HCV core protein has been reported to activate PKR (Delhem 2001; Vyas 2003). Despite this confusion about the mechanism of PKR inhibition, HCV does seem resistant to at least some of the antiviral effects of PKR (Koev 2002; Rivas-Estilla 2002; C. Wang 2003).

Natural Killer Cells

Natural killer cells (NK cells) are part of the early innate response to viral infection. NK cells kill virus-infected cells and secrete IFN-γ. The secretion of IFN-γ is particularly important for recruiting T cells to the site of infection and promoting a T_H1 response. NK cells may also have further roles in mediating the regulation of activated CD4 and CD8 T cells in the liver; these roles are currently under investigation (Rabinovich 2003). The cytotoxic capability of NK cells is stimulated by IFN-α and IFN-β. Some research has documented impairment in the cell-killing ability of NK cells in HCV infection, though conflicting evidence exists (Corado 1997; Duesberg 2001; Pár 2002).

Several factors may contribute to an impairment of NK cells. The HCV envelope protein E2, bound to CD81 receptors on NK cells, effectively shuts down NK cell functions. For reasons that are not clear, E2-CD81 binding on T cells has the opposite effect, and lowers the requirements for stimulating cell activation and proliferation (Crotta 2002; Tseng 2002; Wack 2001). The HCV core protein may also indirectly allow infected cells to avoid bing killed by NK cells. The HCV core protein may increase levels of MHC class I molecule levels on infected cells, sending signals that inhibit NK cell activity (Herzer 2003). A particular HLA type, associated with a decreased chance of HCV clearance, encodes a molecule that sends an inhibitory signal to NK cells (Thio 2002). This

suggests an association between proper NK function and a successful immune response during acute HCV infection.

Finally, dendritic cells and NK cells engage in a reciprocal, regulated pattern of priming and activation, with dendritic cells activating NK cells and enhancing their functions (Moretta 2002). However, dendritic cells from individuals with chronic HCV show defects in their ability to activate NK cells in response to IFN-α stimulation (Jinushi 2003a; Jinushi 2003b). Impaired NK cell activation may also result from deficient levels of IL-15 in people with chronic HCV. IL-15 is produced by dendritic cells in response to IFN-α and is involved in dendritic cell maturation (Jinushi 2003b).

Dendritic Cells

Dendritic cells (DCs) bridge the innate and adaptive immune responses. Dendritic cells are antigen-presenting cells; they capture antigens for presentation to CD4 T cells. Immature DCs scout the body for invading pathogens, such as HCV. When DCs encounter a pathogen, they internalize it in order to process or break apart the pathogen. Dendritic cells undergo maturation during this process. Mature DCs can present epitopes from the pathogen, in the form of a peptide bound to an MHC class II molecule, to CD4 T cells. Successful antigen presentation by mature DCs stimulates, or primes, the CD4 T cells. Mature dendritic cells can also be stimulated during antigen presentation; in turn, mature DCs can prime CTLs in a process dependent on IFN-α and/or IFN-β (Le Bon 2003). The extent and effectiveness of CTL and CD4 T cell priming by DCs depends on the maturation state of dendritic cells. IFN-α and interactions between dendritic cells and NK cells both contribute to DC maturation and promotion of T_H1 responses (Mailliard 2003).

Several groups have reported that dendritic cells extracted from individuals with chronic HCV infection fail to mature *in vitro* and do not effectively prime T cells, though different experimental models have yielded conflicting results (Auffermann-Gretzinger 2001; Bain 2001; Goutagny 2004; Kanto 1999; Longman 2004). Groups citing impaired DC maturation note that individuals who cleared HCV during acute infection or underwent successful HCV treatment showed no impairment in maturation or ability to stimulate T cells (Auffermann-Gretzinger 2001; Bain 2001). Recent research has also identified distinct patterns of selective dendritic cell impairment in people with HCV and HIV, differing by DC subset (myeloid-derived DCs and plasmacytoid-derived DCs) (Anthony 2004). However, a study of DC function in HCV-infected chimpanzees found no correlation between viral persistence and DC impairment; functional defects were seen only in the two chimpanzees with the highest HCV viral loads (Rollier 2003). A more recent report similarly found no signs of DC impairment in chimpanzees infected with HCV (Larsson 2004).

Exposure to the HCV core and NS3 proteins *in vitro* inhibits DC maturation and stimulatory capacity (Dolganiuc 2003). One group genetically engineered DCs to express the HCV core and E1 proteins in order to explore the effects of these viral proteins on dendritic cell function. The engineered DCs displayed impairment in their ability to prime CD4 T cells, further supporting the hypothesis that HCV interferes with antigen presentation and T cell priming (Sarobe 2002).

Notably, the resulting incompletely primed CD4 T cells demonstrated functional impairments similar to those seen in the *in vitro* CD4 T cell response to HCV core protein in chronically infected individuals. This impairment contrasts with normal functioning of CD4 T cell responses

to other (non-HCV) antigens seen in people with chronic HCV infection, indicating that HCV-mediated interference in T cell priming has HCV-specific effects rather than a global deficit in DC function. The impairment may be reversible, and depend on the on-going presence of HCV, since successful HCV treatment restores proper CD4 T cell responses to HCV core proteins. These results were confirmed in a follow-up study of mice immunized with immature DCs engineered to express HCV core and E1. The immunized mice displayed deficiencies in DC maturation and poorer CD4 and CD8 T cell responses, in contrast to mice immunized with mature DCs expressing HCV core and E1 or HCV NS3 (Sarobe 2003).

The actual mechanism of impaired maturation is not certain, though some speculation has focused on the possibility of direct HCV infection of dendritic cells (Bain 2001; Goutagny 2003; Laporte 2003; Navas 2002). Alternately, it has recently been suggested that inhibition may arise from HCV E2 binding to the DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin) molecules expressed on dendritic cells (see Chapter VIII, The Molecular Virology of Hepatitis C) (Pöhlmann 2003; van Kooyk 2003). The environment where dendritic cells acquire antigen can also inhibit the maturation environment. In the context of HCV infection, IFN-α and NK cells may not provide adequate signals to stimulate DC maturation (Bertoletti 2003). Indeed, the environment of the liver itself may require a higher threshold of such signaling for DCs to reach full maturation (Crispe 2003).

No evidence is available yet to support these hypotheses on the cause(s) of potential DC impairment in HCV infection. Regardless of the mechanism of impairment, defects in dendritic cell maturation would have clear implications for the priming of HCV-specific CD4 T cells and CTLs, and may contribute to functional defects in cell-mediated immunity and viral persistence.

Summary of Immune Responses and Future Research Directions

The immunologic determinants of viral clearance—the breadth and magnitude of the HCV-specific CD4 and CD8 T cell response—have been relatively well established, but the reasons for the immune system's frequent failure to control infections that become chronic remain obscure. The majority of the research relating to the immune response in acute infection has examined the contributions of HLA types, immune escape through quasispecies evolution, and functional impairments in components of the immune system and their relation to viral and environmental factors. These broad categories do not exhaust the inventory of possible mechanisms for viral persistence. The influence of genetic factors and potential viral interference with antigen processing and presentation by infected cells, as well as perturbation of T cell activation, proliferation, and function by the HCV core protein, have also been proposed (Asti 1999; Bergqvist 2001; Bergqvist 2003; Herzer 2003; Konan 2003; Soguero 2002; Z. Q. Yao 2001; Z. Q. Yao 2004). Little research has focused on the possible involvement in acute HCV infection of other components of the innate immune system that play important roles in liver immunology. NK T cells (which have properties of both NK cells and T cells), $\gamma\delta$ T cells (a variant of traditional T cells that function similarly to NK cells), and Kupffer cells (a type of macrophage specific to the liver) may all be relevant to the outcomes of HCV infection (Agrati 2001a; Burgio 1998; Deignan 2002; Doherty 1999; Exley 2002; Lucas 2003; Tseng 2001). Yet an inventory of potential immunologic mechanisms for HCV persistence does not explain the relative contribution of these factors or prioritize immunological targets for therapeutic intervention.

A number of factors have frustrated efforts to clarify the immune response to HCV during acute infection:

- Acute HCV infection is rarely identified and often clinically silent. Moreover, acute infection may not be diagnosed until several weeks into the course of infection, making it difficult to track the innate response (Gordon 2003).
- The immune response to HCV is concentrated in the liver, but liver biopsies are not clinically indicated during acute infection. Information about the early intrahepatic immune response to HCV comes from chimpanzee studies; human data has relied on blood samples.
- Chimpanzees are the sole animal model for HCV infection, but due to expense and scarcity, only a small number of chimpanzees have been studied. Chimpanzees may be more likely to successfully clear HCV than humans, for reasons that are unclear (Bassett 1998). Furthermore, hepatic inflammation is generally absent in chimpanzees during chronic liver disease, suggesting differences in pathogenesis and immune response. Experimental conditions used to infect chimpanzees through direct intrahepatic innoculation may further limit the direct applicability of chimpanzee studies to HCV infection in humans (Diepolder 2003).
- Current laboratory methods for studying T cell populations and function have important limitations that can bias results and require careful interpretation. In particular, techniques for the quantitative and qualitative analysis of epitope-specific CD4 T cells lag behind those available for CD8 T cells. Also, clear distinctions and means of identifying various T cell subsets (for instance, effector, memory, terminally differentiated) are still evolving and subject to debate.

Nevertheless, research into the immune response to HCV has made tremendous progress in the last few years. Recent studies of the immune responses to acute HCV infection in chimpanzees have previously cleared HCV provide insights into the correlates of lasting immunity to HCV. Prior resolved HCV infection does not invariably block new infections, which would require the maintenance of an effective, sterilizing antibody response to HCV. Rather, successful protection against HCV re-infection involves early and sustained vigorous virus-specific T cell responses in the liver and blood capable of exerting more rapid control over HCV viremia (Bassett 2001; Nascimbeni 2003; Shoukry 2003; Shoukry 2004).

These data tend to confirm observations of successful immune responses to acute HCV infection, emphasizing the importance of the quality, breadth, magnitude, and durability of HCV-specific T cell responses. Yet recent studies have also challenged and complicated this model:

- **Relevance of cell-mediated immune responses:** A study of chimpanzees infected with the same genotype 1b strain of HCV found no obvious immunologic differences between two chimpanzees that cleared HCV infection and two that developed persistent infection, as measured by HCV-specific CD4 and CD8 T cell responses in the blood and intrahepatic cytokine levels (Thomson 2003).

- **Two stages of T cell response:** Some evidence supports a two-stage model of HCV-specific T cell responses during acute infection, with clearance requiring both induction and maintenance. Induction of T cell responses corresponds to early control of viremia, but maintenance of these responses is required to resolve infection. Diminished or impaired responses during the maintenance stage—particularly HCV-specific CD4 T cell responses—results in viral persistence (Gerlach 1999; Thimme 2001; Thimme 2002). Similarly, some studies have noted qualitative shifts in the HCV-specific CD8 T cell, which may influence the likelihood of viral clearance (Lechner 2000; Thimme 2002). A recent report identified a first and second wave of HCV-specific CD4 T cell responses in a chimpanzee, with the first wave corresponding temporally with viral clearance and the second wave targeting different epitopes at low frequencies (Shoukry 2004).

- **Blood versus liver:** Studies of acute infection in humans have correlated viral clearance with the quality and quantity of HCV-specific T cell responses in peripheral blood. In contrast, a chimpanzee study found that intrahepatic T cell responses, but not peripheral blood responses, correlated with viral control (Thimme 2002).

- **Multiple pathways to clearance and persistence:** Some of the inconsistencies across studies indicate that mechanisms for viral persistence may vary between individuals (Thimme 2001). Clearance of acute infection may similarly follow divergent patterns among groups. African-Americans are less likely to clear acute HCV infection than Whites (Howell 2000). Some variation could be attributable to HLA differences, but other host, viral, and environmental factors may be involved (Thio 2001). A recent report described differences in epitope-specific immune responses between African-Americans and Whites who cleared acute HCV infection. Both groups had similar CD4 T cell responses to HCV non-structural protein antigens that correlated with resolved infection. Yet responses to core protein were only associated with viral clearance in Whites, and not African-Americans (K. Sugimoto 2003a). Immunologic differences may also influence the course of chronic HCV infection, which may be milder overall in African-Americans than Whites and characterized by slower fibrosis progression (Wiley 2002). The recent study found a significantly higher frequency of HCV-specific CD4 T cell responses among African-Americans, but with functional alterations: these HCV-specific CD4 T cells could proliferate, but failed to secrete IFN-γ (K. Sugimoto 2003a).

Some recent reports also call into question the traditional understanding of patterns of acute HCV infection and viral clearance. A Canadian study examined 16 people with resolved HCV infection—five who cleared acute infection, and eleven who achieved sustained virological responses to interferon-based treatment. During up to five years of follow-up, each person maintained undetectable HCV viral loads with no clinical signs of hepatitis. Yet ultrasensitive tests revealed the presence of HCV RNA in all 16 people, with the majority showing signs of active viral replication (detected through the presence of negative strand HCV RNA—see Chapter VIII, The Molecular Virology of Hepatitis C). HCV RNA was found in serum, in peripheral blood mononuclear cells (PBMCs, types of white blood cells that include lymphocytes), and dendritic cells; indicators of viral replication were seen in PBMCs (Pham 2004). The clinical significance of this data is unclear,

but this study challenges assumptions that resolved infections necessarily result in the complete eradication of HCV from the body.

Similar methods led to the detection of HCV RNA in liver biopsy samples from 57 people with signs of liver disease (abnormal liver enzyme levels—see Chapter IV, Diagnostics) despite undetectable HCV viral loads on standard assays and negative results for HCV antibody testing (Castillo 2004). The majority of these biopsy samples (48/57, or 84.2%) also revealed negative strand HCV RNA indicative of active viral replication, and in most cases (40/57, or 70%) HCV RNA was detectable in PBMCs. Based on biopsy results, 35% (20/57) showed signs of liver inflammation, and 17.5% (10/57) had some degree of fibrosis. These cases were characterized as "occult HCV infection" by analogy with a similar, more well established pattern seen with occult hepatitis B infection (Blendis 2003). Due to inherent limitations of RNA detection techniques, both research teams used a rigorous set of controls and measures to rule out contamination, lending credibility to their results, though independent confirmation by other studies is warranted (Lerat 2004).

Other recent reports describe atypical courses of acute infection. A group of four acutely infected Australian prisoners developed HCV-specific T cell responses during viremia, but never converted to HCV antibody seropositivity on standard assays (Post 2004). In another study, researchers identified 25 people with documented exposures to HCV (primarily in health care settings) that did not result in viremic infection or HCV antibody seroconversion. Yet 15 showed significant immune responses targeting HCV; in the majority of cases, increases in HCV-specific T cell responses faded within three months. Notably, about half of the individuals had strong immune responses to HCV detectable at the time of exposure, suggesting a prior exposure resulting in immunologic memory (Heller 2003).

This phenomenon—induction of HCV-specific immune responses without detectable viremia or antibody seroconversion—is reminiscent of the immune responses to HIV found in people described as "highly exposed, persistently seronegative" or "exposed, uninfected" due to the lack of HIV viremia and antibodies to HIV (Kaul 2001; Makedonas 2002; Promadej 2004). A similar pattern has recently been described in HCV antibody negative spouses of people with acute HCV infection; these spouses have HCV-specific CD4 T cell responses, despite undetectable HCV viral loads (Al Tawil 2003). In chimpanzee studies of acute HCV infection, animals exposed to very low levels of HCV also develop HCV-specific T cell responses even in the absence of detectable viremia or antibody seroconversion (Shata 2003). These findings suggest that exposure to low levels of antigen stimulation (relatively small amounts of HCV) can promote effective HCV-specific T cell responses that prevent the emergence of uncontrolled viremia. This area requires further research, validation in other groups, and longer follow-up, but the data collectively provide encouragement for the prospects of a vaccine against HCV (see Chapter X, The Future of HCV Therapy).

Progress in related fields (e.g., hepatitis B, immune tolerance, and chemokine networks) will no doubt inform future studies of the immune response to HCV. The study of the hepatitis B virus (HBV)—a hepatotropic virus cleared by the majority of individuals during acute infection—has generated provocative findings on the role of NK T cells, IFN-γ, and IL-18 (interleukin-18) in viral clearance (Baron 2002; Kakimi 2000; Kakimi 2001; Kimura 2002a; Kimura 2002b; Thimme 2003). Similarly, a growing body of research describes mechanisms of tolerance induction in the

liver that silence immune responses to potential antigens (Crispe 2003; Klugewitz 2002; A. H. Lau 2003a; A. H. Lau 2003b; Lian 2003; Trobonjaca 2001; Trobonjaca 2002). Recent reports have also clarified the role of specific chemokines and chemokine receptors in the recruitment of T cells to the liver and in hepatic inflammation (Ajuebor 2003; Apolinario 2002; Bonacchi 2003; Harvey 2003; Lalor 2002; Lichterfeld 2002; Meng Soo 2002; Mihm 2003; J. W. Park 2001; Shields 1999; Terada 2003). These areas of inquiry provide a basis for many hypotheses worth testing in HCV infection.

The general mechanisms of viral persistence have been the focus of intense research, with implications not only for chronic HCV infection but also for HBV and HIV. Recent research suggests that these viruses may establish persistent infection through a combination of high viral loads and impaired immune responses. In a mouse model of LCMV (lymphocytic choriomeningitis virus) infection, when LCMV-specific CD8 T cells fail to clear acute infection, they undergo a series of functional impairments. The LCMV-specific CD8 T cell population sequentially loses the capacity to kill target cells and produce IL-2, TNF-α, and INF-γ in the face of high viral loads; IL-2 production was also impaired in virus-specific CD4 T cells (Fuller 2003; Wherry 2003). This progressive loss of CD8 function correlates with viral load, and coincides with shifted patterns of immunodominance through the deletion of clonal populations presented with higher levels of epitopes (van der Most 2003; Wherry 2003).

In HCV, as in LCMV, failure to clear acute viral infection is associated with both high viral loads and functional impairments to the virus-specific immune response. The LCMV model bears particular relevance to HCV infection, since LCMV can infect the liver but organ damage results from the immune response rather than viral cytopathicity. As discussed in the next section, much if not all of the liver damage associated with HCV infection derives from the immune response, rather than the virus itself. This suggests a link between viral persistence and pathogenesis; when confronted by high levels of a virus that does not kill infected cells, an overwhelmed immune system may effectively shut down its response through immune exhaustion, rather than maintain a level of activity that threatens the infected organ.

Pathogenesis of Hepatitis C

Chronic HCV is associated with progressive liver disease, marked by fibrosis and cirrhosis (mild-to-moderate and severe scarring of the liver), steatosis (fatty liver), and hepatocellular carcinoma (liver cancer). However, chronically infected individuals experience different degrees of liver damage that proceed at different rates. Steatosis is common but not ubiquitous, cirrhosis is slow to develop and not inevitable and . Hepatocellular carcinoma (HCC) occurs relatively rarely, late in the course of chronic infection, and only in people who have already developed cirrhosis. Fibrosis itself may proceed slowly or not at all in a substantial proportion of cases (see Chapter II, Natural History of Hepatitis C). As with viral persistence, HCV pathogenesis poses two major questions: what are the mechanisms that cause liver damage in chronic HCV infection, and why do they operate differently in different individuals and groups? Tentative answers to these question focus on genetic factors, viral diversity, and most importantly, direct and indirect effects of the virus, the immune response, and the interactions between the two.

Fibrogenesis

Liver damage in chronic HCV disease primarily takes the form of fibrosis. Fibrosis refers to the accumulation of scar tissue (fibrous material composed of matrix proteins such as collagens) in the extracellular matrix, the area surrounding hepatocytes. Extensive fibrosis, or scarring of the liver, that changes the architecture of the liver and severely impairs liver function is called cirrhosis (see Chapter II, Natural History of Hepatitis C). Fibrogenesis—the development of fibrosis—is a general process, not specific to HCV, and results from liver damage. Through fibrosis, the liver seals off injured areas to prevent broader damage caused by inflammation, depositing fibrous material between hepatocytes and circulating blood. As a result, hepatocytes lose access to the flow of blood that carries nutrients (including oxygen) as well as substances requiring metabolism by the liver. Hepatocytes starved of oxygen and nutrients will die, leading to further fibrosis.

Fibrosis can be reversed under some circumstances. The increased deposition of matrix proteins that occurs in fibrogenesis can be counteracted by matrix metalloproteinases (MMPs), a group of proteases that break down matrix proteins. MMP activity can in turn be blocked by other molecules called tissue inhibitors of metalloproteinases (TIMPs). Members of the MMP and TIMP families are distinguished by number (e.g., MMP-1, MMP-2; likewise, TIMP-1, TIMP-2.). The balance between MMPs and TIMPs therefore influences the outcome of fibrogenesis (Lichtinghagen 2003).

Fibrogenesis requires the activation of hepatic stellate cells, vitamin A-storing cells that synthesize collagen and other extracellular matrix proteins in response to liver injury. Damaged hepatocytes activate hepatic stellate cells, as do liver sinusoidal endothelial cells (LSECs). Immune responses can also mediate fibrogenesis. Activated Kupffer cells can activate hepatic stellate cells through release of a cytokine, TGF-β (transforming growth factor-beta). TGF-β plays a major role in fibrogenesis and can also suppress immune responses. Neutrophils (infection-fighting white blood cells recruited to the liver by inflammatory cytokines) can also stimulate fibrogenesis through stellate cells. T cells may also modulate fibrogenesis through patterns of cytokine release. T_H2 responses tend to promote fibrosis to a greater degree than T_H1 responses in a mouse model of liver injury. IFN-γ appears protective against fibrosis, and can inhibit TGF-β activity.

The mechanics of fibrogenesis in HCV infection parallel those seen in other forms of liver disease. Markers of stellate cell activation correlate with the extent of fibrosis in individuals with chronic HCV infection, and successful anti-HCV treatment is associated with a reduction in stellate cell activation (Clouston 2001; Guido 1996; M. T. Levy 2002; Paradis 1999; Sakaida 1999). HCV infection is associated with higher levels of TGF-β both *in vivo* and *in vitro*, and TGF-β levels may correlate with fibrosis progression in chronic HCV (Kanzler 2001; Nelson 1997; Neuman 2002b; S. Ray 2003; Roulot 1995). HCV-related cirrhosis augments the expression in the liver of genes associated with inflammation, fibrosis, and cell death, including TGF-β (Shackel 2002). Some studies have reported alterations in MMP-2 and TIMP-1 levels associated with HCV-related liver disease; the MMP-2/TIMP-1 ratio correlates with degree of fibrosis (Kasahara 1997; Lichtinghagen 2000; Lichtinghagen 2001).

Host factors that modulate fibrogenesis may account for different rates of disease progression observed in HCV infection. Estradiol, an estrogen hormone, protects against fibrosis through

various mechanisms, including the downregulation of hepatic stellate cell activation, which may partly explain the milder rates of fibrosis progression and low incidence of cirrhosis in pre-menopausal women infected with HCV (I. Shimizu 2003; see Chapter II, Natural History of Hepatitis C). Increased insulin resistance has also been associated with fibrosis in HCV, though a causal mechanism to explain this relationship remains uncertain (Hickman 2003; Hui 2002; Hui 2003; Maeno 2003; Petit 2001). High iron concentrations in the liver may augment HCV-related fibrogenesis and inflammation; proposed mechanisms include injury to hepatocytes, direct activation of stellate cells, and alteration of the immune response (Beinker 1996; Casaril 2000; Farinati 1995; Rigamonti 2002; Thorburn 2002; Weiss 1999). Efforts to determine an association between HCV-related fibrosis rates and hemochromatosis, a hereditary disorder causing iron overload, have produced conflicting results (Bataller 2003). Other genetic factors—including variations in CCR5, cytokine-related genes (TGF-β, IL-10, TNF-α, osteopontin) and HLA types—might also have some influence on the fibrogenesis rate in HCV infection (Ahlenstiel 2004; Bataller 2003, Gewaltig 2002; Hellier 2003; Mochida 2004; Powell 2000; Promrat 2003b; Wright 2003; Yee 2000).

Viral cytopathicity: fibrosis, steatosis, and oxidative stress

The central question for HCV pathogenesis is how and why hepatocytes die during HCV infection: are they killed by the virus or by the immune response? If HCV were directly responsible for hepatocyte death, then higher levels of HCV and hepatocyte infection should lead to more liver damage. Yet studies have largely failed to correlate the severity of liver disease with HCV viral load or the proportion of infected hepatocytes (De Moliner 1998; Fanning 1999; McGuinness 1996; Negro 1999; Rodríguez-Iñigo 1999). Research has not entirely excluded the possibility that HCV infection or viral replication is directly cytopathic, but little evidence directly supports a model of liver injury based on HCV cytopathicity. Moreover, multiple mechanisms may influence the cytopathic potential of HCV, including intracellular levels of particular viral proteins, the extent of active viral replication and virion release, and the dynamics of viral-host cell interactions, including viral evasion of cellular defenses. Finally, some evidence suggests that certain HCV proteins may actually inhibit cell death under certain circumstances (see 'Viral proteins and oncogenesis' later in this chapter). HCV may protect its host cells from cell death as a survival strategy, since rapid death of infected cells would diminish the chances of successful viral replication.

Some groups have explored an association between HCV quasispecies complexity and disease severity, though results conflict across studies. Most research in this area has used cross-sectional comparisons at a single time point, grouping individuals by disease stage (i.e., degree of fibrosis) and comparing viral heterogeneity between groups. A more definitive evaluation of the relationship between viral mutation and disease progression would require a longitudinal evaluation of the evolution of HCV quasispecies, correlated with differences in rates of fibrosis progression. Even then, it would not be clear whether viral complexity drives disease progression, or simply reflects ongoing immune pressure corresponding to immune-mediated liver damage.

The cytopathic potential of HCV may vary according to cell type; a recent report found an association between HCV infection of a B cell line and higher rates of cell death (Sung 2003). A few studies have observed high rates of cell death in cultured cell lines or in transgenic mice that express various HCV proteins, including E1 and other structural proteins, but the relevance of

these findings to chronic HCV infection has not been established (Bantel 2003; Ciccaglione 2003; A Honda 1999; Kalkeri 2001). Other research found no cytopathic effects on hepatocytes in transgenic mice expressing HCV structural proteins (Kawamura 1997; Koike 1995; Pasquinelli 1997). These conflicting data complicate the interpretation of the potential cytopathicity of viral proteins. Further clarification of HCV's contribution to cell death will require the development of efficient cell and tissue cultures and small-animal models.

HCV may be directly cytopathic under certain circumstances. HCV may directly induce fulminant hepatic failure, a rare event characterized by the sudden onset of liver failure in the absence of prior liver disease. One report found an association between liver damage and HCV viral load in a case of fulminant hepatic failure, which peaked before the onset of an HCV-specific immune response. The patient died within two months of presumed infection (Farci 1996a). This case, though exceptional, presents a clear correlation between viremia and liver damage, suggesting that HCV can at times directly kill hepatocytes. HCV cytopathicity may also contribute to liver damage in acute HCV reinfection of the liver following a liver transplant (Ballardini 2002). Similarly, some studies associate high viral loads with HCV-related liver damage following liver or kidney transplantation, though evidence is mixed (Einav 2002). The cytopathicity of HCV may depend on genotype, particularly in the case of hepatic steatosis.

Steatosis, or fatty liver, arises from the build up of fats or lipids in the liver, and is common in chronic HCV infection. Individuals with HCV genotype 3 have an increased frequency of steatosis (Adinolfi 2001; Monto 2002; Romero-Gómez 2003; Rubbia-Brandt 2000; Rubbia-Brandt 2001; Westin 2002). In some studies of genotype 3, steatosis has been correlated with intrahepatic HCV levels, suggesting a direct viral effect (Romero-Gómez 2003; Rubbia-Brandt 2000). Furthermore, successful anti-HCV treatment can reduce steatosis in patients with genotype 3, but treatment largely does not affect steatosis in patients with genotype 1 (D. Kumar 2002; Poynard 2003b). Current thinking tends to associate the pathogenesis of steatosis in HCV infection with primarily viral effects in genotype 3, and with metabolic factors—including insulin resistance and type 2 diabetes mellitus—in other genotypes, particularly genotype 1 (Ramalho 2003; Ratziu 2004). The precise mechanism by which HCV genotype 3 induces steatosis has not been determined.

Steatosis may result from a disturbance in lipid metabolism caused by viral proteins, as evidenced by *in vitro* and transgenic mouse studies involving the HCV core protein and the NS5A protein (Barba 1997; Lerat 2002; Moriya 1997; Perlemuter 2002; Sabile 1999; Shi 2002). HCV may also alter glucose metabolism, and insulin resistance is frequently seen in people with chronic hepatitis C (Weinman 2004). In a transgenic mouse model, HCV core protein expression promoted insulin resistance, mediated by increased levels of TNF-α, suggesting a pathogenic mechanism to explain the association between chronic HCV infection and increased incidence of diabetes mellitus (Shintani 2004). An association between steatosis and the presence of detectable intrahepatic levels of HCV core protein in chronically infected individuals strengthens the case for direct viral involvement in steatosis (Fujie 1999). However, viral mechanisms cannot wholly account for the pathogenesis of all HCV-related steatosis, which is more common in obese persons (Adinolfi 2001; Bressler 2003; Hourigan 1999; Monto 2002; Negro 2002).

Some studies have found an association between steatosis and fibrosis progression, though a causal relationship has not been conclusively established (Adinolfi 2001; Asselah 2003; Castéra 2003;

Hourigan 1999; Romero-Gómez 2003; Westin 2002). Oxidative stress may also link fibrosis and steatosis. Oxidative stress results from the action of reactive oxygen species (ROS), including free radicals. These charged and highly reactive molecules can damage cells, though they can be neutralized by antioxidants. ROS can trigger the death of hepatocytes and activate hepatic stellate cells; ROS levels increase during cell death. Oxidative stress can therefore contribute to fibrosis. Oxidative stress can also disrupt lipid metabolism, and ROS levels have been linked to the pathogenesis of steatosis.

Several studies have linked HCV infection with signs of oxidative stress in the liver (De Maria 1996; Farinati 1995; S. K. Jain 2002; Paradis 1997; Romero 1998; Sumida 2000; Togashi 2000). In particular, some research suggests that the HCV core protein can induce oxidative stress when expressed in mouse livers or cell cultures (K. Li 2002; Moriya 2001b; Okuda 2002). Alcohol and iron both promote oxidative stress in the liver, and oxidative stress may play a central role in the pathogenesis of alcohol-related cirrhosis. Oxidative stress may thus explain the association between heavy alcohol consumption and more rapid HCV disease progression (Morgan 2003; Rigamonti 2003).

The biological relevance of these observations is not clear; in cell lines expressing core protein, an upregulation of antioxidant genes accompanies oxidative stress, and prevents cell death (K. Li 2002; Okuda 2002). In transgenic mice expressing HCV core protein, ROS overproduction did not result in signs of inflammation, while *in vitro* experiments using HCV replicons suggest that ROS actually disrupts or inhibits HCV replication (Choi 2004; Moriya 2001b). Yet some research has correlated hepatic ROS levels with disease activity in chronic HCV infection (De Maria 1996; Valgimigli 2002). Oxidative stress and consequent increases in antioxidant activity have also been seen in experiments using subgenomic HCV replicons, and NS5A may trigger intracellular signaling events involved in oxidative stress and antioxidant response (Qadri 2004). Clarification of the role of oxidative stress in HCV pathogenesis would help to establish the potential benefit—or harm—of antioxidant therapies in slowing fibrosis progression.

Immunopathogenesis

Fibrosis has more commonly been attributed to the immune response to HCV than to direct viral effects. HCV infection triggers a pronounced and sustained influx of lymphocytes into the liver. T cells, natural killer cells, γδ T cells, and NK-T cells are all found in HCV-infected livers (Agrati 2001a; Agrati 2002; Amaraa 2002; Boisvert 2003). Increased levels of CD8 T cells are seen in the livers of people chronically infected with HCV in comparison to healthy controls (Leroy 2003). Recruitment of lymphocytes to the liver is governed by chemokines, secreted proteins that direct cell trafficking by binding to chemokine receptors on lymphocytes (Simpson 2003). The chemokine IP-10 (interferon-γ-inducible protein 10, also called CXCL10), secreted at increased levels by liver cells in chronic HCV infection, has been implicated in intrahepatic lymphocyte recruitment (Harvey 2003; Itoh 2001; Mihm 2003; Patzwahl 2001; Shackel 2002; Shields 1999). *In vitro* studies and examinations of biopsy samples from HCV-infected livers have also found high levels of two other chemokines: MIG (monokine-induced by IFN-γ, or CXCL9) and RANTES (Regulated on Activation, Normal T cell Expressed and Secreted) (Apolinario 2002; Leroy 2003; Shields 1999; Soo 2002).

IP-10 and MIG bind to the CXCR3 receptor, found on activated T_H1-type T cells and NK cells. Lymphocytes in the livers of people with hepatitis C express high levels of CXCR3 (Amaraa 2002; Apolinario 2002; Boisvert 2003; Harvey 2003; Leroy 2003). A large proportion of T cells in the liver also express the CCR5 receptor, which binds to RANTES (Amaraa 2002; Apolinario 2002; Boisvert 2003; Shields 1999). Another chemokine, CCL21 (also called secondary lymphoid tissue chemokine or SLC) and its receptor, CCR7, may also play important roles during hepatitis C infection in recruitment of lymphocytes to the liver (Bonacchi 2003). Some evidence suggests that the degree of chemokine secretion influences disease severity, suggesting a model wherein the chemokine/chemokine receptor network directs lymphocytes to the HCV-infected liver, resulting in damage through cell killing and inflammation (Harvey 2003; Narumi 1997).

In one study of individuals with chronic HCV infection, hepatic stellate cell activation and progression of liver disease was associated with a T_H1 response, but not with HCV viral load (Baroni 1999). A predominantly T_H1 response in intrahepatic CD4 T cells and higher levels of intrahepatic T_H1 cytokine expression—specifically IL-2 and IFN-γ—have been associated with disease progression in HCV infection (Dumoulin 1997; Fukuda 1996; Napoli 1996; Sobue 2001). Hepatic levels of other proinflammatory cytokines, including IL-8 and IL-18, have also been associated with liver inflammation (Fukuda 1996; McGuinness 2000; Shimoda 1998). Though T_H1 responses are necessary for viral control, they can also result in inflammation and may cause liver damage. At the same time, IFN-γ can have antifibrotic effects, inhibiting the activation of hepatic stellate cells (Baroni 1996). Some evidence suggests that fibrosis progression corresponds with inadequate CD4 and CD8 T cell responses, while stronger responses are associated with inflammation but not fibrosis (Sreenarasimhaiah 2003). A recent report found that people with intrahepatic CD4 T cells displaying strong T_H1 responses had milder fibrosis progression after eight years of chronic infection (Kamal 2004).

Liver damage may result from the killing of HCV-infected hepatocytes by CTLs. CTLs have three main mechanisms for killing cells:

- Perforin-mediated cytotoxicity: an epitope-specific CTL engages an infected cell, releasing perforin. Perforin forms small holes or pores in the membrane of the infected cell, allowing the CTL to release granzymes into the cell. Granzymes are a type of protease (enzymes that break down proteins) that act inside the target cell to trigger cell death.
- Fas-mediated cytotoxicity: an epitope-specific CTL bears a molecule, the Fas ligand, on its surface. The Fas ligand engages the Fas receptor on a target cell, sending a signal to the cell that triggers cell death.
- TNF-α-mediated cytotoxicity: activated CTLs produce TNF-α, a cytokine that binds to the TNF receptor on infected cells and triggers cell death.

In all cases, cell death occurs through a process called apoptosis, or programmed cell death. CTLs do not directly kill infected cells so much as send an initial signal triggering a cascade of events within the cell culminating in death. Increased rates of apoptosis in the liver have been seen in HCV infection and correlated with liver damage (Bantel 2001; Calabrese 2000; Pianko 2001). Evidence suggests that Fas/Fas ligand interactions, TNF-α, and, to a lesser degree, perforin expression, mediate hepatocyte death in HCV infection (Ando 1997; Galle 1995; Hayashi 1997; Hiramatsu

1994; Ibuki 2002; Iio 1998; Macías 2001; Neuman 2002b; Ono 2002; Pianko 2001; Tagashira 2000; Tordjmann 1998). Research using a mouse model of viral infection in the liver indicates that IFN-γ also facilitates CTL killing through Fas ligand and perforin mechanisms (Roth 2004). A precise correlation between *in vivo* HCV-specific CTL activity and hepatocyte apoptosis during chronic infection has not been found, though some research has examined the immune response in transgenic mice expressing HCV proteins (Wakita 2000). A recent study of transgenic mice expressing HCV structural proteins demonstrated that HCV-specific CTLs killed hepatocytes expressing viral proteins (Takaku 2003). Other lymphocytes, particularly natural killer cells, may also participate in cell killing during HCV infection.

While HCV-specific CTL responses may be responsible for some liver damage during chronic HCV infection, it is not clear that all hepatocyte death can be attributed to the killing of infected cells. At least some hepatocyte apoptosis may reflect a generalized and persistent inflammation in the liver, with higher levels of inflammatory cytokines resulting in the death of infected and uninfected cells. Levels of TNF-α tend to correlate with liver inflammation, and successful anti-HCV treatment lowers TNF-α levels (Crespo 2002; Dumoulin 2001; Neuman 2002a; Neuman 2002b; Neuman 2002c). Similar patterns are observed with the relationship between TGF-β and fibrosis. Low levels of TNF-α and TGF-β predict a successful response to treatment (Kanzler 2001; Neuman 2002a; Neuman 2002b; Neuman 2002c; Tsushima 1999). Inflammation in the liver may also result from expanded populations of activated NK T cells and γδ T cells capable of killing hepatocytes and releasing inflammatory cytokines (Agrati 2001a; Agrati 2002; Exley 2002; Tseng 2001).

Studies of the pathogenesis of HBV infection suggest that liver damage may also result from other non-specific immune responses, including an influx of neutrophils into the liver, and from Fas-Fas ligand interactions that result in inflammation and general hepatocyte apoptosis (Manigold 2003). Moreover, T cell apoptosis may itself contribute to liver damage through Fas-dependent mechanisms. Activated CD8 T cells congregate in the liver, where they undergo apoptosis; the very presence of these dying T cells can cause hepatocyte damage in a mouse model (Crispe 2003; Kennedy 2001). Peripheral T cells may also be more susceptible to apoptosis in HCV infection, and T cell susceptibility to apoptosis may be associated with the severity of liver disease (Emi 1999; Nakamoto 2001; Nakamoto 2002; Toubi 2001).

In short, liver damage may not result exclusively from the targeted killing of HCV-infected hepatocytes. Rather, the persistence of HCV infection creates a continual state of emergency for the immune system, which responds by the constant migration of lymphocytes to the liver and the maintenance of an inflammatory milieu. Intrahepatic T_H1 responses induce a perpetual recruitment of T cells to the liver, where T cell activation and apoptosis further contribute to hepatic inflammation (Apolinario 2002; Crispe 2003; Shields 1999). This vicious circle may exert partial control over viral replication at the expense of cumulative damage to the liver. This model for HCV pathogenesis does not exclude a possible contribution of viral cytopathicity, but the burden of responsibility for inflammation and fibrosis rests upon the sustained immune response to persistent viral infection.

HIV disease and HCV pathogenesis

An emphasis on inflammation could help to explain some otherwise contradictory aspects of the pathogenesis of HCV infection in people coinfected with HIV. The progressive depletion of CD4 T cells and loss of immune response is the hallmark of HIV infection. If HCV disease progression resulted from direct killing of infected hepatocytes, then HIV-related immunodeficiency should result in milder liver damage, as the immune system fails to respond to HCV. Yet HIV coinfection accelerates the rate of fibrosis progression and increases the risk of cirrhosis, particularly at low CD4 T cell counts (Benhamou 1999; Di Martino 2001; Graham 2001; Mohsen 2003; Poynard 2003a; Puoti 2001). HIV coinfection has also been associated with higher HCV viral loads, though studies conflict as to whether increased HCV viremia correlates with low CD4 T cell counts (Bonacini 1999; Di Martino 2001; Martinez-Sierra 2003; Sherman 1993; Thomas 1996).

HIV coinfection alters the immune response to HCV:

- Weak HCV-specific CD4 and CD8 T cell responses: HIV coinfection diminishes T cell responses to HCV, though long-term HIV non-progressors maintain relatively intact HCV-specific immune responses (Alatrakchi 2002; Anthony 2004; Valdez 2000; Valdez 2002). In one study of blood samples from coinfected individuals, CD8 T cell responses to HCV were weak or undetectable, and HCV-specific CD4 T cell responses were entirely absent, even in individuals with high CD4 T cell counts. In contrast, HIV-specific responses were greater and more frequently detected in this group, while CD4 T cell responses to HCV were detectable in nearly half of a control group solely infected with HCV (Lauer 2002a).
- T_H1/T_H2 responses: One study found that pro-inflammatory T_H1 responses to HCV antigens were higher in blood samples from coinfected individuals, compared to samples from people infected with HCV alone. T_H2 responses to HCV were also detected, but only in the coinfected group (Woitas 1999).
- IFN-α levels: A study of intrahepatic levels of interferon-related gene expression found increased levels of IFN-α in people coinfected with HIV, corresponding to HIV viral load. However, responsiveness to IFN-α may be impaired, since levels of the IFN-α receptor (IFN-α receptor subunit 1, or IFNAR1) and of the interferon-stimulated gene PKR, present in people infected with HCV alone, were unde tectable in the livers of those coinfected with HIV and hepatitis C (Abbate 2004).

Overall, these studies suggest that HIV coinfection both impairs and skews the immune response to HCV in ways that cannot be entirely reduced to CD4 T cell depletion.

HIV disease is increasingly thought of not only in terms of immune deficiency, but more broadly as immune dysfunction (Grossman 2002; Letvin 2003; G. Silvestri 2003). Several aspects of HIV immune dysfunction may contribute to the pathogenesis of HCV in coinfected individuals:

- Increases in activated $\gamma\delta$ T cells in the liver, seen in coinfected persons, correspond to hepatic inflammation (Agrati 2001b).

- HIV coinfection may increase levels of TGF-β and alter the balance between MMPs and TIMPs, thereby contributing to fibrosis (L. He 2000; Mastroianni 2002).
- A trend towards increased hepatocyte proliferation and apoptosis was seen in a small study comparing coinfected patients to HCV monoinfected patients, though differences did not reach statistical significance (Talal 2000).
- The gp120 viral envelope protein of HIV can trigger apoptosis of hepatocytes in vitro, potentially adding to liver damage (Vlahakis 2003). Viral envelope proteins HCV E2 and HIV gp120 can together stimulate production of the inflammatory cytokine IL-8 in hepatocyte-derived cell cultures, and collaborate *in vitro* to induce hepatocyte apoptosis (Balasubramanian 2003; Munshi 2003). However, the physiological relevance of *in vitro* studies of gp120's putative effects has recently been questioned on methodological grounds (Klasse 2004).
- HIV coinfection may foster the compartmentalization of different HCV quasispecies in other cells and tissues outside the liver, particularly in monocytes/macrophages and lymphocytes, which are involved in immune responses and are also susceptible to HIV infection (Laskus 1998a; Laskus 2000).

Together, these studies suggest that HIV coinfection may affect multiple parameters of both the immune response to HCV and viral-host interactions relevant to HCV pathogenesis. Other mechanisms may also be involved (Einav 2002):

- HIV preferentially infects activated and proliferating CD4 T cells, especially HIV-specific CD4 T cells (Brenchley 2004; Douek 2002). Chronic HCV infection results in on-going activation and proliferation of HCV-specific CD4 T cells, and these cells may be especially vulnerable to HIV infection.
- HIV is associated with high levels of immune activation, which may increase the trafficking of activated CD8 T cells to the liver and contribute to inflammation.
- In coinfected individuals, higher levels of IFN-γ production in blood stimulated with HCV antigens have been associated with milder fibrosis and inflammation, suggesting that predominantly T_H1-type responses to HCV protect against liver damage (Graham 2003). HIV infection can induce a shift from a T_H1 to T_H2 response; in HIV-HCV coinfection, this may alter or compromise the immune response to HCV and affect cytokine profiles.
- HIV coinfection is associated with both higher HCV viral load and more rapid fibrosis progression, suggesting a partial role for HCV cytopathicity in the context of impaired immune control of viral replication.
- According to one theory based on mouse studies of persistent viral infections, the pool of memory T cells specific to a prior infection can decrease after exposures to subsequent new pathogens (S. K. Kim 2004; H. Liu 2003). In this model, the HCV-specific memory T cell repertoire could be compromised when an individual is later infected with HIV.

Ultimately, the causes for accelerated HCV disease progression in HIV coinfection are most likely multifactorial. The mechanisms of HIV pathogenesis, like those of HCV pathogenesis, are not fully understood, complicating the study of HIV/HCV coinfection. The relative contribution of immunological and viral factors to liver damage in coinfection will require further clarification, and may in turn shed light on the dynamics of HCV pathogenesis.

Pathogenesis of hepatocellular carcinoma

The pathogenesis of liver cancer, or hepatocellular carcinoma (HCC), in chronic HCV infection has been the subject of intense research. HCC involves the formation of tumors, produced by uncontrolled cell division of hepatocytes. HCC can be a consequence of various forms of liver disease and injury, and is not specific to hepatitis C infection. HCV-related hepatocellular carcinoma develops in a relatively small proportion of HCV-infected individuals decades after infection, and almost exclusively in individuals with cirrhosis (see Chapter II, Natural History of Hepatitis C).

Viral factors, and particularly the HCV core protein, have been implicated in the pathogenesis of HCC. However, considerable doubt remains as to whether the hepatitis C virus itself causes HCC. Cirrhosis dramatically increases the risk of HCC in people with HCV and in people with chronic hepatitis B virus (HBV) infection. However, chronic HBV infection can cause HCC even in the absence of cirrhosis, while the development of HCC in chronic HCV infection usually requires the presence of cirrhosis (Idilman 1998). HBV is a DNA virus that replicates within the nucleus of hepatocytes and may directly promote hepatocellular carcinoma; the evidence for HCV's direct role in the pathogenesis of HCC is more ambiguous (Block 2003; Suriawinata 2004; Szabó 2004). Whether hepatocellular carcinoma results primarily from cirrhosis or direct effects of HCV has enormous significance to the development of strategies to prevent and treat HCC.

Chronic HCV infection fosters prolonged and extensive turnover of hepatocytes; the liver attempts to compensate for the death of cells by regenerating itself through the production of new hepatocytes. Ongoing hepatocyte proliferation and apoptosis can eventually foster an environment conducive to the development of HCC through changes and damage to hepatocyte genes (Donato 2001; Farinati 1996; Farinati 2001; Idilman 1998; Lake-Bakaar 2002; Shibata 1998). HCC oncogenesis (the development of cancer) involves the accumulation of multiple alterations during the long course of chronic hepatitis (Thorgeirsson 2002). Alterations include:

- A heightened level of hepatocyte turnover through cell death and proliferation;
- Quantitative changes in the level of expression of key genes;
- Structural changes to genes and chromosomes, including deletion of genes protective against cancer, caused by factors including the accumulation of random mutation during accelerated cell proliferation, faulty DNA repair mechanisms, and oxidative stress.

These events culminate in the transformation of a subset of hepatocytes into malignant, cancerous cells. The course of oncogenesis can vary considerably across liver cancers associated with various types of viral and non-viral chronic hepatitis. However, this process inevitably entails the altering or

transforming of cells through changing the expression of genes regulating cell growth, proliferation, differentiation, and apoptosis. The proteins encoded by these genes perform a range of functions that provide important safeguards against cell transformation. The processes that mediate cell growth and proliferation involve a dense network of factors controlling the expression or inhibition of thousands of genes in the cell in response to various internal and external stimuli. These networks are called cell signaling pathways, and substantial evidence suggests that certain HCV proteins—especially core protein and NS5A—can interact with and disrupt these pathways and may thus contribute to oncogenesis.

Several factors related to HCV infection may influence the risk of hepatocellular carcinoma. Oxidative stress has been implicated in oncogenesis; reactive oxygen species can damage DNA and produce gene mutations (Mahmood 2004; Moriya 2001b). Some host genetic variations may also promote HCC, including variant forms of genes that encode IL-1β, an inflammatory cytokine that stimulates production of TNFα, and various enzymes involved in metabolism (L. Silvestri 2003; Sonzogni 2002; Tanaka 2003; Y. Wang 2003). High copper levels in the liver may also increase the risk of developing HCC (Ebara 2003).

While host and environmental influences play a major role in determining HCC risk, viral factors appear to directly contribute to establishing the conditions that promote oncogenesis. A recent report suggested that the HCV core protein might interfere with the cellular DNA repair mechanism, and a separate study found evidence that HCV infection increases the frequency of mutations to cellular genes (Machida 2004; Naganuma 2004). Other studies have examined HCV quasispecies diversity in relation to hepatocellular carcinoma. One study found an association between HCC incidence and increased genetic diversity in the region of HCV NS5A associated with PKR inhibition, though a later study produced conflicting results (De Mitri 2002; Giménez-Barcons 2001). Some evidence points to differences in HCV quasispecies composition between cancerous tissue and adjacent non-cancerous tissue in individuals with hepatocellular carcinoma, though a clear correlation between viral complexity and HCC pathogenesis has not been established (Alam 2002; De Mitri 1998; Horie 1996; C. K. Park 1997; Ruster 2001; S. Saito 1996; Young 2002).

HCV proteins may contribute to HCC by interfering with apoptosis, and subverting other cell signaling pathways that protect against oncogenesis. HCC develops in transgenic mice engineered to express the HCV core protein in their livers; other viral proteins may also contribute to HCC (Lerat 2002; Moriya 1998). Not all transgenic mice expressing core protein develop HCC, and core protein is not sufficient for oncogenesis, suggesting that genetic variables are important (Koike 2002a; Koike 2002b; M. M. Lai 2002). The core protein also showed *in vitro* oncogenic potential in cell lines (J. Chang 1998; R. B. Ray 1996a; Tsuchihara 1999). The core protein collaborates with cancer-promoting genes, or oncogenes, and may directly induce their expression (R. B. Ray 1995).

The core protein appears to perturb intracellular signaling pathways regulating gene expression, thereby modifying gene expression patterns and levels (Watashi 2003c). *In vitro* and mouse studies have demonstrated that the core protein can alternately activate and repress the expression of cellular genes (Kwun 2003; K. Li 2002; Ohkawa 2003; R. B. Ray 1995; R. B. Ray 1997; Watashi 2003c). HCV core binds to several proteins involved in the regulation of gene expression and the cell cycle (Hsieh 1998; Moriishi 2003; Ohkawa 2004; Tellinghuisen 2002; Tsutsumi 2002a; Watashi 2003b). These functions reflect the presence of various forms of the core protein in both

the cell cytoplasm and the nucleus, and core proteins' interactions with various cellular components (Alisi 2003; S. C. Chang 1994; Falcón 2003; Isoyama 2002; Q. Liu 1997; Lo 1995; Mamiya 1999; R. Suzuki 1995; Yamanaka 2002; Yasui 1998). Other viral proteins, including NS5A, NS3, and NS4B, have also been implicated in oncogenesis (Ghosh 2000; Y. He 2003; J. S. Park 2000; Sakamuro 1995; Zemel 2001).

Viral proteins and oncogenesis

The regulation of hepatocyte apoptosis is vital to the development of hepatocellular carcinoma, since cell transformation and proliferation depend in part on the suppression of cell death. The ultimate defense against cell transformation is apoptosis, and cells have a number of mechanisms to induce apoptosis if they enter into states that could lead to unchecked proliferation. Viral proteins may protect infected cells from apoptosis, though research investigating this possibility has produced a body of evidence rife with apparent contradictions (Disson 2002; Rubbia-Brandt 2002). The majority of these studies focus on the HCV core protein's ability to regulate apoptosis, though its effects are somewhat unclear and inconsistent across studies (C. M. Chen 1997; Goh 2001; A. Honda 2000; Kao 2004; Otsuka 2002; Pavio 2003b; R. B. Ray 1996b; Ruggieri 1997; Yang 2002; T. Yoshida 2002). Conceivably, the core protein may be capable of alternately enhancing or inhibiting apoptosis in different circumstances, perhaps dependent on cellular factors (e.g., cell type or stage of cell cycle) or degree of expression of core protein (N. Zhu 2001). T cells, for example, may be more prone to apoptosis in the presence of core protein (C. S. Hahn 2000; Soguero 2002).

The core protein can bind to both the Fas receptor and the TNF receptor 1, involved in Fas- and TNF-α-mediated cytopathicity (C. S. Hahn 2000; N. Zhu 1998). In theory, this could interfere with the signaling events that culminate in apoptosis, either inhibiting or sensitizing cells to apoptosis. Transgenic mice expressing HCV RNA show resistance to Fas-mediated apoptosis, potentially allowing infected cells to evade HCV-specific CTL killing while contributing to oncogenesis (Disson 2004). The NS2 and NS5A proteins have also been implicated in inhibiting cell growth, blocking apoptosis and promoting cell survival (Arima 2001; Y. L. Chung 2003; Erdtmann 2003; Ghosh 2000; Gong 2001; K. H. Lan 2002; Machida 2001; Majumder 2002; Miyasaka 2003; K. J. Park 2002; K. J. Park 2003; Reyes 2002). The survival of HCV-infected hepatocytes would protect the host cell during the viral replication process. At the same time, suppression of apoptosis leaves hepatocytes susceptible to transformation into cancerous cells, particularly if HCV overrides other cellular defenses. However, mice engineered to express HCV NS5A in the liver did not experience liver cancer after up to 24 months (Majumder 2003).

Some research hypothesizes that the core protein may mediate apoptosis through activating NF-κB (nuclear factor-kappa B), a cellular protein involved in regulating cell proliferation and apoptosis through intracellular signaling events (Marusawa 1999; Tai 2000; L. R. You 1999). NS5A can also indirectly activate NF-κB through multiple mechanisms, including oxidative stress (Gong 2001; K. J. Park 2002; Waris 2002; Waris 2003). Heightened levels of NF-κB were found in the livers of HCV-infected livers and cultured cells expressing core protein, with NF-κB activation associated with reduced apoptosis *in vitro* (Tai 2000).

Hepatocytes in general are relatively resistant to TNF-α-mediated apoptosis due to the protective effects of NF-κB activation and signaling. The modulation of NF-κB signaling pathways also suggests the potential for a perturbation of inflammatory responses, as NF-κB plays a central role in regulating innate and adaptive immune responses (Caamaño 2002; Y. M. Chung 2001). Indeed, the HCV core protein appears to increase the production of a number of inflammatory cytokines, including IL-1β, IL-8 and TNF-α, through NF-κB activation or other mechanisms (Dolganiuc 2003; N. Kato 2000b; Tsutsumi 2002b; H. Yoshida 2001). The multiple diverse effects of NF-κB suggest that its activation through viral proteins may protect infected cells from apoptosis, while potentially increasing the susceptibility of neighboring hepatocytes to cell death by fostering an inflammatory milieu. Bystander cell death, rather than the killing of infected hepatocytes, may therefore play an important role in HCV persistence and pathogenesis (Ando 1997; Lasarte 2003). According to a recent report based on *in vitro* studies, even a small fraction of HCV-infected cells can induce Fas-mediated apoptosis in substantial numbers of bystander hepatocytes, which may account for significant cell death in chronic HCV infection (Gremion 2004).

HCV viral proteins interact and interfere with several other signaling pathways involved in oncogenesis. HCV proteins can activate or inhibit cell proteins that trigger the expression of genes involved in regulating cell growth and proliferation. Viral manipulation of these signaling pathways ultimately disrupts the cell's defenses against transformation and oncogenesis. *In vitro* studies have documented potential interactions between HCV proteins (particularly core and NS5A proteins) and key signaling pathways involving tumor suppressor genes (p53/p21), the mitogen-activated protein (MAP) kinase family, and other pathways including the JAK-STAT pathway (Pavio 2003b).

In transgenic mice constitutively expressing HCV core protein, alcohol increases core-mediated activation of some signaling pathways, presumably via oxidative stress, which may account for part of the synergy between HCV and alcohol consumption in risk of liver disease and hepatocellular carcinoma (Morgan 2003; Tsutsumi 2003). HCV NS5A also interacts with other components of signaling pathway cascades, including AP1 (activating protein-1), Grb2 (growth factor receptor-bound protein 2), and the p85 subunit of PI3K (phosphatidylinositol 3-kinase), with possible implications for cell survival and proliferation (Y. He 2002b; A. Macdonald 2003; Street 2004; Tan 1999). The NS5A protein also contains a domain that may activate cellular gene transcription (N. Kato 1997). The transcriptional activity of NS5A, which induces IL-8 expression *in vitro*, may vary according to mutations introduced via quasispecies evolution (Pellerin 2004; Polyak 2001a).

The results of these studies should be interpreted with caution, since *in vitro* methods do not necessarily reflect the physiological conditions of HCV replication *in vivo*. As a result, these documented interactions between HCV proteins and signaling pathways may not necessarily be clinically relevant. The actual effects of HCV replication and viral proteins on the expression of cellular genes that regulate cell growth, proliferation, and death remain speculative. A recent study examining patterns of gene expression in human hepatoma cell lines found that levels of host cell gene transcription were roughly equivalent in cell lines containing HCV replicons compared to cell lines without replicons (Scholle 2004). The authors argue that these findings call into question hypotheses about viral disruption of host cell regulation, since any HCV protein-mediated changes in apoptosis or cell cycle regulation would most likely affect cellular gene expression.

HCV replicon studies are limited in their ability to reproduce actual HCV infection dynamics. Microarray analysis of gene expression patterns in the livers of humans and chimpanzees infected with HCV have found a variety of differential gene expression patterns, some potentially involved in oncogenesis (Bigger 2001, Iizuka 2002; Iizuka 2003; Okabe 2001; M. W. Smith 2003a; M. W. Smith 2003b). Patterns and outcomes of viral subversion of signaling pathways may differ according to the cellular environment and cytokine milieu (Hosui 2003). These dynamics suggest a complex balance between HCV proteins, cytokines, and signaling pathways that regulates cell death and survival.

Summary of Pathogenesis and Future Research Directions

A constellation of viral, genetic, and immunologic factors converge in the pathogenesis of chronic HCV infection. The presence or absence of any single component involved in liver disease may be less relevant than the global dynamics governing the interactions of all of these factors. The development of tissue culture and small animal models for HCV infection would enable research clarifying the relative contribution of various factors to fibrosis, steatosis, and hepatocellular carcinoma. Genomic techniques, including microarray analysis, have already begun to shed light on the various genes expressed from liver tissue samples and *in vitro* models in response to HCV infection, and their relation to HCV pathogenesis (Aizaki 2002; Bigger 2001, Iizuka 2002; Iizuka 2003; Okabe 2001; Otsuka 2003; Scholle 2004; M. W. Smith 2003a; M. W. Smith 2003b; Sreekumar 2003). Further research on signaling pathways will contribute to the understanding of the role of viral interference in the immune response as well as HCC oncogenesis (Berqvist 2001; Berqvist 2003; Bureau 2001; Meng Soo 2002). Larger studies of differences in disease progression according to age, race/ethnicity, and gender may clarify the immunologic, genetic, and environmental correlates of cirrhosis and HCC risk.

Ultimately, this research may result in new therapeutic strategies protective against HCV-related fibrogenesis and HCC oncogenesis, as well as better predictive and diagnostic tools to guide care and treatment. These developments would be particularly welcome for people coinfected with both HCV and HIV, who face poorer prognoses and difficult choices around balancing treatment for both viruses.

Research Recommendations

Support and intensify research into immune response, persistence and pathogenesis.

Some fundamental aspects of the immune response to HCV and its role in viral persistence and pathogenesis have been established. HCV infection induces a broad CD4 and CD8 T cell response concentrated in the liver. Clearance of acute infection requires a robust, sustained T_H1 immune response, while genetic factors, viral escape mutations, and functional impairments in the innate and adaptive immune responses may impair viral control. Intrahepatic immune responses contribute to hepatic injury during chronic HCV infection, resulting in fibrosis. HIV coinfection, particularly at lower CD4 T cell counts, correlates with accelerated rates of fibrosis and higher risk of cirrhosis.

In spite of the substantial amount of data supporting these assertions, significant questions about immune response, persistence, and pathogenesis have not been fully resolved. Additional research will clarify outstanding questions surrounding the contributions of immune responses and viral factors in HCV persistence and pathogenesis. Some questions warrant particular attention:

- What is the impact of the immunologic milieu of the liver and its promotion of immune tolerance on the response to HCV infection?
- How do intrahepatic lymphocytes and liver-resident antigen-presenting cells con tribute to viral persistence and pathogenesis?
- How do viral and host factors regulate the cytokine and chemokine networks in the liver, and how do these networks mediate immune response and inflammation?
- To what extent does impairment in the components of innate immunity determine the outcome of acute infection?
- Can functional impairments in the HCV-specific cell-mediated immune response be reversed?
- How does the interplay between HCV and the immune response contribute to extrahepatic manifestations of HCV, including autoimmune and lymphoproliferative disorders?
- In HCV/HIV coinfection, does HIV accelerate HCV disease progression through immune deficiency, immune dysfunction, viral mechanisms, or some combination of the three?
- Does viral cytopathicity play a role in pathogenesis, and under what circumstances?
- How do viral interactions with signaling pathways operate *in vivo*, and how do they affect immune responses and pathogenesis?
- How do gene expression patterns change at various stages of HCV infection, and how do interactions between viral and cellular proteins affect cell viability, cell transformation, and cell-cell interactions?

These are not simple questions, and easy answers will not be forthcoming. Some of these topics would benefit from stimulating cross-disciplinary dialogues that draw on the insights of research outside of the HCV field. For example, the understanding of HCV persistence could be considerably advanced by facilitating productive exchanges between researchers studying immune responses to

HCV, basic scientists investigating determinants of viral persistence using murine models of LCMV and other viral infections, and immunologists focused on the liver's role in immunologic tolerance, a field of inquiry traditionally more engaged with the implications for organ transplant than with viral infection.

Ultimately, a greater sustained investment led by the National Institutes of Health in basic research on HCV persistence and pathogenesis could hasten the development of new and long-overdue therapies and vaccines for the prevention and treatment of HCV infection. Continued leadership, coordination, and funding will play a vital role.

List of Terms Used in This Chapter

2′,5′-OAS [(2′,5′)-oligoadenylate synthetase]: a cellular defense against viral infection that activates RNase L, a cellular enzyme that degrades viral RNA. 2′,5′-OAS is part of the interferon response.
Agammaglobulinemia: a rare hereditary disease in which the body cannot produce antibodies.
Anergy: a state of non-responsiveness to stimulation.
Antibodies: small proteins produced by B cells that can target and neutralize circulating virus.
Apoptosis: programmed cell death.
B cells: antibody-producing cells.
CCL21: a chemokine (chemical messenger); binds to the CCR7 receptor.
CCR5: a chemokine receptor; found on the surface of activated T cells; binds to the chemokines RANTES, MIP-1α, and MIP-1β.
CCR7: a chemokine receptor; found on the surface of T cells and dendritic cells; binds to the chemokine CCL21.
CD4 T cell: a T cell responsible for coordinating humoral and cell-mediated immune responses.
CD8 T cell: a T cell responsible for killing infected cells.
CD81: a cell surface receptor involved in immune responses that appears to bind to the HCV envelope protein E2.
Chemokine: a type of cytokine (chemical messenger) involved in cell migration and trafficking.
Clonal anergy: functional deficiencies (e.g. non-responsiveness) in a population of virus-specific T cells.
Clonal deletion: the disappearance (through apoptosis) of a population of virus-specific T cells.
Core: an HCV protein; interacts with cellular proteins and may affect signaling pathways, contributing to hepatocellular carcinoma.
Cytokine: secreted proteins that function as chemical messengers between cells to influence (e.g., stimulate, inhibit) immune responses. Cytokines work by binding to receptors on immune system cells (e.g., T cells, dendritic cells). Cytokines include chemokines, interferons, and interleukins.
Cytotoxic: toxic to cells.
Cytotoxic T lymphocytes (CTLs): a subset of CD8 T cells; directly kills infected cells.
CXCL9 (MIG; monokine-induced by IFN-γ): a chemokine (chemical messenger); binds to the CXCR3 receptor.
CXCL10 (IP-10; interferon-γ-inducible protein 10): a chemokine (chemical messenger); binds to the CXCR3 receptor.
CXCR3: a chemokine receptor; found on the surface of activated T cells and natural killer (NK) cells; binds to the chemokines MIG (CXCL9) and IP-10 (CXCL10).

Dendritic cells: immune cells that sweep through the body looking for pathogens. Dendritic cells capture, process, and present antigens to CD4 T cells.
E1: one of two HCV envelope proteins.
E2: one of two HCV envelope proteins.
Effector cells: T cells that perform specific functions (e.g., effector CD8 T cells are cytotoxic T lymphocytes that directly kill infected cells).
Endoplasmic reticulum stress response: a cellular defense that can shut down protein synthesis and lead to cell death.
Epitope: a specific segment of an antigen recognized by T cells or antibodies.
Escape mutant: a viral strain containing a mutation that allows the virus to evade immune recognition.
Eukaryotic initiation factor 2 (eIF2): a cellular protein involved in viral and cellular translation initiation.
Eukaryotic initiation factor 3 (eIF3): a cellular protein involved in viral and cellular translation initiation.
Extracellular matrix: the material that surrounds cells, including collagen and other proteins.
Fas: cell surface receptor involved in cell death; binds to Fas ligand.
Fas ligand: molecule on cytotoxic T lymphocytes involved in cell killing; binds to Fas.
Fibrogenesis: the development of fibrosis.
Hepatocytes: liver cells.
HLA (human leukocyte antigen): genes that encode major histocompatibility complex (MHC) molecules.
HLA alleles: Individual variations of HLA genes.
HLA type: The set of HLA alleles present within an individual.
Humoral immunity: involves antibodies produced by B cells, which guard against extracellular pathogens, such as viral particles circulating in the blood.
HVR1: the highly variable region 1 of the HCV envelope protein E2.
Hypogammaglobulinemia: a condition in which antibody levels are abnormally low.
IL-1β (interleukin 1 beta): an inflammatory cytokine.
IL-2 (interleukin 2): a cytokine associated with T_H1 responses.
IL-4 (interleukin 4): a cytokine associated with T_H2 responses.
IL-8 (interleukin 8): a cytokine involved with inflammation.
IL-10 (interleukin 10): a cytokine associated with T_H2 responses.
IL-15 (interleukin 15): a cytokine involved in the maturation of dendritic cells.
IL-18 (interleukin 18): a cytokine involved with inflammation.
Immunodominant epitopes: the epitopes that stimulate the strongest immune response.
INF-α (interferon alpha): a cytokine involved in cellular defenses.
INF-β (interferon beta): a cytokine involved in cellular defenses.
INF-γ (interferon gamma): a cytokine associated with T_H1 responses.
IFNAR1 (interferon alpha receptor subunit 1): part of the interferon alpha receptor; binds to interferon alpha (IFN-α).

Interferon response: a range of cellular defenses against infection that include interferon and interferon-stimulated genes.
Interferon-stimulated genes: genes triggered by interferon and involved in cellular defenses (e.g., PKR); part of the interferon response.
Internal ribosomal entry site (IRES): a structure with the HCV RNA 5′ UTR that binds directly to the ribosome to initiate translation.
IP-10 (interferon-γ-inducible protein 10; CXCL10): a chemokine (chemical messenger); binds to the CXCR3 receptor.
Kupffer cells: liver-resident macrophages, a type of immune cell that functions as a scavenger by ingesting dead cells, and presents antigen to T cells.
LCMV (lymphocytic choriomeningitis virus): a mouse virus used as a model for studying immune responses in acute and chronic infections.
Liver sinusoidal endothelial cells (LSECs): endothelial cells that line blood vessels in the liver and separate blood from surrounding organ tissue.
Matrix protein: a component of the extracellular matrix, the material that surrounds cells.
MHC class I (major histocompatibility complex class I): a molecule that can bind to fragments of HCV proteins and present them on the surface of infected cells to CD8 T cells. CD8 T cells only recognize peptides bound to MHC class I molecules. MHC class I molecules are encoded by HLA genes.
MHC class II (major histocompatibility complex class II): a molecule that can bind to fragments of HCV proteins and present them to CD4 T cells. CD4 T cells only recognize peptides bound to MHC class II. MHC class II molecules are encoded by HLA genes.
Microarray: a technique for analyzing and comparing gene expression patterns.
MIG (monokine-induced by IFN-γ; CXCL9): a chemokine (chemical messenger); binds to the CXCR3 receptor.
MIP-1α (macrophage inflammatory protein-1 alpha): a chemokine (chemical messenger); binds to the CCR5 receptor.
MIP-1β (macrophage inflammatory protein-1 beta): a chemokine (chemical messenger); binds to the CCR5 receptor.
MMP (matrix metalloproteinase): an enzyme that breaks down matrix proteins.
MxA: a cellular defense against viral infection that inhibits viral replication; part of the interferon response.
NK cells (natural killer cells): part of the innate immune response; NK cells kill infected cells and recruit other T cells to the site of infection.
NK T cells (natural killer T cells): part of the innate immune response; NK T cells have the properties of both natural killer cells and CD8 T cells.
Neutrophil: a white blood cell that fights infections.
NS3 (Non-structural protein 3): an HCV protein; contains key HCV epitopes.
NS3 helicase: An HCV enzyme that keeps viral RNA strands separated during strand synthesis.
NS3 serine protease: An HCV enzyme that cleaves viral proteins after translation; blocks the interferon response.
NS4A (Non-structural protein 4A): an HCV protein.

NS5A (Non-structural protein 5A): an HCV protein; interacts with cellular proteins and may affect signaling pathways, contributing to hepatocellular carcinoma.
NF-κB (nuclear factor kappa B): a cellular protein involved in signaling pathways regulating cell proliferation and apoptosis as well as inflammatory and immune responses.
Oncogenesis: the development of cancer.
Osteopontin: a cytokine involved with inflammation.
Oxidative stress: destruction caused by free radicals (reactive oxygen species).
p56: a cellular defense against viral infection that shuts down protein synthesis by binding to eukaryotic initiation factor 3; part of the interferon response.
Pathogenesis: the underlying mechanism(s) of disease.
Peptide: a short section or fragment of a protein.
Perforin: a protein secreted by CTLs and NK cells that can punch holes in, or perforate, the membrane of infected cells targeted for destruction.
Phenotype: the functional or maturational state of a T cell (e.g., naïve, effector, memory).
PKR (Double-stranded RNA-dependent protein kinase R): a cellular defense against viral infection that shuts down protein synthesis; part of the interferon response.
Pre-terminally differentiated T cells: a T cell phenotype representing an intermediary stage between memory cells and terminally differentiated effector cells.
Protease: an enzyme that breaks down proteins.
Quasispecies: a dynamic population of closely related but distinct genetic sequences; the population of HCV within an individual, including strains containing mutations.
RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted): a chemokine (chemical messenger); binds to the CCR5 receptor.
Reactive oxygen species (ROS): molecules (e.g., free radicals) involved in oxidative stress.
Regulatory T cells: a subset of T cells capable of suppressing cell-mediated immune responses.
Signaling pathways: networks within cells that direct gene expression and cellular functions (e.g., interferon response, activation, apoptosis).
Stellate cells: cells that play an essential role in the development of fibrosis.
TGF-β (transforming growth factor beta): a cytokine involved in immune responses and fibrosis.
T_H **1 response:** a synonym for cell-mediated immunity, involving CD8 T cells to destroy infected cells.
T_H**2 response:** a synonym for humoral immunity, involving B cells and antibodies to guard against extracellular pathogens, such as viral particles circulating in the blood.
TIMP (tissue inhibitor of metalloproteinases): a protein that blocks the activity of matrix metalloproteinases (MMPs).
TNF-α (tumor necrosis factor alpha): an inflammatory cytokine.
Virion: an individual virus particle.

X. The Future of HCV Therapy: Viral Targets and Drug Development

Introduction

The standard treatment for hepatitis C virus (HCV), combination therapy with pegylated interferon alfa and ribavirin, has limited efficacy, poor tolerability, and significant expense. New treatment options that are more potent and less toxic are desperately needed. Anecdotal reports suggest that many people with HCV are choosing—often based on the advice of their doctors—to defer treatment due to the limitations of interferon and ribavirin, in the hopes that better options will become available in the next few years. The complicated calculus of when and whether to initiate HCV treatment begins with an assessment of one's current disease state and risk of disease progression. However, for many, these considerations are superseded by the difficulties of managing treatment, including factors such as the duration of treatment, the risk of depression, the potential quality of life impairment, and the necessity of injecting interferon. Candidates for treatment who have HCV genotype 1, HCV viral loads exceeding two million, and/or coinfection with HIV must also confront the lower likelihood of achieving a sustained virological response (SVR). Most people in the United States with chronic HCV infection have one or more of these poor prognostic factors. New and better treatments could mitigate at least some of these concerns and make therapy for HCV more acceptable and, ultimately, more successful. Moreover, more effective treatments are an urgent priority for those who relapse or do not respond to current regimens.

The next advances in HCV treatment will improve on success rates but are unlikely to supplant pegylated interferon alfa and ribavirin as the backbone of therapy. Many of the new agents proposed for HCV treatment are being developed for use in combination with these existing drugs, though some compounds are under investigation as substitutes for or improvements over current formulations of interferon alfa and ribavirin. Neither interferon alfa nor ribavirin were developed specifically to treat HCV infection; both had been developed and approved for other indications well before the actual discovery of the hepatitis C virus. The mechanisms of action of interferon and ribavirin as treatment for HCV are not fully understood, and most likely involve multiple immunomodulatory and antiviral effects (J. Y. Lau 2002; Tanabe 2004; Taylor 2001b). Ongoing research aimed at clarifying these mechanisms may shed light on the nature of viral resistance and the causes of treatment failure. Such studies could provide new insights into strategies to improve treatment response and successfully retreat non-responders and relapsers. Studies of viral kinetics and changes in gene expression in individuals on treatment have begun to produce some answers, and have fueled hypotheses about the optimal timing, duration, and dosing of current therapy.

Most current drug development programs are oriented towards antiviral compounds that specifically inhibit HCV by targeting aspects of its replication cycle. Informed by a substantial body of basic research into the molecular virology of HCV, researchers and drug companies are exploring several viral targets and drug candidates. Progress has been delayed by the lack of cell culture and small animal models, standard tools to screen potential drugs. Recent advances in HCV replicon systems have finally enabled *in vitro* testing of a candidate compound's ability to inhibit viral replication, while chimpanzees have been used to study pathogenesis and immune response, yielding data that may contribute to efforts at finding a vaccine against HCV.

Several drugs currently in development target aspects of the HCV replication cycle, including translation initiation (antisense oligonucleotides and synthetic ribozymes), cleavage and processing (serine protease inhibitors), and RNA synthesis (RdRp inhibitors, helicase inhibitors, and nucleoside analogues). Other drugs with broad antiviral and immunomodulatory properties are also under investigation (Pawlotsky 2004b; Tan 2002). Most of these drugs are in very early stages of preclinical or clinical development; many of the most promising candidates, including NS3 serine protease inhibitors, will not be available outside of clinical trials until 2008 at the earliest (McHutchison 2002). A few therapeutic vaccine candidates, aimed at improving immune responses to HCV among people with chronic infection, have entered small clinical trials. Yet for the most part, the development of a vaccine to prevent HCV infection has not advanced beyond a few animal studies, and vaccine research faces significant hurdles due to HCV's genetic diversity (Himoudi 2002; Pancholi 2003).

Government and academic researchers, large pharmaceutical companies, and small biotechnology firms have undertaken dozens of HCV drug and vaccine development programs representing a diverse range of targets and strategies. Beyond the established medical need and public health importance of these efforts, a significant financial incentive underlies the substantial investment by industry in this field. Industry figures estimate the current size of the worldwide hepatitis C treatment market at $2.5 billion. The U.S. HCV market alone is currently calculated at $1.4 billion, representing 100,000 people treated each year. Commonly cited projections of the potential annual market for HCV therapeutics by 2010 typically range from $3 billion to $7 billion or higher. Based on these figures, a new HCV treatment would likely meet the pharmaceutical industry's criteria of a blockbuster drug: anticipated annual sales of $1 billion.

While commercial motives have generally benefited HCV research, they have also at times hindered progress due to the nature of the patent system. During the 1990s, some drug and vaccine studies were delayed by lawsuits initiated by Chiron against other companies, claiming that their drug development programs infringed Chiron's patents related to HCV (Cohen 1999). Similarly, access to ribavirin—a compound originally discovered in 1970—had been limited by exclusive licensing arrangements between ribavirin's developer, ICN Pharmaceuticals (through its former subsidiary, Ribapharm) and Schering-Plough, the manufacturer of standard and pegylated forms of interferon alfa-2b. This limited the ability to study and market potentially superior forms of interferon alfa in combination with ribavirin, while other actions kept the price of HCV therapy artificially high by delaying the introduction of generic, and hence cheaper, forms of ribavirin, which finally reached the market in April 2004. While these issues appear to be largely resolved for now, the potential inhibitory effect of intellectual property disputes on the development of new drugs, vaccines and diagnostics calls for scrutiny and vigilance, especially in emerging therapeutic areas such as RNA interference.

Targeting the HCV Replication Cycle

With better knowledge of the molecular virology of HCV, research has increasingly focused on viral targets specific to HCV, rather than general antiviral and immunomodulatory agents. Compounds identified through this work may ultimately increase sustained virological response rates. Except where noted, these drug candidates will be tested and used, at least initially, in combination with interferon alfa and, in most cases, ribavirin. In part, this relates to the perceived need for

combination approaches to therapy in order to offset the potential for developing drug resistance.

The high mutation rate of HCV poses a particular challenge to therapeutic strategies that directly target sections of the virus. For example, mutations in the NS3 serine protease could potentially abolish the antiviral efficacy of a protease inhibitor. As with immune escape, the selective pressure exerted by a protease inhibitor would favor the survival and replication of HCV virions bearing those mutations. Therefore multiple simultaneous strategies for inhibiting HCV replication—the use of interferon alfa and ribavirin alongside the serine protease inhibitor—would minimize the potential for the emergence of viral resistance. This approach to HCV therapy mirrors the standard treatment for HIV, which combines three antiretroviral drugs. In anticipation of future issues with HCV inhibitor resistance, the NIH's National Institute of Allergy and Infectious Diseases (NIAID) awarded a contracted for the development of a resistance test for HCV protease and RNA-dependent RNA polymerase inhibitors to ViroLogic, a company that markets assays for HIV drug resistance.

Ideally, several new agents will become available over the next several years, increasing therapeutic options. New drugs could ultimately facilitate a shift away from interferon alfa-based treatment regimens, and hopefully allow patients and doctors to construct combinations of anti-HCV agents tailored to individual circumstances. Such a scenario will not conceivably emerge for several years. Even the agents described below that have already entered clinical trials will most likely not be approved by the FDA until the second half of this decade. Yet the broad range of approaches to drug development is encouraging, increasing the odds of finding successful agents. Despite some gaps in research, largely reflecting lingering questions from molecular virology, HCV drug development programs are targeting virtually every stage of the viral replication cycle.

New approaches to HCV drug development can be divided into three general categories: agents that target viral enzymes (protease, RNA-dependent RNA polymerase, and helicase), agents that target viral envelope proteins (E1 and E2), and agents that target viral RNA. Some research suggests that cellular proteins involved in HCV replication may also be appropriate targets for drug development, though little work has been done in this area. In all cases, the goal of therapy is the disruption of the viral replication cycle.

Target: viral enzymes

NS3 serine protease inhibitors

The success of protease inhibitors in HIV treatment has inspired numerous drug development programs aimed at producing a protease inhibitor effective against HCV. Virtually all of these efforts have targeted the NS3 serine protease. Drugs that inhibit the NS3 serine protease would block cleavage, making viral replication impossible. NS3 serine protease inhibitors may further prevent the inhibition of interferon responses by HCV NS3 (Ferenci 2004; Foy 2003). Strategies to inhibit NS2-NS3 protease activity have received less attention, though a collaboration between Merck and Aurora Biosciences had made progress in identifying potential inhibitors prior to Aurora's acquisition in 2001 by Vertex (Waxman 2001; Whitney 2002). The candidate furthest along in clinical development, Boehringer Ingelheim's BILN 2061, has shown potent inhibitory effects both *in vivo* and *in vitro*, generating substantial enthusiasm for the prospects of this class of drugs.

Boehringer Ingelheim's BILN 2061

BILN 2061 is an oral NS3 serine protease inhibitor. Three phase I studies of people infected with HCV genotype 1 have looked at safety and the effect on viral load of a two-day treatment period. Treated subjects in each group received twice-daily 200 mg doses of BILN 2061, administered in a solution of polyethylene glycol and ethanol. In a pilot study, eight subjects with significant fibrosis receiving BILN 2061 all experienced at least a 2-log drop (a 100-fold decrease) in HCV viral load while on treatment; two subjects had a greater than 3-log, or 1000-fold, drop in viral load (Benhamou 2002; Lamarre 2003). A study of cirrhotic patients also showed greater than 2-log drops in viral load in all eight treated subjects (Wedemeyer 2003).

Similar, if less dramatic, results were seen in a third study of individuals with milder liver damage treated at different doses; 7 of 9 who received the 25 mg twice-a-day dose achieved a temporary viral load reduction of at least 1 log (10-fold), while all 8 subjects receiving either 200 mg or 500 mg twice-daily experienced viral load decrease greater than one log (Hinrichsen 2002). A study using similar design but looking at individuals with genotypes 2 and 3 found that BILN 2061 was less effective in non-1 genotypes. Four of the 8 treated subjects experienced viral load declines of greater than 1 log; another subject experienced a smaller drop in viral load, while the remaining three saw no change (Reiser 2003). In all studies, viral loads returned to baseline levels within a week after the two-day treatment period. No significant adverse events were reported in these trials or in dose-escalation studies of healthy volunteers. Based on pharmacokinetic data, BILN 2061 could be dosed twice daily (Lamarre 2003).

Based on these encouraging results, phase II studies of BILN 2061 were scheduled to begin in 2003 in Europe and the United States. These trials have been placed on hold while Boehringer researchers attempt to resolve toxicities observed in monkeys taking high doses of BILN 2061, reportedly related to cardiac abnormalities. As of May 2004, Boehringer has not made any further announcements on the status of their HCV protease inhibitor program.

Other compounds in or nearing clinical trials

Vertex has announced that it plans to bring VX-950, an NS3 serine protease inhibitor originally developed in partnership with Eli Lilly, into clinical trials in the first half of 2004. Initial trials launched in June will examine safety and dosing in healthy volunteers, with plans for a pilot study in people with HCV expected later in 2004. Vertex researchers report that VX-950 appears to inhibit HCV replicons containing a mutation conferring resistance to BILN 2061; similarly, BILN 2061 remains active against VX-950-resistant strains (C. C. Lin 2004).

Schering-Plough also has an active protease inhibitor development program, and has published data on one compound, SCH6, that demonstrated potent *in vitro* inhibitory effects in HCV replicon systems (Foy 2003; J. J. Lu 2003). A related compound has entered phase I clinical trials in healthy volunteers.

Several other companies reportedly have HCV NS3 protease inhibitors in development, including Abbott, Agouron/Pfizer, Bristol-Myers Squibb, Chiron, Eli Lilly, Gilead, GlaxoSmithKline, InterMune (in partnership with Array Biopharma), and Merck. GlaxoSmithKline has evaluated an HCV NS3

protease inhibitor in marmosets (a New World primate species related to tamarins) infected with GB virus B (GBV-B, a virus closely related to HCV—see Chapter VIII, The Molecular Virology of Hepatitis C). In this surrogate animal model for HCV infection, treatment with GW0014X, an HCV NS3 protease inhibitor, for four days (by subcutaneous injection twice daily) resulted in a transient 3 log (1,000-fold) reduction in GBV-B viral load (Bright 2004).

A novel gene therapy approach exploits the protease activity of NS3 to induce apoptosis in HCV-infected cells. Researchers in Canada have developed a modified form of the BID (BH3 interacting domain death agonist) molecule, which causes cells to undergo apoptosis. The molecule has been modified to act as a substrate for the HCV NS3 protease; upon entering an HCV-infected cell, the modified BID is cleaved by the HCV serine protease and thus activated, triggering cell death. In theory, the cells containing HCV would die off, leaving only uninfected hepatocytes. So far, *in vitro* data and studies in chimeric mice bearing human hepatocytes support this hypothesis (Hsi 2003; E.C. Hsu 2003). Further studies are underway.

Drug design issues

In theory, the NS3 serine protease should offer several potential points of intervention for therapeutic development, such as blocking the active site which catalyzes cleavage, interfering with the cofactor activity of NS4A, and inhibiting a zinc-binding site that forms a crucial structural component of properly-folded NS3 (Bartenschlager 1999; De Francesco 1996). The latter two approaches have yielded few leads, though some preliminary work on zinc-dependent inhibition has been conducted by Axys (acquired by Discovery Partners), Celera, and Bristol-Myers Squibb (Sperandio 2002; Yeung 2001). The active or substrate-binding site of the NS3 serine protease poses its own challenges, since the cleft or pocket where the enzyme binds to its substrate (the region targeted for cleavage) is quite shallow, and thus a difficult target for drug design (Lindenbach 2003; McHutchison 2002; Penin 2004). However, a number of compounds with the potential to target NS3's protease activity—at its active site or other susceptible regions—have been identified, including peptidic (peptide-based) inhibitors, non-peptidic inhibitors, and peptidomimetics (Bianchi 2002; Casbarra 2002; Fattori 2002; Ingallinella 2002; Narjes 2003).

These classes of compounds—peptidics, non-peptidics, and peptidomimetics—have different pharmacological properties, which can translate into differences in dosing and metabolism. Peptide-based compounds, which can mimic the NS3 protease's substrate, range from dipeptides, which contain two amino acids, to hexapeptides, composed of a linear chain of six amino acids (Fischmann 2002; Llinàs-Brunet 2000 [Boehringer Ingelheim]; Tan 2002). Peptidic inhibitors face challenges in bioavailability, since they tend to be degraded rapidly in the body. Non-peptidic inhibitors typically have different methods of binding to NS3, and in general have improved bioavailability over peptidic compounds. Peptidomimetics are compounds developed through structure-based design that mimic or antagonize peptides, with non-peptide-like properties that in theory overcome some of the pharmacokinetics limitations of peptides (Poupart 2001 [Boehringer Ingelheim]; Priestley 2000 [DuPont]; X. Zhang 2003 [Bristol-Myers Squibb]). For HIV treatment, all currently approved HIV protease inhibitors are peptidomimetics, though the first non-peptidic HIV protease inhibitor, Boehringer Ingelheim's tipranivir, is in late-stage clinical testing. BILN 2061 and VX-950 are peptidomimetic protease inhibitors, while SCH6 is a peptidic inhibitor.

The risk of viral resistance is likely to pose a major challenge to the development and clinical use of NS3 serine protease inhibitors, as it has with HIV protease inhibitors. A recent *in vitro* study using HCV replicons examined the potential for resistance to Compound 1, a Boehringer Ingelheim agent with protease inhibitor activity. HCV replicons were able to adapt in the presence of Compound 1 and develop resistance to its effects, with several mutations identified that conferred decreased susceptibility to inibition (Trozzi 2003). Mutations conferring drug resistance have also been identified for BILN 2061 and VX-950 (C. Lin 2004; L. Lu 2004). These findings were not unexpected. Indeed, the potential for resistance is virtually guaranteed for compounds targeting the NS3 protease as well as other viral enzymes, given the quasispecies nature of HCV and its aptitude for evolution in response to selective pressure from the immune system. However, the ability to anticipate resistance mutations through such *in vitro* methods will enable the optimization of candidate agents and, in principle, the development of protease inhibitor combinations that offset the risks of resistance (Lindenbach 2003). Ideally, companies with compounds in or approaching early clinical development—such as Boehringer Ingelheim, Schering-Plough, and Vertex—will collaborate on researching combination approaches after initial safety and efficacy has been demonstrated.

Progress in developing NS3 serine protease inhibitors and other classes of drugs directly targeting HCV has been delayed considerably by actions taken by Chiron. In 1998, Chiron filed suit against Gilead Sciences and Agouron (and later Vertex and Eli Lilly) for infringing on Chiron's HCV patents. Chiron filed a number of patents on the basis of its leading role in the discovery of HCV, including patents relating to the NS3 protease. The company maintained that research on protease inhibitors infringed on its patents, and demanded licensing fees from companies conducting research, and a guarantee of royalties from any products reaching the market. These claims had a chilling effect on the field, bringing several companies' drug development programs to a halt pending resolution of legal issues (Cohen 1999). To date, most of the patent disputes have been resolved, with companies that conduct research on protease inhibitors paying licensing fees to Chiron.

NS5B RNA-dependent RNA polymerase and NS3 helicase/NTPase

Both the NS5B RNA-dependent RNA polymerase (RdRp) and the NS3 helicase/NTPase constitute major targets for the development of antiviral therapies (Borowski 2002; McHutchison 2002; Tan 2002; Walker 2002; N. Yao 1998). Inhibition of either enzyme would disrupt HCV RNA strand synthesis, preventing the production of genomic HCV RNA for new virions. Part of ribavirin's mechanism of action may operate at this stage of the viral replication cycle (see 'Antiviral effects of ribavirin: from chain termination to lethal mutagenesis' later in this chapter). Several groups are developing other nucleoside analogues for HCV treatment, with some compounds already in clinical trials. Alternately, the HCV NS5B polymerase enzyme could be targeted directly. The RNA binding cleft of RdRp offers potential for small molecule inhibitors, an approach that has been employed to develop compounds active against the RdRp of bovine viral diarrhea virus (BVDV), a pestivirus closely related to HCV (Baginski 2000; Sun 2003).

Other compounds, described by Shire BioChem Inc. and academic collaborators, are characterized as non-nucleoside inhibitors of HCV replication, binding at a distance from the NS5B RdRp active site and preventing protein conformations necessary for enzymatic activity (L. Chan 2003; Reddy

2003; M. Wang 2003). This strategy is roughly analogous to the presumed mechanism of action of the non-nucleoside reverse transcriptase inhibitor (NNRTI) class of antiretrovirals used in HIV treatment—which include efavirenz (Sustiva®) and nevirapine (Viramune™)—and reflects the functional similarities between HCV RdRp and the HIV reverse transcriptase enzyme, an RNA- and DNA-dependent polymerase (Esnouf 1995; Hsiou 1996; Temiz 2002).

Various targets and candidates for helicase/NTPase inhibition have also been proposed, but none are currently in human clinical trials and no major companies have announced drug discovery programs for these targets (Borowski 2000; Borowski 2001; Borowski 2002; Phoon 2001).

RdRp inhibitors entering clinical trials

Some HCV RdRp inhibitors have recently moved towards clinical trials.

Idenix Pharmaceuticals (formerly Novirio) has an oral nucleoside analogue, NM283, in clinical development. Novartis has an option to jointly develop NM283. NM283 has shown potent anti-HCV activity in a chimpanzee study reported at the 2003 HEP DART meeting. The first data studying NM283 in people with hepatitis C was presented at conferences in the spring of 2004. A phase I/II dose escalation study examined the safety, pharmacologic profile, and antiviral activity of NM283 given for 15 days to people with HCV genotype 1. At the highest dose (800 mg, once a day), study participants experienced on average a 1 log (10-fold) decline in HCV viral load; the most frequent side effects were nausea and vomiting, which generally faded after the first two days of treatment (Godofsky 2004). A follow-up study to be conducted in the summer of 2004 will evaluate combination treatment with pegylated interferon and NM283 for four weeks. A larger, long-term combination therapy trial will begin in the second half of 2004.

JTK-003, an HCV NS5B RdRp inhibitor developed by Japan Tobacco (JT), has entered into phase II trials in Japan. Phase I trials of JTK-003 have been initiated in the United States by JT's U.S.-based subsidiary, AKROS Pharma. Japan Tobacco also has another RdRp inhibitor, JTK-109, in phase I studies.

Rigel Pharmaceuticals has begun clinical testing of its non-nucleoside RdRp inhibitor, R803. An initial phase I study in healthy volunteers was completed in January 2004. Rigel initiated a phase I/II trial of twice-daily R803 at different doses in people with HCV in May 2004, with results expected by the end of the year. Roche also has an HCV polymerase inhibitor, R1479, in phase I studies.

ViroPharma and Wyeth have collaborated in developing HCV RdRp inhibitors for several years. An early candidate called VP-50406, alternately described as a helicase and replication inhibitor, had advanced to phase II trials before ViroPharma and Wyeth discontinued further research on this agent due to poor *in vivo* antiviral activity (McHutchison 2002). ViroPharma used an HCV replicon system to screen for compounds with inhibitory activity against HCV RdRp. A compound dubbed HCV-371 entered phase I trials in January 2003, but ViroPharma terminated development of this compound due to study results showing no effect on HCV RNA in study participants, presumably due to poor pharmacokinetics.

ViroPharma initiated a phase I study in healthy volunteers of another candidate, HCV-086, in early 2004. Pending results from this initial trial, a phase Ib dose-ranging trial among people with HCV is planned for the second half of 2004, with phase II trials anticipated for 2005.

Compounds in preclinical development

Several nucleoside analogues are in development for HCV treatment. Merck, Pharmasset, and Ribapharm have all reported results from preclinical compound screening for anti-HCV nucleoside analogues (Carroll 2003; Murray 2003; Shim 2003; Stuyver 2003a). Roche has a nucleoside analogue, 1048297, in early clinical development. Metabasis Therapeutics received a grant from the National Institute of Allegies and Infectious Diseases to screen for nucleoside analogues active against HCV that specifically target the liver. Merck scientists have described a family of structurally related chain-terminating nucleoside analogues showing *in vitro* synergy with interferon alfa; a mutation conferring high-level resistance to the compounds has been identified (Migliaccio 2003).

A different version of nucleoside analogues, called nucleotide analogues, may also show promise in HCV therapy (Gallois-Montbrun 2003). Nucleosides must undergo phosphorylation (the addition of one or more phosphate groups) before achieving the form that enables them to intervene in strand synthesis. Nucleotides are nucleosides that have already been partly phosphorylated. In 2002, Biota and GlaxoSmithKline formed a collaboration to screen nucleotides for anti-HCV activity.

Merck scientists have identified two classes of nonnucleoside inhibitors of NS5B RdRp and used HCV replicon cultures to describe mutations that confer resistance to one class of these compounds while remaining sensitive to inhibition by other RdRp inhibitors; other types of NS5B polymerase inhibitors have also been described by this group (Summa 2004; Tomei 2003; Tomei 2004). Merck has formed an HCV drug development partnership with Metabasis. Metabasis will apply its proprietary liver-targeting prodrug technology to Merck compounds, which may reduce any systemic toxicities associated with these drugs and improve their efficacy. GlaxoSmithKline researchers have characterized an agent called Compound 4 that inhibits the initiation of RNA synthesis *in vitro* and is synergistic with interferon alpha; an NS5B mutation associated with resistance to Compound 4 has been identified (Gu 2003b; Johnston 2003; Nguyen 2003).

Several other companies have non-nucleoside HCV RdRp inhibitors in preclinical development. Scientists from Pfizer and Boehringer Ingelheim have each recently described non-nucleoside inhibitors of HCV RdRp (Beaulieu 2004; Love 2003; McKercher 2004). Israel's XTL Biopharmaceuticals plans to develop non-nucleoside inhibitors identified and evaluated in preclinical testing by South Korea's B&C Biopharm (Dagan 2003). XTL plans to file an application with FDA in the second half of 2004 to begin clinical trials of a polymerase inhibitor. Genelabs is screening nucleoside analogue and non-nucleoside RdRp inhibitors that can inhibit HCV replication in replicon models. BioCryst is also evaluating compounds identified by rational drug design methods for anti-RdRp activity. Roche recently entered a partnership with the Swedish company Medivir focused on discovery of HCV NS5B polymerase inhibitors. Abbott and Eli Lilly are also pursuing development of HCV polymerase inhibitors.

Target: viral envelope proteins

Alpha-glucosidase inhibitors, entry inhibitors, monoclonal antibodies, and immunoglobulin

The post-cleavage modifications of the HCV envelope proteins E1 and E2 may also present a target for drug development. E1 and E2 both undergo glycosylation (the addition of sugar molecules, or glycans) in the endoplasmic reticulum (ER). The glycosylation of E1 and E2 leaves glucose residues on the N-linked glycans that are trimmed off by cellular enzymes, the ER α-glucosidases. The removal of these extra glucose residues is a prerequisite for proper folding, since it allows the glycoproteins to associate with the chaperone proteins calnexin and calreticulin (Helenius 2001). Therefore, inhibitors of ER α-glucosidases should prevent proper folding and heterodimerization of HCV E1 and E2, ultimately blocking the assembly of infectious viral particles (Branza-Nichita 2001; Dwek 2002). Derivatives of imino sugars, compounds that mimic monosaccharides, have been evaluated *in vitro* as ER α-glucosidase inhibitors, with potent antiviral activity that compares favorably to the effects of interferon alfa and ribavirin (Durantel 2004). Castanospermine, a glucose imino sugar, has potent inhibitory effects on glycosylation. Celgosivir (MBI-3253), a pro-drug of castanospermine, has been licensed by the Canadian company Micrologix from Virogen, a U.K. firm. Micrologix plans to initiate phase II trials of celgosivir as an HCV therapy in 2004.

The plethora of promising drugs in development to inhibit the entry of HIV into target cells, along with the FDA approval of the HIV entry inhibitor, enfuvirtide (Fuzeon™), offers encouragement for antiviral strategies targeting HCV entry. HCV entry inhibitors would likely attempt to prevent HCV from binding to its receptor(s), thus blocking the infection of new cells (Lahm 2002). However, the development of such inhibitors would require better knowledge of HCV receptor usage, binding sites on the E1 and E2 glycoproteins, and conformational changes—alterations in the three-dimensional structure of the proteins—induced by receptor binding (see Chapter VIII, The Molecular Virology of Hepatitis C).

Given the uncertainties about the mechanisms of HCV entry, little research has explored strategies for entry inhibition. A team at Kansas State University has synthesized compounds that block HCV envelope protein E2 from binding to CD81 *in vitro* by mimicking part of the CD81 receptor (VanCompernolle 2003). Compounds related to amantadine that may potentially block HCV binding to CD81 *in vitro* have also been reported by researchers at University of California-Irvine, but it remains unclear whether this mechanism will inhibit HCV entry and viral replication (Wagner 2003). Progenics has expressed an intention to develop therapies that block HCV from binding to L-SIGN (liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin), but the company does not have an active HCV drug development program, and L-SIGN has not been thoroughly validated as a target for HCV entry inhibition (see Chapter VIII, The Molecular Virology of Hepatitis C).

In lieu of targeting receptors used by HCV for cell entry, some researchers have attempted to target the envelope proteins themselves. Several groups have pursued the development of monoclonal antibodies (mAbs) that can neutralize HCV (Cerino 2001; C. Li 2001; Y. H. Zhou 2000). Monoclonal antibodies are derived from a B cell line, sometimes taken from individuals infected with HCV, that is engineered to produce identical antibodies, all targeting the same region of E1 or

E2. Monoclonal antibodies have also been used to identify sites of the properly folded E1-E2 heterodimer susceptible to targeting and neutralization (Burioni 1998; Clayton 2002; Cocquerel 2003a; Habersetzer 1998; Hadlock 2000).

XTL Pharmaceuticals, based in Israel, has begun clinical testing of a human monoclonal antibody (mAb) directed against E2. The mAb, designated HepeX™-C (formerly called XTL-002), is administered by infusion. HepeX-C demonstrated safety and efficacy in phase I trials, and has entered phase II studies to determine whether it can prevent recurrence of HCV viremia in chronically infected patients undergoing liver transplants. XTL has chosen to focus its drug development exclusively on liver transplant recipients, abandoning immediate plans to further research HepeX-C in the larger pool of individuals with chronic HCV infection due to "market conditions" (XTL 2002). In late 2003, XTL indicated that further clinical development will require additional financial support, and that the company will seek a development partner. XTL also halted one of the dosing arms in a HepeX-C study in May 2004, pending further investigation of the death of a trial participant (a liver transplant recipient). XTL has licensed other anti-HCV monoclonal antibodies, also directed against HCV E2, from Stanford University. XTL plans to initiate studies of HepeX-C in combination with one of the newly licensed mAbs.

The Danish company Genmab has an anti-HCV E2 monoclonal antibody, HuMax-HepC, in preclinical development for treating post-transplant HCV re-infection. HuMax-HepC is based on an antibody isolated from an individual with chronic HCV infection and was licensed from Connex GmbH and INSERM, the French National Institute for Health and Medical Research. Genmab is using a proprietary technology to produce fully human monoclonal antibodies from transgenic mice, rather than typical processes that generate monoclonal antibodies in mice which then need to be humanized to remove mouse proteins. Nabi Pharmaceuticals is studying its HCV immunoglobulin Civacir™, an infusion of antibodies to HCV collected from the blood of individuals who are HCV-antibody positive, for the prevention of HCV recurrence following liver transplantation. This approach has proved successful in preventing HBV recurrence; the initiation of a NIAID-sponsored randomized phase I/II trial was announced in December 2002. Nabi announced in February 2004 that preliminary results from this study demonstrated safety and a trend towards reduced ALT levels; further trials are under consideration. A similar effort by the Canadian company Cangene to prevent post-transplant recurrence through anti-HCV hyperimmune serum (blood containing highly reactive antibodies to hepatitis C) was terminated after failure in a phase II trial.

Other viral protein targets: p7

Imino sugar derivatives

The putative viroporin (pore-forming) activity of HCV protein p7, hypothesized to form ion channels on cell membranes, may provide another target for drug discovery. Some *in vitro* research shows that the antiviral drug amantadine can block p7's ability to form ion channels, but amantadine does not appear effective in clinical trials as an HCV treatment (Griffin 2003; Griffin 2004; see 'General Approaches to HCV treatment: Immune modulators and broad-spectrum antivirals' later in this chapter). However, other compounds, including imino sugar derivatives, may effectively target p7. NN-DGJ is an imino sugar derivative that does not inhibit

α-glucosidase, but rather appears to interfere with viral replication by blocking the formation of ion channels by p7 (Pavlović 2003). Recent research suggests that NN-DGJ may also exert antiviral activity through other mechanisms, including induction of cellular defenses that included interferon-stimulated genes such as 2′,5′-OAS (A. S. Mehta 2004; see Chapter IX, Immune Response, Persistence, and Pathogenesis).

While the antiviral potential of imino sugar derivatives has been recognized for several years, development of agents for HCV treatment has been slow, with a lead candidate only recently entering clinical trials. The convoluted path from laboratory to clinic for these compounds began with academic research conducted through collaborations between the Oxford University Glycobiology Institute and the Thomas Jefferson University Medical School in Philadelphia. In an effort to develop therapeutic uses for imino sugar derivatives, these scientists formed a research partnership in 1998—IgX Oxford Hepatitis—with a New Jersey-based biotech, the IgX Corporation, which subsequently changed its name to Synergy Pharmaceuticals. SP231B, a NN-DGJ compound resulting from this work, underwent further development by Synergy, which dubbed this class of imino sugar derivates "alkavirs" based on their alkyl side chains. Synergy, which was acquired by Callisto Pharmaceuticals (formerly Webtronics) in 2003, licensed SP231B to United Therapeutics for development as an HCV treatment. This compound—now called UT-231B— appears to function by blocking p7 ion channels rather than through α-glucosidase inhibition (Pavlović 2003). UT-231B completed phase I trials in healthy volunteers in 2003. A 12-week phase II dose-ranging "proof-of-concept" study in people with HCV who failed standard treatment is underway and expected to be completed by the end of 2004.

Target: viral RNA

Some drug discovery programs have focused on HCV RNA, rather than viral proteins. Conserved HCV RNA elements required for efficient viral replication make an attractive target for inhibition, and approaches that interfere with protein synthesis would disrupt an essential step in viral replication (Jubin 2001; Jubin 2003). This approach underlies Rigel Pharmaceuticals' attempts to develop an inhibitor that targets the HCV internal ribosomal entry site (IRES), thus blocking translation initiation. PTC Therapeutics also has a drug development program aimed at disrupting HCV translation.

Most research to date has largely focused on a field called nucleic acid therapeutics, which studies the antiviral potential of RNA molecules that can bind to HCV RNA sequences. These RNA molecules include ribozymes and antisense oligonucleotides. Most recently, a new technique called RNA interference has generated new excitement for its therapeutic potential, though clinical applications remain years away. These strategies target HCV RNA after its release into the cytoplasm, and aim to block translation of viral proteins.

Ribozymes

Ribozymes are enzymes composed of RNA, unlike most enzymes, which are generally proteins. Ribozymes can bind to and catalyze the cleavage of specific mRNA sequences, thus blocking protein synthesis. Studies in the 1990s showed promising results *in vitro* for ribozymes constructed to target the HCV 5′ UTR and other regions involved in translation and replication (Ohkawa 1997;

Sakamoto 1996; Welch 1996; Welch 1998). More recently, sites accessible to ribozyme-mediated cleavage have been identified on the folded structure of the 5′ UTR, with the suggestion that multiple sites be targeted simultaneously (Nadal 2003). Alternately, trans-splicing ribozymes can introduce new genetic material into a targeted region of RNA (Long 2003). A South Korean group has used this technique to introduce a gene sequence encoding the diphtheria toxin A chain that undergoes HCV IRES-dependent translation, resulting in the death of HCV IRES-containing cells (Ryu 2003).

Heptazyme™, a synthetic ribozyme developed by Ribozyme Pharmaceuticals (RPI) in collaboration with Eli Lilly, targets the 5′ UTR and successfully inhibited replication in cell culture of a chimeric HCV-poliovirus that uses the HCV IRES to initiate translation (Macejak 2000; Macejak 2001a). Heptazyme could be administered by injection subcutaneously or intravenously; a phase I trial found that Heptazyme was relatively well tolerated (Macejak 2001b; Sandberg 2001). However after initiating a phase II trial (put on hold due to primate toxicology—loss of vision in one animal), RPI opted to discontinue development of this drug, presumably due to relatively weak preliminary data on clinical efficacy as well as toxicity issues.

Antisense oligonucleotides

Antisense oligonucleotides are short sequences of RNA or DNA, commonly 15 to 20 nucleotides in length, complementary to a sequence of viral RNA. The antisense oligonucleotide hybridizes with, or binds to, the complementary sequence, blocking translation initiation or targeting the RNA for enzymatic degradation by RNase H. In vitro studies from several research groups in the 1990s have demonstrated the potential of antisense oligonucleotides to identify targets on the 5′ UTR and inhibit HCV translation. Since then, continued evolution in technical knowledge has led to the construction of synthetic oligonucleotides which are themselves resistant to cellular degradation and show a high sequence specificity restricted to their target region (Braasch 2002; Faria 2001; Heintges 2001; McCaffrey 2003b; Tallet-Lopez 2003; Toulmé 2001).

One compound currently under investigation, ISIS 14803, has been developed by Isis Pharmaceuticals (originally in partnership with Elan Corporation) to hybridize with the HCV IRES, and showed safety and efficacy in initial phase II trials (Gordon 2002). In May 2003, Isis announced the initiation of phase II trials of ISIS 14803 (given by injection) in combination with pegylated interferon and ribavirin in a group of 30 patients, all with genotype 1 virus, who did not respond to prior pegylated interferon/ribavirin treatment. A phase I/II study is examining the prospect of intensifying standard pegylated interferon/ribavirin treatment by adding ISIS 14803. People who have not achieved a 2 log drop in HCV viral load by 12 weeks of standard combination therapy or an undetectable viral load by 24 weeks will receive 12 weeks of ISIS 14803 (given twice-weekly as a two hour intravenous infusion) while continuing with pegylated interferon and ribavirin. ISIS 14803 is also being studied as a single agent for HCV treatment. AVI BioPharma also has an antisense compound targeting HCV in preclinical development.

RNA aptamers, another type of oligonucleotide, may also inhibit HCV protein synthesis. RNA aptamers (also referred to as RNA ligands) are short RNA sequences that fold into a particular conformation. This folding allows aptamers to bind to the three-dimensional structures on viral mRNA, such as the stem and hairpin loops found in the HCV IRES. Thus, in contrast to the binding

properties of antisense oligonucleotides, which rely on complementary RNA sequences, RNA aptamers can bind to HCV RNA based on conserved structures on the viral genome. RNA aptamers have been identified for the HCV IRES and the 3′ UTR that can inhibit translation (Aldaz-Carroll 2002; Kikuchi 2003; Toulmé 2003).

RNA Interference

RNA interference (RNAi, also referred to as gene silencing) is an antiviral mechanism first discovered in plants. RNAi relies on small interfering RNA (siRNA—double-stranded RNA sequences barely over 20 nucleotides in length). These siRNA hybridize (bind) to a complementary sequence of mRNA, thereby targeting the mRNA for degradation by the cell. These siRNA can be designed to bind to specific mRNA sequences, and thus may have broad therapeutic potential.

Several groups have recently synthesized siRNAs that inhibit HCV protein synthesis and replication in cell cultures. Inhibition of a chimeric HCV NS5B/luciferase protein using RNAi has been demonstrated in mice (Kapadia 2003; McCaffrey 2002; Randall 2003; P. S. Ray 2004; Wilson 2003). Researchers from Chiron tested siRNAs targeting various regions of the HCV 5′ UTR in an Huh7 human hepatoma cell line, showing strong and specific inhibition of translation (Seo 2003). A recent study identified different degrees of susceptibility to siRNA inhibition across different regions of the HCV 5′ UTR (Krönke 2004). This study also used an engineered retrovirus vector to successfully deliver short hairpin RNA (shRNA, related to siRNA) effectively to cells, suppressing the replication of HCV replicons.

Mouse studies have shown the potential for siRNA as a therapy against liver disease. siRNA has been shown to protect mice from fulminant hepatitis, or acute hepatic failure, and fibrosis through blocking Fas-mediated cell death (E. Song 2003). A similar study protected mice from acute liver failure through siRNA targeting caspase 8, an enzyme involved in apoptosis (Zender 2003). Two reports have demonstrated inhibition of hepatitis B virus (HBV) through siRNA in a mouse model of HBV infection (Giladi 2003; McCaffrey 2003a).

As a new research tool and potential therapeutic target, the mechanics of RNAi are still being explored. Recent reports indicate that some siRNAs, despite their short length, can upregulate interferon-stimulated genes, at least in part via PKR, thus complicating analysis of their antiviral efficacy (Bridge 2003; Sledz 2003). Alterations in the techniques used to synthesize siRNA may reduce their potential for inducing interferon responses (D. H. Kim 2004). Other research has suggested that some siRNA can degrade target mRNA, albeit less effectively, even when slightly mismatched in complementary nucleotide sequences. A recent study also found that siRNA can affect expression levels of a non-targeted gene with a partial overlapping genetic sequence, raising concerns for RNAi therapeutics about selectivity and the risk of inadvertently targeting cellular genes (Jackson 2003; Pusch 2003, Saxena 2003; Scacheri 2004).

These questions have not dampened commercial interest in exploiting RNAi; companies reported to be developing RNAi therapies for hepatitis C include Acacia/CombiMatrix (collaborating with Spain's Fundació irsiCaixa), Alnylam, Australia's Benitec (which recently acquired the California-based Avocel), Nucleonics, and Sirna (Check 2003). Sirna expects to identify a lead candidate for entry into clinical testing in 2004; other companies have announced plans to file IND applications

with FDA as early as 2005.

Intellectual property battles threaten to overshadow the scientific challenges of siRNA therapeutics; a number of companies have filed potentially conflicting patent claims on RNAi technology. In theory, this could stall or jeopardize development efforts, as occurred with HCV drug development in general around Chiron's HCV patents. Indeed, Benitec has already filed patent infringement suits against rival Nucleonics and two other companies, claiming violation of Benitec's patented gene silencing technology. These issues should be resolved through a framework that grants broad and open access to technological innovations with reasonable but not burdensome provisions for licensing fees. If patent disputes restrict drug development efforts and suppress competition, people with hepatitis C will suffer.

Drug design issues

Little of the work on ribozymes and antisense oligonucleotides has translated into clinical research on HCV drug development. This reflects in part the need for more research to clarify the potential for safe and effective uses of these classes of therapeutic agents. For instance, ribozymes risk inadvertently cleaving cellular mRNA if they are not carefully designed to specifically and selectively target viral RNA. Therapeutic ribozymes must therefore be highly selective for viral RNA so as not to disrupt important cellular processes and risk toxicity and side effects. Drug delivery—the effective targeting of therapeutic agents to the appropriate cells and tissues—also poses a substantial challenge for clinical applications of ribozymes, since these molecules are easily degraded in the body before reaching the target cell. Drug delivery and tissue targeting remain important obstacles, though some progress has been made on these technical issues.

By the 1990s, both ribozymes and antisense oligonucleotides had attracted considerable interest and investment as potential therapeutics for a broad range of conditions. Ribozymes and antisense oligonucleotides still play important roles as research tools for molecular biology, but enthusiasm about their clinical value has diminished in many quarters. Some of this apparent retreat from the optimism surrounding RNA-based therapeutics reflects the vicissitudes of the biotech investment market, which punished companies for failing to live up to the excessive hype surrounding these technologies in the early-to-mid 1990s.

Skepticism has also mounted in the face of disappointing results from clinical trials—particularly the failure in a phase III trial of ISIS Pharmaceutical's antisense compound to treat Crohn's disease. The field has not fully overcome concerns about side effects and issues with drug delivery (Dove 2002; Opalinska 2002). To date, FDA has only approved one antisense oligonucleotide (Isis' Vitravene®, for the treatment of CMV retinitis, approved in 1998) for marketing, though several other compounds are in clinical trials. FDA has not approved any ribozyme-based therapies.

The hype that once surrounded ribozymes and antisense oligonucleotides now centers on RNA interference. In some quarters, excitement about RNA interference has all but eclipsed interest in ribozymes and antisense oligonucleotides as potential therapeutics for HCV. Indeed, Ribozyme Pharmaceuticals has shifted its focus entirely from developing ribozymes to RNA interference. The company has gone so far as to rename itself as Sirna Therapeutics, to reflect its new focus on small interfering RNA (siRNA).

Approaches using siRNA, while promising, also face challenges in drug delivery (getting the siRNA to the target cell) and the durability of their therapeutic effect, which tends to be transient using current methods (Kitabwalla 2002). Chemical modifications to siRNA may allow these molecules to resist degradation until they reach target cells (Chiu 2003; Czauderna 2003; Dorsett 2004; Layzer 2004; Muratovska 2004). Based on mouse studies, one group recommends exploring the use of siRNA in a solution of lipiodol (iodine in poppy-seed oil), injected directly into the portal vein of the liver (Zender 2003; Zender 2004). Another potential strategy would involve using viral vectors containing genetic sequences designed to express siRNA; the viral vectors would infect target cells and deliver the genetic sequences enabling intracellular production of siRNA, as in gene therapy (Check 2003; Devroe 2004; Dorsett 2004; Krönke 2004; Yokota 2003). Despite unresolved questions about translating siRNA approaches into therapeutic applications, RNA interference has attracted considerable interest, drawing many researchers into the nascent field, and research is advancing rapidly. Still, the therapeutic potential of siRNA will not be realized for several years, assuming that research overcomes obstacles similar to those facing groups developing antisense oligonucleotides as therapeutic agents (Dove 2002; Jubin 2003; Opalinska 2002; Robinson 2004).

Resistance may also pose a challenge for RNAi therapy. As with ribozymes and oligonucleotide analogues, siRNA molecules must be designed with high specificity for the targeted region of HCV RNA, yet that specificity also makes RNAi approaches vulnerable to the emergence of mutations that escape siRNA hybridization. Such resistance has already been seen *in vitro* during studies of siRNA targeting HIV and poliovirus (Boden 2003; A. T. Das 2004; Gitlin 2002). Resistance could in theory be prevented by using multiple siRNAs with different viral targets (Lieberman 2003; Saksela 2003). Recent *in vitro* research using HCV replicons has demonstrated the viability of this strategy, using multiple siRNAs targeting the HCV 5′ UTR and HCV coding sequences within the open reading frame to inhibit replication (Krönke 2004). It has not been determined whether HCV and other human viruses possess other defensive strategies to evade RNA interference, as has been described with plant viruses (Vargason 2003; K. Ye 2003).

Target: cellular proteins

HCV replication could potentially be blocked by interfering with cellular proteins required for efficient translation of viral proteins or strand synthesis, as an alternative to directly targeting the HCV viral enzymes and HCV RNA. In theory, agents that target cellular factors may reduce the risk of drug resistance from viral mutation, since the human genes encoding a targeted cellular protein would not mutate in response to therapy. However, recent evidence from poliovirus suggests that drug resistant mutations that obviate the antiviral effects of compounds targeting cellular proteins can occur in some situations (Crotty 2004).

A number of cellular proteins have been implicated in HCV replication and may provide effective targets for drug development. The recent recognition that inhibition of geranylgeranylation can disrupt HCV replication *in vitro* suggests that prenylation inhibitors, a broad class of drugs that includes statins, may have potential for HCV treatment (J. Ye 2003; see also Chapter VIII, The Molecular Virology of Hepatitis C). Prenylation inhibitors are currently under investigation for the treatment of the hepatitis delta virus and may have activity against a broad range of viruses (Einav 2003b).

Research targeting cellular factors has attracted little activity to date, in part because the specific cellular proteins involved in HCV replication have not been fully identified, and their roles are still being explored. In addition, strategies that target cellular proteins risk interfering with important cellular processes. Nevertheless, some researchers have pursued strategies aimed at cellular cofactors involved in HCV IRES-directed translation.

A 60-nucleotide RNA molecule isolated from yeast (Saccharomyces cerevisiae) can selectively inhibit the cap-independent translation initiation of various viral IRES, including HCV (S. Das 1998a; S. Das 1998b; Venkatesan 1999). The small RNA molecule, dubbed inhibitor RNA (IRNA), does not appear to disrupt inhibition by binding to viral RNA, as in the case of antisense oligonucleotides. The IRNA has been shown to bind to the La protein, apparently mimicking the region of the HCV IRES to which La binds (S. Das 1996; S. Das 1998b). The researchers hypothesize that HCV translation is inhibited through competition between the HCV 5′ UTR and the IRNA for the La protein, which may be required for efficient protein synthesis (S. Das 1998a).

While the yeast RNA itself may not be readily adopted for clinical use, interference with cellular factors involved in translation regulation may be a viable alternative approach to antiviral drug development. BioZak, Inc., a California-based company, hopes to develop or license BZK111, a peptide 18 amino acids in length derived from the IRNA research. BAK111 apparently inhibits viral protein synthesis through competitive binding to the HCV IRES, which blocks La protein and ribosome binding. Targeting cellular proteins such as La involved in HCV translation provides another strategy for inhibiting viral replication that remains largely unexplored, though Anadys Pharmaceuticals is collaborating with German researchers on identifying potential targets among cellular factors essential for HCV protein synthesis. The German company Axxima is also investigating target host cell proteins involved in regulating HCV replication, including gastrointestinal-glutathione peroxidase (GI-GPx). Hybrigenics, a French company, is mapping viral and cellular protein-protein interactions as part of its HCV drug target identification program.

Researchers associated with Immusol and the University of California San Diego School of Medicine have used ribozymes in research to identify a role for human 20S proteasome α-subunit PSMA7 in HCV translation. Their group found that a hairpin ribozyme designated Rz3'X, originally designed to target the HCV 3′ UTR, apparently exerts its effects on HCV IRES-directed translation by cleaving PSMA7 mRNA (Krüger 2001a; Krüger 2001b). The nature of PSMA7's involvement in HCV translation remains unclear.

Mechanisms of Action for Current HCV Therapy: Interferon and Ribavirin

Introduction

The future of HCV therapy will build on the present—combination therapy with interferon alfa and ribavirin. In most cases, drugs in development for HCV treatment will be used, at least initially, with this combination, or attempt to improve on and substitute for current versions of interferon or ribavirin. Ironically, some of the antiviral properties and effects of the newer anti-HCV agents being developed through rational drug design approaches will, in many cases, be better defined than those of the drugs that have been used in HCV therapy for years. Interferon alfa (originally approved for HCV treatment in 1992) and ribavirin (approved in combination with interferon alfa in 1998) each have a range of antiviral and immunomodulatory effects, making it difficult to pinpoint specific mechanisms associated with response to HCV treatment. Patterns of virological response to combination therapy with interferon alfa and ribavirin also vary significantly according to viral and host factors, indicating that the effect of treatment with these drugs is not necessarily uniform. Nevertheless, a better understanding of the mechanisms through which interferon alfa and ribavirin exert their effects on HCV would aid in designing therapeutic strategies that can augment or refine current combination therapy (Gale 2003). Ultimately, research into the mechanisms of action of current combination therapy may create possibilities for adjunctive therapy that complements their effects, or for safer and more effective alternatives to these drugs.

Some probable mechanisms of action for interferon alfa and ribavirin can be hypothesized, despite much inconclusive or inconsistent research:

- Interferon alfa can trigger cellular defenses that block viral replication.
- Ribavirin may also disrupt or inhibit viral replication during the course of treatment.
- Interferon alfa and ribavirin may both modulate the immune response to HCV, restoring or enhancing an HCV-specific T_H1-type response and/or protecting against inflammation and fibrosis.

Interferon alfa and ribavirin appear to operate synergistically (Buckwold 2004; Tanabe 2004). When used alone, ribavirin does not reduce viral load, though it may reduce ALT levels and inflammation. Similarly, response rates to interferon monotherapy are substantially lower than to combination treatment.

Interferon alfa

Interferon alfa used in HCV treatment is a synthetic form of IFN-α secreted by cells in response to infection*. Endogenous IFN-α is a cytokine, a type of protein involved in immune responses, first discovered in 1957 and named to reflect its role in interfering with the viral replication cycle (see Chapter IX, Immune Response, Persistence, and Pathogenesis).

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* For clarity, 'interferon alfa' will be used to describe formulations used in treatment, and 'IFN-α' will refer to the endogenous substance produced by cells in response to infection.

IFN-α, along with IFN-β and the recently discovered IFN-ω, are classified as type I interferons, while IFN-γ is considered a type II interferon. IFN-α and other type I interferons bind to the IFN-α receptor (IFNAR, composed of two subunits, IFNAR1 and IFNAR2) expressed on cell surfaces. The IFN-α gene family consists of genes encoding over two dozen closely related but distinct IFN-α proteins, referred to as IFN-α subtypes and distinguished by number (IFN-α1, IFN-α2, etc.). For the most part, these subtypes are thought to act similarly, but differences in function and induction (by cell type and by viral stimulus, for example) have been noted (Castelruiz 1999; Foster 1998; Hilkens 2003; Larrea 2001). The liver may primarily express the IFN-α5 subtype, which has also been the predominant subtype found in the blood of individuals with HCV infection, though various other subtypes have also been detected alongside IFN-α5 (Castelruiz 1999; Larrea 2001). The clinical relevance of these observations is unclear; the two forms of alpha interferon most widely used in combination treatment for HCV infection both derive from IFN-α2.

The mechanisms of action of interferon alfa treatment are linked to the anti-HCV activity of endogenous IFN-α. Cells produce IFN-α very early in HCV infection, triggering cellular defenses and invoking immune responses. Endogenous IFN-α does not successfully control viral replication during acute HCV infection (Bigger 2001; Pavio 2003b). Exogenous interferon alfa—interferon alfa used as treatment—presumably operates through the same mechanisms as endogenous IFN-α. Treatment with interferon alfa would thus augment the antiviral and immunomodulatory effects of endogenous IFN-α to the level required for effective control of HCV, and overcome viral resistance to lower levels of IFN-α. The precise nature of the cellular defenses and immune responses induced by alpha interferon therapy remains unclear. Interferon alfa treatment also appears to reduce the risk of hepatocellular carcinoma, even in individuals who do not achieve a sustained virological response, through unknown mechanisms (Hino 2004).

Antiviral effects of interferon: signaling pathways and interferon-stimulated genes

Viral infection of a cell triggers a series of intracellular signaling events that stimulate the expression of genes and results in the secretion of endogenous IFN-α. The IFN-α secreted by the infected cell interacts with neighboring cells through interferon receptors, initiating another cascade of intracellular signals that direct the expression of antiviral genes. The induction of IFN-α expression is coordinated by interferon regulatory factors (IRFs), particularly IRF-3 and IRF-7; IRFs belong to a class of proteins called transcription factors that regulate gene expression. Multiple other signaling pathways also play a role in this process, including the NF-κB, JAK/STAT, and JNK pathways. The particular pathways through which interferon alfa therapy exerts its antiviral effects on HCV replication have not been well established. However, research in this area has already benefited from the increased use of microarray analysis—a powerful new tool for studying changes in gene expression—to examine the effects of interferon alfa in HCV replicon systems and in individuals undergoing HCV treatment.

IFN-α conducts its intracellular defense against HCV through the products of these interferon-stimulated genes (ISGs). The expression of ISGs induces an antiviral state in cells, by establishing conditions within the cell unfavorable for or actively hostile towards viral replication. Strategies used by ISGs that restrict viral replication range from the inhibition of cell growth, to suppression of protein synthesis, to apoptosis. Hundreds of ISGs have been identified, many of which may contribute to antiviral defenses; the full complement of ISGs may extend into the thousands (de

Veer 2001; Grandvaux 2002). Most research has focused on the three "classical" interferon-stimulated pathways: PKR, 2′,5′-OAS/RNase L, and Mx proteins (see Chapter IX, Immune Response, Persistence and Pathogenesis). These pathways, as well as ISG56 (also referred to as p56), have all been explored in relation to their contribution to the antiviral defense against HCV.

Attempts to identify changes in ISG expression levels in chronic HCV infection and during interferon alfa therapy have produced mixed results that are difficult to correlate with treatment outcomes. Most likely the antiviral defense triggered by endogenous and exogenous forms of IFN-α involves multiple pathways and extends beyond the most frequently studied ISGs. Indeed, one group examined blood samples from seven individuals initiating HCV treatment and found changes in the expression of over one thousand genes within three hours after the dose of interferon alfa (Ji 2003). Any single ISG pathway may ultimately be less important than the interactions between the networks of genes regulated by IFN-α. Hopefully future studies will clarify the key ISGs involved in viral suppression and provide a foundation for optimizing interferon alfa-based therapy (Gale 2003). Some research already suggests that interferon alfa may facilitate viral clearance through routes that do not necessarily, or even primarily, lead to apoptosis (Guo 2003). In theory, the identification of key gene expression patterns associated with treatment success could indicate whether current treatments derived from the IFN-α2 subtype induce the most effective cellular defenses.

Antiviral effects of ribavirin: from chain termination to lethal mutagenesis

Ribavirin was first synthesized by researchers at ICN Pharmaceuticals (recently renamed Valeant) in 1970, and was approved for HCV treatment in combination with alpha interferon in 1998 (J. Y. Lau 2002). Ribavirin shows antiviral activity against a broad range of viruses, and is used as monotherapy to treat respiratory syncytial virus and Lassa fever; most recently it has been used with corticosteroids for the treatment of SARS (Koren 2003; Snell 2001). Ribavirin has limited effects on HCV viral load when administered alone, but appears to work synergistically with alpha interferon treatment (Bodenheimer 1997; Di Bisceglie 1995; Dusheiko 1996). However, ribavirin monotherapy may have value in the treatment of non-responders to combination therapy with interferon alfa. A placebo-controlled study of 48 weeks of ribavirin maintenance therapy in 34 individuals who did not respond to combination therapy found reductions in hepatic inflammation in 8 subjects receiving ribavirin (47%) and no members of the control group (Hoofnagle 2003).

Ribavirin belongs to a class of drugs called nucleoside analogues, which block viral replication during strand synthesis. Nucleoside analogues are compounds that mimic nucleotides and therefore can be incorporated by polymerase enzymes during chain elongation. Incorporation of a nucleoside analogue disrupts strand synthesis by terminating the growing chain of nucleotides. This is the mechanism of action of anti-HIV medications such as AZT (zidovudine; Retrovir®) and ddI (didanosine; Videx®), both nucleoside analogue reverse transcriptase inhibitors (NRTIs) (el Kouni 2002). Ribavirin appears to have a direct but modest inhibitory effect on HCV RNA strand synthesis through chain termination (Maag 2001). Ribavirin may also directly inhibit the HCV NS5B RNA-dependent RNA polymerase (Guo 2003).

Ribavirin may also indirectly inhibit RNA strand synthesis by reducing the intracellular supply of guanosine triphosphate (GTP), one of the four nucleotide building blocks for RNA strand synthesis.

GTP levels depend on the activity of a cellular enzyme, IMPDH (inosine-5'-monophosphate dehydrogenase). Ribavirin functions as an IMPDH inhibitor, thus depleting cells of GTP pools (Sintchak 2000). IMPDH inhibition may account for part of ribavirin's immunomodulatory effects. However, IMPDH inhibition does not appear to account for all of the anti-HCV activity of ribavirin, and IMPDH inhibitors do not always have antiviral effects (Lanford 2001b; Markland 2000; S. Zhou 2003).

A recent theory, which has rapidly gained currency, about ribavirin's mechanism of action postulates that ribavirin induces a state called error catastrophe during HCV replication. Under this model, ribavirin is seen as a mutagen, incorporated into the synthesized negative sense HCV RNA strand and leading to mispaired bases in the complementary strand of genomic RNA. Consequently, the mutations introduced in HCV RNA through nucleotide substitutions result in amino acid changes in the proteins synthesized from the new positive sense mRNA strand. HCV, like other RNA viruses, already has a relatively high mutation rate—viral RNA-dependent RNA polymerase-directed strand synthesis is an inherently error prone process (Steinhauser 1992). HCV replication is relatively tolerant of mutations, which may actually promote viral persistence by enabling the viral population to adapt to host cell environments and resist cellular defenses, immune responses, and antiviral therapy. However, some mutations—individually or in combination—will lead to loss of viral protein function and ultimately abolish the replicative efficiency of HCV. From this perspective, a background level of mutation during HCV replication does not impair viral fitness as long as it does not exceed a certain threshold. A mutation rate that exceeds that threshold will lead to the irretrievable loss, or "melting," of genomic information and viral viability, effectively driving the virus into extinction (Cameron 2001; Domingo 2003).

In this model, termed lethal mutagenesis, ribavirin would increase the mutation rate and push HCV over the threshold and into error catastrophe (Graci 2002). Unlike nucleoside analogue inhibition through chain termination, in lethal mutagenesis, ribavirin is (mis)incorporated into the growing chain without interrupting strand synthesis. This role of ribavirin was first observed through *in vitro* poliovirus experiments, where the poliovirus polymerase 3Dpol (analogous to HCV's RNA-dependent RNA polymerase) incorporated RTP while continuing chain elongation. The subsequent increase in the poliovirus mutation rate caused by ribavirin was correlated with inhibition of poliovirus replication (Crotty 2000; Crotty 2001). Subsequent research found evidence of a similar mechanism operative in the anti-HCV activity of ribavirin, though ribavirin misincorporation appears to be a relatively infrequent event (Contreras 2002; Maag 2001; Tanabe 2004; S. Zhou 2003). Further support for the lethal mutagenesis theory came from studies of GBV-B infection in tamarin hepatocytes, a surrogate tissue culture model for HCV, where ribavirin increased replication errors and reduced viral infectivity (Lanford 2001b).

Lethal mutagenesis and IMPDH inhibition may work in tandem, as the depletion of GTP could increase the likelihood of RTP misincorporation (Crotty 2001; Lanford 2001b). Indeed, an HCV replicon study examining ribavirin's effects on viral replication found evidence consistent with both lethal mutagenesis and IMPDH inhibition contributing to antiviral activity. The combination of ribavirin and another IMPDH inhibitor (either mycophenolic acid or VX-497; see 'Ribavirin's Successors' later in this chapter) increased inhibition of replication, but without ribavirin, the IMPDH inhibitors had only modest inhibitory effects (S. Zhou 2003). These findings have opened up new possibilities in developing antiviral drugs that promote lethal mutagenesis, and suggest that

IMPDH inhibitors may increase the efficacy of mutagenic compounds such as ribavirin (Crotty 2002; Daifuku 2003; S. Zhou 2003). Interferon may also operate synergistically with ribavirin as an RNA mutagen (Hong 2003). However, the proposed paradigm of error catastrophe as antiviral strategy has not yet been confirmed through *in vivo* studies of HCV treatment and requires further elaboration to clarify the necessary conditions, constraints, and complexities of these events (Contreras 2002; Eigen 2002; González-López 2004; Grande-Pérez 2002; Pariente 2003; Pfeiffer 2003; Schinkel 2003).

Interferon alfa and ribavirin as immunomodulators

Recent microarray research has documented an upregulation in the expression of dozens of genes associated with cellular immune responses within hours of initiating interferon alfa therapy, suggesting the potential importance in HCV treatment of immunomodulatory mechanisms as well as antiviral effects (Ji 2003). Studies into the immunomodulatory role of interferon alfa and its bearing on response to treatment have produced strikingly inconsistent results, in part because they examine different variables and in some cases use different methods. Observed changes in T cell responses and cytokine profiles during therapy suggest that treatment apparently promotes and augments a T_H1 response to HCV, but studies differ on whether changes in the HCV-specific immune response are sustained or correlate with treatment success (Alvarado Esquivel 2002; Barnes 2002; Cramp 2000; Hempel 2001; Kamal 2002; Sreenarasimhaiah 2003; Z. X. Zhang 1997). Most studies have measured T cells from peripheral blood, though some evidence indicates that robust intrahepatic HCV-specific CD8 cell responses before initiating treatment predict favorable treatment outcomes (Vrolijk 2003).

Ribavirin may also partly function as an immunomodulator, modifying or improving the immune response to HCV (Bergamini 2001b; Cramp 2000; Fang 2001; Tam 1999). The nature of ribavirin's immunomodulatory effects, particularly in the context of interferon alfa therapy, has not been conclusively determined. Several reports describe a shift to a predominantly T_H1 response and suppression of T_H2 responses induced by ribavirin, perhaps mediated by a rise in IL-12 levels or a decrease in IL-4 and/or IL-10 levels (Cramp 2000; Fang 2000; Fang 2001; Hultgren 1998; Ning 1998; Tam 1999). Alternately, some studies indicate that ribavirin counterbalances the immunomodulatory effects of interferon alfa, restoring a proper equilibrium between T_H1 and T_H2 responses and increasing the expression of both IFN-γ and IL-10 (Amati 2002; J. Martín 1998). IMPDH inhibition may account for part of ribavirin's immunomodulatory effects. T cells are particularly dependent on IMPDH when they proliferate in response to antigen stimulation (Fairbanks 1995). Ribavirin can reduce T cell proliferation, which may help suppress both inflammation and the development of T_H2 responses (Heagy 1991; J. Martín 1998).

Overall, research on interferon alfa and ribavirin treatment outcomes tends to support an association between changes in immune dynamics and HCV therapy. Yet studies diverge on the nature and object of the changes in immune parameters induced by interferon alfa, and the relevance of these changes to treatment outcomes. In particular, research has not yet demonstrated that restoration of potent T_H1-type HCV-specific immune responses is necessary or sufficient for sustained virological responses to treatment. Immunomodulatory mechanisms may have different significance in different individuals, perhaps taking on greater importance when pre-treatment

cytokine profiles are more skewed towards T_H2-type responses (Piazzola 2001). However the relationship between cause and effect remains unclear, even in studies documenting an association between response to treatment and improvement in immune responses. Changes in HCV-specific T cell responses, where observed, typically occur during the later stages of treatment, after there have been dramatic reductions in the levels of circulating virus. Perhaps the viral suppression achieved by interferon alfa and ribavirin subsequently enables the re-emergence of HCV-specific immune responses which were previously exhausted by persistent levels of high viral replication.

Treatment failure and resistance

Despite the many potential routes through which interferon alfa and ribavirin therapy suppresses HCV, many people do not experience sustained virological responses to treatment. Viral factors—particularly genotype and viral load—play a major role in treatment failure; HCV infection with genotype 1 and a pre-treatment HCV viral load greater than 2 million copies (about 800,000 international units) are both associated with poorer treatment outcomes. The association between genotype and treatment outcome implies that some HCV strains are more resistant to the effects of therapy. Similarly, if a higher viral load predicts treatment failure, then viral replication dynamics should in part determine treatment outcomes.

Several studies have investigated the possibility that HCV viral proteins interfere with interferon alfa's antiviral activity. The NS5A protein may play a particularly important role in interferon alfa resistance. Some researchers in Japan have described a sequence of the HCV genotype 1b NS5A protein characterized as the interferon sensitivity determining region (ISDR), based on initial studies that found mutations in this region could predict response to interferon alfa treatment (Enomoto 1995; Enomoto 1996). In theory, differences in the genetic sequences encoding NS5A between individuals or across genotypes could therefore account for variations in responsiveness to interferon alfa treatment. While other research in Japan has supported the predictive value of ISDR mutations on treatment outcome, researchers in other countries have been unable to confirm this association, suggesting the existence of subtle strain-specific differences related to geographic distribution of genotype 1b variants (Herion 1997). The role of ISDR mutations in treatment response remains controversial (Schinkel 2004).

Other research has found that the HCV envelope E2 also interacts with and inhibits PKR *in vitro*, though correlates to interferon alfa treatment outcomes have not been identified (Pavio 2002; Taylor 1999; Taylor 2001a). Similarly, research investigating whether greater pre-treatment complexity and diversity of HCV quasispecies populations predicts treatment failure has been inconclusive. Little is known about potential failure to respond to ribavirin, though a mutation in the NS5B region of the HCV genome conferring *in vitro* resistance to ribavirin has recently been identified (Young 2003).

In addition to viral factors, host factors also influence treatment outcomes (B. Gao 2004). Several host variables have been proposed, but the extent of their contribution to impaired responses to interferon alfa therapy is unknown. Exagen Diagnostics is developing a genomic marker test to identify individuals most likely to respond to interferon alfa/ribavirin treatment based on gene expression patterns, as well as a prognostic test to assess risk of liver disease progression. Immunologic variables may influence response to interferon alfa. Differences in immunologic

status could account for lower response rates to HCV treatment among individuals coinfected with HIV, though viral factors—specifically the higher HCV viral load seen in HCV/HIV coinfection—could also influence treatment outcomes. Similarly, African-Americans generally have poorer responses to interferon alfa-based therapy than Whites. Differences in cell-mediated immune responses, as seen in the response to acute infection, may account for part of this disparity (K. Sugimoto 2003a).

Individual genetic variations could also affect the response to interferon alfa treatment. A polymorphism in the interferon-stimulated gene MxA has been associated with HCV treatment outcomes (Knapp 2003). In at least some patient groups, high iron levels and a genetic predisposition to iron overload may predict poorer response rates to HCV treatment, though other studies found that hepatic iron concentrations have no impact on treatment outcomes (Coelho-Borges 2002; Distante 2002; Fargion 2002; Pianko 2002; Shedlofsky 2002). In addition, some genetic polymorphisms related to proteins involved in immune responses (e.g., IL-10) may influence the likelihood of response to interferon alfa treatment (Edwards-Smith 1999; Promrat 2003a; H. Saito 2002; Y. Sugimoto 2002; Yee 2001; Yee 2003).

Finally, pharmokinetic parameters affecting the tissue distribution of interferon alfa in the body may also influence treatment outcomes. In some studies, treatment efficacy is reduced among obese patients, potentially implying that overall concentrations of interferon alfa are lower in individuals with high body mass indices (McCullough 2003). Individual variations in ribavirin concentrations have also been linked to differences in HCV treatment outcomes (Larrat 2003).

Implications for current treatment strategies and future drug development

Ideally a more refined understanding of the mechanisms of action—and reasons for failure—of interferon alfa and ribavirin could lead to improved treatment outcomes. For instance, minor variations in interferon alfa proteins, as seen with IFN-α subtypes, could hypothetically modify the gene expression profile induced by interferon alfa treatment. If a sustained virological response is associated with a particular profile of ISG expression, then it might be possible to identify or design an interferon alfa protein most likely to induce the desired gene expression profile, perhaps with milder side effects than current treatments. Without information about which interferon-stimulated genes correlate with treatment success, it is difficult to evaluate particular interferon alfa variants in order to optimize HCV treatment response rates. Such data could also help to evaluate proposed strategies to increase the efficacy of current treatment through higher induction doses or longer courses of treatment. The development of alternative, less toxic forms of ribavirin would also benefit from a clearer understanding of the desired effects.

Understanding how the immunomodulatory effects of interferon alfa and ribavirin contribute to HCV treatment success could have particular relevance for individuals coinfected with HIV. If interferon alfa-based treatment succeeds through modulating immune responses, its efficacy may require an intact immune system. This logic underlies the suggestion that people coinfected with HCV and HIV may require antiretroviral therapy aimed at reversing immunodeficiency and immune dysfunction prior to initiating HCV treatment. Similarly, if treatment outcomes depend on the enhancement of immune responses targeting HCV-infected cells, then response rates to interferon alfa-based treatment may be improved by adjunctive therapy with other immuno-

modulatory cytokines.

The broad outlines of the potential mechanisms underlying the success of interferon alfa/ribavirin therapy have largely been established. The specific contributions of the multiple effects of these compounds, the nature of their synergy when used in combination, and mechanisms of resistance all require further investigation (Buckwold 2003; Buckwold 2004; Y. He 2002a; Pawlotsky 2004b; Pfeiffer 2003; Tanabe 2004; Taylor 2001b).

Alternate forms of interferon alfa

Consensus Interferon:

Consensus Interferon (CIFN; Infergen®; interferon alfacon-1) is a synthetic form of interferon alfa, developed by Amgen and licensed to InterMune, that is based on a consensus sequence of all IFN-α subtypes. In contrast, interferon alfa-2a (Roferon®-A; Roche) and interferon alfa-2b (Intron® A; Schering-Plough) are recombinant forms of interferon alfa based on a single subtype. By some measures, CIFN demonstrates higher levels of antiviral activity than interferon alfa-2a and interferon alfa-2b *in vitro* (Blatt 1996). Studies of CIFN used as monotherapy for chronic HCV showed efficacy and tolerability comparable to or better than interferon alfa-2b (Jensen 1999; Keeffe 1997; Tong 1998). A preliminary analysis of a thrice-weekly regimen of CIFN in combination with daily ribavirin showed a sustained virological response of 55%, compared with 31% among patients treated with standard interferon alfa-2b and ribavirin (Sjogren 2002). In small, open-label studies, treatment with CIFN and ribavirin produced sustained virological responses in some individuals who did not respond to or who relapsed after prior interferon alfa treatment, suggesting a role as second-line therapy (Barbaro 2002; da Silva 2002). InterMune announced that in the second quarter of 2004, the company will initiate the DIRECT Trial, a phase III study of daily CIFN and ribavirin in non-responders to prior treatment.

The FDA has approved CIFN for the treatment of chronic HCV, though its use in clinical practice is minimal compared to Roche and Schering's pegylated interferons. Use of CIFN was initially limited due to the superiority of combination therapy with interferon alfa and ribavirin. When originally approved, ribavirin was only available for use with Schering's interferon alfa-2b (Intron® A). Schering bundled ribavirin with Intron® A so that ribavirin was not sold separately to be combined with other interferons. While Schering now markets ribavirin separately, standard interferon has been supplanted by more effective pegylated forms (see Chapter V, Hepatitis C Treatment). InterMune initiated a phase I trial of a pegylated version of Infergen, PEG-Alfacon-1, in early 2003. Further development of PEG-Alfacon-1 has been suspended for financial reasons while InterMune seeks a partner to subsidize development costs.

Other interferon alfa variants:

- Albuferon™-alpha, a form of interferon alfa fused to albumin molecules, is in development by Human Genome Sciences. Albumin fusion can extend the half-life of the drug, so that less frequent dosing is possible. Preliminary data from a phase I study indicated that Albuferon has a half-life of up to 157 hours, or nearly a week, suggesting a potential for a once or twice monthly dosing schedule.

The side effect profile was comparable to other forms of interferon alfa (Davis 2002). An on-going phase I/II trial is examining the safety and pharmacologic profile of Albuferon, given as a single dose or in two doses 14 days apart, in 92 people who did not respond to prior HCV treatment.

In May 2004 Human Genome Sciences began a phase II dose-ranging trial in Canada to compare three different doses of Albuferon in people with HCV geno type 1. Study participants will be given Albuferon twice, with 14 days between doses, to evaluate declines in HCV viral load at day 28. The results of this trial will guide the Albuferon dose used in a larger study that will combine Albuferon with ribavirin for 48 weeks in people with genotype 1 who have not had prior HCV treatment.

- Omega interferon, a genetically engineered type I interferon originally discovered by Boehringer Ingelheim and acquired by BioMedicines for development, has entered phase II trials. BioMedicines has announced plans to develop an implantable drug delivery system for omega interferon in partnership with ALZA Corp., as well as an oral prodrug formulation in collaboration with Nobex Corp. An oral drug would provide substantial advantages over currently approved forms of interferon alfa, which require injection, but oral formulations that allow adequate and efficacious drug levels pose significant challenges for drug development. BioMedicines is also researching methods to target omega interferon to the liver, reducing systemic side effects.

- Natural alpha interferon (Multiferon™), developed by Viragen, is a multi-subtype interferon produced from human white blood cells; in contrast, other forms of interferon alfa used to treat HCV are recombinant, synthetic proteins. Multiferon has not been submitted for FDA approval in the United States, though it is marketed in Mexico. Due in large part to its weak financial position, Viragen has no plans to conduct registrational trials that would allow a submission for FDA approval.

Additional forms of interferon alfa are in development. Maxygen is developing an optimized pegylated interferon, which could enter clinical trials for HCV infection in 2005, and recently entered into a partnership with Roche for clinical development and marketing. Amarillo Biosciences is exploring an oral formulation of low-dose interferon alfa, though no trials for HCV are currently planned and the company lacks the resources to conduct clinical research.

Other interferons:

- Interferon beta: Several studies have examined the effects of interferon beta, which might induce an antiviral response similar, though not identical, to the effects of interferon alfa (Cheney 2002). Early studies at low doses showed limited efficacy of interferon beta (Castro 1997; Perez 1995; Villa 1996). More recently, researchers in Japan have used higher doses of interferon beta, administered intravenously—most often as induction therapy before initiating interferon alfa

treatment. Some studies have shown decent rates of treatment success, though at higher doses interferon beta appears to have a side effect profile similar to that seen with interferon alfa therapy (Horiike 2003; F. Ikeda 2000; Kakizaki 1999; Shiratori 2000; F. Suzuki 2001; Watanabe 2002). Serono has been conducting clinical trials of recombinant interferon beta-1a to treat chronic HCV infection in Asians, and is developing a pegylated formulation of interferon beta. Maxygen, now in partnership with Roche, is also developing an interferon beta compound to treat HCV.

- Interferon gamma: IFN-γ can inhibit HCV replication in a replicon model, and has antiviral effects distinct from but overlapping with those of IFN-α (Cheney 2002; Frese 2002; Lanford 2003). InterMune has conducted phase II trials of Actimmune®, a formulation of interferon gamma-1b, as an antifibrotic therapy in people with chronic HCV infection who did not respond to interferon alfa treatment. Results from a 24-week study, which followed twenty patients receiving 200 mcg of interferon gamma subcutaneously thrice weekly, found no overall improvement in fibrosis scores. No serious adverse events were reported, though two study participants dropped out due to side effects (similar to interfer on alfa's, particularly flu-like symptoms) and ALT elevations. Interferon gamma therapy had no effect on HCV viral load (Muir 2003). Another pilot study of a four week course of interferon gamma treatment administered subcutaneously at 100 or 200 μg thrice weekly found no evidence of antiviral efficacy as measured by changes in HCV viral load and ALT levels (Soza 2003).

  InterMune reported in early 2004 that a phase II study of individuals with chronic HCV infection and advanced fibrosis found no evidence of a protective effect of Actimmune on liver histology. Actimmune is being investigated in small studies in combination with Infergen (consensus interferon) to treat non-responders to standard therapy. In 2004 InterMune launched a phase II trial in non-responders that combines daily Infergen with Actimmune three times a week, each given at varying doses. InterMune is also considering conducting studies in 2005 that combine Actimmune with pegylated interferon (Roche's Pegasys and/or Schering's Peg-Intron) for people who have not been previously treated for HCV.

  Some research has also suggested that sequential interferon alfa and interferon gamma therapy may improve T_H1 responses and facilitate viral clearance (Katayama 2001; Kumashiro 2002). InterMune is also collaborating with Maxygen on a more effective, longer-acting form of interferon gamma that is currently in preclinical testing.

Ribavirin's Successors

The relative success of ribavirin as a component of HCV therapy has led to a search for compounds with similar antiviral effects and fewer toxicities (see Chapter V, Hepatitis C Treatment). Drug development in this area can be seen as a range of hypotheses about the mechanism of action of ribavirin as a component of HCV treatment. Candidate compounds fall under four overlapping categories: next-generation forms of ribavirin, IMPDH inhibitors, agents that induce lethal mutagenesis, and other nucleoside analogues (most of which have been described earlier in this chapter, under 'NS5B RNA-dependent RNA polymerase and NS3 helicase/NTPase'; see also the entry for isatoribine in 'General Approaches to HCV treatment: Immune modulators and broad-spectrum antivirals' later in this chapter):

Next-generation forms of ribavirin. This category includes two compounds, viramidine and levovirin, both discovered by Valeant. Viramidine is a pro-drug of ribavirin that targets the liver, meaning that viramidine enters the body in an inactive form until converted to its active ribavirin form in the liver by the enzyme adenosine deaminase (C. C. Lin 2003; J. Z. Wu 2003). Viramidine, developed by Valeant's former subsidiary Ribapharm, is anticipated to have effects similar to ribavirin, but with less toxicity, particularly with respect to anemia. A phase II trial of 180 subjects taking viramidine in combination with pegylated interferon for 48 weeks began in December 2002 (Agora 2002; C. C. Lin 2002). Based on favorable 12-week results in an interim analysis of the phase II study, Valeant announced two phase III trials, VISER1 (launched in late 2003) and VISER2 (scheduled to commence in mid-2004). Each trial will enroll about 1,000 patients and compare viramidine to ribavirin, both used in combination with pegylated interferon.

Levovirin is an L-isomer of ribavirin, meaning that its chemical structure is the mirror image of ribavirin's—the same but reversed; unlike ribavirin, levovirin does not undergo phosphorylation inside cells. Like viramidine, levovirin is thought to have a favorable side effect profile in comparison to ribavirin. Levovirin has no direct antiviral effects against HCV, but shows immunomodulatory properties similar to, and perhaps greater than, ribavirin (Tam 2000). Roche had been investigating levovirin in early phase studies, but discontinued development of levovirin based on unfavorable results from phase I/II trials. Roche also conducted phase I studies of R1518, a pro-drug of levovirin, but further development of R1518 is doubtful.

IMPDH inhibitors. Two IMPDH inhibitors, merimepodib (also denoted VX-497) and mycophenylate mofetil (MMF, marketed by Roche as CellCept®), are being explored as anti-HCV therapies in combination with alpha interferon. Unlike ribavirin, both merimepodib and MMF are non-competitive IMPDH inhibitors—that is, while ribavirin monophosphate mimics inosine 5'-monophosphate and competes with IMP for IMPDH, these new compounds inhibit IMPDH through other mechanisms (Sintchak 2000). In vitro studies suggest that Merimepodib, in combination with alpha interferon, exerts some direct antiviral activity, presumably through depletion of cellular GTP pools (Markland 2000).

Merimepodib, developed by Vertex, entered phase II trials in Europe in 2002 in combination with pegylated interferon and ribavirin. Common side effects attributed to merimepodib in a phase II trial reported at the 2003 HEP DART meeting include diarrhea, abdominal pain, and mild rash, which occurred in up to a quarter of study participants receiving merimepodib, compared to none

in the control arms. Preliminary unpublished data show that 50 mg of merimepodib taken twice a day, in combination with pegylated interferon and ribavirin, increases the likelihood of prior treatment non-responders reaching undetectable HCV viral loads during re-treatment, though data on sustained virological responses have not been presented.

Based on interim phase II safety and efficacy results, Vertex has announced a phase IIb study, the Merimepodib Triple Combination study (METRO), to begin enrolling in the second half of 2004. METRO will be a randomized, placebo-controlled study of 315 prior non-responders who will receive either merimepodib (at 50 or 100 mg twice-daily) or placebo for six months, in combination with pegylated interferon alfa-2a (Roche's Pegasys) and ribavirin. Study participants who have undetectable HCV RNA after six months will continue treatment with Pegasys and ribavirin for another 24 weeks.

Research using an HCV replicon model suggests that merimepodib and MMF, at least in the absence of alpha interferon treatment, may have little antiviral activity on their own but could potentiate the mutagenic effects of ribavirin (S. Zhou 2003). Merimepodib and MMF also have immunosuppressive properties, with MMF already approved for use as part of combination therapy to prevent organ rejection following heart, kidney, and liver transplants (J. Jain 2001; Tossing 2003). T cells and B cells involved in the immune response are particularly dependent on the availability of GTP, so merimepodib and MMF effectively suppress immune responses by inhibiting cell division and proliferation (see Chapter IX, Immune Response, Persistence, and Pathogenesis). Despite initial promising findings, recent studies of HCV recurrence following liver transplantation and MMF as monotherapy tend to indicate that MMF itself has no direct antiviral effect on HCV post-transplant recurrence or viremia (Charlton 2002; Firpi 2003). MMF is currently being studied in combination with alpha interferon in patients who did not respond to prior HCV treatment following favorable preliminary clinical data (Afdahl 2001).

Lethal mutagens. The putative role of ribavirin as an inducer of error catastrophe has prompted a search for nucleoside analogues that may have similar effects on HCV; this approach has also been proposed for HIV drug discovery (Crotty 2002; Daikufu 2003; Loeb 1999). At least one company, Koronis Pharmaceuticals, has made the development of lethal mutagens—which they describe as "stealth nucleosides"—the centerpiece of its drug development efforts, focusing on HCV, HBV, and HIV. No candidates have entered preclinical development yet.

General Approaches to HCV treatment: Immune modulators and broad-spectrum antivirals

The search for more effective HCV therapies and better options for interferon alfa-based treatment non-responders and relapsers has generated a number of candidates with antiviral and immunomodulatory properties. These agents do not specifically target HCV, and their discovery and synthesis typically predate the identification of the hepatitis C virus. In many cases, these compounds have been investigated or even approved and marketed for the treatment of other conditions. Some, such as the immunomodulatory cytokines and amantadine, have yet to prove their value in the treatment of HCV, despite initial promise. Still other agents are at early stages of development. A few drugs, such as thymosin alfa-1 (Zadaxin®) and histamine dihydrochloride (Ceplene™), have entered large, late-stage efficacy studies to determine whether they benefit prior

non-responders or relapsers to interferon alfa-based treatment. For both practical and statistical reasons, trials in non-responders are typically easier to design and less expensive than research in treatment-naïve populations. For smaller companies in particular, this decreases the risk that a drug will fail to reach FDA approval.

Immunomodulatory cytokines:

Several studies have attempted to identify the therapeutic potential of several cytokines involved in mediating T_H1 and T_H2 responses, including IL-2, IL-10, and IL-12. T_H1 cytokine therapy may augment cell-mediated immune responses and facilitate clearance of HCV; T_H2 cytokine therapy may suppress inflammatory responses and reduce fibrosis. As with interferon alfa, all these cytokines are administered subcutaneously and can have systemic side effects. Due to disappointing results in monotherapy studies, none of these cytokines are currently under active investigation for HCV treatment.

- IL-2: A pilot study of 33 individuals with chronic HCV examined the effects of a 12-week course of varying doses of subcutaneous recombinant interleukin-2 (IL-2), a T_H1-type cytokine involved in T cell proliferation. While 4 of 33 (8%) experienced a sustained biochemical response, indicated by normalized ALT levels, decreases in HCV RNA during treatment were transient (Pardo 1997). Research on HCV/HIV coinfected patients receiving IL-2 treatment generally indicate that IL-2 does not significantly affect HCV viral load, though some small studies report that IL-2 therapy may suppress HCV replication in some individuals (Hengge 2000; Schlaak 2002; Thibault 2002; Uberti-Foppa 1999; Valdez 2001). A study combining low-dose IL-2 with pegylated interferon and ribavirin in people with HCV/HIV coinfection found that adding IL-2 to standard combination therapy for HCV provided no benefit in response to treatment and resulted in high drop-out rates (Glesby 2004).

- IL-10: As a T_H2-type cytokine, IL-10 has been investigated for potential antifibrotic activity. A pilot study of 16 patients receiving a 30-day course of IL-10 found that 50% experienced transient normalization of ALT levels during treatment (McHutchison 1999). Another study of a three-month course of IL-10 at different doses in 22 non-responders to interferon alfa treatment found substantial decreases in inflammation (experienced in 19 of 22 subjects, or 86%) and fibrosis (14 of 22 subjects, or 63%) with no elevation in HCV viral load (Nelson 2000). A subsquent study following 28 subjects treated for at least 12 months found decreases in inflammation and fibrosis scores in less than half the treated subjects (13 out of 28 or 46%, and 11 out of 28 or 39%, respectively). HCV viral load increases during therapy averaged 0.5 log, and IFN-γ-producing HCV-specific CD4 and CD8 T cells decreased, with a shift towards a T_H2-type immune response (Nelson 2003).

- IL-12: Endogenous IL-12 promotes T_H1 responses, augments natural killer (NK) cell cytotoxicity, and increases the secretion of IFN-γ. A phase I/II study showed that a ten-week course of IL-12 in 60 patients failed to reduce HCV RNA to

undetectable levels (Zeuzem 1999). A smaller dose-ranging study showed that HCV viral load became undetectable in a few patients receiving the highest dose of IL-12 at the end of twelve weeks of treatment, but all relapsed after therapy (O'Brien 2001). A more recent study of a 48-week course of IL-12 in 225 non-responders to interferon alfa-based treatment was terminated when the proportion of subjects discontinuing treatment due to severe adverse events (7 of 225 or 3%) exceeded the number experiencing a sustained virological response (2 of 160 subjects completing at least 8 weeks of therapy, or 1% [Pockros 2003a]).

Other immunomodulators:

- Thymosin alfa-1 (thymalfasin; Zadaxin®): Thymalfasin is a synthetic peptide derived from human thymus gland extracts (α-thymosins) believed to promote and enhance T_H1 responses. Thymalfasin may also have additional antiviral effects through upregulating MHC class I molecule expression on virus-infected cells, and through reducing oxidative stress. Thymosin alfa-1 was originally developed at George Washington University and licensed to Hoffman-La Roche, which in turn licensed the compound to Alpha 1 Biomedicals. Alpha 1 Biomedicals subsequently licensed thymalfasin to SciClone, which is currently studying the agent as a treatment for HCV in non-responders to prior interferon alfa therapy.

  Like interferon alfa, thymalfasin is administered subcutaneously; standard dosing is twice weekly. Preliminary data from a dose-ranging study of 31 non-responders to prior interferon-based treatment showed that thymalfasin in combination with Roche's pegylated interferon alfa-2a produced an early (12-week) virological response ranging from 20% to 36%, depending on thymalfasin dosage (Iftikar 2002). Two randomized, phase III trials, each enrolling 500 patients in the United States, will study the effects of treatment with pegylated interferon alfa-2a, with or without thymalfasin, in non-responders to prior interferon treatment. One study recruited people with mild cirrhosis, and the other will examine people with no cirrhosis. SciClone expects that these studies will be completed by the end of 2005, with data available in 2006.

  A third European phase III study, announced in May 2004 and conducted by Sigma-Tau (SciClone's European partner) will enroll 550 non-responders to prior pegylated interferon/ribavirin therapy. All study participants will receive Roche's pegylated interferon alfa-2a with ribavirin; in addition, half will receive thymalfasin (1.6 mg twice a week by subcutaneous injection) and half will receive placebo. Study enrollment will commence in late 2004.

  SciClone also announced in May 2004 plans to develop a pegylated version of thymalfasin using pegylation technology from Nektar Therapeutics (the same technology used for Roche's Pegasys). SciClone also has another compound, SCV-07, in preclinical development as a potential oral therapy for infectious diseases with immunomodulatory effects similar to those of thymalfasin.

- Histamine dihydrochloride (HDC; Ceplene™): Developed by Maxim Pharmaceuticals, histamine dihydrochloride binds to receptors on intrahepatic monocytes/macrophages, blocking the release of reactive oxygen species. This reportedly reduces oxidative stress associated with viral infection, protecting NK and T cells and facilitating their activation, possibly in synergy with INF-α and mediated by INF-γ. Like interferon alfa, HDC is administered subcutaneously, though an oral formulation is in development. Clinical development of HDC has been relatively slow; after a brief partnership with Roche, Maxim entered into an agreement with Schering-Plough to study HDC in combination with Schering's interferon alfa-2b. A phase II dose-ranging study of 129 individuals with untreated HCV showed that HDC treatment combined with interferon alfa-2b yielded sustained virological responses between 31% and 38%, depending on HDC dosing regimen (Lurie 2002).

  In July 2003, Maxim completed enrollment of 302 non-responders to prior interferon alfa/ribavirin therapy in a randomized phase II efficacy trial conducted at European and Canadian sites. This study will compare responses to pegylated interferon alfa-2b and ribavirin with or without HDC. In March 2004, Maxim completed an initial phase Ia of an oral formulation of HDC (dubbed HD-O) and plans to focus further clinical development of histamine dihydrochloride on the oral version.

- Isatoribine (ANA245): Anadys has licensed from Valeant two nucleoside analogues, ANA245 (isatoribine) and ANA246, with immunomodulatory properties. Isatoribine reportedly stimulates innate immune responses through interactions with a receptor found on white blood cells called Toll-like receptor 7 (TLR7), thereby increasing INF-α and TNF-α levels and activating NK cells. Isatoribine, administered intravenously, has entered phase I trials, and preliminary data found that isatoribine can reduce HCV viral loads by nearly 1 log (10-fold).

  ANA971, an oral prodrug of isatoribine, began phase I testing in healthy volunteers in early 2004. Anadys has also conducted preclinical research on another oral prodrug of isatoribine, ANA975. Anadys announced in May 2004 that ANA975 will be its lead candidate for HCV treatment. ANA246 appears to promote a T_H1 response, but Anadys is not currently pursuing development of this compound.

- Actilon™ (CPG 10101): Actilon, developed by Coley Pharmaceuticals, is an agonist of Toll-like receptor 9 (TLR9). TLR9 is a pattern recognition receptor found in dendritic cells and B cells. TLR9 recognizes a particular molecular pattern commonly found in bacterial and viral pathogens (CpG motifs, or cytosine-guanine sequences), prompting dendritic cells to release IFN-α and IL-12, promoting a T_H1-type T cell response (Kapsenberg 2003; Vollmer 2004). Actilon is a compound that interacts TLR9 and triggers IFN-α secretion and T_H1 responses. Coley began two phase I studies of Actilon, administered by subcutaneous injection, in early 2004. Coley expects data from a phase I/II study

of Actilon in people with hepatitis C to be available in late 2004, and plans to begin phase II trials in the second half of 2004.

- Imiquimod and resiquimod: Imiquimod and resiquimod are imidazoquinolinamines, a class of non-nucleoside drugs that stimulates the secretion of IFN-α and various T_H1-type cytokines (Dockrell 2001). Imiquimod has been approved as a topical cream to treat genital warts; a pilot study of oral imiquimod as an HIV treatment found a range of toxicities similar to those associated with other cytokine therapies, including fatigue, fever, malaise, and depression (Goldstein 1998). Preliminary data from a phase II study of resiquimod for chronic viral hepatitis failed to demonstrate antiviral activity (Pawlotsky 2004a).

Antivirals:

- Amantadine and rimantadine: Amantadine was the very first antiviral drug, approved by the FDA to prevent influenza in 1966 and marketed as Symmetrel® by Endo Pharmaceuticals. Amantadine has been under investigation for several years as an HCV therapy, having shown some anti-HCV activity *in vitro*, though no specific inhibition of viral protein synthesis, polyprotein processing, or RdRp-directed strand synthesis was observed (Jubin 2000; J. Martín 1999). Despite some initially promising results for amantadine in clinical trials, several studies have shown little or no antiviral effect alone or in combination with alpha interferon or with alpha interferon and ribavirin (Berg 2003; J. Chan 2002; Craxi 2001; Helbling 2002; Mangia 2001; Thuluvath 2004; Zeuzem 2000). Rimantadine, another drug closely related to amantadine and also used to treat influenza A (Flumadine®, Forest Labs), has shown no benefit as monotherapy for HCV treatment and is not currently under investigation as a component of combination therapy (Fong 1999; Sherman 1999).

- Kemin Pharma compounds: Kemin Pharma, a Belgian company, announced in early 2004 that it was evaluating two compounds for anti-HCV activity. One compound, KPE02003002, is a synthetic molecule derived from a plant chemical. KPE02003002 has advanced to phase II trials. Another compound, KPE00001133, is under preclinical investigation. Their mechanisms of action have not been reported.

Anti-inflammatory and anti-fibrotic agents:

- Idun Pharmaceuticals has developed an oral apoptosis inhibitor, IDN-6556, which acts against cellular caspases (proteases that trigger cell death). IDN-6556 has been shown to reduce ALT levels in initial studies of individuals with mild liver disease. In a 14-day study of 40 subjects with HCV, individuals were randomized to receive IDN-6556 at five different doses (ranging from 25 mg once daily to 100 mg twice daily) or to placebo. Compared to placebo, all treatment groups experienced significant transient decreases in ALT and AST levels. Most ALT levels

remained above the upper limit of normal, except in the 100 mg twice-daily group. Mild side effects included headache, dry mouth, and stomach ache; IDN-6556 did not have significant effects on HCV viral load (Pockros 2003b). Animal toxicology studies lasting up to one year have not found evidence that IDN-6556 increases the risk of hepatocellular carcinoma (a potential concern for apoptosis inhibitors, since the pathogenesis of cancer typically involves overriding signals leading to cell death—see Chapter IX, Immune Response, Persistence, and Pathogenesis). Further phase II studies are planned.

- Pirfenidone: Pirfenidone is an oral compound under investigation by InterMune for the treatment of idiopathic pulmonary fibrosis. A pilot study treated 26 cirrhotics, 15 of whom had chronic hepatitis C infection, with pirfenidone for one year. Pirfenidone treatment reduced inflammation, steatosis, and HCV viral load in a subset of study participants, but results were highly variable and no declines in fibrosis scores were observed (Armendariz-Borunda 2003).

- Enzo Biochem has conducted a phase I study of EHC18, an oral compound thought to modulate immune responses to HCV by inducing tolerance, or non-responsiveness to viral antigens, thus potentially reducing inflammation. No fur ther development plans for EHC18 have been announced, though additional studies are under consideration.

- Several complementary and alternative therapies, particular herbal remedies including milk thistle (active agent: silymarin) and licorice root (active agent: glycyrrhizin) have also been proposed to have beneficial effects in chronic HCV infection and other liver diseases, though data on the safety and efficacy of these agents is limited and often ambiguous (Coon 2004; J. Liu 2003; NCCAM 2003).

Other compounds already in clinical use may also have activity against HCV, directly or indirectly. Recent reports suggest that cyclosporin A (CsA), an immunosuppressant used in liver transplant recipients, can inhibit HCV replication *in vitro* through a mechanism apparently unrelated to its immunosuppressive properties (Nakagawa 2004; Watashi 2003a). Though CsA does not appear to control HCV effectively in liver transplant recipients, presumably due to immunosuppressive effects, a study in Japan found that a six-month course of HCV treatment with a combination of CsA and alpha interferon was more effective at achieving sustained virological responses than interferon alone (42/76 [55%] vs. 14/44 [32%]; $p=0.01$) (K. Inoue 2003). Further research is focused on NIM811, a CsA analogue without immunosuppressive activity. In vitro research also shows that sodium stibogluconate, an injectable drug used to treat the parasitic disease leishmaniasis, also inhibits HCV replication through an unknown mechanism (Yeh 2003). Etanercept (Enbrel®), an injectable TNF-α antagonist used to treat rheumatoid arthritis, showed promise in combination with standard interferon and ribavirin in one small study (Zein 2002).

Vaccine Development

With an estimated 170 million hepatitis C infections globally, the development of a vaccine to prevent HCV infection is an urgent priority. Ideally, a prophylatic (preventive) vaccine could block HCV as soon as it enters the body, before it has a chance to establish infection—an immune response called sterilizing immunity. Alternately, a prophylactic vaccine might not fully prevent HCV infection, could facilitate clearance during acute infection, or else attenuate the effects of chronic infection, stimulating protective immunity, so that the virus persisted at low and ultimately harmless levels. Chronically infected individuals could also benefit from a therapeutic vaccine, which could similarly facilitate the suppression of HCV replication (Moingeon 2003).

Vaccines work by stimulating virus-specific humoral (antibody) and/or cell-mediated (cytotoxic T lymphocyte) immune responses (see Chapter IX, Immune Response, Persistence, and Pathogenesis). Effective antibody responses enable sterilizing immunity, since virus-specific antibodies can intercept virions before they are able to infect cells. If a vaccine does not induce antibody responses, cell-mediated immune responses may provide protection against the effects of viral infection, but on their own would not be expected to block infection completely. HCV vaccine research relies on an understanding of the correlates of immunity that enable viral clearance during acute infection. Viral diversity is also an important consideration for vaccine design, since potential antibody and cytotoxic T lymphocyte (CTL) epitopes may vary between genotypes and subtypes, and the quasispecies nature of HCV may allow the virus to escape from vaccine-induced immune responses.

HCV vaccine research has been constrained by its reliance on chimpanzees as the only established model of HCV infection, given the substantial expenses and limited availability of these animals for research (Bukh 2001a). To date, only a handful of published research has studied vaccine efficacy in chimpanzees (Q. L. Choo 1994; Esumi 1999; Forns 2000; Goto 2001; Rollier 2004; Weiner 2001). Mice have been used to study the nature and potency of immune responses to various HCV antigens and prospective vaccine candidates. Rhesus macaques, though not susceptible to HCV infection, have also been used as a primate model to study immunogenicity of potential vaccines (Forns 1999; Q. Li 2003; Polakos 2001).

Researchers have nevertheless made some progress in vaccine design and development, and conducted studies establishing a number of important considerations:

- Prior resolved HCV infection in chimpanzees does not confer sterilizing immunity that prevents reinfection (Farci 1992; Prince 1992).

- Previously infected chimpanzees who resolved prior HCV infection retain HCV-specific memory T cell responses that facilitate viral clearance and/or confine viral replication to low levels, indicative of long-lasting protective immunity (Bassett 2001; Major 2002; Nascimbeni 2003; Shoukry 2003).

- Resolution of HCV infection in vaccinated chimpanzees is associated with the quality of HCV-specific immune responses—specifically a T_H1-type response to E1 and NS3—rather than the quantity, or magnitude, of the response (Rollier 2004).

- Sterilizing immunity to HCV has occasionally been induced in chimpanzees following immunization, but generally does not protect against other strains of HCV; protective immunity is more frequently observed (Abrignani 1998; Q. L. Choo 1994; Esumi 1999; Esumi 2002; Farci 1994; Farci 1996b; Forns 2000).

- Clearance of acute HCV infection can be achieved without the induction of a significant antibody response to HCV. Four cases of resolved HCV infection were documented in an Australian cohort of prisoners; all mounted detectable HCV-specific T cell responses, without conversion to HCV-antibody seropositivity, though weak antibody responses were detected (Post 2004).

- Prior resolved HCV infection may confer some protection against viral persistence following re-infection. A group of injection drug users (IDUs) who previously cleared HCV were less likely to develop chronic HCV infection when reinfected than a comparison group of IDUs with no prior history of HCV clearance who became infected (S. H. Mehta 2002).

- Prior resolved HCV infection may confer protective immunity against other strains of HCV. Four chimpanzees who had previously cleared infection with HCV geno type 1 were each rechallenged with inocula containing HCV genotype 1, a mix ture of genotypes 2 and 3, genotype 4, or a mixture of genotypes 1, 2, 3, and 4. All animals again cleared infection, more rapidly and with lower viral loads than observed during their initial infections (Lanford 2004).

Collectively, these findings support the viability of developing vaccines that elicit protective immune responses—particularly cell-mediated immunity—that facilitate viral control and clearance. However, sterilizing immunity may be difficult to induce, especially against multiple strains (Burton 2002). At the same time, inducing cell-mediated immune responses through vaccination poses considerable scientific challenges in the vaccine field as a whole (Esser 2003; Zinkernagel 2002; Zinkernagel 2003).

In spite of substantial evidence questioning both the relevance of antibody responses to HCV and the prospects of an HCV vaccine inducing sterilizing immunity, the HCV envelope proteins E1 and E2 have been a main focus of HCV vaccine research (Beyene 2002). Attempts to identify and induce effective HCV-specific immune responses directed at E1 and E2 regions, using both *in vitro* methods and *in vivo* mice and chimpanzees models, have yielded promising leads but mixed results (Bichr 2002; Esumi 1999; Esumi 2002; Forns 2000; Heile 2000; Lucas 2003; X Ma 2002; Rosa 1996; Satoi 2001; Seong 2001; Tedeschi 1997; Y. H. Zhou 1999; Y. H. Zhou 2002a; J. Zhu 2002). Future efforts should benefit from new techniques to identify neutralizing antibodies using HCV pseudo-particles (Bartosch 2003c).

Some research has attempted to elicit both antibody and T cell responses, using mimotopes (synthetic peptides mimicking naturally-occurring viral epitopes) for the hypervariable region 1 (HVR1) of E2. Immunization with HVR1 mimotopes elicited broad antibody responses in rabbits (Roccasecca 2001). A study of the immunogenicity of HVR1 mimotopes in blood samples from 40 subjects, half of whom were chronically infected with HCV, demonstrated the induction of

cross-reactive CD4 T cell responses recognizing multiple HVR1 variants (Frasca 2003). Ideally, such mimotopes could offer a potential vaccine strategy to prevent the emergence of viral escape mutations by inducing broad, cross-reactive antibody and T cell responses.

Other HCV proteins, including core and NS3, have also received attention as potential immunogens, particularly for vaccines attempting to induce HCV-specific T cell responses. Vaccine approaches under preliminary investigation in mice include DNA vaccines, subunit vaccines, viral vectors, prime/boost combinations, hepatitis C virus-like particles (HCV-LPs), lipopeptides, and dendritic cell vaccines. While current small-animal models cannot adequately reproduce conditions of natural infection, some of these approaches have displayed impressive immunogenicity (Arribillaga 2002; Brinster 2002; Jiao 2003; K. Murata 2003; Pancholi 2003; Qiao 2003; Racanelli 2004; Youn 2003). Various adjuvants, including IL-12 and GM-CSF (granulocyte/macrophage colony stimulating factor) genes, IL-23, CpG motifs, and ISCOMs (immune-stimulating complexes) are also being examined for their potential to enhance vaccine-induced immune responses (Ha 2004; X Ma 2002; Matsui 2003; Ou-Yang 2002; Polakos 2001; Qiao 2003).

Most current vaccine development efforts are in pre-clinical testing, with only a few candidates in human trials. Chiron, having abandoned an early effort in the late 1990s to develop an HCV envelope-based vaccine, has begun clinical testing of a new vaccine candidate. The compound, developed with researchers at St. Louis University's Center of Vaccine Development, is designed to induce an immune response directed towards envelope proteins for use in preventing HCV infection. Data from a chimpanzee study showed that vaccinated chimpanzees were not protected from infection, but were able to clear HCV. A phase I study currently underway will test the safety and immunogenicity of the vaccine in 45 healthy volunteers. Chiron also has a therapeutic vaccine candidate, administered with the Australian company CSL Limited's ISCOM® adjuvant and designed to stimulate cell-mediate immune responses. Phase I clinical trials were conducted in Australia in 2002, and further testing in people with chronic HCV infection is planned.

Another therapeutic vaccine, based on a purified version of the HCV E1 protein, has been developed by the Belgian-based Innogenetics. Innogenetics has conducted small phase I and II trials among patients with chronic HCV infection, where its vaccine did not show an effect on HCV viral load. However, some improvements in biochemical and histological markers of disease progression were observed, including reductions in ALT levels, in a study of thirty-five subjects. Twenty-six subjects received vaccinations given intramuscularly at weeks 0, 4, 8, 12, and 24; the remaining nine subjects initially received placebo. After nearly a year, thirty-four subjects from both arms received a round of six vaccinations given every three weeks. Twenty-four subjects who received both rounds of vaccination underwent biopsies before vaccination and seventeen months later. Nine subjects experienced modest histological improvements (37%), with stable histology in ten other subjects (42%); biopsy scores worsened in five subjects (21%) (Nevens 2003). These results certainly warrant further study, but are difficult to interpret due to the lack of effect on HCV viral load and the absence of an unvaccinated control group (Ghany 2003). A larger placebo-controlled phase II trial is planned for 2003-4, enrolling 150 patients for whom current HCV treatment is contraindicated or previous treatment was unsuccessful. Innogenetics is also conducting testing in animals of a prophylatic HCV E1 vaccine.

Intercell, an Austrian company, is developing a therapeutic vaccine that incorporates five HCV

peptides primarily targeting non-structural proteins, for use with a proprietary adjuvant. These peptides were selected to present T cell epitopes associated with immune responses that succeed in clearing HCV during acute infection. A phase II dose-escalation study in non-responders to prior interferon-based treatment was initiated in late 2002, with completion expected by the end of 2003. The company has indicated that it hopes to file for approval in 2007; a second-generation HCV vaccine using other epitopes is in preclinical development. Epimmune and Genencor are also collaborating on the development of prophylactic and therapeutic vaccines for HCV, with a focus on stimulating HCV-specific CD4 T cell responses; Genencor transferred its rights under the collaboration agreement to Innogenetics in 2004. The Swedish company Tripep is planning clinical trials for its therapeutic vaccine candidate Chron-VacC™, and is developing a prophylactic vaccine candidate with the Vaccine Research Institute of San Diego. The Canadian firm ViRexx is pursuing preclinical development of HepaVaxx C, a therapeutic vaccine comprised of viral antigen fused to foreign antibodies; clinical studies could begin in late 2005.

HCV vaccine development will require further exploration of the correlates of immunity and the mechanisms of viral persistence. The capability of some people to clear acute infection through successful immune responses certainly supports the concept of prophylactic vaccination. Therapeutic vaccine development may pose different challenges, since HCV-specific immune responses observed during chronic infection shown signs of functional deficits. For example, dendritic cell impairments could prevent the immune system from mounting the desired responses following vaccination. Finally, viral diversity among genotypes and the quasispecies nature of HCV suggest that vaccine development will require careful targeting of highly conserved epitopes common across strains, lest escape mutations emerge.

Vaccine development efforts would no doubt benefit from greater investment and coordination. In addition to scientific challenges, several practical issues will need to be addressed. For example, testing a prophylactic vaccine will require the recruitment and retention of sizeable cohorts of uninfected individuals at elevated risk for HCV infection, such as young injection drug users. Licensure of a vaccine will require outlining clear standards for proof of efficacy by regulatory agencies, primarily the United States' Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medical Products (EMEA). If a prophylactic HCV vaccine does not fully prevent all infections in all vaccinated subjects, how effective does it need to be for approval? Similarly, what parameters—virologic, histologic, immunologic, biochemical, and clinical—would need to change, by how much, and over what amount of time to consider a therapeutic HCV vaccine effective? The answers to these questions will determine not only clinical trial design, but also the willingness of drug companies to invest in vaccine development.

Research Recommendations

Over 14 years after the identification of HCV, treatment options rely exclusively on interferon alfa and ribavirin. By comparison, within 14 years of the identification of HIV, fifteen antiretroviral drugs—six nucleoside analogues, four protease inhibitors, and three non-nucleoside reverse transcriptase inhibitors—had been approved by the FDA. HCV and HIV are different viruses, and as such pose different challenges for drug development, but the disparity in progress is striking. Federal funding for HIV research dwarfs the amounts allocated for HCV; increased and targeted funding to investigate critical research areas in HCV would be welcome. At the same time, HCV research is divided across several institutes within the NIH, and has arguably suffered from a lack of consistent coordination and effective mechanisms for prioritizing and supporting scientific goals and cross-disciplinary research.

Increase funding and coordination of research

The recently established Liver Disease Research Branch within the NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases) should develop into a strong, responsive, and accountable mechanism for advancing basic and clinical research on HCV. Indeed, this development heralds a renewed emphasis on coordination of efforts within the NIH and in collaboration with other Federal agencies (such as the Centers for Disease Control and the Veteran's Administration). The announced preparation of an Action Plan for Liver Disease Research, due in April 2004, will also enable a more thoughtful and thorough assessment of HCV research needs, and should allow for the meaningful involvement of the broadest possible range of stakeholders. Ultimately, the intensification of both basic and clinical research activities will be necessary for further progress in HCV treatment. The six NIH-supported Hepatitis C Cooperative Research Centers (HC CRCs) provide a compelling model for combining basic and clinical research programs. The HC CRCs are jointly funded by the National Institute of Allergy and Infectious Diseases (NIAID), the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), and the National Institute of Drug Abuse (NIDA). Additional resources should be directed to this innovative funding initiative.

Support refinement of *in vitro* and animal models

HCV drug development efforts will also depend on the quality and accessibility of *in vitro* and animal models for HCV infection. The lack of reliable, efficient cell cultures supporting HCV replication, the absence of a small animal model for HCV infection, and the expense of chimpanzee research pose considerable constraints to HCV drug and vaccine development. HCV replicon systems have revolutionized drug screening by offering a viable model for gauging the inhibitory effects of compounds on viral replication, though they do not fully reproduce the dynamics of *in vivo* infection. Further refinements in these models will be crucial for the development of antiviral agents targeting early or late stages of the HCV replication cycle, including attachment, entry, and uncoating as well as virion assembly and release. XTL Biopharmaceuticals in Israel has developed a proprietary mouse model carrying transplanted human liver tissue infected with HCV (Ilan 2002). A Canadian group of academic researchers has been pursuing a similar strategy, producing chimeric mice, transplanted with human hepatocytes, that are susceptible to infection with HCV (Fausto 2001; Mercer 2001). Further work will be

necessary to increase the utility of mouse models. Researchers at GlaxoSmithKline have reported success in using a marmoset model of GB virus B to screen HCV protease inhibitors for antiviral efficacy (Bright 2004). Biomedical research funders, including government and industry, should launch a concerted effort to develop, refine, and validate *in vitro* and animal models for assessing candidate antiviral compounds against HCV.

Promote drug development efforts that study safety and efficacy in real-world populations

Current efforts at antiviral drug development targeting HCV appear promising, but should be complemented by expanded research programs directed at prophylactic and therapeutic vaccine development, the identification of antifibrotic agents, and strategies to prevent and treat hepatocellular carcinoma. The advent of new classes of medications for HCV—in particular the NS3 serine protease inhibitors in clinical and preclinical development—will hopefully usher in a new era for individuals chronically infected with hepatitis C. At the same time, clinical trial design must consider the particular care and research issues of patient populations with high HCV prevalence rates and urgent needs for more effective and better-tolerated HCV treatment regimens. People coinfected with HIV will make up a substantial proportion of candidates for new treatments, as will current and former injection drug users; combined, these groups likely constitute at least half of the domestic market for HCV treatment. If phase II and III studies completely exclude these groups, they decrease their relevance to real-world clinical care and limit the information patients and health care providers receive to make treatment decisions. Drug interaction studies will also be particularly important for these groups. Current interferon alfa and ribavirin therapies have reached the market before the results of studies in HIV coinfected individuals were available; virtually no data exists on the use of these treatments in drug users. This pattern cannot be repeated for future HCV medications.

Initiate partnerships between industry, government, academia, and community

Speeding the development of new therapeutic strategies for HCV will require a coordinated effort involving government, industry, research institutions, private foundations supporting biomedical research, and HCV advocates, especially people infected with hepatitis C. Some models for such efforts already exist; NIAID's Partnerships for Novel Approaches to Controlling Infectious Diseases, a collaboration between government, industry, and academia, has begun to focus on hepatitis B and could be expanded to address HCV. A strategic partnership between public and private sectors could support exploration of new targets and a better understanding of viral-host interactions through techniques such as microarray analysis (Aizaki 2002; Bigger 2001). An intensive research and development program would speed and expand the refinement of tools for rapid and high-throughput screening of candidate compounds, preclinical research, and the establishment of appropriate research infrastructure to facilitate the recruitment of diverse patient groups into clinical trials.

List of Terms Used in This Chapter

3′ UTR (3-prime untranslated region): non-coding region of HCV RNA; site of initiation of negative-sense strand synthesis.
5′ UTR (5-prime untranslated region): non-coding region of HCV RNA; contains the internal ribosomal entry site (IRES); site of initiation of translation.
Adenosine deaminase: a cellular enzyme that converts viramidine to ribavirin.
Alkavirs: a class of imino sugar derivatives containing alkyl side chains.
Alkyl side chains: a chemical modification made to some imino sugar derivatives.
α-glucosidase inhibitor (alpha-glucosidase inhibitor): an antiviral agent that inhibits α-glucosidase, an enzyme involved in the glycosylation of HCV envelope proteins E1 and E2.
Antisense oligonucleotide: short sequences of RNA or DNA that are complementary to a sequence of viral RNA. Antisense oligonucleotides can bind to HCV RNA inside infected cells and block viral replication.
Apoptosis: programmed cell death.
BH3 interacting domain death agonist (BID): a cellular protein involved in apoptosis (programmed cell death).
Calnexin: a chaperone protein involved in the heterodimerization of HCV envelope proteins E1 and E2.
Calreticulin: a chaperone protein involved in the heterodimerization of HCV envelope proteins E1 and E2.
Cap-independent: a form of translation (protein synthesis) using an internal ribosomal entry site (IRES); the method used by HCV to synthesize viral proteins.
Caspases: cellular enzymes involved in apoptosis (programmed cell death).
Castanospermine: an imino sugar that inhibits glycosylation.
Chaperone proteins: cellular proteins (e.g., calnexin and calreticulin) that help HCV envelope proteins E1 and E2 fold into heterodimers.
Chimeric mouse model: mice that have human liver cells transplanted into them. A possible small animal model for testing HCV drugs.
Complementary: an RNA sequence that is the mirror image of a section of HCV RNA; siRNA and antisense oligonucleotides are designed to be complementary to sections of HCV RNA, allowing them to bind to viral RNA, targeting it for destruction.
CpG motifs (cytosine-guanine sequences): molecular patterns commonly found in bacterial and viruses that trigger toll-like receptor 9 and stimulate innate immune responses, including IFN-α production.
Cytokine: secreted proteins that function as chemical messengers between cells to influence (e.g., stimulate, inhibit) immune responses. Cytokines include chemokines, interferons, and interleukins.
Dipeptides: short peptides composed of two linked amino acids.
Endoplasmic reticulum (ER): a membrane within cells; the site of HCV translation (protein synthesis) and strand synthesis.
Glycans: sugar molecules added to HCV envelope proteins E1 and E2 during glycosylation.

Glycosylation: a chemical modification that adds sugar molecules to proteins. HCV envelope proteins E1 and E2 must undergo glycosylation in order to function properly.
Guanosine triphosphate (GTP): one of the four nucleotide building blocks for RNA strand synthesis. GTP levels depend on a cellular enzyme, IMPDH. Ribavirin and merimepodib may work by inhibiting IMPDH, thereby lowering GTP levels and preventing HCV replication.
Helicase: an HCV enzyme, contained within NS3, which unwinds and separates RNA strands during strand synthesis.
Heterodimerization: the formation of complexes joining two different proteins; the HCV envelope proteins E1 and E2 must form heterodimers in order to function properly.
Hexapeptide: short peptides composed of six linked amino acids.
Hybridize: bind to; as in, an RNA molecule (e.g., siRNA or antisense oligonucleotide) hybridizes to the complementary sequence on HCV RNA.
Hyperimmune serum: blood containing highly reactive antibodies to HCV.
Imidazoquinolinamines: a class of drugs that stimulates the secretion of IFN-α and various cytokines.
Imino sugar derivatives: antiviral agents that chemically resemble sugars (monosaccharides).
Immunoglobulin: antibodies.
IMPDH (inosine-5'-monophosphate dehydrogenase): an enzyme that controls guanosine triphosphate levels. Merimepodib (and possibly ribavirin) may work as IMPDH inhibitors.
INF-α subtype: one of the forms of naturally occurring IFN-α molecules produced by the body. Different subtypes may have slightly different antiviral activity. The two FDA-approved forms of pegylated interferon alfa, Pegasys and Peg-Intron, are synthetic molecules derived from IFN-α subtype 2.
INFAR (INF-α receptor): a cell surface receptor that binds IFN-α; composed of two subunits, INFAR1 and INFAR2.
Ion channels: a gateway, composed of one or more proteins, that allows ions (charged atoms or molecules such as calcium) to pass through cell membranes.
IRF-3 (interferon regulatory factor 3): a protein that regulates INF-α-stimulated gene expression and cellular defenses against HCV; the HCV NS3 serine protease blocks the action of IRF-3.
IRF-7 (interferon regulatory factor 7): a protein that regulates INF-α-stimulated gene expression and cellular defenses against HCV.
ISCOMs: immunostimulatory complexes used as vaccine adjuvants.
ISDR (interferon sensitivity determining region): a region of NS5A possibly involved in resistance to interferon alfa treatment.
ISGs (interferon-stimulated genes): genes involved in the interferon response and cellular defense against viruses.
JAK/STAT pathway: a signaling pathway within cells that directs gene expression and cellular functions, including the interferon response.
JNK pathway: a signaling pathway within cells that directs gene expression and cellular functions, including the interferon response.

La protein (La antigen): a cellular protein that binds to the HCV internal ribosomal entry site (IRES) and increases the efficiency of translation (protein synthesis). The La antigen also binds to the HCV 3′ UTR. Possible target for antiviral drugs.
Lipopeptide: a fat molecule (lipid) bound to a peptide (linked amino acids). Lipopeptides are under investigation as possible HCV vaccines.
Luciferase: a gene used in cell culture studies; luciferase lights up and is useful for studies of translation and gene expression.
Mimotopes: synthetic peptides mimicking naturally-occurring HCV epitopes.
Monoclonal antibody (mAb): antibodies derived from a B cell line (sometimes taken from individuals infected with HCV) that is engineered to produce identical antibodies, all targeting the same epitope. Monoclonal antibodies to HCV are under investigation for HCV treatment.
Monosaccharides: sugar molecules. Imino sugar derivatives are synthetic versions of monosaccharides.
NF-κB pathway: a signaling pathway within cells that directs gene expression and cellular functions, including the interferon response.
NN-DGJ: an imino sugar derivative being studied as an HCV drug.
NTPase (nucleotide triphosphatase): an HCV enzyme, contained within NS3, which catalyzes chemical reactions that support the movements and RNA binding of the HCV NS3 helicase.
p7: an HCV protein of unknown function; may act as a viroporin to create ion channels.
Peptide: two or more linked amino acids; proteins are formed from multiple peptides joined together.
Peptidomimetic: a compound that mimics the form of a peptide.
Pharmacodynamics: the study of the effects of a drug on the body, i.e., antiviral efficacy and toxicity.
Pharmacokinetics: the study of the effects of the body on drug levels, i.e., absorption and metabolism.
Poliovirus 3Dpol: the poliovirus polymerase enzyme.
Polymerase: an enzyme that synthesizes new DNA or RNA strands. HCV NS5B contains a polymerase enzyme, the RNA-dependent RNA polymerase, which synthesizes new HCV RNA.
Prodrug: an inactive form of a drug that gets converted inside the body to its active form.
Protease: an enzyme that breaks down proteins. HCV contains two viral protease enzymes: the NS2-NS3 protease, and the NS3 serine protease.
PSMA 7 (proteasome α-subunit 7): a cellular protein that may be involved in HCV translation (protein synthesis); a possible target for antiviral drugs.
RdRp (RNA-dependent RNA polymerase): the HCV polymerase enzyme, contained within HCV NS5B and responsible for synthesizing new HCV RNA strands during viral replicaton.
Ribosome: cellular machinery responsible for translation (protein synthesis); the ribosome "reads" HCV RNA and translates it into HCV proteins.

Ribozymes: RNA molecules that can bind to and cleave (split) HCV RNA inside infected cells.
RNA aptamers: RNA sequences that can bind to HCV RNA inside infected cells, blocking viral replication.
RNAse H: a cellular enzyme that degrades (destroys) RNA after antisense oligonucleotides bind to RNA.
Saccharomyces cerevisiae: a species of yeast.
siRNA: small RNA molecules that can bind to HCV RNA inside infected cells, targeting it for destruction.
Substrate: the region of a protein that binds to an enzyme; e.g., the substrates of the HCV NS3 serine protease are the areas that NS3 binds to and cleaves (splits). Protease inhibitors can be designed to mimic the structure and composition of the HCV NS3 serine protease substrates.
Toll-like receptor 7 (TLR7): a pattern-recognition receptor involved in innate immune responses, including IFN-α production.
Toll-like receptor 9 (TLR9): a pattern-recognition receptor involved in innate immune responses, including IFN-α production.
Viroporin: a viral protein that creates ion channels; HCV p7 appears to function as a viroporin.

Appendix A: Chart of Drugs in Development

Drug Class	Developer	Compound	Stage
HCV NS3 serine protease inhibitors	Boehringer Ingelheim	BILN 2061	Completed phase I; planned phase II on hold pending investigation of toxicities in animals
	Vertex	VX-950	Phase I to begin in 2004
	Schering-Plough	SCH7	Phase I
HCV NS5B RNA-dependent RNA polymerase inhibitors	Idenix	NM283	Phase II in combination with pegylated interferon to begin in 2004
	Japan Tobacco	JTK-003	Phase II
	Japan Tobacco	JTK-109	Phase I
	Rigel	R803	Phase I/II
	Roche	R1479	Phase I
	ViroPharma	HCV-086	Phase I
Imino sugar derivatives	Micrologix	MBI-3253 (celgosivir)	Phase II to begin in 2004
	United Therapeutics	UT231B	Phase II
Monoclonal antibodies	XTL	HepeX-C	Phase II
Antisense oligonucleotides	Isis	ISIS-14803	Phase I/II in combination with pegylated interferon and ribavirin
Interferon variants and alternates	InterMune	Infergen (interferon alfacon-1; consensus interferon)	Phase III study in combination with ribavirin initiated in 2004
	InterMune	pegylated Infergen	Phase I conducted in 2003; further development on hold for financial reasons
	Human Genome Sciences	Albuferon-alpha	Phase II dose-ranging study underway
	BioMedicines	omega interferon	Phase II underway
	InterMune	interferon gamma-1b (Actimmune)	Phase II study in combination with Infergen initiated in 2004
Next-generation ribavirin	Valeant	Viramidine (ribavirin prodrug)	Phase III studies in combination with pegylated interferon
	Vertex	Merimepodib (VX-497, an IMPDH inhibitor)	Phase IIb study in combination with pegylated interferon and ribavirin to begin in 2004

Drug Class	Developer	Compound	Stage
Other immuno-modulators, broad antivirals, and non-specific therapies	SciClone	Zadaxin (thymosin alfa-1; thymalfasin)	Two phase III studies in combination with pegylated interferon; another phase III study in combination with pegylated interferon and ribavirin to begin in 2004
	Maxim	Ceplene (histamine dihydrochloride)	Phase II study with pegylated interferon and ribavirin; phase Ia study of oral formulation completed
	Anadys	isatoribine (ANA 245, a TLR7 agonist)	Phase I; ANA 971 (an oral prodrug of isatoribine) entered phase I in 2004
	Coley	Actilon (CPG 10101, a TLR9 agonist)	Phase I/II underway; phase II to begin in 2004
	Kemin Pharma	KPE02003002	Phase II
	Idun	IDN-6556 (apopotosis inhibitor)	Phase II planned
Vaccines	Innogenetics	therapeutic vaccine	Phase II
	Intercell	therapeutic vaccine	Phase II
	Chiron	therapeutic vaccine	Phase I
	Chiron	preventative vaccine	Phase I

Appendix B: Overview of the Drug Development Process

Drug development is a lengthy and uncertain process. The discovery, development, and testing of a new compound can last over a decade from initial concept to widespread clinical use, and is rife with potential for failure. A number of drugs currently in development for HCV may not succeed in demonstrating efficacy, or may show unacceptably high levels of toxicity. In some cases, the development of antiviral agents is halted or delayed due to financial considerations. Pharmaceutical companies may opt to terminate a development program that seems unlikely to result in a product generating sufficient revenue, while small biotechs may be unable to raise the financing necessary to support large clinical trials. Promising compounds identified by government or academic research may never find a commercial sponsor to conduct the preclinical and clinical research necessary for approval. Despite these odds, the outlook for new HCV therapeutics remains encouraging, with a pipeline of compounds at various stages of development.

Drug development can be roughly divided into three stages: drug discovery, preclinical development, and clinical development. New drugs are only tested in humans during the clinical development stage.

Drug Discovery

This stage of research focuses on identifying compounds that may be active against HCV. Several processes may be involved:

- Target validation: confirming that the target—for example, a particular site on the HCV NS3 serine protease—is appropriate for developing inhibitory strategies;
- Screening assay development: establishing *in vitro* models suitable for testing potential compounds for activity against a target (i.e., that a compound can inhibit NS3 serine protease activity);
- Lead identification: selecting a candidate compound for further preclinical development;
- Lead optimization: examining and potentially improving on the physical and chemical properties of the lead compound with respect to areas that can include potency, toxicity, and pharmacology (drug bioavailability—adsorption, distribution, metabolism, and excretion).

Researchers have a number of methods to pursue these processes, including high throughput screening of compound libraries and structure-based design. Pharmaceutical companies have vast libraries of compounds potentially active against a given target. Automated high throughput screening techniques can rapidly and efficiently identify lead compounds. Rational or structure-based drug design attempts to produce molecules with antiviral potential based on the three-dimensional structure of the target. In HCV drug discovery, the determination of crystal structures of the HCV serine protease, RNA-dependent RNA polymerase, and helicase enzymes has enabled the design of molecules that can bind to active sites on these enzymes. A host of related compounds

with similar targets can be synthesized through combinatorial chemistry techniques, which tweak the chemical structure of a compound to improve its antiviral and pharmacologic properties. All of these methods have been applied to HCV research, identifying and generating lead compounds for targets such as the HCV NS3 serine protease and HCV RNA strand synthesis.

Preclinical Development

Pharmacology and toxicity studies of lead compounds are extended in the preclinical phase to *in vitro* studies and animal models, prior to human testing in the clinical development stage. In vitro models, such as HCV replicons, can help to characterize the antiviral activity of a lead compound. Other *in vitro* systems can help to predict the specificity, toxicity, and pharmacologic profile of a lead compound. Chimpanzees remain the only established animal model for HCV infection, and therefore the best *in vivo* model for drug efficacy prior to human trials. Some recent work has explored the value of mouse models, tamarin hepatocytes, and GB virus B-infected marmosets in validating the antiviral activity of new compounds.

Other animals not susceptible to HCV infection can be used to study the pharmacokinetics (how the body processes a drug) and pharmacodynamics (how a drug affects the body) of a compound. These studies help to establish potential dosing ranges and frequencies, and can define the relative value of different formulations (oral, infusion, etc.) of a compound. Manufacturing processes with appropriate quality control procedures are also developed during this stage. Animal research can also define the safety issues surrounding a drug candidate, and some drug development efforts are halted at this stage due to unacceptable toxicity in animals. Toxicity can arise for various reasons, but particularly when a compound is not highly selective against its target—for instance, if the compound also acts against cellular proteins in a potentially harmful way.

Clinical Development

When an agent's sponsor (the pharmaceutical company) has compiled all of the necessary preclinical data, particularly on safety, they can submit an investigational new drug application (IND) to the FDA outlining plans for safety and efficacy testing in humans. If approved, research advances in phases. Development can be halted at any phase if study results are unfavorable due to high toxicity and/or poor efficacy, poor pharmacologic properties, or other negative safety data emerges from additional animal studies. Clinical development programs can also be stalled or terminated due to economic factors and business decisions.

- Phase I: Small studies of healthy volunteers or, in some cases, individuals with stable HCV infection that determine short-term safety, explore dosing, and determine pharmacokinetics in humans. Phase I trials are not designed to establish the efficacy of a drug, though some information on the drug's activity may be collected. Phase I studies enroll up to a few dozen participants and can be conducted within a year's time.

- Phase II: Medium-sized trials examining longer-term safety and efficacy in the target population—i.e., individuals with chronic HCV infection. Some aspects of phase I and II trials may be combined and are used to identify the optimal dose

of the drug. Phase II studies may enroll up a few hundred participants and can last for up to two years. The majority of development failures for compounds that have entered human studies occur during this phase.

- Phase III: Longer, large-scale randomized clinical trials determining safety and efficacy. These studies can enroll hundreds to thousands of participants and may last for several years. Phase III research is designed to prove that a drug works and is relatively safe, by comparing it to standard of care or placebo. FDA approval is contingent on the results of these trials.

- Phase IV: Post-marketing studies conducted after a drug's approval by the FDA. Intended to gather data about long-term or rare toxicities, and may collect information about the use and efficacy of the drug in various patient groups.

The discovery, preclinical, and clinical stages of drug development increasingly overlap. Companies also increasingly describe their clinical trials by dividing early phases into loosely (and often, inconsistently) defined subcategories. Phase Ia may be used to describe initial tests in healthy (e.g., uninfected) volunteers, while phase Ib sometimes refers to early short-term tests in people with HCV. Phase IIa studies compare the pharmacokinetics of various doses for longer periods, and phase IIb studies may generate initial data about the use of a new agent in combination (e.g., with interferon).

Many drugs that are currently in early (phase I and II) clinical development may not reach the market for several years. Compounds currently in preclinical development may not become available until the next decade. These timetables have particular relevance to people with hepatitis C and their doctors who are currently considering whether to treat now with pegylated interferon/ribavirin, or defer treatment until better drugs become available.

The development of a prophylactic vaccine could take even longer. Even if a strong candidate vaccine was entering clinical trials, testing for efficacy—the ability of the vaccine to prevent new HCV infections—could take several years and thousands of subjects. A preventative vaccine must be tested in uninfected persons, with an endpoint of whether people receiving the vaccine are less likely to develop infection than people who do not receive the vaccine. The amount of people necessary to test vaccine efficacy depends on the rate of new infections in a population; a high-risk population (such as injection drug users) with a high annual incidence of new HCV infections would be a logical group for vaccine efficacy trials. But even among injection drug users, not everyone will become infected with HCV in a given period of time, regardless of whether they receive the vaccine under investigation. Also, ethics dictate that vaccine trial participants receive information, counseling, and tools to reduce their risk of HCV infection—for example, information on the risk of needle-sharing and referral to a syringe exchange program or drug treatment. If people participating in a vaccine trial reduce their overall risk of HCV infection as a result of these interventions, sample size and/or duration of observation will necessarily increase.

For both drug and vaccine development, Phase III trials are enormously costly undertakings, and general require the direct involvement or financing by a large and established pharmaceutical company. Frequently cited (albeit controversial) estimates of the total cost of developing a new

drug from discovery through to FDA approval exceed $800 million (Rawlins 2004). The necessary resources and, to varying degrees, expertise involved in clinical testing and drug approval often exceed the capacities of smaller, start-up biotech firms, which typically operate for several years without substantial revenue until their first products reach the market. As a result, the numerous biotechs with candidate HCV compounds generally have to form partnerships with larger pharmaceuticals to raise the capital for Phase II and III clinical trials.

Many biotechs have particular expertise in a specialized field of drug development (e.g., compound screening; design of prodrugs), and pharmaceutical companies often enter into partnerships with them aimed at HCV drug discovery and lead identification. Companies may also enter partnerships with academic research groups, and compounds and techniques identified through academic research are commonly licensed to industry or spun off into new biotechs.

Many of these partnerships and discovery programs are referenced in chapter X (The Future of HCV Therapy) to provide a glimpse at the scope and extent of HCV research. However, the history of drug development suggests that few of these discovery and preclinical research programs will result in new drugs reaching the market. According to current estimates, only an estimated one in 5,000-10,000 compounds evaluated in initial screening during the discovery and preclinical stages will reach the market. Based on industry averages, only one in three compounds being evaluated in clinical trials will ultimately receive FDA approval (Preziosi 2004).

GLOSSARY RESOURCES

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