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.