NIH-FUNDED AIDS VACCINE RESEARCH
A Critical Review

by Gregg Gonsalves

March 2000

Treatment Action Group

Gregg Gonsalves is the Policy Director for the Treatment Action Group. He is the author of TAG's Basic Research on HIV Infection: A Report from the Front and the co-author of TAG's AIDS Research at the NIH: A Critical Review and Problems with Protease Inhibitor Development Plans. He is a member of the Office of AIDS Research Advisory Council.

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

Acknowledgements. First thanks go to Sam Avrett without whom this report never would have happened. Thanks also go to Mark Harrington, Rose McCullough, Bill Snow, David Gold, Mike Powell and Luis Santiago for reading and commenting on various versions of this report. This report was made possible with a generous grant from the Lloyd Foundation. TAG and AIDS Vaccine Advocacy Coalition (AVAC) conducted joint work to gather data, interview researchers, and analyze data about NIH funded research in 1998. In January 2000, AVAC and TAG agreed both organizations could separately use the jointly developed material in any way that supports their core missions. Uses made by either organization may not reflect the position or policies of the other organization. The joint portion of the work was done by Gregg Gonsalves for TAG and by Sam Avrett, for AVAC.

Getting Involved. If you would like more information on how to get involved in advocacy efforts on AIDS vaccine research contact:

Treatment Action Group
350 Seventh Ave., Ste. #1603
New York, New York 10036
Phone: 212.971.9022
Fax: 212.971.9019
E-mail: ggonsalves@msn.com

First Distribution: March 2000
Design and production: Barry Paddock
Based on a design by Joy Episalla
Figures 2 and 3 (page 18) by John Grimwade

This report is dedicated to
Paul Joseph Corser (1961-1999)

CONTENTS

INTRODUCTION

The first two decades of AIDS vaccine research have been a series of disappointments and setbacks. Indeed, while at the epidemic's outset many predicted that it would be easier to develop a vaccine for HIV than effective treatments, the reverse has been the case. Initial approaches to HIV vaccine development foundered due to the unexpected complexity of HIV-1 as an immunogen and, in part, due to somewhat simplistic research approaches. Now, however, with new insights into HIV pathogenesis, new research tools, new resources, and new commitment from the U.S. government, from research administrators at the National Institutes of Health (NIH), and from the non-governmental and multilateral sectors, AIDS vaccine research is finally getting the attention, resources, and emphasis it deserves.

Our goal in this report is to outline the scientific and practical obstacles in the path of developing a safe, effective, globally deployable AIDS vaccine, to examine the AIDS vaccine research programs funded by NIH -- taking 1998 (the last year for which complete data are available) as a single, comprehensive snapshot -- and to recommend methods of overcoming those obstacles to accelerate the discovery, development, and deployment of an effective vaccine.

We believe that a vaccine is most likely to emerge from a creative and rigorous synthesis of basic research in human and non-human primate immunology and in HIV virology, with animal and clinical studies of vaccine candidates, delivery routes, adjuvants, and the like. We hope that by examining the scientific issues faced in basic, animal, and clinical HIV vaccine research, we can contribute to overcoming the obstacles and thus contribute to a revitalized, accelerated, intensified effort.

Materials & Methods

We surveyed the published scientific literature to get a sense of the progress in the field and the unanswered questions that are facing the scientific community. We also interviewed more than two-dozen leading researchers,[1] asking them what they thought were the most pressing scientific issues in vaccine research and what practical problems might be impeding progress towards developing a vaccine for HIV infection. Finally, we pored over the listings and abstracts of grants funded with AIDS vaccine research dollars during federal fiscal year (FY) 1998. We looked at FY 1998 because that was the most recent year for which complete records were available from the NIH's AIDS Research Information System (ARIS) and Computer Retrieval of Information on Scientific Projects (CRISP) databases.[2] Whenever possible, we have tried to comment on newer projects and programs when they are relevant.

We have divided the report into three major sections: basic research, animal-based research, and clinical trials. Several major recommendations and overarching themes emerged from our analysis. These major themes include advocating for 1) continuing increases in research funding for all three major areas of HIV vaccine research, 2) intensified investment in human immunology, 3) an expanded, more cohesive animal-based research program, and 4) maintaining a strong, scientifically rigorous domestic and international clinical research program.

It is of great concern to us that our findings and recommendations echo many of those made by researchers and advocates about the NIH AIDS vaccine program in the Levine report almost four years ago. While the NIH AIDS vaccine program has grown substantially in recent years and been considerably restructured, there are lingering problems in the program that have yet to be adequately addressed.

MAJOR THEMES & RECOMMENDATIONS

Major Theme I: Increase the Overall Investment in Vaccine Research

Finding a way to control the global AIDS epidemic should be one of the world's highest public health priorities. Although behavioral interventions and public health measures such as safer sex campaigns, antiretroviral therapy initiated during pregnancy, and the surveillance of the blood supply remain our best current options, HIV vaccines may be the only cost-effective way to ultimately end transmission of HIV worldwide.

Despite increased attention in the media, speeches from President Clinton, and increased budgets for HIV vaccine research, government and private investor funding for HIV vaccine research is still relatively small. Funding in 1998 to research and develop HIV vaccines by NIH and all other U.S. agencies amounted to only about 10% of overall AIDS research funding. However, much HIV pathogenesis, natural history, and epidemiology research is also likely to contribute to the development of an HIV vaccine.

The $148 million spent by NIH in 1998 for HIV vaccine research was widely distributed, supporting research through 647 grants and contracts to more than 140 institutions and easily more than 1,000 researchers. NIH funding thus encompassed an enormously broad range of researcher ideas. Although widely distributed, NIH funding wasn't randomly scattered. The 1998 NIH effort included several targeted, concerted funding initiatives to address critical research questions, refine research techniques, make new assays and reagents widely available, and support networks and collaborations.

We believe the NIH AIDS vaccine research effort -- including its basic, animal, and clinical aspects -- can utilize continuing funding increases in line with those obtained in the past few years. Where will this money come from? In recent years, NIH has received unprecedented increases in its annual budget. AIDS research too has continued to grow at a robust rate. With the NIH budget set to double by 2002, additional spending on AIDS vaccine research should be possible without reducing resources devoted to other areas of research on HIV infection, or on other high priority basic and clinical research areas.

HIV VACCINE BASIC RESEARCH

Major Theme II: Increase the Investment in Human Immunology

While great strides have been made in basic research on HIV infection, there are many unanswered questions about what kind of immune response to HIV a successful vaccine will have to induce and about how to engender that response. Right now, we cannot induce long-lasting, broadly cross-reactive, vigorous antibody and cellular immune responses in humans with any of the current vaccine candidates furthest along in development.[3] While there is a healthy investment in immunology at NIH, most of that work is being done in mice. A robust, new investment in clinical immunology is needed to understand how we can generate effector and memory neutralizing antibody and cytotoxic T-cell responses at mucosal sites.[4] [This too was a key, still incompletely implemented, recommendation of the 1996 Levine Committee report.]

RECOMMENDATIONS

1.Increase Support for Research on Mucosal Immunity

NIH should increase resources devoted to mucosal immunology and vaccinology, and should help bring new researchers into the field by initiating new training programs in this area. In addition, clinical research on mucosal delivery of new candidate vaccines should be made a priority by the new Vaccine Trials Network.

2.Increase Support for Studies of Antigen Delivery in Primates and Humans

NIH needs to support additional work on antigen delivery in primates and humans. NIH should expand access to monkeys through supporting new breeding programs and investing in the development and production of reagents for primate experiments. Study sections need to be more generous in the level of funding they will support for primate studies. Resources devoted to clinical and human immunology need to be increased, with a special emphasis on bringing young physicians into the field to study some of these unanswered questions in a clinical setting.

3.Increase Support for Studies of Immunological Memory

With the difficulty in sustaining both antibody and cellular responses with current immunogens, NIH should invest additional resources in the study of immunological memory in humans. NIH should coordinate its non-AIDS work in this area with basic research on HIV vaccines.

4.Accelerate Research on Novel Envelope-based Immunogens

NIH needs to break free of the tether of outdated approaches to virus neutralization using monomeric subunit vaccines in both basic and clinical research. While several groups are working on better envelope-based immunogens, research in this area can be expedited if NIH supports fast-track testing of these new products in macaques and in humans.

HIV VACCINE ANIMAL-BASED RESEARCH

Major Theme III: Create an Expanded, More Cohesive Animal-based Research Program

The SIV and SHIV macaque models offers important tools to address many basic scientific questions about the primate immune system, the immune responses to retroviral infection, and the relative efficacy of different vaccine concepts. Unfortunately, using macaques in research is expensive; these animals are a precious and scarce resource. To date, most of the studies performed in macaques have been small, pilot studies using different assays, different vaccine candidates, different routes of challenge, different challenge viruses, and so on. With small numbers and varying research conditions, it is hard to compare animal-based research studies and get a sense of the big picture. NIH is just now beginning to initiate a large, comparative study of four vaccine approaches in macaques. This is a good first step, but NIH needs to make greater resources available for animal-based research and to encourage a coordinated, rational approach to animal studies. The National Center for Research Resources (NCRR) has been negligent and unresponsive to criticisms from the Levine Committee and others regarding the administration of the Regional Primate Research Centers (RPRCs) and other primate colonies. NCRR should be catalyzing progress in this area rather than defending its bureaucratic turf.

5.Increase Overall Resources for Animal Research

NIH needs to enhance its support for virological and immunological studies of retroviral infections in primates.

6.Increase Resources per Research Study

Program staff at the National Institute for Allergy & Infectious Diseases (NIAID) and at NCRR need to direct additional funding to support studies with sufficient statistical power to offer reliable answers to research questions. Researchers should design studies to include larger numbers of primates (i.e., more than 3-4 per arm) and ask for this funding in their applications. Study sections need to look favorably on larger primate studies.

7.Rationalize the Simian/SIV Model

Large, comparative studies of vaccines in macaques are critical to assess and optimize candidate immunogens for further testing in humans. NIH should streamline the mechanisms for initiating these trials and provide sufficient resources for their expeditious conduct so that the information gathered can be quickly integrated into decision making regarding human studies.

8.Build Understanding of Protection by Live, Attenuated SIV

Live, attenuated SIV offers an important model for understanding the correlates of protection conferred by vaccination. More research is needed to look at a wide range of systemic and mucosal immune response in this model.

HIV VACCINE CLINICAL TRIALS

Major Theme IV: Maintain a Strong, Scientifically Rigorous Domestic and International Clinical Research Program

NIH has supported clinical research on HIV vaccines for more than a decade. The programs supported have evaluated candidate vaccines supplied by industry for both safety and immunogenicity. So far, NIH has not initiated any phase III efficacy studies (a single, industry-sponsored international study of the VaxGen gpl 20 subunit envelope protein is ongoing). The current phase I-II NIH-supported clinical trials programs need to be maintained in the United States and abroad; however, these programs need to refocus their efforts on phase I and II studies of novel vaccine concepts rather than pursuing further study of vaccine candidates that have shown little promise in preclinical, animal, or early human testing. In addition, the clinical trials programs should continue developing closer ties to the basic scientific community to ensure more bi-directional collaboration between bench and clinic in HIV vaccine research.

9.Fund Development of New Vaccine Products and Immunization Strategies

NIH needs to directly fund development of products to feed into clinical evaluation. NIH should expand direct contracting with companies to develop HIV vaccine candidates, such as newer versions of MVA, VEE, AAV, and attenuated salmonella vectors. The NIH should encourage optimization of current vaccine candidates and be willing to discourage work in outdated vaccine concepts that have performed poorly in preclinical, animal or early human studies.

10. Maintain a Solid Clinical Research Infrastructure

NIH should ensure that the new HIV Vaccine Trials Network (VTN) and the vaccine clinical trial activities of the Vaccine Research Center (VRC), the NIH intramural trial site, and the perinatal sites will have the capacity for an adequate number of phase I, II, and III trials during the coming five years. That being said, the clinical research programs for vaccines should be held to the highest scientific standards with adequate oversight through regular review by extramural researchers. The advancement of candidate immunogens along the vaccine clinical development pipeline has to be driven by science and not by political expediency or industry demands.

11.Riqorously Evaluate Immunology & Basic Science within Clinical Trials

A coherent plan with interim goals need to be articulated for the clinical trials program so that clinical trials evaluate immunization strategies in a concerted, systematic manner. Expansion of human immunology research, linked to HIV vaccine clinical trials, is needed. Larger, more intensive phase I and II trials should compare vaccine products and immunization strategies and the resulting immune responses.

12.Support a Strong International Vaccine Development Effort

NIH should work more closely with the Centers for Disease Control (CDC), the U.S. Agency for International Development (USAID), and the Department of Defense (DOD) to support the development and maintenance of biomedical research infrastructure in developing countries.

I. HIV VACCINE BASIC RESEARCH

Basic research provides the groundwork for developing a vaccine against the virus by advancing our understanding of the immune response to HIV. In 1998, NIH funded a large amount of basic research focused on understanding HIV antigens and human responses to those antigens. Major work explored the mechanisms of humoral and cellular immunity, both systemically and at mucosal surfaces. Other important work helped to define the structure and immunogenicity of HIV envelope and other HIV proteins, and to understand the presentation and processing of HIV antigens by the human immune system. About $37 million, or 25%, of the $148 million spent by NIH on HIV vaccine research was dedicated to basic research. This funding mostly came from NIAID (73%), with the remaining funding coming from NCRR (15%), the National Cancer Institute (NCI) (12%) and other institutes. Funding for basic research related to HIV vaccines was largely extramural (95%). More than 70% of the funding went to extramural investigator-initiated grants. Although the intramural AIDS vaccine program at NIH is small, there is a talented cadre of investigators in this area on the NIH campus. The intramural effort is due for significant expansion in the years ahead with the establishment of the new Vaccine Research Center (VRC) in Bethesda. 1998 was an unusual year for grant funding, since two cycles of the AIDS Vaccine Research Committee (AVRC)-initiated Vaccine Innovation Grants (R21 s) were funded in this year, accounting for as much funding as HIV vaccine-related R01 s.

TABLE 1: FY 1998 NIH-SUPPORTED BASIC HIV VACCINE RESEARCH BY RESEARCH AREA, AWARD TYPE, NUMBER OF AWARDS, & FUNDING LEVEL

AWARD TYPE	MUCOSAL IMMUNITY		ANTIGEN PRESENTATION		CTL RESPONSE		ANTIBODY RESPONSE		TOTAL	
	N	$	N	$	N	$	N	$	N	$
K01	—	—	1	93,205	—	—	—	—	1	93,205
K08	1	582,822	1	16,816	4	241,232	—	—	6	840,870
N01	1	289,219	—	—	2	315,719	—	—	3	604,938
P01	—	—	—	—	10	2,083,359	7	1,145,253	17	3,228,612
P51	—	—	—	—	3	256,701	6	766,499	9	1,023,200
R01	6	1,883,418	4	744,845	13	3,302,111	17	4,735,641	40	10,666,015
R03	1	222,000	1	77,000	—	—	—	—	2	299,000
R21	7	2,126,869	11	2,471,850	14	3,286,751	29	6,287,315	61	14,172,785
R29	1	388,440	—	—	—	—	1	96,300	3	484,740
R37	—	—	—	—	—	—	1	361,127	1	361,127
R43	1	100.000	—	—	—	—	1	104,814	2	204,814
S10	—	—	—	—	—	—	1	88,000	1	88,000
U01	9	2,027,433	—	—	2	154,438	—	—	11	2,181,871
Z01	—	—	1	435,734	2	1,565,225	1	700,303	4	2,701,262
TOTALS	**27**	**7,620,201**	**19**	**3,839,450**	**51**	**11,285,221**	**65**	**14,354,752**	**162**	**36,950,439**
	17%	**(20.6%)**	**12%**	**(10.4%)**	**31%**	**(30.5%)**	**40%**	**(38.8%)**		

Basic HIV vaccine research needs a vigorous infusion of new funding support. The figures above indicate the relative paucity of research being done on mucosal immunity and antigen presentation ($7.6 million and $3.8 million, respectively). It is also obvious that the R21 innovation grants have helped attract high-quality investigators to selected topics; in the 1998 funding cycle these R21s

represented 38% of the NIH basic HIV vaccine research portfolio. These are only two-year awards, however, and NIH needs to consider how best to support longer-term research in these target areas and in others indicated throughout this report.

SCIENTIFIC ISSUES

The Mucosal Immune Response

The infection and the immune response to HIV begin together at the frontier between the outside world and the body, which is, in the case of sexual transmission, the mucosal epithelia. HIV can cross the epithelial barrier through a process known as transcytosis or through active transport by M cells which are both notably susceptible to antibody blockade[5,6] although other studies suggest that intraepithelial Langerhans cells are first infected by the virus and initiate its spread[7]. Once past the epithelia, HIV is thought to be picked up by antigen presenting cells (APCs), primarily dendritic cells (DCs). Newer work, however, suggests that HIV directly infects CD4+ T lymphocytes in mucosal associated lymphoid tissue (MALT), establishes a fulminant local infection within a few days, and then spreads quickly throughout the body.[8,9] Emerging data suggest that viral reservoirs are established early during mucosal infection, and thus predict that preventive vaccines must engender a quick and vigorous mucosal immune response.

What kind of mucosal immune response will be needed to block HIV infection, and how might it be achieved? One key issue, still controversial, is whether most mucosal transmission takes place in the form of cell-free or cell-associated virus. Good research exists supporting the physiological relevance of both approaches.[7,10,11] Most likely it is a mix of both mechanisms; however, the relative frequency of each is unclear. Cell-free infection may be easier to block with an antibody response, while a cell-associated infection is likely to require cell-mediated immune responses in addition to antibodies.

Although several non-human primate studies have demonstrated protection from mucosal infection with SIV, SHIV, and HIV-2, none has been able to determine the correlates of this protective immunity.[12,13,14,15,16] Antibody mediated immune responses could potentially block HIV, at the epithelial surface or in intraepithelial space before it infects T cells or is picked up for transport to local lymphoid tissues, by neutralizing virus intracellularly or by interfering with assembly of virus particles, if cells are infected at these sites.[17] While intravenous challenge experiments have established that only high neutralizing antibody titers will protect against infection by this route, the characteristics of a successful mucosal antibody response to HIV or SIV remain to be established.[18] Mucosal IgA is likely to be important based on evidence from other viral infections,[19,20] studies of individuals exposed to, but uninfected with HIV[21,22] and in vitro studies.[23] IgG may be useful, as it is plentiful at mucosal sites, and has been shown to neutralize HIV and to mediate antibody-dependent cellular cytotoxicity (ADCC), at least in serum.[24]

Even if a vaccine-induced antibody response is not sufficient to contain HIV infection by itself, this response may slow the progress of HIV transmission enough to allow vaccine-induced cellular responses to clear the nascent HIV infection. There is some evidence that mucosal cytotoxic T lymphocytes (CTLs) are involved in protection against rectal and vaginal challenge with SIV. Recent work by Cromwell and colleagues[25] has shown that immunization with live, attenuated SIV, which protects macaques from infection with pathogenic strains of the virus, is associated with the presence of CTLs expressing the alpha 4/beta 7 intestinal homing integrin in blood, lymph node, duodenum, and colon. By contrast, immunizations with a combination of DNA and modified vaccinia virus Ankara (MVA) vaccines did not elicit these CTLs.

There may be additional mechanisms of protection from HIV infection at mucosal sites. Recent studies demonstrating protection from rectal challenge with SIV after targeted iliac lymph node immunization with recombinant simian immunodeficiency virus gp1 20 and p27 indicated an association with elevated levels of beta-chemokines, including RANTES, MIP-1 alpha and MIP-1 beta, and a suppressor factor produced by CD8+ T cells.[26]

Since most HIV infections worldwide occur through sexual contact with infected individuals, the immune response in the genital mucosa will be critical to prevent infection. Yet this area of research remains grossly understudied. Basic principles about the mucosal immunology of the genital tract are not well understood: Where do the humoral and cellular immune responses in this part of the body originate? How long do they last? How do we induce local immune responses? Can we immunize systemically and generate a mucosal response? What cell types are key players, and what are the patterns of cytokine and chemokine expression in a healthy host and during inflammation and infection with other micro-organisms?

Antigen Presentation

Both humoral and cellular immune responses depend on the efficient and proper presentation of antigen to T and B lymphocytes. CD4+ T cells recognize fragments of antigen processed through the endocytic pathway and in complex with major histocompatibility complex encoded class II proteins. CD8+ T cells recognize fragments of antigen processed through the cytoplasmic pathway and in complex with MHC class I encoded proteins. For their part, B cells can recognize unprocessed, intact antigen through their immunoglobulin receptors. Antigens are presented to lymphocytes by specialized antigen presenting cells (APCs) including dendritic cells (DCs), by phagocytic cells such as macrophages, and by B cells themselves, which need feedback from CD4+ T cells to differentiate into plasma cells and secrete antibodies.

HIV subverts the antigen presentation process in a multitude of ways. The HIV-1 tat protein inhibits inhibit the production of MHC class I proteins.[27] The HIV-1 nef protein triggers the endocytosis of these proteins from the surface of the cell.[28] The HIV-1 vpu protein induces the degradation of these proteins in the endoplasmic reticulum.[29] Mutations in HIV can interfere with the binding of viral peptides to MHC proteins or to T-cell receptors (TCR); thus, these mutations can permit viral escape from CTL recognition.[30] In addition, these new viral variants also "can differentially activate CTL, act as CTL decoys, induce anergy, distort the CTL repertoire or antagonize the response to normal antigen.[31]

The primary loci for antigen presentation are the lymphoid organs, which are also the primary loci for HIV replication. Follicular dendritic cells (FDCs) in the germinal centers of the lymph nodes and mature germinal center dendritic cells (GCDCs) can transmit virus to CD4+ T cells respectively through virions complexed with antibody and virions captured on Fc receptors.[32,33] In these ways, HIV subverts the machinery of antigen presentation to perpetuate its own spread and survival yet, despite this interference by the virus, antigen presentation remains a key component of the immune response to HIV in infected individuals. The manner in which immunogens are presented to the immune system will likely be an important factor in the success of any AIDS vaccine. DCs are likely to be responsible for initiating the strong CTL responses seen in HIV infection by stimulating CD8+ T cells with HIV peptides complexed with MHC class I proteins. DCs may be infected in MALT, present peptides from noninfectious virions or viral debris, or present peptides from phagocytosed, apoptotic cells.[34,35] Thus, an effective vaccine is likely to require early mobilization of a DC response.

Basic research on antigen presentation offers clues to what kind of vaccine might be best in inducing a protective immune response; however, much of this work to date has been done in mice. Live, attenuated viruses are ideal candidate immunogens because they can present intact, native virions to B cells, inducing neutralizing antibody responses, and shuttling viral proteins into the endocytic pathway for presentation in complex with MHC class II proteins to CD4+ T cells, which drive differentiation and maturation of antibody and CTL responses. Live, attenuated viruses can also infect cells, allowing viral proteins and RNA to enter the cytoplasmic pathway and be presented in complex with MHC class I proteins to CD8+ CTLs. Other forms of vaccines are theoretically less ideal from the standpoint of antigen presentation. Live viral vectors that replicate poorly or for only a single round of replication may not provide enough peptides for uptake by antigen processing mechanisms and may blunt the maturation of DCs by limiting the inflammatory response that accompanies a robust viral infection. DCs play an integral role in DNA vaccination. They present antigen from neighboring, transfected cells and can be directly transfected themselves by the plasmid immunogen. One current challenge is to target more DCs for transfection or to get them to take up more antigen from neighboring cells. [36,37]

The Cellular Immune Response

Most viral infections, perhaps with the notable exceptions of hepatitis B and rabies,[38] are brought under control by the cellular arm of the immune system with or without the assistance of an antibody response. While the correlates of immunity to HIV infection are still incompletely understood, eliciting cytotoxic T-cell (CTL) responses to HIV is likely to be an important goal for a preventive AIDS vaccine. What evidence do we have that suggests this? Studies in macaques vaccinated with a live, attenuated SIV and protected from challenge with a pathogenic strain of the virus appeared to correlate protection with the presence of SIV-specific CTLs, but not of neutralizing antibody.[39,40,41] More recent studies, however, have shown that SIV-specific CTLs rise after viral challenge in protected monkeys[42] which complicates any assumptions about the role of these cells in conferring protection. Thus, the anti-HIV CTL response may be necessary but not sufficient (CD4 T-cell help may be required, for example). Additionally, in both acute and chronic SIV infection, CTLs seem to be critical in controlling viremia. In primary infection, macaques treated with an antibody to CD8+ lymphocytes do not bring initial viremia under control and have a rapid disease course.[43] Monkeys with chronic SIV infection have a burst of viremia if they are treated with anti-CD8+ lymphocyte antibodies.[44] Using the relatively new MHC class I/peptide tetramer-binding assay, the emergence of a single epitope-specific CTL population constituting anywhere from 1.3% to 8.3% of the total peripheral pool of CD8+ T cells was shown to correlate with the clearance of the initial viremia in acute SIV infection of macaques.[45] Using older CTL assays in studies of acute HIV infection, the presence of env-specific CTLs has been shown to be associated with lower viral loads and delayed disease progression.[46] In a cohort of HIV-infected hemophiliacs, gag-specific CTLs were similarly associated with delayed progression.[47] These observations have been confirmed using the new tetramer technology.[48] The importance of the CTL response in two special populations of individuals-long-term non-progressors (LTNPs) and exposed uninfecteds-has also been extensively documented. In several studies, LTNPs have been found to have strong CTL responses to various HIV epitopes in association with low viral loads.[49,50] EU individuals also seem to have robust CTL responses.[51,52,53] Recently, three HIV-infected individuals who stopped antiviral therapy but maintained viral suppression were shown to have vigorous and broad CTL responses with or without strong neutralizing antibody titers.[54]

Despite the evidence suggesting the potential of a vigorous HIV-specific CTL response to prevent infection with the virus, CD4+ T-cell helper responses generally sustain CTL memory responses and

promote active CTL effector responses[55,56,57,58] and may be required for an effective vaccine. The importance of HIV-specific CD4+ Tcell helper responses is underscored by recent work suggesting that these responses help suppress viral replication in LTNPs, and that HIV-specific CD4+ helper cell responses can be rescued from viral destruction by treating acutely infected patients with highly active antiretroviral therapy (HAART).[59] Another more recent study, however, has shown that these HIV-specific CD4+ cell responses persist in untreated patients with chronic, progressive HIV infection and are not correlated with viral load.[60] [The two studies used different assays, the first using lymphocyte proliferation assays (LPA) and the second cytokine staining.] These studies complicate our understanding of the role of CD4+ T cells in the immune defense against HIV.

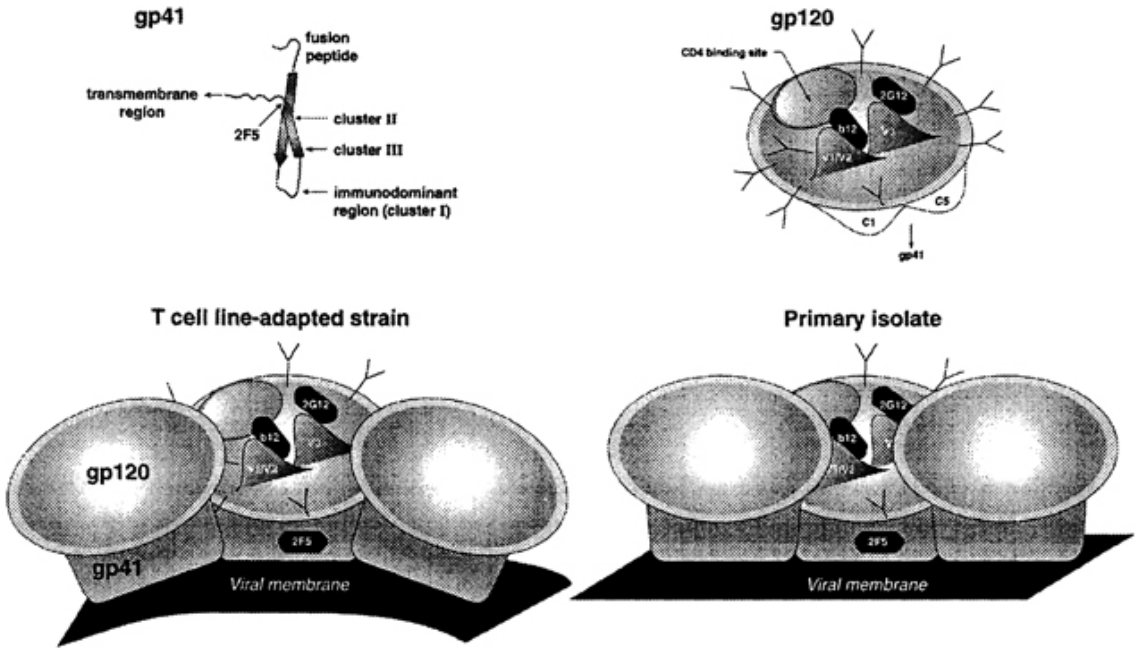
The Antibody Response

Why might it be important to have a potent antibody response to HIV? First of all, important experiments demonstrated that chimpanzees and monkeys can be protected by neutralizing HIV-1 antibodies from infection with lab strains of HIV itself or chimeric SHIV, which carries the core components of SIV and the envelope of primary HIV isolates. In 1992 Emini and colleagues[61] protected chimps from infection with the classic lab strain of HIV-1, HIVIIIB, after passive transfer of neutralizing antibodies directed at the V3 loop of the viral envelope. More recent work by Shibata and colleagues[62] has shown that purified immunoglobulin (IgG) derived from chimpanzees infected with a dual-tropic strain of HIV could block infection of pigtailed macaques by an SHIV with the envelope of that same primary isolate.

Antibody neutralization and prevention of infection by primary HIV isolates, those strains most closely resembling viruses encountered in naturally occurring infections, has also been shown in hu-PBL-SCID mice. Gauduin and colleagues[63] were able to completely protect mice from infection with two primary HIV isolates via passive transfer of a monoclonal antibody, IgGlbl2, several hours after viral challenge.

Although protection of animals by antibodies has been accomplished in several settings, repeating the same success in humans will be a difficult task. Why is this the case? Primary isolates of HIV are notoriously difficult to neutralize. Even antibodies recovered from people with HIV have only a weak ability to neutralize their own autologous viruses or a panel of other primary isolates.[64] There are several reasons for this phenomenon. First, the viral envelope is swathed in relatively non-immunogenic glycoproteins that may provide a shield for the virus against recognition by the immune system.[65] Second, the envelope proteins of primary isolates of HIV are distinctly different in conformation or structure from strains of the virus that have been passaged in culture in the laboratory. The envelope of lab strains, or T cell line-adapted (TCLA) strains of HIV, seems to have more exposed epitopes or sites for immune attack than the strains that circulate in people with HIV. In fact, only three epitopes have been found to be accessible for neutralization on a broad range of primary viruses-bl12, 2012, and 2F5. Figure 1 below represents the envelopes of TCLA and primary isolates of HIV and the various epitopes that are available for neutralization or are structurally obscured.[66] The envelope of the primary isolates seems to have parts of the V1/V2 and V3 loops, as well as the CD4 binding site, masked from recognition. Others have also suggested that the V1/V2 and V3 loops might obscure the CD4 binding and the chemokine receptor binding sites, respectively.[67]

Figure 1: Conformational Differences in HIV-1 Membrane Glycoproteins



Third, the humoral immune response in vivo may be directed to viral debris rather than the envelope of intact virions. [68] Many antibodies from people with HIV bind to unprocessed gpl 60 envelope precursor, "shed" monomeric gpl 20 dissociated from the virion/infected cell surface, and gp41 exposed on the virion/infected cell surface after shedding of gpl 20. Most of these antibodies have extremely low affinity for binding to mature oligomeric envelope.

Most current vaccine candidates elicit at best disappointing levels neutralization to primary HIV isolates. Most subunit vaccines based on monomeric gp120 or gpl 60 fail to induce neutralizing antibody responses to mature envelope proteins on intact virions from primary isolates because epitope exposure on the recombinant proteins differs significantly from that on circulating virions. Moreover, other vaccine strategies such as live recombinant vectors (e.g., poxviruses) and naked DNA have failed to demonstrate success in generating relevant antibody responses.[69] Several strategies are being pursued to design immunogens that will induce better humoral immune responses. One is to try to design envelope proteins mimicking the oligomeric native envelope on primary isolates while removing features of the molecule that help it evade the immune response.[70,71] Some labs are trying to remove the hypervariable loops (i.e., V1/2 and V3) to expose hidden epitopes and to redirect the immune response away from these highly mutable regions-with varying degrees of success.[72,73] Others are trying to strip away the sugar molecules from the envelope to expose the more immunogenic epitopes below.[74,75] Recently, Nunberg and colleagues at the University of Montana devised an immunogen based on a transitional form of the envelope protein that induced neutralization of 23 out of 24 strains of HIV representing primary isolates from North America, Europe, Africa, India, and Thailand.[76] Nunberg crafted his immunogen by taking cells expressing an envelope protein from a T-cell-tropic strain of virus from an HIV-infected person in an Amsterdam cohort study and another set of cells expressing both the CD4 and CCR5 receptors, letting them begin to fuse together and then freezing them in this transitional state with formaldehyde. All these efforts are indebted to the recent groundbreaking work elucidating the structure of the viral envelope by several teams of researchers around the country.[77,78,79]

In addition to defining targets on primary isolates susceptible to attack by antibodies and designing immunogens that can induce neutralization of these viruses, there may be a need to make sure that these humoral immune responses are relatively long-lasting. In a recent study, the antibodies induced by repeated immunizations with a recombinant gpl 20 lingered only for 40-60 days.[80] Live viral or bacterial vectors expressing HIV envelope may be needed in addition to simple recombinant proteins in order to sustain antibody responses over the long haul.

BASIC RESEARCH RECOMMENDATIONS

1. Increase Support for Studies of Mucosal Immunity

HIV infection is largely (~ 80-90%) a mucosally transmitted disease,[17] and the induction of potent immune responses in the genital tract may be essential in the development of an AIDS vaccine. Still, basic principles about the mucosal immunology of the genital tract are not well understood: Where do the humoral and cellular immune responses in this part of the body originate? How long do they last? How do we induce local immune responses? Can we immunize systemically and generate a mucosal response?

Despite the importance of achieving protection at mucosal sites, most human vaccine studies to date have involved intramuscular or intraveneous inoculation. The few completed studies using mucosal delivery have not shown immunogenicity or antibody production in local secretions.[81] The huge, indigenous microflora in mucosa sites may make it difficult for vaccine candidates with low expression of HIV antigens to engender strong immune responses there; again, a vigorous, local infection may need to be set up to initiate a vigorous immune response.

NIH has several programs in mucosal immunity, including NIAID's Cooperative Mucosal Immunology Group for Investigations on AIDS Vaccines, and other more specific initiatives, focusing on the oral and gastrointestinal mucosa, at the National Institute for Dental Research (NIDR) and the National Institute for Diabetes, Digestive and Kidney Diseases (NIDDK), but the scope of NIH's efforts in this area is dwarfed by the need for increased understanding of immunology at mucosal sites and the need for better tools to assess mucosal responses.

NIH should increase resources devoted to mucosal immunology and vaccinology and should endeavor to bring new researchers into the field by initiating new training programs in this area. Clinical research on mucosal delivery of new candidate vaccines should be made a priority by the new Vaccine Trials Network.

2. Increase Support for Studies of Antigen Delivery in Primates and Humans

What is needed to initiate and sustain an immune response to HIV? How are HIV antigens taken up and expressed? Can this process be upregulated or optimized? How much HIV antigen needs to be expressed and for how long? These questions about antigen presentation and the questions outlined above regarding immunological memory are inextricably linked. While much basic research on antigen presentation is being supported by NIH, the vast majority of this work is being done in mice. In the era of molecular biology, a lot of resources are devoted to antigen identification, cloning genes and expressing protein while far fewer resources are devoted to the process of targeting antigen for delivery to macrophages and dendritic cells.

NIH needs to support additional work in antigen delivery in primates and humans. NIH should expand access to monkeys through supporting new breeding programs and investing in the development and production of reagents for primate experiments. Study sections need to be more generous in the level of funding they will support for primate studies. Resources devoted to clinical and human immunology need to be increased with a special emphasis on bringing young physicians into the field to study these unanswered questions in a clinical setting.

3. **<u>Increase Support for Studies of Immunological Memory</u>**

While eliciting broadly cross-reactive and long-lived CTL responses is one of the major goals of basic AIDS vaccine research, there is considerable uncertainty about how to accomplish this task. Do persistent HIV antigens need to be provided to maintain active, effector T cells against the virus, or will a robust initial expansion of T cells engender a robust memory response? One thing is certain, we will need new vaccine candidates that can induce a vigorous, initial infection (with HIV, rather than vector, antigens dominating the immune response) because the CTL responses generated with the immunogens furthest along in clinical testing have been disappointing. However, the newer, live vectors such as MVA and AAV and some DNA-based immunogens have shown promising results in monkey models.[82]

There are many persistent questions about the role of CTLs in mediating protection from HIV infection or disease progression, although the measurement of CTL responses is clearly undergoing a revolution. Researchers can now actually quantitate CTL responses in vivo with the new tetramer and elispot assays, but does the binding of cells to MHC-peptide tetramers correlate with actual CTL killing? New studies suggest there may be striking functional defects even in CTLs that can bind tetramer and stain positive in intracytoplasmic cytokine assays.[83,84] More work is clearly required to refine the new assays to better understand what is going on in the cellular compartment of the immune system, while simultaneously trying to bring standards of measurement to the field.

Recent, persuasive work in mice has been done which establishes that CD8+ and CD4+ memory T cells do not need constant ongoing antigenic stimulation to persist and recognize their cognate antigen (in these cases, murine lymphocytic choriomeningitis virus, LCMV, and pigeon cytochrome).[85,86] The relevance of this work to humans, the nature of the positive signals that allow memory T cells to survive, and whether we can manipulate the cells or innate immune processes to increase their numbers need to be established.

In 1996, the Levine report noted a dearth in funding for human immunology and made a major recommendation to NIH to boost work in this area of research.[87] In addition, the 1998 Jordan report outlined several areas of scientific opportunity in basic immunology that could advance our search for vaccines for many diseases, including HIV.[88] NIH has made several important new efforts to increase its efforts in human immunology, but they are still too small. NIH now supports a handful of Centers for Excellence in Human Immunology, but the awards for these sites are limited (approximately $3 million in total funding for seven centers has been allocated for this initiative). A single round of innovation grants in human immunology has also been supported by the OAR with $6 million from its discretionary fund.

With the difficulty in sustaining both antibody and cellular responses with current immunogens, NIH should invest additional resources in the study of immunological memory in humans. NIH should coordinate its non-AIDS work in this area with basic research on HIV vaccines.

4. Accelerate the Development of New Envelope-based Immunogens

Eliciting antibodies that neutralize a broad range of primary isolates is one of the major goals of basic AIDS vaccine research, and will be especially needed if an intransigent pool of virus is established early after transmission. This task may not be accomplished simply by learning how to present envelope proteins in their mature, native form. While the recombinant subunit vaccines produced to date have only offered monomeric proteins in their non-native conformation, just mimicking what is expressed on the viral envelope in vivo is unlikely to elicit a protective response. The envelope may have to be modified to make it more immunogenic and molecularly tinkered with to induce strong, neutralizing responses. Additional structural studies of the envelope in conjunction with cellular receptors could be very important in reaching this goal. In the studies described earlier by Shibata et al., the serum raised against the primary isolate in chimps had to be able to neutralize 100% of the challenge SHIV virus in vitro before protection was achieved in macaques passively infused with the antibody preparation. This daunting figure clearly demonstrates how difficult it will be to develop an effective HIV vaccine whose only mode of activity is the induction of neutralizing antibody responses. Furthermore, if a potent antibody response against vulnerable epitopes develops too slowly, it may not be capable of preventing the virus from gaining a foothold in the body.

NIH has used the Innovations Grants program (expedited two-year awards with a maximum of $150,000 awarded per year) to spur new work on the viral envelope, but it ended up initially rejecting support for Jack Nunberg's pioneering work (which was initially funded by amfAR, the American Foundation for AIDS Research).

NIH needs to break free of the tether of outdated approaches to virus neutralization using monomeric subunit vaccines both in basic and clinical research. While several groups are working on better envelope-based immunogens, research in this area can be expedited by supporting fast-track testing of these new products in macaques and in humans.

II. HIV VACCINE ANIMAL-BASED RESEARCH

Animal-based HIV vaccine research evaluates the safety, immunogenicity, and efficacy of immunization strategies in animal/virus models that resemble human HIV exposure, infection, and disease as closely as possible. During past years, NIH has annually funded more than $30 million of HIV vaccine research in animal models. In 1998, the funding level rose to $37 million, constituting by far the largest source of worldwide funding for HIV vaccine research in animal models. Use of animal models in HIV vaccine development has greatly increased our understanding of possible immunization approaches, but the effort still needs increased resources, rationalization of the many animal models used, and further investigation of the results seen in current studies.

SCIENTIFIC ISSUES

Modeling Human HIV Infection and AIDS

Animal models, particularly primate models, provide an opportunity in HIV vaccine research for defining potential approaches for HIV immunization, for gaining insight into the mechanisms of inducing immune responses, and for acquiring some sense of potential safety and immunogenicity in humans. No viral infection in an animal exactly duplicates human HIV infection and disease, but several models demonstrate similar patterns of viral infection, replication, spread, mechanisms of disease, expression of disease, and immune response. Certain animal models may ultimately predict the dynamics of HIV in humans in immune system control and protection against mucosal or intravenous infection. Until protection is demonstrated in humans, however, any correlation or predictive value of animal protection to human protection will remain unknown.

Of Monkeys, Mice and Men

TABLE 2: SELECTED PRIMATE MODELS OF HUMAN/HIV INFECTION AND DISEASE

ANIMAL	VIRUS	VIRAL REPLICATION	DISEASE MANIFESTATION
Rhesus macaque	SIVsm PBj14	High	<1 yr progression to AIDS
	SHIV KU2	High	1-2 yr progression to AIDS
	SIVmac239	High	2 yr progression to AIDS
	SIVmac251	High	2 yr progression to AIDS
	SHIV 89.6P	High	2 yr progression to AIDS
	SIVsmE660	High	3 yr progression to AIDS
	HIV-2	Low	Transient infection
Chimpanzee	HIV-1 JC	High	High viremia, rapid CD4 decline
	HIV-1 DH12	High	Persistent infection; no disease
	HIV-1 5016	High	Persistent infection; no disease
	HIV-1 LAI	Medium	Persistent infection; no disease
	HIV-1 SF2	Low	Transient infection

So far, the closest model for in vivo human HIV infection and disease has been the infection of macaques with SIV and with chimeric SHIV (made up of the replication machinery and core proteins of SIV with envelope and some regulatory genes from HIV-1).[89,90,91] African SIV isolated

from mangabeys causes disease in Asian macaque species, including rhesus, cynomolgus, and pigtailed macaques, in a way similar to HIV in humans. This model, therefore, has been important for work in understanding early viral infection and disease, as well as antiviral immune responses. Furthermore, the envelope structure, core proteins, and coreceptor-facilitated entry of SIV are similar to HIV.[92] Thus, candidate vaccines can be formulated with SIV antigens for evaluation in the macaque/SIV model, and with both SIV and HIV antigens in the macaque/SHIV model.[93]

In the macaque/SIV model, there is a range of infectivity, virulence, and disease outcomes. In terms of virulence and disease outcomes, SIV variants range from those causing rapidly lethal disease, such as an SIVsm variant designated PBj14[94,95] to those causing no short-term disease at all, such as an SIVmac attenuated with genetic deletions.[96] Pathogenesis might be different in neonate macaques than in adults, as demonstrated in a study of attenuated SIVmac showing lethality in neonates.[97] Like HIV, SIV is inefficient at infecting across mucosal surfaces.[98] For evaluation of SIV vaccines in affecting viral replication and disease progression in large groups of macaques, researchers have generally used SIVmac or SIVsm, such as SIVmac251, SIVmac239, SIVsmB670, or SIVsmE660, that cause progressive AIDS-like disease within two to three years. Researchers can also directly evaluate the immunogenicity and efficacy of certain HIV-1 vaccines in macaques by using pathogenic SHIV viruses as disease-inducing challenges.[99,100,101,102]

Aside from the macaque/SHIV system, no animal model exists to directly evaluate immune responses generated by HIV-1 vaccines against a virus causing an AIDS-like disease. Furthermore, except for reagents for macaques and mice, development of complex and specific reagents to detect immune responses is limited. Nevertheless, work continues in several other animal species. Some HIV-1 strains can cause an AIDS-like disease in chimpanzees[103,104] and more pathogenic strains could be developed, although this model is limited by the high cost, the restricted availability of animals, and ethical concerns. Baboons can be infected in a limited way by HIV-1 and HIV-2.[105,106] Cats are infected with a lentivirus called FIV, goats with a CAEV, and horses with an EIAV. FIV-infected cats sometimes develop an AIDS-like illness. However, these viruses are much more distantly related to HIV than SIV, and background immunology and immune reagents are limited for these species.

Attempts are being made to develop transgenic mice and rabbits.[107,108,109] If successful, this would create a small (and inexpensive) animal model of HIV infection and replication. Because immunologic reagents for mice and inbred strains of mice are readily available, researchers might be able to use transgenic mice in better understanding the mechanisms of antibody, CTL, and other immune responses in controlling HIV infection and replication. It is unclear, however, how predictive small animal studies will be of human immune responses.

Protection Achieved, but How?

Research in animals has demonstrated that vaccines can induce protection against HIV-like infections. In macaques with SIV, a range of vaccine approaches have been able to clear challenge SIV, although only live, attenuated SIV is able to provide this level of protection against the more virulent SIVmac251[110] and SIVsmB670.[111] Viral load reduction or delayed disease progression against these SIV strains has been demonstrated by vaccination with combination DNA plus vaccinia vector,[112] vaccinia vector plus subunit,[113] and attenuated vaccinia vector plus subunit vaccines.[114]

It remains unclear from these studies which immune responses are responsible for protection, but researchers are developing tools to answer questions about the mechanisms of immune protection

against pathogenic virus. In the macaque/SIV model, researchers are defining macaque MHC,[115] and developing reagents to detect cytokines,[116] chemokines,[117] costimulatory molecules,[118] and other immune responses.

Building Momentum for Discovery & Development

The animal research field is poised to evaluate and compare a range of new HIV immunization strategies in animals, to measure immune responses and outcomes, and to evaluate mechanisms of protection. To move the field forward, momentum must be maintained in three areas of the NIH effort with respect to animal studies:

- Building upon and improving animal models of the human-HIV system, including further development of the macaque/SHIV model, work to MHC type and clone macaques, establishment of specific pathogen-free macaque colonies, and further work on primate and non-primate immunology.
- Use of existing animal models to gain understanding of safety and immunogenicity of newly developed and reformulated candidate immunization strategies.
- Use of existing animal models, particularly the macaque/SIV model, to increase understanding of protection against lentivirus infection.

Figure 2 : Utilization of HIV Vaccine Animal Research Resources

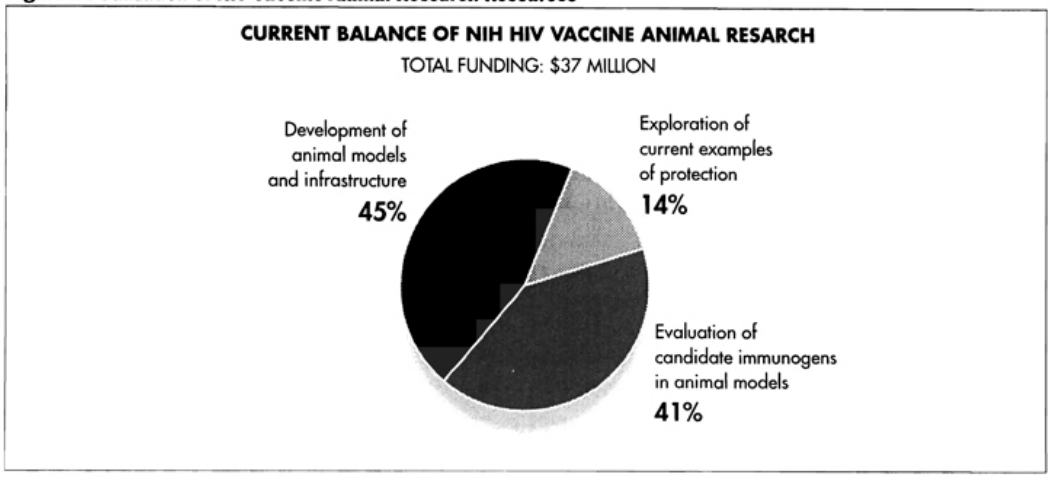
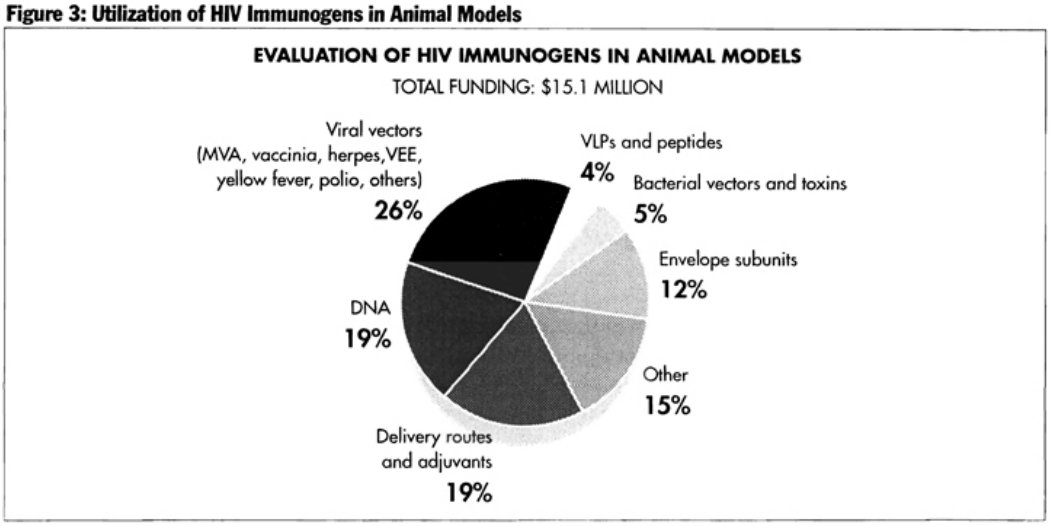


Figure 3: Utilization of HIV Immunogens in Animal Models



In 1998, NIH spent about $37 million on HIV vaccine research involving animals. Most of this funding came from NIAID ($22.7 million, or 61%), NCRR ($5.9 million, or 16%), and NCI ($3.66 million, or 10%). NIAID and NCRR accounted for 74% and 21% of primate-related spending. Overall, 1998 funding was balanced between researcher grants (46%) and extramural and intramural contracts (54%). Programmatically, in 1998, nearly 45% ($16.8 million) of the NIH's $37 million effort in animal-based vaccine research was spent in developing and improving animal models of the human/HIV system. $11 million was spent on macaque breeding contracts and macaque immunology through contracts to the Regional Primate Research Centers (RPRCs) and Simian Vaccine Evaluation Units (SVEUs) and awards to other institutions. About $4 million went to development of mouse-based models of HIV infection (Figure 2). About $5.1 million of NIH funding was dedicated to work on live, attenuated SIV and elucidation of mechanisms of protection and potential pathogenesis in adult and neonatal macaques. $15.1 million was allocated for direct evaluation of immunization strategies in animals, including work on mucosal routes of immunization,[119,120] work on envelope subunits,[121,122,123] and work on viral vectors (Figure 3).[124]

ANIMAL RESEARCH RECOMMENDATIONS

5. Increase Overall Resources for Animal Research

As the above discussion and the basic research section indicate, NIH needs to enhance support for virological and immunological studies of retroviral infections in primates.

6. Increase Resources per Research Study

Despite some efforts within and outside of NIH to address it, the number of animals in any particular HIV vaccine study often remains too limited to demonstrate statistically valid results from any immunization approach. Animal models are an expensive resource, and researchers, funding review committees, and program staff continue to be overly conservative in allocating funding to grants and contracts, particularly for primates.

NCRR and NIAID program staff need to designate additional funding to grants and contracts to support studies with sufficient statistical power to offer reliable answers to research questions. Researchers need to study larger numbers of primates (i.e., more than 3-4 per arm) and request funding for this in their applications. Study sections should look favorably on larger primate studies.

7. Rationalize the Simian / SIV Model

Although ultimate demonstration of comparative immunogenicity and efficacy will have to be done in human volunteers, comparison of vaccine approaches in a standardized primate model would be useful to help further optimize vaccine concepts. But standardization is a challenge. Reliable, standardized assays to measure antibody, CTL, and other immune parameters are needed. Standardized challenge stocks need to be chosen that are "hot" enough to reliably infect and cause disease in a short period of time, but that still mimic the infectivity of HIV in humans. Finally, comparable vaccine antigens and immunization routes need to be selected.

In 1998, NIH funded extensive work to breed specific pathogen-free macaques, to MHC-type macaques, and to develop new SIV and SHIV challenge stocks. NIH also developed a large primate trial to compare different vaccine approaches against each other. Now the primates are out of quarantine and ready, the trial design is complete, and the standardized challenge stock and

immune assays have been chosen. The trial will compare gag protease and env antigens in a wildtype vaccinia vector, an MVA vector, a fowlpox vector, and a DNA construct.

Large, comparative studies of vaccines in macaques are an important tool in assessing and optimizing candidate immunogens for further testing in humans. NIH should streamline the mechanisms for initiating these trials and provide sufficient resources for their expeditious conduct so that the information gathered can be quickly integrated into decision-making regarding human studies.

8. Develop a Clearer Understanding of Protection by Live, Attenuated SIV

In the case of protection of macaques against a pathogenic SIV by immunization with a live, attenuated virus, more research is needed to look at a wide range of systemic and mucosal immune responses. Is there truly sterilizing immunity or transient infection, or does the challenge virus re-emerge? How does control of viral infection happen, and is it correlated with antibody titers, antibody binding and neutralizing capability, timing of early CTLs, CTL epitope specificity and avidity, presence of memory CTLs, other viral suppressive activity, chemokine and cytokine production patterns, some combination of these, or other as yet unknown factors[125,126] Live, attenuated SIV offers an important model for understanding the correlates of protection conferred by vaccination.

III. HIV VACCINE CLINICAL TRIALS

HIV vaccine clinical trials aim to evaluate the safety, immunogenicity, and efficacy of a range of candidate immunization strategies in human volunteers with the ultimate goal of finding a safe and effective vaccine that can be used around the world. Since 1987, NIH has funded clinical evaluation of HIV vaccines, at about $30 million per year since 1995. Much has been accomplished during the past twelve years of clinical testing of vaccine concepts, but concerns continue to be voiced among researchers and NIH program staff about the limits of the clinical trials effort so far. These concerns include the limited number of vaccine strategies tested to date, the limits to insights gained from past trials, and the amount of work yet to be done to be truly ready for large scale international phase III trials.

SCIENTIFIC ISSUES

Safety, Efficacy, Applicability

Ideally, a vaccine to prevent HIV infection and disease should be safe enough to use in millions of people, and effective enough to protect against several routes of infection over a long period of time with as few doses as possible. For global use, an ideal vaccine would be easily administered, inexpensive, and easy to transport and store. To date, most NIH HIV vaccine clinical trials have focused on strategies to prevent infection and disease in adults; some work, however, has evaluated immunization strategies for inducing maternal and infant immune responses to prevent perinatal transmission and HIV disease in children.

Defining and Measuring "Protection"

Efficacy and activity of candidate HIV vaccines can be measured by several clinical and virologic endpoints. If an immunization strategy could completely prevent HIV infection or rapidly clear or control local infection, well-designed, controlled clinical trials carried out in large at-risk populations might be able to demonstrate this in a few years. If an immunization strategy failed to prevent dissemination and establishment of viral infection, but controlled long-term disease progression and transmission, clinical studies would need to follow viral load levels, immune responses, and clinical outcomes in vaccinated-but-infected trial volunteers for many years. In either case, extensive long-term follow-up would be required to define the duration of protection and the long-term safety of the immunization strategy.

Evaluation of potential efficacy of HIV vaccines in clinical trials is complicated by several scientific factors. One is that, in any given clinical trial, an immunization strategy might have a varied protective effect depending on the route of exposure and dose of infecting virus, thus muddying the statistical evidence of short-term protection. The second is that correlates of protection, such as the characteristics of antibody or cellular responses, won't be known definitively until some protection in humans is demonstrated. The third is that longterm evaluation of viral load levels, immune responses, and clinical outcomes in vaccinated-but-infected trial volunteers will be more difficult to interpret in situations where volunteers have broad access to effective antiviral treatments.

Data from vaccine clinical trials provide important information for basic research and product development. Information gained from clinical trials about vaccine administration, antigen processing and expression, and resulting immune responses will help further refine and optimize immunization strategies.

The First Thirteen Years of Trials

Clinical trials of candidate HIV vaccines were begun by NIH through the AIDS Vaccine Evaluation Group (AVEG) in late 1987. The first trials focused on the safety and immunogenicity of recombinant HIV envelope proteins and recombinant vaccinia vector expressing gpl 60.[127,128] During the ensuing thirteen years, the AVEG initiated more than 30 Phase 1 trials evaluating more than three dozen vaccines and vaccine combinations[129]. The most extensive clinical studies evaluated recombinant gpl 20 and gpl 60 envelope subunit proteins, recombinant peptides with B and T cell epitopes, and vaccinia and canarypox vectors. Trials were also done of recombinant p24 and p55 proteins, lipopeptide conjugate, and salmonella and DNA vectors expressing envelope and core antigens. These were tested with a range of adjuvants, delivery routes, and dosing schedules. Immune responses were measured with standard assays for antibody binding, neutralization, fusion inhibition, CD4 binding inhibition, antibody-dependent cellular cytotoxicity (ADCC), and CTL and lymphocyte proliferation.

During 1998, more than ten NIH-sponsored clinical protocols were launched or continued in the United States, concurrent immunology work was done using clinical trial samples and data, and preparation was done for phase 1 and 2 clinical trials in Uganda and elsewhere.

The largest of these NIH sponsored trials, a Phase 2 trial begun in 1997, enrolled 420 participants in order to evaluate the safety and immunogenicity of a combination of recombinant canarypox vaccine (containing genetic copies of HIV-1 env, gag, and protease) with boosts of recombinant gpl 20. Through the AVEG, NIH also funded follow-up analyses of a 1992-94 phase 2 trial of gpl 20 (AVEG protocol 201),[130] and follow-up of all vaccine trial volunteers with break-through infections.[131]

Five phase 1 trials were initiated or continued to evaluate canarypox vectors: a low-dose vs. high-dose vCP205 with rgpl 20 SF2 (AVEG 022 and 022A), vCP205 administered mucosally (AVEG 027), vCP205 with GM-CSF adjuvant (AVEG 033), vCP 300 with rgpl 20 (AVEG 026), and a comparative trial of three canarypox products vCP205 vs. vCP1433 vs. vCP1452 (AVEG 034). The intensity of the NIH effort on canarypox virus vectors is due partly to the availability of the products from a company, Pasteur-Merieux Connaught (PMC), but also due to the fact that canarypox vectors so far induce the best CTL responses seen so far in humans. These CTL responses are far from optimal, with only 15 to 30% of volunteers showing CTL responses at any one time during studies. A lot more needs to be known about the duration and kinetics of these CTL responses and their relation to protective efficacy. More research is being done to evaluate immune responses generated by canarypox vectors with multiple genetic inserts, administered mucosally, and in combination with other vaccine products.

Beyond canarypox vectors, the AVEG network conducted trials to identify new immunization strategies that are safe and generate strong, durable CTL responses. These included a phase 1 safety and immunogenicity trial of a Therion vaccinia vector with env/gag/pol inserts (AVEG 014C), a phase 1 trial of an Apollon gag/pol DNA vaccine (AVEG 031), a phase 1 trial of an orally administered attenuated salmonella vaccine (expressing env) in combination with gpl 20 (AVEG 028), and a phase 1 trial of the adjuvant QS21 with two different gpl 20 vaccines (AVEG 036). Perinatal work through the Pediatric AIDS Clinical Trials Group (PACTG), the Women and Infants Transmission Study (WITS), and General Clinical Research Center (GCRC) sites coded as HIV vaccine research evaluated the safety and immunogenicity of gpl 20 vaccines in mothers and infants. [132] This work demonstrated safety of gpl 20 in infants less than six months old, and stronger antibody responses than adults to low doses of gpl 20.

Researchers also continued to develop and refine new assays for use in clinical trials to define humoral, cellular, and other immune responses to HIV, and to define in vivo processing and expression of viral vector and DNA vaccine antigens. Clinical researchers are now proposing phase 1 and 2 studies to define the comparative safety and immunogenicity of newer envelope designs, such as proteins derived from primary isolates, newer viral vector or DNA constructs with multiple epitopes (env, gag, pol, nef), and various prime-boost schedules and doses of vector-subunit combinations.

Envelope is Safe

In the first decade of clinical trials, evaluation of recombinant envelope subunit proteins had found that:

- Immunization with HIV envelope proteins is safe in humans.[133]
- Recombinant envelope subunits can induce antibodies that recognize native envelope structure.[134]
- Recombinant subunit vaccines can induce functional antibody, with neutralizing activity and fusion inhibition.[135]
- Antibodies induced by the tested envelope subunit vaccines do not neutralize primary isolates in vitro, and have differences in specificity to antibodies found in natural infection.[136]
- Immune responses in gp120 vaccinees with subsequent breakthrough HIV infection do not appear to affect early viral load.[137]

Some Vectors are Safe and Can Induce Cellular Responses

Clinical trials of viral vector vaccines demonstrated:

- Recombinant vaccinia and canarypox vector vaccines are safe, can induce CTL responses, and can augment antibody responses to subunit vaccines. [138,139,140,141]
- Antibodies induced by candidate vector vaccines do not neutralize primary isolates in vitro, and levels of neutralizing antibody responses induced by current vaccines are 5 to 10 times lower against T cell line adapted viruses than levels found in HIV-infected persons.[142]

Trial Sites are Being Prepared

Finally, establishment of HIV vaccine trial sites and development of volunteer cohorts by the NIH HIV Vaccine Efficacy Trials Network (HIVNET) had adequately shown that suitable high-risk and low-risk cohorts can be recruited for HIV vaccine trials in the United States and in Africa, Asia, the Caribbean, and South America. Clinical infrastructure, laboratory infrastructure and staff, and trained investigators were funded through the international HIVNET in many countries for Phase 3 efficacy trials. HIVNET sites identified, recruited, and retained volunteers at high risk for HIV infection, and provided high quality risk-reduction interventions. Finally, long-term political relationships, critical to the success of HIV vaccine research, have been established by NIH with governments, researchers and communities in several countries.

CLINICAL RESEARCH RECOMMENDATIONS

9. Fund Development of Novel Vaccine Products & Immunization Strategies

The major limitation of the clinical trials effort is the lack of products and immunization concepts to be tested. Major advances were made in 1997 and 1998 in understanding HIV envelope structure and function, and yet the clinical trials networks are still reliant on recombinant gpl 20 envelope subunit products that pre-date these findings. NIH has funded pre-clinical work for years on novel viral and bacterial vectors, such as Venezuelan equine encephalitis virus (VEE), modified vaccinia virus Ankara (MVA), bacillus Calmette-Gu6rin (BCG), herpesvirus vector, attenuated salmonella, adeno-associated virus (AAV), and yellow fever virus, but the clinical trial networks have focused trials on canarypox vectors as the only vaccine vector with a fully developed and company-supported development plan. Vaccine companies have recently increased investment in DNA and vector vaccines.

NIH needs to directly fund development of products to feed into clinical evaluation. NIH should expand direct contracting with companies to develop HIV vaccine candidates, such as newer versions of MVA, VEE, AAV, and attenuated salmonella vectors. The NIH should encourage optimization of current vaccine candidates and be willing to discourage work in outdated vaccine concepts that have performed poorly in preclinical, animal or early human studies.

10. Maintain a Solid Clinical Research Infrastructure

During the coming decade, NIH clinical trial programs need to continue rapid phase 1 and 2 assessment of a broader range of candidate immunization strategies for adult and perinatal use, push the basic science to increase comparative understanding of elicited immune responses, and prepare for phase 3 evaluation of candidate vaccines in the US and internationally. This will require an ongoing commitment to a productive, efficient clinical trials infrastructure.

The total 1998 funding from NIH designated for HIV vaccine clinical research was approximately $42 million, about 28% of the total vaccine program. Of this, about $11 million went to the AVEG network, about $11 million to the US HIVNET, $7.6 million to the international HIVNET and international clinical training programs, and about $2.2 million to perinatal and pediatric clinical immunization studies. An additional $5.2 million was steered to establishing the new NIH Vaccine Research Center. (Table 3) More than 90% of 1998 clinical trial funding came from NIAID, with most of the remainder from NCRR to support for adult and pediatric trial activities through General Clinical Research Centers (GCRCs).

TABLE 3: HIV VACCINE CLINICAL TRIALS PROGRAM SPENDING, FY 1998	
CLINICAL TRIALS IN THE US IN ADULTS	**$22.0 M**
AVEG (plus GCRC grants)	$11.1 m
HIVNET–U.S.	$10.9 m
CLINICAL TRIALS IN THE US — PERINATAL	**$2.2 M**
Woman Infant Health Study (WIHS)	$.9 m
Other Grants and Contracts	$1.3 m
INTRAMURAL NIH AND OTHER	**$10.3 M**
Vaccine Research Center	$5.2 m
Lab Support, Repository, Reagents, etc.	$5.1 m
INTERNATIONAL HIV/NET PLUS FOGARTY TRAINING	**$7.9 M**
HIVNET–International	$5.4 m
Fogarty	$2.5 m
TOTAL	**$42.5 M**

NIH should ensure that the new HIV Vaccine Trials Network and the vaccine clinical trial activities of the Vaccine Research Center, the NIH intramural trial site, and the perinatal sites will have the capacity for an adequate number of phase 1, 2 and 3 trials during the coming five years. That being said, the clinical HIV vaccine research programs should be held to the highest scientific standards with adequate oversight through regular review by extramural researchers. The advancement of candidate immunogens along the vaccine clinical development pipeline must be driven by science and not political expediency or industry requests.

11. Strengthen Links between Clinical Trials, Immunology & Basic Science

Clinical research goals continue to be heavily influenced by advances in basic science. Elucidation of the HIV envelope 3-D structure in 1998 helped to reformulate the thinking on what vaccine antigens might induce primary isolate neutralization. [143,144] As canarypox vectors were able to elicit cross-clade cellular responses, optimism about the feasibility of developing vaccines to induce CTL responses has increased, although -- as we noted above -- those particular CTL responses were far from optimal.

The first era of HIV vaccine clinical trials was characterized by a limited understanding of HIV pathogenesis and human immunology, less sophisticated assays, and limited numbers of volunteers in each phase I vaccine study. Thus, the two dozen phase I clinical trials described above yielded some conclusive (negative) results, but provided few insights for new vaccine development efforts beyond learning what doesn't work. Researchers are challenged to design phase I trials that can generate new understanding about how to elicit potentially protective immune responses.

NIH funding supports important immunology research on the CTL, antibody, and cytokine immune responses of phase I trial volunteers, including analysis of cross-clade activity of antibodies and CTLs from combination vaccines,[145] further development of assays to measure CTL responses,[146] and further analysis of antibody neutralization capabilities.[147] More studies in the clinical setting are needed to learn about mucosal and other routes of vaccine administration, in vivo antigen processing and expression, and mechanisms and quantitative measures of humoral, cellular, and mucosal immune responses.

A coherent plan with interim goals need to be articulated for the clinical trials program, so that clinical trials evaluate immunization strategies in a concerted, systematic manner. Expansion of human immunology research, linked to HIV vaccine clinical trials, is needed. Larger and more intensive phase I and II trials should compare vaccine products and immunization strategies and the resulting immune responses.

12. Support a Strong International Vaccine Development Effort

An important NIH effort during past years has been the support of the first-ever phase I HIV vaccine trial in Africa, and support of clinical trial site preparation in Asia, Africa, Latin America, and the Caribbean.[148] During 1998, NIH funding built on-site laboratory capacity in Uganda to evaluate CTL responses in vaccine volunteers. Through its international HIVNET network, NIH has supported preparation for a phase II trial in Brazil, Haiti, and Trinidad and Tobago, and supported characterization of cohorts, CTL responses, and viral strains in many countries. This international clinical trials effort is significant for several reasons. The safety, efficacy, and practicality of HIV immunization strategies will need to be studied in populations with different disease burden, nutritional status, access to health care, level of exposure to HIV, and other environmental factors. More needs to be learned about the effectiveness of vaccine-induced immune responses against various routes of HIV exposure and to different strains of HIV. Finally, vaccine research efforts by the countries most affected by the global AIDS epidemic must overcome barriers of infrastructure for intensive immunologic research and clinical care, and must also secure long-term political and economic support.

International clinical trial site readiness takes years of investment, training, and relationship-building. The continuity of NIH's investment and support of other countries' clinical trial infrastructure has been an important part of the successful launch of a phase I trial in Uganda, and of the preparedness of trial sites in Thailand, Trinidad, Haiti, Brazil, India, South Africa, and elsewhere. NIH should be careful that the recompetition of clinical trial sites does not undermine the years of investment already committed to those countries.

NIH should work more closely with the Centers for Disease Control (CDC), the United States Agency for International Development (USAID) and the Department of Defense (DOD) to support the development and maintenance of high-quality biomedical research infrastructure in developing countries.

IV. THE NIH AIDS VACCINE RESEARCH PROGRAM

ANATOMY OF FISCAL YEAR 1998 NIH FUNDING FOR HIV VACCINE RESEARCH AND DEVELOPMENT

In 1998, NIH invested about $148 million in HIV vaccine research and development. This HIV vaccine funding was dispersed to intramural research activities at NIH (19%) and externally (81%) through 374 awards to 135 research institutions.

TABLE 4: NIH HIV VACCINE RESEARCH BY STAGE OF DEVELOPMENT

	BASIC RESEARCH	TARGETED RESEARCH	CLINICAL RESEARCH
Extramural Grants(283 awarded)	$31,106,131	$26,956,013	$5,382,113
Extramural Contracts (68 awarded)	$9,243,797	$17,356,033	$17,554,400
Intramural Contracts (23 awarded)	$8,404,627	$4,075,929	$5,191,759
NIH Administrative Overhead (RMS)	$6,702,670	$2,066,443	$998,786
Total Vaccine-coded Funding	**$55,457,225**	**$50,454,418**	**$41,644,308**

The National Institute of Allergy and Infectious Diseases (NIAID) was responsible for 80% of 1998 HIV vaccine research, with nearly $100 million distributed extramurally and $14 million spent on intramural research. The National Cancer Institute (NCI) and the National Center for Research Resources (NCRR) each accounted for an additional 6% of 1998 HIV vaccine dollars, with the remainder spent by the Fogarty International Training Center, other institutes, and the Office of AIDS Research (OAR).

TABLE 5: VACCINE-CODED FUNDING BY INSTITUTE

	TOTAL VACCINE-CODED FUNDING	NUMBER OF AWARDS	NUMBER OF INSTITUTIONS FUNDED	AVERAGE SIZE OF AWARD
NIAID	$118,239,651	269	120	$439,553
NCI	$8,771,328	22	14	$398,697
NCRR	$8,382,857	42	32	$199,592
Fogarty	$2,611,662	13	13	$200,897
Other	$9,550,452	28	20	$341,088
Total	**$147,555,950**	**374**	**135**	**$394,535**

More than a third of NIH vaccine funds (38%, or $55 million) went to research coded as basic science related to HIV vaccines. Another third (34%, or $50 million) funded targeted research involving work in animal models on preclinical product development. The remainder (28%, or $42 million) funded adult and perinatal clinical research and development of clinical trial infrastructure.

NIH funding is dispersed through a range of grant and contract mechanisms. In 1998, NIH funded 283 HIV vaccine grants (average size, $235,000) and 91 intramural and extramural contracts (average size, $779,000). The following is a summary of the major types of grants and contracts awarded in 1998.

GRANTS

Traditional Research Project Grants (RO 1) - 16%

R01 s are four- to five-year grants, commonly called "investigator-initiated awards" because they are unsolicited by the NIH and are funded solely on the basis of merit as determined by peer review. In 1998, 16% of NIH funding for HIV vaccines was awarded through ROl1s, with 86 R01 s funded at an average size of $274,000. These vaccine research grants were awarded primarily by the National Institute of Allergy and Infectious Diseases (NIAID), the National Heart, Lung and Blood Institute (NHLBI), the National Cancer Institute (NCI), and the National Center for Research Resources (NCRR), and were widely distributed to a total of 56 research institutions in the United States and internationally. Eighty percent of RO1 HIV vaccine funding in 1998 was coded as basic and targeted research; 20% of R01 funding supported clinical research.

Innovation Grants (R21) - 14%

In 1997, the AIDS Vaccine Research Committee chaired by Nobel laureate David Baltimore of the California Institute of Technology issued a call for proposals for innovation grants on the topic of the HIV envelope, antigen presentation, and novel vaccine approaches. In 1998, NIH spent a total of $21 million to fund 94 research teams at 61 institutions with these two-year awards, all of which focused on basic research and targeted research topics related to HIV vaccine development.

Research Program Project Grants (P01) - 6%

P01 s support broadly based, multidisciplinary research programs with a specific major objective or theme. P01 awards are large awards; the average size of a vaccine P01 in 1998 was $867,000 per award. P01 awards involve large groups working under the leadership of a senior, established scientist, and can support core facilities used by all the investigators working on the project. In 1998, NIH funded eleven AIDS vaccine P01 grants for a total of $9.5 million. These P01 awards were coded as basic vaccine research but were meant to focus on questions related to the translational aspects of HIV vaccine development through a program called Integrated Preclinical/Clinical AIDS Vaccine Development (IPCAVD) program. The institutions receiving P01s in 1998 were the Aaron Diamond AIDS Research Center, Emory University, Harvard University, Scripps Research Institute, Therion Biologics, University of Alabama, University of Maryland, University of Pennsylvania, University of Pittsburgh, University of Washington, and Yeshiva University in New York.

Primate Research Center Project Grants (P51, P41) - 3%

P51 and P41 grants are awards from the NCRR to the Regional Primate Research Centers (RPRCs) for support for RPRC researcher, for maintenance of primate colonies, and for research by affiliated researchers outside the centers working in collaboration with RPRC personnel. These are large, targeted research grants, with an average size for 1998 vaccine P51s of $466,000 per award. NCRR spent $3.7 million in 1998 for eight of these awards to support activities at RPRCs in California, Georgia, Louisiana, Massachusetts, Oregon, Washington, and Wisconsin.

Fogarty International Training Program Grants (D43) - 2%

The Fogarty International Training Center supports researchers from outside the United States to study and work at leading U.S. universities through its D43 grants. In 1998, the Fogarty Center spent $2.6 million to support 13 institutions in building international HIV vaccine clinical trials capacity, with an average size of $200,000 per award. The participating institutions included Case Western University, Emory University, Johns Hopkins University, Harvard University, University of Alabama, University of California-Los Angeles, University of Maryland, University of Miami, University of North Carolina, University of Pittsburgh, University of Washington, and Yale University.

General Clinical Research Center (GCRC) Grants (M01) - 1%

MOl1 grants are used by the National Center for Research Resources (NCRR) to fund research at its network of General Clinical Research Centers (GCRCs). In 1998, NCRR spent $1.4 million to support work at 14 of its GCRC sites, primarily for a joint study by researchers of the Pediatric AIDS Clinical Trial Group (PACTG) into the safety and immunogenicity of gpl 20 vaccine in HIV-uninfected newborns.

Center for AIDS Research (CFAR) Grants (P30) - 1%

P30 awards are NIAID grants to support basic science research at its Centers for AIDS Research. In 1998, NIAID spent $1.3 million in P30 grants, averaging $79,000 per award, to support basic research related to HIV vaccines at 17 CFAR sites. These sites included the Aaron Diamond AIDS Research Center, Dana Farber Cancer Research Institute, Duke University, New York University Medical Center, University of California-San Francisco, and University of Washington.

Other Grants - 2%

The remaining 40 HIV vaccine-related grants from NIH in 1998 totaled $3.4 million, averaged $85,000 per award, and reflected an NIH effort to meet a range of funding needs in preclinical research. These included Small Grants (R03s), limited in both duration and funding levels and designed to offer support in generating preliminary data in a specific program area; First Awards (R29s), now discontinued, but designed to provide initial support to new researchers to allow development of research experience and preliminary data to further R01 s and other standard grant funding; Merit Awards (R37s), providing long term funding to outstanding researchers; and Small Business Innovation Research (SBIR) Grants (R43s), made to small companies and designed to establish the feasibility of an R&D project for further development.

CONTRACTS

Research and Development Contracts (NO 1, N02) - 32%

N01 awards are large contracts from the NIH focused on particular research and development tasks. For 1998 HIV vaccine research and development, N01 contracts were used by NIAID to support the sites and associated reagent and lab work of the primate research SVEU (Simian Vaccine Evaluation Unit) sites, and the domestic and international clinical trial networks of the AVEG (AIDS Vaccine Evaluation Group) and HIVNET (HIV Network for Vaccine and Prevention Trials). In 1998, N01 funding totaled nearly $47 million, a full 32% of all NIH vaccine dollars. Forty-five N01 contracts were awarded at an average of $1 million per award, with 31 institutions as

primary "master" contractors and many more as subcontracts. More than half (56%, or $26 million) of this N01 HIV vaccine funding was related to clinical research in the AVEG and HIVNET. A third (34%, or $16 million) was devoted to primate research in the SVEUs and related sites. The remaining ten percent of N01 contract funding was coded as basic research, going mostly to contractors such as Quality Biological, Advanced Bioscience Laboratories, and Science Applications International to provide reagent and repository services in support of the primate and clinical networks.

Cooperative Agreements (U01, U 19) - 2%

NIAID also contracts with research centers and institutions through a "U-" series of cooperative agreements to conduct specific research and development projects. In 1998, NIAID funded ten of these awards, totaling $3.5 million. Research under these contracts included basic science and preclinical development related to live, attenuated vaccines (Harvard), development of herpes vector vaccines (Harvard), development of vaccinia vector vaccines (Therion and University of Washington), development of peptide vaccines (Duke University), and perinatal evaluation of gpl 20 vaccines (through sites of WITS, the Women Infant Transmission Study).

Primate Breeding Contracts (U42) - 1%

NCRR funds the breeding and maintenance of primate colonies at the Regional Primate Research Centers (RPRCs) through U42 contracts. In 1998, about $1 million of this funding was coded as HIV vaccine-related, with each of the RPRCs in California, Georgia, Louisiana, Massachusetts, Oregon, Washington, and Wisconsin receiving about $128,000 to support the breeding and care of primates for use in HIV vaccine research.

Intramural Contracts (ZO1) - 12%

In addition to external contracts, NIH supports a number of researchers on the NIH campus with intramural funding. In 1998, funding for intramural HIV vaccine research totaled $17.8 million. Most of this was distributed to the establishment of the NIH Vaccine Research Center ($9.4 million), to the labs of NIAID researchers such as Vanessa Hirsch, Bernard Moss, and Malcolm Martin ($4.5 million), and to the labs of NCI researchers such as Robert Beneviste, Jay Berzofsky, Genoveffa Franchini, and Marjorie Robert-Guroff ($3.5 million).

PREVIOUS REVIEWS OF THE NIH AIDS VACCINE RESEARCH PROGRAM

The NIH's AIDS vaccine research program was reviewed in detail during 1995-1996 by the NIH AIDS Research Program Evaluation Working Group under the leadership of Dr. Arnold Levine, then of Princeton, now President of Rockefeller University[149]. The Levine Committee report, covering the NIH's entire AIDS research program, was commissioned by the Office of AIDS Research Advisory Council (OARAC). A Vaccine Research and Development Area Review Panel chaired by Dr. Dani Bolognesi of Duke University prepared the specific findings and recommendations on vaccine research.[150]

The Vaccine Research and Development Area Review Panel had six major questions that drove its work:

- Is basic research providing the fundamental information necessary to design and evaluate AIDS vaccines? Are there gaps in scientific areas that should be addressed?

- How can NIH encourage additional high-caliber investigator-initiated research pertinent to HIV vaccines?
- How successful are targeted research programs for HIV vaccine design? How successful are they for vaccine development?
- Is effective use being made of animal resources for preclinical vaccine evaluation?
- To what level should established infrastructures for evaluating vaccines in people be maintained considering the limited number of new vaccine candidates available for testing?
- What other valuable research might be accomplished within these units until promising vaccine candidates are available for testing?

The Levine report identified some important problems in the NIH vaccine effort and led to a sweeping reorganization of the whole enterprise. The key vaccine-related recommendation of the Levine report was to establish a trans-NIH vaccine program with leadership and oversight by an AIDS Vaccine Research Committee (AVRC) composed of distinguished, non-governmental scientists.

The area review panel's report identified several areas for increased emphasis in the NIH's scientific portfolio, including research on human and primate immunology, correlates of protection from HIV, and male and female genital tract immunology. It also identified problems in the peer review of vaccine-related research proposals that tended to fare poorly in study sections. To address these issues, the panel recommended the following:

- Create an initial review group (IRG) that would be dedicated to broad aspects of vaccine research, including HIV and other pathogens.
- Continue NIH/NIAID efforts to encourage and solicit the research community to submit applications in vaccine biology and immunology.
- Selectively target vaccine-related research areas for special consideration during review and funding.
- Increase the access of basic research scientists who are interested in HIV interactions with the human immune system to clinical materials emerging 1) from studies with the existing networks, such as the Multicenter AIDS Cohort Study (MACS), the AIDS Vaccine Evaluation Group (AVEG), and the HIV Network for Efficacy Trials (HIVNET); and 2) from certain animal model studies.

The report also criticized the existing experimental work in primate models, recommending fewer, more intensive studies with greater numbers of animals in the future. The panel also asked for a review of the human clinical trials infrastructure for vaccine evaluation at NIH. With a paucity of promising vaccine candidates, the panel thought that a redirection of resources and objectives was needed in clinical research with a new focus on phase I and II testing of new candidates, more intensive immunological studies of vaccinees, and clear guidelines for moving vaccines into phase III efficacy testing.

In 1998 and 1999, the AIDS Vaccine Advocacy Coalition (AVAC) issued two reports on the search for an AIDS vaccine, 9 Years and Counting, Will We Have an AIDS Vaccine by 2007?[151] and 8 Years and Counting, What Will Speed the Development of an AIDS Vaccine?152 These reports surveyed the entire domestic AIDS vaccine development program in both the private and public sectors, with large sections devoted to the NIH's vaccine research program.

The 1999 AVAC report made the following major recommendations concerning NIH:

- NIH should renew its commitment to the Innovation Grants program, increasing the number of awards to new investigators on important, understudied topics in AIDS vaccine research.
- NIAID should increase product development funding to generate vaccine candidates that are optimized for comparative primate studies.
- NIH should, as promised, provide the director of the new Vaccine Research Center with the authority and resources needed to staff the center with the best and brightest vaccine scientists, whether they are selected from within NIH or brought in from the outside.
- NIAID should increase the number of clinical trials and ensure that the new clinical trial networks are adequate to evaluate candidate vaccines at all stages, from initial safety studies through large-scale trials needed for licensure.

TABLE 6: 1998 NIH VACCINE FUNDING

		TOTAL VACCINE-CODED FUNDING	AVERAGE SIZE OF AWARD	NUMBER OF AWARDS	NUMBER OF INSTITUTIONS FUNDED
All Vaccine-Coded Funding		$147,555,950	$394,535	374	135
Vaccine-Coded Funding by Institute					
NIAID		$118,239,651	$439,553	269	120
NCI		$8,771,328	$398,697	22	14
NCRR		$8,382,857	$199,592	42	32
Fogarty		$2,611,662	$200,897	13	13
OAR and other		$9,550,452	$341,088	28	20
Vaccine-Coded Funding by Mechanism					
Extramural Grants					
R01	NIAID, NHLBI NCI, NCRR	$23,532,324	$273,632	86	56
R21	NIAID	$20,883,510	$222,165	94	61
R03, R29, R43, other	NIAID	$2,509,442	$96,517	26	25
P01	NIAID, NCI	$9,540,344	$867,304	11	11
P30	NIAID	$1,343,979	$79,058	17	17
P41, P51	NCRR	$3,727,112	$465,889	88	
D43	Fogarty	$2,611,662	$200,897	13	13
K02, K08	NIAID	$510,321	$56,702	9	9
M01	NCRR	$1,416,189	$101,156	14	14
Other	NCRR, NCI	$394,162	$78,832	5	5
Extramural Contracts					
N01, N02	NIAID, NCI	$46,561,326	$1,034,696	45	31
U01, U19	NIAID	$3,476,282	$347,628	10	9
U42	NCRR	$1,023,606	$127,951	8	7
Y01, Y02	NIAID	$2,032,505	$406,501	5	5
Intramural Funding					
Z01 NIAID		$13,908,967	$637,707	9	2
Z01 NCI		$3,476,290	$347,629	10	1
Z01 Other		$419,310	$104,828	4	2
RMS* NIAID		$4,905,505			
RMS NCI		$1,118,080			
RMS Other		$4,165,034			

*RMS=Research management & support (NIH program staff)

TABLE 7: 1998 NIH VACCINE FUNDING BY PROGRAM AREA

PROGRAMMATIC FOCUS AREA:		BASIC	TARGETED	EARLY CLINICAL	PERINATAL	EFFICACY
OAR PLAN CODE:		4A	4B	4C	4D	4E
All Vaccine-Coded Funding		55,457,225	$45,158,935	$21,905,289	$10,590,965	$14,443,536
Vaccine-Coded Funding by Institute						
NIAID		$44,058,886	$35,239,825	$18,280,004	$8,898,774	$11,762,162
NCI		$5,594,790	$2,912,034		$264,504	
NCRR		$229,769	$6,715,072	$31,970	$1,381,334	$24,712
Fogarty						$2,611,662
OAR and other		$5,573,780	$292,004	$3,593,315	$46,353	$45,000
Vaccine-Coded Funding by Mechanism						
	Extramural Grants					
R01	NIAID, NHLBI NCI, NCRR	$7,436,740	$10,411,727	$3,766,381	$1,381,615	$535,861
R21	NIAID	$11,508,279	$9,375,231			
R03, R29, R43, other	NIAID	$938,314	$1,508,708		$42,420	$20,000
P01	NIAID, NCI	$9,540,344				
P30	NIAID	$1,343,979				
P41, P51	NCRR		$3,727,112			
D43	Fogarty					$2,611,662
K02, K08	NIAID	$125,518	$308,123		$76,680	
M01	NCRR		$2,879	$31,970	$1,381,340	
Other	NCRR, NCI	$212,957	$181,205			
	Extramural Contracts					
N01, N02	NIAID, NCI	$4,870,687	$12,591,379	$12,130,601	$6,130,061	$10,838,598
U01, U19	NIAID	$2,346,605	$222,358		$907,319	
U42	NCRR		$1,023,606			
Y01, Y02	NIAID	$2,026,505		$3,000		$3,000
	Intramural Funding					
Z01	NIAID	$5,399,650	$3,317,55	$5,191,759		
Z01	NCI	$2,877,671	$334,115		$264,504	
Z01	Other	$127,306	$292,004			
RMS*	NIAID	$2,667,440	$882,991	$703,421	$260,679	$390,974
RMS	NCI	$916,826	$201,254			
RMS	Other	$3,118,404	$778,685	$78,157	$146,347	$43,441

**RMS=Research management & support (NIH program staff)*

REFERENCES

1. Interviews were conducted with the following scientists: Rafi Ahmed; Deborah Birx; Ronald DesRosiers; Thomas Evans; Ralph Steinman; Mark Feinberg; Patricia Fultz; Ronald Germain; Barney Graham; Beatrice Hahn; Barton Haynes; Eric Hunter; R. Paul Johnson; Norman Letvin; John Mascola; Thomas Matthews; Kenneth Mayer; John McNeil; Jiri Mestecky; David Montefiore; Mark Mulligan; Harriet Robinson; Kenneth Rock; Sarah Rowland-Jones; Ruth Ruprecht; Robert Siliciano; Joseph Sodroski; and Kent Weinhold.
2. We have made the listing of FY 1998 NIH AIDS vaccine research awards available on the TAG website (www.aidsinfonyc.org/tag/reports/nihl 998grants.pdf). Abstracts for each of these awards is available on the CRISP database at www-commons.cit.nih.gov/crisp/index.html.
3. Burton, DR; Moore, JP. Why do we not have an HIV vaccine and how can we make one? Nat. Med. 1998 May 4(5): 495-498.
4. This recommendation is also shared by the 1998 Jordan Report. See Hackett, CJ. Immunological Basis of Vaccine Research and Development in The Jordan Report, Accelerated Development of Vaccines, B. Gellin, ed. Available: http://www.niaid.nih.gov/publications/pdf/jordan.pdf.
5. Hocini H; Bomsel M Infectious human immunodeficiency virus can rapidly penetrate a tight human epithelial barrier by transcytosis in a process impaired by mucosal immunoglobulins J Infect Dis. 1999 May;179 Suppl 3:S448-53.
6. Neutra MR. HIV transmission and immune protection at mucosal surfaces. Adv Exp Med Biol. 1998;452:169 -75.
7. Miller, CJ, HU J. T cell-tropic SIV and SHIVs are readily transmitted by vaginal inoculation of rhesus macaques, and Langerhans' cells of the female genital tract are infected with SIV. J Infect Dis. 1999 May; 179 Suppl 3: S413-7.
8. Spira AI; Marx PA; Patterson BK; Mahoney J; Koup RA; Wolinsky SM; Ho DD Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques J Exp Med. 1996 Jan 1;183(1):215-25.
9. Stahl-Hennig C; Steinman RM; Tenner-Racz K; Pope M; Stolte N; Matz-Rensing K; Grobschupff G; Raschdorff B; Hunsmann G; Racz P Rapid infection of oral mucosal-associated lymphoid tissue with simian immunodeficiency virus. Science. 1999 Aug 20;285(5431): 1261-5.
10. Fultz PN, May P, Stallworth J, Su L, Mahoney J, Muchmore L, Girard M. Cervical infection of chimpanzees with cell-free and cell-associated HIV-1. Natl Conf Hum Retroviruses Related Infect (1st). 1993 Dec 12 -16;:109.
11. Sodora DL, Gettie A, Miller CJ, Marx PA. Vaginal transmission of SIV: assessing infectivity and hormonal influences in macaques inoculated with cell-free and cell-associated viral stocks. AIDS Res Hum Retroviruses 1998 Apr;14 Suppl 1:S1 19-23.
12. Cranage MP, Whatmore AM, Sharpe SA, Cook N, Polyanskaya N, Leech 5, Smith JD, Rud EW, Dennis MJ, Hall GA Macaques infected with live attenuated SIVmac are protected against superinfection via the rectal mucosa. Virology 1997 Mar 3;229(1):143-54.
13. Lehner T, Wang Y, Cranage M, Bergmeier LA, Mitchell E, Tao L, Hall G, Dennis M, Cook N, Brookes R, Klavinskis L, Jones I, Doyle C, Ward R Protective mucosal immunity elicited by targeted iliac lymph node immunization with a subunit SIV envelope and core vaccine in macaques. Nat Med 1996 Jul;2(7):767-75.
14. Putkonen P, Makitalo B, Bottiger D, Biberfeld G, Thorstensson R Protection of human immunodeficiency virus type 2-exposed seronegative macaques from mucosal simian immunodeficiency virus transmission. J Virol 1997 Jul;71 (7):4981-4.
15. Miller CJ, McChesney MB, Lu X, Dailey PJ, Chutkowski C, Lu D, Brosio P, Roberts B, Lu Y Rhesus macaques previously infected with simian/human immunodeficiency virus are protected from vaginal challenge with pathogenic SIVmac239 J Virol 1997 Mar;71 (3):1911-21.

16. Quesada-Rolander M, Makitalo B, Thorstensson R, Zhang YJ, Castanos-Velez E, Biberfeld G, Putkonen P Protection against mucosal SIVsm challenge in macaques infected with a chimeric SIV that expresses HIV type 1 envelope. AIDS Res Hum Retroviruses 1996 Jul 20;12(11):993-9.
17. Kresina TF; Mathieson B Human immunodeficiency virus type 1 infection, mucosal immunity, and pathogenesis and extramural research programs at the National Institutes of Health. J Infect Dis. 1999 May;179 Suppl 3:S392-6.
18. Moore JP; Burton DR HIV-1 neutralizing antibodies: how full is the bottle? [news; comment] Nat Med 1999 Feb;5(2):142-4.
19. Brandtzaeg, P; Berstad, A.E.; Farstad, I.N.; Haraldsen, G.; Helgeland, L; Jahnsen, F.L.; Johansen, F.E.; Natvig, I.B.; Nilsen, E.M.; Rugtveit, J. Mucosal immunity-a major adaptive defence mechanism. Behring Inst. Mitt. 1997:1-23
20. Mazanec MB; Nedrud JG; Kaetzel CS; Lamm ME A three-tiered view of the role of IgA in mucosal defense Immunol Today 1993 Sep;14(9):430-5
21. Mazzoli S; Lopalco L; Salvi A; Trabattoni D; Lo Caputo S; Semplici F; Biasin M; BI C; Cosma A; Pastori C; Meacci F; Mazzotta F; Villa ML; Siccardi AG; Clerici M Human immunodeficiency virus (HIV)-specific IgA and HIV neutralizing activity in the serum of exposed seronegative partners of HIV-seropositive persons J Infect Dis. 1999 Sep;180(3):871-5.
22. Kaul R; Trabattoni D; Bwayo JJ; Arienti D; Zagliani A; Mwangi FM; Kariuki C; Ngugi EN; MacDonald KS; Ball TB; Clerici M; Plummer FA HIV-1-specific mucosal IgA in a cohort of HIV-1-resistant Kenyan sex workers. AIDS 1999 Jan 14;13(1):23-9.
23. Hocini H; Belec L; Iscaki S; Garin B; Pillot J; Becquart P; Bomsel M High-level ability of secretory IgA to block HIV type 1 transcytosis: contrasting secretory IgA and IgG responses to glycoprotein 160 AIDS Res Hum Retroviruses. 1997 Sep 20;13(14):1 179-85.
24. Janoff EN; Scamurra RW; Sanneman TC; Eidman K; Thurn JR Human immunodeficiency virus type 1 and mucosal humoral defense. J Infect Dis. 1999 May;179 Suppl 3:S475-9.
25. Cromwell M; Veazey R; Altman J; Allen T; Watkins DI; Lackner A; Johnson RP Immunization with live attenuated simian immunodeficiency viruses induces virus-specific CTL that express the alph4/beta7 intestinal homing integrin. 12eme Colloque des Cent Gardes, 1999 Oct 25-26: 84
26. Lehner T; Wang Y; Ping L; Bergmeier L; Mitchell E; Cranage M; Hall G; Dennis M; Cook N; Doyle C; Jones I. The effect of route of immunization on mucosal immunity and protection. J Infect Dis. 1999 May;179 Suppl 3:S489-92.
27. Howcroft TK; Strebel K; Martin MA; Singer DS Repression of MHC class I gene promoter activity by two-exon Tat of HIV. Science. 1993 May 28;260(5112):1320-2.
28. Schwartz O; Marechal V; Le Gall S; Lemonnier F; Heard JM Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein. Nat Med. 1996 Mar;2(3):338-42.
29. Kerkau T; Bacik I; Bennink JR; Yewdell JW; Hunig T; Schimpl A; Schubert U The human immunodeficiency virus type 1 (HIV-1) Vpu protein interferes with an early step in the biosynthesis of major histocompatibility complex (MHC) class I molecules J Exp Med. 1997 Apr 7;185(7):1295-305.
30. McMichael AJ; Phillips RE Escape of human immunodeficiency virus from immune control. Annu Rev Immunol. 1997;15:271-96.
31. Sewell AK; Purbhoo MA; Ogg G; Harcourt GC; Goulder PJ; Price DA; Phillips R Antigenic variation and cytotoxic T lymphocyte responses in HIV-1 infection. HIV Pathog Treat Conf. 1998 Mar 13-19:107 (abstract no. 4105).
32. Heath SL; Tew JG; Tew JG; Szakal AK; Burton GF Follicular dendritic cells and human immunodeficiency virus infectivity [see comments] Nature. 1995 Oct 26;377(6551):740-4.
33. Grouard G; Clark EA Role of dendritic and follicular dendritic cells in HIV infection and pathogenesis [see comments] Curr Opin Immunol. 1997 Aug;9(4):563-7.

34. Steinman RM; Germain RN Antigen presentation and related immunological aspects of HIV-1 vaccines. AIDS. 1998;12 Suppl A:S97-112.
35. Albert ML; Sauter B; Bhardwaj N Dendritic cells acquire antigen from apoptotic cells and induce class I- restricted CTLs. Nature 1998 Mar 5;392(6671):86-9
36. Steinman, 597-112.
37. Takashima A; Morita A Dendritic cells in genetic immunization. J Leukoc Biol 1999 Aug;66(2):350-6
38. Perry LL; Lodmell DL Role of CD4+ and CD8+ T cells in murine resistance to street rabies virus. J Virol 1991 Jul;65(7):3429-34.
39. Nixon DF; Kakimoto WM; Donahoe SM; Gettie A; Ho DD; Marx P; Connor R Longitudinal Analysis of CTL Precursor Frequency in Rhesus Macaques Immunized with Live Attenuated SIVmac239 [DELTA]nef and Challenged with Pathogenic SIVmac251. Sixth Conference on Retroviruses and Opportunistic Infections, 1999 Jan 31-Feb 4 (abstract 31).
40. Almond N; Rose J; Sangster R; Silvera P; Stebbings R; Walker B; Stott EJ Mechanisms of protection induced by attenuated simian immunodeficiency virus. I. Protection cannot be transferred with immune serum J Gen Virol 1997 Aug;78 (Pt 8):1919-22.
41. Johnson RP, Lifson JD, Czajak SC et al. Highly attenuated vaccine strains of SIV protect against vaginal challenge. J Virol. 1999 June; 73(6):4952-61.
42. Nixon DF; Donahoe SM; Kakimoto WM; Samuel RV; Metzner KJ; Gettie A; Hanke T; Marx PA; Connor RI. Simian immunodeficiency virus-specific cytotoxic T lymphocytes and protection against challenge in rhesus macaques immunized with a live attenuated simian immunodeficiency virus vaccine. J. Virol 2000 Jan 5;266(1):203-10
43. Schmitz JE; Kuroda MJ; Santra S; Sasseville VG; Simon MA; Lifton MA; Racz P; Tenner-Racz K; Dalesandro M; Scallon BJ; Ghrayeb J; Forman MA; Montefiori DC; Rieber EP; Letvin NL; Reimann KA Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science. 1999 Feb 5;283(5403):857-60.
44. Jin X; Bauer DE; Tuttleton SE; Lewin S; Gettie A; Blanchard J; Irwin CE; Safrit JT; Mittler J; Weinberger L; Kostrikis LG; Zhang L; Perelson AS; Ho DD Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. J Exp Med. 1999 Mar 15;189(6):991-8.
45. Kuroda MJ; Schmitz JE; Charini WA; Nickerson CE; Lifton MA; Lord Cl; Forman MA; Letvin NL Emergence of CTL coincides with clearance of virus during primary simian immunodeficiency virus infection in rhesus monkeys. J Immunol. 1999 May 1;162(9):5127-33.
46. Musey L; Hughes J; Schacker T; Shea T; Corey L; McElrath MJ. Cytotoxic-T-cell responses, viral load, and disease progression in early human immunodeficiency virus type 1 infection N Engl J Med. 1997 Oct 30;337(18):1 267-74.
47. Greenough TC; Brettler DB; Somasundaran M; Panicali DL; Sullivan JL Human immunodeficiency virus type 1-specific cytotoxic T lymphocytes (CTL), virus load, and CD4 T cell loss: evidence supporting a protective role for CTL in vivo. J Infect Dis. 1997 Jul;176(1):118-25.
48. Ogg, GS, Jin, X, Bonhoeffer, S. et al. Quantitation of HIV-1 specific CTL and plasma load of viral RNA. Science 1998; 279:2103-6.
49. Harrer T; Harrer E; Kalams SA; Elbeik T; Staprans SI; Feinberg MB; Cao Y; Ho DD; Yilma T; Caliendo AM; Johnson RP; Buchbinder SP; Walker BD. Strong cytotoxic T cell and weak neutralizing antibody responses in a subset of persons with stable nonprogressing HIV type 1 infection AIDS Res Hum Retroviruses. 1 996 May 1;1 2(7):585-92.
50. Rinaldo C; Huang XL; Fan ZF; Ding M; Beltz L; Logar A; Panicali D; Mazzara G; Liebmann J; Cottrill M; et al. High levels of anti-human immunodeficiency virus type 1 (HIV-1) memory cytotoxic T-lymphocyte activity and low viral load are associated with lack of disease in HIV-1-infected long-term nonprogressors. J Virol. 1995 Sep;69(9):5838-42.

51. Rowland-Jones S; Sutton J; Ariyoshi K; Dong T; Gotch F; McAdam S; Whitby D; Sabally S; Gallimore A; Corrah T; et al. HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women Nat Med. 1995 Jan;1(1):59-64.
52. Langlade-Demoyen P; Ngo-Giang-Huong N; Ferchal F; Oksenhendler E Human immunodeficiency virus (HIV) nef-specific cytotoxic T lymphocytes in noninfected heterosexual contact of HIV-infected patients J Clin Invest. 1994 Mar;93(3):1293-7.
53. Goh WC; Markee J; Akridge RE; Meldorf M; Musey L; Karchmer T; Krone M; Collier A; Corey L; Emerman M; McElrath MJ Protection against human immunodeficiency virus type 1 infection in persons with repeated exposure: evidence for T cell immunity in the absence of inherited CCR5 coreceptor defects J Infect Dis. 1999 Mar;179(3):548-57.
54. Ortiz GM; Nixon DF; Trkola A; Binley J; Jin X; Bonhoeffer S; Kuebler PJ; Donahoe SM; Demoitie MA; Kakimoto WM; Ketas T; Clas B; Heymann JJ; Zhang L; Cao Y; Hurley; Moore JP; Ho DD; Markowitz M. HIV-1-specific immune responses in subjects who temporarily contain virus replication after discontinuation of highly active antiretroviral therapy. J Clin Invest. 1999 Sept, 0(1999): R13-R18.
55. Matloubian M; Concepcion RJ; Ahmed R CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection J Virol. 1994 Dec;68(12):8056-63.
56. Hasenkrug KJ; Brooks DM; Nishio J; Chesebro B Differing T-cell requirements for recombinant retrovirus vaccines. J Virol. 1996 Jan;70(1):368-72.
57. von Herrath MG; Yokoyama M; Dockter J; Oldstone MB; Whitton JL CD4-deficient mice have reduced levels of memory cytotoxic T lymphocytes after immunization and show diminished resistance to subsequent virus challenge J Virol. 1996 Feb;70(2):1072-9.
58. Battegay M; Moskophidis D; Rahemtulla A; Hengartner H; Mak TW; Zinkernagel RM. Enhanced establishment of a virus carrier state in adult CD4+ T-cell- deficient mice. J Virol 1994 Jul;68(7):4700-4
59. Rosenberg ES; LaRosa L; Flynn T; Robbins G; Walker BD Characterization of HIV-1-specific T-helper cells in acute and chronic infection Immunol Lett. 1999 Mar;66(1-3):89-93.
60. Pitcher CJ; Quittner C; Peterson DM; Connors M; Koup RA; Maino VC; Picker U. HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression Nat Med. 1999 May;5(5):518-25.
61. 9 Emini EA; Schleif WA; Nunberg JH; Conley AJ; Eda Y; Tokiyoshi S; Putney SD; Matsushita S; Cobb KE; Jett CM; et al. Prevention of HIV-1 infection in chimpanzees by gp120 V3 domain-specific monoclonal antibody. Nature, 1992 Feb 20;355(6362):728-30.
62. Shibata R; Igarashi T; Haigwood N; Buckler-White A; Ogert R; Ross W; Willey R; Cho MW; Martin. M. Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. Nat Med. 1999 Feb;5(2):204-10.
63. Gauduin MC; Parren PW; Weir R; Barbas CF; Burton DR; Koup RA. Passive immunization with a human monoclonal antibody protects hu-PBL-SCID mice against challenge by primary isolates of HIV-1. Nat Med. 1997 Dec;3(12):1389-93.
64. Moog C; Fleury HJ; Pellegrin I; Kirn A; Aubertin AM. Autologous and heterologous neutralizing antibody responses following initial seroconversion in human immunodeficiency virus type 1-infected individuals. J Virol. 1997 May;71(5):3734-41.
65. Reitter JN; Desrosiers RC. Identification of replication-competent strains of simian immunodeficiency virus lacking multiple attachment sites for N-linked carbohydrates in variable regions 1 and 2 of the surface envelope protein. J Virol. 1998 Jul;72(7):5399-407.
66. Burton, DR. A vaccine for HIV type 1: The antibody perspective. Proc. Natl. Acad. Sci. USA 1997 Sept; 94: 10018-10023.

67. Sattentau QJ; Moulard M; Brivet B; Botto F; Guillemot JC; Mondor I; Poignard P; Ugolini S Antibody neutralization of HIV-1 and the potential for vaccine design. Immunol Lett. 1999 Mar;66(1-3):143-9.
68. Parren PW; Burton DR; Sattentau QJ HIV-1 antibody--debris or virion? Nat Med. 1997 Apr;3(4):366-7.
69. Burton, Nat Med. 1998 May;4(5 Suppl):495-8.
70. Farzan M; Choe H; Desjardins E; Sun Y; Kuhn J; Cao J; Archambault D; Kolchinsky P; Koch M; Wyatt R; Sodroski J Stabilization of human immunodeficiency virus type 1 envelope glycoprotein trimers by disulfide bonds introduced into the gp41 glycoprotein ectodomain. J Virol. 1998 Sep;72(9):7620-5.
71. Binley JM; Sanders RW; Clas B; Schuelke N; Master A; Guo Y; Kajumo F; Anselma DJ; Maddon PJ; Olson WC; Moore JP. A recombinant human immunodeficiency virus type 1 envelope glycoprotein complex stabilized by an intermolecular disulfide bond between the gpl 20 and gp41 subunits is an antigenic mimic of the trimeric virion-associated structure. J Virol 2000 Jan;74(2):627-43
72. Wyatt R; Sullivan N; Thali M; Repke H; Ho D; Robinson J; Posner M; Sodroski J Functional and immunologic characterization of human immunodeficiency virus type 1 envelope glycoproteins containing deletions of the major variable regions. J Virol. 1993 Aug;67(8):4557-65.
73. Lu S; Wyatt R; Richmond JF; Mustafa F; Wang S; Weng J; Montefiori DC; Sodroski J; Robinson Immunogenicity of DNA vaccines expressing human immunodeficiency virus type 1 envelope glycoprotein with and without deletions in the V1/2 and V3 regions. AIDS Res Hum Retroviruses. 1998 Jan 20;14(2):151-5.
74. Binley JM; Wyatt R; Desjardins E; Kwong PD; Hendrickson W; Moore JP; Sodroski J Analysis of the interaction of antibodies with a conserved enzymatically deglycosylated core of the HIV type 1 envelope glycoprotein 120. AIDS Res Hum Retroviruses. 1998 Feb 10;14(3):191-8.
75. Reitter J; Czajak S; Desrosiers Identification of a replication competent SIV variant lacking five sites for Nlinked glycosylation in its envelope protein. RC 4th Conf Retro and Opportun Infect. 1997 Jan 22-26;:81 (abstract no. 90).
76. LaCasse RA, Follis KE, Trahey M, et al. Fusion-competent vaccines: broad neutralization of primary isolates of HIV. Science. 1999 Jan;283(5400):357-62.
77. Wyatt R; Kwong PD; Desjardins E; Sweet RW; Robinson J; Hendrickson WA; Sodroski JG The antigenic structure of the HIV gpl 20 envelope glycoprotein. Nature 1998 Jun 18;393(6686):705-11.
78. Kwong PD; Wyatt R; Robinson J; Sweet RW; Sodroski J; Hendrickson WA Structure of an HIV gpl 20 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature. 1998 Jun 18;393(6686):648-59.
79. Chan DC; Fass OD; Berger JM; Kim PS. Core structure of gp41 from the HIV envelope glycoprotein. Cell. 1997 Apr 18;89(2):263-73.
80. Connor, RI, Korber, BTM, Graham, BS, et al. Immunological and virological analyses of persons infected by HIV while participating in trials of rgpl 20 vaccines. J Virol 1998; 72: 1552-1576.
81. WahI SM; Smith PD; Janoff EN Human immunodeficiency virus type 1 infection, mucosal immunity, and pathogenesis: comments and conference summary. J Infect Dis. 1999 May;179 Suppl 3:S397-400.
82. Letvin NL. Vaccine-elicited SIV-specific immunity: CTL and protection. 1 2eme Colloque des Cent Gardes, 1999 Oct 25-26: 144.
83. Rowland-Jones S. HIV-specific CD8+ T-cells: what are we measuring. 12eme Colloque des Cent Gardes, 1999 Oct 25-26: 51.
84. Andersson J, Behbahani H, Lieberman J, Connick E, Landay A, Patterson B, Sonnerborg A, Lore K, Uccini S, Fehniger TE Perforin is 4not co-expressed with granzyme A within cytotoxic granules

in CD8 T lymphocytes present in lymphoid tissue during chronic HIV infection. AIDS 1999 Jul 30;13(11):1295-303
85. Swain SL; Hu H; Huston G Class II-Independent Generation of CD4 Memory T Cells from Effectors Science 1999 November 12; 286:1381-1383.
86. Murali-Krishna K; Lau LL; Sambhara S; Lemonnier F; Altman J; Ahmed R Persistence of Memory CD8 T Cells in MHC Class I-Deficient Mice Science 1999 November 12; 286: 1377-1381.
87. Report of the NIH AIDS Research Program Evaluation Working Group (Levine Report). Available: http://www.nih.gov/od/oar/public/pubs/levine.pdf. 1996, March 13.
88. Hackett, http://www.niaid.nih.gov/publications/pdf/jordan.pdf.
89. Harouse JM, Gettie A, Tan RC, et al. Distinct pathogenic sequelae in rhesus macaques infected with CCR5 or CXCR4-utilizing SHIVs. Science. 1999 April; 284(5415):816-819.
90. Liu ZQ, Muhkerjee 5, Sahni M et al. Derivation and biological characterization of a molecular clone of SHIV KU2 that causes AIDS, neurological disease, and renal disease in rhesus macaques. Virology, 1999 Aug; 260(2):295-307.
91. Miller, J Infect Dis. 1999 May; 179 Suppl 3: 5413-7.
92. Deng HK, Unutmaz D, Kewal Ramani VN, et al. Expression cloning of new receptors used by simian and human immunodeficiency viruses. Nature 1997;388:296-300.
93. Cayabyab M, Karlsson GB, Etemad-Moghadam BA, et al. Changes in HIV-1 envelope glycoproteins responsible for the pathogenicity of a multiply passaged SHIV HXBc2. J Virol. 1999 Feb; 73(2):976-84.
94. Fultz PN, McClure HM, Anderson DC, et al. Identification and biologic characterization of an acutely lethal variant of SIV from sooty mangabeys. AIDS Res Hum Ret 1989;5:397-409.
95. Tao B, Fultz PN. Pathogenicity and comparative evolution in-vivo of the tranistional quasispecies SIVsmPBj8. Virology. 1999 Jun; 259(1):1 66-75.
96. Desrosiers RC. HIV with multiple gene deletions as a live attenuated vaccine for AIDS. AIDS Res Hum Ret 1992;8:411-421.
97. Ruprecht RM, Baba TW, Liska V. Attenuated HIV vaccine: caveats. Science 1996; 271:1790-1791.
98. Warren JT, Levinson, MA. Preclinical AIDS vaccine development: formal survey of global HIV, SIV, and SHIV in-vivo challenge studies in vaccinated non-human primates. J Med Primatol 1997;26:63-81.
99. Karlsson GB, Halloran M, Li J, et al. Characterization of molecularly cloned SHIV viruses causing rapid CD4+ lymphocyte depletion in rhesus monkeys. J Virol 1997;71:4218-4225.
100. Joag SV, Li Z, Foresman L, et al. Characterization of the pathogenic KU-SHIV model of AIDS in Macaques. AIDS Res Hum Ret 1997;13:635-645.
101. Lu YC, Salvato MS, Pauza CD, et al. Utility of SHIV for testing HIV-1 candidates in macaques. J AIDS Hum Ret 1996;12:99-106.
102. Alexander L, Du Z, Howe AY, et al. Induction of AIDS in rhesus monkeys by a recombinant SIV expressing nef of HIV-1. J Virol. 1999 July; 73(7):5814-25.
103. Novembre FJ, Saucier M, Anderson DC, et al. Development of AIDS in a chimpanzee infected with HIV-1. J Virol 1997;71:4086-4091.
104. Santra S, Fultz PN, Letvin, N. Virus-specific CTLs in HIV-1 infected chimpanzees. J Virol. 1999 Aug. 73(8):7065-9.
105. Barnett SW, Murthy KK, Herndier BG, et al. An AIDS-like condition induced in baboons by HIV-2. Science 1994;266:642-646.
106. VanCott TC, Mascola JR, Loomis-Price LD, et al. Cross-subtype neutralizing antibodies induced in baboons by a subtype E gpl 20 immunogen based on an R5 primary HIV-1 envelope. J Virol. 1999 June. 73(6):4640 -50.

107. Wieder KJ, Chatis P, Boltax J, et al. HIV-1 entry into murine cell lines and lymphocytes from transgenic mice expressing a gpl 20-binding mutant mouse CD4. AIDS Res Hum Ret 1996;12:867-876.
108. Felber B, Valentin A, Rosati M, et al. Use of mice to model HIV disease: lack of CD4 cell depletion in SCIDhu mice infected by a rev-independent HIV-1. 1997 NCVDG Conference. 1997 May.
109. Yamamura Y, Kotani M, Chowdhury MI, et al. Infection of human CD4_ rabbit cells with HIV-1; the possibility of the rabbit as a model for HIV-1 infection. Intern Med 1991;3:1183-1187.
110. Wyand MS, Manson KH, Garciamoll M, et al. Vaccine protection by a triple-deletion mutant of SIV. J Virol 1996;70:3724-3733.
111. Cole KS, Rowles JL, Jagerski BA, et al. Evolution of envelope-specific antibody responses in monkeys experimentally infected or immunized with SIV and its association with the development of protective immunity. J Virol 1997;71:5069-5079.
112. Fuller DH, Simpson L, Cole KS, et al. Gene gun-based nucleic acid immunization alone or in combination with recombinant vaccinia vectors suppresses viral burden in rhesus macaques challenged with a heterologous SIV. Immunol Cell Biol 1997; 75;389-396.
113. Ahmad 5, Lohman B, Marthas M, et al. Reduced viral load in rhesus macaques immunized with rgpl 60 and challenged with SIV. AIDS Res Hum Ret 1994;10:195-204.
114. Abimiku AG, Robert-Guroff M., Benson J, et al. Long-term survival of SIVmac251-infected macaques previously immunozed with NYVAC SIV vaccines. J AIDS Hum Ret 1997;15(Suppl):S78-S85.
115. Lekutis C, Letvin NL. HIV-1 envelope-specific CD4+ T-helper cells from SHIV-infected rhesus monkeys recognize epitopes restricted by MHC class II DRBI*0406 and DRB*W201 molecules. J Immunol 1997;159:2049 -2057.
116. Villinger F, Brar ss, Mayne A, et al. Comparative sequence analysis of cytokine genes from human and nonhuman primates. J Virol 1995; 155:3946-3954.
117. Zou W, Lackner AA, Simon M, et al. Early cytokine and chemokine gene expression in lymph nodes of macaques infected with SIV is predictive of disease outcome and vaccine efficacy. J Virol 1997;71:1227 -1236.
118. Zhang D, Johnson RP. Molecular cloning and comparative analysis of the rhesus macaque costimulatory molecules CD80 (B7-1) and CD86 (B7-2). Cell Immunol 1997;177:9-17.
119. Imaoka K, Miller CJ, Kubota M, et al. Nasal immunization of nonhuman primates with SIV p55gag and cholera toxin adjuvant induces Thi /Th2 help for virus specific immune responses in reproductive tissues. J Immunol. 1998 Dec; 161(11):5952-8.
120. Marinaro M, Boyaka PN, Jackson RJ, et al. Use of intranasal IL-12 to target predominantly Th1 responses to nasal and Th2 responses to oral vaccines given with cholera toxin. J Immunol. 1999 Jan; 162(1):114-21.
121. LaCasse, 357-62.
122. Lallos LB, Laal S, Hoxie J, et al. Exclusion of HIV coreceptors CXCR4, CCR5, and CCR3 from the HIV envelope. AIDS Res Hum Ret. 1999 July; 15(10): 895-7.
123. Polacino PS, Stallard V, Klaniecki JE, et al. Rose of immune responses against the envelope and core antigens of SIVmne in protection against homologous cloned and uncloned virus challenge in macaques. J Virol. 1999 Oct; 73(10):8201-15.
124. Belyakov IM, Moss B, Strober W, et al. Mucosal vaccination overcomes the barrier to recombinant vaccinia immunization caused by pre-existing poxvirus immunity. Proc Natl Acad Sci. 1999 April; 96(8):4512-7.
125. Wyand M, Manson K, Montefiori DC, Lifson JD, Johnson RP, Desrosiers RC. Protection by live attenuated SIV against heterologous challenge. J Virol. 1999 Oct; 73(10):8356-63.
126. Johnson, 4952-61.

127. Dolin R; Graham BS; Greenberg SB; Tacket CO; Belshe RB; Midthun K; Clements ML; Gorse GJ; Horgan BW; Atmar RL; et al The safety and immunogenicity of a human immunodeficiency virus type 1 (HIV-1) recombinant gpl160 candidate vaccine in humans. Ann Intern Med. 1991 Jan 15;114(2):119-27.
128. Graham, BS; Belshe, RB; Clement, ML; et al. Vaccination of vaccinia-naive adults with HIV-1 gpl 60 recombinant vaccinia in a blinded controlled randomized clinical trial. J. Infect Dis 1992; 166; 244-252.
129. Graham, BS; Karzon, DT. AIDS Vaccine Development. Textbook of AIDS Medicine. D. Bolognesi, ed. 1998; 689-724.
130. McElrath, MJ, Corey, L, Montefiore, D, et al. A phase 2 study of two HIV-1 envelope vaccines comparing their immunogenecity in populations at risk for acquiring HIV-1 infection. J Infect Dis 1999 (in press).
131. Connor, 1552-76.
132. Lambert JS, et al; Safety and immunogenicity of HIV recombinant envelope vaccines in HIV-infected infants and children. National Institutes of Health-sponsored Pediatric AIDS Clinical Trials Group (ACTG-21 8). J Acquir Immune Defic Syndr Hum Retrovirol, 1998 Dec.
133. Keefer, MC; Wolff, M; Gorse, GI; et al. Safety profile of phase 1 and 2 preventive HIV-1 envelope vaccination: experience of the NIAID AIDS Vaccine Evaluation Group. AIDS Res Hum Ret. 1997; 13(14):1163-1177.
134. Gorse GJ; Patel GB; Newman FK; Belshe RB; Berman PW; Gregory TJ; Matthews TJ Antibody to native human immunodeficiency virus type 1 envelope glycoproteins induced by IIIB and MN recombinant gpl 20 vaccines Clin Diagn Lab Immunol. 1996 Jul;3(4):378-86.
135. Graham BS; Keefer MC; McElrath MJ; Gorse GJ; Schwartz DH; Weinhold K; Matthews TJ; Esterlitz JR; Sinangil F; Fast PE Safety and immunogenicity of a candidate HIV-1 vaccine in healthy adults: recombinant glycoprotein (rgp) 120. A randomized, double-blind trial. Ann Intern Med. 1996 Aug 15;125(4):270-9.
136. Mascola JR; Snyder SW; Weislow OS; Belay SM; Belshe RB; Schwartz DH; Clements ML; Dolin R; Graham BS; Gorse GJ; Keefer MC; McElrath MJ; Walker MC; Wagner KF; McNeil JG; McCutchan FE; Burke DS Immunization with envelope subunit vaccine products elicits neutralizing antibodies against laboratory-adapted but not primary isolates of human immunodeficiency virus type 1. J Infect Dis. 1996 Feb;173(2):340-8.
137. Graham, BS, McElrath MJ, Connor, RI, et al. Analysis of intercurrent HIV-1 infection in phase 1 and 2 trials of candidate AIDS vaccines. J Infect Dis 1998; 177: 310-319.
138. Clements-Mann, ML, Weinhold, K., Matthews, TJ, et al. Immune responses to HIV-1 induced by canarypox expressing HIVmn gpl 20, HIV sf2 gpl 20, or both vaccines in seronegative adults. J Infect Dis 1998; 177: 1230-1246
139. Evans, TG, Keefer, MC, Wolff, M, et al. A canarypox recombinant containing HIV-1 env, gag, pol, and nef genes given with SF2 rgpl 20 elicits broad, durable CD8+ CTL responses in seronegative volunteers. JID 1999; 180: 290-8.
140. Corey, L, McEIrath, MJ, Weinhold, K, et al. CTL and neutralizing antibody responses to HIV-1 envelope with a combination vaccine regimen. J Infect Dis 1998; 177:301-309.
141. McEIrath, MJ, Corey, L, et al. HIV-1 vaccine induced CTL responses in phase I clinical trials: detection, characterization, and quantitation. AIDS Res Hum Ret, Feb 1997.
142. Belshe, RB, Gorse, GJ, Mulligan, MJ, et al. Induction of immune responses to HIV by canarypox virus (ALVAC) and gpl 20 SF-2 vaccines in uninfected volunteers. AIDS 1998; 12:2407-2415.
143. Wyatt, Nature 1998 Jun 18;393(6686):705-11.
144. Kwong, 648-59.
145. Ferrari G, Humphrey W, McEIrath MJ, et al. Clade B-based HIV-1 vaccines elicit cross-clade CTL reactivities in uninfected volunteers. Proc Nat'l Acad Sci USA. 1997: 94; 1396-1401.

146. Ogg, 2103-6.
147. Zolla Pazner S, et al; Neutralization of a clade B primary isolate by sera from human immunodeficiency virus-uninfected recipients of candidate AIDS vaccines [see comments] J Infect Dis, 1997 Apr
148. Nitayaphan S, Brown AE. Preventive HIV vaccine development in Thailand. AIDS 1998;12 Suppl B:S155 -61.
149. Report of the NIH AIDS Research Program Evaluation Working Group (Levine Report). Available: http://www.nih.gov/od/oar/public/pubs/levine.pdf. 1996, March 13.
150. Vaccine Research and Development Area Review Panel Report. Available: http://www.nih.gov/od/ oar/public/pubs/vaccine.pdf. 1996, March 13.
151. AIDS Vaccine Advocacy Coalition. 9 Years and Counting.....Will We Have an HIV Vaccine by 2007? Available: http://www.avac.org/avacsite/index.html. 1998, May 18.
152. AIDS Vaccine Advocacy Coalition. 8 Years and Counting.....What Will Speed the Development of an AIDS Vaccine? Available: http://www.avac.org/counting/index.html. 1999 May.

Treatment Action Group 350 Seventh Avenue, Suite 1603 New York, NY 10001 phone: (212) 971-9022 fax: (212) 971-9019