



## Basic Research

Basic research in HIV pathogenesis, microbiology, immunology, virology, and animal model development lays the foundation for advancing research in HIV treatment and prevention. At NIAID, this research is conducted primarily through investigator-initiated research as well as a number of targeted programs and several large cohort studies.

This past year, as a result of advances in basic science, researchers have learned that infection with a second strain of HIV (superinfection) is possible, despite a potent immune response to the initial infecting virus. The lack of cross-protective immunity for closely related HIV strains has important implications in the areas of HIV pathogenesis, treatment, and vaccine development.

In the past, studies of HIV infection have shown that a small number of high-risk individuals who engage in unprotected sexual activity with HIV-infected partners do not show evidence of infection with HIV. That is, they do not show clinical signs of HIV infection, do not develop antibodies to HIV, and do not have detectable levels of HIV in their blood or lymphoid tissues. However, recent studies by NIAID-funded investigators of HIV-exposed, seronegative individuals found that some of these individuals carry extremely low levels of HIV DNA. This suggests that some individuals can control HIV infection, although the mechanisms underlying this control are not completely understood. Possibilities include initial exposure to only a small amount of virus, exposure to a less pathogenic HIV variant than that found in the majority of HIV-infected individuals, and/or the initiation and

maintenance of an effective immune response. Future studies will examine HIV-exposed, seronegative individuals to determine the prevalence of low-level HIV infection and further investigate the mechanisms responsible, which will help inform development of a safe and effective vaccine.

HIV disease is characterized by a gradual deterioration of immune function. Most notably, crucial immune cells called CD4+ T cells are disabled and killed during the typical course of infection. Currently, drugs available for the treatment of HIV attack various aspects of its life cycle, including critical steps in viral replication and the entry of the virus into the CD4+ T cells. Nucleoside reverse transcriptase inhibitors and protease inhibitors are antiretroviral drugs that inhibit specific aspects of viral replication, while fusion inhibitors are the class of antiretroviral drugs that prevent the virus from penetrating the host cell membrane, thereby preventing viral replication within that host cell. Although sustained reduction in the amount of circulating virus in an HIV-infected individual can be achieved when these drugs are used in appropriate combinations (so-called HAART), drug-resistant viruses that limit the effectiveness of HAART for many patients can and do emerge. Therefore, it is important to develop alternate approaches for attacking HIV that will limit the emergence of viral resistance. One potential target that has yet to be exploited is the basic protein structure of the virus, termed the “capsid,” which is a cone-shaped structure that encloses the viral RNA. The ability of the virus to infect the cell requires an intact capsid. Using powerful computers and high-resolution nuclear magnetic resonance imaging technology, NIAID-supported investigators

have identified compounds that bind to a specific site on the proteins that combine to form the capsid, inhibiting its assembly inside an infected cell. This resulted in the production of abnormal HIV virus particles that could not infect new cells. These findings lay the groundwork for the development of capsid assembly inhibitors as a new class of therapeutic agents for HIV.

Although current antiretroviral therapy (ART) regimens can eliminate most HIV replication, a reservoir of HIV-infected cells can persist in a resting state (latent reservoir) in which HIV is present but not actively replicating. NIAID-funded researchers recently discovered that polyamides (synthetic small molecules containing modified amino acids) activate HIV genes. Increased HIV gene expression in latently infected cells that are exposed to polyamides makes the cells more vulnerable to the effects of ART and the immune system. These studies could lead to the development of therapies directed at eliminating the persistent reservoir of HIV infection within resting immune cells.

To search for ways to enhance the effectiveness of ART, NIAID funds research to explore a potential role for vaccination in treatment regimens. In a recent study, investigators found that vaccination, which increased virus-specific T cell responses in ART-treated, simian immunodeficiency virus-infected macaques, resulted in a significantly lower steady-state viral load (viral set point) for at least 4 months after ART was discontinued. Although the effect of decreasing viral load was transient, and more potent vaccine strategies may be needed to obtain longer term benefits, the study provided proof that therapeutic vaccination before stopping ART may be a feasible approach in the management of HIV.

Although much has been learned, questions still remain about (1) the molecular interactions involved in the regulation of HIV expression and replication; (2) why the host immune response is not fully effective in controlling the infection; (3) how reservoirs of infection persist in the body despite HAART; and (4) the role of viral, host, environmental, and social factors in disease progression. Answering basic scientific questions about how the virus attacks the body and how the body defends itself is critical to provide additional potential targets against which therapeutic interventions and vaccines can be directed.

## Vaccine Research

An HIV vaccine that is simple to administer, inexpensive, and induces long-lasting immunity against all HIV subtypes is the ideal prevention strategy and critical to the effective control of the global spread of HIV. It is, therefore, one of NIAID's highest priorities, albeit one of the most difficult challenges in HIV/AIDS research. NIAID supports a full spectrum of HIV vaccine research and development, including basic research (discovery), preclinical screening and animal model development, product development and manufacturing, and clinical research. The scope and breadth of these programs and resources continue to significantly advance global HIV vaccine development efforts.

Over the years, NIAID-supported HIV vaccine research has led to the identification of new and innovative HIV vaccine designs, improvements in vaccine delivery, development of innovative laboratory techniques and animal models for evaluating vaccines, and evaluation of 40 vaccine candidates in clinical studies. Additional

studies have already been initiated or are being planned to evaluate the safety and immunogenicity of a number of new candidate vaccines, including lipopeptide vaccines alone and in combination with a canary pox vaccine, a VEE replicon vector vaccine, novel DNA vaccines, a recombinant nonreplicating adenovirus vaccine, modified vaccinia Ankara (MVA) vectored and other novel pox vector-based vaccines, and a CTL multi-epitope peptide (MEP) vaccine.

In addition, through NIAID's HIV Vaccine Trials Network, the international infrastructure to evaluate candidate vaccines worldwide has been established and expanded to meet the demands of the growing number of candidate vaccines currently in the pipeline for which large efficacy trials may be needed.

Because rapidly identifying a safe and effective HIV/AIDS vaccine will require unprecedented cooperation among governments throughout the world, private sector vaccine developers, academic researchers, nonprofit organizations, and affected communities, NIAID continues to strengthen existing collaborations and develop new partnerships. Specifically, NIAID and the U.S. Army Medical Research and Materiel Command (USAMRMC) of the Department of Defense (DoD) continue to work together on the integration and coordination of their HIV vaccine research efforts. This collaboration combines the collective strengths and capabilities of NIAID and DoD in the planning and conduct of future HIV vaccine studies, and includes collaboration with the Thai Royal Government in the conduct of a large phase III efficacy trial that was recently initiated in Thailand.

In addition, NIAID has forged a new, innovative collaboration called the Partnership for HIV/AIDS Vaccine Evaluation (PAVE), which includes the HIV vaccine program of the Centers for Disease Control and Prevention (CDC), the U.S. Military HIV Research Program, and several nongovernmental organizations active in HIV vaccine development. PAVE will help ensure coordination and efficiency of U.S. Government agencies and their partners and accelerate HIV vaccine research and development in and outside the United States, including in developing countries. (See the Vaccine Research and Development section on page 139 for additional vaccine information.)

### **Nonvaccine Prevention Research**

To control the HIV epidemic, new and more effective methods and strategies are needed to prevent HIV infection. Until a highly efficacious vaccine that can be widely distributed and used is developed, control of the epidemic will require a combination of prevention approaches. NIAID's HIV Prevention Trials Network (HPTN) develops and tests promising nonvaccine strategies to prevent the spread of HIV/AIDS, including

- Drugs or vaccines that are practical and easy to use to prevent mother-to-child transmission of HIV, including prevention during breastfeeding;
- Microbicides to prevent sexual transmission of HIV;
- ART that may reduce the spread of HIV from infected persons to their sexual partners;
- Measures to control other sexually transmitted diseases and thereby decrease the risk of co-infection with HIV;

- Interventions to reduce behavior that exposes people to HIV; and
- Programs to curb the spread of HIV by reducing intravenous drug abuse.

NIAID-funded research that makes use of the HPTN has led to important scientific advances that increase our understanding about the transmission of HIV. These findings provide a foundation for developing and testing innovative prevention strategies. For example, NIAID-funded researchers recently reported that infants who received a single dose of the inexpensive antiviral drug nevirapine (NVP) soon after birth—and whose mothers took one dose of the same drug during labor—were 41 percent less likely to acquire HIV at birth or during breastfeeding than infants in infant/mother pairs who were treated with a multidose regimen using zidovudine (AZT). This benefit was largely sustained until the children reached 18 months of age, with few serious side effects attributable to NVP. This finding was based on the continued follow-up of the infants in a study that NIAID initiated in November 1997 (known as HIVNET 012) in Uganda and offers compelling new evidence that short-course NVP effectively and safely reduces the number of children who become infected with HIV. Because the cost of the NVP regimen is significantly lower than other regimens and easy to administer, these results could have far-reaching implications in resource-poor countries.

In other prevention research, preliminary data are available from the Project EXPLORE study, which is a national HIV prevention behavioral trial involving nearly 4,300 men who have sex with men. Project EXPLORE is one of the largest behavioral studies of its kind and is designed to examine whether an intensified program of counseling on high-risk

behavior helps to prevent men who have sex with men from getting HIV. Recent publications have described the prevalence of high-risk behaviors of the Project EXPLORE study participants prior to their enrollment in the behavioral intervention and the types of behavioral interventions used in the study.<sup>2,3</sup>

Prevention research involving topical microbicides is described in the Sexually Transmitted Infections section on page 124.

## Therapeutics

One of the primary goals of HIV/AIDS therapeutic research is to evaluate innovative therapeutic strategies for both HIV/AIDS and the complications of therapeutic interventions, in all stages of HIV infection. As a result of HAART, the life expectancy of HIV-infected individuals has increased. As the number of individuals living with HIV disease increases, many develop a host of complications resulting from their therapeutic regimens including the development of drug resistance and metabolic abnormalities and toxicities. Moreover, the immune system only partially recovers during HAART treatment. Thus, new therapies, and ways to expand the clinical benefit of currently approved therapies, are still urgently needed. NIAID's therapeutics research programs and networks are focusing on these issues.

NIAID is currently conducting a long-term study to address important questions about the most appropriate use of currently available antiviral drugs for the treatment of HIV/AIDS. This study, Strategies for Management of Anti-Retroviral Therapies (SMART), is designed to determine which of two common HIV treatment strategies results in a better outcome over time. The SMART study is among the first long-term studies of its kind

and is being conducted through the Terry Bein Community Programs for Clinical Research on AIDS (CPCRA). This study will be conducted over a 9-year period and will eventually involve 6,000 patients. To date, more than 1,260 HIV-infected people have been enrolled. The SMART study is designed to provide information that will help physicians and their patients make informed treatment decisions. For more information about the SMART study, please visit [www.smart-trial.org](http://www.smart-trial.org).

Hoping to find better treatment strategies for people with treatment failure and multidrug-resistant HIV, CPCRA conducted a study of structured treatment interruption in HIV patients with advanced disease and multidrug resistance. In this specific population, however, structured treatment interruption was found to possibly hasten disease progression. The study was significant because of its large number of participants, the length of follow-up, and the fact that there was a randomized comparison with a control group. These factors allowed an assessment of the overall impact of structured treatment interruption, including the effects on AIDS-related illnesses, HIV viral load, CD4+ T cell count, and quality of life.

NIAID-funded researchers recently identified the prevalence of transmitted drug resistance among individuals with primary HIV infection who had never received treatment. The investigators found that the proportion of new HIV infections with drug-resistant virus increased significantly in North Americans between 1995 and 2000, and that initial ART was more likely to fail in patients newly infected with drug-resistant virus. These findings suggest that resistance tests should be used routinely before making treatment

decisions for patients with recently acquired HIV.

Using a novel assay to characterize the types of antibodies that are produced in patients with HIV, NIAID-funded researchers demonstrated that most people generate potent neutralizing antibodies soon after infection. The antibodies that patients generated first were specific to each individual's infecting HIV strain. After that, however, the virus rapidly evolved variants with mutations in the envelope glycoprotein gene, and thereby disguised itself to escape the body's immune defenses. Future studies may elucidate mechanisms through which the virus is able to engage in this immunologic game of cat and mouse. A better understanding of how HIV is recognized by the immune system and how it evades the attack mounted against it may lead to the development of a broadly protective vaccine or a novel ART.

A major goal of NIAID intramural researchers and their collaborators is to discover new therapies for AIDS that are less expensive or less toxic than current therapies and can therefore be used more widely. Several such new approaches are under study in NIAID's Division of Intramural Research (DIR). For some HIV-infected patients whose plasma levels of virus have fallen to undetectable levels while on HAART, it may prove feasible to move from a continuous HAART regimen to intermittent therapy in which an individual discontinues and then resumes HAART in a preplanned cyclic fashion. This cyclic approach to treatment, known as structured intermittent therapy, might enable an HIV-infected person to have regular HAART-free periods while maintaining a minimal viral load and adequate levels of CD4+ T cells.

To test this concept, DIR researchers conducted a pilot study in which the regimen of structured intermittent therapy (7 days on HAART, 7 days off) maintained suppression of both viral particles in the blood and HIV replication in reservoir sites while preserving CD4+ T cell counts.<sup>4</sup> In addition, study volunteers experienced a decrease in important toxicity parameters and significantly lower cholesterol and triglyceride levels, which are often abnormally elevated in patients on HAART. The researchers also conducted a trial involving longer therapy interruption (8 weeks on HAART, 4 weeks off); however, this cycle of longer therapy interruption does not look promising because of the emergence of genetic mutations in a few patients.<sup>5</sup> Larger clinical trials are under way in the United States and Africa to address the efficacy and impact of short-cycle intermittent therapy.

Although HAART has dramatically improved the clinical outcome for many HIV-infected patients, the associated cost, toxicity, and development of drug resistance underscore the need for additional therapeutic strategies. Strategies aimed at enhancing the ability of the immune system to fight HIV infection are currently being investigated by NIAID intramural scientists as potential supplements to ART. These immune-based strategies include treatments that stimulate or suppress a particular part of the immune system, infusion of additional immune system cells, and therapeutic immunizations. NIAID's long-term basic research into the function of interleukin-2 (IL-2), a protein that makes CD4+ T cells mature and multiply, and clinical studies of its safety and efficacy for HIV therapy have led to promising results. Ongoing randomized phase III clinical studies by NIAID intramural

investigators, as well as two large international studies, should clarify the effect of IL-2 on viral load and CD4+ T cell counts to assess long-term clinical outcomes.<sup>6</sup>

The next generation of antiviral therapeutics may include compounds that prevent HIV from entering CD4+ T cells. Infection of CD4+ T cells by HIV is a complex process that starts with binding of the virus to a CD4 protein molecule on the outer surface of the cell. Past attempts to develop compounds that block viral binding and entry have not been successful, in part because they were unable to efficiently prevent HIV from binding to the CD4 protein molecule. In fact, at low concentrations, these inhibitors actually enhanced the ability of HIV to infect CD4+ T cells.

NIAID researchers, however, have recently constructed a compound that inhibits entry of HIV into CD4+ T cells and does not enhance HIV entry under any conditions. This compound is a large protein that binds specifically to the part of HIV that attaches to the CD4+ T cells. The protein exhibited extraordinarily strong binding to HIV, and relatively small amounts were able to neutralize HIV samples from a broad range of infected patients. In addition, the protein activates natural killer (NK) cells, which are an important defense against the virus. The specificity for both HIV envelope and the NK cell receptor may promote NK cell-mediated killing of HIV-infected cells. Based on these observations, NIAID scientists are evaluating the compound for its potential as both a therapeutic agent and a vaccine adjuvant.