Reexamining AIDS Research Priorities

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Human immunodeficiency virus (HIV), the causative agent of AIDS, has penetrated virtually every population on the globe. More than 17 million children, women, and men are infected, with the greatest number being in sub-Saharan Africa (*1*). The epidemic is rapidly gathering force in Asia where the number of new cases of AIDS increased eightfold in a single year. In several Asian nations, a public health disaster is in the making. We must also anticipate that parts of the world where there are now very limited numbers of infected individuals will soon become battlegrounds in our confrontation with the virus.

In the United States, the number of HIV-infected individuals continues to increase. Furthermore, the epidemic has undergone striking demographic changes. In 1993, more than 50% of the new cases of AIDS among men, more than 75% among women, and 84% among children occurred in minority populations, particularly within African-American and Hispanic communities (*2*). As a tragic example of these changing demographics, the risk of infection of an African-American woman was 15 times that of a white woman. The continued development of the epidemic and the high rate of infection in many disadvantaged communities highlights the responsibility of the federal government to craft an effective response.

The first decade of research on AIDS emphasized the nation's commitment to respond promptly and vigorously. Much has been achieved. We have attained some understanding of the pathogenesis of the disease. A class of useful anti-retroviral drugs, the reverse transcriptase inhibitors, has been introduced. The likelihood of transmission of HIV infection from a pregnant woman to her child can be markedly diminished by treatment with one of these agents, zidovudine (*3*). Treatments for opportunistic infections have made impressive inroads and have prolonged and improved the lives of people living with AIDS.

Nonetheless, these achievements have not provided us with the robust therapies that had been hoped for nor is a highly effective preventive vaccine in sight. Our ability to alter risk-taking behaviors is still very limited. We do not understand major aspects of the virus' interaction with the infected individual and the nature of the host response to the virus is far from clear. A turning point has now been reached. Simple continuation of the policies of the past is likely to bring us only slow, fitful progress.

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A New Emphasis on the Biology of HIV Infection and AIDS

Thoughtful scientists analyzing the current status of our progress against the disease and the state of research have concluded that the limited progress made thus far is due to an inadequate knowledge base. They argue that the emergency effort to find therapeutics and preventives without a penetrating understanding of the dynamics of the infection and of its progression has, inevitably, led to disappointment. Many, most notably Professor Bernard Fields of the Harvard Medical School (*4*), have issued a call for a striking increase in the support of research on the basic mechanisms underlying HIV infection and disease progression and on the nature of immune responses that might control such progression. This proposal has been coupled with a recommendation for increased support of research on model systems that may help to elucidate the key principles that HIV follows in its induction of disease.

New legislation has given the Office of AIDS Research (OAR) at the National Institutes of Health (NIH) the responsibility of creating a comprehensive research plan that sets the scientific priorities to be used in the development of the entire NIH AIDS research budget. The OAR agrees that increased investment in the fundamental science underpinning the AIDS research effort is essential. However, a "back-to-basics" approach cannot be instituted by large-scale reduction of current efforts to find agents that provide benefit for infected individuals and those now living with AIDS. The OAR is committed to a strategy that allows both a rededication to fundamental research on HIV and AIDS and the maintenance of efficient efforts in the spheres of drug discovery, clinical trials, vaccine development and behavior modification.

Investigator-Initiated Research Versus Dedicated Resources

A strong tension exists in the effort to develop an enhanced program of basic research targeted at HIV and AIDS. Inevitably, NIH research program directors seek, and are given, advice about promising research approaches that need to be emphasized. In turn, NIH administrators commonly issue requests for applications (RFAs) or requests for proposals (RFPs) that ask scientists to submit grant or contract proposals aimed at studying a particular subject. To ensure funding for some of these proposals, an NIH institute will reserve money to support successful applications. Generally, these reserved funds diminish the pool of money that would have been available to support unsolicited, investigator-initiated research grant applications, exacerbating the already substantial difficulties that scientists find in raising support for such programs. This is a particular problem for AIDS research. The fraction of the AIDS research budget used to support unsolicited grant applications is less than 50% of the fraction of the non-AIDS budget used to support such grants (*5*).

Such central direction of the AIDS research program certainly could have been defended in view of the dramatic increase in funds for this purpose from zero in 1982 to more than $1.3 billion in 1995, in response to the recognition of the public health emergency presented by AIDS, and the lack of a broad base of scientists that were already working in the area. As we now enter a phase requiring the development of new basic knowledge and in which research budgets are no longer rapidly increasing, I am committed to increasing that fraction of the AIDS budget that supports unsolicited investigator-initiated research grants. In developing the consolidated NIH AIDS budget, the OAR has instructed the institutes to prepare budget requests that call for the use of RFAs and contracts only where absolutely essential. In its review of budgetary requests, the OAR will place a very high priority in making funds available to the institutes to support innovative, investigator-initiated research proposals. In the future, the NIH interest in an AIDS research approach will more often be indicated by the issuance of program announcements. Such program announcements do not commit funds to a given area but they do indicate to the scientific community that NIH believes that there is considerable reason to anticipate that work in this area can make an important contribution to the overall AIDS research effort. However, the funding decision is fully determined by the procedures of peer review as part of the entire competitive process.

Dedicated Resources Are Required in Certain Situations

Nevertheless, dedicated resources are necessary in some special circumstances. It will be possible to mount an effective approach to government-sponsored drug and vaccine clinical trials and to related types of pathogenesis research only if a coordinated program is in place. Programs such as the adult and pediatric AIDS Clinical Trials Groups (ACTGs) will continue to require dedicated support. The level of such support must be predicated upon the tasks these groups will confront and by the opportunities that exist to test new, promising therapeutic agents and vaccines that are not being adequately evaluated by their corporate sponsors. It is essential that such groups be funded based on their capacity to contribute to scientific research, as judged by a process of peer review. These funds cannot be regarded as subsidies for clinical care, except in so far as that care is essential for the conduct of the research program.

It is understandable, but unfortunate, that these research programs have come to be regarded in their communities as treatment resources. In turn, this has led to attempts to have considerations other than the ability to carry out the needed research, as determined through a competitive, peer-review process, play a major role in funding decisions. Recognizing that the goal of these funds is to carry out research aimed at bettering the lot of all infected individuals and of all individuals at risk of infection, such nonresearch-based considerations must be resisted.

Scientific Opportunities in AIDS Research

Primate research must play a central role in understanding pathogenesis. An area in which we foresee an expanded effort is the study of the pathogenesis of AIDS and of immunity to HIV based on macaques infected with simian immunodeficiency virus (SIV) or with chimeric SIV/HIV viruses. Since no truly adequate small animal model for HIV infection and AIDS exists, the macaque takes on a particularly important role. There is already an active program of such work. Such research is largely funded through direct support of the Regional Primate Research Centers (RPRC) and through grants to principal investigators, many of whom are RPRC staff members. This has sometimes deterred other investigators from carrying out research programs involving macaques. We will strongly encourage investigators outside of primate centers to form alliances with RPRC scientists and to submit grant applications on any topic related to AIDS including studies of pathogenesis, therapeutics, and vaccine research. In view of the complexity of primate research and the need for specialized centers in which to carry out such work, vigorous approaches to stimulate such collaborations will be essential.

What forms of immunity would be most effective against HIV? It is of the utmost importance to do further research to determine the nature of the immune responses that would be most effective in preventing infection and in containing the virus once infection has occurred. In most infectious diseases, a state of acquired immunity exists among individuals who have recovered from that infection providing important information on the requirements for such protection. The apparent absence of such a clear state of acquired immunity to HIV deprives us of key information on the nature of protective anti-HIV responses. This inevitably has led to the examination of the responses to HIV that do occur with the hope that enhancing them might prevent infection or control infection once it has occurred. Such an analysis may prove misleading. In particular, we need to recall that the types of immunity that can prevent infection may be quite different from those that control the virus once infection has occurred.

Some scientists have argued that only antibodies capable of neutralizing a microorganism can prevent infection and that only vaccines that generate such antibodies can be truly preventive. As an example, one may cite the experience in the development of a vaccine that protects against infection with the obligatory intracellular microorganism, *Salmonella typhi*. Antibodies that block the action of a key virulence factor, the salmonella Vi polysaccharide, prevent infection. A vaccine consisting of this polysaccharide is highly protective (*6*). Furthermore, high titers of antibodies to other infectious agents in the fluids that bathe mucosal surfaces can be induced by appropriate immunization strategies (*7*). Surely, prevention of infection at the sites of entry of HIV would be the most desirable of goals.

On the other hand, it is well known that natural infections to many intracellular pathogens are controlled by vigorous T cell responses, but these must be of the appropriate type. For control of infection with the protozoan parasite *Leishmania major* (*8*), T cell responses dominated by interferon γ (IFN-γ) production are critical for the development of curative immunity. If a similar mechanism of immunity applies to HIV infection, a thesis about which there is considerable controversy, efforts to emphasize cellular immunity (IFN-γ production and generation of cytotoxic T cells) may require immunization strategies that are quite different from those aimed at developing the highest titers of neutralizing antibody.

Although it may be possible to temporize and to develop immune responses in which both cellular immune responses and high-titer neutralizing antibody are achieved, generally the same immunization conditions do not result in optimization of both types of responses (*9*). Clearly, we would be best served by understanding what type of immunity would more likely provide protection. Here we need results from animal models or actual clinical trials. Preliminary highly focused macaque studies are a high priority, even if the data may not fully extrapolate from SIV to HIV and from macaques to humans. The development of an appropriate small animal model could provide an even more powerful tool to achieve these goals. The latter must remain a high priority, despite the need to overcome the inability of HIV to naturally infect non-primate cells, even if the cells express human CD4 (*10*).

Protective immunity—does it occur and under what circumstances? Despite the importance of animal models, we must ultimately draw our lessons from the natural history of the disease and from the perturbations that occur in individuals treated with potent anti-retroviral agents. Provocative inferences have been drawn from the study of infected individuals and individuals at high risk of infection. It has been suggested that it is possible to have an encounter with SIV or HIV, to develop protective immunity, and to control the virus. The best documented example of protective immunity is provided by the observation that an SIV defective in the gene *nef* both fails to induce disease in macaques (although it does infect them) and induces a state of resistance to infection with virulent SIV (*11*). The nature of this immunity is still not fully understood but its analysis is of utmost importance.

Clinical observations have been offered in support of the view that individuals exposed to HIV may develop an immune response capable of fully eliminating the virus. Among a group of HIV-exposed health care workers who have remained seronegative, some possess T cells that secrete interleukin-2 (IL-2) upon stimulation with HIV envelope peptides (*12*). HIV cannot be detected in these individuals. Since cytokine-producing T cells recognize viral peptides that have been loaded, intracellularly, into newly synthesized class I or class II major histocompatability complex molecules, virus-infected cells are very likely to have induced this state of immunity. There is a group of commercial sex workers in Nairobi who have remained uninfected despite repetitive, long-term exposure to the virus. These women may have acquired a similar state of protective immunity (*13*).

Results from the analysis of cohorts of

individuals who have been infected for 10 years or more but have sustained normal numbers of $CD4^+$ T cells suggest that immune responses may control the virus even in instances in which it is not actually eliminated (*14, 15*). The disruption of lymphoid tissue that characterizes progressive HIV infection does not occur in most long-term nonprogressors (*14*). Some of these long-term nonprogressors may have been infected with a virus of low virulence. However, virus can be recovered from lymphocytes of other long-term nonprogressors that is competent to infect activated peripheral blood T cells. In many cases, $CD8^+$ T cells drawn from long-term nonprogressors exert an inhibitory effect on the ability of HIV to infect $CD4^+$ T cells in vitro (*15*). This $CD8^+$ T cell–mediated inhibition may be due to destruction of virally infected cells or inhibition of viral replication (*16*). These long-term nonprogressors appear to have successfully contained the virus; understanding the nature of their immune response may provide critical insights into how HIV can be controlled in other infected individuals.

Is the cytokine environment critical in determining protective value of immune responses? The striking disparities in the course of infection in different individuals suggest that fundamental differences in the response to HIV occur. Studies of responses of different inbred strains of mice to infection with the protozoan parasite *L. major* have led some to suggest a possible basis for such differences. As already noted, immune responses to *L. major* (dominated by IFN-γ production by $CD4^+$ T cells) result in clearance of the parasite infection (8). BALB/c mice, which succumb to infection with *L. major*, display a vigorous $CD4^+$ T cell response dominated not by production of IFN-γ but rather by the production of IL-4. If BALB/c mice are prevented from producing an IL-4–dominated response, they develop leishmania-specific T cells that produce IFN-γ, develop a protective immune response, and survive (8).

Does the difference between most infected individuals and the small cohort of long-term nonprogressors depend upon the cytokines that dominate their responses? That is, do long-term nonprogressors develop an IFN-γ–dominated response whereas most other individuals develop a response in which IL-4 dominates? This subject has been a matter of considerable controversy, with evidence being offered on both sides (*17–20*). A major obstacle in deciding whether the quality of the immune response determines the outcome of infection is that it has been difficult to measure T cell cytokine production in response to HIV antigens. There are particularly few examples of such measurements done early in responses, when it would be most important to evaluate them. A high priority will be to examine in detail the nature of the immune response in the period immediately after HIV infection.

Efforts to alter the balance of IFN-γ and IL-4 produced in the course of infections with various pathogens in experimental animals or in HIV-infected humans have not yet yielded convincing results. The bulk of evidence drawn from in vitro studies and from studies of animal models is that, once determined, the cytokine-production pattern of primed T cells does not change (9). Thus, a major perturbation in the dynamics of lymphocyte production in infected individuals, leading to the development of populations of naïve, antigen-specific T cells available for priming, may be required to alter the established pattern of cytokines in an infected individual. Recent studies suggesting that viral burden can be strikingly lowered by HIV protease inhibitors (*21*) may provide an opportunity to fundamentally alter the balance of cytokines produced in response to the infection. This might be achieved either through natural replacement of T cells from the thymus, or through the intentional introduction of naive T cells.

The central engima: What accounts for the immunodeficiency in AIDS? What accounts for the immunosuppression and the loss of $CD4^+$ T cells in HIV infection? Some scientists have taken the view that the relatively few $CD4^+$ T cells that appear to harbor HIV proviral DNA or RNA cannot account for the deficiency in the overall numbers of $CD4^+$ T cells nor for their apparent deficient immunologic function. It is now apparent that more cells are infected than had initially been estimated based on examination of $CD4^+$ cells in blood (*22*). Furthermore, recent progress in clarifying the means through which tissue damage may occur in many viral infections and in understanding the regulation of T cell function provides ample means through which noninfected lymphoid cells might be destroyed or through which their function might be impaired.

It is clear that engagement of T cell receptors can lead to cellular inactivation rather than activation. In general, such inactivation results if receptor engagement occurs in the absence of a costimulatory signal, often provided by the interaction of cell surface ligands (expressed by antigen-presenting cells) with complementary receptors on T cells (*23*). One such ligand receptor pair involves a member of the B7 family, expressed on antigen-presenting cells, and CD28 on T cells (*24*). In the absence of a B7-CD28 interaction (or a comparable interaction), engagement of the T cell receptor may lead to long-lived T cell "anergy," the incapacity of the T cell to respond even to competent stimuli. In addition, there is growing evidence that receptor-mediated T cell activation can result in the overexpression of *fas* on T cells and the subsequent death of these cells through apoptosis when they encounter the *fas* ligand (*25*). Other forms of apoptotic death may also occur. Evidence has been obtained that the interaction of HIV-infected cells with activated T cells may result in apoptotic death (*26*).

We still do not fully understand the relative contributions of these (or other) processes to the actual immunodeficiency of HIV disease. If the direct cytopathic effect of the virus is responsible for only a portion of cellular death or inactivation, the control of the dominant effectors may relieve the immunodeficiency and thus have a major impact on the clinical course of the disease.

New classes of drugs give hope for effective combination chemotherapy. Drug discovery provides additional opportunities that must be exploited. HIV protease inhibitors, now entering efficacy trials, may provide us with a second class of effective antiretroviral agents. This could be very good news for patients who have become resistant to reverse transcriptase inhibitors, but it may be particularly valuable in the primary treatment of infected individuals in combination with reverse transcriptase inhibitors. If the cancer model holds a lesson for HIV, it is that combinations based on independent types of drugs may further suppress virus and, by lowering viral load and viral replication, may diminish the likelihood that resistant organisms will take over the population. The effort to develop additional independent classes of drugs, such as integrase inhibitors, must be vigorously pursued.

An essential aspect of drug discovery efforts is an understanding of the functions of the viral gene products in the economy of the cell. We still have an incomplete knowledge of the function of some of these gene products. A good example is Nef. Although Nef has been extensively studied and has been used as a major target in the preparation of an attenuated SIV for vaccine purposes, its precise function is not known. Nef-deficient HIVs and SIVs generally replicate at a very reduced rate compared to wild-type virus. Nonetheless, the recent observation by Ruprecht and her colleagues (*27*) that an attenuated SIV, in which *nef*, *vpr*, and *nre* were deleted, was capable of inducing disease when it was administered orally to neonatal macaques strongly implies that we do not fully understand how Nef mediates its functions.

Thus, the effort to develop inhibitors of particular targets must be based on a sophisticated understanding of the roles of these gene products in the viral life cycle and in viral pathogenesis. Linked to this

should be the development of assays that reflect biologically important functions of these targets so that inhibitors that are developed will have a reasonable likelihood of being effective as anti-retroviral drugs. Information on the detailed molecular structure of the target can, potentially, be of major help in the development of inhibitors. The recent description of the three-dimensional structure of the catalytic domain of HIV integrase (*28*) gives hope that progress toward developing potent inhibitors of this important enzyme will soon be forthcoming.

Behavioral research is an essential component of building the knowledge base on AIDS. Since we understand how HIV is transmitted, it should be possible for individuals to protect themselves against infection by avoiding behaviors that place them at risk. Educational programs aimed at informing at-risk populations of strategies through which they can diminish their risk of infection have been initiated in many areas and have often achieved considerable success. Nonetheless, even among the young, gay, male population in San Francisco, a population that has been provided with some of the most effective prevention programs, the prevalence of the disease continues to rise, although much less dramatically than in the past (*29*).

This sobering fact calls for efforts to develop more effective strategies to aid individuals and population groups to modify behaviors that place them at risk. Such efforts must include the use of already recognized strategies for behavior modification at the individual level and in the context of a group. However, it must also involve a substantial investment in fundamental behavioral research, with an emphasis on gaining insight into motivational aspects of the major behaviors that carry increased risk—unprotected sex and intravenous drug use. These are daunting challenges that will certainly require a deepening in our understanding of fundamental aspects of human behavior.

Prevention of STDs and female barrier methods can diminish HIV transmission. There is clear evidence that in populations in which there is a high incidence of sexually transmitted diseases (STDs), individuals are at greater risk of acquiring HIV infection. The OAR recognizes that a vigorous program aimed at developing better techniques to prevent and treat STDs is a critical component in programs aimed at preventing HIV infection. In addition, we are committed to supporting the development of better barrier methods for women to allow them to protect themselves against infection.

Fundamental Research as Source of Hope

This brief summary is by no means intended to be an exhaustive discussion of areas of promise and excitement in the fields of AIDS research. It reflects my scientific background, being that of an immunologist with a major interest in cytokines. Nonetheless, the critical importance of immune responses both before and after infection indicates to me that concerted efforts to understand how the immune system can be mobilized to control HIV is of the highest priority.

I emphasize that great opportunities for gaining the knowledge we need lie before us and that such knowledge should provide the basis for effective treatments and preventives. Far from being a time of pessimism, this should be an era of hope. But hope is only useful if it leads us to those actions that will translate it into reality. To accomplish this, the OAR has launched a reexamination of the entire NIH AIDS research endeavor. This evaluation, by a panel of eminent scientists led by Dr. Arnold Levine of Princeton University, will form the basis of recommendations for the future AIDS research agenda.

AIDS is a new disease for much of the world. While it would be far from prudent to be overly optimistic, if we can learn to master the disease, it is conceivable that we could permanently purge HIV infection from human populations. While the final elimination of AIDS is an ambitious goal, it is to this end that our efforts must be devoted.

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