macrophage-tropic non-SI HIV to the LESTR receptor used by T cell line adapted strains.

To speculate one step further, I postulate that the "antigenic" diversity of the HIV envelope is driven primarily by adaptation to new coreceptors and only secondarily by immune selection. The coreceptors delineated thus far (*2*, *3*) belong to the 7tm family. Thousands of these receptor genes exist in the human genome; for example, the nasal epithelium alone expresses several hundred such receptors, which serve as chemoreceptors in the discrimination of odor (*21*). Receptors for β-adrenergic neuropeptide transmitters, some cytokines (interleukin-8), and chemokines triggering chemotaxis and allergic responses also belong to the 7tm family (*22*). Some members of the 7tm receptor family are restricted to strictly defined cell subsets, such as basophils or macrophages (*23*). It is becoming evident from the subtle changes in HIV tropism that LESTR (*2*) and CC CKR5 (*3*) are not the only coreceptors exploited by HIV. HIV-2 can infect a broader range of CD4-positive cells than HIV-1 can (*24*) and may use a number of different coreceptors. Some HIV-1 strains can adapt to novel cell types in culture (*16*, *17*). During the long period of infection in each person, the HIV "quasispecies" has the opportunity to colonize new cellular environments, possibly by adaptation to an array of 7tm receptors, thus gradually invading different subsets and clones of hematopoietic cells as well as the brain.

The discovery of this new class of HIV receptor is opening up a field in which many questions can now be addressed at the molecular level. Do the V1/V2, V3, and other domains of gp120 interact directly or indirectly with 7tm receptors? Which epitopes on the receptors are crucial for gp120 recognition? Do the conformational changes that follow binding to CD4 expose 7tm-interacting domains? Does gp120 interaction with 7tm receptors affect their downstream signaling? Does the limitation on the number of infectible T cells at any one time (*25*) reflect receptor expression as a marker of immune activation? Could variation between individuals' CD4 lymphocytes in susceptibility to HIV infection (*26*) be due to blocking by β-chemokines (*3*) or to genetic polymorphism of the coreceptor? Will small animals transgenic for both human CD4 and human coreceptors be useful for AIDS research (*2*) (but if my hypothesis is correct, such animals may not generate such a high degree of HIV variation)? Will ways of blocking HIV and coreceptor interaction be found that will lead to new therapeutic approaches to control of HIV infection?

Many years ago, Sir David Smithers wrote, "Cancer is no more a disease of cells than a traffic jam is a disease of cars. A lifetime of study of the internal combustion engine would not help anyone to understand our traffic problems" (*27*). Yet it was just those studies of internal workings of the cell that revealed the dysregulation of oncogenes, tumor suppressor genes, signal transduction pathways, cyclins, DNA repair, and apoptosis. In AIDS as in cancer, cell biology illuminates the population dynamics of pathogenesis.

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HIV Therapeutics

Douglas D. Richman

After years of small but measurable advances that temporarily delayed disease progression, the chemotherapy of human immunodeficiency virus (HIV) infection now promises to suppress indefinitely the progression of disease, and conceivably to eradicate it altogether. Three factors have converged to provide important advances in antiretroviral chemotherapy: (i) improved understanding of the pathogenesis of HIV infection, (ii) the availability of standardized reliable quantitative assays of HIV RNA in the plasma (present as genomic RNA in virions), and (iii) more potent antiretroviral drugs. Each of these three factors has affected the others.

Surprisingly large amounts of virus are present in the extensive lymphoid tissues of HIV-infected individuals, even in asymptomatic patients early in the disease process (*1*, *2*). The levels of virus in blood are less than in lymphoid tissues and almost certainly reflect the spillover from replication in that tissue. This large amount of virus turns over rapidly, as measured in blood, with a virion half-life of approximately 6 hours and an estimated 10 billion (10^{10}) virus particles generated daily (*3*). Within a year after seroconversion, each infected individual establishes his or her own "set point" of quasi–steady-state levels of plasma HIV RNA (between 10^2 and 10^6 copies per milliliter of plasma) that largely determines the rate at which CD4 T lymphocytes are lost. At sufficiently low levels of CD4 cells, patients are susceptible to opportunistic infections, malignancies, and death. Levels of HIV replication, measurable as plasma HIV RNA, thus drive the rate of immune destruction and in fact can be used to predict the natural history of the disease (*4*).

The measurement of plasma HIV RNA has been instrumental in elucidating this pathogenetic process, as well as in characterizing the magnitude and durability of antiviral activity of investigational drug regimens (*5*). Moreover, this reduction in measured viral "load" appears to account for most of the clinical benefit as measured by opportunistic events or death in study patients (*6*, *7*). With the regimens of nucleoside analogs, even a reduction of plasma HIV RNA by severalfold corresponds to

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the reduced rates of disease progression observed with the natural history studies.

Several large trials with clinical endpoints in both adults and children have documented that a number of regimens containing combinations of nucleoside reverse transcriptase inhibitors are superior to zidovudine (3′-azido-3′-deoxythymidine, AZT) monotherapy (8, 9). The controversy raised by older studies about early versus delayed AZT monotherapy is moot. These newer nucleoside regimens reduce plasma HIV RNA levels to approximately one-tenth their initial value and on average maintain these levels below those present at the initiation of treatment for at least 2 years. This reduction is sufficient to delay the rate of disease progression, but not to prevent it.

In a field with such rapid accrual of new information and of drug approval (at least in the United States), clinical practice must proceed with new drug regimens even when long-term effects are still uncertain. Large phase III clinical endpoint trials (as yet unpublished) have been completed with only three nucleoside reverse transcriptase inhibitors [AZT, zalcitabine (dideoxycytosine, ddC), and didanosine (dideoxyinosine, ddI)] and one protease inhibitor (ritonavir), whereas five nucleosides and three protease inhibitors have received regulatory approval in the United States. An international panel recently generated consensus recommendations to provide guidance for practitioners while investigation proceeds (*10*).

Several inhibitors of a second viral enzyme, the aspartyl protease, have provided evidence of even more potent activity, with average reductions of plasma HIV RNA to between one-thirtieth and one-hundredth their initial value; in many patients even greater reductions have been seen. Suboptimal doses of these potent drugs result in loss of suppression after several months, which is associated with the cumulative acquisition of multiple mutations in the protease gene that confer high-level drug resistance (*11*). Patients with sustained suppression do not develop resistance, presumably because some level of replication must be maintained for generation of drug-resistant mutants that can emerge in the presence of the selective pressure of drug treatment (*12*).

HIV, and most single-stranded RNA viruses, undergo approximately 3×10^{-5} mutations per nucleotide per replication cycle (*13*). What drives the appearance of genetic variation of HIV within patients is the persistent, high levels of virus replication (*14*). With the production of perhaps 10 billion virions daily and a genome size of 10^4 nucleotides, virtually all possible mutations, and perhaps many combinations of mutations, are generated in each patient daily. Moreover, recombination that rapidly selects for combinations of resistance mutations can occur rapidly with the diploid genome of HIV (*15*). Although most mutations compromise virus replication to confer a selective disadvantage, the range of possible genetic variants confers adaptive advantages in the face of varying host cells, immune responses, and drug treatments. The outgrowth of resistant variants can be prevented only with potent chemotherapeutic regimens effective against a broad range of potential mutations.

The combination of two nucleoside analogs, AZT and lamivudine (3TC), and a potent protease inhibitor (indinavir) has reduced virus levels in blood from between 20,000 and 1,000,000 RNA copies per milliliter of plasma to below the levels of detection as measured by polymerase chain reaction (PCR) and by culture. This reduction has lasted for periods up to 1 year in at least 90% of treated patients (*16*). (HIV can be cultured and plasma HIV RNA can be detected in the blood from all but a few percent of untreated, infected patients. The threshold of detection of plasma HIV RNA with current assays is 200 to 400 copies per milliliter.) Encouraging results have been seen with a number of combination regimens containing various nucleoside and protease inhibitors. The levels of virus in the blood, as measured by virus culture and PCR for HIV RNA, are below those seen in the individuals (termed long-term nonprogressors) who have remained clinically stable without treatment for over a decade after being infected with HIV (*17*, *18*).

The clearance of any evidence of virus replication raises a number of questions that previously could not be considered or at least experimentally addressed: (i) Does the magnitude of reduction in the circulation reflect that in the lymphoid tissue? (ii) Given the greater concentrations of HIV in lymphoid tissue, if no detectable virus is in the circulation and virus replication in these two compartments is cleared in parallel, then how much additional reduction will be necessary to suppress all replication in the lymphoid tissue? (iii) If replication can be completely suppressed, will infection be eradicated? There are several corollaries to this last question: (i) Is there a pharmacologic sanctuary that requires special consideration, such as the central nervous system? (ii) Is there a long-lived, latently infected cell population (quiescent lymphocytes and stem cells) that may reactivate infection upon withdrawal of suppressive chemotherapy?

If eradication is possible, then aggressive early treatment becomes a relatively easy decision. This prospect raises the issue of the susceptibility of such cured patients to reinfection, with great implications for vaccine development. If eradication cannot be achieved, the proper implementation of chronic suppression must consider several competing factors. The current practice of adding one additional drug to the treatment regimen of individuals as they progressively deteriorate is ideally suited to select for resistant virus and preclude the benefits of potent combination therapy. The premature use of such potent regimens involves a financial commitment, risk of toxicity, and patient inconvenience. Treatment can be considered premature only if the pathologic process is sufficiently reversible to justify delay. Arguments for earlier use of potent regimens are that chemotherapeutic success with many diseases occurs in a greater proportion of patients and can often be less aggressive when the disease is less extensive. Moreover, the immunopathology of HIV infection is progressive and not completely reversible. The progressive deletion of the immunologic repertoire, at least in adults (who normally have reduced thymic function), is a concern that has not been sufficiently characterized. The progressive fibrosis and loss of normal nodal histology has been well characterized (*2*). The convenience of chronic suppression will be enhanced if a simple maintenance regimen to sustain chronic suppression can be identified.

In less than a year, the prospects for treatment of HIV-infected patients, at least those socioeconomically privileged, have improved dramatically. Important new questions about HIV pathogenesis can now be asked and investigated. Nevertheless, the prospects of drug resistance, the toxicities of current drugs, and the need for even greater antiretroviral activity will require the discovery and development of still more and better drugs.

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A Perspective on AIDS Vaccines

Barry R. Bloom

At the 11th International Conference on AIDS, a formal debate is scheduled on the question of whether "more fundamental research on vaccine development is required prior to the implementation of phase III trials of certain HIV vaccines." After more than a decade of research without a single HIV vaccine deemed worthy of large-scale efficacy trials, it may be useful to reconsider some general questions about AIDS vaccines that are commonly, or uncommonly, asked (*1–3*).

Is a vaccine against AIDS really needed? This question is appropriate in light of the dramatic reductions in viral burden and increased survival recently achieved with multiple drug therapy (and the probability of new therapeutics). Clearly, childhood vaccines are among the most cost-effective medical interventions to prevent death and disease (*4*). Assuming the best cases for therapeutic efficacy and lack of drug resistance, the inhibitors of reverse transcriptase and protease will remain chemically complex and enormously expensive (*5*). The 90% of people infected with HIV who live in the developing world, and many in industrialized countries, will not have access to them. The best long-term hope for preventing AIDS in the United States and globally must be an effective vaccine.

Why is it so difficult to develop AIDS vaccines? As part of the National Institutes of Health (NIH) Office of AIDS Research (OAR) review of NIH AIDS programs, I met with graduate students and postdocs to discuss barriers in attracting the brightest young scientists into AIDS research. It was my impression that research on AIDS was considered more applied than basic, and that research on vaccines was not thought to be particularly intellectually challenging. My own perception is that AIDS vaccines represent the most formidable vaccine challenge of any infectious disease. In most natural viral infections, illness occurs, an immune response develops, and if the illness is not acutely fatal, recovery ensues. In AIDS, most patients develop antibodies and even killer T cells against the virus, yet they fail to clear the virus and inexorably succumb to AIDS. The challenge is how to achieve something that nature has not succeeded in doing. The hurdles are daunting: (i) HIV infection places the immune system in double jeopardy, from the virus and from immune attack. (ii) There are multiple genetic types or clades of the virus, and with the high mutation rates of RNA viruses, there are even more antigenic subtypes and variants against which to engender protection. (iii) A vaccine may have to protect not only against transmission of free virus but also against virus-infected cells that can transmit infection, an immunological task comparable to selective rejection of tumor cells. (iv) Animal models of HIV that are faithful to the human disease are lacking, and experiments with different models often yield conflicting findings. (v) The possibility of generating inappropriate immune responses that might enhance infection or cause pathology cannot be ignored. (vi) Any population in which a vaccine might be tested for efficacy must ethically be provided with the best counseling on preventing transmission and reducing high-risk behavior, and such counseling is likely to compromise the statistical power of any trial.

Is there hope for an effective vaccine? Long-term survivors of HIV infection who have controlled the virus for more than a decade (*6*) and high-exposure, uninfected sex workers who show no detectable virus or disease but have HIV-specific immune responses (*7*) have been identified. Encouraging data indicate that individuals infected with HIV-2 have some cross-protection against HIV-1 infection (*8*). A small number of children infected at birth with HIV appear to clear their virus (*9*). Understanding the mechanisms that contribute to the well-being of these individuals could provide valuable insights relevant to vaccines. Although the diseases may differ, effective vaccines against the feline leukemia virus, a related retrovirus, have been available for years (*10*). Finally, long-term protection has been achieved in macaques with live, genetically attenuated simian immunodeficiency virus (SIV) strains (*11*).

How good must an AIDS vaccine be? We commonly think of vaccines as preventing infection and producing "sterilizing" immunity. In fact, relatively few vaccines prevent infection of host cells. Rather, they confer protection by reducing the initial burden of pathogen, by accelerating clearance of the infection, or by preventing recurrence, sequelae, and transmission. In the context of a fatal disease that is devastating a major proportion of young people in many countries, even a partially protective AIDS vaccine—20 to 40% as effective as measles or polio vaccines—would save and extend millions of lives and would reduce secondary transmission to offspring and contacts. Public health use of a vaccine will be decided not only by its efficacy, but also by the magnitude and urgency of the problem in different countries.

What do we have to know to develop an effective vaccine? Historically, successful vaccines were created with little understanding of the molecular basis of pathogenicity. Pickled proteins, viruses and bacteria, or spontaneously arising attenuated strains worked in the past. Empirical trial and error is indeed crucial in the development of vaccines but can no longer be the paradigm. For AIDS vaccines, there is a clear need to better understand the mechanisms of pathogenesis and protection.

In a rational world, to design an effective vaccine one would like to know: (i) What are the necessary and sufficient immune responses required for protection? (ii) What are the antigens or immune targets to which they are directed? (iii) What is the best way to deliver the appropriate antigens to engender the protective immune responses? In the real world, that's not how it usually works. More likely, an antigen is identified, then patented and licensed, and the developer tries to show that it will produce some immune responses in some animal models. If any responses are seen in animals and then in a limited number of humans, the plea is inevitably made that the antigen might protect against the disease if only the federal government would run a large efficacy trial.

I would argue that two issues have been confounded: questions of fundamental knowledge and questions of vaccine efficacy. The question of what immune responses are essential for protection against HIV is fundamental. It is similar to, but distinct from, the practically important question of what the "correlates" of protection are—that is, what tests can be measured in a test tube that correlate with protection. Responses may correlate but may not be necessary for protection. Experiments that can

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