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Progress toward an AIDS vaccine: Prospects for protective immunity

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Abstract

Much can be asked of an AIDS vaccine. Ideally, vaccination would pre-empt the initial viremia associated with HIV infection, preventing illness, transmission, and the establishment of long-term viral reservoirs. If this goal of "sterilizing immunity" is not attainable, the vaccine-induced immune response would at least mitigate the effects of HIV infection by limiting viral replication. An optimal AIDS vaccine would induce long-lasting protection not only against the dominant viral subtype (clade) in a particular geographic region but against the nine or more clades of HIV-1 distributed around the world, as well as interclade recombinant strains, and would be effective regardless of the HLA genotype of the recipient. A great deal of attention and effort is also being devoted to the important work of building an international infrastructure to support the testing and ultimate availability and distribution of an AIDS vaccine. But the scientific questions remain: Can any AIDS vaccine currently in development be expected to achieve what is being asked? And how are we to decide which of the growing number of vaccine candidates should receive priority for evaluation in human trials? This article reviews key studies published over the past year, as well as research efforts presented at the international research conference "AIDS Vaccines 2001," held from 5 to 8 September in Philadelphia, that represent progress toward answering these questions.

Introduction: HIV-specific responses and protection

The immunologic correlates of protection against HIV in humans have yet to be characterized. Because the virus integrates into the host genome and persists for the lifetime of the infected cell and its progeny, it would appear that a humoral immune response directed against the viral envelope would provide the best chance for sterilizing immunity by preventing initial cellular infection. However, despite evidence of protection in a chimpanzee model (*1*), the development of envelope subunit vaccines has fallen on controversy with the recognition that these vaccines fail to elicit neutralizing antibody responses to primary HIV isolates (as opposed to tissue-culture laboratory adapted strains) in humans (*2*). Preliminary results are expected within the next year from two phase III efficacy studies of envelope subunits, one based in the United States and one in Thailand (*3*). Basic researchers continue to pursue the development of envelope-based constructs that might elicit broadly neutralizing antibodies (such as oligomeric structures or variable-loop deletions). However, the majority of human vaccine trials currently in development are based on evidence that cellular immunity may play a protective role even in the absence of neutralizing antibodies.

New evidence from monkeys

HIV-specific cellular responses

Several studies in nonhuman primates published or presented in the past year support the

correlation between vaccine-induced, HIV-specific cellular immunity and protection against subsequent challenge with pathogenic SHIV (SIV core/HIV-1 envelope) recombinants. For example, in macaques immunized with a replication-incompetent adenovirus vector expressing SIV Gag, protection against SHIV challenge correlated with prechallenge cytotoxic T lymphocyte (CTL) response to an immunodominant CTL epitope (*4*). In macaques immunized with *gag/env* DNA augmented by a plasmid expressing an IL-2/Ig hybrid cytokine, HIV-specific CTL responses correlated with viral load set point following challenge; in some animals, clinical progression was delayed more than 1.5 years following challenge (*5*, *6*). Macaque trials of a regimen consisting of *gag/pol/env* DNA priming followed by boosting with a recombinant modified vaccinia Ankara vector expressing Gag-Pol-Env found similarly that vaccine-induced cellular responses correlated with control of viral load following challenge (*7*, *8*). With regard to route of administration, intrarectal immunization of macaques with HIV/SIV peptides resulted in the induction of SIV-specific CTL and lymphoproliferative responses in the gut and was associated with control of viral load, both in the blood and in gut mucosal reservoirs, following intrarectal challenge (*9*, *10*).

Mucosal Immunity

Because the vast majority of HIV infections occur via mucosal routes, mucosal immunity may be of particular importance in screening vaccine candidates for probable efficacy. In addition to the intrarectal peptide vaccine described above (*9*, *10*), induction of protective immunity against mucosal challenge with SIV or SHIV has recently been reported in several macaque models, including an intranasal recombinant poliovirus vector encoding SIV proteins followed by intravaginal challenge (*11*), and subcutaneously administered Venezuelan equine encephalitis replicons containing SIV genes followed by intrarectal challenge (*12*).

Recent histologic studies in macaques show that viral replication remains confined to the endocervical mucosa and submucosa for approximately 7 days after vaginal inoculation with SIV, with dissemination to local and distant lymph nodes subsequently occurring by day 12 (*13*). These data raise the intriguing possibility that sterilizing immunity might be achieved even without neutralizing antibodies, if infection remains localized long enough to permit the vaccinated (or immunologically fortunate) individual to mount an effective cellular response. Although these animal results cannot be assumed to hold true for humans exposed to HIV, they are consistent with studies (discussed below) showing mucosal immune responses in exposed, uninfected individuals. Whether such individuals have contained rather than eradicated infection is unclear. Although it seems unlikely that an anemnestic effector response could arise without at least one round of cellular infection (and the attendant risk of establishing long-lived proviral reservoirs), CTL activation by dendritic cells presenting HIV antigens in the absence of viral replication was recently described in vitro (*14*).

Lasting vaccine-induced cellular immunity would require the activation of memory T cells upon subsequent exposure to HIV, and these animal studies suggest that such a response might indeed control subsequent viral activity. Nevertheless, the specifics of immunopathogenesis are expected to differ between SHIV-macaque models and actual HIV infection in humans. In the absence of a reliable animal model for HIV infection, the efficacy of a vaccine in humans can not be predicted solely on the basis of animal studies. However, results correlating retrovirus-specific cellular immune responses, particularly at the mucosal surface, with protection in monkeys are consistent with evidence for the existence of effective cellular responses to HIV-1 in humans.

HIV-specific cellular immunity in humans

HIV-specific cellular responses have been identified and partially characterized both in people who are HIV-exposed but persistently seronegative (HEPS) and in HIV-infected individuals who control viral replication in the absence of antiretroviral medications. Consistently undetectable viral activity in individuals from both groups suggests that protective cellular immune responses, while perhaps exceptional, do indeed occur and might provide a basis for vaccine design.

Lymphoproliferative or cytotoxic T cell responses to HIV-1 have been reported in a variety of HEPS cohorts, including a predominantly male group with high-risk sexual exposures (15), homosexual men (16), commercial sex workers (17), infants born to HIV-infected mothers (18, 19), and health care workers with occupational exposures (20, 21). A recent report also described a correlation between HIV-specific CD8 responses and protection from infection among injection drug users sharing needles (22). In addition, an enzyme-linked immunospot (ELISPOT) assay using autologous dendritic cells detected HIV-specific CD8 responses not previously observed by standard methods in a cohort of North American, heterosexually exposed, seronegative women (23). Finally, a study of HEPS heterosexual males found HIV-specific immunoglobulin A (IgA) in seminal fluid, as well as cellular responses in blood (24).

Perhaps the most extensive data on specific immune responses in HEPS individuals derive from the Pumwani sex worker cohort in Nairobi, Kenya (25). Of those cohort members remaining HIV-seronegative after more than 3 years of follow-up, nearly half were found to have HIV-specific CTL responses (17). In contrast with HIV-infected subjects, CD8+ response frequencies (by ELISPOT) in these HEPS women were higher in cervical mucosal specimens than in blood (26). Mucosal IgA capable of neutralizing HIV of several clades was also detected and appeared to occur independent of HIV-specific cellular responses (27). The relative protective activity of cellular versus antibody responses in mucosa remains unclear.

The prospect of cross-clade protection

Sequence analysis has found that CTL epitope-rich regions of the HIV-1 genome are likely to be conserved, suggesting that a single vaccine based on conserved sequences may be effective in a variety of geographic settings. In contrast, epitope-poor regions of the viral genome tend to be poorly conserved, suggesting that the virus can and does evolve to evade immune responses directed at peptides encoded by these regions (28). In the Nairobi sex workers cohort, CTL responses were detected using conserved peptides derived from North American clade B epitopes, even though exposure would have been primarily to African clades A, C, and D. However, CTL activity was found to be higher against homologous peptides derived from clade A or D sequences, even though these differed from clade B sequences by one or at most two amino acid residues. Moreover, the clade B epitopes chosen for evaluation were identified in the context of HLA molecules predominating in Caucasians, and the Kenyan women were selected for these studies based on the presence of at least one class I HLA molecule for which clade B epitopes had previously been defined (17). Whether additional epitopic regions not obvious by homology to clade B might prove protective in the context of HLA subtypes common in Africans, and whether such HLA subtypes might fail to present epitopes identified in Caucasians, are unresolved issues with major implications for vaccine design. Nevertheless, the existence of cross-clade responses in HEPS individuals lends support to the genetic sequence analyses favoring the prospect of a broadly protective vaccine.

That a vaccine can in fact produce cross-clade responses to conserved epitopes has been reported, although such responses vary from one person to another, depending in part on HLA allelic expression (29-31). Whether these responses correlate with protection remains unknown.

Which epitopes?

The identification of protective epitopes is problematic for several reasons. First, there is no experimental model for human protection. Second, even investigations in humans may not be generalizable because, as discussed above, the immunogenicity of any given epitope may vary according to the precise sequence of the virus and characteristics of the individual immune response, including, but probably not limited to, the HLA alleles present. Comparison of CTL epitopes recognized by infected or HEPS individuals controlling viral replication to those seen in infected individuals without effective immune control may have the potential to distinguish protective from nonprotective epitopes. It has been shown, for example, that immune responses

present and even dominant in chronic infection are not necessarily those present in the early response to HIV-1 infection (32). In the Nairobi sex workers cohort, the proportion of subjects with responses to certain CTL epitopes differed between HEPS subjects and seropositive controls matched for the relevant HLA class I allele, and seroconversion of previously HEPS women was associated with a change in CTL specificities (33).

Immune escape

Even if studies of this kind can elucidate which epitopes are likely to be protective, however, it is becoming clear that evolution of the virus during the course of infection can result in escape from the immune response, and that mutations that interfere with HLA binding of previously immunogenic epitopes play a role in this process (34, 35). It was recently shown that a single human passage can result in mutations in the HLA-binding anchor residues of an HLA-B27-restricted dominant Gag epitope (36). Following mother-to-child transmission of such CTL-escape mutants, infants were found to target a subdominant HLA-B27-binding epitope and showed impaired control of viral load. Further, these anchor mutations did not revert to wild-type when grown in the absence of immune selection.

Immunologic pressure, then, can select stable mutants of HIV-1 epitopes that, if transmitted to individuals with a common HLA allele, can continue to elude presentation and result in further impairment of immune control. Similarly, a single amino acid change in HLA class I molecules that alters their peptide-binding specificity has a substantial effect on the rate of progression to AIDS in infected individuals (37). In Boston, the high incidence among sexually transmitted cases of HIV of mutations in an immunodominant epitope presented by HLA-A3 (38) illustrates that immune escape can be expected to shape not only the course of HIV infection in the individual but the course of the epidemic in populations.

Duration of effectiveness of an HIV-specific immune response

In a cohort of 14 individuals receiving antiretroviral treatment early in the course of HIV infection and subsequently undergoing treatment interruption, several were able to control viral load for an extended period without further treatment and developed HIV-specific cellular responses of increased magnitude and breadth (39). Neutralizing antibodies to autologous virus also developed in some of these individuals following treatment interruption (40). At AIDS Vaccines 2001, immunological and viral genetic analyses were reported from a different group of three individuals identified prior to seroconversion and followed for more than 3 years. Two had narrow CTL responses to HIV (targeting eight or nine epitopes) and showed CTL escape within weeks and subsequently high viral loads, while the third showed CTL responses to 29 different epitopes, developed no detectable escape mutations, and maintained low viral loads (41). However, a presentation at the same conference (38) showed that two participants in the treatment-interruption cohort experienced viral recurrence following more than 12 months of suppression without medication. In one case, viral load rebounded despite the presence of CTL responses to 30 HIV-1 epitopes. Thus, breadth of CTL response, although desirable, may not be sufficient to control viral replication in every case.

The detection of enduring CTL responses in chronically exposed sex workers, but not following acute needle-stick injury or perinatal exposure, suggests that ongoing exposure may be required to maintain an effective immune response. Several of the HEPS Nairobi sex workers subsequently became infected, which has been attributed to waning of HIV-specific CTL responses, perhaps associated with a period of reduced exposure to HIV (42).

Therefore, whereas a vaccine that either completely suppresses or substantially reduces viral replication would be considered a success, either outcome is potentially uncertain in the long term. On one hand, initially suppressive vaccine-induced responses may wane over time in the absence of antigen. On the other, partial suppression permitting some level of viral replication

may select escape mutants. In either case, the duration of effectiveness of any vaccine found to be effective in the short term will have to be determined, and strategies for boosting or for providing continuous exposure to antigen may become paramount.

Conclusion: next steps

One possible approach to vaccine design, then, would be to focus on epitopes recognized in cases of apparent protective immunity, such as in HEPS individuals or long-term nonprogressors. To increase the chances of activity in diverse host and virus combinations, the epitopes should be conserved across clades and presented by multiple HLA types. To minimize the likelihood of immune escape, as many such epitopes as possible should be included. A form and route of administration that would elicit mucosal immune responses might permit early control of infection. At this time, however, few such epitopes have been characterized. Moreover, potentially important humoral immunity might be poorly induced by a vaccine limited to T cell epitopes.

The more immediately practical approach planned by the National Institutes of Health-sponsored HIV Vaccine Trials Network (HVTN) is to test a vaccine regimen known to elicit a high rate of HIV-specific responses in a trial large enough to detect correlations between these responses and protection (*43*). In such a design, even if the overall efficacy of the vaccine is not demonstrated, information on correlates of protection would be gained provided that an adequate proportion of the vaccine-induced responses actually turn out to be protective and would facilitate future selection of vaccine candidates. Vaccines similar to components of the regimen currently being considered by the HVTN (a combination of an attenuated canarypox vector containing multiple HIV-1 sequences, together with a recombinant envelope subunit boost) have been shown to induce cross-clade CTL responses (*29*, *30*). However, such responses appear to depend on HLA allelic expression (*31*) and were not found to contribute significantly to control of viral load following antiretroviral treatment interruption in a group of 14 patients treated within 4 months following acute HIV infection (*44*).

Whatever approach is undertaken in upcoming efficacy trials, the questions of immune escape and duration of protection will almost certainly remain: If protective responses are obtained, will they be maintained at levels capable of providing long-term protection? And if the vaccine elicits immune responses that control, but do not eliminate, infection, will the virus evolve to escape the immune response? The questions of diversity are also likely to persist: Will regional and even individual variation in HLA alleles and viral subspecies exclude many people from the benefit of any single vaccine?

The past year has brought to light some limitations of the immune responses studied so far, but also certain causes for optimism. We are asking a lot of an AIDS vaccine. We must continue to do so, for as long as no vaccine exists, AIDS will remain a global crisis of devastating magnitude.

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