Perspective

HIV Vaccine Research: Problems and Progress

At the Chicago International AIDS Society–USA course in April 2002, Richard A. Koup, MD, reviewed ongoing efforts to develop prophylactic anti-HIV vaccines. Among the approaches he discussed were vaccines that induce neutralizing antibodies to HIV and those that employ cytolytic T lymphocytes.

The urgent need for effective anti-HIV vaccines is most immediately felt when considering the scope of the HIV pandemic in such regions of the world as Africa, Southeast Asia, and China. Figure 1 shows HIV seroprevalence in South Africa from 1990 through 1999; in some areas of sub-Saharan Africa, HIV seroprevalence rates currently approach 50% (Schwartlander et al, *Science*, 2000). Figure 2 shows the projected population structure of Botswana in 2020 as it reflects the effects of AIDS deaths. Most HIV infections in the world occur in areas where antiretroviral therapy is unavailable or has only recently become available in some measure. In any case, antiretroviral therapy does not cure HIV infection. Population-based methods for preventing the spread of HIV infection are needed everywhere in the world.

Objectives in Vaccine Development

Based on the current understanding of HIV infection and immune response to infection, there are a number of potential immune correlates to be investigated in vaccine development (Letvin, *J Clin Invest*, 2002; Letvin et al, *Annu Rev Immunol*, 2002; Mascola and Nabel, *Curr Opin Immunol*, 2001). Vaccines may be used to stimulate antibodies that could bind virus and to neutralize or stop virus from infecting target cells, thereby eliminating free virus before cellular infection is established. Vaccines could also be employed to increase cytolytic T-lymphocyte (CTL) responses targeting virus-infected cells or to stimulate antiviral factors elaborated by these cells. In the case of HIV infection, the ability to elicit or augment immune responses at locations in addition to the circulation may be of importance; since HIV enters the body at mucosal surfaces and replicates in lymphoid tissue, induction of immune response at these sites may be necessary as preventive approaches.

There are different levels or types of protection at which vaccine development can be aimed. In sterilizing immunity, infection is never established, and there is no seroconversion to nonvaccine antigens, no detectable HIV in the host at any time, and no risk of transmission of HIV to others. Although this is a desired goal, no vaccine against any human pathogen has ever stimulated sterilizing immunity. A successful vaccine might permit transient infection, in which there is transient detection of HIV at mucosal sites or in the blood but no detectable virus at later time points (eg, 6-12 months) with maintenance of immune response. HIV seroconversion might or might not occur, and risk of transmission of infection might be time-limited or completely prevented. Vaccines might also result in long-term controlled infection, in which virus is undetectable or at very low levels throughout life and in which there is no harmful drop in CD4+ cell count and no immunodeficiency disease. Seroconversion in this case is likely, and risk of transmission might be prevented or greatly reduced. Another potential aim of vaccine development, albeit a relatively undesirable one, might be an "altruistic" vaccine. In this case, the vaccine might provide no protection from infection or disease in those vaccinated, but would reduce viral load in mucosal secretions such that risk of transmission would be reduced or eliminated.

Is Vaccine-Induced Immunity Against HIV Possible?

There are a number of reasons to be optimistic about the potential for inducing immunity to HIV. HIV transmission is relatively inefficient. The primary mode of transmission worldwide is sexual, and transmission usually occurs only after

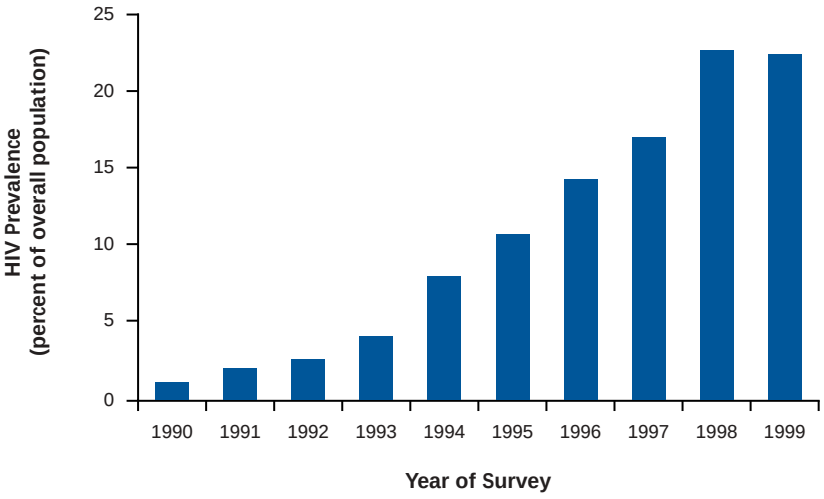

Figure **1.** HIV seroprevalence in South Africa, 1990-1999. Adapted with permission from Schwartlander et al, *Science,* 2000.

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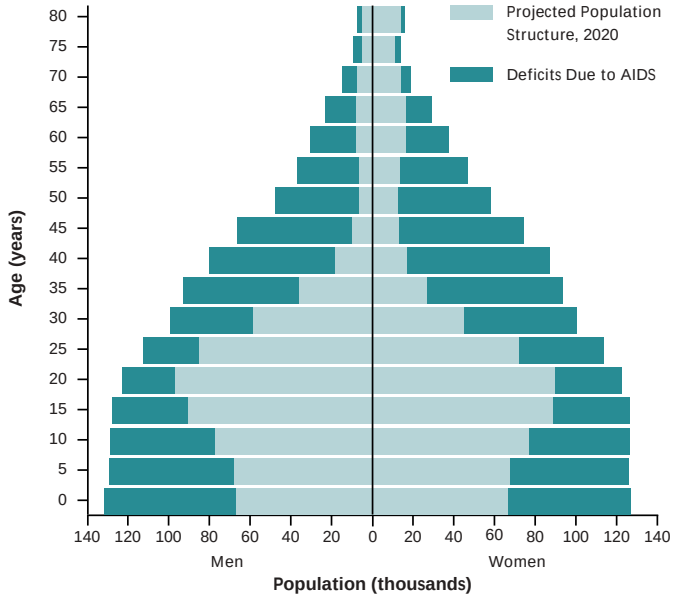

Figure **2.** Projected population structure of Botswana in 2020 as affected by AIDS deaths. HIV seroprevalence rates were 20% to 25% in 1998 and 1999. Adapted with permission from Schwartlander et al, *Science,* 2000.

multiple exposures (Downs and De Vincenzi, *J Acquir Immune Defic Syndr Hum Retrovirol,* 1996; Mastro and Kitayaporn, *AIDS Res Hum Retroviruses,* 1998). It is known that only a small number of virions establish infection during exposure (Zhu et al, *Science,* 1993); the likelihood that inoculum size is a factor in transmission suggests that reduction of the inoculum through vaccination might prevent infection. Transmitted viruses may have limited structural and genotypic features, reducing the genetic variability that would need to be covered by an effective vaccine. In addition to these factors, there are examples of natural immunity to infection that suggest that prevention is possible, including highly exposed individuals who remain uninfected and individuals with long-term nonprogressive infection (Cao et al, *N Engl J Med,* 1995; Rowland-Jones et al, *Nat Med,* 1995). Individuals with HIV-2 infection do not exhibit rapidly progressive disease, and they may have some degree of protection against HIV-1 infection (Travers et al, *Science,* 1995). Finally, there is evidence of vaccine-induced protection in animal models—eg, the simian immunodeficiency virus (SIV) macaque model (Amara et al, *Science,* 2001; Barouch et al, *Science,* 2000).

Although there is reason for optimism regarding the ability to induce immunity, there are also substantial biological challenges facing vaccine development (Letvin et al, *Annu Rev Immunol,* 2002). HIV infection is characterized by early establishment of cellular integration and latency; once infection occurs, latency is very likely and elimination of the virus is improbable. The virus preferentially infects and depletes key immune mediators (Douek et al, *Nature,* 2002), including CD4+ T-helper cells and antigen-presenting cells. HIV has other immune evasion strategies: HIV Nef downregulates major histocompatibility complex (MHC) class I expression, potentially interfering with immune recognition of HIV-infected cells; the immune response to infection includes failure to mount a good neutralizing antibody response; and a wide genetic variation in the virus population in established infection enables the virus to escape host immune responses.

Inducing Neutralizing Antibody Response

It is difficult to induce broadly reactive neutralizing antibodies to HIV by immunization. The HIV envelope glycoprotein features loop domains with high variability, which permit the virus to evade antibody recognition. The envelope is also heavily glycosylated, with the glycosylation moieties shielding the regions of the envelope protein gp120 that are targeted by neutralizing antibodies. The conformational changes in gp120 during CD4 binding reveal the viral binding site for the cellular CCR5 coreceptor; although this domain of gp120 is a potential antibody target, access may be blocked by steric hindrance. The gp120 is also a flexible protein, which makes it a more difficult target for antibodies than a rigid protein would be. The conformational change that occurs in the envelope glycoprotein gp41 during binding reveals additional epitopes that can serve as antibody targets; monoclonal antibodies targeting these regions of gp41 are being developed in the attempt to prevent fusion of virus with target cell membrane. HIV exhibits rapid escape from neutralizing antibodies, and continual escape from these antibodies is observed in individuals with chronic HIV infection.

There are reasons to be optimistic about inducing immunity to HIV, but there are also substantial biological challenges in vaccine development

Studies with vaccines to induce neutralizing antibodies generally have shown that the antibody produced in animals or human subjects exhibits high neutralization titers against laboratory (T-cell-line-adapted) HIV but poor titers against primary HIV isolates from infected individuals (Mascola et al, *J Infect Dis,* 1996). For example, investigation in human subjects of the recombinant ALVAC vaccine, a vaccinia-like vaccine

that expresses gp120, showed that all of the neutralizing antibodies generated in response to vaccination were type-specific and that response was of relatively short duration (Belshe et al, AIDS, 1998). The neutralizing titers were 5 to 10 times lower than sera from individuals with long-term nonprogressive HIV infection, and the antibody did not neutralize primary HIV isolates.

A number of strategies for improving neutralizing antibody responses are being pursued. These include attempts to improve protein expression in vaccines, unmask the neutralizing antibody epitopes on gp120 by removing the glycosylation sites that shield the neutralizing domain or removing the variable loop domains of the glycoprotein, express native trimeric forms of gp41 and gp120, express a rigid (neutralization-sensitive) form of gp120, and express a fusion-competent form of the glycoprotein.

CTL-Based Vaccines

Among the advantages of a CTL-based vaccine approach is the fact that CTLs recognize HIV-infected cells. Although antibodies would be expected to have their primary effect on free virus, they may also be used to target productively infected cells and promote elimination of these cells through activity of complement or targeting by other natural killer cells. If latently infected cells express some viral protein, it is also possible that this approach could have an effect on the latent HIV reservoir. Overall, targeting of HIV using a CTL-based approach may be more efficient than with antibodies, since CTLs recognize multiple linear epitopes.

The reliance of the CTL-based vaccine approach on recognition of HIV-infected cells, however, may also constitute a disadvantage, since the antiviral effect would only occur after cellular infection had taken place. Other potential disadvantages include the requirement that active memory cells be present in sufficient amounts, the potential downmodulation of MHC by HIV that can enable infected cells to escape detection, and the fact that access to infection sites is more limited for CTLs than for antibodies.

Evidence that CTLs are important for control of HIV and SIV includes a negative correlation between CTL numbers and viral load (Ogg et al, *Science,* 1998), an increase in SIV viremia observed with CD8+ cell depletion in the SIV-infected macaque model (Jin et al, *J Exp Med*, 1999; Schmitz et al, *Science*, 1999), and an association between the appearance of CTL activity and a decline in HIV viremia in acute infection (Borrow et al, *J Virol*, 1994; Koup et al, *J Virol*, 1994). Studies in HIV-infected individuals have shown that 2% to 20% of total CD8+ cells are specific for HIV antigens, suggesting a strong CTL response (Betts et al, *J Virol*, 2001).

Status of Vaccine Development

Traditional approaches to viral vaccine development have consisted of using live, attenuated virus or whole, killed virus. There are safety concerns with the former approach for HIV vaccines in that the live, attenuated forms of HIV or SIV that have been tested appear to be pathogenic (Baba et al, *Nat Med*, 1999; Greenough et al, *N Engl J Med*, 1999). There are also safety concerns with the use of whole, killed virus, in addition to concerns regarding the potential lack of adequate production of CTL using this approach. Most HIV vaccines currently in development are the products of recombinant DNA technology. In this approach, DNA encoding 1 or more viral proteins can be used to transfect cells in the laboratory to produce antigen that can be used as a vaccine. The DNA can also be delivered as a vaccine through a viral vector, such as vaccinia virus or adenovirus, with the antigens thus being expressed in vivo. The DNA can also be directly injected (ie, the "naked" DNA approach) to stimulate in vivo antigen production.

Selected vaccine strategies that are currently being tested in clinical trials are shown in Table 1. A gp120 envelope subunit vaccine is currently in phase 3 evaluation. A canarypox-vector vaccine is in phase 2 testing. Vaccines using adenovirus-vector, DNA, vaccinia-vector,

Table **1.** Vaccine Strategies Tested in Clinical Trials

Vaccine Antigens	HIV-1 Strain of Origin	Adjuvant, Conjugate, or Delivery System	Route of Delivery
V3 loop of gp120	Numerous	Alum, microspheres, incomplete Freund's adjuvant	Intramusclar, oral
gp120	MN, SF-2 GNE8, A244	Alum, others	Intramuscular
gp160	LAI, MN	Alum, alum plus deoxycholate	Intramuscular
Env Env, Gag Env, Gag, Pol Env, Gag, Pol, Nef	LAI, MN	Vaccinia, canarypox, *Salmonella,* granulocyte macrophage colony-stimulating factor, adenovirus	Intramuscular, intrarectal, intravaginal, intranasal, oral, intradermal, combined
Env, Rev Gag, Pol	LAI, MN, polyepitope	DNA	Intramuscular
gp160, p24	MN	Virus-like particle	Intramuscular
p24	LAI	Self-assembling particle	Intramuscular, intrarectal, combined
Gag (p24)	MN	Lipid conjugate	Intramuscular

and peptide approaches are in phase 1 or phase 1/2 evaluation. The final results of the phase 3 trial of the gp120 subunit vaccine are due in the fall of 2002; expectations are not high that the vaccine will prove to be protective, particularly since the gp120 used in the vaccine is monomeric and the vaccine does not appear to be effective in inducing neutralizing antibodies. A canarypox-vector vaccine was assessed by the Human Vaccine Trials Network of the National Institutes of Health (NIH) and did not produce levels of immunogenicity deemed adequate to warrant going forward with phase 3 trials; nevertheless, this vaccine is being examined in the phase 3 setting by the US Army (Cohen, *Science*, 2002). A modified-vaccinia-Ankara-based vaccine strain is currently being examined in both phase 1 and phase 2 studies.

A vaccine in which Gag-DNA administration is followed by an adenovirus boost has produced intriguing results in animal studies. The aim of this vaccine is to induce pure cellular immunity rather than to induce neutralizing antibody response; although the vaccine currently being tested expresses Gag protein, it could be modified to contain other or additional HIV proteins. The DNA/adenovirus approach is promising because it has stimulated the strongest and most cross-reactive T-cell immunity (in nonhuman primates and human volunteers) of any vaccine approach tried to date (Emini, Keystone Symposium, 2002). Testing in monkeys has shown a strong CTL response (Shiver et al, *Nature*, 2002). In human studies, strong CTL responses with cross-HIV clade recognition have also been stimulated, and it appears that preexisting immunity to the adenovirus vector can be overcome by increasing the dose of adenovirus in the vaccine (Emini, Keystone Symposium, 2002). The vaccine was found to be partially protective against challenge with simian-human immunodeficiency virus. The NIH Vaccine Research Center is also pursuing a strategy of DNA followed by adenovirus. The vaccine currently being tested uses a construct of clade-B HIV Gag and Pol. In a dose-escalation trial examining the safety and immunogenicity of this vaccine, 3 groups of 7 patients each are to receive 3 doses of 0.5, 1.5, or 4.0 mg via a needle-free injection system, with 2 patients receiving placebo at each dose level. Future Vaccine Research Center initiatives are to involve additional viral antigens (eg, Gag, Pol, Nef, and Env), envelope modifications, and antigens from clade A, B, and C viruses; use of a prime/boost method for the adenovirus vector; and use of cytokine adjuvants to increase T-cell memory.

The DNA/adenovirus approach is promising because it has stimulated the strongest and most cross-reactive T-cell immunity

Presented in April 2002. First draft prepared from transcripts by Matthew Stenger. Reviewed and updated by Dr Koup in July 2002.

Financial Disclosure: Dr Koup has no affiliations with commercial organizations that may have interests related to the content of this article.

Suggested Reading

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