Biological Considerations in the Development of a Human Immunodeficiency Virus Vaccine

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Over the last 12 years, many human immunodeficiency virus (HIV) vaccine candidates have been tried in humans, with disappointing results. In particular, recombinant envelope proteins have failed to elicit strong cellular immune responses or neutralizing antibody against many wild-type isolates of HIV-1. Attenuated strains of simian immunodeficiency virus (SIV), although capable of protecting against virulent strains of SIV, often retain residual pathogenicity. These difficulties suggest that it will be necessary to address a number of biological questions that underpin the rational development of an AIDS vaccine: (1) Will natural infection with HIV protect against superinfection? (2) Is partial protection induced by an HIV vaccine adequate to prevent AIDS? (3) What are the immune correlates of protection for an AIDS vaccine? (4) Will a monotypic HIV-1 vaccine confer cross-clade immunity? (5) Is mucosal immunity important for an effective AIDS vaccine? (6) Is there a rationale for therapeutic immunization? Ongoing research that is addressing these questions should lead to the formulation of a safe and effective AIDS vaccine.

When human immunodeficiency virus type 1 (HIV-1) was isolated in 1984, it was predicted that a vaccine would be developed within a few years, assuming an adequate investment in research. Yet, 15 years later, prospects for an AIDS vaccine still appear quite remote. Herein, we will consider why it has been so difficult to formulate an effective vaccine and then to review some of the underlying biological questions [1, 2]. This discussion will also illustrate that our research investment has, in fact, led to an impressive, although still incomplete, understanding of the biology of HIV and the pathogenesis of AIDS in human and nonhuman primates [3–6]. First, it is pertinent to recapitulate some recent history.

In the mid-1980s, the most recent achievement in human vaccinology was the hepatitis B virus (HBV) vaccine, which depended for its efficacy upon the ability of the surface glycoprotein of the virus (HBsAg) to induce antibodies that neutralized the virus. The greatest challenge for HBV vaccine was to protect newborns born to infected mothers. Without intervention, a high proportion of such infants became chronic carriers of the virus, putting them at jeopardy of developing hepatocellular carcinoma many years later [7, 8]. When HBV vaccine was administered to such newborn infants, it did not induce sterilizing immunity, but it changed the nature of the infections, so that vaccinated newborn infants underwent transient virus replication (with a boost to their active immune responses) and then cleared their infections rather than becoming carriers [8].

This successful experience with immunization against a persistent virus infection of humans with purified or recombinant surface glycoprotein led to the expectation that a similar approach might be effective for prevention of AIDS. The only phase III efficacy trials for AIDS vaccines that have been undertaken utilize immunogens consisting of the gp120 envelope glycoprotein of HIV-1 [9, 10]. These trials have just begun, and their outcome will not be known for several years. However, expectations for this approach have been dampened by phase I and II trials, which indicate that although vaccinees develop measurable serum antibody, the antibodies have little if any ability to neutralize most wild-type HIV isolates [11, 12]. Furthermore, in phase II trials, there has been little difference between virus loads for immunized and control subjects among vaccinees undergoing breakthrough infections [13, 14].

Live attenuated viruses offer both a more complex immunogen and an alternative to nonreplicating viral antigens for the formulation of an effective AIDS vaccine. Substantial evaluation of the potential for attenuation of HIV has been explored using the simian immunodeficiency virus (SIV) model [15–18]. Studies have shown that it is possible to engineer mutations in many nonstructural and structural genes of SIV, all
of which confer some degree of attenuation [15, 19–25]. These and other attenuated viruses have been tested extensively in monkeys and have been shown to confer a considerable level of protection against challenge with virulent SIV strains; indeed, the degree of protection has been greater than that provided by other vaccine formulations [18, 19, 26–32]. However, some attenuated strains of SIV retain pathogenicity, as evidenced by the ability to produce AIDS with long incubation periods in adult animals or with shorter incubation periods in newborn macaques [33–35]. The relevance of these animal observations has been bolstered by studies of the “Sydney cohort,” a small group of patients who were inadvertently infected by blood transfusions from a single donor prior to 1984, when testing for HIV was first available [36, 37]. The Sydney cohort is remarkable because the donor and recipients are all infected with an HIV strain bearing naturally occurring deletions in the nef gene [38]. Because nef-deleted SIV is attenuated in macaques, this group of about 10 human subjects could be construed as an unplanned trial of the safety of a nef-deleted attenuated strain of HIV. For >10 years, the subjects in the Sydney cohort experienced a relatively benign infection, as judged by the absence of an AIDS-defining illness and relatively high numbers of CD4 T lymphocytes. However, beginning at about 12 years after infection, the CD4 cell levels have declined in a number of these subjects, and several have developed an AIDS-defining illness [36].

The parallel between human and animal studies of nef-deleted mutants of SIV have strengthened confidence that SIV can be used as a model for HIV attenuation. However, these observations also have had a chilling effect on the outlook for a safe, attenuated HIV strain that replicates sufficiently to be an effective immunogen.

In summary, initial efforts to develop an effective monovalent env-based immunogen or a more complex, safe, attenuated strain of HIV have been impeded by experimental and clinical observations that have suggested very significant problems for each strategy. There is a continuing need to go “back to the basics” to understand not only the virology, as suggested by the late Bernard Fields [39], but also the immune responses that can provide protection against infection. In this brief review, we will discuss some of these important biological issues.

Why Does AIDS Present Special Challenges for Vaccine Formulation?

The pathogenesis of HIV infection has been dissected in some detail, and, considering its complexity, we probably understand it more thoroughly than the pathogenesis of most other virus infections of humans [3, 4, 6]. Initially, there is an acute phase of infection marked by a peak in viremia that is brought under control within 2 months, suggesting that the host response is quite effective. Nevertheless, immune defenses do not eliminate HIV-1, as occurs in most acute virus infections. Herein lies the first problem. An AIDS vaccine might blunt the acute infection with wild-type HIV-1 so that it was rendered asymptomatic, equaling the efficacy of poliovirus or measles vaccines. However, if the infected vaccinee does not eliminate the virus, it could persist, albeit at a lower level than in an unimmunized person.

Of course, there are many human viruses that persist without causing disease in most infected persons—herpes simplex virus, cytomegalovirus, Epstein-Barr virus, varicella zoster virus, BK papovavirus, and others—unless the individual is immune suppressed. Therein lies the second problem. HIV-1 infections, in contrast to other persistent viruses of humans, directly attack the immune system and appear to cause disease in 100% of infected persons, as determined on the basis of cohort studies of long-term nonprogressors, all of whom appear destined eventually to develop AIDS from the persisting virus [40, 41]. It remains a crucial question whether an AIDS vaccinee who undergoes a diminished infection with a wild-type strain of HIV-1 will eventually develop illness. This issue is discussed below.

Will Natural Infection with HIV-1 Protect against Superinfection?

For acute viral infections, a first infection often provides immunity of lifelong duration. When reexposure occurs, reinfection will result but with markedly reduced replication and dissemination, so that the host is protected against disease while undergoing an anamnestic immune response. Protection conferred by natural infection is often used as a reference standard against which to judge candidate vaccines. It is assumed that a vaccine will, at best, equal the protection conferred by natural infection. Conversely, if natural infection does not protect the host against the pathogenic consequences of reinfection (with the same immunotype of the virus), then the outlook for a vaccine may be questionable.

Can this “litmus test” be applied to HIV-1? This question is best addressed from an epidemiologic perspective in selected areas of sub-Saharan Africa, where several clades (genotypes) of HIV-1 are circulating simultaneously. Unfortunately, there are few longitudinal studies in sub-Saharan Africa designed to identify serial infections with HIV-1. However, genetic studies of isolates from regions where multiple clades have been circulating for some time indicate that a substantial number—perhaps 50%—of isolates appear to be recombinants between 2 clades. An examination of such recombinants indicates that each one represents a distinct genetic chimera [42]. This suggests that multiple infections of a single person may not be uncommon in high-risk environments. However, these observations could represent an accumulation in the population of viral recombinants with optimal transmission and replication capacity. They do not address the question of whether an individual who has an infection with 1 HIV-1 strain and then has time (perhaps 6–12 months) to develop a “mature” immune response to that virus will, if infected with a second strain, develop AIDS because of the second superinfecting strain [43].
Ourmanov et al. [48].

titer and the slower course to AIDS in the immunized animals. After protection is documented by the 100-fold reduction in mean viremia subsequently were challenged intravenously with SIV smE660. Partial pol, and virus expressing the envelope of HIV on an SIV genetic back-

various candidate immunogens, using SIV or SHIV (a chimeric against AIDS?

may be predicted that vaccinated persons will experience in-
exceptions, confer only partial protection against natural infection with wild-type virus. Assuming that an AIDS vaccine will not exceed the performance of other successful vaccines, then it may be predicted that vaccinated persons will experience infection when exposed to HIV-1. Will such vaccines be protective against AIDS?

Data are now accumulating about the protection induced by various candidate immunogens, using SIV or SHIV (a chimeric virus expressing the envelope of HIV on an SIV genetic back-

ground) in rhesus macaques. A typical result with a promising vaccine candidate is shown in figure 1. The data in figure 1 are results from monkeys that were immunized with several doses of a recombinant attenuated vaccinia virus (modified vaccinia Ankara) that expresses many of the structural proteins of SIV. Clearly, the immunized animals were infected, but the viremia titer were ~100-fold lower than those seen in the naive control macaques. SIVsmE660, the challenge virus, is very virulent when injected intravenously, and the control animals developed disease in 3–12 months (rapid progressors or progressors). Immunized animals, however, were partially protected against disease, since 5 of 6 were classified as nonprogressors within the limited time of observation [48]. Thus the question of the eventual outcome among partially protected animals in whom viremia is suppressed is not resolved.

One perspective on this question is provided by studies of cohorts of subjects infected with HIV-1. Figure 2, which shows an important data set from such a cohort, illustrates the very significant relationship between viremia set point (6–9 months after infection) and time to AIDS [49]. The cohort with the highest set point (29–250 RNA copies/μL plasma) had a 50% AIDS-free survival of ~3 years. This survival compares with a 50% AIDS-free survival of ~14 years that is projected for the cohort with the lowest set point (0.5–4 RNA copies/μL plasma). The differences in set point between these 2 cohorts was ~100-fold, which is similar to the effect of the SIV vaccine illustrated in figure 1. What implications does this have for an AIDS vaccine that provides partial protection? Will vaccinees be at risk of developing AIDS but with an increased incubation period?

Another possibility also is illustrated in figure 2, which shows the AIDS-free survival curve for persons infected with HIV-2. Epidemiologic studies in West Africa, where HIV-2 is most prevalent, have established that HIV-2 is a less aggressive agent; it is estimated that >75% of those infected with HIV-2 will never develop AIDS [50]. Could an AIDS vaccine down-regulate viremia and viral replication in cells in some vaccinees so that they undergo a lifelong subclinical infection with HIV-1?

What Are the Immune Correlates of Protection for an AIDS Vaccine?

The development of established vaccines was greatly enhanced by the demonstration of their ability to elicit an immune

Is Partial Vaccine-Induced Protection Adequate to Prevent AIDS?

As noted above, established vaccines, with occasional exceptions, confer only partial protection against natural infection with wild-type virus. Assuming that an AIDS vaccine will not exceed the performance of other successful vaccines, then it may be predicted that vaccinated persons will experience infection when exposed to HIV-1. Will such vaccines be protective against AIDS?

Data are now accumulating about the protection induced by various candidate immunogens, using SIV or SHIV (a chimeric virus expressing the envelope of HIV on an SIV genetic back-

| Table 1. Ability of serum from monkeys infected with mutant or wild-type simian immunodeficiency virus (SIV) to neutralize deglyco-

sylated mutant and wild-type parent SIV strains. |
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<tr>
<td>Immunizing virus</td>
<td>Wild-type SIV</td>
<td>Mutant SIV</td>
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<tr>
<td>Wild-type</td>
<td>–60 (40–80)</td>
<td>450 (1–1500)</td>
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<tr>
<td>Mutant</td>
<td>450 (100–1000)</td>
<td>1500 (50–5500)</td>
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NOTE. Data are median (range) of neutralizing titers. After Reitter et al. [77].
response (most frequently neutralizing antibody) that was assumed to be a correlate of protection. In some instances (e.g., poliomyelitis), justification for this assumption was based on experiments using passive antibody. Animals were given graded doses of serum pooled from immune animals and then were challenged with a potentially pathogenic virus to determine the level of antibody that conferred protection against disease. For example, this level was \(1:8\) for poliomyelitis, and, perhaps surprisingly, the experimental titer was similar to that associated with protection in vaccinated humans, as determined in the 1954 field trial of inactivated poliovirus vaccine (IPV) [51].

In development of IPV and oral poliovirus vaccine, Salk and Sabin used this correlate to compare the immunogenicity of different vaccine formulations and immunization schedules [52, 53].

Obviously, it would greatly assist the development of an AIDS vaccine if a single immune correlate could be identified. Many attempts have been made to identify such an immune correlate, mainly on the basis of studies in the SIV model, in which a variety of immune responses were measured and related to protection after challenge with pathogenic viruses [16, 18, 30, 31, 54–56]. Different studies and investigators have come to various conclusions about correlates of protection, and it may safely be stated that no consensus has been reached on this somewhat controversial point.

One possible explanation for the inconsistent conclusions from studies of SIV and SHIV models is that protection does not correlate with any single immune response but is conferred by a barrier created by the sum of several immune defenses. Evidence to support the need for multiple immune components of the immune response to confer protection has been derived from studies of the immune responses to other viruses in mouse models, which can be manipulated to dissect protective responses. One example is Friend leukemia virus (FLV), a retrovirus that causes erythroleukemia in mice. Mice may be immunized by infecting them with an attenuated variant of FLV. Furthermore, protection may be transferred by adoptive immunization of naive mice with spleen cells from immune mice. When such spleen cells are fractionated, protection is conferred by a mix of 3 populations—CD4 T lymphocytes, CD8 T lymphocytes, and B cells; however, any single cell population or combination of 2 populations confers only partial protection [57].

Parallel results can be seen in studies of monkeys challenged with SIV or with virulent or avirulent SHIVs, even though the experimental manipulations are more restricted than in the mouse model. It is possible to protect against an SIV or SHIV challenge by several different modes of immunization [58–63]. Immunization with attenuated SIV induces cellular immunity against the SHIV proteins except for the envelope protein that is derived from HIV. Conversely, DNA immunization against the HIV envelope protein induces neutralizing antibodies against the SHIV and cytolytic T cells that recognize envelope epitopes, but there is no immune response against the SIV proteins [60]; however, both kinds of immunogens protect against HIV infection [64].

Table 2. Clade-specific and cross-clade neutralizing antibody responses in 10 subjects naturally infected with human immunodeficiency virus (HIV) from different clades.

<table>
<thead>
<tr>
<th>Sera from individuals infected with clade</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>F1</th>
<th>E</th>
<th>F2</th>
<th>G</th>
<th>H</th>
<th>O2</th>
<th>O3</th>
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<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>0</td>
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<td>0</td>
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<td>11</td>
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<td>7</td>
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<td>O3</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
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NOTE: Natural infection with HIV-1 induces antibody responses against viral proteins, but this antibody usually has very limited neutralizing activity when tested against wild-type HIV isolates. As evidenced above, in infrequent instances when a patient’s serum does neutralize the homologous virus, there is evidence of considerable cross-clade neutralization. Boldface type indicates homologous neutralizing antibody responses. After Nyambi et al. [81].

Figure 2. The time course to AIDS of patients infected with human immunodeficiency virus (HIV), according to the titer of viremia (virus set point) determined 6–9 months after infection. The curves with filled circles are based on a study of HIV-1 infection, in which infected individuals were divided into 4 quartiles, according to their set point (RNA copies per microliter of plasma), and the Kaplan-Meier survival curve was plotted for each quartile [49]. The \(\sim 100\)-fold difference in virus level between the top and bottom quartiles was associated with striking differences in the patients' AIDS-free survival curves. The curve with open squares is based on a study of HIV-2 infection, in which the set points are lower, and it may be estimated that \(>75\%\) of infected persons never develop an AIDS-defining illness [50].
CD8 cell levels and viremia rapidly returned to their pretreatment values. During the period of CD8 cell depletion, viremia levels rose in both of the monkeys but more dramatically in one instance. With the cessation of treatment, CD8 cell levels and viremia rapidly returned to their pretreatment values. After Schmitz et al. [66].

...levels of passive antibody administered 1 day before challenge can mediate protection [69±74]. Figure 4 demonstrates that low levels of cellular immunity is correlated with a sharp but transient increase in plasma viremia titers (figure 3) [64±66].

...CD8 T lymphocytes appear to play an important role in the control of simian immunodeficiency virus (SIV) viremia levels. In this study, macaques with ongoing SIV infection were treated with a CD8 cell-specific monoclonal antibody that temporarily reduced CD8 cell levels to <5% of their pretreatment values. During the period of CD8 cell depletion, viremia levels rose in both of the monkeys but more dramatically in one instance. With the cessation of treatment, CD8 cell levels and viremia rapidly returned to their pretreatment values. After Schmitz et al. [66].

...an SHIV challenge. The most parsimonious interpretation is that different immune responses that are directed against different viral proteins can provide protection [56]. Given these results, it seems unlikely that there is a single immune correlate of protection. Instead, it may be possible to build a protective barrier by inducing a mix of immune responses, a mix that could vary with different vaccine formulations.

...The role of cellular immunity in control of HIV infection has been inferred by longitudinal studies of HIV-infected subjects: the studies show an inverse correlation between various measures of cellular immune responses and virus load as measured by viral RNA copies per microliter of plasma. Thus, long-term nonprogressors tend to have more consistent cellular immune responses than their rapid progressor counterparts [40]. This interpretation is bolstered by recent studies of SIV infections in macaques, in which a transient reduction in virus-specific levels of cellular immunity is correlated with a sharp but transient increase in plasma viremia titers (figure 3) [64–66].

...The potential role of antibody in prophylactic immunization should not be underestimated. Antibody plays a role in the protection afforded by many effective viral vaccines, a view that is supported by passive antibody studies for several viruses (e.g., poliovirus and hepatitis B virus) [7, 67, 68]. Of importance, antibody has the potential to provide interim protection during the interval of several days after exposure of a vaccinated subject to a wild virus, an interval in which an anamnestic response is elicited by the wild virus challenge. Studies in the SIV and SHIV models have also demonstrated that passive antibody can mediate protection [69–74]. Figure 4 demonstrates that low levels of passive antibody administered 1 day before challenge can alter the course of infection with a pathogenic strain of SHIV [73]. Of particular relevance is the observation that levels of antibody that are too low to totally block infection appear to down-modulate it, indicating that antibody could synergize with other immune modalities of protection. In addition, antibodies that do not have a strong neutralizing potential in vitro can have a profound effect on virus load and pathogenesis in vivo [72].

HIV infection routinely induces an antibody response to many of the major viral proteins, but this antibody has limited ability to neutralize wild-type isolates [11, 12, 75]. There is considerable evidence that the difficulty in inducing neutralizing antibody is associated with the structure of the gp120 protein, the surface protein of the virus envelope. Strong indirect support for this view comes from studies of SIV that show that the virus incorporates major histocompatibility complex (MHC) class II molecules into its envelope. Antibodies directed against class II molecules can protect animals and, in the presence of complement, can readily neutralize SIV while antibodies against the env proteins cannot [76].

In considering a structural explanation of the neutralization resistance of the envelope protein, it has been suggested that the large number of carbohydrate side chains on the gp120 molecule may shield domains that are potential targets for antibody binding to the surface of the virus and for neutralization. Evidence for this view comes from studies of SIV mutants that have been engineered to remove some of the 24 N-linked glycosylation sites [77] (table 1). Mutants lacking 2 or 3 selected glycosylation sites are more sensitive to neutralization. Furthermore, such mutants induce antibody that has increased neutralization activity against the wild-type virus. More recent studies indicate that other mutants, for instance those lacking the V1 and V2 loops of the virus envelope, are more sensitive to neutralization by antibodies and may be more effective at inducing such antibodies (R. Desrosiers, Division of Microbiology and Molecular Genetics, Harvard Medical School; personal communication).

In addition to these efforts to alter the “static” structure of the envelope glycoprotein, another approach has been taken by researchers who have focussed on “fusion intermediates” as

Table 3. Mucosal immunization provides better protection against mucosal challenge than does systemic immunization.

<table>
<thead>
<tr>
<th>Assessment site</th>
<th>Subcutaneous</th>
<th>Rectal</th>
<th>Not immunized</th>
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<tbody>
<tr>
<td>Splenic T cell response</td>
<td>68</td>
<td>45</td>
<td>ND</td>
</tr>
<tr>
<td>Peyer’s patches, %</td>
<td>3</td>
<td>35</td>
<td>ND</td>
</tr>
<tr>
<td>Lamina propria, %</td>
<td>5</td>
<td>30</td>
<td>ND</td>
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<tr>
<td>Rectal challenge</td>
<td></td>
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<tr>
<td>viral titer, log10 per ovary</td>
<td>8.2</td>
<td>4.3</td>
<td>8.3</td>
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NOTE. In this rather contrived example, mice were immunized by either subcutaneous or intrarectal routes with immunodominant peptides adjuvanted with cholera toxin. ND, not done. After Belyakov et al. [93].
Figure 4. Neutralizing antibody can play a role in the control of SHIV (a chimeric virus expressing the envelope of human immunodeficiency virus [HIV] on a simian immunodeficiency virus genetic background) infection. In this study, monkeys were treated with anti-HIV antibody, which was raised in a chimpanzee, and then were challenged with a pathogenic SHIV, DH12, bearing the homologous HIV envelope protein. A prechallenge passive antibody titer of 5–8 provided sterilizing protection against the challenge virus, and a titer of 2.5–4 provided partial protection with attenuated viremia titers (compared with untreated control animals). After Shibata et al. [73]. NAB titer, neutralizing antibody titer; PBMC, peripheral blood mononuclear cells.

Will a Monotypic HIV-1 Vaccine Confer Cross-Clade Immunity?

Some human viruses, such as measles, rubella, and variola, exist as a single immunotype, whereas other viruses, such as poliovirus and influenza virus, are composed of several distinct immunotypes. Many methods have been used to construct an antigenic classification of virus subtypes, but probably most pertinent to this discussion is the ability of infection with 1 immunotype to confer protection against infection and disease with another immunotype. Protective immunotyping has traditionally been used to determine whether a vaccine should consist of 1 or several different virus strains. With HIV, no single neutralization procedure and no single stock preparation of virus resolves the issue of antigenic diversity.

Extensive sequencing of HIV-1 strains from many continents and countries forms the basis for dendrograms depicting the genetic classification of HIV isolates. Overall, most strains fall into a major group (group M) that consists of ~10 genotypes or clades, whereas a few isolates are classified as either group O (outlier) or group N (new). By analogy with classification systems for other viruses, it was surmised that the major clades might represent different immunotypes of HIV-1 that could be distinguished by serotyping. However, panels of human monoclonal antibodies against a few of the viral proteins have indicated that if HIV-1 strains are grouped into immunotypes, these groups would not correspond to the major clades [80].

Is it necessary to formulate a different vaccine for each HIV-1 clade, or are there a sufficient number of conserved epitopes to provide cross-clade protection? For several reasons, this is a difficult question to address. First, the low levels of neutralizing activity induced during natural HIV-1 infection limit the analysis of cross-clade neutralization. Second, it is not clear how antibodies to different regions of the gp120 and gp41 contribute to neutralizing activity. Nevertheless, there are sufficient data to provide some insights. Table 2 shows antibody tests from subjects naturally infected with isolates representing a broad range of clades. Results are shown only those few individuals who raised substantial neutralization responses to the isolate with which they had been infected. Among these 10 subjects, 2 showed neutralization that was quite clade specific, whereas 8 produced a broad cross-clade response [81].

Similar questions can be raised with respect to cross-clade cellular immunity. It is difficult to address this issue in a definitive way, since MHC-specific immunodominant epitopes for cytolytic T lymphocytes have not been defined for a sufficient
number of viral proteins and MHC haplotypes. Some preliminary impressions can be garnered from studies, such as that shown in figure 5, which compares cytolytic T lymphocyte (CTL) activity in 10 subjects, half of whom were infected with clade B viruses and half with clade E viruses. Most subjects showed CTL responses against the heterologous clade, but CTL activity generally was lower than that against the virus from the same clade [82]. Cross-clade immune responses have been reported by many laboratories [83–87]. Of note, vaccination has been shown to induce cross-clade CTL responses [85] (figure 5).

These data suggest that the issue of cross-clade immunity must be carefully considered when planning future trials of candidate vaccines. For instance, it would be possible to include in future vaccine trials 2 products, one matched for the local clade and the other unmatched. A multivalent product also could be compared with a univalent product. Conversely, if trials compare only a placebo arm to a clade-matched vaccine, and the vaccine appears to be partially protective, it could be difficult to decipher whether clade matching is needed for future vaccines.

Is Mucosal Immunity Important for an Effective AIDS Vaccine?

Worldwide, most HIV infections are acquired through sexual transmission. After transmission, AIDS viruses quickly infect T lymphocytes in the tissue at the portal of entry [88, 89] before spreading to local lymph nodes. Therefore, a local barrier at the site of initial infection might provide an important adjunct to systemic immunity, particularly for a virus that is so resistant to protective vaccination.

One other hypothetical advantage of mucosal immunity is its potential effect on viral shedding by infected vaccinees. For example, oral poliovirus vaccine provides more protection than does IPV against enteric replication of poliovirus at the same levels of circulating neutralizing antibody. This effect on viral shedding produces a degree of herd immunity in the population, and such immunity played an important role in the disappearance of wild poliovirus from an immunized population, despite the presence of a significant minority of susceptible unvaccinated individuals [90]. In theory, an AIDS vaccine that induced mucosal immunity could produce an “altruistic” effect for the population that was greater than that conferred by a vaccine that only induced systemic protective immunity in an individual.

Is there evidence for the induction of a local immune barrier in persons who have undergone natural infection with HIV? Most HIV-infected patients have developed systemic immunity, making it difficult to detect any hypothetical incremental protection due to mucosal immunity. However, the enigmatic group of subjects classified as “exposed uninfected” (EU) offers potential insight. EU individuals are persons who are repeatedly exposed to many partners, either through their personal lifestyle or because they are commercial sex workers. Yet, despite their putative frequent HIV exposure history, these persons lack the stigmata of systemic infection, since they do not have anti-HIV serum antibody nor can the viral genome be detected in their blood by polymerase chain reaction. However, some of these individuals exhibit evidence of prior local infection by virtue of HIV-specific IgG or IgA antibody in mucosal secretions or CTLs in blood (or both) [91, 92].

Although the EU data are fragmentary, similar observations have been made by a number of investigators. Furthermore, the EU phenomenon has been modeled experimentally in macaques, either by mucosal exposure to minimal SIV inocula and/or by infection followed by postexposure antiretroviral treatment. Taken together, these observations suggest that it might be possible to induce mucosal and systemic immunity to HIV.

Can mucosal immunity be induced by vaccination? In the presence of induced systemic immunity, this can be difficult to determine, as illustrated in a mouse model (table 3) in which an HIV immunogen was applied subcutaneously (systemic immunization) or intrarectally (mucosal immunization) [93]. When CTLs were assayed in spleen and intestinal tissues (lamina propria and Peyer’s patches), the mucosal CTL activity was higher in animals immunized by the rectal route. Furthermore, rectally immunized mice resisted infection by rectal challenge more effectively than did mice immunized by the subcutaneous route. Although this is a somewhat contrived experimental model, it clearly demonstrates the principle of inducing mucosal versus systemic immunity. Exploratory experiments in macaques have been initiated [31, 61] (C. Miller, Department of Pathology, Microbiology and Immunology, University of California, Davis; personal communication), but more extensive studies are required to address the potential for mucosal immunization.

Is There a Rationale for Therapeutic Immunization?

Several important observations have been made in recent years that provide a rationale for therapeutic immunization (that is, immunization of persons who are already infected with HIV). First, there is a large body of observations that document an inverse relationship between HIV-specific cellular immunity (measured either on fresh or cultured peripheral blood mononuclear cells) and HIV load (as measured by HIV RNA copies per milliliter of plasma) [94, 95]. Another set of studies has documented that virus load is the single best predictor of the clinical course or the incubation period to AIDS. This strongly suggests that CD8 cells play an important role in the control of HIV infection. Second, extensive experience with highly active retroviral therapy (HAART) indicates that HIV-infected patients whose virus loads are reduced to minimal levels experience a gradual decline in their anti-HIV cellular immune response.
responses, as determined by using newer tetramer assays to measure circulating levels of memory CD8 T lymphocytes [96–98]. This finding suggests that the most effective quantitative reduction in HIV antigen burden produced by HAART is sufficient to reduce the intensity of the immune response against the virus. Third, when HAART is terminated in patients whose virus loads have been reduced to minimal levels, even for periods of years, almost all individuals undergo a reemergence of patent viral replication, and often this replication is to high set points. This occurs even in patients whose overall CD4 cell counts are quite high (well over 400/µL) [4]. This observation indicates that even if the balance between host and virus is tilted against the virus by effective therapy, the host immune response may be inadequate—without specific stimulation—to maintain virus suppression once therapy has been stopped.

Taken together, these 3 observations suggest that it might be possible to boost the HIV-specific cellular immune response of infected patients receiving HAART and that the subsequent enhanced response might be more effective at suppressing viral replication after termination of HAART. This hypothesis has been tested in the experimental SIV model, in which all variables can be subject to controlled evaluation (G. Franchini, Division of Basic Sciences, National Cancer Institute, National Institutes of Health; personal communication). Macaques were infected with a pathogenic strain of SIV, treated with a combination of antiretroviral drugs, and immunized with a highly attenuated recombinant vaccinia virus expressing the gag, pol, and env genes of SIV. Therapy was terminated, and the animals, together with unimmunized controls, were followed for SIV viremia. All immunized animals controlled their viremias quite successfully, as did some of the control group. This initial experiment indicates that further efforts to explore therapeutic immunization are justified.

Future Prospects

The formulation of an effective AIDS vaccine constitutes a daunting challenge for a number of reasons, including the following: (1) the ability of the virus to persist, to replicate in the face of a vigorous immune response, and, ultimately, to destroy the integrity of the immune system by an attack on CD4 helper T lymphocytes; (2) the question of whether partial immunity will suffice to protect vaccinees against eventual disease; (3) the absence of a single clear-cut immune correlate of protection; (4) the difficulty of inducing neutralizing antibodies; (5) the necessity of defining and inducing CTL epitopes that are immunodominant for each of many different MHC class I haplotypes; (6) the question of whether a vaccine formulated on a virus of a single clade will protect against infection with viruses of other clades; (7) the question of whether an effective vaccine must induce mucosal immunity; and (8) the difficulty of developing an attenuated virus strain that is both safe and immunogenic.

The body of knowledge developed over the last 10 years has permitted the definition of issues and impediments that need to be addressed. In itself, this represents a major advance toward our goal. Today, there is a considerable research agenda directed toward each of the problems noted above, and most of them should eventually yield to scientific inquiry. Furthermore, nonhuman primate models offer a major opportunity to study some of these issues and systematically to compare various candidate immunogens for their ability to protect against an SIV or SHIV challenge that resembles HIV/AIDS in humans [99]. At present, several candidate immunogens (e.g., recombinant poxviruses and recombinant alphaviruses) have shown promise in their ability to provide partial protection of macaques against highly pathogenic SIV challenges. Further empirical trials of vaccine formulation and immunization schedules will probably improve the degree of protection with these existing immunogens, particularly against the mucosal route of infection. It seems reasonable to assume that some of these optimized vaccines (reformulated for HIV) will induce immune responses in humans that are sufficiently promising to justify phase III efficacy trials.

Looking beyond candidate immunogens that have already been tried in nonhuman primates, there are many other vectors and adjuvants that are still in the early stages of development. They include vectors, such as adeno-associated virus, adenovirus, flaviviruses, and picornaviruses; cytokine adjuvants; intestinal bacteria, such as recombinant attenuated Salmonella, Shigella, and Listeria strains. In addition, genetically engineered variants of the virus envelope proteins may be able to elicit more potent antibody responses, and defined, immunodominant MHC-specific CTL epitopes may induce more consistent cellular immune responses. It may be predicted that these experimental approaches will lead to additional effective methods for the induction of protective immunity.

In summary, it seems likely that safe HIV vaccines will be developed, perhaps in several generations of increasingly effective formulations. Probably the greatest question remaining is not whether but when will there be an AIDS vaccine that can be deployed in high-risk populations around the world.

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References


