Clinical Trials of HIV Vaccines

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INTRODUCTION

HIV-1 was identified in 1983 and subsequently has been shown to be the etiologic agent of the acquired immunodeficiency syndrome. The HIV-1 pandemic has grown to become one of the greatest infectious disease threats to human health and social stability that the world has ever encountered. An estimated 35 million persons were living with HIV-1 infection at the beginning of this millennium and more than 12 million people have already died from HIV-induced disease. Although effective anti-retroviral therapy has attenuated the expansion of the epidemic in some industrialized countries, worldwide there are still an estimated 16,000 new HIV infections occurring daily. In addition to the vast personal suffering, the loss of young adult parents, caretakers, and wage-earners, HIV has created an unprecedented strain on the social and economic infrastructure of many developing countries, particularly in Subsaharan Africa. These are the facts that make it imperative that the epidemic be controlled as rapidly as possible through prevention of new infections. Although education and available public health approaches should be vigorously pursued, development of a preventive vaccine is the best hope of controlling the HIV epidemic.

New molecular tools in virology and immunology, new adjuvants, new gene expression systems, new antigen delivery systems, recent discoveries in HIV entry and pathogenesis, evidence that natural immunity is achieved in rare instances, and promising studies of candidate vaccines in animal models have provided reasons to hope that developing a safe and effective AIDS vaccine will be possible. However, some have argued that preventive vaccination for AIDS will not be possible (1), and the complex biology of HIV-1 makes this a daunting task.

This review will present current immunologic concepts and assumptions that form a framework for developing approaches to vaccine development, relevant data from studies of pathogenesis and vaccines in humans and animal models, the current status of vaccine concepts being evaluated in clinical trials, and additional questions that must be addressed. A more comprehensive review of the issues involved in HIV vaccine development has been recently published (2).

ASSUMPTIONS RELEVANT TO HIV VACCINE DESIGN

Vaccine-induced immunity is possible. While there are many challenges remaining, the following observations suggest that vaccine development is achievable: 1) HIV-1 transmission is relatively inefficient, 2) transmitted virus may have a restricted set of structural and genotypic features that are susceptible to immune interference, 3) most infections are probably initiated with very few virions, 4) a relatively small impact on transmission efficiency of HIV between individuals may have a major influence on the epidemic within a population, 5) there are demonstrations of HIV-specific immune responses in some exposed-but-uninfected persons that suggest it may be possible for natural immunity to clear virus infection, 6) there are examples of vaccine-induced immunity in primate models of lentivirus infection, 7) there is evidence that HIV-2 infection confers partial protection against HIV-1 infection (3), 8) vaccine-induced immune responses include CTL activity against multiple clades of primary HIV-1 isolates, 9) continued gains in our knowledge of antigen presentation, cytokines, chemokines, co-stimulatory molecules and their ligands will lead to new vaccine approaches, and 10) new vaccine approaches promise to improve the immunogenicity of vaccine antigens and their delivery into appropriate immunological compartments.

Timing is everything.

There are several parameters of the vaccine-induced immune response that will determine its ability to protect the host from infection or disease including specificity, functional properties, magnitude, and compartmentalization. However, the most critical factor is timing. The timing of the immune response with respect to initial virus infection and spread is particularly important in the case of HIV-1 infection. One reason for this is that the longer HIV-1 replicates in the host, the more diverse variants evolve that allow escape from subsequent immune responses. In addition, once HIV-1 resides in the extracellular space of lymph node germinal centers and in latently infected cellular reservoirs, and is sequestered in the central nervous system and other sites that are relatively protected from immune responses, it is unlikely that it can be fully eliminated from the host. At this stage of infection the immune response to HIV-1 infection includes a number of potent effector responses that at best achieve a steady state in which virus clearance matches virus production. This balance results in a viral load “set point that correlates with the rate of immune system destruction. The important advantage of vaccine- induced

immune responses is that they are induced prior to infection and can be recalled more rapidly than primary effector mechanisms. Therefore the success of vaccination may hinge upon altering events that occur in the early hours following HIV-1 exposure.

Vaccines work through induction of adaptive immune responses. Preventive vaccines work through establishing immunologic memory for antigenic structures presented by the pathogen or by infected cells. Therefore, the immunologic "tool box accessible for vaccine-induced immunity only includes elements of the adaptive immune response. The basic cellular elements of adaptive immunity include the B and T lymphocytes. The primary effector mechanisms important for protection against viruses are antibodies produced by B cells and cytolytic activity mediated primarily by CD8+ T cells. In addition, there are soluble factors produced by activated CD4+ and CD8+ T cells that have anti-viral activity and can influence the differentiation, expansion, and duration of T cell responses. Elements of the nonadaptive immune system are important during the initial phases of antigen presentation and development of the cytokine microenvironment. However, immunity against subsequent infection will be determined by adaptive immune responses with memory for key antigens and functional effector activities that can neutralize the pathogen and rapidly eliminate infected cells.

Neutralizing antibody and cytolytic T cells are the major effectors of anti-viral immunity. The correlates of immunity against HIV-1 have not been defined in an absolute sense, but much is known about HIV-specific immune responses associated with long-term survival and maintenance of low viral loads. In addition, there is a general understanding about how different elements of the adaptive immune response should work and these concepts can be tested against observations made in studies of the natural history of HIV infection in humans or experimental data from animal models (4). Alternative vaccine-inducible effector mechanisms mediated by the adaptive immune response may ultimately be shown to have a role in protection (5–11) but in this review I will focus on classical neutralizing antibody and CD8+ cytolytic T cell activities. There is often debate and speculation about which component of the adaptive immune system is most important for immunity. However, there is abundant evidence for HIV and other virus infections that both antibody and CD8+ CTL are important and perform complementary roles in protection from and control of infection. CD4+ T cells are also of obvious importance, especially for influencing differentiation patterns and expansion of selected lymphocyte populations, but their role as a direct effector of virus clearance in less clear. Therefore, another assumption is that CD4+ T cells will be induced in the process of achieving the appropriate antibody and CD8+ CTL responses, and will not be specifically addressed in this paper.

Antibody is the only component of the adaptive immune response that can neutralize a virus particle prior to infection of a cell and is the only immune response associated with protection for any currently licensed vaccines. Antibody titers can be sustained at high levels in serum and in mucosal secretions and be present at the time of infection. This is unlike T cells which only recognize virus in the context of an already infected cell, and require a few days for activation and expansion of memory populations to respond. Therefore, an effective neutralizing antibody response will be a critical component of vaccine induced immunity, because it can prevent infection and thereby reduce inoculum size and establishment of latently infected cells.

Neutralization is defined as the ability to reduce infectivity of cell-free virus usually measured in susceptible cells in culture. While this aspect of antibody activity is thought to be the key function associated with protection from infection. There is some debate about the mechanism of neutralization. There are reports of specific neutralizing epitopes suggesting the site of antibody binding is important (12–16). Another view is that neutralization occurs when a threshold level of the virion surface is covered by antibody that binds the native envelope oligomer regardless of specificity (17). In either case, it is clear that T cell line-adapted viruses are more susceptible to neutralization than primary field isolates which poses a major hurdle for achieving this immunologic endpoint (18, 19).

T cells recognize virus infected cells by specific interactions between the T cell receptor and 8- 10 amino acid peptides processed from viral antigens and presented in the context of major histocompatibility complex (MHC) molecules. Therefore, T cells can only clear virus effectively after infection has occurred. The recognition is restricted by the MHC molecule, which means that the particular epitopes recognized by a given individual will depend on the set of inherited alleles encoding the MHC molecules. While each person should have the capacity to recognize multiple epitopes among the antigens included in HIV-1, the hierarchy of recognition or epitope dominance may vary even among individuals who share MHC haplotypes. These issues suggest that the epitope repertoire in a vaccine will need to have enough breadth to encompass all the relevant MHC haplotypes of potential vaccinees. In addition, it will be important to induce a broad response in each individual against several viral antigens to diminish the possibility of immune escape through genetic variation and to allow for host selection of dominant epitopes. The need for CD4+ T lymphocytes to initiate the adaptive immune response presents a dilemma since these cells are the major target for HIV-1 infection. The problem is how to effectively induce protective immunity against HIV-1 without putting vaccine-

induced HIV-specific CD4+ T cells at risk of infection. This emphasizes the need for effective immune responses, preexistent at the time of HIV exposure, so that virus clearance can be accomplished before the burden of infected cells is sufficient to maintain persistent infection. While CD4+ T cells may have some capacity for lysis of HIV-infected cells (20) and production of anti-viral cytokines, the major role is in shaping the immune response by establishing a microenvironment with a particular cytokine composition. For HIV and most other viruses, induction of a Type 1 cytokine profile (production of IL-12, IL-2, and IFN-) is more likely to provide protection than induction of Type 2 cytokines (IL-4, IL-5, IL-13). Initial priming with vectors and the use of adjuvants other than alum (which promotes Type 2 responses) would provide an advantage in this regard.

CD8+ T cells are the principal effector mechanism of the adaptive immune response to clear virus infected cells. This has been demonstrated exhaustively in murine models of LCMV, Sendai virus, influenza virus, respiratory syncytial virus, ectromelia, herpes simplex, and others (21–27). The CD8+ lymphocyte recognizes a virus-infected cell through a cognate interaction between the T cell receptor and a processed peptide epitope presented in the grove of a MHC class I molecule. The lysis of the infected cells occurs through the production and secretion of perforin and granzymes that penetrate the target cell membrane and induce apoptosis. FasL is also upregulated on the activated CD8+ T cell which can bind Fas on the target cell and induces apoptosis through other pathways. CD8+ T cells also produce cytokines with anti-viral properties like IFN- and TNF- , in addition to other soluble factors that may play a role in virus inhibition. The T cell response causes cytopathology not only of the virus-infected cell but to a varying degree in bystander cells. This again points to the importance of clearing virus rapidly to diminish the overall cytopathology and illness associated with the immune response to infection.

Acceptable definitions of vaccine-induced immunity. The outcome of infection in the setting of vaccine-induced immunity could range from complete prevention of infection to immune-mediated enhancement of disease. None of the currently licensed vaccines for other viral pathogens are known to fully prevent infection, and most are effective because they limit the replication and spread of the pathogen below the threshold for clinical expression of disease. Ideally, a vaccine against HIV-1 will either prevent infection or result in a transient infection that is rapidly cleared before the establishment of latently infected cells or widespread dissemination. Vaccine-induced immune responses may not be sufficient to prevent persistent infection. However, if low virus loads could be maintained to protect the individual from disease and to limit transmission to others, this would also be an acceptable outcome for vaccine-induced immunity.

DATA FROM STUDIES IN HUMANS AND ANIMAL MODELS

Antibody can prevent HIV infection. It has been directly proven using passive antibody studies in nonhuman primate models of lentivirus infection that sufficient levels of neutralizing antibody can prevent infection. Studies evaluating polyclonal anti-HIV-1 antiserum (28) or monoclonal anti-V3 antibody in HIV-1 infected chimpanzees (29) or polyclonal serum in SIV-infected macaques (30) have shown that when sufficiently high antibody titers are present prior to intravenous challenge that lentivirus infection can be prevented. Importantly, antibody mediated protection has also been demonstrated against SHIV with an envelope glycoprotein derived from a dual tropic primary HIV isolate, and the protection could be correlated with *in vitro* neutralizing activity (31). More recently, passive prophylaxis using HIV immune globulin combined with two monoclonal antibodies has protected macaques from vaginal challenge with SHIV (32), and a mixture of three neutralizing monoclonal IgG1 antibodies given to pregnant macaques has protected their infants from SHIV oral challenge (33). Definitive evidence of antibody-mediated protection in studies of active immunization has been more difficult to demonstrate, but there is an example from early studies performed with whole inactivated SIV vaccines that is provocative. In these studies it was shown that antibodies to cell constituents incorporated into virions during production of challenge stocks were the best correlate of protection (34–37). When the virus used to produce vaccine was grown in human cells, and the virus challenge stock was grown in the same human cells, allogenic responses to the human proteins incorporated by the virus were the dominant mechanism of protection (34,38,39). Studies done with vaccine produced in monkey cells did not show consistent protection. Even though the antibody response was not specific for virus-encoded antigens, this represents an example of vaccine-induced antibody-mediated protection suggesting that protection through induction of virus-specific antibodies may be achievable. When SIV immune globulin was given one day after intravenous challenge with SIV, infection was not prevented, but disease progression was delayed in some animals (40). This again illustrates that the timing of immune responses are critical to the outcome of infection and that preexisting immunity gives the host a distinct advantage.

T cells can control HIV infection.

Control of the initial viremia associated with primary HIV infection temporally correlates with the appearance of CD8+ cytotoxic T lymphocytes

(41,42), and mutations in specific CTL epitopes can be detected in the residual virus population (43–47). In addition, HIV-specific CD8+ CTL activity has been demonstrated in a small subset of uninfected, seronegative commercial sex workers in The Gambia and in Kenya suggesting transient infection may have occurred inducing protective immunity mediated by CD8+ CTL (48,49). In persons who remain uninfected despite significant occupational exposure to HIV-1 contaminated material, studies have also focused on HIV-specific T cell responses. Although HIV-specific antibodies can not be detected, PBMCs show lymphoproliferative activity when stimulated with HIV-specific peptides (50). HIV-specific CTL responses have also been seen in this cohort (51), suggesting that transient infection may have occurred and been cleared with natural immune defenses. Another subset of persons infected with HIV-1 have persistent infection, but do not progress to AIDS for greater than 12 years. Some of these individuals are infected with virus isolates that replicate poorly (52,53). However, others are infected with viruses that have normal replication capacity, but have maintained a strong and broad set of humoral and cellular HIV-specific immune responses that appears to be responsible for their delayed disease progression. This has been associated with HIV-specific CD4+ T cell proliferation (54) and strong CD8+ CTL activity against multiple epitopes (55,56). Another clue to the importance of T cell responses in the control of HIV has come from the evaluation of HIV- infected persons treated with highly active anti-retroviral therapy (HAART) soon after primary infection. When these persons undergo structured treatment interruptions there is a transient rise in the virus load which results in a boost of functional T cell activity and subsequent control of virus load without HAART (57).

The most compelling evidence for the importance of CD8+ CTL for controlling lentivirus infection comes from studies of pathogenesis and vaccine evaluation in nonhuman primate models. The CD8+ CTL response is the best correlate of viremia control after primary SIV infection in macaques, similar to the findings in HIV-infected humans as discussed above (58). There are now several studies using nucleic acid or other recombinant vector approaches that have demonstrated induction of CD8+ CTL responses with a weak or absent antibody response, does not protect from lentivirus infection, but reduces viral load and delays disease progression. One of the early demonstrations of this was in macaques immunized with recombinant MVA (modified vaccinia Ankara) prior to challenge of macaques with SIV. Vaccination did not prevent infection, and the CTL cell response was associated with delayed disease progression (59). Subsequent studies have shown similar patterns (60–67). As approaches are taken to optimize the CD8+ CTL response, such as the addition of an IL-2 adjuvant to a recombinant DNA vaccine regimen, nearly complete control of subsequent SHIV infection can be achieved (67). These data are consistent with the premise that vaccines able to establish a preexisting expanded population of HIV-specific CD8+ CTL are likely to delay disease progression in HIV-infected persons.

CLINICAL TRIALS OF CANDIDATE HIV VACCINES

Overview of concepts evaluated. Clinical trials in seronegative volunteers have been performed to evaluate the safety and immunogenicity of candidate AIDS vaccines in more than 3500 subjects. Several recombinant envelope products, rgp120 or rgp160, produced in insect, yeast or mammalian cells formulated with a variety of adjuvants have been evaluated in clinical trials. Peptides tested to date have been derived from envelope V3 loop or gag sequences of clade B or multiple clades. They have been presented conjugated to an oligolysine backbone, as a lipopeptide conjugate, mixed with adjuvant, or as a fusion protein with the self-assembling yeast protein Ty as a particle. They have been administered intramuscularly in the deltoid or anterior thigh (to target lymph nodes that also drain the rectal mucosa), rectally and orally as Ty-gag virus-like particles, and orally encapsulated in polylactide co-polymers. Live recombinant vectors including vaccinia, canarypox, and salmonella have been evaluated as well as nucleic acid based vaccines. These vectors have been delivered by a variety of routes and have been constructed to express both single or multiple HIV-1 antigens. In addition, there have been studies evaluating schedule of administration and combination approaches using more than one product in the immunization regimen. These studies are listed in Table 1 and referenced when possible. This review will summarize the findings of the studies to date without including the details of each individual product. Because there have been no significant safety concerns other than unacceptable local reactogenicity associated with a few selected adjuvants, I will focus on vaccine immunogenicity particular the ability to induce neutralizing antibody and CD8+ CTL responses. Vaccine-induced antibody responses in clinical trials. Neutralizing antibody responses have been induced by immunization with recombinant envelope glycoproteins alone or in combination with poxvirus vectors. The antibody response to immunization with rgp120 alone is in general maximal after the third or fourth injection, is dose dependent, and can be attenuated unless there is a several month interval between injections. Serum antibody titers have a relatively short half-life, and while they can be boosted the titers generally achieve their peak level after the third or fourth injections. Repeated boosting does not prolong the half-life significantly. Therefore, it is likely that recombinant envelope glycoprotein products may find their greatest utility in boosting antibody responses in subjects primed with recombinant vector vaccines (99), or other strategies

that can induce MHC class I-restricted CTL responses. This combination approach not only adds the CD8+ CTL component to the immune response, but results in a more durable antibody response. The initial recombinant envelope glycoprotein products were derived from sequences of syncytium-inducing, T cell line-adapted (TCLA), CXCR-4 utilizing X4 viruses from clade B. Newer products, such as the VaxGen B/B product incorporate sequences from primary isolates which utilize CCR5 (R5) combining the rgp120 from HIV-1MN and the rgp120 from HIV-1GNE8 (110). Phase I and II studies have defined how parameters of dose, schedule, and formulation affect immunogenicity of purified protein subunit preparations as primary immunogens and as booster immunogens given in combination with other vaccine modalities. The principal findings related to vaccine-induced antibody responses in clinical trials of candidate HIV vaccines include:

1 While type-specific neutralization can be induced, particularly to the vaccine antigen, neutralization of typical primary R5 HIV isolates is not induced (111). There are some reports of neutralization of selected R5 HIV strains, but these are strains that are easier to neutralize in general, and how this will translate into protection against more typical neutralization resistant strains is not known.

2 Antibody is induced that can bind oligomeric, R5 virus presented on the surface of virus-infected cells (112). This suggests that the monomeric envelope products currently being tested can produce antibody that recognizes oligomeric envelope structures, even though the affinity and specificity is not sufficient to result in virus neutralization.

3 Antigens produced in mammalian cells induce higher titer of neutralizing antibody against TCLA virus than those produced in baculovirus or yeast systems (69,73,77,78).

4 Recombinant gp120 products induce less binding antibody, but more neutralizing antibody than rgp160 products (71,77,78). As noted above, the neutralizing activity does not include primary isolate R5 viruses.

5 A four dose immunization regimen using envelope glycoprotein is more effective for antibody induction when there is a several month interval between doses (77,78). Intervals of at least 3 to 4 months between the second, third, and fourth immunization increase the magnitude of response.

6 A rapid (every month) vaccination schedule using envelope glycoproteins alone results in attenuation of antibody responses after the fourth dose (77). Titers of both binding and neutralizing antibody activities are reduced after a monthly immunization schedule using rgp120 in MF59 (77). The attenuating effect of rapid dosing is not as apparent with other adjuvants (72).

7 A fifth dose of rgp120, regardless of interval, does not boost antibody response, but only returns it to previous level.

8 The half-life of vaccine antigen-specific antibody titers is $<$3 months in subjects receiving only rgp120 envelope glycoprotein, regardless of number of doses. The half-life is extended with gp160 antigens, and is also more prolonged when rgp120 immunization is preceded by priming with poxvirus vectors. The factors underlying antibody maintenance have not been defined.

9 Priming with one subtype and boosting with another demonstrates subtype-specificity in antibody response (102). When a subject is initially immunized with rgp120 derived from a clade B, TCLA X4 HIV strain, subsequent boosting with another clade B strain does not broaden the response significantly, and does not boost the response to the new envelope antigen as well as to the original rgp120.

10 Antigen dose effects on magnitude of antibody production are dependent on the adjuvant formulation. QS21 appears to allow a reduction in the antigen dose by more than 10–100 fold without affecting the magnitude of antibody response (113).

11 Peptide vaccines in general have been weakly immunogenic with one exception. This was a complex peptide that contains T helper epitopes from the C4 domain of gp120 and neutralizing antibody and CTL epitopes from the V3 domain. Peptides from four strains of HIV-1 were combined in incomplete Freunds adjuvant and administered intramuscularly. Neutralizing activity against HIV-1MN was detected in 75% of subjects after the second dose. However, the study was terminated prematurely because of the development of sterile abscesses in a few vaccinees.

12 Recombinant gp160 vaccinia immunization induces an antibody response to the HIV envelope that is slow in developing, often not being detectable until $>$100 days after inoculation (100),

13 Recombinant vaccinia effectively primes for antibody responses elicited by subsequent boosting with purified recombinant envelope glycoprotein formulations (98,99),

14 Specificity of the antibody response in subject primed with recombinant vaccinia then boosted with recombinant envelop glycoprotein is determined more by the initial antigen expressed by the recombinant vector than by the subsequent envelope antigen given as booster (101,102),

15 Priming immunization with recombinant vaccinia induces type-specific memory for the recombinant gene product that is not boosted by subsequent immunization with heterologous recombinant envelope glycoproteins (102).

16 In subjects immunized with recombinant HIV-1LAI gp160 vaccinia and boosted with HIV- 1LAI gp160 produced in baculovirus and formulated with alum, the dominant antibody response in vaccinees was directed against a gp41 epitope (aa 720–740) that was not a major target for antibodies produced by HIV-1LAI-infected persons (114).

17 The HIV-specific antibody response after recombinant canarypox immunization alone is weak, but subsequent boosting with purified recombinant envelope subunit protein induces HIV-specific antibody titers of the same magnitude and quality as 3 or 4 inoculations of the purified recombinant envelope subunit protein alone (105,106).

In summary, neutralizing antibody responses against TCLA viruses induced by the most immunogenic formulations are still 5–10 fold lower than those produced by HIV-1 infection. The responses are type-specific with a relatively short half-life, and are unable to neutralize typical primary isolate R5 viruses.

Vaccine-induced CD8+ CTL responses in clinical trials. Induction of HIV-specific CD8+ CTL responses requires the delivery of vaccine antigens into the cytoplasmic compartment of an antigen presenting cell (APC) for display in a MHC class I molecule on the cell surface. Therefore, vector-based approaches or nucleic acid vaccines that rely on antigen production within the target cell are most effective. Delivering vaccine antigens as purified proteins or even whole inactivated virus will primarily access the endocytic pathway for antigen presentation and lead to CD4+ T cell activation. While this is critical for antibody production and important for supporting CD8+ CTL development, it is not sufficient for inducing CD8+ CTL. In some cases a novel adjuvant or delivery system is able to provide access for these types of vaccines into the cytoplasmic compartment, but in general vector-based vaccines, including nucleic acids, are more potent methods for inducing CD8+ CTL. One exception is the use of peptides which incorporate a T cell epitope that can bind directly to an MHC class I molecule on the cell surface and induce CD8+ CTL responses. Vector-based vaccines, beginning with recombinant vaccinia products, were first evaluated in clinical trials in the late 1980‘s with the expressed purpose of achieving vaccine-induced CD8+ CTL responses. The induction of CD8+ CTL responses has been a primary focus of clinical trials since the mid 1990’s. The principal findings related to vaccine-induced CD8+ CTL responses in clinical trials of candidate HIV vaccines include:

1 Recombinant vaccinia expressing envelope glycoprotein only, or multiple antigens has consistently induced long-lived CD8+ CTL responses in vaccinia-naive subjects (98,102,115,116).

2 HIV-specific CD8+ CTL can be detected in a majority of subjects receiving recombinant poxvirus vectors, and in a subset CTL activity is detectable for >18 months. The activity is at the threshold of detection in classical 51Cr release assays requiring *in vitro* stimulation and is only detected in 15–30% of subjects at any given time point. However, unlike antibody responses, vaccine-induced CTL responses are broadly reactive (117). CTL induced by recombinant canarypox vectors has been shown to lyse target cells infected with primary R5 HIV-1 isolates from multiple clades (117). CD8+ CTL effectors have also been isolated from rectal mucosa from vaccinees suggesting that T cells induced by parenteral vaccination may provide some level of protection at mucosal surfaces (McElrath *et al.*, unpublished observations). Not only is classical MHC class I-restricted cytolytic activity induced, but vaccine-induced noncytolytic CD8+-mediated suppression of HIV-1 replication (118) has been demonstrated in recipients of recombinant canarypox vaccines (119).

3 Envelope subunits can induce CD4+ CTL, but rarely induce CD8+ CTL even when formulated with novel adjuvants (77,120). This is an expected result of obligate processing through the endocytic pathway leading to MHC class II presentation. New adjuvants and delivery systems may improve MHC class I presentation of purified protein antigens, but it is unlikely to ever approach the efficiency of antigens produced intracellularly by approaches such as nucleic acid immunization, live recombinant vectors, or live attenuated vaccines.

4 In one study with the Ty-gag-VLP without alum CD8+ CTL responses were detected in a few subjects (84).

In summary, vaccine approaches that are currently being evaluated in clinical trials can induce HIV-specific CD8+ CTL activity that is durable and can lyse cells infected with typical primary R5 HIV-1 isolates from multiple clades.

FUTURE DIRECTIONS

Timeline for HIV vaccine development. Although the need for an effective vaccine against HIV is urgent, what is a realistic timeline for identifying the approaches and products needed to achieve vaccine-induced immunity? In a May 18, 1997 commencement address at Morgan State University President Clinton challenged the scientific community to have a vaccine within 10 years. Thus, the vaccine development effort is motivated by political as well as humanitarian, social, ethical, and scientific purposes. To meet this timeline, I believe the following goals will have to be achieved: 1) identification of antigenic structures that can induce neutralizing antibody activity against primary R5 HIV-1 isolates, 2) development of new approaches to optimize the breadth

and magnitude of vaccine-induced memory CTL, and 3) performance of a large scale trial to test the concept that a vaccine-induced CD8+ CTL response will modify infection rates or alter the course of naturally acquired HIV infection.

Induction of R5 neutralizing antibody activity. Many R5 viruses are difficult to neutralize even with high titer, type-specific serum. The basis for the relative neutralization resistance is an active area of current research. Empiric approaches are being pursued to find envelope glycoprotein structures that can induce broad neutralizing antibody and include: 1) triggering the envelope glycoprotein into an intermediate structure present during the membrane fusion process, 2) producing envelope glycoproteins with selected mutations that alter glycosylation or folding patterns, 3) production of oligomeric envelope structures, 4) expression of envelope glycoproteins in vectors and virus-like particles, and 5) multi-epitope combinations. In addition, a more systematic approach combining information from structural biology, molecular biology, and epitope mapping to produce novel envelope structures is being pursued. Solving the problem of how to produce an antigenic structure that can induce a neutralizing antibody response effective against typical R5 HIV-1 isolates represents an important step toward addressing additional questions about the antibody response including: 1) will local induction of mucosal antibody be necessary for protection from HIV infection or will high titer serum antibody be sufficient, 2) can broadly neutralizing antibody be induced with a single antigenic structure, or will mixtures of envelope glycoprotein structures be required, 3) can durable antibody responses be induced, or will repeated booster immunizations be required?

Duration, magnitude, kinetics, and breadth of CTL response. There is evidence from studies in both humans and animal models that a robust CD8+ CTL response can control HIV infection. However, the character of those responses and relative degree of protection for a given level and breadth of CTL response have not been defined. There are candidate vaccines currently in Phase II clinical trials that can induce CD8+ CTL responses in a portion of subjects. Whether this level of CTL is sufficient for control of HIV infection is not known. However, based on studies in nonhuman primates, it is likely that vaccine induction of a robust CD8+ CTL response will lower the viral load set point. The large number of vector-based vaccine delivery approaches, emergence of cytokine adjuvants to specifically direct selected immune responses, and advances in methods used to enumerate T cell responses promise that strategies for consistent vaccine-induction of high magnitude CTL responses are within reach in a time frame of about 5 years. Future studies may need to address the following issues: 1) how broad does the CTL response need to be to control viremia, in terms of number and dominance hierarchy of epitopes, 2) does the CTL response measured in peripheral blood accurately predict control of viremia, or will specific induction of CTL responses in mucosal tissue or lymph nodes be required, 3) can the kinetics of the CTL response or efficiency of killing be influenced by vaccine formulation and delivery to improve protection, or is the outcome only determined by the magnitude of the response, and 4) will maintenance of sufficient CTL memory require booster immunizations?

The importance of efficacy trial evaluation. One of the next major steps in vaccine development will be the performance of larger scale trials in higher risk populations. The appropriate timing, vaccine approach, trial design, and trial location for such a study are issues of current controversy and debate. The performance of a Phase III clinical trial should be based on: 1) its potential for defining a biological impact of the vaccine on HIV-induced disease based on results from animal model studies and Phase I/II trials, 2) its potential for answering questions about correlates of immunity, and 3) the importance of establishing a benchmark against which future vaccine design and development can be measured. For example, if a Phase III study can be designed and executed that will show whether CD8+ CTL can control HIV viremia, the current vaccine strategies with potential CTL-inducing capacity would be accelerated with a focus on optimizing the magnitude of CD8+ induction. If induction of CD8+ CTL is not associated with any level of protection, then issues of breadth and compartmentalization of responses will need to be addressed more rigorously in animal models.

The ultimate vaccine that can prevent persistent HIV-1 infection will probably require a conceptual breakthrough in the understanding of how to elicit broadly neutralizing antibody against primary R5 HIV-1 isolates, and will also involve a number of iterative steps to achieve optimal HIV-specific CD8+ CTL responses. However, a vaccine aimed at control of viremia, delayed disease progression, and reduced transmission, based on induction of HIV-specific CD8+ CTL could have a significant impact on the AIDS epidemic, and may be within our grasp using currently available technology.

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Vaccine Antigen (HIV strain)	Production method	Adjuvant or vehicle	Vaccine developer	Site of study or AVEG/HVTN** protocol number(s)*	Reference for clinical trial
PHASE I STUDIES					
ENVELOPE PROTEINS					
rgp160 (LAI)	Baculovirus/insect cells	Aluminum Phosphate	MicroGeneSys	National Institutes of Health, Bethesda, MD	68
rgp160 (LAI)	Baculovirus/insect cells	Aluminum Phosphate	MicroGeneSys	003, 003A, 003B	69,70
rgp160 (IIIB)	Vaccinia/vero cells	$Al(OH)_3$ + Deoxycholate	IMMUNO-AG	004, 004A, 004B	71,72
Env 2-3 (SF-2)	Yeast	MF59 Å MTP-PE	Chiron/Biocine	005A, 005B, 005C; Geneva, Switzerland	73,74
rgp120 (IIIB) rgp120 (MN)	CHO cells	$Al(OH)_3$	Genentech	006	75
rgp120 (SF-2)	CHO cells	MF59	Chiron/Biocine	Univ. California-San Francisco	76
rgp120 (SF-2)	CHO cells	MF59 Å MTP-PE	Chiron/Biocine	007A, 007B, 007C	77
rgp120 (MN) rgp120 (IIIB)	CHO cells	$Al(OH)_3$	Genentech	009	78
rgp160 (MN)	Vaccinia/vero cells	$Al(OH)_3$ + Deoxycholate	IMMUNO-AG	013A	79
rgp160 (MN) rgp120 (IIIB)	Vaccinia/vero cells CHO cells	$Al(OH)_3$ + Deoxycholate $Al(OH)_3$	IMMUNO-AG Genentech	013B	80
rgp160 (MN/LAI) V3 loop of gp120 (MN)	Vaccinia/BHK-21 cells Synthetic linear peptide	$Al(OH)_3$ Mineral oil + mannose monooleate (IFA)	Pasteur-Merieux Connaught	Paris, France	81
rgp120 (SF-2)	CHO cells	Multiple Adjuvants†	Chiron/Biocine	015	Manuscript in preparation‡
rgp120 (MN)	CHO cells	QS-21 and/or $Al(OH)_3$	Genentech (VaxGen)	016, 016A, 016B, 036	Manuscript in preparation
rgp120 (SF-2)	CHO cells	MF59	Chiron/Biocine	024	control for AVEG 022a
rgp120 (MN/A244) (Clades B & E)	CHO cells	QS-21 and/or $Al(OH)_3$	VaxGen	036	in progress

rgp120 (SF-2)	CHO cells	MF59	Chiron/Biocine	WRAIR§/Thailand	82
rgp120 (W61D) (1o R5X4, Clade B)	CHO cells	3D-MPL + QS21 in oil-in-water emulsion (SBAS) $Al(OH)_3$	Smith-Kline Beecham Biologics	UK	83
NONENVELOPE PROTEINS					
Ty p17/p24 VLP (LAI)	Yeast transposon	None	British Biotech PLC	UK	84
Ty p17/p24 VLP (LAI)	Yeast transposon	None or $Al(OH)_3$	British Biotech PLC	019	Manuscript in preparation
p24 gag	CHO cells	MF59	Chiron	University of Nebraska	NA
Tat (LAI)	E. coli	NA	B. Ensoli	Italy	in progress
PEPTIDES					
Gag lipopeptide P3C541b	Synthetic linear peptide	lipid conjugate	United Biomedical, Inc.	021	Manuscript in preparation
HGP-30 (LAI p17)	Synthetic linear peptide	KLH	Viral Technologies	George Washington University Medical Center, Washington, DC	85-87
V3 loop of gp120 (MN)	Synthetic octameric peptide	$Al(OH)_3$	United Biomedical, Inc.	011; Yunnan, China; St. Vincent's Hospital, Sydney, Australia; Bangkok, Thailand	88-91
V3 loop of gp120 (MN)	Synthetic linear peptide	PPD	A. Rubinstein	Albert Einstein College of Medicine	92
V3 loop of gp120 (15 strains)	Synthetic octameric peptide	$Al(OH)_3$	United Biomedical, Inc.	017	Manuscript in preparation
V3 loop of gp120 (MN)	Synthetic octameric peptide $\mathring{A}$ encapsulation	$Al(OH)_3$	United Biomedical, Inc.	018	Manuscript in preparation
V3 loop of gp120 (MN)	Synthetic octameric peptide $\mathring{A}$ encapsulation	$Al(OH)_3$	United Biomedical, Inc.	023	Manuscript in preparation
HIV-1 gp120 C4-V3 peptides (MN, EV91, RF, CANO)	Hybrid polyvalent synthetic peptides	Mineral oil + mannose mono-oleate (IFA)	Wyeth-Lederle Vaccines with Barton Haynes, Duke University	020	Manuscript in preparation

Epitopes from nef, gag (LAI), and V3 from envelope of BX08	mixture of synthetic linear peptides	lipid conjugate in micelles	BACHEM Feinchemikalien, Bubendorf, Switzerlandand & Sterilyo, Saint-Amand-les-Eaux, France	France	93
DNA VACCINES					
env/rev (APL 400-003)	GeneVax® DNA plasmid backbone	Bupivacaine ("facilitator")	Apollon	National Institutes of Health, Bethesda, MD	in progress
gag/pol (APL 400-047) (HXB2) Canarypox-HIV gp120, TM gp41, gag, protease, (MN/LAI) (vCP205) or Canarypox-HIV gp120, TM gp41, gag, protease, nef, pol (MN/LAI) (vCP1452)	GeneVax® DNA plasmid backbone Recombinant canarypox	Bupivacaine ("facilitator")	Apollon Aventis Pasteur	031 031 amendment	in progress in progress
env/rev (APL 400-003)	GeneVax® DNA plasmid backbone	Bupivacaine ("facilitator")	Apollon	University of Pennsylvania & Hahnemann MCP University	94
gag	plasmid		Merck Research Laboratories	Multiple sites in U.S.	in progress
Multiple HIV-1 epitopes (HIVA)	pTHr plasmid vector		Medical Research Council, Andrew McMichael, Oxford, UK	UK, Kenya	in progress, 95
gp160 (MN) Canarypox-HIV gp120, TM gp41, gag, protease (MN/LAI) (vCP205)	GeneVax® DNA plasmid backbone Recombinant canarypox	Bupivacaine ("facilitator")	Apollon Aventis Pasteur	WRAIR	in progress
POX VIRUS RECOMBINANTS/COMBINATION					
Vaccinia-gp160 [HIVAC-1e] (LAI)	Recombinant vaccinia		Bristol-Myers Squibb/Oncogen	Zaire	96
Vaccinia-gp160 [HIVAC-1e] (LAI) rgp160 (LAI)	Recombinant vaccinia Baculovirus/insect cells		Bristol-Myers Squibb/Oncogen MicroGeneSys	Univ. of Washington-Seattle	97,98
Vaccinia-gp160 [HIVAC-1e] (LAI) rgp160 (LAI)	Recombinant vaccinia Baculovirus/insect cells	$Al(OH)_3$	Bristol-Myers Squibb/Oncogen MicroGeneSys	002, 002A, 002B	99-101
Vaccinia-gp160 [HIVAC-1e] (LAI) rgp120 (SF-2) or Env 2-3 (SF-2)	Recombinant vaccinia CHO cells or yeast	MF59	Bristol-Myers Squibb/Oncogen Chiron/Biocene	008	Manuscript in preparation

Vaccinia-gp160 [HIVAC-1e] (LAI) rgp120 (SF-2) or rgp120 (LAI) or rgp120 (MN) or rgp160 (MN)	Recombinant vaccinia CHO cells Vaccinia/vero cells	MF59 $Al(OH)_3$ $Al(OH)_3$ + Deoxy-cholate	Bristol-Myers Squibb/Oncogen Chiron/Biocine Genentech IMMUNO-AG	010	102
Canarypox-gp160 (MN) (vCP125) rgp160 (MN/LAI)	Recombinant canarypox	PCPP	Pasteur-Merieux Connaught	Hopital de l'Institut Pasteur, Paris, France	103,104
Canarypox-gp160 (MN) (vCP125) rgp120 (SF-2)	Recombinant canarypox CHO cells	MF59	Pasteur-Merieux Connaught Chiron/Biocine	012A, 012B	105
Vaccinia-HIV Env/gag/pol (TBC-3B)	Recombinant vaccinia		Therion Biologics	014A	in progress
Vaccinia-HIV Env/gag/pol (TBC-3B) rgp120 (MN)	Recombinant vaccinia CHO cells	$Al(OH)_3$	Therion Biologics VaxGen	014C	in progress
Canarypox-HIV gp120, TM gp41, gag, protease (MN/LAI) (vCP205) rgp120 (SF-2)	Recombinant canarypox CHO cells	MF-59	Pasteur-Merieux Connaught Chiron/Biocine	022, 022A	Manuscript in preparation
Canarypox-HIV gp120, TM gp41, gag, protease, CTL epitopes in pol and nef (MN/LAI) (vCP300) rgp120 (SF-2)	Recombinant canarypox CHO cells	MF59	Pasteur-Merieux Connaught Chiron/Biocine	026	106
Canarypox-HIV gp120, TM gp41, gag, protease (MN/LAI) (vCP205)¶ rgp120 (MN)	Recombinant canarypox CHO cells	$Al(OH)_3$	Pasteur-Merieux Connaught VaxGen	027	in progress
Canarypox-HIV gp120, TM gp41, gag, protease (MN/LAI) (vCP205) rgp120 (SF-2)	Recombinant canarypox CHO cells	MF59	Pasteur-Merieux Connaught Chiron/Biocine	029	107
Canarypox-HIV gp120, TM gp41, gag, protease (MN/LAI) (vCP205) APL 400-047 HIV-1 core structural proteins (HXB2 - gag/pol)	Recombinant canarypox GeneVax® DNA plasmid backbone	GM-CSF Bupivacaine ("facilitator")	Pasteur-Merieux Connaught Immunex Apollon	033 033 amendment	in progress
Canarypox-HIV gp120, TM gp41, gag, protease (MN/LAI) (vCP205) rgp120 p24	Recombinant canarypox CHO cells	MF59	Aventis-Pasteur Chiron	032	manuscript in preparation
Canarypox-HIV gp120, TM gp41, gag, protease, (MN/LAI) (vCP205)					
Canarypox-HIV gp120, TM gp41, gag, protease, (MN/LAI) (vCP205)	Recombinant canarypox		Aventis Pasteur	034	in progress

Canarypox-HIV gp120, TM gp41, gag, protease (MN/LAI) (vCP205) p24/V3 peptide	Recombinant canarypox synthetic peptide		Aventis-Pasteur	France	108
Canarypox-HIV gp120, TM gp41, gag, protease, (MN/LAI) (vCP205) (o)gp140	Recombinant canarypox vaccine/vero cells	PCPP or $Al(OH)_3$	Aventis Pasteur	WRAIR	manuscript in preparation
Canarypox-HIV gp120, TM gp41, gag, protease, (MN/LAI) (vCP205)	Recombinant canarypox		Aventis Pasteur	Case Western Reserve University/Uganda	in progress
Canarypox-HIV gp120 (92TH023), TM gp41(LAI), gag/protease (LAI) vCP1521 (Clade E) (o)gp140 (92TH023/LAI) or rgp120 (SF-2/CM235)	Recombinant canarypox vaccinia-infected Vero cells CHO cells	PCPP MF59	Aventis-Pasteur Aventis Pasteur Chiron	WRAIR/Thailand	in progress
Canarypox-HIV gp120 (92TH023), TM gp41(LAI), gag/protease (LAI) vCP1521 (Clade E) rgp120 (MN/CM240)	Recombinant canarypox CHO cells	Alum	Aventis-Pasteur VaxGen	WRAIR/Thailand	in progress
Modified Vaccinia Ankara (MVA) with HIV-A polyepitope insert	Recombinant MVA		Andrew McMichael	UK	in progress
SALMONELLA TYPHI RECOMBINANT/COMBINATION					
Salmonella typhi CVD 908 - HIV-1 LAI rgp120 (VVG 203) rgp120 (MN)	Live attenuated recombinant Salmonella typhi CHO cells	$Al(OH)_3$	University of Maryland Center for Vaccine Development VaxGen	028	in progress

PHASE II STUDIES

ENVELOPE PROTEINS					
rgp120 (SF-2)	CHO cells	MF59	Chiron/Biocine	201	109
rgp120 (MN)	CHO cells	$Al(OH)_3$	Genentech	201	109
rgp120 (SF-2/CM235) (1o clade E)	CHO cells	MF59	Chiron	WRAIR/Thailand	manuscript in preparation
POX VIRUS RECOMBINANTS/COMBINATIONS					

Canarypox-HIV gp120, TM gp41, gag, protease (MN/LAI) (vCP205) rgp120 (SF-2)	Recombinant canarypox CHO cells	MF59	Pasteur-Merieux Connaught Chiron/Biocine	202	manuscript in preparation
Canarypox-HIV gp120, TM gp41, gag, protease, nef, pol (MN/LAI) (vCP1452) rgp120 (MN and GNE8)	Recombinant canarypox CHO cells	$Al(OH)_3$	Aventis Pasteur VaxGen	203	in progress
Canarypox-HIV gp120, TM gp41, gag, protease, nef, pol (MN/LAI) (vCP1452) rgp120 (MN)	Recombinant canarypox CHO cells	$Al(OH)_3$	Aventis Pasteur VaxGen	HIVNET 026, Haiti, Trinidad, Brazil	in progress

PHASE III STUDIES

ENVELOPE PROTEINS					
rgp120 (MN and GNE8) Clade B	CHO cells	$Al(OH)_3$	VaxGen	Multi-Center VaxGen	Interim analysis 11/2001
rgp120 (MN and A244) Clades B & E	CHO cells	$Al(OH)_3$	VaxGen&Thailand	Interim analysis 2002	

NA = Not Available

* Multiple protocol numbers indicate that significantly different doses, schedules, or administration routes were evaluated. More details about AVEG/HVTN trial design can be found on the internet at http://scharp.org.

** AVEG = AIDS Vaccine Evaluation Group, HIVNET = HIV Network for Prevention Trials, now both incorporated into the HIV Vaccine Trials Network (HVTN)

• rgp120(SF-2) administered with one of seven adjuvants ($Al(OH)_3$, Monophosphoryl lipid A [MPL, Ribi ImmunoChem Research], liposome-encapsulated MPL with Alum [Walter Reed Army Institute of Research], MF59 [Chiron Vaccines], MF59 + MTP-PE [Chiron Vaccines], SAF/2 [Chiron Vaccines], SAF/2 with MDP [Chiron Vaccines]) to 15-16 volunteers. The rgp120 (SF-2) + MPL group was not offered an 18-month immunization.

¶ Alternate routes of administration: IM, oral, intranasal, intrarectal, intravaginal, IM+intranasal, IM+intrarectal

‡ Unpublished studies that have not been performed within the AVEG/HVTN system may not be listed.

• WRAIR = Walter Reed Army Institute of Research

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