

PROGRESS TOWARD AN HIV VACCINE

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■ **Abstract** The development of an HIV vaccine is proving to be an unprecedented challenge. The difficulty in creating this vaccine arises from the enormous genetic variation of the virus and the unusual importance of cytotoxic T lymphocytes (CTL) in controlling its spread. Whereas traditional vaccine strategies are unlikely to confer safe and effective HIV protection, novel strategies for eliciting CTL have provided substantial clinical benefits in nonhuman primate model systems. These vaccine strategies, including plasmid DNA and live recombinant vectors, are currently being evaluated in human clinical trials.

INTRODUCTION

An effective vaccine is needed to stop the continuing spread of HIV worldwide. However, the development of an AIDS vaccine is proving to be an enormous scientific challenge. This article reviews aspects of the biology of HIV that make it a uniquely difficult target for vaccine protection. I summarize the reasons that traditional approaches to creating an antiviral vaccine have failed to provide protection against HIV infection. I then describe novel vaccination strategies being explored as potential approaches to generating protective immunity. Finally, I review experimental findings in nonhuman primate models suggesting that currently available vaccine technologies may allow the elicitation of cellular immune responses that can attenuate the clinical disease induced by HIV, even if they do not actually prevent infection.

THE BIOLOGY OF HIV INFECTIONS

A number of unique characteristics of the biology of HIV infections make the creation of an HIV vaccine particularly challenging. HIV is spread both venereally and by contaminated blood products. It is reasonable, therefore, to assume that an effective HIV vaccine must elicit mucosal immune responses to contain venereal spread and systemic immune responses to control hematogenous spread. Moreover,

HIV is probably transmitted both as cell-free and as cell-associated virus. Because cell-free virus can only be eliminated through binding to neutralizing antibody and cell-associated virus by cell-mediated immune responses, a vaccine may have to elicit both types of immune responses to protect against HIV. Most troubling, high levels of viral replication persist in infected individuals despite ongoing humoral and cell-mediated immune responses to HIV. This persistence of viral replication suggests that it may simply not be possible to generate immune protection against the virus.

A worrying aspect of the biology of HIV for those attempting to create an AIDS vaccine is the extraordinary genetic diversity of the virus (1). This diversity is apparent both in a single infected individual and at a global level in geographically disparate infected people. Because of the inaccuracy of the replication machinery of the virus, new mutations are introduced into virtually every virion generated in an infected individual. As many as a billion new and unique viral particles can be created each day in an infected person, so the virus population in an individual must be considered a swarm or quasi-species. Viruses can be so diverse that a particular antibody may neutralize one virion but not another in the same infected individual. At a global level, the genetic diversity of the virus is manifest in distinct HIV subtypes or clades that cluster epidemiologically in distinct geographic regions. The AIDS epidemic in the Western hemisphere and Western Europe is caused by clade B viruses, while the epidemic in sub-Saharan Africa and the Indian subcontinent is due to clade C viruses.

NONHUMAN PRIMATE MODELS FOR HIV VACCINE EVALUATION

Powerful nonhuman primate models have been developed for assessing HIV vaccine strategies. HIV is a member of a large family of primate lentiviruses. Several African nonhuman primate species are endemically infected with species-restricted lentiviruses known as simian immunodeficiency viruses (SIVs) (2). Interestingly, these SIVs usually do not cause disease in their natural host species. Recent evidence indicates that isolated wild populations of chimpanzees are infected with viruses very similar to HIV (3). These chimpanzee viruses appear to have evolved through recombination of distinct SIV isolates. This HIV-like virus does not usually cause disease in chimpanzees, but it appears to have infected humans zoonotically to initiate the AIDS epidemic.

In view of the very close relationship between these various primate lentiviruses, it is not surprising that AIDS can evolve in a number of primate species following infection with selected SIVs. Some SIV isolates obtained from African monkey species can infect and induce AIDS in Asian macaque species (4). Further, chimeric viruses have been created in the laboratory that express various HIV envelopes on an SIV backbone. Some of these chimeric viruses, referred to as simian human immunodeficiency viruses (SHIVs), also cause AIDS in Asian monkeys (5). The

SIV- and SHIV-infected macaques have provided useful models for the study of AIDS pathogenesis and vaccine strategies to prevent HIV infection.

IMMUNE CONTROL OF HIV REPLICATION

HIV replication in acutely infected individuals follows a stereotypic pattern. A dramatic burst of replication occurs within a few weeks of virus exposure. Partial containment of the replicating virus is then almost always seen in the ensuing weeks. This limited inhibition of virus spread appears to be a manifestation of a partially successful antiviral immune response. Considerable effort has been devoted to characterizing the immune mechanisms that mediate this partial viral containment.

Antibodies that bind and neutralize HIV, blocking its ability to infect cells, can be shown to contain the spread of the virus under experimental conditions. Monoclonal antibodies have been generated that bind to diverse HIV isolates and block their ability to infect cells *in vitro* (6). If very high levels of these antibodies are achieved *in vivo* by passive administration, SHIV infections can be prevented in monkeys (7). However, in the setting of natural infections, evidence of an important role for HIV-neutralizing antibodies in viral containment has proved elusive (8). Antibody responses in HIV-infected individuals have only low titers of virus-neutralizing activity. Moreover, the emergence of an HIV-neutralizing antibody response following an HIV infection can only be detected several weeks after the partial containment of virus replication. The absence of a temporal correlation between early viral control and the development of a neutralizing antibody response has called into question the importance of antibody in early HIV control.

Nevertheless, making the reasonable assumption that neutralizing antibody will be needed to block the establishment of infection by cell-free virus, investigators have attempted to create subunit immunogens that elicit neutralizing antibody responses. They have, however, been unsuccessful in creating vaccines that elicit antibodies that bind to the neutralization-sensitive domains of the virus. The creation of an immunogen that can induce an antibody response capable of neutralizing a variety of HIV isolates remains a major challenge in the development of an HIV vaccine.

HIV differs from viruses for which successful vaccines have been developed in that it is controlled predominantly by a cellular rather than humoral immune response. Diverse evidence supports the importance of the cellular immune response in HIV containment. CD8⁺ T lymphocytes can inhibit the replication of HIV in CD4⁺ T lymphocytes *in vitro*, probably through direct cytotoxicity and the production of soluble factors including beta chemokines (9, 10). The clinical status of infected individuals is associated with the level of virus-specific CD8⁺ cytotoxic T lymphocytes (CTL) in their peripheral blood; high levels are predictive of good clinical status (11). The early containment of HIV replication in acutely infected individuals coincides with the emergence of an HIV-specific CTL response (12).

The most direct evidence for the importance of CD8+ lymphocytes in controlling HIV replication comes from studies in monkeys (13). Monkeys depleted of CD8+ lymphocytes by administration of a monoclonal anti-CD8 antibody and then infected with SIV never controlled viral replication and died with an accelerated disease course. These accumulating studies make a compelling argument for the importance of CD8+ CTL in controlling HIV replication and suggest that an effective HIV vaccine should elicit high-frequency CTL responses.

The extraordinary rate of mutation of HIV has a profound impact on immune control of viral replication. Recent studies have demonstrated that HIV continually accrues mutations that enable it to escape recognition by neutralizing antibodies in chronically infected individuals (14, 15). Interestingly, however, there is no evidence of an association between this process and abrupt clinical deterioration in infected subjects. Mutations have also been shown to result in a loss of HIV and SIV recognition by CTL in both acutely and chronically infected individuals (16, 17). In many of the documented instances of viral escape from CTL recognition, this escape phenomenon is associated with an abrupt increase in viral replication and decrease in immune function in the infected individuals. Thus, the phenomenon of viral mutation away from recognition by antibodies and CTL appears to be a central reason why the immune system eventually fails to contain HIV replication.

TRADITIONAL VACCINE STRATEGIES

Given the unique biology of HIV and its interactions with the immune system, it is not surprising that traditional strategies for vaccination are not proving useful in protecting against HIV infection. Live attenuated virus, inactivated virus, and recombinant protein strategies for vaccination safely protect against a variety of viral pathogens in humans. However, these strategies are not likely to be useful for vaccinating against HIV.

Many viruses can be mutated through tissue culture passage such that they remain infectious but lose their pathogenic potential. Such pathogenically attenuated viruses have provided safe and effective protection against smallpox, measles, and polio. Data in monkeys indicated that this strategy protects against SIV infection (18). Large deletions made in certain of the nonstructural genes of SIV resulted in the virtual elimination of early pathogenic consequences of infection in adult monkeys. Moreover, the monkeys infected with these gene-deleted viruses were protected from subsequent infection with pathogenic SIV. However, adult monkeys infected with these pathogenically attenuated viruses were found to develop disease with a delayed onset, and newborn monkeys developed disease soon after infection (19). Similarly, a cohort of humans who received a blood product infected with an HIV isolate that had a large genetic deletion were at first reported to be free of disease. Later, however, these individuals did develop AIDS (20). Thus, accumulating experience has raised serious questions concerning the safety of this vaccine modality for HIV, and there is little enthusiasm among investigators at the present time for pursuing this vaccine approach for AIDS.

Physically or chemically inactivated virus immunogens provide effective immunity in humans against influenza and polio. Inactivated virus vaccines have also provided effective immunity in monkeys against SIV (21). However, this immunity has been very restricted in the diversity of viral isolates against which it protects. Moreover, the duration of the protection conferred by vaccination with inactivated virus immunogens was quite short. Because vaccines of this type will not elicit antibody responses that neutralize diverse HIV isolates and these immunogens do not generate CTL, there is little optimism that this type of approach will ultimately prove useful. Nevertheless, particle immunization strategies are being pursued with the hope that these immunogens may provide benefit if combined with another vaccine modality. At this time, studies are being done with noninfectious virus-like particles rather than virions, since virus-like particles are readily manipulated and are safer than inactivated virus.

Highly purified viral protein that is expressed in mammalian or bacterial cells using recombinant DNA technologies can be used as a vaccine antigen. This is the approach that produces the immunogen used in the successful hepatitis B vaccine. Because recombinant protein vaccines are easy to produce and safe to administer, a good deal of effort has been devoted to evaluating recombinant envelope glycoprotein-based HIV vaccine strategies. This avenue of HIV vaccine development has, however, been quite controversial. Proponents point to the success of this approach in the creation of other vaccines. Critics point out that these immunogens, like inactivated virus immunogens, fail to elicit antibodies that neutralize diverse HIV isolates and cannot elicit CTL responses. They argue, therefore, that recombinant protein vaccines have not been shown to induce either of the immune responses that might contain HIV replication. Although a broad scientific consensus was never reached for pursuing a recombinant-protein-based HIV vaccine strategy, a biotechnology company raised sufficient investment capital to produce and test such a vaccine. Data from two recently concluded efficacy trials of these vaccines in the United States and Thailand show no evidence of protection against HIV infection (22). These disappointing results bolster the argument for moving forward in the future with scientifically defensible HIV vaccine strategies.

NOVEL VACCINE STRATEGIES

In light of the limitations of traditional vaccine strategies for preventing HIV infections, investigators have been exploring novel approaches. The most promising of these are the use of plasmid DNA and live recombinant vectors.

It was shown approximately a decade ago that intramuscular inoculation of a plasmid encoding a viral gene under the control of a potent promoter elicits both antibody and cell-mediated immune responses in laboratory animals (23). This vaccine modality is a safe and effective means of eliciting CTL responses in nonhuman primates (24). Although plasmid DNAs have been less immunogenic in early-phase clinical testing in humans than in laboratory animals, a number of changes in plasmid DNA constructs have been shown to increase their

immunogenicity. These changes include altering the nucleotides of the viral genes to optimize the ability of the plasmids to produce viral proteins in mammalian cells, and altering the regulatory elements in the plasmids. Exploratory work is also being pursued to increase the immunogenicity of these vaccines using plasmid cytokines and novel formulations with polymers.

Live recombinant microorganisms are also being evaluated as potential HIV vaccines. Genes of HIV and SIV can be engineered into microorganisms that have proved safe and effective as live attenuated vaccines, such as the smallpox vaccine virus vaccinia or the tuberculosis vaccine BCG (25, 26). As these engineered viruses and bacteria replicate in the inoculated individual, immunity is developed to both the vector and the HIV gene product. Importantly, since these are live, replicating organisms, both humoral and cellular immune responses are generated in vaccine recipients.

The best studied of the live vectors are the poxviruses. Although vaccinia virus might be an effective vector for HIV genes, safety issues preclude its use as an HIV vaccine. Vaccinia virus was reported to disseminate and cause a fatal encephalitis in an immunosuppressed HIV-infected individual (27). There is a well-founded concern that if a worldwide HIV eradication effort were mounted, a recombinant vaccinia virus vaccine would cause fatal disease in undiagnosed immunosuppressed individuals in areas where HIV infection is endemic. Therefore, most work using poxviruses as vectors for HIV immunization has been done with viruses that undergo an abortive replication cycle in human cells. These viruses, which include modified vaccinia Ankara (MVA) and the avian poxviruses canarypox and fowlpox (FPV), can produce sufficient HIV protein during an abortive cycle of replication to initiate both humoral and cellular anti-HIV immune responses. These recombinant vectors have proved immunogenic in nonhuman primates (28, 29). A trial in Thailand is assessing the efficacy of a recombinant canarypox vaccine delivered with a recombinant HIV envelope protein, although its immunogenicity has been quite disappointing in human trials to date (30). Early-phase human clinical trials with recombinant MVA and FPV HIV constructs are ongoing.

Studies with recombinant adenovirus vectors have been particularly promising (31). Adenovirus serotype 5, made replication-incompetent by deletion of its E1 and/or E3 gene(s), has proved highly immunogenic as a vector for HIV gene products in small laboratory animals and nonhuman primates (32). In fact, such HIV vaccine constructs are now entering advanced-phase human clinical trials. Studies are demonstrating that recombinant adenovirus can elicit HIV-specific T cell immune responses in human volunteers. Problematic with this vaccine approach, however, is the emerging evidence that pre-existing immunity to the vector significantly decreases the immunogenicity of these constructs (33). Several strategies are being explored to circumvent this problem. Vaccination with plasmid DNA constructs prior to recombinant adenovirus boost is being evaluated as a means of augmenting vaccine-elicited immunity in individuals with pre-existing antibody responses to adenovirus serotype 5 (34). Work is also ongoing to explore the immunogenicity of rare-serotype human adenoviruses and chimpanzee adenoviruses

as HIV vaccine vectors; the reasoning is that the properties of these viruses as vectors should resemble those of serotype 5 adenovirus, but human populations should not have significant pre-existing immunity to these viruses.

Other live recombinant vectors are also being explored as potential HIV vaccine modalities. These include single-strand RNA alpha viruses (Semliki forest virus and Venezuelan equine encephalitis virus) and the parvovirus adeno-associated virus. The pathogenically attenuated mycobacterium *Bacille Calmette-Guerin* is also being evaluated as an HIV vector in preclinical and early-phase human testing.

Although plasmid DNA and recombinant live vector strategies can elicit cellular immune responses, a protein immunogen will probably be required to elicit an antibody response capable of neutralizing the virus. The creation of such a protein immunogen is, however, an enormous challenge. A number of strategies are being pursued to create an immunogen that will generate a broadly neutralizing antibody response. Although a protein immunogen can be designed to elicit an antibody that neutralizes a single HIV isolate, that antibody is unlikely to neutralize a significant number of other HIV isolates due to their extreme genetic diversity. Because regions of the envelope that are genetically conserved appear to be shielded from access to antibodies by highly variable loop structures and sugars, envelope proteins with variable loops and glycosylation sites deleted are being evaluated as immunogens (35, 36). New data on the structure of native virions indicate that the envelope glycoprotein exists as a trimer (37). Therefore, trimeric envelope immunogens are being assessed for their ability to elicit broadly neutralizing antibodies. Since monoclonal antibodies have been generated that can neutralize a wide spectrum of HIV isolates, there is reason to assume that it will ultimately be possible to configure an immunogen that can elicit a broadly neutralizing antibody response (38, 39).

VACCINE IMMUNITY AND PREVENTION OF CLINICAL DISEASE

All viral vaccines in use today in humans provide sterilizing immunity against infection through elicitation of neutralizing antibody responses. No immunogen yet created can elicit an antibody response that neutralizes diverse HIV isolates, so there is little reason to suppose that any currently available vaccine might provide broad protection against HIV infection. However, recent studies in nonhuman primates suggest that existing vaccine technologies might confer important clinical benefits in humans, although they fall short of providing complete protection against infection.

Monkeys that received plasmid DNA and/or live recombinant vectors developed high-frequency CTL responses that achieved partial control of AIDS virus replication (40, 41). Vaccine-elicited memory CTL rapidly expanded upon exposure to the replicating virus, blunting early peak viral replication and substantially

decreasing the long-term setpoint level of viral replication. As a result of this decrease in viral replication, the vaccinated monkeys demonstrated a dramatically slowed progression of clinical disease.

Although virus-host interactions in the nonhuman primate models differ in important ways from those in HIV-infected humans, there is reason to suppose that a similar CTL-based partial containment of HIV replication might occur in humans receiving these types of vaccines. Lowered setpoint viral loads in HIV-infected individuals should be associated with slowing of disease progression. Moreover, with low levels of replicating virus, such individuals might be expected to have low levels of virus in their secretions and therefore transmit virus inefficiently (42). If that proves to be the case, a CTL-based vaccine may slow the spread of HIV in human populations. A vaccine that slows both the progression of AIDS and the spread of the virus would be of enormous benefit, particularly in regions of the world with inadequate financial resources and medical infrastructure for the distribution of highly active antiretroviral drugs.

However, recent nonhuman primate studies demonstrate the limitations of this CTL-based vaccine approach in disease protection. Vaccinated monkeys that demonstrated early containment of SHIV and SIV replication with associated benign clinical courses developed abrupt rises in viral replication and clinical deterioration (43, 44). In all of these cases, viruses in the infected monkeys had developed mutations that allowed them to escape recognition by circulating CTL. These mutant viruses became the predominant viruses in the infected animals, were not controlled by the cellular immune responses, and eventually caused immune deterioration and death. Thus, viral escape from CTL may be a general mechanism for vaccine failure with CTL-based vaccines. It is hoped that the accumulation of such mutations by HIV will be slow if the vaccine-elicited immune responses control viral replication at low levels.

DIFFICULTIES ASSOCIATED WITH CLINICAL TRIALS OF HIV VACCINES

As we move HIV vaccine candidates into advanced-phase efficacy trials in human volunteers, the daunting difficulties associated with these trials are becoming clear. Efficacy trials of usual viral vaccine candidates have used vaccine-elicited antibody responses and acquisition of infection as the sole endpoints. Measuring these outcomes is relatively easy. However, the most promising HIV vaccine candidates at this time are immunogens that elicit CTL, and the hoped-for benefit is containment of acquired virus rather than sterile protection. Therefore, the endpoints of the upcoming HIV vaccine trials are likely to include measures of vaccine-elicited cellular immune responses and viral control following infection. Measures of viral control will include plasma viral RNA levels and peripheral blood CD4+ T lymphocyte counts. Quantitation of virus-specific cellular immune responses in lymphocyte samples from vaccinated individuals in multiple developing-world sites is proving a challenge. Further, the clinical endpoints of viral load and CD4+

T lymphocyte counts would be significantly altered if subjects who become infected during the trial commence treatment with antiretroviral drugs. The acquisition and precise quantitation of these clinical parameters will be challenging.

Because the worldwide AIDS epidemic is concentrated in the developing world, vaccine trials must be carried out in sub-Saharan Africa and Asia. Vaccine efficacy trials have never been done before in these regions, so the infrastructure to carry out this work does not exist there. The development of that infrastructure is difficult and expensive. Nevertheless, these challenges must be met to carry out the upcoming trials of HIV vaccines.

CONCLUSION

Although neutralizing antibodies should be able to prevent HIV infection, vaccine immunogens that elicit antibodies that neutralize diverse HIV isolates have not been created. With a growing appreciation for the importance of CTL in containing HIV replication, efforts are being made to develop immunogens that elicit high-frequency HIV-specific CTL responses. Such vaccines are not likely to confer sterile protection against HIV infection. However, nonhuman primate studies have suggested that these vaccines may elicit immune responses that contain the spread of HIV and slow the progression of disease in individuals who become infected. Considerable effort is now being devoted to creating such vaccines and testing them in the developing world.

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