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Review Article

New vaccine strategies for chronic viral infections

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CONTENTS

Abstract

Vaccination is the most efficient and cost-effective strategy to prevent viral diseases and control infections. Through vaccination it has been possible to achieve unprecedented results, *e.g.*, the worldwide eradication of very serious infectious diseases such as smallpox. Most vaccines currently available are effective against viruses associated with acute infections. The development of vaccines against chronic viruses appears to be more complex. Increasing knowledge in the fields of virology, immunology, biotechnology and molecular biology is essential for the development of new vaccines, in particular those against chronic viral infections. This review primarily focuses on the different strategies employed towards the development of an anti-HIV vaccine, in order to possibly control the global AIDS pandemic. It also discusses the many different approaches that have been tried and tested for the development of an HIV vaccine, from live attenuated to recombinant vaccines. Although such a vaccine is not yet available, the efforts that have been made thus far have provided many important clues that will be essential for developing new vaccine approaches.

Introduction

A vaccine induces immunological memory that protects from subsequent encounters with a given pathogen. Vaccines confer different degrees of protection. A good vaccine should be able to confer a "sterilizing" immunity that provides complete protection from an initial infection. This goal is not easy to achieve, and a vaccine that allows the clearance of both the pathogen and infected cells, resulting in an abortive infection, is also useful. Finally, a vaccine may not protect from the infection but may control it, resulting in a persistent infection that does not evolve to disease. Several antiviral vaccines prevent the disease but not the infection.

The use of vaccines in the history of medicine began without any knowledge of how protection was achieved. Today, advances in immunology allow us to better understand what a vaccine stimulates and how it mediates a protective effect (1). The first antiviral vaccines were live attenuated viruses. Due to safety concerns, viruses inactivated by chemical or physical agents were subsequently developed, followed by vaccines that used purified or synthetic proteins. Progress in molecular biology has facilitated the development of new vaccines using DNA and viral vectors.

Traditional vaccines comprised of attenuated or inactivated infectious agents, as well as certain new vaccines that employ protein subunits, induce humoral immunity. Neutralizing antibodies are fundamental for inducing vaccine-mediated protection against most viruses. Neutralization results when antibodies bind to the viral particle and subsequently inhibit virus attachment to the membrane of the target cell (2). Protection may also be achieved by immune mechanisms other than neutralization, such as phagocytosis and activation of CD8+ cells. Vaccines that prevent chronic infections generally induce neutralizing antibodies. Antibodies are fundamental for preventing chronic infection, while cell-mediated responses control the infection by protecting against disease progression (1).

From an immunological point of view, infectious agents can be divided into two categories. The first consists of agents causing acute infections ("hit and run"), such as smallpox, poliovirus, measles, rubella, diphtheria, tetanus, Ebola, SARS coronavirus or influenza, which are normally cleared during resolution of the natural disease. The second category includes agents causing chronic

Table I: Existing and future vaccines.

Vaccine	"Hit and run" viruses (acute infection)	"Hit and stay" viruses (chronic infection)
Existing	Poliovirus Measles virus Mumps virus Rubella virus Influenza virus Hepatitis A virus Yellow fever virus Japanese encephalitis virus Rabies virus Rotavirus Smallpox virus Tick B encephalitis	Hepatitis B virus Varicella-zoster virus Adenovirus
Future	Respiratory syncytial virus Parainfluenza virus Dengue virus West Nile virus SARS coronavirus	Papillomavirus Cytomegalovirs Herpes simplex virus Epstein-Barr virus Hepatitis C virus HIV

infections ("hit and stay"), such as Epstein-Barr virus, cytomegalovirus, herpes simplex, varicella-zoster, hepatitis B and C viruses and human immunodeficiency virus (HIV), which establish a long-lasting, chronic infection even after symptoms of disease have resolved (Table I).

Viruses associated with acute infections initially cause severe disease and induce a strong immune response that ultimately clears the infection (3). The initial infection by chronic viruses generally does not cause severe disease and thus does not induce a strong immune response. This initial low-level response controls the infection and long-term disease is caused by the low level of continuing replication over time (*i.e.*, immunodeficiency for HIV, or cirrhosis and hepatocarcinoma for hepatitis B and C viruses).

Currently available vaccines are effective mostly against viruses responsible for acute infections and mediate protection by inducing neutralizing antibodies. Many vaccines currently in use (polio, tetanus, diphtheria, measles, hepatitis B, influenza, etc.) prevent disease without actually preventing infection. They reduce the number of invading infectious agents and increase the rate of clearance, preventing the secondary effects of the infection, such as the disease and its transmission. Chronic virus infections are primarily controlled by cell-mediated immune responses, although the presence of neutralizing antibodies may help in preventing infection (1). The immune response against viruses causing chronic infections is generally not sufficient to confer protection. Such viruses have evolved numerous mechanisms to evade the immune system, and thus a vaccine designed to mimic the natural infection is generally ineffective (3).

Information from the fields of virology, immunology, biotechnology and molecular biology needs to be focused toward the development of vaccines that are more effective than the natural infection itself in inducing a strong immune response that will prevent chronic viral infections (3, 4). Antibodies are needed as an initial defense to prevent or limit the infection, while the cellular response clears infected cells (5). Neutralizing antibodies are thought to be the immune correlate of protection from infection, while cell-mediated responses are thought to be the correlate of protection from disease.

The possibility of developing an effective vaccine depends upon the biology of the infectious agent. For some infectious diseases, such as tuberculosis, malaria and AIDS, preventive or therapeutic vaccines are inadequate for controlling the disease (6).

This review is focused on HIV and emphasizes the difficulty in developing a vaccine posed by the biology of this virus. The increasing spread of AIDS, especially in developing countries, highlights the urgent need for an anti-HIV vaccine. Despite the tremendous progress in treatment, antiretroviral therapy cannot eradicate the infection, has to be administered continuously, causes major side effects, is very expensive and is not affordable in developing countries. Vaccines are the most cost-effective means for the control and eradication of infectious diseases and a vaccine represents the only realistic approach to controlling the global HIV epidemic, in particular in developing countries (7).

Despite unprecedented efforts to develop an anti-HIV vaccine, the correlates of protective immunity are still unknown. No HIV-infected patient has developed an immune response that has cleared the infection. Moreover, not a single case of recovery from HIV infection has been documented, despite the existence of some long-term nonprogressor (LTNP) individuals who are HIV-infected but do not develop AIDS.

An ideal anti-HIV vaccine should: 1) prevent transmission by both mucosal and parenteral routes by inducing humoral immunity in the form of neutralizing antibodies, cellular and mucosal immunity; 2) be effective after a single dose; 3) provide long-term protection over a period of

Table II: New vaccine approaches.

Approach	Pros	Cons
Live attenuated	Highest immunogenicity	Pathogenicity of the vaccine
Inactivated virus	Immunogenicity	Only humoral immunity, no CTLs, isolate specificity
Subunit	Safe and well tolerated Useful in prime-boost regimens	No neutralizing antibodies, isolate specificity
Live recombinant vectors	CTL immunity against both structural and nonstructural proteins	Cannot prevent first wave of cell-free virus infection
-Vaccinia	Induce potent and persistent immune responses	Dangerous in immunodeficient patients
-NYVAC, MVA	Useful for the induction of mucosal immunity	May have limited immunogenicity Ineffective in the presence of pre-existing immunity
-Canarypox, fowlpox	Do not replicate in mammalian cells	Difficulty to generate GMP-grade lots
-Adenovirus	Well-known model studied for gene therapy	Pre-existing immunity common
-Alphaviruses, adeno-associated virus, Semliki Forest virus, poliovirus	Ease of engineering and production, stability, replication-defective, safe	Under development, limited experience in humans
-Bacterial vectors (*Mycobacterium*, *Salmonella*, *Streptococcus*, *Listeria*)	Induction of mucosal immunity, low cost	Escape from CTLs
DNA-based	Antigens presentes in the context of MHC-I	Limited immunogenicity in humans Concerns about inducing tolerance or anti-DNA antibodies Integration into the host genome is an important theoretical risk
Pseudovirions	Do not replicate, form virus-like particles	May be difficult to engineer

years; 4) be low in cost; 5) be easy to administer; and 6) be effective against different viral strains (8). Such a vaccine will be very difficult, if not impossible, to develop. It is highly unlikely that a single vaccine can achieve the multiple goals necessary for total protection against HIV, and a combination of different vaccines may be necessary (9).

The most optimistic goal would be a vaccine that prevents the infection regardless of the route of acquisition. It is not known whether a vaccine that is effective against sexually transmitted virus would be effective for parenterally transmitted virus.

During HIV infection, cell-mediated immunity alone cannot efficiently control virus replication. Neutralizing antibodies are effective against free virus particles but not against virus-infected cells. Neither humoral nor cellular immunity is active against latently infected cells (1). The limited efficacy of both the humoral and the cellular immune response against HIV is related in part to the rapid selection of new immune-escape variants. A small reservoir of memory T-cells that bear latent HIV infection are resistant to elimination by either drugs or the immune response (8). HIV infection therefore persists and progresses despite an initially functioning immune system that fails to control and clear the infection. The high turnover of CD4+ cells may exhaust the immune system and deplete cytotoxic T-lymphocytes (CTLs), resulting in the development of AIDS.

Strategies (Table II)

Live attenuated vaccines

Historically, live attenuated vaccines have been the most useful in inducing complete and long-lasting immunity. They generate an immune response that mimics that induced by infection with the natural pathogen, resulting in both neutralizing antibodies and cell-mediated immunity.

An HIV vaccine based on live attenuated strains is unlikely because a live virus may revert to a more virulent form or possibly cause malignancy (10). Preliminary studies in macaques infected with simian immunodeficiency virus (SIV) showed that such a vaccine caused persistent infection and some animals developed AIDS-like symptoms (11). Attenuated SIV deletion mutants still caused AIDS in neonatal macaques and have been transmitted to other individuals (12). Individuals in an Australian cohort who were infected with a *nef*-deleted HIV strain, initially described as nonprogressors, ultimately developed AIDS (13), further confirming the high-risk nature of the live attenuated vaccine approach. Finally, the ability of HIV to integrate into host DNA poses the risk of insertional mutagenesis (14).

Thus, the development of attenuated vaccines may require even further attenuation of the parental strain by deleting accessory genes or by including a suicide gene conferring susceptibility to antiviral agents. Because of

the challenge involved, there is very little enthusiasm in pursuing an attenuated vaccine strategy.

Whole inactivated vaccines

Effective vaccines for polio (Salk) and influenza are based on whole inactivated virus. These vaccines elicit neutralizing antibodies, but not CTLs. However, inactivation of HIV bears the risk of either being incomplete or altering the structure of existing neutralizing epitopes. Preferential covalent modification by oxidation of internal proteins for inactivation of virions (15) abolishes detectable infectivity, although concerns about the possibility of reversibility remain.

The risk of immunopotentiation should also be considered. A whole inactivated vaccine for feline infectious peritonitis virus increased the severity of clinical symptoms by a mechanism known as antibody-dependent enhancement (16). A gp120-depleted immunogen that was evaluated in a limited human immunogenicity trial (17) gave disappointing results because it did not elicit neutralizing antibodies. Another inactivated HIV vaccine, known as Remune® (Immune Response Corp.), has been tested as an HIV vaccine but afforded no significant protective effect (18, 19).

Classic vaccination strategies based on attenuated or killed viruses may ultimately be ineffective for HIV because the virus has evolved numerous mechanisms to elude the activity of neutralizing antibodies (2). New strategies are therefore needed for the development of an effective HIV vaccine. The new strategies should be based on the knowledge accumulated and the technologies developed in the last two decades in the field of immunology, molecular biology and biotechnology.

Recombinant subunit vaccines

Purified or synthetic proteins as vaccines selectively induce neutralizing antibodies, but not CTLs. A highly effective vaccine against hepatitis B virus (HBV) (20) consists of subunits and is currently the only licensed vaccine of this type (3). A major advantage of a subunit vaccine is its safety, as exemplified by the licensed anti-HBV vaccine.

A humoral immune response mediated by neutralizing antibodies is fundamental for conferring protection against viral infections. An anti-HIV vaccine needs to target viral envelope glycoproteins on the surface of the viral particle to induce neutralizing antibodies that block membrane fusion and thus neutralize infectivity. Unfortunately, neutralizing antibodies elicited by subunit gp120 were limited to homologous, generally laboratory-adapted strains (7). HIV mutates rapidly because the viral reverse transcriptase has no “proof-reading” function. The introduction of new nucleotides results in new variants in the population, as well as in the same infected individual. Hypervariable and heavily glycosylated regions are exposed on the exterior of envelope glycoproteins. Conserved epitopes are located either in recessed molecular cavities or become exposed only after binding to the CD4 receptor. Antibodies developed against monomeric gp120/gp41 or against gp160 do not recognize the trimeric structure of the envelope glycoproteins, and thus they cannot neutralize primary HIV isolates (21, 22). Finally, unprocessed gp160 acts as a decoy, selecting antibodies that do not efficiently neutralize the virus. Antibodies against monomeric gp120 are generally too specific and do not protect against unrelated variants. Antibodies that neutralize one isolate fail to neutralize other isolates, even if from the same patient.

The induction of neutralizing antibodies is essential for blocking virus entry into cells. There is evidence that some antibodies can neutralize several unrelated HIV strains (23, 24) and confer protection by passive transfer of IgG in animal models (25), suggesting that antibodies with a broad range of action could be developed.

A candidate subunit vaccine (VaxGen) based on recombinant envelope proteins conferred impressive protection in chimpanzees in preclinical studies. However, it was ineffective in large phase III clinical efficacy trials in the U.S.A. and Thailand (26, 27). Subunit vaccines developed so far lack the broad spectrum and potency needed to achieve sterilizing immunity against the wide variety of HIV isolates. The development of an immunogen that elicits broad-spectrum HIV antibodies and decreases the possibility for viral immune escape is one of the most challenging tasks for the development of an anti-HIV vaccine and remains one of the most difficult unsolved problems (7).

Another possible target is the HIV regulatory protein Tat, found in the sera of infected individuals. Tat acts as an extracellular toxin. Protection from simian/human immunodeficiency virus (SHIV)-induced disease was observed in a monkey model (28), although Tat alone was not always found to be protective (29). Multicomponent vaccine strategies may benefit from the inclusion of Tat.

Oligomeric gp120

The use of monomeric gp120 does not represent a viable vaccine strategy. The early vaccine efforts based on the use of gp120 considered neither the trimeric structure of the envelope proteins in their native form, nor the complexity of the CD4 and co-receptor binding sites (8). Monomeric gp120 generates neutralizing antibodies to laboratory-adapted strains, but not to fresh clinical isolates (21). The trimeric structure of gp120/gp41 shields neutralizing epitopes (26). Soluble trimeric envelope glycoproteins derived from primary isolates elicited neutralizing antibodies when used as immunogens (30, 31).

New means of inducing broad-spectrum HIV-neutralizing antibodies are therefore topics of active research. Efforts are focused on the development of antibodies

against conserved epitopes on the trimeric structure which are similar among all viral variants that use the same entry mechanism, and that could not mutate without resulting in the loss of functionality of gp120/gp41 (3).

The regions of the envelope glycoproteins binding to CD4 and to the co-receptor are conserved, but the recessed nature of the CD4 binding site makes antibody binding difficult. Moreover, the co-receptor site is shielded by hypervariable regions and is exposed only transiently before docking on the co-receptor (8).

Envelope glycoproteins undergo several conformational changes during the fusion process between the viral envelope and the cellular membrane, which results in transient exposure of conserved epitopes. Approaches used to expose such epitopes on fusion intermediates include carbohydrate removal (32), the use of CD4-independent gp120 (33), the deletion of variable loops on gp120 (34-36), constructs based on gp41 (37, 38), strategies to induce antibodies against conserved regions of gp120 such as the co-receptor binding site (39), and the use of flexible linkers (40, 41).

Fab and single-chain Fv antibody fragments can neutralize primary HIV isolates better than intact antibody molecules, suggesting that fusion complex antibodies may be very difficult to prepare due to kinetic and steric constraints (26). Spatial limitation at the neutralization site and rapid disappearance of the transient neutralization targets on the fusion complex may be limiting factors for efficient antibody binding (42).

Peptide and lipopeptide vaccines

Peptide-based vaccines, especially when chemically attached to a carrier with adjuvant activity such as toxoids, can elicit immune protection. Immunization with mixtures of peptides representing both B- and T-cell epitopes confers both long-term and cross-strain immunity against the influenza virus (43). Potential components of a peptide-based HIV vaccine formulation are regions of the CD4 molecule that bind gp120 and unmask conserved neutralization epitopes (44). Selection of CTL epitopes for inclusion in polyepitope vaccines, matched to the major histocompatibility complex (MHC) alleles of a given population, has the potential to induce responses to subdominant epitopes and confer a broader immune response.

Peptides, including CTL epitopes from Nef and Gag, included in a lipopeptide preparation combined with the tetanus toxoid induced CTL responses after immunization of macaques (45). Peptide- and lipopeptide-based vaccines are being tested in combination with the ALVAC vector in trials sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), the HIV Vaccine Trials Network (HVTN), Aventis Pasteur and the French Agence Nationale de Recherches sur le Sida (ANRS).

Live recombinant vectors

Live recombinant vectors being actively studied as vehicles for gene therapy are possible vectors for AIDS vaccines. Live recombinant vectors have the advantage of presenting the relevant antigen in the correct states of conformation, glycosylation and oligomerization, of increasing the duration of the immune response and inducing a CD8+ CTL response. On the other hand, live recombinant viral vectors are potentially dangerous in immunocompromised hosts because they can disseminate and cause severe illness. In immunocompetent hosts, pre-existing immunity against the vector could render a vaccine less effective.

Recent studies on poxviruses using highly attenuated vaccinia, such as the modified vaccinia Ankara (MVA), the gene-deleted NYVAC and avipoxes, such as the canarypox ALVAC (Aventis Pasteur) and the fowlpox, show that they cannot replicate in mammalian cells but can produce foreign proteins (46). Vectors based on poxviruses can accept large segments of HIV cDNA that could code for multiple antigens. Poxvirus vectors also induce neutralizing antibodies and T-cell responses to HIV in macaques (47). Poxvirus-based vaccines face the problem of generating large GMP-grade lots of vector to high titers (7). Efficacy may be blunted even further by an eventual smallpox revaccination campaign as an antibioterrorism measure (48), resulting in a population that is immune to the vector.

Adenovirus vectors induce both mucosal and systemic immunity. In animal studies, these vectors induced humoral and cellular responses, resulting in lower viral titers after HIV challenge (49). The most frequently used adenovirus vector is the gene-deleted serotype 5 (Ad5; Merck Research Laboratory), widely used in gene therapy studies. It has good immunogenicity in murine and primate models (50). Pre-existing group-specific immunity to adenovirus serotypes could reduce their effectiveness, however. The immunogenicity of gene products inserted into this vector may be improved when used as a booster for DNA-based prime vaccine.

Live attenuated poliovirus vaccines, in which the capsid gene has been substituted with the HIV *gag* gene, resulted in the formation of virus-like particles (VLPs) that were able to induce a mucosal humoral response in animal models (51).

The vesicular stomatitis virus (VSV) has been engineered to express HIV antigens and used in preclinical trials in macaques administered by a mucosal route. It is being developed through the HVDDT (HIV Vaccine Design and Development Teams) program at the NIAID and by Wyeth Pharmaceuticals.

The Venezuelan equine encephalitis virus (VEE) is an alphavirus being studied as an attenuated vaccine vector. This vector allows high levels of expression of foreign genes, does not encounter pre-existing immunity in humans and has the potential for targeting dendritic cells. Studies in macaques showed partial protection from pathogenic SIV. This vaccine approach is being developed by

AlphaVax with support from the International AIDS Vaccine Initiative (IAVI) and the NIAID.

The adeno-associated virus (AAV), a nonpathogenic parvovirus, has been tested for expression of the gene for G1b12, a potent monoclonal antibody with broad-spectrum neutralizing activity. Direct expression of the antibody by the vector will circumvent difficulties in producing cross-neutralizing antibodies by immunization. High levels of expression of the neutralizing antibody were demonstrated in treated animals (52).

The rabies virus is another possible vector for use in an HIV vaccine. The rabies N protein can act as a superantigen, serving a role similar to that of adjuvants (53). A careful assessment of safety is necessary before considering possible clinical trials.

The yellow fever virus has been manipulated to construct a live attenuated virus vector to induce protective immune responses against viruses such as the West Nile virus, Japanese encephalitis and dengue. The recombinant chimeric virus, known as ChimeriVax™ (Acambis), has been shown to be safe, immunogenic and protective in primates (54-56).

Recombinant *Salmonella* strains, in particular attenuated strains such as CVD908 or aroA, induce CTL immunity in some studies (57), but in other studies, *Salmonella* vaccines did not induce CTL responses, neutralizing antibodies or protection from infection (58, 59).

Recombinant bacterial strains should be useful for the development of a vaccine that induces mucosal immunity and this approach is under development by the Institute of Human Virology (IHV) supported by the IAVI. Delivery vehicles and adjuvants that are under study to direct vaccine antigens to mucosal sites include modified bacterial toxins such as cholera and *Escherichia coli* labile toxins (60). The avirulent strain of *Mycobacterium* BCG (Bacille Calmette-Guerin) has a good safety record and confers long-lasting immunity, may be administered as a single dose and has a low cost (61). However, although BCG vectors induce CTL responses, they do not provide protection from HIV infection.

Live vectors that are also being studied include the influenza virus, alphaviruses such as Semliki Forest virus, parvovirus and other AAVs, as well as intact recombinant yeasts and an attenuated strain of *Listeria* (62). The multitude of possible vectors, coupled with the complexity of recently introduced prime-boost strategies, necessitate a careful selection of the most promising candidates for large-scale efficacy trials in humans.

DNA vaccines

DNA-based vaccines are plasmid DNA vectors expressing the proteins of interest under the control of a strong promoter. Such DNA is immunogenic when inoculated intramuscularly. DNA vaccines are considered subunit genetic vaccines because they are based on parts of the pathogens. The vectors use the transcription and translation machinery of the host cells to produce the immunogen. The immunogens produced by DNA vaccines are processed through the MHC-I presentation pathway and elicit CD8+ CTLs. DNA-based vaccines induce either no or very low levels of antibodies, although the response may be improved by a boost with a subunit vaccine (63). For safety reasons, DNA vaccines should not integrate into the genome of cells of vaccinees, to avoid risks due to insertional mutagenesis (64). DNA vectors generate a strong CTL response in mouse models, but a less vigorous CTL response in primates.

The efficacy of DNA-based vaccines can be improved by including genetic adjuvants such as genes coding for cytokines, chemokines or T-cell co-stimulatory molecules (65-67), thus modulating and increasing the immune response (68). Further improvements may be obtained by the use of codons most frequently found in human genes, the use of relevant CpG motifs (69), the removal of inhibitory instability elements (INS) to stabilize mRNAs and increase expression levels, the optimization of sequences to promote secretion and stimulate humoral immunity, and the improvement of protease degradation, which would enhance MHC-I peptide exposition (70). Methods to increase the uptake of DNA, such as electric pulses using electrodes, biojectors, gene gun, adsorption onto cationic microparticles, topical dermal application or bacterial carriers, would also be helpful (64).

To improve the broad-spectrum response of the vaccine, immunogens based on consensus and ancestral sequences derived by computer analysis are being evaluated (71), although this extrapolation based on currently known sequences may have a high degree of uncertainty (64). Cocktails of antigens of defined composition are needed for vaccines that provide the required efficacy (72).

Pseudovirions

Pseudovirions are defective VLPs engineered to produce the viral proteins necessary for virion assembly while being replication-incompetent. Pseudovirions may substitute for live attenuated or inactivated virus, because they are not associated with the concerns that impact the use of these vectors.

Virus-like particles coated with the L1 protein of the human papillomaviruses HPV-16 and HPV-18 are currently being tested in a phase III trial as a vaccine that is giving promising results for the prevention of cervical cancer (73).

Subvirion particles may be obtained by expressing HIV *gag* or *gag-pol* genes in recombinant viruses or transfected mammalian cells. Nonreplicating VLPs carrying viral antigens are thus formed. Virus-like particles bearing gp120 on their surface have been evaluated alone or in DNA/VLP prime-boost protocols (74) that induce CTLs and neutralizing antibodies and are effective when administered through mucosal immunization.

Animal models

Studies in nonhuman primates have been very important for the development of an anti-HIV vaccine. Primate studies have given conflicting results because some species do not develop AIDS when infected by HIV. To circumvent this limitation, chimeric lentiviruses have been developed that contain the backbone of SIV with other genes, generally for the envelope, of HIV. Such SHIV constructs cause a rapidly progressive AIDS-like disease in the macaque model that is being used for vaccine trials.

Prime-boost strategies

Combination of at least two different vaccine strategies, whereby one induces humoral and the other cellular immune responses, has been tested in recent years (9). The most recent vaccine trials are based on prime-boost protocols. The first vaccination generates cellular immunity and a boost with another vaccine may induce a humoral response. Prime-boost strategies using heterologous vectors represent the best available strategy for eliciting an anti-HIV response (3).

Recombinant viral vectors are among the most promising because they are safe and can trigger all branches of the immune response. Because immunity to the vector may be present, they are usually administered in combination with inactivated virus, pseudovirions, proteins, peptides or DNA (75). DNA vectors are very effective as the initial immunogen in a prime-boost strategy (76), particularly when followed by boosting either with a viral vector vaccine to induce CTLs or with recombinant proteins to induce neutralizing antibodies. The use of a bimodal vaccine approach in which two immunogens with complementary modalities are combined is a new, radical change from traditional vaccines (77).

Adjuvants

An appropriate adjuvant is critical to the success of a vaccine. An adjuvant was defined by Ramon G., in 1924, as a “substance used in combination with a specific antigen that produced a more robust immune response than the antigen alone” (78). Despite its name, an adjuvant plays a primary role in the vaccination process and its nature is fundamental to a vaccine formulation (79). Adjuvants are important because they help to provide maximum efficacy, reduce the number of doses of vaccine, facilitate ease of delivery and are safe. Adjuvants are not licensed as stand-alone products, but always in combination with a particular vaccine. Preclinical studies need to be done to evaluate the safety profile of every adjuvant/vaccine combination. The action of an adjuvant/vaccine combination is dependent upon multiple factors. Responses obtained with one combination cannot be extrapolated to other antigens, or even to the same combination when administered by a different route (80).

Biological adjuvants, such as cytokines and chemokines, may be used to enhance T-cell activity to improve the efficacy of a vaccine, but more studies to verify activity are needed (81). CpG oligodeoxynucleotides are new, potent adjuvants that act by directly activating B-lymphocytes and plasmacytoid dendritic cells (DCs). They indirectly activate monocyte-macrophages and the secretion of both cytokines and chemokines to increase the adaptive immune response (82).

Clinical vaccine trials

More than 20 years after the discovery of HIV as the etiological agent responsible for AIDS, a vaccine still remains a distant hope despite unprecedented development efforts.

The first phase I clinical trial of an HIV vaccine was conducted in 1987 in the U.S.A. More than 30 vaccine candidates have been tested since then in over 70 phase I/II trials. In 1993, the first trial was conducted in developing countries, followed by 18 other phase I/II trials and the first 2 phase III clinical trials that were conducted in western countries (VAX003) and in Thailand (VAX004). The protein subunit vaccine used in the first phase III trials was ineffective in preventing disease and did not elicit cross-clade neutralizing antibodies (3, 27). So far, candidate vaccines have failed because they were either too or not sufficiently attenuated (6). According to the IAVI, there is only 1 large phase III trial ongoing in high-risk populations in Thailand and 3 mid-size phase II trials in low- and high-risk populations testing vaccine safety and immunogenicity. Many different approaches to vaccines are currently being tested internationally in phase I trials.

It may be important to match the local (83, 84) or at least country-specific (85) clades for the development of effective HIV vaccines. The first tested AIDS vaccines were based on clade B viruses, the dominant subtype in western countries, although clade C is the most prevalent worldwide (85). The demonstration of cross-clade neutralizing antibodies suggests that neutralization may not align with clade assignments (86).

Future perspectives

The first HIV candidate vaccines based on recombinant envelope glycoproteins and aimed at inducing neutralizing antibodies gave disappointing results. This failure was likely because most of the antibodies elicited by these vaccines were directed against regions unavailable for binding in the native structure of primary HIV strains. Due to the difficulty of eliciting broad-spectrum neutralizing antibodies against HIV, the second-generation vaccine candidates were focused on eliciting a cellular immune response, exemplified by generation of CTLs.

Current vaccine research is aimed at the optimization and induction of both humoral and cellular immune responses.

A vaccine against HIV should elicit both mucosal and systemic immunity and be effective in containing both sexually and parenterally transmitted virus. An effective vaccine should also elicit both humoral and cellular immunity to block the transmission of both virions and cell-associated virus (84).

Strategic aims of an effective vaccine include prevention of mucosal transmission, blockade of attachment of virus to epithelial cells and elimination of locally infected cells (87). HIV is never completely cleared due to persistent replication and the presence of latently infected cells, even in the presence of a potent immune response. Because the virus is so completely embedded, the possibility exists that no vaccine will ever be able to fully eliminate HIV, or even contain its replication indefinitely (77).

The analysis of the efficacy of a candidate vaccine is biased by the fact that massive doses of challenging virus are administered in animal trials to ensure that all controls are infected. Humans are exposed to lower doses in the course of natural infection. A vaccine that has little or no efficacy when tested with high doses may be protective when tested with lower challenge doses (8). Studies on sex workers in Kenya who remained uninfected despite multiple and regular contact with HIV-infected individuals suggested that a CTL response might be fully protective. When some of those subjects ceased sex work, they become infected, suggesting that regular contact with the virus is needed to maintain protection. These findings indicate the need to maintain the CTL response in a state of persistent activation, which may be achieved only with repeated immunizations (8). A similar problem is presented by the BCG tuberculosis vaccine, which is effective for only 1-3 years (6).

Different strategies are currently under study to improve vaccines against infectious agents that have evolved ways of evading the immune response. For viruses that have evolved sequences with poor affinity for MHC molecules, epitope enhancement allows the modification of such sequences in order to make them more effective in vaccines. The use of cytokines, chemokines and other co-stimulatory molecules, as well as CpG-rich oligonucleotides, may help to activate the innate immune response and improve vaccine efficacy (81). The blockade of molecules that negatively regulate the immune system may also improve the vaccine response (3).

The existence of broad-spectrum neutralizing antibodies to HIV validates the possibility that a vaccine that is effective in neutralizing HIV infection via antibody-mediated mechanisms may be feasible (26). A useful approach to produce immunogens would be to study neutralizing antibodies to identify the area of binding on the antigen, by analyzing peptides or structural libraries, and then use the identified "structures" as immunogens (88). Such an approach is known as "reverse vaccinology", since vaccines are developed from antibodies rather than generating antibodies from a vaccine. A "directed molecular evolution" approach may also be useful for designing new vaccine antigens. The method, developed by Maxygen, is based on DNA shuffling and mimics natural selection. Small fragments of DNA derived from variants of the gene coding for the antigen of interest will recombine. The sequences of the HIV *env* gene have been "shuffled" to generate recombinant forms that produce a strong antibody response (89). If antibodies directed against different neutralizing determinants act synergistically, the antibody concentration needed for effective neutralization could be reduced (90).

Vaccine protocols based on a prime-boost strategy are giving promising results. To broaden the CTL response, it has been proposed that more viral protein be added to the DNA and/or live recombinant virus construct. This approach might not give the expected results because the CTL response is limited to relatively few epitopes and adding more proteins to a construct may not broaden the immune response. A good strategy might be to immunize with constructs that each express a different viral protein. The immune system would then respond as if each construct was an individual pathogen, providing a broader response (8).

Dendritic cells

Dendritic cells (DCs) are the most efficient antigen-presenting cells of the immune system (91). Vaccine efficacy will likely be increased if the immunogenic principle is targeted to DCs.

Dendritic cells are efficiently activated by CpG sequences and oligonucleotides. The "*ex vivo* education" of autologous DCs may represent a novel vaccine strategy. In this approach, peripheral blood DCs are exposed *in vitro* to a canarypox vaccine vector containing HIV antigens. Such "trained" cells are then used for inoculation. While preliminary proof-of-concept experiments are promising, this approach to widespread vaccination would be difficult to implement.

Antigens may be delivered directly to Langerhans cells or other dermal DCs (92) using a novel topical immunization known as DermaVir (Research Institute for Genetic & Human Therapy) that mimics the expression of virus in DCs. DermaVir has reduced rebound viral load to undetectable levels (92).

An experimental DC-based vaccine has proved effective for the treatment of chronic HBV infection (93).

Mucosal immunization

Mucosal surfaces are a target for sexually transmitted HIV infection. Therefore, focusing on a mucosal immune response may be one of the most effective approaches to immunization (94). Mucosal immunity may protect from virus dissemination and eventually help to clear the virus (95). Mucosal vaccination appears to be more effective than a systemic vaccine (94). Vaccination into the salivary

gland using plasmid DNA (96), transcutaneous immunization (*i.e.,* application of vaccines on the skin), new mucosal adjuvants and targeting to lymph nodes draining mucosal sites (94) have been proposed as methods for eliciting mucosal immunity.

Consequences of only partial protection by vaccines

Due to the particular biology of HIV, a vaccine that controls and contains viral replication, resulting in protection from disease rather than from infection, may be an achievable and more realistic goal. Such a vaccine could result in an extended asymptomatic period, similar to the status of LTNPs, by conferring protection against the persistent high level of HIV replication, and thus may slow the progression of the disease. Furthermore, reduced replication and a lower viral load may reduce the probability of transmission, ultimately slowing the spread of the infection in the population. Epidemiological models suggest that a partially effective vaccine might significantly decrease the incidence of infection in high-risk regions (97).

Therapeutic vaccines

A therapeutic vaccine could be useful for the control of chronic infection by broadening and reinforcing immune defenses. Therapeutic immunization is very effective for the control of infections caused by viruses that do not establish latency, such as polio, measles or the hepatitis A viruses.

The lack of immune control or the development of inappropriate immune tolerance could be blunted by therapeutic vaccines, not only for HIV, but also for other chronic diseases such as HBV-initiated cirrhosis and the malignancies induced by HPV (75). Therapeutic vaccines may be useful when antiviral agents are either not available or are too toxic for long-term administration.

A therapeutic vaccine controlling HIV infection could result in a prolonged asymptomatic period, but, even more importantly, may decrease the risk of transmission. The generation of an HIV-specific immune response by therapeutic immunization in infected patients may help suppress viral replication and allow the reduction, or even temporary discontinuation, of therapy (98).

Therapeutic vaccines need to be administered as early in the infection as possible, when the number of T-helper and memory cells is sufficient to produce an antiviral immune response. Therapeutic vaccines should also be able to induce immunity to epitopes that have not been recognized by the host immune system or that are newly arisen due to virus mutation to avoid immune escape (99).

To improve the efficacy of a therapeutic vaccine it is important to understand the immune correlates of protection. For example, we do not know whether continuous immune stimulation will lead to immune exhaustion. Finally, such a vaccine should deal with immune evasion strategies used by viruses that cause chronic infection. Therapeutic vaccination may be most effective when viral titers are low to avoid the inefficacy, or even dangers, that may result from administration in advanced stages (75).

Obstacles to therapeutic vaccines could be a state of immunodeficiency too advanced to allow an effective immune response, along with the possibility of augmenting immunological escape and both defective antigen presentation and insufficient T-helper cell function, which are consequences of HIV-induced immunodeficiency. A successful protocol could include initial treatment with antiretroviral therapy to reduce viral load and restore some immune function, followed by a therapeutic vaccine to boost the immune response to HIV. Such a protocol might be expected to attenuate disease progression during treatment interruptions and reduce the toxicity and the cost of antiviral drugs. Initial trials based on therapeutic immunization with DNA vaccines showed some immunogenicity in HIV-positive patients (100).

Several vaccine candidates are being evaluated for therapeutic use (96). Therapeutic vaccines should help to prevent complications of chronic infections, but their efficacy has yet to be demonstrated. While sterilizing immunity to HIV is unlikely, the possibility of containing virus replication, prolonging disease-free survival and reducing spread may be achievable goals. A therapeutic vaccine could also be useful against HCV, by reducing viral load and therefore the risk of the onset of a chronic infection. Persistent HCV infection is present in about 55-85% of infected patients and is responsible for significant morbidity and mortality (101). Therapeutic vaccines targeting HPV oncoproteins may help reduce the risk of dysplasia and cervical cancer progression that are related to persistent infection.

Emphasis must be placed on the safety of vaccines that will be administered to a healthy population. A greater risk level may be acceptable for therapeutic vaccines if the benefit of the vaccine is substantial.

The establishment of a nonprofit international network dedicated to the development of therapeutic vaccines and immunization programs against HIV was recently proposed (102).

Plant-derived edible vaccines

A successful AIDS vaccine, particularly in developing countries, must be both easy and cheap to deliver. Edible plants may be safe and inexpensive delivery vehicles. Plants may be used as bioreactors because the system permits appropriate modification of foreign proteins, enables convenient oral delivery through consumption of edible plant tissues, and scale-up production is easy. Vaccine antigens accumulate within cellular compartments, where they are protected by the thick walls enclosing plant cells from digestion in the stomach. This bioencapsulation will facilitate delivery and targeting to the intestinal lymphoid tissue.

The HIV-1 *tat* gene, with its broad, non-subtype specificity, has been inserted in the tobacco mosaic virus vector and expressed in plants. The protein retained full immunological reactivity against *tat*-specific monoclonal antibodies (103). ProdiGene has developed a maize-based source of HIV gp120 to be used as an edible vaccine. Russian scientists from the State Scientific Center for Virology and Biotechnology, in collaboration with the Siberian Institute for Plant Physiology, have expressed HIV antigens in tomatoes. HIV antigens have already been expressed in nonedible plants such as tobacco, or in plants that need to be thermally processed, such as potatoes. Tomatoes would be an ideal vector for vaccine development because they are consumed raw and can be rapidly cultivated. The plant approach has also been used for the expression and production of HBV surface antigens (104) and for human cytomegalovirus glycoprotein B (105).

Conclusions

At the recent AIDS Vaccine 2004 conference, held in Lausanne, Switzerland, at least 10-15 billion euros were requested for the next decade to develop HIV vaccines, as well as for collaboration on research to bring vaccine candidates to phase III clinical trials. Efforts to develop an HIV vaccine need to be strengthened, both by coordinating scientific research and by setting up well-planned clinical trials. The unsuccessful empirical approaches used so far and the failure of the first phase III clinical trial clearly indicate that a rational approach, based on basic knowledge and understanding of correlates of immunity, is fundamental to develop a successful vaccine (106). Good planning is needed to avoid expensive clinical trial failures and to reverse the fading optimism for an HIV vaccine (106).

Basic research needs to be well funded and should take advantage of any new knowledge of the intricate interplay between HIV and the human immune system (107). Basic studies are needed in order to understand how to induce protective neutralizing antibodies against the different HIV strains. None of the vaccines so far tested in clinical trials has elicited such broad-spectrum antibodies. Optimization of envelope-based immunogens is needed for exposing neutralizing epitopes and for masking non-neutralizing epitopes that elicit ineffective antibodies (5).

Strategies based on whole killed virus or attenuated vaccines may have been prematurely dropped and should be re-evaluated (108). A recent, long-term retrospective study showed delayed progression to AIDS in patients treated with the Remune® immunogen (109). The study of the Remune® vaccine, which is based on inactivated whole particles, was interrupted before highly active antiretroviral therapy (HAART) became available; Remune® was inefficient in controlling HIV infection, but may be useful as a therapeutic vaccine within the context of HAART.

The monkey model should be used in large-scale trials that are sufficiently uniform for cross-comparisons of results. A consensus on which parameters are associated with efficacy and/or protection from a vaccine needs to be reached.

Finally, the commitment of industry is not sufficient. Without profit incentives and some protection from risk, it is difficult to convince the pharmaceutical industry to invest the necessary resources to support candidate vaccines and the necessary clinical trials. The European Commission recently implemented the European Development Countries Clinical Trials Partnership (EDCTP) as a consortium for testing vaccines for HIV, malaria and tuberculosis in clinical trials. Human clinical trials conducted in a coherent manner are fundamental to assess the value of a candidate HIV vaccine, although great attention should be afforded to ethical issues, including clear informed consent, lack of coercion and confidentiality. Since vaccinees will develop HIV-positive status in serological assays, there must be assurance that the healthcare rights of volunteers will be preserved (9).

Existing vaccines are both safe and immunogenic, but the immune responses obtained are generally of narrow spectrum and short duration (9). The promise of an HIV vaccine will not be fulfilled until there is further research and support to identify specific immunological and virological issues that impede efficacy (7).

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Sources of information on HIV vaccines

Ongoing and planned clinical trials:
http://www.iavi.org/trialsdb/basicresearchform.asp
http://chi.ucsf.edu/vaccines
http://www.scharp.org/public/indesx.htm
http://www.niaid.nih.gov/daids/vaccines
http://www.unaids.org
http://www.clinicaltrials.gov/ct/gui/c/r

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