The potential of vaccines for the control of AIDS

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MI JOHNSTON, PE FAST, MC WALKER, D HOTH. Potential of vaccines for the control of AIDS. Can J Infect Dis 1994;5(Suppl A):36A-41A. The goal of a prophylactic human immunodeficiency (HIV) vaccine is to elicit immune response(s) that will, upon subsequent exposure to HIV, prevent infection and/or disease. On the other hand, therapeutic administration of a vaccine to an individual in whom infection is already established might benefit the individual by augmenting existing functional immune responses or inducing new ones. Development of vaccines for the prevention of AIDS offers unique challenges. Concerns regarding the safety of attenuated and whole-killed products have led to the pursuit of alternative designs, including recombinant proteins, vectors and particles, synthetic peptides and naked DNA. Seven recombinant envelope, two recombinant vector and four other candidate vaccines that have entered into phase 1 trials in noninfected individuals have proven safe to date, and have differed in their ability to induce functional antibody and cytotoxic T lymphocytes. Two recombinant envelope products have recently progressed to phase 2 testing. Five envelope-based and six other products have entered trial in HIV-infected individuals and have appeared to be safe. Evidence of new antibody, increased T cell proliferation and increased cytotoxic T lymphocyte activity have been reported. Additional placebo controlled trials will be required to evaluate the impact of therapeutic vaccination on CD4 cell count, viral burden and clinical endpoints. The status of HIV/AIDS vaccine development is reviewed, with emphasis on the challenging task of finding an efficacious, safe, prophylactic vaccine.

Key Words: AIDS, Clinical trials, Human immunodeficiency virus, Vaccines

Potentiel des vaccins dans la lutte contre le SIDA

RÉSUMÉ : Le but d'un vaccin contre le virus de l'immunodéficience humaine (HIV) est de déclencher des réactions immunitaires qui, lors d'une exposition subséquente au HIV, préviendront l'infection ou la maladie. Autre part, l'administration thérapeutique d'un vaccin à une personne déjà infectée pourrait offrir à cette personne l'avantage de renforcer sa propre réponse immunitaire ou d'en déclencher de nouvelles. Le développement de vaccins pour la prévention du SIDA offre un potentiel unique. Les craintes qu'inspirent les produits testés ou tués à l'égard de l'innocuité ont stimulé la recherche en vue de trouver des modèles différents, notamment des protéines, des vecteurs et des particules recombinantes, des peptides synthétiques et de l'ADN dénué. Parmi les vaccins qui sont entrés dans des essais de phase I chez des sujets non infectés et qui se sont révélés sans danger jusqu'à présent, notons-en sept à base d'enveloppe recombinante, deux à base de vecteur recombinant et quatre d'autres types. Leur capacité à induire la production d'anticorps et de lymphocytes T cytotoxiques fonctionnels est variable. Deux des produits à base d'enveloppe recombinante ont récemment progressé vers des essais de phase II. Cinq produits à base d'enveloppe et six produits d'autres types font l'objet d'essais auprès de sujets infectés au HIV et semblent également être sans danger. On a pu observer l'existence de nouveaux anticorps, la prolifération accrue de cellules T et une activité plus grande des lymphocytes T cytotoxiques. D'autres essais contrôlés avec placebo seront nécessaires pour évaluer les conséquences d'une vaccination thérapeutique sur la numération des cellules CD4, sur la charge virale et les paramètres cliniques. L'évolution des vaccins contre le HIV/SIDA est passée en revue et l'on insiste sur le défi que représente la découverte d'un vaccin prophylactique sûr et efficace.

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Since the discovery of AIDS and its etiological agent, the human immunodeficiency virus (HIV), researchers from all sectors of the scientific community have been challenged to find effective therapies to treat HIV/AIDS and a vaccine to curb its spread (1-5). HIV/AIDS has grown to be one of the major causes of death in the United States. Approximately one million individuals in the United States are infected (6, 7), almost one quarter million have been diagnosed with AIDS, and approximately 65% of those individuals diagnosed with AIDS have died (8). The impact of HIV/AIDS on other parts of the world, particularly parts of Africa and Asia, will likely be more devastating than the impact on the United States if current trends continue (9). Since behavioral intervention approaches have not proven entirely successful and access to antiretroviral therapies is generally limited to developed countries, the major hope for curbing the HIV/AIDS epidemic worldwide is the development of an efficacious, inexpensive, easy to administer vaccine.

The combination of scientific challenges in HIV vaccine development is unparalleled in the field of vaccine development. First, the great degree of genetic diversity of HIV suggests that broadly cross-reactive immune responses will be needed to protect an individual from all subtypes of virus to which the individual might be exposed. Five major clades or subtypes of HIV-1 have been identified (10-12). The virus in each clade differs from virus in other clades by 30 to 35% in the genetic sequence of env and gag genes. Antigenic variation, which has hindered the development of the influenza vaccine, may prove to be an even more significant obstacle in the case of HIV-1. Second, the virus exists in both free virion and cell-associated forms. Vaccines that induce both neutralizing antibodies, which block infection by free virions, and cytotoxic T lymphocytes (CTL), which may eliminate infected cells, may be required. However, the nature and amount of HIV antigen expression on the surface of cells that are not actively producing virus remains unknown; latently infected cells might not be vulnerable to attack by either antibody or CTLs. Third, the major mode of transmission of HIV-1 is through sexual contact; an efficacious vaccine may require induction of HIV-1-specific mucosal immune responses through novel antigen presentation methods. Fourth, there is no documentation that individuals can completely clear HIV-1 from the body once infection is established. Thus, the immune response(s) that correlates with protection from HIV-induced disease is not known. Fifth, although monkeys infected with the simian immunodeficiency virus (SIV) have proven useful in evaluating vaccine concepts, there is as yet no animal model that can be employed to determine the effectiveness of an HIV-1 candidate vaccine in preventing HIV-1-induced disease (13). HIV-1 can establish a chronic infection in chimpanzees, but does not cause immunodeficiency or disease. Thus, this expensive model can be used to evaluate the ability of candidate HIV-1 vaccines to block primary infection, but it will not provide information on the impact of the vaccine on disease induction or progression. Recent results with Macaca nemestrina suggest that this species can be acutely infected with HIV-1 (14); data are being accumulated to document the degree and reproducibility of this infection. Infection of monkeys with an HIV-SIV chimera, known as SHIV, may also prove valuable in the evaluation of certain candidate HIV vaccines (15).

Most viral vaccines licensed in the United States are whole-killed (inactivated) or live-attenuated products (16). Because of safety concerns associated with administration of a live-attenuated retrovirus vaccine or killed HIV virions to noninfected, healthy individuals, most vaccine manufacturers have elected either not to pursue HIV vaccine development or to pursue alternative approaches to antigen delivery. These approaches have included: recombinant viral and bacterial vectors engineered to express one or more HIV antigens; purified recombinant HIV proteins; pseudovirions and other noninfectious particles that lack HIV genomic RNA; synthetic HIV peptides; and other approaches (Table 1). Many of these candidate HIV vaccines have entered early stages of clinical trial in noninfected and/or infected individuals.

The most studied antigen in candidate HIV vaccines is the HIV envelope protein (17). The majority of neutralizing antibodies in HIV-infected individuals are directed against the HIV envelope (18-23). HIV envelope, as well as gag, pol and nef gene-encoded proteins, possess determinants recognized by CTL from HIV-infected individuals (24-32).

The HIV gp120 envelope protein is an extracellular glycoprotein that mediates the binding of HIV to the CD4 receptor of susceptible cells. Gp160 is a precursor protein that is cleaved during HIV replication into the mature extracellular gp120 and the transmembrane gp41 proteins. Gp160 and gp120 have been prepared as recombinant proteins from one or more expression systems employing insect, yeast and/or mammalian cells (33-40). The HIV env gene has also been engineered into recombinant vaccinia virus, either alone or along with gag and pol genes (41,42). More recently, recombinant canarypox vectors, which express gp160, entered clinical trial, and two envelope peptide-based vaccines entered clinical trial in 1993 (43-45). Other candidates being evaluated in noninfected individuals are based on the HIV p17 (HGP-30) (46) and p24 (Ty-gag) core (47) proteins.

Phase I testing in noninfected volunteers conducted by the National Institute of Allergy and Infectious Diseases (NIAID) AIDS Vaccine Evaluation Group (AVEG) has yielded valuable information concerning the safety and immunogenicity of many of these first generation candidate vaccines (48-56). All HIV-1 vaccine candidates evaluated by the NIAID AVEG in noninfected individuals...
TABLE 1
Types of candidate HIV vaccines

| Candidate vaccine | Expression system/production methods | Developer |
|-------------------|--------------------------------------|-----------|
| Recombinant proteins |
| rgp160<sup>+</sup> | Baculovirus/insect | MicroGeneSys |
| rgp160<sup>+</sup> | Monkey kidney/vaccinia | ImmunoAG |
| rgp160<sup>+</sup> | Mammalian/vaccinia | Pasteur/Merieux Serums et Vaccins |
| rgp120 (env 2-3)<sup>+</sup> | Yeast | Biocine |
| rgp120<sup>+</sup> | Mammalian | Biocine |
| rgp120<sup>+</sup> | Mammalian | Genentech |
| Peptides |
| HGP-30<sup>+</sup> | Synthetic p17 gag peptide | Viral Technologies, Inc |
| p24<sup>+</sup> | Baculovirus/insect | MicroGeneSys |
| Tp24.VLP<sup>*</sup> | Yeast transposon product | British Bio-tech, Ltd |
| V3-MAPS<sup>*</sup> | Synthetic | United Biomedical, Inc |
| MNV3-PPD<sup>*</sup> | Synthetic conjugated to purified protein derivative | Swiss Serum & Vaccine Institute |
| Recombinant poxviruses |
| vac-gp160<sup>+</sup> | Recombinant vaccinia | Bristol-Myers Squibb |
| TBC-3B | Recombinant vaccinia | Therion Biologics |
| CP-gp160<sup>+</sup> | Recombinant canarypox | Pasteur/Merieux Serums et Vaccins |
| Other |
| Whole-inactivated HIV<sup>+</sup> | β-propiolactone and gamma irradiation | Immune Response Corp |
| rCD4<sup>+</sup> | Mammalian | Biogen |
| anti-gp120 Ig<sub>G</sub> | Monoclonal antibody | IDEC |

*Ccurrently in trial in noninfected individuals; †currently or previously in trial in infected individuals. HIV Human immunodeficiency virus.

have been safe and well tolerated. Observed side effects such as local pain and tenderness, low grade fever and minor systemic symptoms, some of which also occur in placebo recipients, are typical of those observed with other vaccines. (When the immune modulator muramyl tripeptide-phosphatidyl ethanolamine is added, fever and malaise have been more prominent.) No major side effects such as renal, hepatic or neurological toxicity have been reported.

With respect to immunogenicity, almost all products evaluated induce binding antibody lasting from weeks to months (48,49,51,55,56). Almost all induce some level of functional antibody, usually against the strain of virus on which the vaccine is based. Functional antibody is defined here as antibody that can neutralize virus in an in vitro acute HIV infection assay and/or inhibit fusion (syncytia formation) between noninfected and infected cells in culture. Recent preliminary results suggest that gp120 may induce more functional antibody than gp160.

Recombinant (r) gp160 has also been evaluated in combination with recombinant live vaccinia virus genetically engineered to express the gp160 envelope protein (vac-env) (54,56). After two priming doses of vac-env, little antibody was produced. Following subsequent immunization with gp160, binding and functional antibody was elicited. Indeed, a higher percentage of individuals produced functional antibody to the vac-env plus gp160 combination than to gp160 alone in comparable immunization protocols (56).

Memory T cells may be essential to induction of a rapid protective immune response upon subsequent exposure to virus. HIV-specific lymphoproliferative responses to HIV antigen, indicative of HIV-specific T cell memory, have been induced by the candidate vaccines in every trial from which data are available (52-55). CTLs may be needed to clear virus-infected host cells and produce a 'sterilizing immunity'. Thus far, CD8<sup>+</sup> major histocompatibility complex (MHC) class I-restricted cytotoxic T lymphocytes (CD8<sup>+</sup> CTL) have been observed only in volunteers immunized with vac-env followed by boosts with rgp160 (57,58; personal communication). CTLs cloned from selected volunteers demonstrated cytolytic activity specific for HIV-infected cells in vitro (57). The vac-env and gp160 combination also elicited CD4<sup>+</sup> MHC class 2-restricted CTLs (CD4<sup>+</sup> CTL). In one study, CD8<sup>+</sup> CTL clones from these volunteers were more potent than CD4<sup>+</sup> CTL in lysing Epstein-Barr virus-transformed autologous B cells transfected with a eukaryotic expression vector carrying the HIV envelope gene (57). One CD8<sup>+</sup> clone lysed target cells at effector:target ratios as low as 0.03:1 (57). Furthermore, CD4<sup>+</sup> CTL clones, but not CD8<sup>+</sup> CTL clones, lysed gp120-pulsed noninfected cells, suggesting that CD4<sup>+</sup> CTL might have a detrimental effect in the presence of significant levels of gp120, although the physiological significance of this observation remains unknown (57).

Peripheral blood lymphocytes isolated from selected volunteers primed with vac-env and then boosted with rgp160 were able to protect severe combined immuno-
deficiency mice from HIV infection (59). Protection correlated more closely with T cell proliferation responses than with the level of antibody in the donor. However, 100% protection was not achieved, and the number of protective cells waned with time after the gp160 boost.

A number of novel adjuvants are being developed for use in human vaccine formulations (60,61). Recent meeting reports of animal studies (62-64) suggest that, in addition to increasing the breadth, magnitude and duration of the antibody response, some of these novel adjuvants show promise in stimulating CD8+ CTL. Several of these adjuvants are expected to be employed in prototype HIV vaccine formulations in clinical trials in the near future, in hopes that both humoral and cellular HIV-specific immune responses can be elicited or augmented.

All products evaluated to date are based on HIV-1 LAI, HIV-1 MN or HIV-1 SF2, which are all laboratory strains of the virus that belong to the same clade or subtype of HIV-1. A high research priority is to determine the degree to which antibodies induced by these candidate vaccines recognize HIV isolated from infected individuals in the United States and other countries. Multiple products or cocktails of products may be required to achieve sufficiently broad immune responses.

THERAPEUTIC VACCINES

Approximately 11 products have entered phase 1 testing in HIV-infected volunteers. These include whole-killed HIV depleted of gp120, rgp160, rgp120 and other CD4 or antibody-based approaches (17,48-56). Five phase 2 trials are in progress or soon to begin.

Most available information is from trials of a candidate HIV vaccine, rgp160, which include the only completed, double-blind, placebo controlled trials of rgp160 in infected individuals (65). Candidates for which safety information has been made available have not elicited any significant toxicities (65,66). Furthermore, rgp120 and rgp160 have been shown to elicit both new humoral and new cellular immune responses in HIV-1-infected individuals. Specifically, rgp160 has elicited new antibody responses directed against the C1, C2, C3 and V3 regions of the HIV envelope, augmented levels of HIV-specific CTLs, induced delayed-type hypersensitivity responses to HIV envelope and increased gp160-specific lymphocyte proliferative responses.

Ongoing and planned placebo controlled phase 2 and 3 trials will help determine if any of these immune responses lead to changes in viral load and/or stabilization of CD4 cell counts. However, it is uncertain whether these virological markers prognosticate clinical outcome. Furthermore, clinical end-points in HIV-infected individuals with more than 500 CD4 cells/mm3 would take an inordinate time to reach. These considerations have led to a proposal that larger trials should begin in the absence of phase 2 data demonstrating that immunization leads to positive changes in progression of infection (e.g., CD4 stabilization and viral load reduction) (67).

SUMMARY

Candidate HIV vaccines are being developed in hopes that one or more will protect noninfected individuals from infection upon subsequent exposure to virus, and/or will elicit new responses of therapeutic benefit in individuals in whom infection is already established. Safety concerns associated with the more traditional approaches of vaccine design, combined with the opportunities provided by advances in biotechnology, have resulted in the pursuit of numerous HIV-1 vaccine designs. The techniques of molecular biology are being applied to an unprecedented extent in the design and delivery of candidate HIV-1 vaccines. Most prototype vaccines are purified recombinant HIV envelope proteins. Results with these products have been encouraging. All products for which information is available have proven safe and immunogenic in noninfected and/or infected individuals. Additional information on the duration, breadth and functionality of the immune responses will be needed before efficacy trials in noninfected individuals are likely to begin. Important information on the benefit of HIV vaccines in infected individuals will likely emerge in the next few years.

Additional novel candidate vaccines are in earlier stages of development, entering human trials in 1993 and beyond. These include viral and bacterial vectors that express one or more HIV proteins, peptide-based approaches, new particle designs and others. In addition, the success of live-attenuated SIV in protecting monkeys from infection by SIV has resulted in renewed interest in live-attenuated designs as prophylactic vaccines (68,69). Should problems or disappointing results with current prophylactic candidates arise, the risks and benefits of live-attenuated products will have to be reconsidered. In the interim, additional preclinical and safety and efficacy studies of live-attenuated and other novel designs is of high priority.

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