VIROLOGICA SINICA, December 2007, 22 (6):486-492
CLC number: R511 Document code: A Article ID: 1674-0769(2007)06-0486-07

HIV Vaccine-Challenges and Opportunities*

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Abstract: The need for an efficacious HIV/AIDS vaccine remains the highest priority of the world HIV/AIDS agenda. The generation of an efficacious HIV/AIDS vaccine proves an enormous scientific challenge. This article reviews the neutralizing antibody problem, elusive immune protection, immunogen design, pre-existing anti-vector immunity and design of phase 3 vaccine trials and the challenges and opportunities in development of HIV/AIDS vaccine are discussed.

Key words: Neutralizing antibody; Cellular immune; Protective immunity; Immunogen; Efficacy studies

Currently available vaccines are mostly effective against acute viruses, which are normally cleared during natural infection, such as smallpox, polio, measles, mumps, rubella and influenza, protection is indeed conferred through neutralizing antibodies. In the meantime, all of the vaccines are able to prevent chronicity during natural infection are associated with the generation of virus-specific neutralizing antibodies. HIV establishes chronic infection, in the end causes AIDS. There is not even an example of clear virus clearance in natural HIV infection. The need for an efficacious HIV/AIDS vaccine is desperately needed (5, 8, 11, 12, 18, 21).

NEUTRALIZING ANTIBODY PROBLEM

Primary isolates of HIV-1 from different genetic subtypes can be neutralized by some broadly reactive human monoclonal antibodies such as b12, 2G12, 2F5 and 4E10. In non-human primate models of AIDS, systemic passive transfer of such neutralizing monoclonal antibodies (under conditions to achieve high antibody concentrations *in vivo*) can provide protection to macaques against simian-HIV (SHIV) challenge. Given the practical limitations of achieving passive HIV prophylaxis via monoclonal antibody cocktails, the neutralizing antibody problem is one of the most difficult problems to be solved in the design of a successful HIV vaccine. The array of protective structural carbohydrates that covers the antigens on the HIV virus surface is one of the main barriers to antibody neutralization. The conserved neutralizing epitopes in the native gp120 are not accessible or recognized by the immune system. The most interesting results come from a novel variant of trimeric Env

Received: 2007-07-18, Accepted: 2007-10-16

* Foundation item: National High Technology Research and Development Program of China (2005AAZ18040)

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containing a deletion in the second variable loop (ΔV2-Env), which was developed and tested in preclinical models. Vaccination with ΔV2- Env protein induced cross-clade neutralizing antibody in rabbits using a DNA-prime/protein-boost regimen, and controlled infection in monkeys following virus challenge (4, 6, 15, 18).

The International AIDS Vaccine Initiative has established the Neutralizing Antibody Consortium (NAC), in expect to accelerate solving the critical vaccine design problems. The NAC is placing great emphasis on the determination of antibody and Env structures as a major starting point from which to apply rational vaccine design, with the progress in structural genomics for glycoprotein crystallization, including mimicing the mature Env trimer, generating engineered Env to better present Nab epitopes, producing epitope mimics of the broadly neutralizing mAbs determined from structural studies of the antibody-antigen complexes (2).

IMMUNOLOGICAL CORRELATES OF PROTECTION

Under certain conditions HIV-1-specific cell-mediated immunity may effectively control virus replication during established chronic infection. The administration of a highly attenuated antibody, neutralization-sensitive mutant of SIV with complete deletion of the V1-V2 region of the envelope protein has conferred potent protection against intravenous challenge by wild-type, pathogenic SIVmac239. Protection has been achieved in some monkeys despite the fact that antiviral immune responses measured in peripheral blood are extremely weak or undetectable at the time of challenge. Those results provide important lessons for AIDS vaccine development. It is demonstrated that high antiviral immune responses, measured in the peripheral blood by standard assays, are not necessarily required for achieving vaccine-mediated protection from pathogenic SIV strains that are closely analogous to HIV. It also provided encouraging evidence that an effective HIV vaccine can be developed (9).

In current preclinical and clinical trials, CTL function is currently measured with assays such as IFN-γ enzyme-linked immunospot (Elispot) assay and intracellular staining by flow cytometry (ICS). Although Elispot is robust, sensitive and inexpensive for rapid screening of antigen-specific CTLs and epitope mapping, it does not provide information regarding the immune-phonotype of responder lymphocytes and only measures production of a single effector molecule, IFN-γ. In contrast, ICS correlates better with CTL responses in the multi-parameter format and it can provide more information simultaneously about the specific T-lymphocyte subset, its maturational phenotype and multiple functional profiles. Recent data have reported that IFN-γ is not consistently associated with the control of HIV-1 replication and that examination of IFN-γ secretion alone will underestimate the total of anti-HIV-1 specific $CD4^+$ T lymphocytes. Secretion of IFN-γ is typically happens during the early phase of immune response generation but will decrease with antigen clearance. In contrast, IL-2 secretion is typical of the long-term memory (predominantly CD4+) T-cell response and thus it may be an important cytokine signature in long-term memory T cells. Thus, the assessment of vaccine-specific responses must be extended to detect IL-2 secreting cells.

Other potential assays that have been used for measurement of use in vaccine end-point measurements include the tetramer assay that directly test the frequency of $CD8^+$ T lymphocytes with a specific receptor and, if combined with multicolor flow cytometry, allow phenotype identification. However, the drawback is that reagent preparation requires knowledge of the subjects HLA type. Another assay that measures cell proliferation is also gaining use because recent data suggest that it is the proliferative capacity, rather than the quantity of CTLs that is most relevant to controlling HIV-1 replication. $CD8^+$ T lymphocyte proliferation appears to be coupled to perforin expression, which contributes to the killing ability of infected cells. Future immunogenicity tools are likely to include microarrays to identify specific gene profiles that might be associated with developing protective immune responses against HIV-1 upon vaccination (16, 17, 19).

PRECLINICAL VACCINE EFFICACY STUDIES

At the early stage of evaluating T cell-based vaccine strategies for control of virus load, the most rigorous vaccine responses were directed against the Gag antigen, which induced substantial proliferative as well as IFN-γ and IL-2 responses, as assessed by enzyme-linked immunospot assay (ELISpot). In contrast to Env, which induced somewhat weaker IFN-γ and IL-2 responses but a substantial IL-4 response, as assessed by ELISpot assay. Protection from $CD4^+$ T cell loss was often seen when the T cell response to Gag was dominant. However in the absence of Gag, improved DNA plasmids encoding Env, Tat and negative factor (Nef), when delivered by gene gun, generated 'preferential' IFN-γ and IL-2 responses to the proteins that they encoded. Whereas when primed or boosted with protein, IL-4 $CD4^+$ T cell responses became predominant. In a large comprehensive DNA-Ad5 vaccine study designed to evaluate the efficacy of Gag-Pol, Env alone, Gag-Pol plus Env or Ad5 alone, after challenged by SHIV-89.6P virus, Gary Nabel found that the most important correlation of survival seemed to be not the vaccine antigens but instead the persistence of $CD4^+$ central memory T cell counts, that is, Gag-specific $CD8^+$ central memory T cells but not $CD8^+$ effector memory T cells. Data indicates that systemic vaccination can (in part) protect from considerable loss of $CD4^+$ memory T cells in the acute period in mucosal as well as other compartments (7, 13, 14).

Pre-existing immunity to AdHu5, commonly found in humans, changed the homing pattern of vaccine-induced T cells. In AdHu5 pre-exposed rhesus monkeys models，frequencies of transgene-specific T cell were higher in spleens than in blood, when vaccinated with the chimpanzee Ad vectors, but it was exceptional high in livers when vaccinated either with AdHu5 vectors or chimpanzee adenovirus vectors. The latter results indicate that analysis of T cell responses solely from blood mononuclear cells of vaccine recipients may not suffice to compare the potency of different vaccine regimens. However, the correlations of protection involved in preventing disease progression during natural infection with HIV-1 do not necessarily reflect those that would be protective in the presence of pre-existing vaccine-induced immunity. It is necessary to expand heterotypic vaccination challenge studies with a much larger pool of monkeys of different major histocompatibility complex type. The increasing scarcity of

Indian rhesus monkeys calls for development AIDS model by using Chinese rhesus monkey, which needs knowledge of the detailed analysis of genotype of major histocompatibility complex. We need more clinical trials, it is difficult to predict exactly how pre-existing immunity would affect the outcome of HIV-1 infection.

IMMUNOGEN SELECTION

The set of HIV antigens and, by analogy, SIV antigens that must be included in an effective vaccine to confer protection against HIV and SIV respectively, remain unclear. Several antigens are potential targets for cell-mediated immune responses, and the envelop glycoproteins gp41and gp120 are the principal targets for neutralizing antibodies. Designing an immunogen to elicit broadly neutralizing antibodies to HIV remains a chief scientific challenge. The various HIV vaccine candidates now in human clinical trials focusing on the induction of cell-mediated immune responses express incomplete subsets of the full complement of HIV antigens. Furthermore, none of the candidates matche the set of analogous antigens incorporated in live attenuated SIV and none confers the level of protection obtained with live attenuated SIV. The Live Attenuated Consortium (LAC) of the International AIDS Vaccine Initiative (IAVI) is addressing the importance of antigen persistence in conferring protection by systematically comparing the immune responses elicited by live attenuated SIV, to viral vectors known for their capacity to persist. All the relevant systems to be compared will carry a set of functional genes similar to those found in live attenuated SIV (10).

In addition to six structural proteins [group-specific antigens (Gag), polymerase (Pol), envelop protein (Env), protease (Pro), reverse transcriptase (RT) and integrase (Int)], the HIV genome encodes the small regulatory proteins Tat, Rev and four accessory proteins (Nef, Vif, Vpr and Vpu). HIV-1 Rev, Tat and Nef play important roles in pathogenesis of HIV/ AIDS disease by facilitating transmission across the mucosal barrier, viral replication and dissemination, whth impairing immune response. Besides Rev, Tat and Nef pathogenetic effects, which constitute a solid substrate for their inclusion in vaccine formulation, Tat and Nef (Nothing is know for Rev) also display immunomodulatory features that make them attractive adjuvants for other antigens. Interestingly humoral and cellular responses to Rev, Tat and Nef are present during the asymptomatic phase of infection and correlate with the non-progression to AIDS (3).

Epidemiological and experimental evidence support the development and evaluation in Phase I trials of an entirely new generation of vaccines based on the rational combination of HIV-1 non-structural (Rev, Tat, Nef) and structural (ΔV2-Env) gene products for induction of effective, long-lasting and possibly sterilizing antiviral immunity. However, due to potential antigen interference and immunodominant effects of Env, the new vaccine design, formulation and immunization protocol must be evaluated and optimized before proceeding to human trials.

CURRENT CLINICAL TRIALS

In general terms, there are now three schools of HIV vaccine development. The first aims to develop HIV-1 vaccine candidates that will induce broad cross-neutralizing antibodies to conserved B cell epitopes of the HIV-1 envelope. This has proven extremely dif ficult.

As two VaxGen efficacy trials of monomeric gp120 have failed. There are relatively few vaccine candidates of that type in clinical trial. For the first kind of vaccine, neutralizing antibodies to Env have occasionally been found in the few studies in which protection from infection has been noted. The second focused on development of T cell-mediated HIV vaccine candidates, it will reduce viral load and CD4 T cell loss, prolong survival of cells, and reduce transmission of virus. The challenge of that approach is to generate $CD8^+$ T cell responses to conserved HIV-1 epitopes in populations with a diverse HLA background and being exposured to a wide variety of HIV-1 genotypes. The third utilize a combined approach, in which broad T helper responses should be induced to drive B cell responses to conserved neutralizing epitopes and CTL targets of HIV. As neutralizing antibodies are the immune responses that confer protection against infection. Cell-mediated immune responses are the immune responses that confer protection against disease (9).

Adenoviruses are attractive and highly promising, because they infect the epithelial cells lining mucosal surfaces. It is able to elicit both systemic and mucosal cellular immune. A recombinant nonreplicating adenovirus vector is in two phase 2b trials each of which will each enroll 3 000 people at high risk for HIV infection. In human history no vaccine has previously been developed or licensed based on cellular immune responses, and thus major scientific, regulatory, and logistic challenges are undoubtedly ahead for T cell-based vaccines. The objective of phase 2b proof-of-concert studies is to evaluate immune correlates of protection in humans, which will provide valuable guidance for the future optimization and evaluation of vaccine candidates. Prevention of HIV-1 infection as well as reduction of peak and setpoint viral loads will be evaluated as endpoints in these studies. Therefore, a detailed qualitative and functional characterization, rather than the simple detection of the vaccine-specific immune response, will be required to determine the immune correlates of protection against HIV (1, 20).

As a unique feature of HIV is that a reservoir of latently infected resting $CD4^+$ T cells is established very early during primary infection. The long-term goal for an effective HIV vaccine is to prevent the establishment of such HIV infection. So far it has been achieved only by live attenuated SIV vaccines in monkeys. The design of HIV vaccines that mimic and improve the efficacy of live attenuated SIV vaccines will probably require both systemic testing of scientific hypotheses and innovation in immunogen design. A HIV vaccine that prevents the establishment of chronic infection would probably need to induce immune responses that halt the local expansion of HIV prevent dissemination of HIV to distal lymphatic tissue within the first week or two ot both. A vaccine that acts at later stages might still reduce or prevent the destruction of $CD4^+$ T cells, and it helps in controling infection and to prolonging disease-free survival time. It is now accepted that broadly neutralizing antibodies, T helper cell, cytotoxic activities, innate immunity, long-lived memory T cell response, long-lived memory B cell responses are all important for protection and control of disease progression. Combined this kind of 'trial and error' and limited rational design are the main endeavor on the path to a successful HIV vaccine until we have a more clear understanding of its pathogenesis mechanism.

However, on September 21 2007, vaccination in a phase II clinical trial of Merck's investigational HIV vaccine is being discontinued because the vaccine was not effective. The once promising leading experimental AIDS vaccines not only failed to prevent test subjects from becoming infected with HIV, but it didn't offter any indication it might delay the onset of full-blown AIDS, which had been a key hope. The Merck vaccine did stimulate the immune system's T-cells--a notable development-but not in a way that helped infected test subjects control the virus. Now, researchers will try to figure out why. The ultimate fear among researchers is that the whole theory underlying the Merck vaccine might be flawed, which, if true, could doom an entire class of experimental vsaccines.

CLINICAL TRIALS IN CHINA

In China, until now, some progress has been achieved, especially in pre-clinical AIDS vaccine researches based on vaccinia vector vaccine of Tiantan strains, adenovirus vector, adeno-associated virus vector vaccine, Sendai vector, peptide epitope vaccine, protein vaccine etc.

In cooperation with Europe, the Chinese Center for Disease Control and Prevention had developed AIDS vaccine of the recombinant vaccinia virus vector, and results from Phase I clinical studies showed that the vaccine is safe and reliable.

In fact, the Phase I clinical trial of the first HIV vaccine in China, had been formally initiated in 2005 by Jilin University and Guangxi CDC. After180 days observation on 49 volunteers. it had been completed on August of 2006. It showed there was no serious adverse reactions. HIV-1 virus-specific cellular immune responses had been elicited after 15 days of vaccination, particularly in the high-dose group subjects.

However, it appears that AIDS vaccine research in China exerted limited impact on AIDS vaccine impact on AIDS- vaccine development sin the world. Furthermore China need effective vaccines which intellectual property.

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