SII-4

HIV-1 Vaccine Development: Need For New Directions.

Michael W. Cho, Ph.D.

ABSTRACT

The AIDS epidemic continues unabated in many part of the world. After near two decades, no vaccine is available to combat the spread of this deadly disease. Much of the HIV-1 vaccine effort during the past decade has focused on the viral envelope glycoprotein, largely because it is the only protein that can elicit neutralizing antibodies (Nabs). Eliciting broadly cross-reactive Nabs has been a primary goal. The intrinsic genetic diversity of the viral envelope, however, has been one of the major impediments in vaccine development. We have recently completed a comprehensive study examining whether it is possible to elicit broadly acting Nabs by immunizing monkeys with mixtures of envelope proteins from multiple HIV-1 isolates. We compared the humoral immune responses elicited by vaccination with either single or multiple envelope proteins and evaluated the importance of humoral and non-humoral immune response in protection against a challenge virus with a homologous or heterologous envelope protein. Our results show that (1) Nab is the correlate of sterilizing immunity, (2) Nabs against primary HIV-1 isolates can be elicited by the live vector-prime/protein boost approach, and (3) polyvalent envelope vaccines elicit broader Nab response than monovalent vaccines. Nonetheless, our findings clearly indicate that the increased breadth of Nab response is by and large limited to strains included in the vaccine mixture and does not extend to heterologous non-vaccine strains. Our study strongly demonstrates how difficult it may be to elicit broadly reactive Nabs using envelope proteins and sadly predicts a similar fate for many of the vaccine candidates currently being evaluated in clinical trials. We have started to evaluate other vaccine candidates (*e.g.* genetically modified envelope proteins) that might elicit broadly reactive Nabs. We are also exploring

other vaccine strategies to elicit potent cytotoxic T lymphocyte responses. Preliminary results from some of these experiments will be discussed.

PRESENTATION SUMMARY

According to 1999 UNAIDS report over 33 million people are currently living with HIV/AIDS worldwide. More than 16 million people have already died since the start of the epidemic. Reports of drug-resistant variants, debilitating side effects and high cost of the drugs are putting serious doubts in the long-term success of currently available combination antiretroviral therapies. It is a widely held belief that a vaccine is the only cost-effective measure of containing the AIDS epidemic. The progress of AIDS vaccine development has been frustratingly slow. The difficulty lies from the fact that HIV-1 is drastically different from other human pathogens we have encountered. It includes the lack of documented natural immunity, the ability of the virus to integrate its genetic codes into host's genome, and the ability of the virus to infect and kill immune cells that play a central role in eliciting immune response against pathogens. High antigenic variation of its surface envelope glycoprotein, the lack of a good animal model, and unknown correlates of protection make the vaccine development even more difficult.

Neutralizing antibodies (Nabs) are critical components of the immune response that controls a variety of viral infections. However, the protective role(s) of Nabs directed against HIV-1 and other primate lentiviruses, which become detectable following acute infections, has been debated over the years and remains unresolved. Nonetheless, passive immunization experiments have demonstrated the protective effects of Nabs against subsequent challenges with primate lentiviruses. However, the development of immunogens capable of prospectively eliciting broadly reactive Nabs against multiple virus isolates has been

frustratingly unproductive. None of the envelope glycoprotein-based vaccine candidates tested in primates thus far have been able to elicit broadly reactive Nabs, especially against primary isolates.

The HIV-1 envelope glycoprotein contains five highly variable regions. These variable regions very likely cover significant portions of the exposed surface on the trimeric gp120 complex, as suggested from antigenic probing with monoclonal antibodies and crystallographic data of the envelope core. The variable regions of HIV-1 and SIV gp120 have long been known to be targeted by Nabs. In contrast, the conserved domains of gp120 are either extensively shielded by carbohydrate moieties, obscured beneath the variable regions, or hidden due to intermolecular protein-protein interactions and do not elicit antibodies that neutralize virions.

Conserved neutralizing epitopes, present on the unmodified native gp120, have been nearly impossible to identify. To date, only two human anti-gp120 monoclonal Nabs, which exhibit relatively broad neutralizing activity, have been isolated. Immunization with a variety of envelope glycoprotein preparations (*e.g.* monomeric gp120, soluble gp160, oligomeric gp140, virions or virus-like particles) and using different vaccine strategies (*e.g.* whole-inactivated virus, subunit, live vector, DNA vector) usually results in extremely narrow and/or immunogen-specific Nab responses.

Most lentivirus vaccine studies have utilized envelope glycoproteins from either one or, at most, two virus isolates; protective efficacy has usually been assessed using a homologous virus challenge (*i.e.* the virus isolate used to challenge animals contains the same gp120 used for immunization). In reality, however, vaccinated individuals would be expected to encounter an HIV-1 isolate/viral quasispecies containing a gp120 unrelated to the immunogen used for vaccination (heterologous challenge). In our study, we have evaluated a

vaccine regimen, based solely on envelope glycoproteins, which utilizes individual or mixtures of both recombinant vaccinia viruses and gp120 boosts to address the following questions pertaining to protection against heterologous virus strains: (i) Is it possible to elicit a broader Nab response by immunizing with a mixture (polyvalent) of envelope glycoproteins? (ii) If so, does the breadth of the Nab response extend beyond the virus isolates included in the immunization cocktail? (iii) How does the protective efficacy of the immune response elicited by a polyvalent envelope vaccine compare to that elicited by a monovalent (homologous or heterologous) envelope vaccine? And (iv) will there be antigenic competition between the different envelope glycoprotein components of the polyvalent envelope vaccine cocktail?

Macaques were challenged with $SHIV_{DH12}$ following priming with recombinant vaccinia virus(es) expressing gp160(s) and boosting with gp120 protein(s) from: i) LAI, RF, 89.6, AD8 and Bal (Polyvalent); ii) LAI, RF, 89.6, AD8, Bal, and DH12 (Polyvalent-DH12); iii) 89.6 (Monovalent-89.6); and iv) DH12 (Monovalent-DH12). Animals in the two polyvalent vaccine groups developed Nabs against more HIV-1 isolates compared to those in the two monovalent vaccine groups ($p=0.0054$). However, the increased breadth was directed almost entirely against the vaccine strains. Resistance to $SHIV_{DH12}$ strongly correlated with the level of Nabs directed against the virus on the day of challenge ($p=0.0008$). Accordingly, the animals in the Monovalent-DH12 and Polyvalent-DH12 vaccine groups were more resistant to the $SHIV_{DH12}$ challenge than the macaques immunized with preparations lacking a DH12 component (*viz.* Polyvalent and Monovalent-89.6) ($p=0.039$). Despite the absence of any detectable Nab, animals in Polyvalent vaccine group, but not those immunized with Monovalent-89.6, exhibited markedly lower levels of plasma

virus compared to those in the control group, suggesting a superior cell-mediated immune response induced by the polyvalent vaccine.

In attempts to explore other vaccine candidates for eliciting broadly reactive Nabs, we have generated recombinant vaccinia viruses and plasmid constructs that encode gp160 and gp120, respectively, with deletions of the variable loops. By removing the variable loops, we are hoping to (1) expose any hidden conserved neutralization epitopes and/or (2) eliminate immunodominant variable regions so that the immune response can be redirected towards more conserved regions of the protein. In addition to variable loop deleted envelope proteins, we have also developed a system that expresses envelope proteins containing random amino acids in the variable loops V1/V2 and V3. Finally, we are also exploring vaccine strategies that can elicit potent cytotoxic T lymphocyte response, including a live attenuated virus vaccine. Although these experiments are very preliminary, the design and prospects will be discussed.