

# Advances in AIDS Vaccines

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*HIV/AIDS vaccine research reported at the conference was marked by optimism on future prospects for novel vaccine approaches but also by continued concern regarding the limited success of products currently in clinical trials and an increasing sense of urgency at the continued spread of HIV infection. Substantial hope was offered by the wide range of vaccine approaches now being evaluated in nonhuman primates that are able to consistently induce cytotoxic T lymphocyte (CTL) responses, a goal that was frustratingly elusive several years ago, and by the expanded range of quantitative techniques to measure cell-mediated immune responses, thereby allowing more accurate comparisons of different vaccine approaches. However, translation of these novel vaccine approaches into clinical trials has been time-consuming and results from these early trials have only addressed questions of safety and immunogenicity, while issues of efficacy remain unanswered.*

## National Institutes of Health Vaccine Research Center

In the keynote lecture, Nabel, director of the newly formed National Institutes of Health Vaccine Research Center (VRC), provided a broad overview of the state of HIV vaccine development and the desired characteristics of an effective AIDS vaccine (Abstract L2). He highlighted the importance of inducing CTL responses, as well as the desirability of strategies able to provide long-lived presentation of vaccine antigens. He also provided an update on the formation of the VRC, which will open in the fall of 2000. The VRC will include a broad-based spectrum of expertise, ranging from basic science to nonhuman primate and human clinical trials, and recruitment of investigators for the VRC is underway.

## Novel Vaccine Approaches

Shiver (Abstract S4) reviewed the strategy that he and his colleagues have developed for standardized assessment of T cells through development of short-term stimulation with peptide pools and ELISPOT assays that detect cytokine-secreting cells. Studies in HIV-infected individuals showed that ELISPOT assays can reproducibly and quantitatively measure cell-mediated responses to a variety of HIV proteins, including Gag, reverse transcriptase, Nef, Rev, and Tat. Preclinical research in macaques was presented using DNA vaccines developed by codon optimization and utilization of strong promoters to bypass the requirement for Rev and the *rev* responsive element and thereby enhance expression of HIV genes. Immunization of macaques 4 times with low doses (1 mg) of "optimized" HIV-1 *gag* DNA has generated strong interferon- $\gamma$  ELISPOT responses to selected peptide pools. These responses in macaques appeared to be at the lower range of the responses seen in HIV-infected subjects.

The oral presentation sessions described several exciting new products and strategies for HIV/AIDS vaccines. Barnett (Abstract 431) demonstrated that the use of polylactide galactide (PLG) particles results in improved delivery of DNA vaccines and makes it feasible to deliver 500-fold lower doses of DNA while still inducing strong immune responses. In addition, in collaboration with Stamatatos and colleagues (Barnett et al. Abstract 828; Lian et al. Abstract 799) they have immunized macaques with a recombinant gp120 from a primary isolate (SF162) that has a deletion of a portion of the V1/V2 variable region that contains numerous glycosylation sites. Sera from vaccinated macaques were able to neutralize about half of the primary isolates in the National Institutes of Health (NIH) panel at the 50% level and several even at the 90% level. Also, Gardner and colleagues (Abstract 432) report-

ed early impressive immunogenicity data on Sindbis (alpha) virus replicons that appear to be targeting dendritic cells for antigen presentation. The combination of these approaches is being explored in an attempt to further boost production of neutralizing antibodies.

Other presentations in the session reinforced the potent immunogenicity of dendritic cell-based vaccine approaches. Expression of HIV antigens in dendritic cells by either transfection of RNA-encoding HIV Gag (Weissman et al. Abstract 433) or by infection with canarypox (ALVAC) vectors expressing simian immunodeficiency virus (SIV) proteins (Ignatius et al. Abstract 434) resulted in the induction of both CD4+ T-cell and CD8+ T-cell responses.

There have been several attempts to identify and explore the possibility of using mimetopes or synthetic epitopes to induce functional antibody responses against HIV. Hanson and colleagues (Abstract 432A) presented preliminary data using an approach that has identified an epitope that is detected by a monoclonal antibody to human CD4. The details of the antibody have been previously published, and the presentation focused on the data that have been generated from immunizations of guinea pigs and swine to this epitope mimic. Neutralization was observed against multiple strains of HIV, including those representing primary isolates from different clades of HIV.

A number of posters provided information about new forms of antigens that are being developed for HIV vaccines. Srivastava and colleagues (Abstract 822) presented information about a newly developed oligomeric gp140 product from the non-syncytium-inducing, R5 isolate US4 that has shown some promise in small animal immunogenicity testing with RIBI adjuvant formulation. Other oligomeric products such as the one presented by Binley and colleagues, in which gp120 is stably linked to gp41 by a disulfide bond (Abstract 824), await immunogenicity testing.

## Induction of Mucosal Immune Responses

The ability of candidate AIDS vaccines to induce mucosal immune responses is likely to be an essential feature of an effective AIDS vaccine, not only because of the importance of mucosal transmission of HIV, but also because of the large pool of activated T cells in the gastrointestinal tract that serve to support HIV replication after infection. Because of the compartmentalization of systemic and mucosal immune systems, systemic immunization has been notoriously inefficient at inducing immune responses in mucosal sites, prompting an intensive effort to develop novel vaccine approaches able to induce mucosal immune responses. Many of these approaches have yielded promising results in mice, only to fail to live up to expectations in primate experiments. Wang and colleagues (Abstract 106) evaluated the immunogenicity of a DNA vaccine that produces genetically inactivated SIV particles following different combinations of systemic and mucosal immunizations. Mucosal DNA immunization, administered intrarectally (via liposomes), resulted in impressive levels of SIV-specific IgA in rectal secretions with lower levels of systemic SIV-specific IgG or CTLs. Although levels of protection following intrarectal challenge with the pathogenic SIV<sub>mac239</sub> strain were unimpressive (2 of 7 animals protected), the study provides a convincing proof of concept of the ability to induce mucosal immune responses in primates using DNA immunization. Further optimization of this vaccine approach should improve the ability to induce mucosal IgA responses in concert with more vigorous systemic humoral and cellular responses.

## Live Attenuated AIDS Vaccines

Despite impressive results in nonhuman primates, concerns over the safety of live attenuated HIV vaccines have proved to be an all but insurmountable obstacle to extending this approach to human trials. Several reports documenting the ability of SIV to compensate for mutations or deletions in *nef* further highlighted the challenges facing this approach. Previous data evaluating the development of AIDS following infection of neonatal or adult

macaques with attenuated SIV strains had not defined the extent to which evolution of changes in the viral genome can compensate for deletion of *nef* or other accessory genes. Alexander and colleagues (Abstract 150) presented results from the New England Regional Primate Research Center showing that passage of 2 separate lineages of viruses obtained from animals infected with SIV strains with a 182-base pair deletion in *nef* resulted in moderate to high virus loads in the recipient animals. Sequencing of the viruses obtained from these from animals revealed a number of changes, including in the cytoplasmic domain of gp41, shortening of the U3 region of the long terminal repeat (LTR), or duplication of the NFκB transcription activation site. Inoculation of monkeys with molecular clones with different combinations of these mutations revealed that changes in the cytoplasmic domain of gp41, either alone or in combination with the other mutations, were able to induce high virus loads.

Further evidence for the ability of SIV to compensate for *nef* deletions was presented by Ye and colleagues (Abstract 445), who described evolution of pathogenic SIV in animals that had been infected with a SIV strain with a 150-base pair deletion in *nef*. The development of pathogenic strains was associated with evolution of a truncated Nef formed by reversion of a stop codon to restore the open reading frame, ultimately resulting in expression in a truncated form of Nef that was still able to interact but not activate the Nef-associated kinase. The remarkable ability of primate lentiviruses to compensate for even large genetic deletions in *nef* is likely to be a difficult obstacle to surmount in the quest for the development of an attenuated AIDS vaccine.

Debate regarding the potential use of live attenuated HIV vaccines in human clinical trials has often failed to account for the potential benefit of even a risky vaccine in a high-risk population. Blower and colleagues (Abstract 812) applied a novel transmission model to carry out a risk/benefit analysis that takes into consideration the uncertainty of both the protective efficacy and safety of a hypothetical attenuated HIV vaccine. In high-risk populations such as in Zimbabwe, a

live attenuated vaccine could be beneficial, whereas in an area with lower incidence of HIV transmission (eg, Thailand) the risks of vaccine-associated AIDS would outweigh the benefits.

## Nonhuman Primate Trials

Direct comparisons of the ability of different vaccine approaches to include CTL responses has been difficult, in part due to the limited ability of standard functional CTL assays to precisely measure the magnitude of responses. Recent advances in the ability to quantitate cell-mediated immune responses via ELISPOT assays, intracellular cytokine staining, and major histocompatibility complex (MHC) tetramers, which allow the precise measurement of T cells able to recognize this specific epitope by flow cytometry, have now permitted a more precise comparison of the immunogenicity of different vaccine approaches. In a symposium on AIDS vaccines, Letvin provided a direct comparison of the ability of different AIDS vaccine approaches to induce SIV-specific CD8+ T cells in rhesus macaques, as assessed by functional CTL assays and by the use of MHC tetramers (Abstract S5). Immunization of monkeys expressing the Mamu-A\*01 MHC class I allele with a canarypox vector (ALVAC) encoding the SIV *gag* and *pol* genes revealed relatively low levels of tetramer-binding cells after 5 vaccinations (0.1% to 0.3% of all CD8+ T lymphocytes). Challenging these animals demonstrated no evidence for a decrease in viral load in vaccine recipients compared with naive controls.

In contrast, immunization with a modified vaccine Ankara (MVA) expressing the SIV *gag* and *pol* genes resulted in peak levels of tetramer binding cells up to 5% after 2 immunizations, although these responses rapidly waned after each boost. Subsequent boosts were able to transiently elevate the number of tetramer-binding cells, but were not able to induce sustained increases in SIV-specific CD8+ T-cell responses. Following challenge with a pathogenic SIV strain (SIV<sub>smE660</sub>), MVA-vaccinated animals had a nonsignificant trend toward lower viral loads than controls.

The potential pitfall of relying solely on levels of tetramer-binding cells to

evaluate AIDS vaccines was provided by results from a similar study involving immunization of macaques expressing Mamu A\*01 with a codon-optimized SIV DNA developed by Shiver and colleagues. Levels of tetramer-positive cells induced by DNA immunization were relatively low (0.1% to 0.9%), and as with the other modalities, decayed rapidly following booster immunization. However, despite these modest levels of SIV-specific CD8+ T cells, viral loads following challenge with SIV<sub>sm</sub>E660 were significantly lower than those in unvaccinated controls. The reason for this discrepancy is not clear and suggests either qualitative differences in the antiviral efficacy of tetramer-binding cells induced by different vaccine approaches or the effect of the immune responses other than tetramer-binding cells. Finally, a summary of experiments employing an interleukin-2 (IL-2)/immunoglobulin fusion protein demonstrated that the IL-2-IgG protein, either as a recombinant protein or as DNA immunization, was able to increase levels of SIV-specific humoral responses and CD8+ T cells. Overall, these results are encouraging in documenting the ability of a variety of different vaccines to induce SIV-specific CTL and the utility of the newer techniques of quantitation. Further use of these vaccines with other approaches able to induce better levels of humoral responses show promise for the future.

### Human Clinical Trials of Prophylactic AIDS Vaccines

Graham (Abstract S7) presented a review of the status of vaccines now in clinical trials in the AVEG/HIVNET program. Currently an ALVAC vector, vCP205, combined with a recombinant gp120 product, is under evaluation in 435 volunteers. CTL responses to Gag or Env or both were detected at week 14 in 6 of 16 individuals (37%) and at any time in 14 of 22 individuals (64%) studied. Encouraging evidence has been generated by these early trials. CTLs that are generated by these vaccines recognize viral proteins from other clades of virus (Ferrari et al. 1997). The frequency of individuals with CTL responses that can be detected at extended time points after vaccination (up to 18 months) with the ALVAC vectors

appears to be stable or increasing (Evans et al. 1999). In addition, the combination use of a recombinant protein boost is highly effective in inducing a rapid and relatively high titer antibody response. Studies are continuing to evaluate improved vectors, alternative recombinant envelope products for boosting, and different regimens to optimize the peak and duration of immune responses.

Weinhold and colleagues (Abstract 657) provided an update on the assessment of CD8+ T-cell responses in seronegative vaccine recipients using a series of complementary assays, including conventional CTL assays, interferon- $\gamma$  ELISPOTS, and MHC class I tetramers. One of the most striking observations was that HLA-A\*201 individuals with strong CTL responses to Gag do not appear to recognize an epitope in *gag* (SLYNVATL) that has been reported to be recognized by approximately 70% of chronically infected individuals. Responses to this epitope were not detected even after *in vitro* stimulation of peripheral blood mononuclear cells (PBMCs) from the subjects with specific peptide. These results were similar to those reported by Betts and colleagues (Abstract 655) in HIV-infected subjects and reinforce the need for caution in depending on the analysis of responses to single epitopes in assessing immune responses to complex immunogens.

The initiation of Phase III clinical trials using recombinant gp120 has garnered considerable attention, although debate continues on whether antibody responses induced by these recombinant proteins are likely to prevent or reduce infection. Berman (Abstract 823) presented recent data from individuals vaccinated with the clade B/E dual component gp120, which is currently being used in clinical trials in Thailand. The clade B/E immunogen was able to induce neutralization of clade E R5 isolates in a subset of volunteers. Definitive evidence on the efficacy of this vaccine approach awaits the results of ongoing Phase III trials.

### Therapeutic Vaccination

Increasing evidence of the limitations of highly active antiretroviral therapy

(HAART) and emerging results from a rapidly expanding body of evidence on structured treatment interruption (STI) have prompted renewed interest in therapeutic immunization. Interpretation of data from a number of ongoing human clinical trials analyzing the effects of therapeutic immunization are complicated by a variety of factors, including variations in the stage of disease, heterogeneity in viral strains, and different regimens employed, among others. Studies in non-human primates offer the opportunity to rigorously address such questions as the effect of therapeutic immunization on host immune responses and their efficacy at different stages of infection.

Franchini and colleagues from the National Cancer Institute presented results from a study examining the effect of therapeutic immunization with an attenuated poxvirus (NYVAC) with and without HAART (Abstract S6). Monkeys were treated with PMPA (a potent reverse transcriptase inhibitor), didanosine, and stavudine 2 weeks after infection with SIV<sub>mac</sub>251 and then received either 3 vaccinations with a NYVAC-expressing SIV Gag/Pol or control NYVAC vector during 6 months of antiretroviral therapy. NYVAC/SIV vaccination resulted in significant levels of proliferative responses to both SIV Gag and gp120, as well as transient increases in the level of SIV-specific CD8+ cells (as assessed by MHC tetramers). Following discontinuation of treatment, 5 of 6 animals had transient spikes in viremia, but all 6 animals were able to contain SIV replication to less than 5000 copies/mL per animal for up to 40 weeks after infection. These animals with controlled viremia had increases in the strength in SIV-specific T-helper responses and transient increases in the levels of SIV-specific CD8+ T cells. However, 3 of 7 that received the control vaccinia were also able to control viremia to levels below detection limits, suggesting that early treatment alone, even without immunization, was able to improve the ability of the host to contain SIV replication. Regardless of vaccination status, the presence of proliferative responses to SIV p27 antigen were significantly associated (before or after STI) with control of viremia.

Overall, these results reinforce the hypothesis that early antiretroviral treat-

ment can significantly improve the ability of the host to contain AIDS virus replication. Further studies both in humans and macaques will be necessary to clearly demonstrate whether therapeutic immunization per se can improve the ability of host immune responses to contain viral replication and to delineate the relative importance of T-helper and CTL responses.

Several different human clinical trials provided encouraging evidence of the immunogenicity of different therapeutic vaccine regimens. Vaccination with a whole, inactivated HIV that is depleted of gp120 (Remune) resulted in increases in the frequency of HIV-specific CD4+ T cells determined by intracellular cytokine staining following in vitro stimulation with HIV antigens (Abstract 347). These boosts and T-helper responses were also demonstrated by increases in in vitro proliferative responses to HIV antigens. Similar results were obtained by investigators at the Henry Jackson Foundation (Abstract 348), who observed 4-fold increases in p24-specific antibodies and 6-fold increases in HIV-specific proliferative responses following immunization with whole inactivated HIV. Neither of

these groups presented any evidence regarding whether immunization with whole inactivated HIV was associated with improved virologic control (with or without interruption of therapy).

Jin and colleagues (Abstract 346) analyzed the effect of immunization with canarypox expressing the HIV *gag-pol*, *env*, and *nef* genes, in combination with recombinant gp160. In patients with AIDS on HAART, they observed 2- and 3-log increases in the level of gp120-specific antibodies and a significant minority of patients who had increases in proliferative responses to either envelope or Gag and/or increases in CD8+ T cells as measured by ELISPOT assays or tetramer staining. Preliminary data regarding control of viremia after discontinuation of HAART in 4 of these subjects was provided in a late breaker abstract (Abstract LB12). Two of these vaccinated subjects had delayed rebounds in viremia following discontinuation of HAART, which first rose to detectable levels 2 to 3 months after discontinuation of HAART, and ultimately resulted in steady state levels of plasma viremia between 330 and 5600 copies/mL. Delayed rebounders had increases in CTL responses to HIV antigens

following vaccination to more than 1 protein, whereas the rapid rebounders either did not exhibit a CTL response to vaccination or responded to Gag alone. Although these data are encouraging, the lack of a control group that was not vaccinated or knowledge of what would have been the viral "set point" of these patients precludes any conclusions regarding the effect of vaccination per se. Further studies of larger groups of patients with appropriate controls will be necessary to determine whether therapeutic vaccination can in fact affect viral set points following discontinuation of HAART.

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### Additional Suggested Reading

Evans TG, Keefer MC, Weinhold KJ, et al. A canarypox vaccine expressing multiple human immunodeficiency virus type 1 genes given alone or with rgp120 elicits broad and durable CD8+ cytotoxic T lymphocyte responses in seronegative volunteers. *J Infect Dis.* 1999;180:290-298.

Ferrari G, Humphrey W, McElrath MJ, et al. Clade B-based HIV-1 vaccines elicit cross-clade cytotoxic T lymphocyte reactivities in uninfected volunteers. *Proc Natl Acad Sci USA.* 1997;94(4):1396-1401.