

HIV Pathogenesis and Vaccine Development

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Cautious optimism was a recurring theme of many of the AIDS vaccine-related presentations at the 13th annual Conference on Retroviruses and Opportunistic Infections. Several investigators suggested that the ability of HIV to escape cytotoxic T-lymphocyte responses may be more limited than previously thought, and encouraging results were presented regarding the ability of consensus ancestral sequences or polyvalent vaccines to increase the breadth of induced immune responses. A number of studies highlighted the potential efficacy of neutralizing antibodies: data from 2 groups suggested that neutralizing antibodies may play a role in preventing superinfection and previously unrecognized neutralizing epitopes were identified in the membrane proximal external region of envelope. Two studies documented that immunization with polyvalent simian immunodeficiency virus vaccines can induce sustained control of viremia following repeated low-dose mucosal challenge with pathogenic SIVmac strains and provided hope for the potential of T-cell-based vaccines to slow disease progression.

HIV Sequence Variation and its Implication for Vaccine Design

The remarkable genetic diversity displayed by HIV represents one of the major barriers to the development of an AIDS vaccine. In the Bernard Fields Memorial Lecture, Korber highlighted the intricate interplay between HIV sequence variation and host immune responses (Abstract 13). Since its introduction into the human population approximately 70 years ago, HIV has developed tremendous genetic diversity, resulting in the evolution of multiple distinct clades of viruses that can vary by more than 38% in the envelope gene. The precise nature of the selective pressures that have fostered the evolution of these clades remains a matter of debate. One leading theory is that selective pressure exerted by T-cell responses may have been a major factor guiding the evolution of these distinct clades. By generating phylogenetic trees using only the silent bases in HIV that do not direct the synthesis of amino acids, Korber demonstrated that a similar clade

structure is evident, thus strongly suggesting that selection by human leukocyte antigen (HLA) molecules (which would be expected to select for non-synonymous substitutions encoding escape mutations) is not the major driving force in the evolution of these clades. However, Korber noted that this finding does not exclude a role for immune selective pressure on selected portions of the genome.

The fact that the current HIV diversity has arisen as a result of progressive divergence from common viral ancestors has important implications for the design of AIDS vaccines: this observation implies that a common ancestral sequence may be closer to divergent contemporaneous sequences than any of these contemporaneous sequences are to each other. This hypothesis has prompted intensive efforts over the past several years to develop consensus or central sequence vaccines that have been artificially constructed to mimic the predicted ancestral sequence. An envelope consensus sequence (designated Con6) has been created by Korber, Hahn, and colleagues for the M group clade. Initial results demonstrate that the Con6 envelope can bind to CD4 and CCR5, can mediate viral entry, and can be recognized by neutralizing antibodies. Recent unpublished results reveal that

mice vaccinated with this M group consensus envelope develop significant levels of cross-clade T-cell responses and that the elicited neutralizing antibodies may have a greater breadth of neutralization than those induced by wild-type envelope.

Although the increased breadth of T-cell and antibody responses induced by the consensus vaccines to date are encouraging, they are modest and may only be partly effective in combating HIV sequence diversity. An alternative approach would be to generate a mosaic or polyvalent T-cell vaccine that contains a mixture of variant peptides at key positions that vary in natural infection, which Korber termed toggle peptides. The design of these toggle peptides is facilitated by the fact that in relatively conserved HIV proteins such as p24 or Pol, the variable amino acids are limited in repertoire and tend to occur in only a subset of positions. Use of these synthetic toggle peptides to detect T-cell responses in HIV-infected subjects resulted in a 2.5-fold increase compared with consensus peptides. Korber postulated that the creation of a mosaic cocktail of overlapping viral peptides might serve as a very effective means to generate a diverse array of immune responses. Although this approach clearly has theoretical advantages, the key test will be whether immunization with a mosaic vaccine is able to induce a T-cell response with greater breadth than monovalent vaccines.

Korber also provided a reanalysis of a previously reported association between HIV mutations in Pol and specific HLA alleles that suggested that T-cell-mediated selection pressure was a major force in the evolution of HIV diversity. Korber presented compelling data that many of these apparent associations between sequence mutations and HLA alleles were in fact the result of association with distinct clades rather than a result of HLA-driv-

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en mutations. Of more than 300 potential sites of selective evolution, only 6 mutations were clearly identified as being likely to have been mediated by cytotoxic T-lymphocyte (CTL) escape. This reanalysis suggests that the complex array of selective pressures on HIV evolution, including fitness constraints and selection pressure exerted by diverse HLA alleles, is likely to minimize the probability of fixation of CTL escape mutations at the population level, an encouraging finding with respect to vaccine design.

Guts, Germs, and AIDS

Over the past several years, AIDS immunologists have progressively become more “gut-centric,” prompted by a number of high-profile reports that have vividly documented the fact that HIV and simian immunodeficiency virus (SIV) infections rapidly deplete CD4+ T cells from the gut and other mucosal sites early in the courses of infection. These reports have raised the question of whether the acute depletion of gut CD4+ T cells, which is not apparent by examination of total CD4+ T-cells in peripheral blood, may be a necessary step in the progression to AIDS. There has thus been considerable interest as to whether depletion of gut CD4+ T cells also occurs in the natural hosts of SIV that do not develop AIDS. Reports from several laboratories clearly demonstrated that depletion of gut CD4+ T cells occurs commonly during infection of natural hosts.

Gordon (Abstract 36) analyzed both naturally and experimentally infected sooty mangabeys. As assessed by rectal biopsies and by bronchial alveolar lavage, SIV-infected mangabeys showed a significant and in some cases profound depletion of CD4+ T cells in mucosal sites, with some animals having less than 1% CD4+ T cells in mucosal-associated lymphoid tissues. These findings were observed in longitudinal studies of experimentally infected animals and in cross-sectional studies of naturally infected animals. Interestingly, analysis of SIV-uninfected mangabeys demonstrated that a lower

percentage of mucosal CD4+ T cells expressed CCR5 than in either rhesus macaques or humans, raising the possibility that this represents an adaptive mechanism of natural hosts to minimize the effects of lentiviral infection on mucosal immunity. However, the ability of SIVsm to induce gut CD4+ T-cell depletion despite the lower level of CCR5 expression suggests that SIVsm either uses coreceptors other than CCR5 or that it is able to infect cells with levels of CCR5 not detectable by flow cytometry. Although some degree of CD4+ T-cell repletion was observed at mucosal sites following institution of antiretroviral therapy in SIV-infected sooty mangabeys, levels remained significantly below those of uninfected animals.

Pandrea (Abstract 37) presented a similar picture in African green monkeys. As observed in sooty mangabeys, SIV-uninfected African green monkeys displayed relatively low levels of CCR5+ CD4+ T cells in mucosal sites. However, following infection with SIVagm, rapid depletion of CD4+ T cells in the intestines was observed by 21 days after infection, resulting in a more than 6-fold decrease that was sustained for more than 400 days after infection. A similar depletion at early time points was observed in rhesus macaques infected with SIVagm, although these animals subsequently controlled viremia and had a slow but partial reconstitution of gut-associated CD4+ T cells. Taken together, these 2 abstracts suggest that depletion of gut-associated CD4+ T cells is not a unique feature of pathogenic SIV infection, although they do not exclude the possibility that depletion of gut-associated CD4+ T cells may play a major role in the pathogenesis of AIDS in susceptible hosts.

The specific mechanisms that foster the robust replication of HIV or SIV in mucosal CD4+ T cells remain incompletely understood. Initial reports suggested that the high frequency of activated CD4+ T cells expressing CCR5 in mucosal sites was a major factor. However, subsequent reports that replication occurs substantially in

CD4+ T cells that are not activated (at least by assessment of conventional markers such as CD69) have raised the possibility that other distinctive characteristics of the gut microenvironment may play a role. Brechley (Abstract 38) investigated mechanisms that might mediate depletion of gut CD4+ T cells in HIV-infected patients. Analysis of individuals on highly active antiretroviral therapy (HAART) with undetectable viral loads and excellent restoration of peripheral blood CD4+ T cells revealed continued profound depletion of CD4+ T cells in gut-associated lymphoid tissue. CD4+ T cells obtained from the gut of individuals on HAART had relatively high levels of HIV DNA, suggesting that HIV continued to infect mucosal CD4+ T cells despite the apparently effective suppression of viral replication by HAART in the periphery. Analysis of the expression of P-glycoprotein, a protein pump that can mediate resistance of cells to antiretroviral drugs, did not reveal any clear difference in P-glycoprotein expression in CD4+ T cells in the gut and peripheral blood. However, Brechley and colleagues did find evidence of a less effective HIV-specific CD8+ T-cell response in the gut. The frequency of HIV-specific cells as measured by HLA tetramers was lower in the gut than in peripheral blood. In addition, cytokine secretion in response to antigenic stimulation was markedly lower in the gut than in peripheral blood. These data suggest that factors other than the availability of activated T cells in the gut may contribute to the ability of mucosal tissues to provide a fertile field for HIV or SIV replication, including the possibility of a less effective CD8+ T-cell response.

Toward a Better Definition of Protective Immunity

Lack of definitive information on the nature of protective immunity against HIV infection has long been a significant hurdle in the quest for an HIV vaccine. Several presentations at this year's conference provided a more detailed understanding of the relative

roles of humoral and cellular immune responses.

Although there is now convincing evidence that many HIV-infected individuals generate neutralizing antibodies against autologous viral sequences, the role that neutralizing antibodies play in controlling viral replication in chronic HIV infection and their potential to protect against infection (or superinfection) remains unclear. Two presentations suggested that the presence of neutralizing antibody responses may help prevent against superinfection. Smith and colleagues (Abstract 91) performed a case-controlled study of 3 well-characterized subjects who were superinfected within the preceding 13 months. The 3 subjects all had significantly lower titers of neutralizing antibodies to common laboratory strains of HIV (JRCSF and NL4-3) than 11 matched individuals who also had primary infection and ongoing sexual exposures. In addition, there was a trend for weaker neutralizing responses to autologous HIV sequences in the superinfected patients. These differences in the frequency of neutralizing antibody responses were not explained by unusual genetic dissimilarity of either the pre-existing or superinfecting sequences. Increases in both homologous and heterologous neutralizing antibody responses were observed following superinfection, but the levels of neutralizing antibodies still remained lower than in those subjects without superinfection. Comparable data were reported by Grant and colleagues (Abstract 92) who identified 4 cases of superinfection in a cohort of 104 patients with recent HIV infection. These 4 subjects had either no neutralization or only weak neutralization of autologous virus, whereas analysis of 12 individuals without superinfection revealed relatively strong autologous and heterologous neutralization. All 4 of these individuals were superinfected 1 to 3 years after their initial primary infection, leading the authors to propose that subjects who lack broad neutralizing activity may be particularly susceptible to superinfection early after primary infection. Superinfected

individuals also had lower neutralization titers to laboratory strain JRCSF.

The frequent occurrence of HIV escape mutations that evade host CD8+ T-cell responses has cast doubts on whether T-cell-based vaccines would be able to mediate sustained control of HIV infection. Walker (Abstract 98) suggested that the predictable occurrence of immune escape might be exploited to facilitate the design of improved HIV vaccines. Underscoring the challenges inherent in studying HIV-infected individuals with a diverse array of infecting genotypes and host HLA alleles, Walker focused on several selected experimental settings. He first described analysis of HIV-specific CD8+ T-cell responses and immune escape in a pair of monozygotic twins who were infected simultaneously from the same donor. Genome-wide analysis of HIV-specific responses revealed a remarkable concordance of T-cell responses, which differed in only 2 of the 10 to 14 epitopes recognized by each subject. CD8+ T-cell responses were correlated not only in specificity but in magnitude as well. Both twins demonstrated characteristic evolution of escape mutations in the same immunodominant HLA-B*40-restricted response to a Pol epitope. Analysis of autologous neutralizing titers also revealed significant concordance between the infected twins. Overall, these data suggest that the evolution of HIV-specific humoral and cellular responses can be relatively predictable in the setting of a similar genetic background and viral inoculum. Transitioning from a microanalytic to a macroanalytic approach, Walker next described a detailed analysis of epitope recognition in a cohort of 515 HIV-infected subjects from South Africa. Through the combination of high-resolution HLA typing and precise epitope mapping, Walker and his colleagues were able to demonstrate that recognition of a subset of HIV peptides was highly associated with expression of specific HLA alleles such as HLA-B15. Analysis of the relationship between HLA alleles and plasma viremia revealed a strong associa-

tion of B alleles with lower levels of viremia, whereas no significant association was noted with A alleles. To better understand the relationship of HLA alleles and control of viremia, Walker went on to analyze a cohort of 104 subjects with acute HIV infection who were tested 2 months after infection for recognition of a panel of HIV-optimal epitopes selected based on the donor's HLA type. Of an average of 30 peptides tested per subject, only 10% to 15% of all defined optimal epitopes were recognized, suggesting that only a minority of potential epitopes are involved in control of acute infection. Interestingly, the immunodominance of specific epitopes was affected by the presence of other HLA alleles. Certain HLA alleles such as A24, which are normally responsible for presenting immunodominant peptides, could be "trumped" by the presence of even more immunodominant alleles such as B27 and B57. HLA alleles associated with prolonged survival (eg, B27, B57) contributed disproportionately to the early T-cell response, raising the question of which qualities were distinctive about these early responses associated with more effective control. Analysis of the affinity of T-cell responses to these epitopes revealed relatively high affinity responses in acute infection, whereas lower affinity responses were observed in chronic infection, a pattern of evolution that stands in contrast with what has been typically observed in murine infections. This suggests the possibility that the high-affinity clonotypes may be selectively lost over time in HIV-infected subjects.

Further pursuing the analysis of potential qualitative difference among CTL epitopes, Walker and colleagues performed a comprehensive analysis of more than 1000 South African individuals infected with HIV clade C viruses. They identified 8 immunodominant epitopes presented by high-frequency HLA alleles that were used to create HLA tetramers, and then correlated the frequency of tetramer-binding cells with plasma viremia in a subset of 113 subjects.

Remarkably, a negative association between the frequency of tetramer-binding cells and viral load was only observed for 1 of 8 of the epitopes. Two of the epitopes showed a positive correlation with viral load, and for 5 of the 8, no significant relationship was observed. Escape was only observed in 1 of the epitopes. These results suggest that a significant fraction of HIV-specific T-cell responses may be relatively ineffectual, as demonstrated by their inability to either mediate viral escape or suppress viral replication. Walker highlighted another example of qualitative differences between epitopes: the distinct characteristics of CD8+ T-cell responses to an immunodominant Gag epitope that is recognized by B*5701- and B*5703-positive individuals. Evolution of escape in this Gag epitope is commonly observed in subjects who express B*5703 but not B*5701. Comprehensive analysis of the T-cell receptor (TCR) repertoire to this epitope revealed that the response was relatively narrow in subjects that express B*5701, and more diverse in subjects with B*5703. This apparently paradoxical result stands in contrast to previously reported results in SIV-infected macaques. Walker and colleagues postulate that the highly conserved TCRs in HLA-B*5701+ patients are better able to recognize the escape variants, whereas the more diverse repertoire in B*5703+ subjects is less able to recognize variants.

The Quest for Neutralizing Antibodies Against HIV—Is the Membrane Proximal External Region the Achilles Heel of HIV?

Only a handful of monoclonal antibodies are able to mediate significant neutralization activity against primary isolates of HIV-1, and many of these antibodies bind to a conserved region of gp41 that is close to the transmembrane region, termed the membrane proximal external region (MPER). An entire symposium at this year's conference was dedicated to recent advances in the analysis of neutralizing antibod-

ies to the HIV-1 MPER. Three neutralizing antibodies that bind to epitopes in the MPER have been characterized to date (2F5, 4E10, and Z13). Although the MPER represents a potentially attractive target for neutralizing antibodies, a number of issues have arisen in attempts to induce antibody responses to this region, including difficulties with accessibility, relatively low immunogenicity, the requirement that MPER epitopes be complexed with lipid for effective binding, and the question of whether generation of antibodies to MPER epitopes might be limited by self mimicry.

Zwick and colleagues (Abstract 111) presented new structural data on the nature of binding of the MPER-binding antibody Z13, which has been less studied than the 2F5 and the 4E10 antibodies. Detailed peptide-binding studies revealed that the Z13 epitope is similar to that recognized by 4E10 but shifted to the N-terminal region of the MPER. Using an *in vitro* affinity maturation technique, Zwick and colleagues were able to generate a variant antibody fragment (Fab), termed Z13 E1, with a 100-fold greater affinity than that of the wild-type Z13, which was associated with a corresponding increase in neutralization efficiency. Like the other MPER antibodies 4E10 and 2F5, Z13 E1 has a long hydrophobic CDR H3 loop, which may play a crucial role in allowing the antibody access to the hydrophobic environment close to the virion membrane. As with the other MPER-specific monoclonal antibodies, Z13 E1 does exhibit some non-specific binding to self-antigens such as cardiolipin, reinforcing the issues addressed by Haynes and colleagues (see below) regarding whether the potential mimicry of the MPER by host molecules may serve as a barrier to the induction of antibodies to this target. Detailed mapping of this specificity of Z13 E1 compared with 2F5 and 4E10 revealed significant differences, which reinforces the existence of multiple potential targets for neutralization within the MPER. However, the issue of how to reliably induce antibody responses to this epitope by immunization remains.

Although many groups have noted the difficulty in inducing neutralizing antibodies to the MPER by immunization, the mechanisms responsible for this lack of success have remained controversial. A provocative hypothesis advanced by Haynes and colleagues proposes that structural similarities between the MPER and other host antigens may in part underlie this difficulty (Abstract 112). Haynes considered several potential explanations for the difficulty in inducing anti-MPER antibodies, including holes in the antibody repertoire, the inability of immunogens to mimic the native conformation of the MPER, the possibility that antibodies that recognize MPER may be structurally unusual and difficult to generate, and the possibility that they may be derived from a polyspecific pool of B cells that is usually either deleted or tolerized. Focusing on the latter possibility, Haynes referred to recently published work that documented that both 2F5 and 4E10 antibodies were able to bind several self-antigens, particularly lipid molecules such as cardiolipin. If polyspecificity and the ability to recognize self-antigens are common characteristics of MPER-specific neutralizing antibodies, then these antibodies might be deleted or tolerized during normal B-cell differentiation. Comparison of the 2F5 and 4E10 antibodies with 2 well-characterized anticardiolipin monoclonal antibodies revealed a number of significant binding and structural similarities, suggesting that the ability of MPER-specific antibodies to bind a subset of host antigens may be related to their neutralizing activity. Examination of the kinetics of association of the 2F5 and 4E10 antibodies with either linear MPER peptides or peptide-lipid complexes led Haynes to propose that these antibodies require a 2-step conformational change in order to efficiently neutralize HIV. In the first step, weak interactions with lipid bring the antibody in close proximity to the MPER; in the second step, a conformational change leads to a more stable, high-affinity interaction of the antibody with the envelope trimer.

Although elicitation of antibodies able to bind the MPER during HIV infection occurs commonly, there have not been good data on how frequently neutralizing antibodies directed to the MPER are generated during natural infection. Shaw and colleagues (Abstract 113) grafted epitopes, or various portions of the MPER, of HIV-1 into an HIV-2 backbone to address this question. Because of the relatively high sequence divergence between HIV-1 and HIV-2 and the lack of cross-neutralizing antibodies, the HIV-2 envelope provides a convenient scaffold to examine the effects of MPER sequences without the confounding effects of neutralizing antibodies to other envelope determinants. They examined neutralization of a panel of HIV-2 envelopes that were engrafted with either the entire 23-amino-acid HIV-1 MPER or several truncations of the MPER, including truncations limited to the defined 2F5 and 4E10 epitopes. Plasma was obtained from 217 subjects infected with 10 different HIV-1 subtypes or circulating recombinant forms. Of these 217 individuals, only 3 had any significant neutralizing antibody titer against either the 2F5 or 4E10 epitopes, and these were only at low levels. The remarkable rarity of neutralizing antibodies to the 2F5 and the 4E10 epitopes was compatible with work presented by Johnson and colleagues (Abstract 94) who were unable to detect any specific neutralization of either the 2F5 or the 4E10 epitopes when engrafted onto an SIV backbone. Interestingly, however, Shaw and colleagues did identify anti-HIV-1 MPER activity to regions other than those represented by the 4E10 and 2F5 epitopes in one third of study subjects. The specificity of these antibodies mapped to several distinct regions of the MPER and in some instances required the complete 23-amino-acid MPER sequence for recognition. The demonstration of these novel neutralizing epitopes in the MPER in a significant minority of HIV-infected patients suggests that this region may represent a valuable target for vaccines. However, a number of issues need to be

addressed in more detail, including the relative potency of these antibodies, their breadth of neutralization, and the ability to induce immune responses to these novel MPER epitopes by vaccination.

The ultimate test of the efficacy of MPER antibodies should be reflected in their ability to suppress viral replication in HIV-infected patients. Such a trial was reported at last year's CROI and subsequently published by Trkola (Trkola, *Nat Med*, 2005). Fourteen HIV-infected patients received infusions of the broadly neutralizing antibody 2G12—which recognizes a carbohydrate epitope in V3—and 2 MPER antibodies, 4E10 and 2F5, while receiving antiretroviral therapy. After interruption of antiretroviral therapy, only 3 patients had sustained suppression of viral replication until the time of antibody washout; the remainder of the patients had rebound viremia during the period when infused neutralizing antibodies were still present. Although the antibody infusions provided some delay in the rebound of viral replication, one of the major questions surrounding this trial was how the virus was able to replicate even in the presence of what appeared to be significant levels of neutralizing antibodies in vivo. At this year's conference, Trkola and colleagues (Abstract 114) provided a detailed analysis of potential mechanisms that might be responsible for the limited efficacy of the MPER antibodies in vivo. Although evolution of viral strains resistant to the 2G12 antibody was observed in the majority of patients, no significant evolution of escape mutations in the 2F5 and the 4E10 epitopes was identified, a finding that was confirmed by sequencing of viral isolates as well as by sequencing of plasma viral RNA. This observation suggests that the MPER antibodies might be less effective in vivo than predicted based on their in vitro efficacy. Analysis of the pharmacokinetics of each of the 3 antibodies revealed that although the distribution half-lives of all of these antibodies were equal, the elimination half-lives of the 2

MPER antibodies were approximately 4-fold shorter than that of the 2G12 antibody. This was not due to the development of endogenous antibody response against the infused antibodies and may reflect the ability of the MPER antibodies to bind phospholipid or other self-antigens in vivo. Similar results were reported by Mehndru and colleagues (Abstract 178), who also observed consistent induction of viral variants resistant to 2G12 but not 4E10 and 2F5 in patients who received infusions of all 3 monoclonal antibodies and then underwent treatment interruption. To address the question of whether the observed concentrations of antibodies were effective in vivo, Trkola and colleagues calculated the relative contributions of the infused neutralizing antibodies against the patient's endogenous neutralizing antibodies based on the measured antibody concentration in plasma and analysis of the ability of the monoclonal antibodies to neutralize each patient's autologous viral sequence. In responding patients, a significant contribution of the infused antibodies to the total neutralizing antibody titer in plasma was observed, whereas in non-responding patients, there was little contribution of the infused antibody to total neutralization activity. An alternative explanation that the investigators pursued was that the fitness cost of escape mutations to the MPER antibodies was so great as to render the escape viruses nonviable. In vitro experiments demonstrated that escape to 2G12 rapidly occurs in vitro, as opposed to escape to the MPER antibodies, which was relatively infrequent and appeared to be associated with less replication-competent viruses. This conclusion was confirmed by examining the growth kinetics of in vitro viruses that had the 4E10 and 2F5 escape mutations inserted into their envelope sequences. Notably, the 4E10 escape mutation significantly increased the sensitivity of the envelope to autologous neutralizing antibodies. Thus, several factors appear to contribute to the infrequent observation of escape to the MPER antibod-

ies, including the fitness cost of these escape mutations, the low frequency of nonsynonymous mutations occurring in the 4E10 epitopes, and the fact that these escape mutations may increase the susceptibility of these viral variants to other neutralizing antibodies. Overall, passive immunization with these antibodies was only infrequently able to increase neutralization titers above the patients' own autologous neutralizing antibody responses.

Preclinical AIDS Vaccine Studies—Is Less More?

In the majority of studies conducted to date, T-cell-based vaccines have had only limited success in protecting against infection or disease progression in macaques challenged with pathogenic SIVmac strains. (The primary exceptions to this general observation have been in macaques challenged with rapidly pathogenic, CXCR4-tropic simian-human immunodeficiency viruses, but there is controversy as to how well these strains are likely to model HIV pathogenesis.) Although these discouraging results using the SIVmac-challenge model have induced some pessimism about the potential use of T-cell-based AIDS vaccines, it has also been suggested that the use of high-dose challenges in nonhuman primates may overwhelm a potentially protective effect. There has therefore been increasing interest in the utility of repeated low-dose SIV challenges in nonhuman primate vaccine studies. Gauduin and colleagues (Abstract 174) reported the ability of a multigenic DNA prime, modified vaccinia Ankara (MVA)-boost regimen to protect against a repeated low-dose vaginal SIV challenge. Macaques were immunized with an optimized DNA vaccine encoding for 6 SIV proteins, followed by a peripheral boost with MVA vectors expressing a similar complement of SIV proteins. Levels of enzyme-linked immunospot (ELISPOT) responses induced by this regimen were robust and generally comparable to those induced by other regimens.

When examined 2 weeks after the MVA boost, this systemic immunization regimen was able to induce levels of SIV-specific CD8+ T cells in vaginal and rectal tissues similar to those in peripheral blood. Following a repeated low-dose challenge, the authors observed an approximately 30-fold reduction in peak viremia and a 100- to 300-fold reduction in viremia in vaccinees compared with controls, a difference that was sustained to more than 25 weeks after infection. Vaccinees also had significantly better preservation of CD4+ T-cell counts and lower mortality. Taken together with the results reported by Watkins (see below), these data suggest that the ability of T-cell-based vaccines to provide sustained protection against disease progression in nonhuman primates challenged with SIVmac may have been underestimated by traditional high-dose challenge models. However, whether these results will necessarily prove predictive of those obtained in human clinical trials awaits the results from ongoing phase IIb and III trials.

Watkins (Abstract 180) described similar results in macaques vaccinated with DNA encoding SIV Nef, Tat, and Rev followed by an adenovirus boost, which resulted in quite robust SIV-specific CD8+ T-cell responses comprising 1% to 24% of all CD8+ T cells at peak after boost. Following a repeated low-dose rectal challenge, the vaccinees had an approximately 1- \log_{10} decrease in peak plasma SIV RNA level and had a sustained 30-fold decrease in set-point viremia, which was maintained almost 1 year after infection. This significant and sustained decrease in viral load was accompanied by preservation of total CD4+ T-cell counts and CCR5+ memory cells.

Watkins went on to readdress the theme of qualitative difference in T-cell responses previously introduced in Walker's presentation. Watkins's model consisted of analysis of a subset of rhesus macaques that were able to control replication of the highly pathogenic SIVmac239 strain to less than 1000 plasma RNA copies/mL for several years. Control of viremia in

these animals was highly statistically associated with specific major histocompatibility complex (MHC) class I alleles, primarily B*17 and, to a lesser extent, A*01. Compelling evidence for the role of CD8+ T-cell responses in controlling viral replication in these animals arose from the observation that following CD8+ T-lymphocyte depletion with a monoclonal antibody, viremia promptly rebounded by 2- to 4- \log_{10} plasma RNA copies/mL, and subsequently came under control when the CD8+ T-cell responses returned. After analyzing the immunodominance of specific epitopes during initial viremia and during subsequent rebound following CD8+ T-cell depletion, Watkins concluded that recognition of only a subset of all CTL epitopes originally targeted were involved with control of viremia after CD8+ T-cell depletion. Moreover, most of these epitopes represented formerly subdominant responses and were commonly found in Nef and Vif. These data, coupled with the data from Watkins' Nef/Tat/Rev vaccine trial, suggested that induction of T-cell responses against a subset of normally subdominant epitopes, particularly in proteins such as Nef, Vif, and Gag, may be an especially attractive strategy for vaccine development.

Immunity to adenovirus serotype 5 (Ad5) vectors represents a significant limitation to the otherwise notable successes of the adenovirus-based vectors. Barouch and colleagues (Abstract 179LB) described the construction of novel chimeric Ad5 vectors designed to circumvent anti-Ad5 humoral responses. Previous work has shown that much of the antibody response against the Ad5 vectors is directed against the Ad5 hexon protein. The authors replaced the short hypervariable regions of the Ad5 hexon protein with the corresponding hypervariable regions from the relatively rare adenovirus serotype 48. In mice and in monkeys, these recombinant chimeric Ad5 vectors displayed immunogenicity comparable to that induced by the Ad5 parent. Similar levels of immune responses were generated in Ad5-

seropositive animals, demonstrating that the chimeric vectors were able to bypass the effect of pre-existing antibodies. Previous attempts to utilize adenovirus vectors based on serotypes other than Ad5 have been limited in part by the suboptimal immunogenicity, whereas this strategy, if successful in humans, would retain the proven safety and immunogenicity of the Ad5 vectors while also offering the opportunity to bypass pre-existing immunity.

In their quest to develop a vaccine able to induce effective antibody responses against HIV, investigators have engineered a number of mutant envelopes, which have largely been unsuccessful in inducing broadly neutralizing antibodies. As an alternative approach, Frost and colleagues (Abstract 176) turned to natural HIV envelope sequences, hypothesizing that infection with neutralization-sensitive HIV strains may result in stronger neutralizing antibody responses. Drawing on a population of 38 recently HIV-infected individuals who were not treated with antiretroviral therapy, Frost and colleagues correlated the results of autologous neutralizing antibody titers to heterologous neutralization using benchmark sera which displayed broad cross-neutralization. Subjects who were infected with a virus that was more susceptible to neutralization by the broadly cross-neutralizing sera had an approximately 4-fold increase in the rate of neutralizing antibody responses, which was associated with an approximately half- \log_{10} increase in set-point plasma viral RNA levels. The authors concluded that infection with inherently more neutralization-sensitive viruses is more effective in eliciting better neutralizing antibody responses and suggested that these envelope strains may prove useful in inducing neutralizing antibody responses.

AIDS Vaccine Trials—Where Do We Stand?

Corey, Director of the HIV Vaccine Trials Network (HVTN), provided a frank and comprehensive assessment

of past progress and future challenges in the AIDS vaccine field (Abstract 56). Twenty-five years after the identification of HIV, much progress has been made: 23 distinct AIDS candidate vaccines are now in various phases of development and large-scale clinical trials are underway. However, progress toward an effective vaccine remains frustratingly slow. Corey started by highlighting disappointing results from a number of different vaccine candidates in phase I and II trials during 2005, including lackluster immunogenicity results observed with a number of peptide or lipopeptide vaccines, as well as several DNA vaccines. He also highlighted the need to define true maximal tolerated doses of candidate vaccines in phase I trials as opposed to the tendency to employ doses dictated by manufacturing limitations.

Despite these setbacks, the past year was also notable for the success of several vaccine strategies employing adenovirus vectors, either in the form of a recombinant Ad5 vector alone or when used as a boost following initial priming with DNA immunization. Ad5-based vaccines have proved effective in both nonhuman primate studies and phase II clinical trials in inducing immune T-cell responses in more than 60% of vaccinees. The factors associated with the proven immunogenicity of the adenovirus-based vectors are not well-defined but may be related in part to the ability to produce these vectors at sufficiently high titers to allow dosages of 10^{10} to 10^{12} infectious units per dose, which exceed the currently employed doses used for other viral vectors by 3 to 4 \log_{10} units per dose. However, the presence of pre-existing immune responses to adenovirus vectors represents an important limitation. The presence of antibodies to Ad5, currently the most commonly used backbone employed in clinical trials, results in a decreased frequency of response and decreased breadth of the response in seropositive vaccinees. However, this diminution can be overcome in part by increasing the

vector dose, albeit at the expense of increased vector reactogenicity. The use of a DNA prime and the use of adenovirus vectors other than Ad5 are also being pursued as alternative strategies to deal with pre-existing immunity. The potential effect of pre-existing immunity is particularly important in areas of high HIV prevalence where rates of Ad5 seropositivity are typically even higher than those observed in the United States.

Corey also elucidated the rationale for the current emphasis on proof-of-concept or phase IIb vaccine trials. The phase IIb trials are designed to demonstrate proof-of-principle (ie, can a T-cell-based vaccine reduce the risk of HIV infection or decrease set-point viremia in infected individuals) but is not of sufficient size to result in licensure. Distinct advantages of the IIb approach are the significant decrease in cost (10% to 30% of that for a standard phase III trial), the use of a significantly smaller study population, and the accelerated timeline, which can shave 1.5 to 2 years from the time required to complete a phase III trial. These features have been employed in the design of the currently ongoing HVTN 502 trial, which will examine the efficacy of an Ad5 trivalent vaccine expressing Gag, Pol, and Nef, and is currently enrolling a targeted number of 3000 patients at sites predominantly in North and South America and the Caribbean. One significant limitation of the HVTN 502 study design is the relatively small number of women who will be enrolled, a situation that is likely to be remedied by the plans to move forward with HVTN 503, which will examine the safety and efficacy of the Ad5 trivalent vaccine in South Africa. Encouraging results in phase II trials have also been obtained with a vaccine approach directed by the National Institutes of Health (NIH) Vaccine Research Center, which involves a polyvalent DNA prime with the *gag*, *pol*, and *nef* genes from clade B strains and the envelope from clade A, B, and C, followed by an Ad5 boost expressing the 3 different HIV-1 envelopes, Gag, and Pol. Initial analysis of T-cell responses in

subjects who have received a combination DNA prime and adenovirus boost are encouraging, and stand in contrast to the results observed using several other DNA vaccines. This approach is currently being investigated in phase II studies and is targeted to enter a phase IIb proof-of-concept trial in early 2007.

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Additional Reference

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