

HIV Pathogenesis and Vaccine Development

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New information on the crystal structures of the HIV and the simian immunodeficiency virus (SIV) envelopes represented one of the scientific highlights of the 12th Annual Conference on Retroviruses and Opportunistic Infections. Numerous presentations also underscored the increasing recognition of the central role of gut-associated lymphoid tissue in AIDS pathogenesis and helped reveal a better understanding of the multiple mechanisms underlying CD4+ T lymphocyte depletion in AIDS. Progress on vaccine development was largely incremental but was strongly influenced by the impact of an expanding array of flow cytometric assays that have revealed significant functional and phenotypic differences in virus-specific CD8+ cells. The interplay between host cellular and humoral immune responses and virus evolution was another prominent theme, and it underscored the challenge facing host immune responses and vaccine developers in attempting to thwart an ever-mutating virus.

New Insights into Envelope Structure and HIV-specific Neutralizing Antibodies

Designing strategies to foil the ability of HIV to evade neutralizing antibodies represents one of the primary (and still elusive) goals of HIV vaccine research. The crystal structure of HIV-1 gp120 published in 1998 analyzed the conformation of a modified envelope stripped of most of its sugar residues and bound to CD4 and to an antibody that mimicked the chemokine coreceptor binding site (ie, a liganded envelope). Although this structure has been quite valuable for advancing our understanding of HIV envelope function, it does not provide information on several key points, including the structure of the envelope in its native state prior to binding to CD4, the point at which it is most susceptible to neutralizing antibodies.

Using crystals of a fully glycosylated SIV gp120 (which was more stable than the HIV version) stripped of the V1-V2 and V3 variable loops, the Harrison lab determined the crystal structure of the unliganded envelope (Abstract 7). The most notable finding was the dramatic conformational shift observed in the inner domain of the unliganded envelope compared with the previously determined liganded envelope structure.

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In the unliganded form, the tip of the CD4 binding site is exposed at the top of envelope. Following initial contact with CD4, the resulting conformational change brings together the other residues of the CD4 binding site as well as the chemokine receptor binding site, locking in the remodeled envelope around CD4, and exposing the newly formed chemokine receptor binding site. These changes shift a portion of the V1-V2 loop that interacts with gp41, thereby helping to release gp41 for subsequent fusion with the host cell.

The investigators also identified a deep hydrophobic pocket in the inner domain of the unliganded envelope where resistance mutations have been mapped for a newly identified antiviral compound (BMS-78806) that has been shown to inhibit HIV entry. This class of inhibitor is therefore likely to inhibit entry by binding to this pocket and stabilizing the unliganded form of gp120. Although significant conformational changes in the envelope were clearly anticipated prior to the determination of this structure, the availability of a 3-dimensional model to visualize these changes should help guide efforts to develop new means to interfere with viral entry by pharmacologic or immunologic means.

Another gap in our understanding of envelope structure has been the lack of solid information on the V3 loop, which was deleted in the molecule used for the previously reported crystal structure. Kwong (Abstract 110) described recent success in generating crystals of HIV-1 envelopes containing an intact V3 loop.

After screening different combinations of gp120 core proteins with an intact V3 loop complexed with CD4 and different antibodies able to bind to the envelope only after CD4 binding (CD4-induced antibodies), the Kwong laboratory identified a crystal that could be used for structural analysis. Several features of the resulting predicted structure of the V3 loop in the context of an envelope trimer help explain its relative immunogenicity, including the fact that this loop is relatively exposed in the envelope trimer and the lack of intermolecular hydrogen bonds, a feature that confers more flexibility on the loop to interact with antibodies.

Only a handful of monoclonal antibodies are able to provide relatively broad neutralization of primary HIV isolates. Based on the premise that elucidation of the structure of these rare antibodies could provide clues to the design of modified envelope immunogens that would be more effective in inducing broadly neutralizing antibodies, the structures of several of these antibodies have been determined over the past several years. However, to date, the hope that this information would lead to better vaccines has yet to be realized. Most of these antibodies (notably b12 and 2G12) have relatively unusual structures that are likely to be difficult to elicit by immunization. However, Kwong highlighted the fact that the recently determined structure of the 2F5 monoclonal antibody might provide a logical path to a better immunogen. The antigen-binding region of 2F5 contains an extended hydrophobic region that allows the antibody to bind to a hydrophobic region of gp41 that is relatively close to the virion surface. In fact, binding of 2F5 to the HIV envelope is strongly enhanced by the presence of membrane, suggesting that presentation of the 2F5 epitope in the context of membrane may be a key factor in eliciting similar antibodies.

This observation suggests that use of a modified envelope immunogen, which is locked into place (by introduced disulfide bonds) and presented in the context of a membrane (eg, by a virus-like parti-

cle or proteoliposome), might be a more effective means to induce antibodies to this epitope.

Alternative approaches to understand how to elicit broadly neutralizing antibodies have focused on analysis of antibodies in HIV-infected people that bind to conserved functional targets, such as the chemokine coreceptor binding site. Decker (Abstract 87) utilized a novel approach to screen for antibodies in patient sera able to bind to the chemokine receptor binding site by preincubating either HIV-1 or HIV-2 reporter viruses with soluble CD4 to expose the coreceptor binding site.

These CD4-induced neutralizing antibodies were able to mediate neutralization across numerous HIV-1 clades and even neutralization of HIV-2 at titers of up to 1:100,000 or more. Competition experiments with an antibody (19c) known to bind to the chemokine receptor binding site confirmed that this antibody activity largely or exclusively reflected antibodies to the chemokine receptor binding site. These results probably have greater significance for understanding of envelope function and evolution than vaccine design, because CD4-inducible neutralizing activity is only detected after the virus binds to CD4. Thus, the access of antibodies to this binding site is sterically inhibited following the interaction between HIV and CD4+ target cells. However, these data suggest that viruses are largely constrained to CD4 dependence in order to avoid neutralization by these relatively potent antibodies.

Virtually all studies that have attempted to understand protective immunity against HIV in humans have relied on correlative studies, and there have been relatively few opportunities to examine the ability of specific immune responses to block HIV replication *in vivo*. Trkola (Abstract 94LB) investigated whether 3 potent neutralizing antibodies (2G12, 2F5, and 4E10) could suppress viral rebound in HIV-infected subjects with either acute ($n=6$) or chronic ($n=8$) infection who underwent interruption of their antiretroviral therapy. These antibodies represent 3 of the 5 well-characterized antibodies with broadly neutralizing activity against HIV-1: 2G12 recognizes a carbohydrate epitope on gp120; 2F5 and 4E10 both recognize epitopes on highly conserved regions of

gp41. As noted above, these antibodies are atypical, both with respect to their structure and ability to mediate relatively broad neutralization of multiple HIV strains, and are not representative of neutralizing antibodies found in most HIV-infected subjects. The subjects, who were preselected for HIV isolates sensitive to these antibodies, received all 3 neutralizing antibodies 1 day prior to discontinuation of antiretroviral therapy and received weekly infusions for a total of 12 weeks. The 8 chronically infected patients had undergone previous treatment interruptions, thereby allowing a comparison of the increase in viremia following monoclonal antibody treatment with that observed following previous interruptions.

A significant delay in the rebound of viremia was observed in 2 of the 8 chronically infected patients. The 6 acutely infected patients had such rebound significantly later than did a retrospective control group of patients with acute HIV infection who underwent treatment interruption and were not treated with neutralizing antibodies. Interestingly, the development of resistance in the resurgent virus to the 2G12 monoclonal antibody was observed in 11 of 13 patients. Overall, 7 of 14 patients had either a delayed or a decreased rebound in viremia after interruption of therapy. These data provide a proof-of-principle demonstration of the ability of neutralizing antibodies to help contain viral replication in a subset of HIV-infected patients. However, the fact that relatively high amounts of 3 potent neutralizing antibodies in a highly idealized setting had no effect on viral rebound in at least half of the study subjects serves as a compelling reminder of the challenge of neutralizing HIV *in vivo*. Also, the observation that evolution of resistance to 2G12 occurred in some patients without evidence of significant suppression of viremia suggests the possibility that neutralizing antibodies might exert selective pressure on envelope sequence diversity while mediating only modest suppression of viral replication.

The Guts of HIV Pathogenesis

Although CD4+ cell depletion has long been recognized as the defining feature of AIDS, the mechanisms that mediate this relentless depletion have remained

controversial. In a plenary lecture, Douek (Abstract 127) provided an insightful and entertaining review of different mechanisms contributing to CD4+ cell depletion at different stages of HIV infection. Several studies in SIV-infected monkeys had previously documented depletion of CD4+ cells in the gut within 2 weeks after infection. However, there were some who interpreted these studies as representing peculiarities of the SIV/ macaque model rather than insights into HIV pathogenesis. Recent studies in HIV-infected patients, including one from the Douek laboratory, have provided compelling evidence for rapid and profound depletion of CCR5+CD4+ cells in the gut of patients in the first several weeks of HIV infection. This depletion of activated CCR5+CD4+ cells occurs most noticeably in the gut, the major reservoir for activated CD4+ cells in the body, but based on studies in macaques, also occurs at other mucosal sites, including the pulmonary tract and female reproductive tract. Whether these cells are killed by direct infection or indirect mechanisms has been much debated. Douek presented data from acutely infected macaques documenting SIV infection of 30% to 60% of memory CD4+ cells. Although this high level of infection was most striking in the gut, it was also observed in peripheral blood lymphocytes as well. Interestingly, memory CD4+ cells that appeared to lack surface expression of CCR5 were also found to be infected with SIV at relatively high rates. These extraordinary rates of infection are over 100-fold higher than those observed in chronic infection. These data document the remarkable finding that approximately 50% of all the body's memory CD4+ cells are killed within 2 weeks after infection, a loss which appears to be largely, if not exclusively, due to the direct effects of viral infection.

However, distinct mechanisms appear to contribute to the depletion of CD4+ cells during chronic HIV infection, in which levels of infected memory CD4+ cells are much lower. Several mechanisms contribute to the progressive CD4+ cell depletion in chronic HIV infection, including the induction of an inflammatory state in lymph nodes associated with the deposition of collagen, suppression of thymic output, and chronic immune activation with its asso-

ciated increased rates of CD4+ and CD8+ cell apoptosis. Douek also proposed that breakdown of local immune responses at mucosal sites could contribute to chronic immune activation. Taken together, these observations paint a complex picture of CD4+ cell depletion in AIDS, in which direct infection of CD4+ cells leads to a substantial depletion of memory CD4+ cells within the first couple weeks of infection and in which multiple direct and indirect mechanisms contribute to the depletion of CD4+ cells during the chronic phase.

Protective Immunity Against HIV: Lessons from Macaques

Lack of solid information on protective immunity against HIV remains one of the major impediments to the development of an AIDS vaccine. Watkins (Abstract 95) provided an overview of insights into protective immunity from studies in macaques. He rejected the notion that it was realistic to expect that an AIDS vaccine could provide sterile protection and instead highlighted the importance of trying to suppress viral replication in the postacute phase of infection to less than 1000 copies/mL, a benchmark that would reduce transmission and significantly delay disease progression.

He also highlighted the fact that macaques infected with attenuated viruses such as SIV Δ nef, which has provided some of the strongest protection in the SIV/macaque model to date, are distinguished not so much by high-frequency CD8+ cell responses but by their relatively high-frequency and broadly directed SIV-specific CD4+ cell responses. He further postulated that the induction of strong virus-specific CD4+ cell responses would likely prove to be a crucial characteristic of an effective AIDS vaccine, especially with regard to the ability of CD4+ cell responses to provide help for antibody and CD8+ cell responses. He also highlighted insights into the role of CD8+ cell responses in controlling SIV replication gleaned from the study of 161 macaques at the University of Wisconsin. Of these animals, 11 were termed elite controllers: animals able to achieve sustained control of plasma viremia to less than 1000 copies/mL. Seven of these 11 animals express the relatively infrequent major histocompati-

bility class I allele Mamu-B*17, which has been shown to present a number of relatively conserved SIV CD8+ cell epitopes. These animals do possess relatively broadly directed SIV-specific CD4+ and CD8+ cell responses, but the frequency of these responses does not necessarily distinguish these animals from other animals who have not achieved long-term control of SIV replication. Watkins postulated that there may be distinct differences in the ability of SIV-specific CD8+ lymphocytes specific for different epitopes to suppress SIV replication both in vitro and in vivo, and that these differences may be observed in settings where no cytotoxic T lymphocyte (CTL) escape has occurred.

Escape from HIV- and SIV-specific CTL Responses

The ability of HIV and SIV to rapidly mutate to escape CTL responses has been well documented in numerous settings. There is increasing evidence that immune selection pressure is shaping the sequence of HIV on a population level as well.

Altfeld (Abstract 91) analyzed CTL recognition of an HLA-A2–restricted epitope in Vpr. Most HLA-A2–positive individuals do not generate a response to the consensus sequence of this epitope. However, a subset of individuals infected with a less-frequent variant (I₆₀L) did mount a response against this epitope that was detectable relatively early in the course of infection. This I₆₀L variant epitope had a higher affinity for HLA-A2 and was better recognized by CTL than the consensus sequence, suggesting that this immunodominant epitope may have already been lost due to virus escape driven by the relatively common HLA-A2 allele.

A similar scenario was presented by Leslie (Abstract 92), who demonstrated a negative association between the presence of HLA-B*57/*5801 and conservation of a consensus glycine at residue 83 of Nef. Most previous studies on immune escape from CTL have focused on the documentation of a positive association between the presence of a specific HLA allele and a change from the consensus HIV-1 sequence, whereas these authors reported the opposite effect—that is, the presence of a specific HLA allele was associated with a preser-

vation of the consensus sequence. They went on to demonstrate that the Nef 83 residue lies in a previously undefined B*57/B*5801-restricted epitope and appears to represent an escape mutation that has increased in frequency to become the consensus sequence. A similar situation was demonstrated for an HLA-B*51-restricted epitope. In several instances, they documented transmission of these escape sequences to subjects who lacked the restricting allele and did not observe reversion of the sequence, an observation that strongly suggests that the presence of this mutation does not exact a cost to the virus in terms of decreased fitness.

The dynamic relationship between CTL escape and replication fitness was reinforced by macaque studies reported by Barouch (Abstract 131). Using a virus stock derived from monkeys that expressed the Mamu-A*01 major histocompatibility complex (MHC) class I allele and contained an escape mutation in the dominant A*01-restricted Gag epitope p11c, Barouch observed consistent reversion to wild-type p11c epitope sequences in monkeys that did not express A*01. These data confirm prior reports that escape mutations in this epitope exact a significant fitness cost to the virus. Interestingly, in A*01-positive animals, transient reversions to wild-type p11c sequences were observed, which then triggered an increase in the frequency of CTL specific for this epitope, followed by an increase in the frequency of CTL escape variants.

Taken together, these data reinforce the notion that HIV and SIV have evolved both in individuals and on a population level to develop mutations in immunodominant CTL epitopes. The implications for vaccine design would be that vaccines should attempt to elicit responses to epitopes for which escape mutations exact a significant fitness cost to the virus and are therefore less likely to become fixed in the general population.

Natural Hosts of Primate Lentiviruses

Natural hosts of primate lentiviruses, such as sooty mangabeys and African green monkeys, rarely develop immunodeficiency, despite viral loads that are often as high as those in HIV-infected people or SIV-infected macaques with

AIDS. Notwithstanding the intense interest surrounding this topic, the mechanisms that underlie the lack of disease progression in natural hosts of SIV remain obscure. Previous studies from several groups have shown that species such as sooty mangabeys lack the generalized immune activation and increased T-cell turnover found in HIV-infected humans or SIVmac-infected macaques.

One interpretation of the absence of generalized immune activation in natural hosts of SIV is that this reflects a marked attenuation of the host response to SIV and would be associated with weak or absent SIV-specific CD8+ cell responses. Two oral presentations presented differing views of this basic question.

Silvestri (Abstract 155) described the results of intracellular cytokine staining using lymphocytes from 74 infected sooty mangabeys that were stimulated with peptide pools corresponding to the SIVmac239 Gag, Pol, Env, and Nef proteins. Although SIV-specific CD8+ cell responses were detected in 65% of all animals tested, responses were low (<0.2%) or absent in 78% of all animals tested. No correlation between the magnitude of the CD8+ cell response and plasma viremia was found.

In contrast, Wang (Abstract 154) described a more robust SIV-specific CD8+ cell response in naturally infected sooty mangabeys using a combination of enzyme-linked immunospot (ELISPOT) and intracellular cytokine staining assays. Positive ELISPOT responses to SIV peptide pools were detected in 25 of 25 SIV-infected mangabeys studied, with the highest responses observed to SIV structural proteins. The overall magnitude of the total SIV-specific ELISPOT response ranged from 240 to 5200 spot-forming cells per 10⁶ peripheral blood mononuclear cells, a value comparable to that obtained in SIV-infected rhesus macaques.

Reasons for the apparently discrepant results were not immediately clear but may be related to the greater sensitivity of ELISPOT assays in the detection of responses in sooty mangabeys. Although this group also noted no correlation between the overall magnitude of the response and the control of viremia, these results are in fact similar to those observed in SIV-infected macaques or HIV-infected humans. Although these 2 groups arrived at dif-

ferent conclusions, their data on the magnitude of SIV-specific CD8+ cell responses were in fact overlapping rather than diametrically opposed and clearly documented that most infected animals generate a significant virus-specific CD8+ cell response. This debate highlighted the limitations of trying to draw conclusions on the role of virus-specific immune response by correlative and phenotypic studies and emphasized the need for additional interventional studies such as CD8+ lymphocyte depletion in these animals to better assess the role of CD8+ cells.

An alternative hypothesis to explain the lack of immune activation and CD4+ cell depletion in naturally infected hosts suggests that the early interaction of SIV with the host innate immune responses and dendritic cells may differ fundamentally from those observed in susceptible hosts. Staprans (Abstract 152) analyzed differences in myeloid and plasmacytoid dendritic cell subsets during acute SIV infection of sooty mangabeys and rhesus macaques. Acutely infected macaques had significantly higher levels of expression of the chemokine receptor CCR7 on myeloid and plasmacytoid dendritic cells, but little CCR7 was observed on either dendritic subtype in acutely infected mangabeys. Consistent with the known role of CCR7 in mediating homing of lymphoid cells to secondary lymphoid tissue, there was a preferential accumulation of dendritic cells in lymph nodes of acutely infected rhesus macaques but not in mangabeys.

A significant difference among species was also observed in regard to the response of their dendritic cells to SIV stimulation. Both rhesus macaque and human dendritic cells produced relatively large amounts of interferon (IFN)- α following stimulation with SIV, but sooty mangabey dendritic cells had relatively little response. These data suggest that the lack of dendritic cell activation and type 1 IFN production in SIV-infected sooty mangabeys may lead to an attenuated inflammatory response and a significant reduction of the indirect bystander effects of SIV infection. The relative lack of induction of a type 1 IFN response to SIV infection was also verified by microarray analysis of peripheral blood lymphocytes from uninfected and infected mangabeys (Abstract 323). In

contrast, activation of type 1 IFN responses was observed in lymphocytes from HIV-infected subjects.

Previous work from Veazey and colleagues has demonstrated that the intestinal tract is a major target for SIV infection and CD4+ cell depletion in SIV-infected macaques. Could the relative absence of these activated T cells in gut-associated lymphoid tissue of natural hosts in part underlie their resistance to immunodeficiency?

To address this issue, Veazey (Abstract 153), compared CD4 and CCR5 expression in lymphocytes from the intestinal tract, lymph nodes, and blood of uninfected sooty mangabeys, African green monkeys, and rhesus macaques. As previously reported, approximately 50% of T cells in the intestines of macaques express CD4 and 50% of these CD4+ cells express CCR5. However, less than 10% of T cells in the intestines of African green monkeys express CD4 and of these, only approximately 12% also express CCR5. The frequency of CD4+ cells in the gut of sooty mangabeys was higher than in African green monkeys, but essentially none of these cells express CCR5.

These observations suggest the hypothesis that natural hosts of SIV infection may have evolved to reduce the number of CD4+CCR5+ cells in gut-associated lymphoid tissue that serve as the primary fuel for SIV replication. Although this observation would initially appear to be at odds with the fact that many of the natural hosts have viral loads that are as high or higher than those observed in susceptible hosts, it is possible that viral replication in gut-associated lymphoid tissue may be responsible for initiating the indirect effects of CD4+ lymphocyte depletion. As suggested by Douek's plenary talk, this process may be initiated by immune activation induced by increased mucosal inflammation associated with the depletion of mucosal CD4+ lymphocytes.

Update on the Quest for a Safe and Effective AIDS Vaccine

Recent advances in HIV vaccine research were highlighted in a symposium encompassing basic science, nonhuman primate trials, and human clinical trials. The past decade has witnessed a significant expansion in the number of viral vectors able to induce cell-mediated immune

responses. Johnson (Abstract 111) summarized the strengths and shortcomings of viral vectors currently under study as candidate AIDS vaccines.

Notable advantages of these vectors include their track record in inducing relatively robust cellular immune responses in mice and nonhuman primates, as well as their ability to deliver multiple viral antigens. However, he highlighted the challenge of overcoming immune responses to these vectors induced by prior administration or natural infection. This limitation has been well documented in a trial of an adenovirus serotype 5 (Ad5) HIV-1 Gag vaccine, which demonstrated a significant decrease in the frequency of ELISPOT responses in subjects with high levels of titers of neutralizing antibodies to the Ad5 vector backbone, especially when administered at lower doses.

Several different options for overcoming preexisting or induced antivector immunity are being pursued. In some cases, the effect of preexisting immunity can be overcome by increasing the vector dose, as has been demonstrated for the adenovirus vectors. However, the necessity of increasing the vaccine dose by 100-fold to 1000-fold has a significant impact on vaccine cost and production, as well as reactogenicity.

Alternative strategies that are being pursued include the use of less-frequent serotypes of viral vectors (eg, adenovirus serotypes 11 and 35 or adenoviruses from other species, such as chimpanzees) and the use of viral vectors derived from viruses that infrequently infect humans (eg, vesicular stomatitis virus or Venezuelan equine encephalitis virus). A final strategy that is being pursued is that of boosting with heterologous viral vectors, an approach that is currently being pursued in a collaborative trial that is examining the immunogenicity of a combined canarypox (ALVAC) and adenovirus vaccine regimen.

McElrath (Abstract 112) summarized CD8+ cell responses elicited in several recent candidate AIDS vaccine trials. A trial jointly sponsored by Oxford University and the International AIDS Vaccine Initiative (IAVI) examined the safety and immunogenicity of an HIV-1 clade-A DNA vaccine followed by boosting with an HIV-1 clade-A modified vaccinia Ankara (MVA) vaccine in seronegative volunteers. Although preclinical trials

with a comparable SIV construct yielded significant levels of SIV-specific CD8+ cell responses in nonhuman primates, the overall level of immunogenicity of the HIV-1 clade-A constructs was disappointing: regardless of the DNA dose or the MVA boosting schedule, only 14% to 20% of volunteers had a positive ELISPOT response, and the majority of these responses were not sustained. More encouraging results have been obtained with the human trials of adenovirus vectors. McElrath particularly highlighted recent results from the Ad5 Gag/Pol/Nef vaccine, which were also reported in detail in a subsequent oral presentation (Abstract 135). This vaccine was immunogenic in approximately 70% of subjects, and the majority of responders developed a response to more than 1 vaccine antigen.

McElrath also addressed the issue of heterogeneity of virus-specific CD8+ cell function. Although much of recent AIDS vaccine development has been focused on increasing the magnitude of HIV-specific CD8+ cells induced by vaccination, she highlighted a number of qualitative aspects of CD8+ cell function that might affect efficacy, including the breadth of responses, epitope avidity, and their ability to home to mucosal sites, as well as to proliferate following antigenic stimulation.

As one approach to determine what parameters of HIV-specific CD8+ cell function might correlate with control of viral set point after acute infection, 21 patients with primary HIV infection were studied in detail with respect to the magnitude, breadth, and avidity of their HIV-specific CD8+ cell response. In fact, none of these parameters correlated with viral set point, and the functional avidity of individual epitopes did not correlate with the magnitude of the response for a given epitope.

Although it remains unclear which parameters of CD8+ cell function best correlate with control of HIV replication, McElrath underscored the finding that HIV-specific CD8+ cells from long-term nonprogressors typically maintain a high perforin content and the ability to proliferate following antigen stimulation. Thus, although HIV-specific CD8+ cells generally persist with disease progression and retain the ability to secrete IFN- γ or tumor necrosis factor (TNF)- α , their functional characteristics with respect to prolif-

eration and cytolytic ability are often impaired.

Although nonhuman primates have served as a primary platform for preclinical evaluation of AIDS vaccines, the ability of monkey studies to predict results in humans has been a source of recurring debate. Letvin (Abstract 113) reviewed evidence as to whether results from nonhuman primate studies are likely to be predictive of either the immunogenicity or efficacy of AIDS vaccines in humans. He highlighted 2 studies—the Oxford/IAVI DNA/MVA study described in McElrath's talk and a DNA/recombinant Ad5 study sponsored by the National Institutes of Health Vaccine Research Center—in which disappointing immunogenicity results had been obtained in humans. In each case, although initial testing of similar SIV vaccines had yielded promising results in macaques, subsequent testing of the HIV vaccine constructs that had yielded poor results in humans also demonstrated low levels of cell-mediated immune responses in macaques. These results highlight the fact that HIV immunogens should be evaluated for immunogenicity in macaques even without the ability to carry out an effective challenge.

Answering the question of whether vaccine efficacy trials in macaques will predict efficacy results in humans will await results from additional phase 2B or phase 3 efficacy studies of candidate AIDS vaccines. There has been considerable controversy as to whether the use of CXCR4-tropic simian/human immunodeficiency virus (SHIV) strains such as SHIV 89.6p, which induces rapidly progressive depletion of naive CD4+ cells in 2 to 4 weeks, are likely to serve as a better predictor of vaccine efficacy in humans than the use of CCR5-tropic viruses such as SIVmac239 or SIVmac251, which induce a slower progression to AIDS over a period of 1 to 2 years.

Letvin presented data from a DNA prime/adenovirus boost vaccine trial in SIV-infected macaques using a regimen that had previously generated significant protection from disease induced by SHIV 89.6p. When challenged with SIVmac251, vaccinated animals had an approximate 1-log_{10} decrease in viral load and improved survival. However, only a modest and marginally statistically significant effect against CD4+ cell

depletion was observed, in contrast to dramatic protection against CD4+ cell depletion induced by a similar vaccine following challenge with SHIV 89.6p.

These results provide some encouragement that T-cell–based vaccines may provide protection against disease in a setting of challenge viruses such as SIV-mac251, which appear likely to provide a better model of HIV-induced disease in people. However, they still leave open the question of which of the different SIV and SHIV challenge models is most likely to predict results of vaccine efficacy in humans.

Most vaccine trials in nonhuman primates have focused on the use of a single high-dose challenge. However, there has been increasing interest in the use of repeated low-dose mucosal challenges that may better model the relative inefficient transmission of HIV through sexual contact observed in humans. Butera and colleagues (Abstract 134) analyzed the ability of a DNA prime/MVA boost regimen to protect against repeated low-dose rectal challenge. Animals were vaccinated with the Gag, Pol, and Env antigens of an HIV-1 CRF02_AG primary isolate using a DNA/MVA regimen previously shown to induce relatively strong cell-mediated immune responses. Vaccinated animals and naive controls then had a repeated low-dose rectal challenge with a recombinant SHIV isolate expressing an R5 clade-B envelope (SHIV SF162p3).

Although most vaccinated animals were ultimately infected after repeated intrarectal exposure to SHIV, there was a significant increase in the number of intrarectal exposures required to infect the vaccine recipients as opposed to naive controls. Naive controls were infected after an average of 3.5 rectal exposures, but 44% of vaccinated animals remained virus free after 10 intrarectal exposures and 5 of these remained uninfected after a total of 18 exposures.

Vaccine Alternatives: Microbicides and Preexposure Prophylaxis

In light of the clear challenges to developing an effective HIV vaccine in the next decade, there has been a resurgence of interest in microbicides. Veazey (Abstract 128) reported on the ability of a CCR5 coreceptor inhibitor (CMPD-167, which is no longer in the clinical

pipeline) to block vaginal infection of macaques with the R5 SHIV strain 162p3. Previous studies of a lower dose of this compound had demonstrated that a low intravaginal dose (0.6 mg) of CMPD-167 was able to decrease peak viremia in macaques but was only rarely able to induce complete protection. Following a reformulation of the compound to allow a 5-fold increase in dose, the investigators observed complete protection from SHIV infection in 7 of 8 animals studied, whereas all 5 placebo-treated control animals were infected. The relatively high doses of this compound (and other vaginal microbicides such as PSC-RANTES) that are required suggest that diffusion of these compounds into the vaginal epithelium or submucosa may be required for inhibition of viral entry.

Promising preliminary findings for an alternative microbicide approach were reported by Cristofaro (Abstract 129), who demonstrated the ability of liposomal delivery of small interfering RNA (siRNA) to downregulate vaginal expression of the nuclear membrane protein lamin A/C (which was selected as a proof-of-principle target) or CCR5 in mice. Sustained downregulation was observed for up to 7 days after a single application. Although these results provide an encouraging demonstration of the feasibility of using siRNAs as a microbicide, practical questions related to toxicity, cost, and optimal dosing schedules will have to be addressed, including nonhuman primate studies, before this strategy can move forward to clinical trials.

Although the concept of providing preexposure prophylaxis to people at increased risk for acquisition of HIV infection has been controversial, given the grim prospects of developing an AIDS vaccine, this approach has received increasing attention and provoked ongoing controversy. Grant (Abstract 137) reviewed the rationale behind preexposure prophylaxis. The planned or recently initiated clinical trials of preexposure prophylaxis have employed tenofovir, which has a number of characteristics well suited for this purpose, including once-daily dosing and an excellent safety profile.

Several trials of tenofovir in macaques employing relatively high doses (20–30 mg/kg) had demonstrated

essentially complete protection against mucosal or even intravenous infection with SIV. However, more recent studies using lower doses of tenofovir (4–10 mg/kg/day) have demonstrated more modest protection against oral or rectal challenge, although they have still demonstrated a statistically significant reduction in the risk of infection per exposure. Although several clinical trials of preexposure prophylaxis employing tenofovir have been planned or initiated, controversy regarding these studies has resulted in some of these studies being cancelled, notably in Cambodia, Cameroon, and Nigeria. Grant highlighted the fact that the assessment of the effects of preexposure prophylaxis should extend beyond simply measuring acquisition rates of HIV infection and include assessment of the development of viral resistance to the drug(s), potential effects of drug resistance mutations on viral replication and transmission fitness, potential effects on risk-taking behavior, and whether abortive infections might induce immune responses that could subsequently protect against infection (as has been observed in macaque studies).

Given the controversy and difficult ethical questions that have surrounded these trials, it is also clear that involvement of community members in the planning stages of future studies of preexposure prophylaxis will be a key factor in their successful execution.

Therapeutic Vaccination

The prospect that immunization of HIV-infected individuals could improve their ability to contain viral replication has generally offered more promise than hard results. Two studies provided support for the ability of therapeutic immunization regimens to improve control over viremia but also highlighted the significant challenges that this approach faces. In a late breaker (Abstract 133LB), Levy offered a report on the ANRS 093 trial through 100 weeks of follow-up.

In this trial, HIV-infected subjects with CD4+ counts greater than 350 cells/ μ l and HIV-1 RNA levels less than 50 copies/mL were randomized to antiretroviral therapy alone or to antiretroviral therapy plus immunization with the canarypox vector ALVAC vCP1433 (which expresses HIV-1 *gag*, *pol*, *env*, and *nef*) and HIV-1 lipopeptides in association

with low-dose subcutaneous interleukin (IL)-2. All subjects then underwent a treatment interruption at week 40 and were restarted on therapy if HIV-1 RNA levels rose to more than 50,000 copies/mL at 4 weeks or more than 10,000 copies/mL at subsequent time points. In the second phase of this trial, patients who had resumed antiretroviral therapy underwent a second treatment interruption with the restart criteria as defined above. Vaccinated patients did have a significant increase in their time off treatment as compared with controls (177 days vs 89 days) and also had slightly lower levels of viremia during the interruptions. Better control was associated with a positive lymphoproliferative response to one of the HIV peptides used for vaccination and with higher HIV-specific ELISPOT responses.

Although these results clearly document the potential of this therapeutic vaccination regimen to enhance control of viral replication in the setting of treat-

ment interruptions, the magnitude and duration of benefit was relatively modest.

Additional evidence for the potential of therapeutic immunization was provided by a presentation from Pavlakis (Abstract 132) on SIV-infected macaques that received an SIV DNA vaccine while on antiviral therapy. SIVmac-infected macaques were treated with a combination of tenofovir, didanosine, and stavudine for 13 to 23 weeks. During treatment, the animals received intramuscular injections with optimized DNA vectors, and in a subset of animals, with an IL-15 DNA vector as well. Compared with unvaccinated controls that underwent a similar treatment interruption regimen, vaccinated animals had an approximate 1-log_{10} decrease in viral load off therapy, and a subset of 3 animals had prolonged control of viremia off therapy. Animals with better control had relatively high levels of Gag and envelope-specific ELISPOT responses. Although these results also suggested that the benefit of

therapeutic immunization is generally short lived, they do offer some hope that more potent therapeutic immunization regimens may be able to offer an extended drug-free period to at least a subset of patients. Whether this increased period of time off therapy will justify the effort of therapeutic immunization is at present unclear.

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A list of all cited abstracts appears on pages 45 to 50.

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