

HIV Pathogenesis and Vaccine Development

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Continuing the trend of recent years, the 10th Conference on Retroviruses and Opportunistic Infections offered a strong and diverse array of presentations on AIDS pathogenesis and vaccine development. Recent advances in pathogenesis research highlighted efforts to understand how HIV persists in the face of a vigorous immune response and underscored the importance of assessing functional properties of cellular immune responses. This year's conference also witnessed a resurgence of interest in neutralizing antibody responses, and numerous efforts to translate new insights into the structure of the HIV-1 envelope and neutralizing antibodies to the development of new vaccine approaches able to induce broadly neutralizing antibodies. Nonhuman primate vaccine studies offered encouraging results of novel approaches able to induce protection against pathogenic simian immunodeficiency virus (SIV) isolates but also offered concerning caveats regarding the potential for viral escape and the impact of host genotype on vaccine protection.

Immune Control of HIV: Size is Not Enough

In the ongoing quest for an effective AIDS vaccine, advancing our understanding of the barriers that impede immune control of viral replication in HIV-infected individuals is of paramount importance. Although the association of

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long-term nonprogression (LTNP) with strong HIV-specific CD4+ and CD8+ T-cell responses is well-established, the factors associated with progressive HIV infection are not well-understood.

Using a panel of 410 overlapping peptides spanning all HIV proteins of a clade B consensus sequence, Kaufmann and Draenert presented results of a comprehensive investigation of the total HIV-specific CD4+ and CD8+ T-lymphocyte response in different cohorts of HIV-infected subjects. Kaufmann (Abstract 31) presented an analysis of HIV-specific CD4+ T-cell responses in 32 HIV-infected subjects who either were on highly active antiretroviral therapy (HAART) or were off therapy and had relatively low plasma HIV-1 RNA levels (median, 2850 copies/mL). HIV-specific CD4+ responses were focused on relatively few epitopes, predominantly found in Gag and Nef. CD4+ T-cell responses to at least 1 HIV peptide were detected in 78% of patients, and a median of 4.5 peptides were recognized by each subject. Despite the presence of disparate human leukocyte antigen (HLA) class II alleles, 7 peptides (5 in Gag and 2 in Nef) were recognized by 75% of the subjects, including 1 that was recognized in 56% of subjects. This finding suggests a relatively promiscuous presentation of CD4+ T-cell epitopes by different HLA class II alleles. In contrast to the CD4+ T-cell response, the CD8+ T-cell response in this cohort of HIV-infected subjects was 10-fold greater in magnitude and broadly directed, targeting reverse transcriptase (RT), integrase, and Env in addition to Gag and Nef. A similar pattern of vigorous and broad HIV-specific CD8+ T-cell responses was also observed by Draenert (Abstract 35) in 26 chronically HIV-infected subjects with late-stage HIV infection (mean plasma HIV-1 RNA level, 186,271 copies/mL, and mean CD4+ count, 141 cells/ μ l). A median of 13.5 peptides (range, 2-39) were recognized by individual progressors in this group, and the median magnitude of

HIV-specific CD8+ T cells was 5705 spot-forming cells/ 10^6 peripheral blood mononuclear cells (PBMCs) (range, 185-25,000). Although the plasma HIV-1 RNA levels and CD4+ T-cell counts in this group showed a weak inverse correlation with the breadth and magnitude of the CD8+ T-cell response, a striking overlap in the magnitude and breadth of CD8+ T-cell responses was observed between progressors and LTNPs. Thus, in 25 LTNPs, recognition of a median of 16 peptides (range, 5-58) with magnitudes ranging between 1046 and 43,000 spot-forming cells/ 10^6 PBMCs (median, 7980) did not differ significantly from progressors.

Since T-lymphocyte responses to HIV have been routinely measured using nonautologous viral sequences, the real breadth and magnitude of the HIV-specific T-cell response as gauged by autologous virus sequences is likely to be even greater. Using peptide sets from a clade B consensus sequence, Altfeld and colleagues (Abstract 314) showed that the virus-specific T-cell response clustered in conserved regions of the genome. However, when responses to peptides spanning the consensus and autologous virus sequences of Tat, Vpr, and p24-Gag were compared, the autologous sequences demonstrated broader and significantly stronger responses, particularly those directed against the variable regulatory proteins. Thus, differences between controllers and non-controllers of HIV infection may be better appreciated using autologous virus sequences. However, Migueles (Abstract 318) showed that the frequency of CD8+ T lymphocytes secreting interferon- γ in response to stimulation with autologous virus-infected CD4+ T cells was equivalent in LTNPs and progressors. In addition, variant peptides with autologous virus sequences were well-recognized, demonstrating that loss of immune control can occur in the presence of persistent recognition of autologous virus sequences.

These data highlight the paradox

that despite the fact that HIV infection is highly immunogenic, induction of a strong and broad anti-HIV response is not in itself sufficient for effective immune control. This finding raises the possibility that factors other than the overall magnitude of cellular immune responses may be critical in the ability of the host to control HIV infection. Are there distinct functional properties of the HIV-specific T-cell response associated with an effective immune response? Presentations by Walker (Abstract 164) and Connors (Abstract 165) addressed this key issue.

The ability of cytotoxic T lymphocytes (CTLs) to control HIV replication may be dependent on a variety of factors, including genetic background, presence of CTL escape, and defects in CTL maturation or function. In his presentation, Walker (Abstract 164) proposed that one of the reasons underlying lack of effective viral control with strong CTL responses may be related to epitope-specific or allele-specific qualitative differences among CTLs. These variations may lead to differences in the propensity for and the consequences of CTL escape. Thus, loss of certain CTL responses may be more important than loss of others. One such example is the consequence of CTL escape in a single B27-restricted epitope (KK10). Although the presence of HLA-B27 is highly associated with nonprogression in HIV-infected individuals, escape in this particular CTL epitope is strongly correlated with loss of immune control of viral replication. Often, factors other than sequence variation in known CTL epitopes appear to be responsible for non-control. Thus, in a noncontroller, only 4 of 13 peptide responses identified by enzyme-linked immunospot (ELISPOT) assays differed from the viral sequence of the autologous virus. In longitudinal studies of sequence variation in the whole HIV genome in 2 HIV-infected subjects, Walker showed that although increasing sequence variation with time was associated with increasing plasma HIV-1 RNA levels, amino acid variation in as few as 15 amino acids of all HIV proteins was temporally associated with the loss of immune control. In both individuals, more than half of the amino acid changes were not at sites of previously defined CTL epitopes. These may

represent novel CD8 + T-cell epitopes in the autologous virus or sites of escape from CD4 + T lymphocytes or antibodies.

Loss of immune control in the setting of a strong and broad HIV-specific T-lymphocyte response was also observed in subjects undergoing structured treatment interruptions (STIs) following institution of HAART in acute HIV infection. Walker presented an update on 14 such subjects. Substantial augmentation of cellular immunity was observed following each episode of STI. In 1 individual, the number of peptides recognized increased from 2 pre-STI to 25 at the end of the second STI. Post-STI responses were also broader, encompassing both structural and regulatory proteins. Although the pre-STI responses were exclusively in Gag, Env, or Nef, following STI, 100% of the responses targeted Nef, Env, and Pol; 88% targeted Gag; and 38% to 60% were directed toward regulatory proteins. However, even this augmentation in breadth was not sufficient to sustain inhibition of viral replication, and eventually immune control was lost. No benefit in survival was observed in an intent-to-treat analysis. However, there was some benefit in terms of plasma HIV-1 RNA levels, in that the time to reach greater than 30,000 HIV-1 RNA copies/mL in plasma was slowed in individuals on STIs.

What qualitative defects are correlates of poor immune control? In studies presented by Connors (Abstract 165), reduced proliferative capacity of HIV-specific CD8 + T cells was the single most dominant defect that differentiated progressors from nonprogressors, as well as distinguishing viremic and aviremic episodes in HIV-infected subjects on intermittent HAART. The HLA-B*5701 allele was present in 15 of 17 patients in a cohort of LTNP. The magnitude of the HIV-specific CD8 + T-cell response, as assessed by interferon- γ secretion, did not differ between HLA-B*5701-positive and -negative progressors. Neither did it differ between HLA-B*5701-positive progressors and non-progressors. The difference in immune control between the 2 groups was not due to escape of HLA-B57-restricted CTL epitopes from immune control. Instead, HLA-B57-restricted CTLs showed a

marked inability to proliferate in response to antigen-specific stimulation in progressors but not in LTNPs. This defect in proliferative capacity was linked to a lack of perforin expression in the nonproliferating antigen-specific CD8 + T cells. A similar defect in proliferative capacity has been seen in HIV-specific CD4 + T cells in individuals on STI during viremic episodes.

Price and colleagues (Abstract 32) compared the physical enumeration of HIV-specific CD8 + T lymphocytes by tetramers or by clonotypic quantitative polymerase chain reaction for the T-cell receptor B (TCRB) locus of defined HIV-specific CTL clones with functional enumeration of interferon- γ producing cells during viremic and aviremic episodes in 133 HIV-infected subjects enrolled in the Swiss HIV Cohort Study. In this study, individuals were subjected to cycles of 8 weeks on and 2 weeks off therapy, and treatment was discontinued after 4 such cycles. During "off-treatment" phases, increases in plasma HIV-1 RNA levels were associated with decline in interferon- γ ELISPOT responses in the presence of stable or increased frequencies of tetramer-positive cells. In contrast, the frequency of tetramer-positive and interferon- γ -positive cells approximated each other in aviremic individuals. Functional and nonfunctional CD8 + T cells had the same clonality and were not preferentially infected with HIV.

A novel method of assessing the function of CD8 + T cells was presented by Betts and colleagues (Abstract 306). They assessed degranulation of cytotoxic granules in CTLs by flow-based measurement of lysosomal-associated membrane glycoproteins CD107a, CD107b, and CD63. These proteins are expressed only on the membranes of cells that have recently undergone degranulation following antigen-specific stimulation, and may serve as a surrogate marker for cytolytic ability of antigen-specific CD8 + T lymphocytes. Functional heterogeneity with regard to cytokine-secreting and degranulating ability existed within CD8 + T-cell clonotypes specific for a single antigen. How lack of function demonstrated by 1 technique correlates with lack of function by other techniques (eg, decreased cytokine secretion vs defec-

tive granulation vs decreased perforin vs decreased proliferative ability) remains to be determined.

Phenotypic differences among antigen-specific T lymphocytes that reflect distinct stages of maturation have been correlated with their functional ability. Harari and colleagues (Abstract 33) detected CD45RA⁺ CCR7⁻ HIV-specific CD4⁺ T lymphocytes in LTNP but not in progressors and observed an inverse correlation between viremia and frequency of CD45RA⁺ CCR7⁻ HIV-specific CD4⁺ T lymphocytes. These differences appeared to be restricted to HIV-specific T lymphocytes, since cytomegalovirus (CMV)-specific CD4⁺ T lymphocytes with a CD45RA⁺ CCR7⁻ phenotype were present in both LTNP and progressors. Complementary findings for CD8⁺ T cells were observed by Draenert (Abstract 35), who showed an association between the presence of increased frequencies of CD45RA⁺ CCR7⁻ (mature effector phenotype) HIV-specific CD8⁺ T cells and viral control.

Boutboul and colleagues (Abstract 34) used complementary DNA (cDNA) microarray analysis to compare gene expression in PBMCs from 5 healthy donors and 27 HIV-infected subjects at different stages of disease. Interferon- α and perforin genes were upregulated, while the interleukin-7 receptor (IL-7R) gene was downregulated in resting PBMCs from HIV-infected individuals with late-stage disease compared with PBMCs from HIV-seronegative individuals. The degree of upregulation or downregulation of these genes was comparable to that observed in normal PBMCs activated *in vitro* by CD3/CD28 stimulation. Flow cytometric analysis revealed that CD8⁺ T lymphocytes with high CD127 (IL-7R) expression were negative for perforin, and reciprocally, that cells expressing high levels of perforin were CD127-negative or -low. These findings suggest the possibility that low expression of IL-7R on CD8⁺ T lymphocytes in HIV infection may represent cells that are lytic but that have lost their ability to proliferate.

An additional determinant of immunologic control is the presence of adequate numbers of antigen-specific T lymphocytes at tissue sites of viral replication. Cromwell and colleagues

(Abstract 30) analyzed the frequency and phenotype of SIV-specific CD8⁺ T lymphocytes targeting the Mamu-A*01-restricted SIV Gag₁₈₁₋₁₈₉ epitope in the peripheral blood and reproductive tissues of female SIV-infected rhesus macaques. The frequency of tetramer-positive cells was increased an average of 14-fold in genital mucosa compared with that in peripheral blood. Mucosal CD8⁺ T lymphocytes had a high frequency of CXCR3⁺ cells compared with peripheral blood. The increased expression of CXCR3 on vaginal CD8⁺ T lymphocytes correlated with expression of its ligand CXCL9 in lymphoid aggregates and lamina propria of the vaginal mucosa, and may be a mechanism for homing of antigen-specific T lymphocytes to mucosal sites of viral replication.

The inability of most HIV-infected subjects to generate potent neutralizing antibodies effective against primary HIV isolates has led many to question whether antibodies play a significant role in controlling viral replication. Shaw (Abstract 166) described analysis of neutralizing antibody responses using an assay in which autologous envelope sequences are used to generate pseudotyped HIV particles expressing a reporter gene. This process in turn allows analysis of neutralizing antibody titers to autologous viral sequences using a single-round infectivity assay. Using this assay, he was able to demonstrate emergence of autologous neutralizing antibodies as early as 72 days after seroconversion and titers that reached as high as 2500. However, variant viral sequences resistant to concurrent neutralizing antibodies rapidly emerged, such that neutralizing antibody titers to autologous envelope sequences at the time virus was isolated were relatively low. Analysis of sequence changes associated with the evolution of escape viruses supported the concept of a "glycan shield," in which an ever-shifting umbrella of sugar residues blocks the ability of antibodies to bind to envelope oligomers.

Effect of Viremia on Immune Function

It is now abundantly clear that in addition to depletion of CD4⁺ T cells, HIV

induces widespread immune activation and dysfunction that affects numerous arms of the immune system. In a plenary lecture, Fauci (Abstract 119) outlined results from studies on the effect of HIV viremia on CD4⁺ T lymphocytes, B lymphocytes, and natural killer (NK) cells performed on HIV-infected individuals during viremic and aviremic stages of infection. In viremic subjects, a number of abnormalities in CD4⁺ cells, B cells, and NK cells were detected. Resting CD4⁺ T lymphocytes in viremic subjects had spontaneous virus production, whereas those in aviremic subjects did not. Microarray analysis revealed upregulation of 493 genes involved in transcriptional regulation and RNA processing in resting CD4⁺ T cells from viremic subjects. Fauci proposed that the effect of viremia on CD4⁺ T cells appears to be mediated by both cytokines and the HIV envelope.

Effects of viremia were also demonstrated on B cells and NK cells. B lymphocytes in HIV-viremic subjects were characterized by loss of CD21 expression and decreased proliferation in response to CD4⁺ T-cell help due to downregulation of CD25 expression. As reported in more detail in a subsequent oral presentation by Malaspina (Abstract 128), B cells from HIV-viremic subjects have a reduced capacity to stimulate CD4⁺ T cells through CD80/86-CD28 interactions. B cells of HIV-infected viremic subjects had decreased induction of CD80/86 on activated B cells and a reduced capacity to induce proliferation of allogenic CD4⁺ T cells from healthy HIV-seronegative subjects. This functional abnormality was corrected following reduction of plasma HIV-1 RNA levels by effective antiretroviral therapy and was associated with upregulation of CD80/86 on the B lymphocytes. NK cells from viremic HIV-seropositive subjects had a reduced ability to secrete beta-chemokines and suppress HIV replication *in vitro*. The activating NK receptors NKp46, NKp30, NKp44, and NKG2D were markedly reduced, and some killer inhibitory receptors were upregulated on NK cells of viremic as opposed to aviremic HIV-infected subjects.

A novel mechanism of viremia-induced immune evasion was proposed

by Brainard and colleagues (Abstract 29). Previous work from this laboratory had demonstrated that the chemokine SDF-1, while serving as a chemoattractant at low concentrations, was able to mediate repulsion of T lymphocytes (termed fugetaxis) in a CXCR4-receptor-mediated manner at higher concentrations. Their current data suggest that increased concentrations of gp120 may induce fugetaxis of HIV-specific CTLs. Fugetaxis induced by gp120 was CD4-independent and inhibited by anti-CXCR4 and pertussis toxin. By altering cell density and performing CTL assays in flat-bottomed wells, these investigators demonstrated decreased efficacy of cell killing at lower cell densities, suggesting the possibility that CTL migration may affect killing efficacy. According to this scenario, gp120-mediated inhibition of CTL migration toward sites of increased viral replication may reduce CTL efficacy *in vivo*.

Host Genetics

Host genetic polymorphisms can affect the outcome of HIV infection at the level of virus entry, virus dissemination, and immune control of viral replication. In a symposium, 4 presentations discussed the impact of host genetics on HIV-1 variability and outcome of HIV infection. In large population studies, Ahuja (Abstract 53) presented data on the impact of genetic variation in the CCR5 gene and MCP-1 gene on HIV-1 transmission and disease progression. In a cohort of 649 children exposed perinatally to HIV and followed for 4 years, multiple polymorphisms in the CCR5 gene other than the well-described $\Delta 32$ genotype were identified that were associated with either increased or decreased risk of mother-to-child transmission. CCR5 polymorphisms that were either deleterious or beneficial had a similar impact on disease progression in both adults and children. The prevalence of disease-modifying CCR5 haplotypes was different in populations of different ethnicities, emphasizing race as a confounding factor in therapeutic efficacy studies. Two polymorphisms and 3 haplotypes were also identified in the MCP-1 gene. A 2578G single-nucleotide polymorphism, although associated with a decreased risk of acquiring HIV

infection, was linked to decreased survival and increased risk of AIDS-associated dementia once HIV infection was established. The deleterious effect of this polymorphism was possibly related to increased transcription of MCP-1 and increased monocyte recruitment to the brain. Kaslow (Abstract 56) summarized the current knowledge of host genetic polymorphisms that have consistently been shown to have an effect on HIV transmission or disease progression. At the level of HLA alleles, the most consistent deleterious effects on disease progression have been seen with HLA homozygosity at any locus and with the presence of certain allelic variants of HLA-B35 and HLA-B*5301. In contrast, alleles B*5701 and B*5703 are consistently associated with control and delayed progression of HIV-1 infection across HIV subtypes A, B, and C. With regard to impact on HIV acquisition, sharing of HLA alleles by transmitter and recipient at 1 or more loci can be associated with an increased risk of horizontal transmission. In a study of 221 serodiscordant couples in Zambia followed up for 5 years, there was a strong deleterious effect on transmission of sharing alleles at the HLA-B locus. The effect of the HLA-A locus on clinical outcome or HIV transmission has not been consistent across studies. The HLA-A2/A*6802 supertype has been associated with a decreased risk of horizontal and mother-to-child transmission in white and African American populations infected with clade A or B virus. Surprisingly, the same alleles were shown to be significantly associated with an increased risk of transmission in a Zambian cohort infected with clade C virus.

The effect of HLA polymorphisms in driving HIV-1 sequence variability was elegantly presented by John (Abstract 54). HIV adapts to the host at 2 levels. At 1 level the accumulation of mutations is driven by immune pressure, which for CTL epitopes is evident as HLA class I allele-specific polymorphisms at the population level. However, the emergence of mutations at each residue is modulated by variable genetic barriers, including functional or structural constraints or cost to replicative fitness. In a full-length HIV genome analysis in 175 patients, adaptation of HIV to HLA poly-

morphisms was strongly predictive of plasma HIV-1 RNA levels, with greater viral adaptation being associated with higher HIV-1 RNA level. Although univariate analysis showed a significant association between the presence of HLA B*5701 or HLA-B*2705 and low plasma HIV-1 RNA levels, this effect was no longer apparent on multivariate analysis. Instead, low plasma HIV-1 RNA level was strongly associated with less HIV adaptation. It is possible that the protective effect of HLA B*5701 and B*2705 is causally related to a decreased propensity for their CTL epitopes to accrue mutations because of the presence of genetic barriers.

Beneficial and deleterious effects of HLA class I haplotypes on HIV may also be mediated via its effect on innate immunity. Carrington (Abstract 55) presented data on associations between killer immunoglobulin-like receptors (KIRs) and HLA that can modulate HIV-1 disease. The KIR genes, located on chromosome 19q13.4 in the leukocyte receptor complex, encode a group of receptors that regulate inhibition and activation of NK cells. Binding of inhibitory KIR receptors on NK cells to major histocompatibility complex (MHC) class I molecules on target cells inhibits NK-mediated cytotoxicity, whereas the absence of MHC class I molecules on target cells is associated with NK cell activation and killing. The presence of 1 activating KIR allele, KIR3DS1, in combination with a specific subset of HLA-B alleles (those containing the Bw4 serological epitope that also have an isoleucine at position 80, designated as HLA-B Bw4-80I), was shown to have a highly protective effect against progression to AIDS. This protective effect was not observed with HLA-B Bw4-80I in the absence of KIR3DS1. Further, KIR3DS1 alone shows a deleterious effect on survival. HLA molecules expressing the Bw4 serologic epitope are a ligand for the inhibitory KIR receptor encoded by KIR3DL1. Since the extracellular domains of 3DL1 and 3DS1 are very similar, they may interact with similar ligands. The model to explain the protective synergistic effect of HLA-B Bw4-80I and KIR3DS1 proposes that KIR3DS1 may preferentially bind to Bw4-80I, leading to NK cell activation and killing of HIV-infected target cells.

Swift NK cell activation mediated through KIR molecules early in infection may positively impact viral control, not only for HIV, but also for other pathogens.

Dendritic Cells

Although DC-SIGN, a C-type lectin that is highly expressed in monocyte-derived dendritic cells (DCs), is believed to be a key molecule facilitating dendritic cell-mediated HIV infection of CD4+ T cells, the precise mechanisms underlying this function are not known. KewalRamani (Abstract 110) summarized research on DC-SIGN from his laboratory. Stable expression of DC-SIGN in the monocytic cell line THP-1 resulted in transmission of HIV-1 at efficiencies similar to those of primary immature DCs. In transwell experiments, cell contact between THP-1/DC-SIGN cells and CD4+ T lymphocytes was required for HIV-1 transmission. When other transformed cell lines (K562 and 3T3) stably transfected and expressing DC-SIGN at levels comparable to those of the THP-1/DC-SIGN cells were examined, they were not able to transmit HIV-1, even though their ability to bind HIV-1 or intracellular adhesion molecule 3 (ICAM3) was not impaired. This finding led to a search for cell molecules required for DC-SIGN-mediated HIV transmission. As observed for immature DCs, HLA-DR and leukocyte function antigen 1 (LFA-1) were expressed on THP-1 cells permissible for HIV transmission, but these molecules were not expressed on K562 and 3T3 cells. However, expression of HLA class II molecules and LFA-1, singly or in combination, in the nonpermissive K562 and 3T3 cell lines did not reverse the defect in HIV transmission, suggesting that DC-SIGN expression is not sufficient for efficient HIV-1 transmission and that other cofactors are likely to play a role. Identification of these specific cofactors should provide valuable insights into DC-SIGN-mediated transmission of HIV.

Studies using live-cell video microscopy to examine transfer of HIV from DCs to CD4+ T lymphocytes were presented by Hope (Abstract 113). Using fluorescent labeling of HIV by incorporation of a fusion protein of GFP

with Vpr, investigators showed that in monocyte-derived DCs, HIV was recruited to sites of cell contact with T cells. At the same time, in the target cell, CD4 and CXCR4 were concentrated at the CD4+ T cell/DC interface. The localized concentration of HIV, CD4, and coreceptors (termed an “infectious synapse”) may play a critical role in facilitating infection of T cells by dendritic cells.

Innate Immunity

In addition to acting as a conduit for HIV transmission, DCs are the principal antigen-presenting cells in the body and essential for mounting an effective adaptive immune response. Loré and colleagues (Abstract 81) demonstrated that innate immune recognition mediated by binding of the microbial pattern recognition Toll-like receptors (TLRs) on DCs to their natural ligands resulted in maturation of DCs and enhancement of adaptive virus-specific immune responses *in vitro*. CD11c+ myeloid DCs expressing TLRs 3, 4, and 7 and CD123+ plasmacytoid DCs expressing TLRs 7 and 9 were activated on exposure to their respective ligands: CpG (TLR9 receptor), imidazoquinolones (TLR7), LPS (TLR4), and poly I:C (TLR3). TLR-ligand-mediated activation of plasmacytoid or myeloid DCs resulted in an enhanced capacity to generate antigen-specific T lymphocytes. The ability to modulate adaptive immunity makes TLR ligands a potentially useful immunologic adjuvant for HIV vaccines.

Unutmaz (Abstract 112) outlined the role of natural killer T cells (NKT cells) in HIV infection. NKT cells are a distinct subset of human effector T lymphocytes that express an invariant T-cell receptor and recognize glycolipid antigens presented by the nonpolymorphic MHC class I molecule CD1d. Using flow cytometry to identify NKT cells in humans ($V\alpha 24 + V\beta 11 +$ or CD1d tetramer + $V\beta 11 +$), 20% to 90% of NKT cells were found to be CD4+ and to express CCR5. Autologous DCs pulsed with the glycolipid alpha-galactosylceramide were able to expand NKT cells, which secreted IL-4 and IL-5 and were highly susceptible to infection with R5-tropic HIV. HIV-infected subjects had

marked declines in NKT cells, which may be mediated by direct infection. Decline in CD4+ NKT cells but not total NKT cells correlated inversely with plasma HIV-1 RNA level.

Natural Hosts of SIV Infection

An expanding number of nonhuman primates such as sooty mangabeys and African green monkeys serve as natural hosts for SIV infection. Despite lifelong infection and levels of viremia that can in some species equal or exceed those in HIV-infected subjects with AIDS, these hosts remain asymptomatic and do not develop AIDS. Increasing attention over the past several years has been devoted to trying to elucidate immunologic or virologic mechanisms associated with the lack of pathogenicity of primate lentiviruses in their natural hosts. Barry and colleagues (Abstract 120) proposed that a limited SIV-specific CD8+ T-cell response may play a causal role in the lack of immunodeficiency in sooty mangabeys naturally infected with SIVsm. Analysis of several markers of T-cell activation (eg, CD69, CD25, and HLA-DR) or cell proliferation (Ki67) revealed only minor increases in SIVsm-infected sooty mangabeys compared with those observed in uninfected mangabeys. Similar findings were observed even in the setting of acute SIVsm infection of sooty mangabeys, whereas acute infection of rhesus macaques with the same stock of SIVsm resulted in significant increases in T-cell activation, increased proliferation of CD8+ and CD4+ T cells, and progression to AIDS. Depletion of CD8+ T cells using a murine CD8-specific monoclonal antibody resulted in only 3-fold increases in plasma viremia, leading Barry and colleagues to conclude that a weak or absent SIV-specific CD8+ T-cell response may be an important factor in the lack of immunodeficiency in SIV-infected sooty mangabeys.

A contrasting view was reported by Kaur (Abstract 121), who described the results of a study in which CD8+ T cells were depleted in SIV-infected sooty mangabeys using a chimeric mouse-human CD8-specific monoclonal antibody (cM-T807). This antibody has been widely used in nonhuman primate

experiments and generally induces more prolonged and complete depletion of CD8+ T cells than has been obtained with murine monoclonal antibodies. Following a depletion of CD8+ T cells that often extended to 3 weeks, Kaur and colleagues observed up to 100-fold increases in plasma viremia in 5 of 6 animals studied, whereas no significant increases in viremia were observed in 4 animals that received a control chimeric monoclonal antibody. Further evidence for SIV-specific CD8+ T-cell responses was provided by the finding of significant ELISPOT responses to SIV proteins in most SIV-infected mangabeys. Reasons for the apparent discrepancy in findings between these groups was not immediately clear, but may relate to differences in the efficacy of CD8+ T-cell depletion induced by the 2 different monoclonal antibodies.

Prophylactic AIDS Vaccines

Neutralizing Antibody Responses

After several years of being overshadowed by research on cell-mediated immune responses to HIV, research on neutralizing antibodies has recently enjoyed a renaissance prompted by improved information on the structural basis for neutralization, new assays for measuring neutralizing antibodies, and continued hope that structurally modified envelope immunogens will enhance the induction of broadly reactive neutralizing antibodies to HIV. Leading off a symposium devoted solely to neutralizing antibodies, Burton (Abstract 184) reviewed the evidence that supports the view that neutralizing antibodies represent the small fraction of envelope-specific antibodies that are able to bind the oligomeric envelope spike. According to this viewpoint, broadly reactive neutralizing antibodies are therefore the subset of antibodies that are able to bind to conserved sites on the oligomer. Burton also noted that although passive transfer of neutralizing antibodies in animal models has provided compelling proof-of-principle demonstration of the ability of neutralizing antibodies to protect against primate lentiviruses, efforts to elicit broadly neutralizing antibodies by vaccination have consistently met with failure.

Describing one approach to better understand why it has been so difficult to induce broadly neutralizing antibodies by vaccination, Burton discussed detailed structural studies of 2 neutralizing monoclonal antibodies, b12 and 2G12. The first is a well-characterized neutralizing antibody that was initially isolated by a phage display library derived from an asymptomatic HIV-infected individual. Previous work had demonstrated that b12 recognized a conserved site of gp120 that overlaps with the CD4 binding site. A subsequent analysis of the crystal structure of b12 revealed a long protrusion at the tip of the antibody that inserts into the recessed CD4 binding site of gp120. In an effort to enhance production of antibodies with similar structure to b12, Burton and colleagues have tried to block production of antibodies to other common epitopes on the gp120 oligomer by hyperglycosylation of regions such as the CD4 binding site and the V3 loop. Immunization studies with the hyperglycosylated gp120 molecule are in process. A similar structural analysis has been carried out for the 2G12 antibody, which recognizes an epitope in the C4/V4 region of gp120 and has been previously shown to recognize a cluster of oligomannose glycans in this region of gp120. Crystal structure analysis of 2G12 revealed that instead of forming the typical Y shape of most immunoglobulin G (IgG) molecules, the hypervariable heavy chains (V_H) formed a tight cluster in which the 2 V_H domains are directly juxtaposed, a phenomenon called domain swapping. The unusual structures of both b12 and 2G12 provide at least 1 reason for the difficulties in generating broadly neutralizing antibodies against HIV. Whether this understanding will ultimately facilitate elicitation of such antibodies by the use of modified immunogens will be a major challenge for research in this area for the next several years.

After binding to cellular receptors, gp120 undergoes a series of conformational changes ultimately resulting in insertion of an oligomeric form of gp41 into the host cell membrane and subsequent fusion of the viral particle with the host cell membrane. Whether antibodies can be successfully generated to

these intermediate forms of envelope, thereby blocking virus entry, has been controversial. Weiss (Abstract 185) reviewed efforts to generate antibodies able to block viral fusion. She identified 3 distinct stages at which fusion inhibitors may inhibit virus entry: prevention of conformational changes, blocking close opposition of the virus and host cell membrane, and prevention of fusion pore assembly. One of the intermediate steps of viral entry involves conformational changes in the ectodomain of gp41, in which 2 heptad repeats of gp41 are brought together to make up the prehairpin intermediate. This hairpin intermediate subsequently generates a 6-helix hairpin structure that catalyzes formation of the fusion pore. Peptides mimicking the gp41 heptad repeats are able to potentially inhibit HIV replication, which suggests that antibodies might also be able to block this critical step in virus entry. Inherent challenges to this approach include steric hindrance (ie, whether antibodies can fit in the small gap between the virus particle and the host cell membrane) and whether sufficient time exists during viral fusion to allow the antibody to bind. Immunization with recombinant immunogens that mimic either the prehairpin or 6-helix bundle conformation of gp41 resulted in antibodies able to bind either early intermediates or late intermediates, respectively, and that were able to inhibit viral entry. These data provide an important proof-of-principle demonstration of the feasibility of inducing antibodies able to bind to these fusion intermediates.

Generation of antibodies able to bind the chemokine coreceptor binding site of gp120 has been another leading approach for the induction of broadly neutralizing antibodies against HIV-1. Sodroski (Abstract 186) described recent efforts to define the structural characteristics of antibodies able to bind the chemokine receptor binding site. Two specific regions of gp120 make up the chemokine receptor binding site: the variable V3 loop and the beta19 strand, a more conserved region of the bridging sheet that is protected by the V3 loop. Because the length of IgG molecules (approximately 115 angstroms) is significantly longer than that of the space between gp120 and

the host cell membrane after initial virus attachment (approximately 85 angstroms), antibodies able to interact with the chemokine receptor binding site need to have a flexible and extended region to reach beyond the adjacent V3 and V1/V2 loops. Indeed, these are the characteristics of one of the best-characterized chemokine receptor binding antibodies, 17b. Another distinctive characteristic of many chemokine receptor binding antibodies (although not 17b) is the sulfation of tyrosine residues in the antigen binding site. As described in more detail in the presentation by Farzan (Abstract 28), this sulfation mimics the post-translational modification of tyrosine residues of CCR5 that interact with positively charged residues of gp120 and thus play a critical role in gp120-CCR5 interactions.

Despite the disappointing failure of efforts in the 1980s to generate neutralizing antibodies effective against primary isolates by V3 loop immunization, there has been continued interest in the potential utility of the V3 loop as a vaccine immunogen. Zolla-Pazner (Abstract 107) described efforts to elicit V3-specific antibodies with a broader range of neutralization. Previous studies using antibodies specific for linear determinants of V3 had demonstrated that neutralization mediated by these antibodies was quite type-specific. However, recent work from the Zolla-Pazner laboratory has demonstrated that monoclonal antibodies specific for conformational determinants of V3 were able to mediate broader neutralization of primary isolates. These data presented a paradox: given the variability of the V3 loop, how were V3-specific antibodies able to neutralize diverse HIV-1 isolates? The answer appears to lie in part in the structural constraints of the V3 loop. Nuclear magnetic resonance analysis of the structure of the V3 loop bound to the V3-specific antibody 447 suggested at least 2 alternative conformations of V3: 1 able to bind to CCR5, the other able to bind to CXCR4. Selection pressure on the V3 loop to maintain these 2 alternative conformations may also provide sufficient conservation to allow neutralization by conformation-sensitive V3-specific antibodies.

Nonhuman Primate Studies: The Good News

Much of the newfound optimism in the AIDS vaccine field over the past 2 years has arisen as a result of nonhuman primate studies employing the simian-human immunodeficiency virus (SHIV) 89.6p as a challenge strain. Although this strain is pathogenic and results in rapid CD4+ T-cell depletion over a matter of weeks, concerns have been raised that because of its atypical disease course and ease of neutralization, 89.6p may not be predictive of protection against clinical HIV isolates. Two presentations highlighted vaccine approaches able to induce partial protection against challenge with isolates from pathogenic SIV of macaques (SIVmac), against which it has been historically quite difficult to induce protection.

Robert-Guroff (Abstract 77) described the results of a trial in which macaques were vaccinated mucosally with a replication-competent adenovirus. Animals were vaccinated with a recombinant adenovirus expressing Env and Rev administered either by itself or in combination with recombinant adenoviruses expressing other SIV proteins (Gag or Nef) or a recombinant subunit boost. Relatively strong cell-mediated immune responses as determined by ELISPOT assays were observed against Gag and Env, with weaker responses observed against other SIV proteins. Neutralizing antibody responses were also observed in groups that received a recombinant subunit boost. Following intrarectal challenge with SIVmac251, a significant (20-fold) reduction of set-point viremia was observed in animals that had been immunized with the SIV Env/Rev construct in combination with one of the other SIV recombinants. A subset of animals was able to control viremia to undetectable levels. Protection correlated in part with ELISPOT responses on day of challenge, but other responses, such as proliferative responses, neutralizing antibody responses, and CD8+ antiviral responses, may have played a role in mediating protection as well.

Previous work from Shiver and colleagues had demonstrated impressive protection against SHIV 89.6p challenge

in macaques immunized with a recombinant replication-defective adenovirus vector expressing SIV Gag either alone or when given following DNA priming. In this initial study, to facilitate evaluation of cellular immune responses using MHC tetramers, all macaques were selected to express a specific MHC class I allele (Mamu-A*01). Although these results were clearly encouraging, questions were raised as to whether they could be replicated using other challenge stocks and in animals with a more diverse MHC background. In a late-breaker abstract (Abstract 851b), Shiver presented follow-up studies in 2 groups of macaques to address these questions. The first study involved Mamu-A*01-negative macaques that were immunized with adenovirus vectors encoding either SIV Gag, a heterologous envelope (JRFL), a homologous envelope (89.6p), or the combination of Gag and the heterologous envelope. Following intravenous challenge with SHIV 89.6p, immunized animals had at least a 100-fold decrease in set point viremia, and combined immunization with adenovirus vectors expressing Gag and Env appeared to give better protection than that obtained with either antigen alone, reinforcing observations previously made by the Robinson laboratory at Emory. Interestingly, the level of protection observed in this experiment appeared to be less complete than that observed in previous Mamu-A*01-positive vaccines. The second study involved immunization of Mamu-A*01-positive and -negative macaques with an adenovirus SIV-Gag vector with or without DNA priming. Following an intrarectal challenge with the pathogenic SIVmac239 virus, Mamu-A*01-positive animals that received DNA priming and the adenovirus Gag boost had a significant 10- to 30-fold reduction in plasma viremia at 150 days compared with controls. However, this effect was not observed in Mamu-A*01-positive animals that received adenovirus alone or in Mamu-A*01-negative animals that received the DNA prime/adenovirus-boost regimen. Although the demonstration of a significant protective effect on challenge with a pathogenic SIVmac strain in Mamu-A*01-positive animals is encouraging, the absence of a clear significant effect

in Mamu-A*01-negative animals is concerning. These results further illustrate the difficulties in interpreting challenge data derived from nonhuman primate models and raise numerous questions. Is SIVmac239 too rigorous a challenge? Which macaque model will ultimately prove to be the best predictor of efficacy in human clinical trials? Answers to these questions will be ultimately dependent on results from phase 3 clinical trials involving approaches able to induce potent cellular immune responses and are thus at least 4 to 7 years away.

Nonhuman Primate Studies: The Bad News

Even more grounds for caution was provided by oral presentations from Barouch and Staprans. Barouch (Abstract 76) analyzed the frequency of escape from CTL surveillance in vaccinated macaques. These studies were prompted in part by a report that appeared last year that described escape in a dominant CTL epitope in a single DNA-vaccinated animal that had been challenged with SHIV 89.6p. In the present study, Barouch described breakthrough viremia and disease progression in 3 of 4 Mamu-A*01-positive macaques that had been vaccinated with a DNA *gag* vaccine and challenged with the pathogenic SIV stock SIVsmE660. Emergence of CTL escape mutants in multiple epitopes correlated with decreases in epitope-specific responses as determined by MHC tetramers, increased viremia, and CD4+ T-cell depletion. These results reinforce the frequent occurrence of CTL escape in SIV-infected macaques previously described by other groups and suggest that initial gains in decreasing plasma viremia by vaccination may be lost over time due to viral escape.

Over the past several years, several investigators have raised the question of whether an ineffective AIDS vaccine might in fact worsen disease progression, either by production of antibodies that enhance instead of neutralize viral replication or by induction of virus-specific CD4+ T-cell responses that would serve to facilitate HIV replication. Although there has fortunately been lit-

tle evidence to date for such an in vivo effect in macaque studies, Staprans (Abstract 80) described results of a study that raised a concern in this regard. In this study, macaques were immunized either with a live attenuated varicella-zoster virus (VZV-OKA) expressing the SIVsmH4 envelope or with the parental VZV-OKA strain. In immunized animals, the authors observed relatively disappointing SIV-specific immune responses, which consisted primarily of virus-binding antibodies and limited CTL responses but no neutralizing antibodies. After a prolonged rest, animals were reboosted and challenged rectally with SIVsmE660. A dramatic difference was observed between the groups. Animals that had been vaccinated with the VZV-OKA envelope vaccine developed significantly higher levels of plasma viremia and more rapid CD4+ T-cell depletion compared with controls immunized with the unmodified VZV-OKA. Increased rates of viral replication were correlated with increased percentages of proliferating CD4+ T cells (as assessed by expression of the proliferation marker Ki67) 3 days after challenge, although SIV-specific CD4+ T-cell responses were not directly measured. These results raise the concerning possibility that induction of virus-specific CD4+ T-cell responses in the absence of other effective immune responses might accelerate instead of impede disease progression. However, several significant caveats should be noted. There was no direct measurement of virus-specific CD4+ responses after challenge. Also, the spontaneous suppression of viremia in the control animals challenged with the pathogenic SIVsmE660 strain is unusual; most groups have observed significantly higher levels of viremia with this stock. Although this study raises important concerns, these results will have to be replicated with more intensive analysis of virus-specific immune responses before any hard conclusions regarding potential adverse effects of vaccination can be drawn.

Human Clinical Trials

In contrast to the broad array of novel findings presented from nonhuman pri-

mate AIDS vaccine studies, progress reported in human clinical trials was more modest. (The much-anticipated results from the phase 3 VaxGen study were not reported until 10 days after the close of the conference). Hammer (Abstract 108) provided a broad overview of the current status of HIV vaccine clinical trials. After detailing the numerous challenges facing the development and testing of HIV/AIDS vaccines, he highlighted results from several recently completed or ongoing vaccine trials. HVTN 203 examined the immunogenicity of a third-generation canarypox vector (vCP1452) that expressed HIV-1 Gag and Env. Induction of CTL responses to Gag or Env was disappointingly infrequent, occurring in only 13% and 7% of vaccines, respectively. This relative lack of immunogenicity was significantly below the milestone that had been set for progression of this approach to phase 3 clinical trials in the United States. Results from the ongoing Merck vaccine trials have been more encouraging. At the highest doses studied, 42% of subjects receiving a DNA *gag* vaccine had developed ELISPOT responses to Gag after 4 immunizations. Initial trials of the Merck replication-defective adenovirus vector expressing Gag are still being analyzed, but preliminary results indicate that 78% of subjects receiving the highest dose of adenovirus had positive ELISPOT responses after 2 doses. The National Institutes of Health Vaccine Research Center is pursuing a similar approach utilizing DNA priming with a recombinant adenovirus vector boost and will utilize as immunogens a clade B Gag/Pol/Nef given in combination with 3 different envelopes (clades A, B, and C). Although results from phase 3 efficacy trials of these approaches remain several years away, these early results demonstrating relatively robust induction of cellular immune responses offer some grounds for optimism.

As one approach to try to increase the immunogenicity of canarypox vectors, Goepfert and colleagues (Abstract 82) examined whether increasing the dose of the recombinant canarypox vector vCP1452 up to 10^8 50% tissue culture infectious dose (TCID₅₀) could improve induction of cellular immune responses. While previous lower doses

of this vector have been relatively well-tolerated, at the increased dose, the vast majority of subjects (>85%) developed both local and systemic symptoms. No improvement in interferon- γ ELISPOT responses was observed, and the overall frequency of responding subjects remained disappointingly low (18–24%).

Novel Approaches

Although vaccinology in general (and the study of adjuvants in particular) has been criticized frequently for being a largely empirical science, numerous poster presentations highlighted novel approaches utilizing the rational use of molecular adjuvants. Huang et al (Abstract 396) analyzed the ability of the NKT cell ligand α -galactosylceramide (α -GalCer) to serve as an adjuvant for DNA vaccination in mice. When α -GalCer was administered in combination with suboptimal doses of DNA vaccines, a 5-fold increase in CD8+ T-cell responses and a 2-fold increase in CD4+ T-cell responses was observed. An alternative approach to increasing immunogenicity was presented by Zhang and colleagues (Abstract 397), who created a fusion protein of CTLA4 and SIV Gag in order to target B7-expressing, antigen-presenting cells such as dendritic cells. Immunization with CTLA4/SIV Gag resulted in a 100-fold increase in Gag-specific antibody responses. A complementary approach was described by Ross and colleagues (Abstract 400), in which DNA constructs were created that encoded fusions of Env with C3d. C3d is a component of the complement cascade that specifically binds to complement receptor 2 on B cells and therefore targets C3d fusion proteins to B cells. DNA immunizations with the Env-C3d complex resulted in 10^3 - to 10^5 -fold increases in antibody titers compared with results of immunizations with DNA expressing Env alone. Although it will be important to see whether these encouraging results in mice can be replicated in primates, the proliferation of these studies suggests that rational approaches to optimizing immunogenicity may ultimately bear fruit over the next several years.

A third approach to antigen targeting was reported by Rosati (Abstract 448).

Codon-optimized SIV *gag* and *env* genes were fused to either the secreted chemokine MCP-3 (which directs proteins to the secretory pathway) or to the β -catenin peptide (which directs proteins to the proteasomal degradation pathway). Immunization of animals with the MCP/*gag/env* construct resulted in increased humoral responses. Macaques immunized with a combination of the unmodified MCP-3 and β -catenin fusion proteins showed a broad range of immune responses, including CD4+ T-cell, CTL, and antibody responses. After mucosal challenge with SIVmac251, vaccinated animals had significantly lower viral loads compared with those of naive animals, a difference that was maintained out to 30 weeks postchallenge.

Although DNA vaccination has proved to be a very effective means to induce cellular immune responses in rodents, results have been less impressive in macaques and even more disappointing in humans. Whether these differences reflect true species-specific differences or largely differences in the modes of administration is not clear. For instance, when expressed on a milligram per kilogram basis, doses of DNA administered to mice are significantly higher than those used in comparable studies in nonhuman primates or humans. To address the issue of whether modifications in the method of administration could affect immunogenicity, Gardiner and colleagues (Abstract 452) analyzed whether division of a constant amount of DNA among 1, 2, 3, or 4 limbs in mice altered cellular immune responses as determined by interferon- γ ELISPOT assays. Dividing a 20 μ g DNA dose among 4 extremities significantly increased cellular immune responses to a level similar to that provided by a single 100 mg dose of DNA in one leg. These data suggest that very basic issues regarding amount of DNA and number of immunization sites need to be more carefully addressed in nonhuman primate and human studies.

Although live attenuated SIV vaccines have proved to be one of the most effective means to induce protection against infection with pathogenic SIV isolates in nonhuman primates, safety concerns have precluded pro-

gression of this approach to human clinical trials. Two presentations analyzed the use of single-cycle SIV or HIV constructs (essentially nonreplicating lentiviral vectors) as a potential vaccination approach. Evans (Abstract 78) described results of immunization with a single-cycle SIV generated by introduction of a nucleotide substitution in the *gag/pol* frameshift site and providing Gag-Pol expression in trans. A single intravenous injection of single-cycle SIV in macaques resulted in peak levels of 10^4 to 10^5 copies/mL of plasma viremia that rapidly decayed to undetectable levels and low but significant SIV Gag-specific responses detected by ELISPOT and MHC tetramers. A second inoculation resulted in significantly lower levels of viremia (presumably reflecting the effect of preexisting SIV-specific immune responses) and transient boosting of SIV-specific cellular immune responses. Tung (Abstract 79) described a complementary approach involving production of replication-defective, vesicular stomatitis virus G (VSV-G)-pseudotyped HIV vectors with a truncation of the *pol* gene. Systemic and mucosal inoculation resulted in virus-binding antibodies and virus-specific CTL responses. Challenge of animals with the SHIVku-2 strain resulted in lower levels of viremia in vaccines as compared with levels in a concurrent control animal. These data support further study of single-cycle lentiviruses as a vaccine modality in nonhuman primates, but it remains to be seen whether this approach will have sufficient advantages to outweigh the safety concerns regarding the use of an integrating virus for a preventive vaccine.

Evaluation of cell-mediated immune responses in HIV/AIDS vaccine trials has increasingly relied upon assessment of secretion of cytokines such as interferon- γ using ELISPOT and intracellular cytokine staining assays. In many instances, whether secretion of interferon- γ accurately reflects the majority of antigen-specific cells has not been well-addressed. Using 12-color flow cytometry, De Rosa (Abstract 405) analyzed the range of cytokines secreted by antigen-specific cells following either tetanus or hepatitis B immunization. Remarkably, cells secreting interferon- γ represented only a small minority of the antigen-

specific response. With fresh cells, IL-2 was the dominant response, whereas with frozen cells, cells producing MIP-1 β were the dominant response, exceeding the frequency of cells secreting interferon- γ by 10-fold or more. These provocative results will need to be confirmed with other antigens, but if validated, would have significant implications for evaluation of antigen-specific responses in both vaccine trials and HIV/AIDS pathogenesis studies.

Therapeutic Vaccination

Since immune control of HIV replication is not achieved in most subjects, despite the presence of a readily detectable HIV-specific immune response, could therapeutic augmentation of HIV-specific immunity by vaccination potentiate existing immune responses and generate novel responses sufficient to induce immune control?

Walker (Abstract 164) presented results of a pilot vaccine trial using a whole inactivated Env-depleted vaccine of clade A/G Zairian isolate in an adjuvant to immunize chronic HIV-infected subjects. Using stringent criteria for augmentation of immunity (5-fold increase

in stimulation index and at least an increase of 500 counts per minute in at least 2 antigens on numerous occasions), a significant increase in HIV-specific proliferative responses, but not CD8+ T-cell responses, was observed in vaccinated subjects. Unfortunately, this augmentation was not associated with any clinical benefit. Several presentations (Abstracts 60, 61, and 62) discussed results of therapeutic vaccination using modified vaccinia Ankara (MVA) or recombinant canarypox-HIV (ALVAC) vectors in HIV-infected subjects on HAART. In a phase 1 trial of a MVA vector expressing the HIV-1 *nef* gene, administration of 3 doses of the vaccine to 14 subjects on HAART with plasma HIV-1 RNA levels below 50 copies/mL and CD4+ counts above 400 cells/ μ L induced CD8+ and CD4+ T cells to recognize new epitopes in 10 of 16 subjects (Harrer, Abstract 60). Interruption of HAART resulted in rebound viremia in all subjects, although the plasma HIV-1 RNA levels remained lower than pre-HAART levels in 7 of 14 vaccinated subjects. In a randomized control study (Levy, Abstract 62), 70 patients on HAART for at least 1 year were randomized to continue HAART alone (n = 37)

or to receive HAART in combination with vaccination with ALVAC HIV vCP1433 (a recombinant canarypox expressing HIV-1 *env*, *gag*, and *nef* genes) and Lipo-6T (consisting of 6 peptides in Nef, Gag, Env, and Pol and a helper TT epitope). Five doses were administered at monthly intervals. Following the last dose, the vaccinated group had a significantly higher frequency of subjects with detectable proliferative responses to p24 antigen. Four weeks after stopping HAART, 2 of 37 patients in the control group and 8 of 33 patients in the vaccinated group had controlled viremia. However, this effect was not sustained. At present, although some boosting of immune responses has been observed following therapeutic vaccination, clear clinical benefits have yet to be realized.

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