Highlights From the 9th Retrovirus Conference
HIV Pathogenesis and Vaccine Development

R. Paul Johnson, MD, and Amitinder Kaur, MD

Introduction

Presentations at the 9th Conference on Retroviruses and Opportunistic Infections, held in Seattle, February 24 to 28, 2002, continued to shed light on the complex interactions between HIV and the immune system. Major topics of discussion included natural hosts of simian immunodeficiency virus (SIV) infection, the dynamics of T lymphocytes, the myriad mechanisms utilized by HIV to avoid host immune responses, and the central role of dendritic cells in HIV pathogenesis. Promising new data emerged from initial results of a candidate HIV-1 vaccine able to induce more potent cellular immune responses, raising hopes for the new generation of vaccines currently entering human clinical trials.

Natural Hosts of SIV Infection

There is now little doubt that the global pandemic of HIV infection originated as a result of cross-species transmission from nonhuman primates infected with SIV; thus analysis of the biology of nonhuman primate lentiviruses may offer important insights on AIDS pathogenesis and the potential for new human lentiviral infections. In the Bernard Fields Memorial Lecture, Hahn provided an interim analysis of one of the most comprehensive ongoing surveys of the extent of SIV infection in nonhuman primates in Africa (Abstract L1). Analysis of

Dr Johnson is Associate Professor of Medicine at the Division of Immunology, New England Regional Primate Research Center, Harvard Medical School, in Southborough, Mass, and the Infectious Disease Unit and Partners AIDS Research Center, Massachusetts General Hospital, Harvard Medical School, in Charlestown, Mass. Dr Kaur is Instructor in Medicine at the Division of Immunology, New England Regional Primate Research Center.

specimens obtained from bushmeat (ie, monkeys killed for food) and pet monkeys revealed that antibodies to SIV were present in 13 of 16 species sampled. These results documented infection in 4 species not previously known to be infected with SIV, and molecular analysis of viral sequences amplified by polymerase chain reaction identified 5 new SIV lineages. The overall seroprevalence in all species analyzed was 20% and exceeded 90% in some sexually active nonhuman primate adults. These data dramatically reinforce the existence of a diverse reservoir of SIV strains in nonhuman primates and underscore the potential for new introductions of primate lentiviruses into the human population.

From evidence primarily obtained from captive animals, chimpanzees infected with SIV_{cpz} have long been suspected of being the primary origin of the HIV-1 epidemic. However, there have been no solid data on the prevalence of SIV infection in chimpanzees in the wild, an elusive goal that has been frustrated by the difficulties inherent in trying to obtain samples from an endangered and reclusive species. Armed with improved techniques to document SIV infection in the urine and feces from chimpanzees living in East Africa and West Africa, Hahn was able to confirm the existence of SIV-infected chimps in the wild, although the seroprevalence was surprisingly low. Only 1 in 152 animals initially tested was found to be infected with SIV_{cpz}. Interestingly, the one chimpanzee found to be SIV-infected in the initial survey was from a troop in Tanzania's Gombe National Park that has been studied for decades by Goodall and her associates. Expanded testing of chimpanzees in Gombe identified 3 additional animals infected with SIV.

These new data extend the boundary of SIV_{cpz} infection eastward into Tanzania and also document that 2 different chimpanzee subspecies *(Pan troglodytes* and *Pan troglodytes schweinfurthii)* can serve as reservoirs of infection. The relatively infrequent infection of chimpanzees with SIV in the wild suggests that chimpanzees may have been more recently infected by another nonhuman primate species, a possibility reinforced by recent data obtained from a survey of greater spot-nosed monkeys (Courgnaud et al, Abstract LB1). Genetic analysis of 2 complete sequences of SIV_{gsn} genomes revealed a close relationship to SIV_{cpz} in envelope, suggesting the possibility that SIV_{cpz} may have been derived from infection of chimpanzees by greater spot-nosed monkeys.

The fact that natural hosts of primate lentiviruses do not develop AIDS has prompted an intensive effort into defining the virologic and immunologic mechanisms that underlie the lack of disease. One of the best-studied natural

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hosts of SIV is the sooty mangabey, a species native to West Africa. Despite SIV plasma viral levels of 10^5 to 10^7 copies/mL, sooty mangabeys remain immunologically normal. However, cross-species transmission of the sooty mangabey virus (SIV_{sm}) into people (resulting in HIV-2) or Asian macaques (resulting in SIV_{mac}, the most widely used virus for nonhuman primate studies) induces AIDS.

Feinberg described recent efforts to better understand host-virus interactions in sooty mangabeys (Abstract S24). Debunking the theory that the lack of pathogenicity in this species may be due to a lack of cytopathic effect of the virus in vivo, he noted that administration of the reverse transcriptase

inhibitor PMPA (now approved for use in humans as tenofovir disoproxil fumarate [tenofovir]) induced a rapid decay of plasma viral levels similar to that observed in HIV-infected people and SIV-infected macaques, a result that strongly suggests the rapid death of sooty mangabey SIV-infected CD4+ T lymphocytes in vivo. Despite the loss of infected CD4+ T lymphocytes, the key to the sooty mangabey's ability to maintain normal immune function appears to be its ability to avoid widespread immune activation and the associated increased rates of CD4+ and CD8+ T-cell apoptosis. The precise mechanisms responsible for the lack of generalized immune activation are not clear, but may include a combination of decreased SIV-specific immune responses and lower rates of indirect cell activation and death (ie, death of cells not directly infected with SIV).

This latter point was reinforced by a presentation from Kaur and colleagues (Abstract 22), who analyzed T-cell turnover in sooty mangabeys using the nucleoside analogue bromodeoxyuridine (BrdU). Interestingly, despite the rapid turnover of SIV-infected CD4+ T cells described above, no differences in uptake or decay rates of BrdU-labeled CD4+ or CD8+ T lymphocytes were observed between SIV-infected and uninfected mangabeys. This result reinforces the concept that in this natural host of SIV infection, the virus fails to induce indirect mechanisms of accelerated T-cell activation and destruction that ultimately result in CD4+ T-cell depletion.

T-Cell Dynamics

Although loss of CD4+ T lymphocytes is a hallmark feature of HIV disease, the precise mechanisms that lead to depletion of CD4+ T cells remain incompletely understood and have often provoked contentious debate. In recent years, there has been increasing consensus that the rates of CD4+ and CD8+ T-cell turnover in HIV-infected people and SIV-infected macaques are increased by 2- to 5-fold compared with uninfected controls. However, the effects of antiretroviral therapy on T-cell turnover and the relative roles of increased CD4+ T-cell destruction and impaired T-cell produc-

tion in CD4+ T-cell depletion have remained controversial. A conference symposium on T-cell turnover and thymic function brought together several of the leading scientists in this field to attempt to resolve these issues.

Perelson (Abstract S9) highlighted the utility of mathematical modeling in the analysis of T-cell turnover data generated in HIV-1-infected subjects who underwent a 7-day infusion of deuterated (^{2}H) glucose. Accurate modeling of CD4+ and CD8+ T-cell labeling and decay kinetics required the use of a 2-compartment model including a source of unlabeled lymphocytes. In this model, proliferation and death rates of CD4+ T cells in untreated subjects were increased by 6.3- and 2.9-fold, respectively, compared with normal controls. The calculated mean proliferation rate of CD8+ T cells was 7.7-fold higher in HIV-infected subjects, although the death rate of CD8+ T cells was not increased. Institution of highly active antiretroviral therapy (HAART) resulted in significant decreases in CD4+ and CD8+ lymphocyte proliferation and death rates after 5 to 11 weeks.

Similar data were presented by Kovacs (Abstract S10), who studied rates of labeling and decay of BrdU-labeled lymphocytes in 17 HIV-infected volunteers. Mathematical modeling of the decay of BrdU-labeled cells supported the existence of 2 populations of lymphocytes, one rapidly proliferating, the other slowly proliferating. The magnitude of plasma HIV RNA levels correlated with the size of the proliferating pool but, interestingly, not with the death rate of lymphocytes. The major effect of HAART was to decrease the size of the proliferating pool. Data supporting the existence of 2 pools of short-lived and long-lived T cells were also provided by Hellerstein and colleagues using metabolic labeling with deuterated glucose or water (Abstract 102). Hellerstein's group also observed an expansion of the short-lived, rapidly turning over lymphocyte pool in untreated HIV infection.

Miedema and colleagues (Abstract S11) addressed the multiple perturbations of T-cell homeostasis in HIV-infected patients, which include altered distribution of cells between peripheral and lymphoid compartments, increased

rates of lymphocyte turnover driven by immune activation, and increased turnover of naive T cells. HIV-1 strains able to induce syncytia and to utilize the CXCR4 coreceptor (SI/X4 strains) were uniquely able to infect and lyse naive CD4+ T cells. McCune (Abstract S12) emphasized the interplay between the thymus and the peripheral T-lymphocyte pool in attempting to maintain T-cell homeostasis in the face of HIV-mediated CD4+ T-cell destruction. Interleukin (IL)-7 is one molecule that appears to mediate feedback between the peripheral lymphoid pool and the thymus, serving to increase naive T-cell production. However, therapeutic use of this molecule is likely to be limited by its ability to increase HIV replication, and so recent studies are beginning to examine the utility of growth hormone, which has increased thymic output in rodent models. Preliminary results from 2 pilot studies (Napolitano et al, Abstract 511-M; Pires et al, Abstract 513-M) suggested beneficial effects of growth hormone on thymic tissue size and circulating naive T cells in HIV-infected subjects, but more detailed clinical studies will be required to more rigorously evaluate the utility of growth hormone in accelerating the production of naive T cells.

Taken together, these presentations highlighted the fact that untreated HIV infection results in accelerated peripheral destruction of CD4+ T cells and an associated increase in CD4+ T-cell production. CD4+ T-cell depletion is therefore likely to result from an imbalance in these rates of T-cell production and death, rather than an absolute decrease in the rate of CD4+ T-cell production. There was also a consensus regarding the ability of HAART to decrease rates of CD4+ T-cell proliferation and death, most likely by decreasing the size of the short-lived, rapidly turning over pool of T lymphocytes. These presentations also represent a shift in the debate from the magnitude of T-cell turnover in HIV infection to a renewed focus on how HIV infection perturbs T-cell homeostasis, a topic that will probably provoke ongoing controversy for years to come.

The contribution of the thymus in maintaining T-cell homeostasis was subsequently examined by Arron and colleagues (Abstract 101). Based on the decay of T-cell receptor excision circles

(TRECs) following thymectomy of juvenile rhesus macaques, the authors estimated a daily thymic production rate of approximately 10^7 T cells per day, representing about 0.01% of all T cells in a macaque. Although SIV infection reduced TREC levels in both thymectomized animals and sham-operated controls, no differences in disease progression or lymphocyte naive and memory subsets were observed between these groups. These data reinforce the notion that peripheral destruction of CD4+ T lymphocytes, rather than reduced thymic output, plays a major role in inducing CD4+ T-cell depletion in untreated SIV (and presumably HIV) infection. However, they still leave open the question of whether de novo production of naive CD4+ T cells in the setting of HAART may play a beneficial role in restoring normal immune function.

Immune Responses to HIV

One of the significant challenges in the development of an HIV vaccine has been the difficulty in generating potent and broadly neutralizing antibody responses. In his plenary talk, Wyatt (Kwong et al, Abstract L4) provided a detailed analysis of the mechanisms utilized by HIV to evade neutralizing antibody responses. A variety of structural characteristics of the HIV envelope facilitate immune evasion, including its remarkable degree of glycosylation, conformational changes of the envelope that occur during virus binding, steric barriers, shedding of the viral envelope, and the high mutation rate in the variable domains. Wyatt highlighted in particular the recessed nature of the CD4 binding site of gp120, which represents an exceptionally difficult site for antibodies to block. Binding of CD4 to gp120 induces a conformational change in the envelope protein that may be mimicked by structure-based mutants. Use of these conformationally fixed glycoproteins may prove to be effective in inducing more broadly neutralizing antibody responses.

The analysis of neutralizing antibody responses to HIV has been problematic, plagued by considerable variations in techniques and the difficulty of generating a reproducible source of autologous virus. Using a novel approach involving neutralization of recombinant HIV strains expressing envelope proteins derived from patient samples, Richman and colleagues (Abstract LB5) described the evaluation of neutralizing antibody responses to autologous virus sequences in a cohort of 15 subjects with primary HIV infection. Although 13 of 15 subjects generated strong neutralizing antibody responses to autologous virus sequences, there was rapid evolution of envelope to escape the concurrent neutralizing antibody response. In other words, antibody responses at 25 weeks after infection were able to neutralize virus isolated at the time of infection but not virus isolated at later time points. Reproducing the finding of "original antigenic sin" previously observed in

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Most individuals can produce antibodies able to neutralize virus early in infection

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influenza virus infection, neutralizing antibody titers able to neutralize the initial virus sequences continued to rise with time, despite the inability to neutralize concurrent HIV-1 isolates. These data provide compelling evidence that most individuals are capable of producing antibodies able to neutralize virus at time points early in infection, but that continued evolution of the virus results in a series of mutant viruses that are not efficiently neutralized by autologous antibody responses.

A leading explanation for the relatively weak CD4+ T-cell response to HIV has been that these cells are selectively infected by HIV-infected antigen-presenting cells, yet little direct evidence has been presented to support this hypothesis. Douek and colleagues (Abstract LB7; *Nature, 2002*) analyzed the frequency of HIV infection in HIV-specific CD4+ T cells identified by intracellular cytokine staining. Compared with cytomegalovirus-specific CD4+ T cells or total memory CD4+ T cells, HIV-specific CD4+ T cells were preferentially infected

by HIV. However, only a small fraction of HIV-specific CD4+ T cells were infected (0.01%-1%), an interesting finding that suggests that a substantial proportion of virus-specific CD4+ T cells are able to proliferate after they encounter antigen-presenting cells expressing HIV epitopes, yet escape infection. In individuals undergoing structured treatment interruptions (STIs), the increase in HIV infection of CD4+ T cells occurred predominantly in HIV-specific CD4+ T cells. In one such subject, up to 50% of all HIV-infected cells were HIV-specific. These data help to document one mechanism for the loss of HIV-specific CD4+ T-cell responses and also raise questions about whether STIs in chronically infected subjects will ultimately prove beneficial in boosting virus-specific T-cell responses.

An alternative explanation for relatively weak HIV-specific CD4+ T-cell responses may lie in the intrinsic immunogenicity of the envelope protein. Grundner and colleagues described the results of immunization of mice and rabbits with recombinant core gp120 proteins from which V1 and V2 and portions of the N- and C-termini had been deleted (Abstract 105). Antibody responses to the core subunit of 2 primary isolates, but not the laboratory isolate HXBc2, were relatively weak, but could be boosted by the addition of a heterologous T helper epitope. Similar results were obtained with deglycosylated envelope proteins. Although it will be important to extend this analysis to macaques and humans, these results suggest that the envelope sequences of primary isolates may have evolved to minimize recognition by CD4+ T cells, and that inclusion of heterologous T helper sequences may enhance vaccine-induced responses against gp120.

HIV-specific CD4+ T-cell responses were also the subject of a number of poster presentations. Work from numerous laboratories clearly documented that HIV-specific CD4+ T-cell responses are present during acute and chronic infection, but are generally not detected using standard proliferation assays (Malhotra et al, Abstract 203-T; Malhotra et al, Abstract 204-T; Palmer et al, Abstract 208-T; Yassine-Diab et al, Abstract 215-T; and Iyasere et al, Abstract 216-T). This phenomenon was elegantly demon-

strated by Yassine-Diab and colleagues (Abstract 215-T), who identified HIV-specific CD4+ T cells using human leukocyte antigen (HLA) class II tetramers and showed that fluorescently labeled cells failed to divide following in vitro stimulation.

Research on CD8+ T-cell responses to HIV and SIV over the past several years has increasingly focused on defining mechanisms used by these viruses to evade a relatively vigorous cytolytic T-lymphocyte (CTL) response. O'Connor and colleagues (Abstract 98; *Nat Med,* 2002) described the results of a comprehensive study analyzing complete viral genomes of 21 macaques infected with the SIV$_{mac239}$ molecular clone. By 4 weeks after infection, escape from at least 1 CTL epitope had occurred in 19 of 21 animals. Escape mutations appeared to occur selectively in high-avidity CTL epitopes. Together with the data on neutralizing antibody responses presented by Richman and colleagues (Abstract LB5), these presentations dramatically underscored the role that viral mutation plays in evading both humoral and cellular immune responses.

A role for replication senescence of HIV-specific CD8+ T cells was proposed by Brenchley and colleagues (Abstract 217-T). Using a combination of intracellular cytokine staining and fluorescent labeling to track cell division in vitro, these authors demonstrated that HIV-specific CD8+ T cells expressing CD57 were markedly impaired in their ability to proliferate following antigenic stimulation. Evidence for replicative senescence was provided by the fact that these cells expressed lower levels of TRECs.

Immune Reconstitution

In a plenary talk, Autran (Abstract L3) highlighted the advances that have been made in understanding the complex effects of HAART on immune function. The initial phase of CD4+ T-cell recovery after institution of HAART consists primarily of peripheral memory T cells. Thereafter, a slower, sustained increase in naive CD4+ T cells is observed, which most likely reflects de novo production from the thymus. A balanced recovery of both naive and memory T cells is necessary for restoration of a diverse T-cell repertoire able to maintain T-cell memory and also to respond to new pathogens.

However, in spite of these beneficial effects of HAART, reconstitution of HIV-specific immune responses remains incomplete. HIV-specific CD4+ T-cell responses are not found in most chronically infected individuals after HAART (at least as detected by standard proliferation assays; see discussion in previous section). Moreover, HIV-specific CD8+ T-cell responses decline with the loss of antigenic stimulation. In contrast to patients with acute HIV infection, efforts to use STIs to boost host control of viral replication in chronically infected individuals have generally been unsuccessful, and have also resulted in a loss of HIV-specific proliferative responses. These results have placed a renewed emphasis on therapeutic vaccination. An ongoing European trial is examining whether vaccination with a highly attenuated poxvirus (ALVAC vCP1452), inactivated HIV (HIV Immunogen [Remune, Immune Response Corporation, Carlsbad, Calif]), or both, in combination with HAART, will be able to induce stronger immune responses that may be able to better contain HIV replication.

A key question for immune reconstitution is whether HIV-infected individuals can mount cellular immune responses to new epitopes. Altfeld and colleagues (Abstract 107) addressed this question in the context of patients undergoing STIs. The investigators examined ELISPOT responses in peripheral blood and lymph nodes to a panel of multiple epitopes selected on the basis of HLA type. The characteristic expansion of HIV-specific CD8+ T cells in peripheral blood seen with the rebound of viremia occurred largely as a result of the expansion of preexisting HIV-specific CD8+ T cells in lymph nodes rather than as an induction of new responses. Future studies will need to address this question in the context of therapeutic vaccination as well.

Intermittent infusions of IL-2 in combination with antiretroviral therapy have been well-documented as leading to sustained increases in CD4+ T-cell counts. However, detailed information on the mechanisms underlying this expansion of CD4+ T cells has been lacking. Analysis by Kovacs and colleagues of lymphocyte proliferation using deuterium labeling revealed that patients receiving IL-2 had substantial proliferation of both CD4+ and CD8+ T lymphocytes and, surprisingly, a markedly prolonged survival of CD4+ T cells (Abstract 103). This latter finding may well explain why relatively infrequent IL-2 infusions may lead to prolonged increases in CD4+ T cell counts. A companion presentation by Sereti and colleagues (Abstract 104) demonstrated that the expansion of CD4+ T cells following IL-2 therapy is predominantly composed of a population of CD25+ T cells that expresses some naive markers but has a lower content of TRECs, and therefore most likely represents an expansion of peripheral T cells rather than recent thymic emigrants.

Dendritic Cells and Mucosal Immunology

The past few years have seen an explosion of research into the multifaceted properties of dendritic cells (DCs) and the critical role they play in HIV transmission, replication, and efficient induction of antigen-specific CD4+ and CD8+ T-cell responses. In a plenary talk, Pope (Abstract L2) provided a comprehensive overview of the current state of research on interactions between DCs and T cells in the context of HIV and SIV infection.

There are 2 phenotypically distinct types of DCs currently known. Myeloid DCs are CD11c+, express toll-like receptors (TLRs) 2 and 4, and respond to IL-4, granulocyte macrophage colony-stimulating factor (GM-CSF) and type I interferons. Plasmacytoid dendritic cells are CD11c–, TLR 9+, and are unique in expressing the IL-3 receptor CD123, which makes them responsive to IL-3. When stimulated, as with an initial encounter with a pathogen, plasmacytoid DCs secrete abundant type I interferons, which in turn can stimulate myeloid DC and thus provide a bridge between innate and pathogen-specific immunity. Unlike plasmacytoid DCs, which are present only in the blood and lymph nodes, myeloid DCs are also abundantly present at body surfaces, including mucosal sites. Since mucosal transmission is the predominant mode of HIV spread in the majority of infected

individuals, myeloid DCs are the first cells encountered by the virus. Since early events are important determinants of the ultimate outcome of HIV infection, the initial interactions between virus and DCs are an important area of research.

Previous work by Pope had demonstrated that in the absence of Nef, SIV replication in immature DCs is severely curtailed. She now presented data showing that the presence of Nef in immature DCs induces production of the chemokines MIP-1α, MIP-1β, and RANTES, and the cytokine IL-12. Further, Nef also enhances the ability of immature DCs to induce syngeneic T-cell proliferation. These effects of Nef may modulate the extent of activated T-cell recruitment and viral replication at the site of entry and thereby influence pathogenicity.

Several presentations addressed the expression of dendritic cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN), an HIV/SIV adhesion molecule, and its closely related homologue, DC-SIGNR, on DCs in vivo at different anatomical sites. DC-SIGN is a C-type lectin primarily expressed on myeloid DCs, which binds with high affinity to gp120 of HIV and SIV. Once virus is bound to DC-SIGN, it can be transmitted efficiently to susceptible T cells expressing CD4 and the coreceptors CCR5 or CXCR4.

Iwasaki (Jameson et al, Abstract 28) presented data on immunohistochemical analysis of human and rhesus macaque intestinal and genital tissue using monoclonal antibodies to DC-SIGN and DC-SIGNR. DC-SIGN was not expressed on plasmacytoid DCs. In the ileum, clusters of DC-SIGN+ cells that were CD11c- and CD123-negative were detected subepithelially in the dome and interfollicular regions of Peyer's patches. DC-SIGNR expression was observed on endothelial cells in the ileum. Myeloid DCs expressing high levels of DC-SIGN were present throughout the entire thickness of human rectal mucosa, and DC-SIGN+ cells co-expressing CD4 and CCR5 were localized just beneath the luminal epithelium. In contrast, Langerhans' cells (LCs) in the vaginal epithelium did not express DC-SIGN. Instead, DC-SIGN was moderately expressed in subepithelial DCs in the lamina propria of the vaginal mucosa.

Similar findings were observed in rhesus macaques. These results provide a physical basis for the observation of a higher risk of HIV transmission via rectal as compared to vaginal intercourse, and suggest that the proximity of DCs expressing DC-SIGN to luminal epithelium is important for mucosal transmission of primate lentiviruses.

Expression of DC-SIGN, along with CD4 and coreceptors, can also act in *cis* to promote HIV infection and replication in the DC-SIGN-expressing cells. Although DC-SIGN is almost exclusively detected in myeloid DCs, there are some reports of its presence in non-myeloid DC cells, most notably on a subset of alveolar and placental macrophages (Soilleux et al, Abstract 109), and in perivascular macrophages and micro-

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The similarities between human and macaque DCs open the way to exciting future studies

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glial cells in the brain (Shawver et al, Abstract 63). The expression of DC-SIGN on non-DCs may be one mechanism of expanding the tissue tropism of HIV or SIV in infected hosts. Further, molecules other than DC-SIGN may also mediate HIV transmission. This was highlighted by the finding that SIV can infect and replicate in macaque DCs in the presence of blocking antibodies to DC-SIGN (Pope, Abstract L2). The demonstration of the similarities between human and macaque DCs and the generation of macaque-specific reagents has opened the way to exciting future studies that can directly address the in vivo role of DCs in the early events of HIV transmission.

Although HIV contact with DCs clearly facilitates transmission and viral replication, DCs are also required for the generation of an effective anti-HIV immune response. Macaque DCs pulsed with infectious or inactivated SIV were

capable of eliciting interferon-γ secretion from SIV-specific T cells (Pope, Abstract L2). However, if HIV infection were to impair DC function, this could abrogate HIV-specific immunity and provide yet another mechanism of promoting HIV replication. Several investigators provided evidence that HIV infection can affect the number and function of myeloid and plasmacytoid DCs (Abstracts 99, 252-T, 253-T, 255-T). Both myeloid DCs and plasmacytoid DCs are decreased in individuals chronically infected with HIV-1 (Barron et al, Abstract 252-T; Jones et al, Abstract 253-T). Myeloid DCs from untreated HIV-1-infected individuals with high viral loads had an impaired ability to stimulate allogeneic T-cell proliferation in a mixed leukocyte reaction (Donaghy et al, Abstract 255-T).

Further, in one study (Loré et al, Abstract 99), mature DCs from subjects with acute HIV infection appeared to have impaired activation, as suggested by low levels of expression of CD80 and CD86 molecules, when compared to DCs isolated from individuals with acute Epstein-Barr virus infection. In all, these data suggest that impairment of DC function does occur in HIV infection and may contribute to the inability of the host to clear HIV infection.

Despite the central role that viral shedding from mucosal surfaces plays in transmission of HIV, there has been relatively little information on what cell types are predominantly responsible for release of HIV into mucosal secretions. Brodie and colleagues (Abstract 27) analyzed the distribution of HIV RNA and DNA in rectal lymphocytes, macrophages, and dendritic cells from men who were shedding HIV rectally. Although mucosal T lymphocytes were the most common cell expressing HIV RNA, HIV-infected macrophages had significantly higher levels of HIV RNA, especially in subjects with advanced disease. Rectal shedding of HIV was also detected in 6 subjects on HAART, 2 of whom had plasma HIV-1 RNA levels of less than 200 copies/mL at the time of study. These data suggest that macrophages may be a major source of viral shedding from mucosal sites, even in some patients on potent antiretroviral therapy with plasma HIV-1 RNA below detection levels.

Vaccines

In a plenary talk (Abstract L5), Emini presented data on the results of ongoing phase 1 human clinical trials with 2 candidate HIV vaccines, one a plasmid DNA, and the other a replication-defective adenoviral type 5 (Ad5) vector containing codon-optimized HIV-1 clade B *gag* DNA. The vaccines have been designed with the aim of inducing vigorous Gag-specific CD4+ and CD8+ T-lymphocyte responses. The plasmid HIV-1 *gag* DNA vaccine has been tested in 109 HIV-1-seronegative volunteers. Twenty-four were administered placebo, 42 received 1 mg DNA, and 43 received 5 mg of the DNA vaccine at 0, 4, 8, and 26 weeks. Weak interferon-γ ELISPOT responses were detected in less than 50% of individuals after 4 immunizations. Stronger and sustained interferon-γ ELISPOT responses were observed in 6 of 9 volunteers who received 3 doses of 10^8 viral particles of the Ad5-Gag vaccine. Cross-clade recognition of Gag from clades A and C HIV-1 was demonstrated in more than 75% of the responders, suggesting that this vaccine may be immunogenic across diverse populations. Subjects with preexisting high neutralizing antibody titers to Ad5 were less likely to develop Gag-specific immune responses following immunization. This barrier may be overcome by using higher doses of the Ad5-Gag vaccine, or using a DNA prime/Ad5 boost approach. Both of these modalities are currently being tested. Encouragingly, both vaccines were well tolerated and few minor adverse effects were reported.

Emini discussed a survey of HIV-1-infected subjects in the United States, Brazil, Thailand, and Malawi that showed a remarkable degree of similarity among diverse population groups in the major proteins targeted by anti-HIV-1 T-cell responses and in the magnitude of T-cell responses. A high degree of cross-clade recognition for T-cell responses against HIV-1 Gag and Nef was observed between clades A, B, and C. These results are encouraging and suggest, as mentioned previously, the possibility that vaccines encoding structural HIV proteins of one clade may be immunogenic across diverse populations.

Although promising in terms of immunogenicity, Ad5 vector-based vaccines may be limited in their utility in populations with a high prevalence of preexisting antibodies to the adenovirus type 5. One potential way out of this conundrum is with the use of adenoviruses derived from another primate species. Ertl (Fitzgerald et al, Abstract LB4) presented data on a novel replication-defective adenoviral vaccine carrier based on E1-deleted recombinants of the chimpanzee serotype 68 (AdC68). The AdC68 construct encoding the HIV-1 Gag protein induced a vigorous Gag-specific CD8+ T-cell response which was not inhibited in animals pre-immune to human Ad5.

Although the HIV or SIV regulatory proteins are attractive vaccine candidates and have yielded promising results in previous nonhuman primate studies, a trial using HIV-1 IIIB or 89.6p Tat or Tat toxoid did not show any protection in rhesus macaques, despite induction of vigorous Tat-specific humoral and cellular responses in a subset of animals (Silvera et al, Abstract 289-W).

Dendritic cells are highly immunogenic and thus provide an attractive vehicle for delivery of a vaccine antigen candidate. This approach was tested in rhesus macaques (Abstracts 73, 286-W, and 312-W). In a presentation by Zhu and colleagues (Abstract 73), autologous myeloid DCs generated in vitro after positive selection and culturing with IL-4 and GM-CSF were pulsed with chemically inactivated SIV$_{mne}$ and matured in vitro. Mature autologous DCs were infused subcutaneously 48 hours after the antigen pulse. Six infusions of DCs were given and the macaques were challenged with pathogenic SIV$_{mne}$ 3 weeks after the last DC infusion. Seven of 10 challenged macaques had lower viral loads, and all animals maintained their CD4+ T-cell counts, in contrast to the control macaques. Surprisingly, T-cell responses as assessed by proliferative and ELISPOT assays were observed only in a subset of vaccinated animals and were generally weak. Further studies are warranted to optimize this potentially promising approach.

Lisziewicz and colleagues (Abstracts 286-W and 312-W) have developed another novel vaccination approach designed to enhance mobilization of antigen-specific DCs in vivo. They used a plasmid DNA vaccine encoding a replication- and integration-defective simian-human immunodeficiency virus (SHIV; DermaVir, Georgetown University Research Institute for Genetic and Human Therapy, Washington, DC), which is formulated in polyethylenimine-mannose (PEIm). Topical application of the DNA vaccine to shaved skin results in mobilization of LCs to the dermis. PEIm facilitates transduction of LCs in the epidermis and uptake of DNA by the LCs. DNA-expressing LCs were shown to migrate to the T-cell areas of the draining lymph node and elicit a vigorous SIV-specific CD8+ T-cell response (Abstract 286-W). When macaques with late-stage AIDS that were receiving STI-HAART were immunized topically with DermaVir, there was a dramatic decline in the rate of viral load rebound so that viral load gradually declined to undetectable levels (Abstract 312-W). Viral control was associated with induction of vigorous SIV-specific T-cell responses. These results suggest that there is a reserve of functional DCs even in late-stage AIDS, and that use of autologous DCs as a vehicle may be a promising approach. Further studies will be required to determine the efficacy of these vaccine approaches in protecting or mitigating the effects of HIV/SIV infection.

Conference Abstracts Cited in the Text

The full text of the abstracts is available online at www.retroconference.org.

22. Dynamics of T-Lymphocyte Turnover in Sooty Mangabeys, a Nonpathogenic Host of Simian Immunodeficiency Virus Infection. A. Kaur, A. F. Barabasz, M. Rosenzweig, H. McClure, M. B. Feinberg, and R. P. Johnson.
27. Intestinal Mucosal Macrophages Are a Principal Reservoir of HIV Replication and Sustain High Levels of Infectious Virus in Persons with Chronic Progressive HIV Infection. S. Brodie, S. Tabet, J. Krieger, R. Haggitt, D. Nickle, A. Johnson, R. W. Coombs, J. Mullins, L. Corey, and C. Celum.
28. Expression of DC-SIGN by Intestinal and Genital Mucosal Dendritic Cells in Humans and

Rhesus Macaques. B. Jameson, F. Baribaud, S. Pöhlmann, D. Ghavimi, F. Mortari, R. Doms, and A. Iwasaki.

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286-W. A Novel Topical DNA Vaccine (DermaVir) for the Induction of T-Cell Immunity. J. Lisziewicz, J. Xu, J. Trocio, L. Whitman, P. Markham, S. Arya, J-P. Behr, and F. Lori.

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L1. SIV Reservoirs and Human Zoonotic Risk. B. H. Hahn.

L2. Dendritic Cell Responses and Host Interactions. M. Pope.

L3. Immune Reconstitution and Immunologic Responses to Antiretroviral Therapies: Implications for Therapeutic Strategies. B. Autran.

L4. Analysis of HIV-1 Envelope Glycoproteins and Neutralizing Antibodies. P. Kwong, S. Majeed, S.-H. Xiang, R. Gupta, L. Wang, M. Doyle, D. Casper, W. Hendrickson, J. Sodroski, and R. Wyatt.

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LB4. A Novel Adenoviral Vaccine Based on a Chimpanzee Derived Isolate as a Vaccine Carrier for HIV-1 Gag. J. Fitzgerald, G.-P. Gao, J. Wilson, and H. C. J. Ertl.

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S10. Identification of Dynamically Distinct Subpopulations of T Lymphocytes that are Differentially Affected by HIV. J. A. Kovacs.

S11. Role of Immune Hyperactivation and Failing Thymic Homeostasis in the Pathogenesis of AIDS. F. Miedema, M. D. Hazenberg, S. A. Otto, H. Schuitemaker, and R. J. de Boer.

S12. Mechanisms of T-Cell Depletion in HIV Disease. M. McCune.

S24. Nonpathogenic Infections in Animal Models. M. Feinberg.