ADVANCES IN THE UNDERSTANDING OF HIV PATHOGENESIS

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There has been an accelerated increase in our understanding of HIV pathogenesis in the past year, ranging from host to viral to immunologic factors. In the same way that the 1996 International AIDS Conference in Vancouver will be remembered for delivering stunning results with antiviral combinations including protease inhibitors, the 1999 Retrovirus Conference in Chicago will be remembered for numerous demonstrations on just how much the immune system contributes to control of viremia. The prospects of turning this knowledge into new intervention strategies affords room for great optimism.

HOST FACTORS IN HIV PATHOGENESIS

One of the most important questions surrounding HIV pathogenesis is why some persons progress rapidly while others progress slowly. It has long been speculated that genetic polymorphic HLA class I molecules may be involved because they present viral peptides to the immune system for recognition. Numerous previous studies have suggested that particular HLA class I alleles may be associated with greater risk of progression. In an extensive genetic analysis of persons from several longitudinal cohorts, O'Brien et al **(Abstract S14)** reported that individuals with greater heterozygosity in class I alleles fare significantly better. This seems reasonable, since more heterozygosity at class I alleles should allow for a more diverse array of epitopes to be presented. Coupled with other data showing the importance of cytotoxic T lymphocytes (CTL) in controlling viremia (see below), these studies suggest that prognosis may depend on the breadth of the immune response. In addition, these cohort studies revealed that certain HLA alleles are particularly associated with rapid disease progression, including Cw4 and B35. One possible conclusion of O'Brien's study and that of Kalsow et al **(Abstract 565)** is that the particular HLA alleles used to present epitopes may vary in their efficiency of presentation. Future analysis of the link between MHC alleles, immune responses, and disease course will be particularly important.

Advances in characterization of macaque MHC alleles and CTL responses may afford an animal model to help dissect these influences. Evans et al **(Abstract 255)** showed that MHC alleles are important in directing the CTL responses. Their studies indicate that more broadly directed responses appear to be associated with a better outcome and that considerable immune selection pressure is applied through these responses, with subsequent development of immune escape viral variants.

The Conference also featured additional data on the role of chemokine receptor polymorphisms in disease progression. The link between (32 CCR5 heterozygosity and slower disease progression was confirmed in adults and extended to children **(Abstract 269)**. Kostrikis et al presented data indicating that CCR5 polymorphisms may be related to the risk of maternal-fetal transmission **(Abstract 263)**. Paxton et al **(Abstract 258)**, testing pre-seroconversion blood samples, reported that higher levels of RANTES production by activated CD4+ cells but not CD8+ cells correlated with slower disease progression and lower viral loads at set point. Individuals heterozygous for the CCR5 delta 32 mutation had higher levels of RANTES production, offering an intriguing explanation for the slower disease progression in persons with this genotype. The potential role of the 3'A polymorphism in SDF1 (the chemokine ligand for CXCR4) on disease progression remains contested **(Abstracts 259, 568)**.

VIRAL FACTORS

There remains little doubt that viral attenuation can be associated with less rapid disease progression, and that attenuated strains of SIV can confer protection against challenge with pathogenic viruses. Understanding the immune responses that are operative in these animals is critical, and progress is being made. Nixon et al reported that better protection was achieved with longer duration between immunization and challenge (7 of 8 animals protected at 15 weeks versus 3 of 8 at 5 or 10 weeks), and strong CTL responses were detected in 2 of 3 protected animals that were studied **(Abstract 31)**. These data are consistent with previous reports, as was the finding that neutralizing antibodies did not appear to be correlated with protection.

An intriguing potential link between viral phenotype and pathogenicity was reported by Stoddart et al **(Abstract 4)**. They showed marked differences in thymic pathology when comparing protease inhibitor-sensitive with protease inhibitor-resistant viruses in a SCID-hu Thy/Liv mouse model. Protease inhibitor-sensitive clones of virus replicated to high levels in the thymus and caused T-cell depletion. The same virus with a

patient-derived protease resistance domain exhibited severely impaired thymic replication and did not cause T-cell depletion. This observed lack of viral fitness in vivo in primary lymphoid tissue is encouraging and could be invoked to help explain anecdotal reports of lack of expected disease progression in persons who have protease inhibitor-resistant virus and who continue on the therapy. However, since it has been shown in vitro that protease inhibitor mutations impair replicative capacity but that compensatory mutations in protease and gag cleavage sites restore more wild-type replication, more studies are clearly needed to determine the biologic significance of these findings.

Immunologic Factors

Not long ago a widely held view was that the immune system contributed little in the fight against HIV and SIV, which was supported by the demonstration that these viruses essentially represent infections of the immune system itself. From some of the most dramatic advances reported at the Conference, it is now clear that the immune system plays a major role in contributing to the viral set point. Moreover, the correlates of protective immunity, or at least those immune responses that are associated with control of viremia, are being better defined. In contrast to earlier Conferences in which the focus was on neutralizing antibodies, cellular immune responses took center stage at this year's Conference.

Advances in understanding the role of CTLs in HIV infection come in part from newer assay techniques involving direct visualization of CTL by flow cytometry using HLA-peptide tetramers, and in part from cohort studies of persons with long-term nonprogressing infection. The magnitude of the CTL response in acute infection is likely higher than previously appreciated **(Abstract 25)**, and additional data confirm the presence of a vigorous CTL response in persons who are controlling viremia without antiviral drug therapy **(Abstract 562)**. A potential reason for the lack of better efficacy of CTLs in vivo was provided by Andersson et al **(Abstracts 62, LB3b)**, who reported that CTLs found in lymph nodes contained granzyme but not perforin, leading the authors to conclude that these cells were not fully functional.

The critical role of CD8+ cells in controlling HIV replication was demonstrated in experiments involving in vivo depletion of CD8+ cells in SIV-infected macaques with a monoclonal antibody. Schmitz et al reported that transient depletion of CD8+ cells in either acutely or chronically infected animals resulted in dramatic increases in viral load, which subsequently declined as CD8+ cells returned **(Abstract 252)**. The rise in viremia also correlated with depletion of CTLs as determined by tetramer analysis. Similar results were reported by Xin et al, who concluded based on preliminary mathematical modeling that the changes in viremia could not be due to cytolysis alone, but might also include a soluble factor **(Abstract 253)**. This does not rule out that the effector cells are CTLs, since CTLs are known to inhibit viral replication by both cytolytic and noncytolytic mechanisms. Together these reports provide the strongest evidence to date that the cellular immune system is directly involved in the dynamic equilibrium at viral setpoint.

Further evidence for an antiviral role of CTLs was provided by Brodie et al **(Abstract 26)** in studies of adoptive transfer of CTL clones in infected humans. CTLs specific for Gag epitopes were infused and shown to migrate to sites of infected cells in lymphoid tissue. Moreover, the transfers were associated with transient decreases in peripheral blood mononuclear cell (PBMC) viral load. That the antiviral effect was not long-lived was speculated to be due to lack of sufficient CD4+ T-helper cell function, and future studies will need to address this issue. Clonal analysis of CTL responses in MHC-matched macaques also support an antiviral role for CTLs, since immune selection pressure by CTLs could be clearly demonstrated in this experimental infection **(Abstract 255)**.

That the cellular immune system plays a major role in HIV-1 infection should not be surprising based on animal models of chronic viral infections, as outlined beautifully by Ahmed in a plenary lecture **(Abstract L6)**. CTLs are clearly required to maintain control of viremia, and it is clear that these responses are critically dependent on CD4+ T-helper cells. Even transient depletion of CD4+ cells in murine lymphocytic choriomeningitis virus infection leads to impaired CTL responses and lack of control of viremia during the chronic phase of infection. New data in mice suggest that CTLs can exist in vivo in a nonfunctional state, and that this phenotype is more pronounced in settings of CD4+ T cell deficiency, as is seen with HIV and SIV infections.

Emerging data also extend the understanding of HIV-1-specific T-helper cell responses in HIV pathogenesis, but some controversies are emerging as additional data are generated. Numerous laboratories have now confirmed that Gag-specific T-helper cell responses are more robust in persons with nonprogressing infection **(Abstracts S41, S44, 23, 30, 562)**, and at least 2 labs have reported a strong negative correlation between viral load and Gag-specific T-helper cell responses **(Abstracts S41, S44)**. Although responses are lower in persons with chronic infection, a new and more sensitive assay described by Picker et al **(Abstract 27)**, which

relies on gamma interferon production assessed by flow cytometry, indicates that these cells may be present in chronic infection in greater numbers than previously anticipated. Binley et al **(Abstract 30)** reported no association between Gag-specific T-helper cell responses and viremia, using a slightly different assay technique. It remains to be determined whether stronger HIV-1-specific T-helper cell responses are causally related to lower viral loads in those studies in which this association has been observed.

The Special Case of the Exposed Seronegative Person

Historically, one of the major arguments used in favor of the immune system playing a major protective role has been the report of persons who have been heavily exposed to HIV and yet have remained seronegative. Investigators at previous Conferences have reported the detection of cellular immune responses in at least a subset of such persons who appear not to be infected, based on negative virus culture results, no detectable viral load, negative quantitative DNA PCR, and negative ELISA and Western blot tests. The finding of HIV-1-specific CTL and sometimes T-helper cell responses has raised the question of whether these persons might have cleared a transient or abortive infection. With the report of Zhu et al **(Abstract 8)**, it appears that at least some of these persons may actually harbor virus after all. Zhu et al performed an exhaustive analysis for HIV-1 DNA in a cohort of heavily exposed yet persistently seronegative persons at the University of Washington. They reported the persistent detection of gag, pol, and env sequences in such persons during a 2-year follow-up in which they remained seronegative. In a majority of persons studied, there was marked sequence homogeneity, and phylogenetic analysis revealed that the detection of HIV sequences was not due to laboratory contamination. In one individual low levels of sequence evolution were observed, suggesting persistent low-level virus replication. These very interesting results will need to be confirmed.

Immune Reconstitution

The final symposium of the Conference was devoted to the topic of immune reconstitution, and the data presented provided a degree of justified optimism for the future. The entire field of immune reconstitution has come of age with the advent of potent antiretroviral therapy. The ability to control viremia in newly infected infants has resulted in the ability to respond robustly to immunogens, providing hope that effective HIV-specific immune responses might be able to be induced with therapeutic vaccination **(Abstract L2)**. Prolonged therapy has already resulted in restoration of cytomegalovirus-specific (CMV) immune responses in adults **(Abstract 250)** and in reconstitution of MAC-specific immunity **(Abstract 248)**. These and other data **(Poster sessions 45 and 46)** provide hope that HIV-specific immunity might also be able to be induced with continued viral suppression. Although regeneration of HIV-specific immune function with antiviral therapy alone has been infrequent, the first reports of restitution of HIV-specific T-helper cells came to this meeting from Lori et al **(Abstract 401)**, in which 5 of 11 persons on prolonged didanosine/hydroxyurea therapy generated significant Gag-specific T-helper cell responses.

HIV clearly has a negative impact on the regenerative capacity of bone marrow and thymus **(Abstracts 22, S43)**. The fact that the thymus remains active well into adult life **(Abstracts 21, S42, S43)**, and that potent antiretroviral therapy results in increases in recent thymic emigrants **(Abstracts S42, LB1)**, suggests that prolonged therapy may result in repopulating with cells that may be able to be educated to mediate HIV-specific immunity. This notion is also supported by more extensive longitudinal data indicating that the fraction of naive cells continues to increase with prolonged antiviral therapy **(Abstracts S44)**.

One of the most fundamental questions related to the prospect for immune reconstitution has to do with whether the immune system can ever successfully control the virus. Data presented at this conference confirm that some individuals are able to control viremia in the absence of antiretroviral drug therapy, and that this occurs in the setting of strong virus-specific cellular immune responses **(Abstracts S41, 562)**. Furthermore, at least 3 groups reported that early intervention with potent antiretroviral therapy, particularly when instituted prior to seroconversion, is associated with the development of strong virus-specific T-helper cell responses **(Abstracts S41, S44, 23)**. The critical question of how long after seroconversion one can wait to initiate therapy and still see recovery of these responses remains to be answered. The fact that all persons treated prior to seroconversion had detectable responses **(Abstract S41)**, whereas these responses were less predictable in persons treated in the early stages of chronic infection, suggests that there may be better success with earlier therapy. However, any clinical benefit to patients from restoration or augmentation of these responses

remains to be determined.

These cases of early therapy resulting in the generation of potent antiviral immune responses raises the obvious question of whether these persons might be able to control viremia on their own in the absence of ongoing drug therapy. A corollary to this question is whether these more robust immune responses might allow the immune system to be more specifically boosted with some type of therapeutic vaccine. A number of anecdotal cases presented at the conference provide room for cautious optimism. The sentinel case of such potential immune control following early therapy was presented by Lori **(Abstract LB5)** and involved a patient in Berlin who stopped therapy intermittently over the first 6 months of treatment. At the first discontinuation approximately 1 month into therapy, viremia immediately recurred, but was controlled with reinstitution of drug therapy. On the second transient discontinuation of therapy approximately 2 months later, there was no documented rise in viral load. The patient finally discontinued therapy at 6 months and has now been followed up for an additional 24 months with continued plasma viral load levels of <1000 copies/mL. In this case, the low viral load is associated with persistent strong virus-specific T-helper cell and CTL responses, consistent with immune control of viremia. This case has suggested that early therapy, perhaps with immune boosting, might result in immune containment.

Additional anecdotal cases presented at the Conference further support the possibility that the immune system might be harnessed to contain HIV replication. Ortiz et al **(Abstract 256)** reported on 4 patients who discontinued therapy on their own; in 2, consistently low viral loads were maintained. These low viral loads were associated with strong and broadly directed CTL responses, whereas those persons who were unable to contain viral replication had narrower or absent virus-specific CTL. Neutralizing antibody responses and T-helper cell responses, which might be expected to play a role as well, are yet to be reported.

The possibility that immune control might be achieved following early therapy of acute infection is being tested in at least 1 clinical trial, with others undoubtedly on the way. Rosenberg et al **(Abstract S41)** intentionally discontinued therapy in a patient who had been treated for 17 months from the time of acute infection. Prior to stopping therapy, this person had strong virus-specific T-helper cell responses and strong CTL responses. Once therapy was stopped, virus slowly re-emerged but with kinetics that were much slower than previously observed in persons who discontinue therapy in chronic infection. Viral load was rapidly contained with reinstitution of therapy, and recently therapy was stopped for a second time in the same person to see if intermittent therapy can boost existing responses to some threshold needed for persistent control of viremia. The possibility that intermittent therapy might lead to enhanced immune responses was also suggested by data presented by Lori et al **(Abstract LB5)**, in which they observed a progressive delay in viral rebound with successive interruptions in therapy in person's started on therapy within a year of seroconversion. The theory being tested in both of these trials is that endogenous virus might be used as a vaccine. The availability of potent antiretroviral therapy allows the administration of somewhat regulated doses of a live replicating vaccine, namely the person's own endogenous virus. However, it is important to note that these trials are in their earliest stages and no conclusions can yet be made. It is important that such trials be conducted under very controlled circumstances, particularly given the fact that most persons with chronic infection who discontinue therapy will experience a high viral load.

Other attempts at immune reconstitution involved administration of IL-2, adoptive transfer of antigen-specific cells, and therapeutic vaccination. Chun et al report that 2 persons treated with prolonged courses of IL-2 in the context of potent antiretroviral therapy no longer had culturable virus, and they are discontinuing therapy on these persons to determine whether viremia will recur **(Abstract 496)**. Follow-up is as yet too short to draw conclusions. Hege et al **(Abstract 33)** are infusing genetically engineered cells that express a chimeric T-cell receptor comprising the extracellular portion of the CD4 molecule coupled to the T-cell receptor zeta chain, which allows for cells expressing this receptor to lyse gp120-expressing target cells. Virologic results from this trial are still awaited. Valentine et al **(Abstract 346)** reported follow-up data from Geneva on a cohort of persons given therapeutic immunization with an envelope-depleted whole inactivated virus vaccine. Astounding levels of virus-specific proliferative responses were observed in those receiving the vaccine compared with those who received adjuvant alone, demonstrating that infected persons can be induced to generate these responses. However, a clinical benefit from induction of these responses is yet to be shown and will require specifically designed trials that are likely to be conducted in the next year.

In addition to these investigations of adoptive therapy involving cellular immune responses, there were some studies examining the potential role of antibody responses in immune reconstitution. The hurdles faced in harnessing more potent neutralizing

antibody responses were well outlined by Sodroski in the opening session **(Abstract L1)**. In particular, many sites on the virus envelope are hidden from antibody-mediated immune attack by heavy glycosylation. Mascola et al **(Abstract 257)** reported that combinations of neutralizing monoclonal antibodies greatly enhance the antiviral effect achieved, and in the same way that combination antiviral therapy ushered in a new era in treatment, combining different neutralizing antibodies may afford significant advances in harnessing effective humoral immunity.

Viral Reservoirs

Any attempt to control viremia has to address the issue of latent viral reservoirs. Although it had earlier been suggested that viral eradication might be an achievable goal, data on numerous fronts suggest that this is not going to be readily achievable. A major question is whether, in fact, it is even necessary. It is clear that immune control of viremia in those few persons who are successfully controlling the virus without antiretroviral therapy is associated with the persistence of replication competent virus. In fact, it may be that some degree of viral turnover is required in order to maintain the strong cellular immune responses that are observed. Attempts to flush out persistent reservoirs have provided a mixed picture. Prolonged courses of IL-2 and potent antiretroviral therapy have been associated with the inability to culture virus in a subset of treated persons **(Abstract 496)**. It seems more likely that this strategy may have allowed for immune boosting with autologous virus induced to replicate from the IL-2-activated cells rather than actual elimination of infectious virus. An attempt to flush out latent virus from persistent reservoirs by anti-CD3-mediated activation of T cells resulted in significant toxicity that will severely limit this approach **(Abstract LB6)**. In fact, any approach that requires activation of 1 million uninfected cells to induce 1 infected cell is likely to cause significant toxicity. Other more targeted strategies are awaited.

Conclusions

Recent advances in understanding HIV pathogenesis now provide clear evidence for the tremendous potential of the immune system directed against this virus. The fact that some persons are able to control HIV replication in the absence of antiretroviral drug therapy has to be seen as tremendously positive, and provides substantial ground for optimism that an effective vaccine will ultimately become available. Given what we now know, it seems most likely that a vaccine that would attenuate the effects of infection is the most realistic short-term goal. Such a vaccine would have tremendous impact where the epidemic is spreading most rapidly and where antiretroviral drug therapy remains inaccessible. The coming year should provide important advances in understanding the immune parameters required for successful control of virus, and this should directly facilitate vaccine development. We are clearly making progress.

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