Advances in the Understanding of HIV Pathogenesis

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Host Factors in HIV Pathogenesis

Different rates of disease progression among individuals indicate that viral, genetic, and immunologic factors all play a part in determining HIV disease course. Although viral load and CD4+ T-cell count remain the stalwarts of disease progression assessment, important genetic factors that impact prognosis have been identified. One set of polymorphic gene products, the human leukocyte antigen (HLA) complex, influences immune responses by guiding T cells to certain recognition structures. The HLA class I molecules present peptides to CD8+ T cells, whereas the class II molecules present peptides to CD4+ T cells. Various associations between HLA alleles and long-term nonprogression (LTNP) or rapid development of HIV disease have been reported over the past few years, and these have been assumed to be related to how different HLA alleles influence the HIV-specific cytotoxic T-lymphocyte (CTL) response.

The HLA-B locus has at least 96 different alleles, with 2 "public epitopes," Bw4 or Bw6 (serologically-defined epitopes located at residues 77-83 [alpha 1] on the class 1 HLA-B molecule). Previous work had linked HLA-B*35 with rapid progression and HLA-B*57 with slow progression. In an analysis of 116 patients with well-defined seroconversion dates, Goldfeld and colleagues (Abstract LB4) reported that LTNP was strongly associated with the HLA-Bw4 supertype (P=0.0001). This association was still significant when known class I alleles associated with LTNP, such as HLA-B*57, were removed from the analysis, indicating a true association with the Bw4 supertype. Interestingly, the Bw4 sequence motif not only influences the binding of antigenic peptides, but also functions as a natural killer (NK) cell receptor ligand, indicating a potential involvement of NK cells in LTNP, as well as class 1 restricted CD8+ T cells.

Further evidence for the influence of genetic polymorphisms on the rate of disease progression came from new data on chemokine receptors. The importance of mutations of CCR5- 32, CCR2-641 or CCR5P1 in the rate of disease progression has been established, but additional mutations in other chemokine receptor genes may also have a major impact. Faure and colleagues (Abstract LB3) identified common mutations in the fractalkine receptor CX3CR1, and patients homozygous for I249M280 progressed faster to clinical AIDS. The complex nature of chemokine receptor effects was illustrated by data from Mangano and colleagues (Abstract 448). Five CCR5 haplotype pairs were associated with altered rates of transmission of HIV-1 from mother to child: 3 pairs associated with enhanced, and 2 with reduced, susceptibility.

New technological developments have been utilized this year to contribute to the understanding of genetic influences in HIV disease. The measurement of α1-T-cell receptor-rearrangement excision DNA circles or "α1-TRECs" has been an important tool in the assessment of cell turnover rates, but interestingly, the amount of α1-TRECs can also influence the disease progression rate. Hatzakis and colleagues (Abstract 123) reported on a study of 127 Greek participants in the Multicenter Hemophilia Cohort Study whose baseline α1-TREC values were lower in those who progressed to AIDS compared with those long-term nonprogressers who did not. The relative hazard for death per 10-fold increase in α1-TREC, adjusted for plasma viral load, CD4+ T-cell count, and age at seroconversion was 1.52 (1.12 to 2.06; P=0.007). If α1-TREC measurements really reflect an increase in thymic output, stimulating the production of T cells will become a major therapeutic goal. New assays involving complementary DNA microarrays were presented, and will help further elucidate the relationship between HIV and the infected host (Abstracts 148 and 149).

Viral Reservoirs

Perhaps the biggest disappointment in the last 2 years in HIV research has been the realization that virus can persist in a latent viral reservoir of resting CD4+ T cells. Unfortunately, as described by Blankson and colleagues (Abstract 137), the early initiation of highly active antiretroviral therapy (HAART) does not prevent the establishment of the latent reservoir, but early HAART does accelerate the rate of decay of latently infected cells. They also showed a positive correlation between viral loads and the frequency of latently infected T cells in acute seroconverters.

Zhu and colleagues (Abstract 136) showed that the latent reservoir appears to be continuously seeded from activated CD4+ T cells and monocytes even with continued apparent viral suppression through HAART. However, the clinical relevance of the latent reservoir has not been established.

Immunologic Factors

The central role that HIV-specific cellular immune responses play in containing viral replication is now well established, but there are still surprises. The HIV-specific CD8+ CTL response has been shown to provide the major immunologic containment of viral replication, but as beautifully outlined by McMichael in a plenary lecture (Abstract L8), in HIV infection these cells may lack the ability to function normally. HIV-specific CD8+ T cells in chronic HIV infection appear to lack perforin both in peripheral blood (Abstract 594) and lymph node (Abstract 368). The lack of perforin could result in a defect in the ability of those cells to lyse

targets (Abstract 600). Interestingly, Folkvord and colleagues (Abstract 191) found no evidence of aggregation of CD8+ T cells around HIV-producing cells in lymphoid tissues, which could indicate an inability of HIV-specific CTLs to recognize virus-producing cells. Collectively, these results point toward the intriguing possibility that HIV-specific CTLs may not function adequately under certain conditions. This might also be true for CD4+ T cells, as 3 groups found that HIV-specific CD4+ T cells can be maintained in infected people but without the ability to proliferate in response to HIV antigens (Abstracts 186, 607, and 578). Wilson and colleagues (Abstract 578) also showed that proliferative responses to HIV antigens were difficult to detect in progressing patients, but that the patients' HIV-specific CD4+ T cells still produced cytokine after specific stimulation.

Over the past few years several key technologies have been introduced in cellular immunology, and these are now being utilized to more accurately assess the quantity and function of T cells in HIV infection. The first of these newer methods is the development of stable major histocompatibility complex (MHC)-peptide complexes that can bind to specific T-cell receptors. The use of MHC class I tetrameric peptide complexes in animal models of viral infections has changed our understanding of CD8+ T-cell biology. With these reagents it has been shown that the acute antiviral CD8+ T-cell response can be massive, with half or more of the CD8+ T cells specific for the infecting virus.

At this conference, Spina and colleagues reported on the magnitude of HIV specific CD8+ T cells in primary HIV infection, and found HIV Nef-specific CTL responses can make up as much as 13% of the total CD8+ cell pool in peripheral blood, as measured by MHC-peptide tetrameric complexes (Abstract 593). The construction of HLA class II tetramers has proven to be technologically challenging, but at this conference the first 2 reports were presented of MHC class II (HLA-DR1) tetramers manufactured with HIV-specific peptides representing T-helper-cell epitopes (Abstracts 190 and 583). The direct measurement of helper T cells with these tetramers will help resolve the question of what happens to HIV-specific CD4+ T cells after HAART.

The second assay newly utilized to study cellular immunologic questions is an enzyme linked immunoassay, ELISPOT, that measures the secretion of cytokine in response to an antigenic stimulus. This was shown to be particularly useful in rapidly quantifying both CD8+ and CD4+ T-cell responses, and has become the assay of choice in vaccination studies because this assay, unlike the tetramer assay, can be performed without prior knowledge of the patient's HLA haplotype. (Abstracts 579, 586, and 656).

The third assay is the cytokine flow cytometry or intracellular cytokine production assay. In a novel application of this technique, Betts and colleagues used peptide pools of 95 optimally defined, HIV-derived CTL epitopes to measure the production of cytokine from CD8+ T cells in HIV-infected individuals (Abstract 655). They showed that many individuals recognized multiple peptides, and that epitopes previously described as "immunodominant," such as the "SLYNTVATL" HIV Gag CTL epitope, may not really reflect the true breadth of a CTL response. This would indicate the need for caution when interpreting results using MHC tetrameric peptide complexes based upon a single epitope.

The durable effects of suppressive HAART on viral replication are well known, but the immunologic consequences, especially on HIV-specific immune responses, are not. At this conference Malhotra and colleagues (Abstract 610) showed that HIV Gag-specific T-helper responses were restored with early combination therapy, and Ortiz and colleagues showed that the HIV-specific CD8+ T cell cytokine responses also increased when HAART was started early (Abstract 596). Early treatment with HAART sustained functional activity of HIV-specific CD8+ T cells, and this correlated with HIV-specific T-cell help.

Immunotherapy

Although regeneration of HIV-specific immune responses has been reported in patients treated early with HAART, the majority of chronically infected patients appear to have impaired immunologic responses, and so trials that combine HAART therapy with immune stimulation have been initiated. Prominent at this conference were reports on the effect of immune boosting strategies, either with therapeutic immunogens or cytokine therapy or combinations of both. Hardy and colleagues (Abstract 341) reported that therapeutic immunization with a gp120-depleted immunogen (Remune), in combination with HAART, increased HIV-specific proliferative responses, and that some synergy also occurred with the addition of interleukin-2 (IL-2). Maino and colleagues (Abstract 347) used cytokine flow cytometry to measure the HIV-specific CD4+ T-cell response in 18 chronically infected patients before and after immunization with the gp120-depleted immunogen, and found an increase in HIV-specific memory T cells after each immunization, a result similar to that of Loomis-Price and colleagues (Abstract 348). Whether the gp120-depleted immunogen can boost HIV-specific CTL responses, as well as CD4+ responses, remains to be determined.

Mucosal Immunology and Immune Responses That May Protect Against Infection

Sexual transmission of HIV across the mucosal barrier is the most common mode of transmission for HIV, yet very little is known about natural immune responses at mucosal surfaces or how these might contribute to the prevention of establishment of HIV infection. In an experimental model of acute simian immunodeficiency virus (SIV) infection, Veazey and colleagues found that up to 26% of CD8+ cells were tetramer-positive for the Mamu-A*01 restricted p11C, C-M CTL epitope in the gut-associated lymphoid tissue (GALT) of one animal, while the frequency of tetramer-positive cells in the peripheral blood was much lower (Abstract 113). HIV infection is associated with a significant mucosal inflammation in both GALT (Abstract 116) and vagina (Abstract 117). Smith and colleagues (Abstract 119) made a striking observation on the effect of estrogen on the thickness of vaginal epithelium. In an experimental primate model of vaginal transmission of SIV, they evaluated implanted progesterone, implanted estro-

gen, or no implant in ovariectomized macaques (6 per group). Animals were challenged intravaginally with $SIV_{mac}251$ and studied serially to establish the presence or absence of infection. None of the estrogen-treated animals became infected, and vaginal epithelial thickness was inversely correlated with susceptibility to infection.

It is intriguing to speculate that hormonal influences affected transmission of HIV to "resistant" Nairobi prostitutes. Several laboratories have identified groups of high-risk, exposed but seronegative (ESN) individuals participating in commercial sex work. One of the cohorts best studied is a group of Nairobi prostitutes. Kaul and colleagues (Abstract 489) reported on their findings on 10 sex workers from this group who had recently seroconverted. The only significant association between these cases and matched resistant controls was a greater than 12-month break from sex work (P=0.004). The majority of these women had prior evidence of HIV-specific cellular immune responses and Kaul and colleagues speculate that the sex workers might have become infected because they lacked protective CTL responses, preexisting CTL responses waned through lack of constant exposure, or because of viral escape via mutations of previously recognized epitopes. HIV-specific cellular immunologic responses were also found in exposed but seronegative individuals by Shacklett and colleagues (Abstract 595), who identified weak to moderate interferon-g production from CD8+ T cells in 76% of ESN women studied.

Structured Treatment Interruptions

Following anecdotal case reports last year of HIV-infected individuals in whom viral replication could be suppressed to low levels or levels below the limits of detection for prolonged periods after stopping HAART, this year's conference had preliminary results from trials designed to mimic this effect in patients. The dynamic between antigen drive and strength of immune response has been exploited as a strategy to boost or autoimmunize the HIV-specific immune response prior to drug discontinuation. Clinical trials that involve planned interruptions in drug therapy, or structured treatment interruptions (STIs), were reported in early-treated patients, in those with chronic infection, and as part of a salvage strategy. This strategy is not without potential risk, and careful attention was given to CD4+ T-cell depletion, symptomatic illness, development of drug resistance, or repopulation of the latent reservoir.

In early-treated patients undergoing 2 rounds of STI, Altfeld and colleagues (Abstract 357) noted an increase in both the frequency and breadth of HIV-specific CTL responses with sequential STIs. This remarkable result indicates that this strategy may be useful in helping broaden an immune response. Patients later in the course of disease are more likely to have immunologic compromise than early-treated patients, and the resurgence of virus might have very different consequences in those with cycling therapy started late compared with early.

Boosting of both CD4+ and CD8+ HIV-specific T-cell responses with STI was also seen in patients with chronic infection (Abstracts 353 and LB11), but this effect was not seen with short (7-day) periods of interruption (Abstract 356). The majority of patients who discontinued therapy had rebound of plasma viremia to their original viral load set point (Abstracts 349 and 351). However, Garcia and colleagues (Abstract LB11) reported that after more than 2 rounds of STI a delayed rebound and decrease in the viral set point of up to 1.6 log could be observed in some patients. They found a strong significant association between the reduction in viral load set point and boosted HIV-specific immune responses, strengthening the association between immunologic control and suppression of viral replication. These results highlight the mixed results from STI trials so far, but indicate that further understanding of the length of time of interruption required to boost HIV-specific immune responses will need to be established. Too short a boost may not provide sufficient antigenic stimulation to the immune system, while too prolonged an interruption could lead to substantial CD4+ T-cell depletions. These parameters might best be established in a primate model.

Very encouraging data were presented from an acute SIV infection model, where STI led to prolonged suppression of plasma viremia in the majority of animals in 2 independent studies (Abstracts S6 and 113). Collectively, these studies show that boosting of the HIV specific immune responses through STI is achievable, but these trials should only take place in structured settings where close monitoring can occur.

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