# HIV PATHOGENESIS

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*New findings related to HIV pathogenesis were largely extensions of the dramatic advances made during the past year. This review will focus on several key areas that are of particular relevance in terms of clinical interventions. These include new advances in our understanding of the correlates of protective immunity, new insights into the persistence of tissue reservoirs of virus, and the impact of potent antiretroviral therapy on immune function in HIV-1-infected persons.*

## CORRELATES OF IMMUNE PROTECTION IN HIV-1 INFECTION

One of the central unresolved issues related to disease pathogenesis is the identification of protective immune responses in HIV-1 infection. Letvin **(Abstract L3)** presented data from acute infection of rhesus macaques with a pathogenic simian immunodeficiency virus (SIV) isolate, providing new insights into the dynamics of the early cellular immune response. By day six after experimental infection, approximately 5% to 10% of all T cells in the regional lymph nodes are replicating virus, and by day 16 this robust replication has already been significantly contained. An oligoclonal cytotoxic T-lymphocyte (CTL) response can be detected in the regional lymph nodes within 72 hours of mucosal exposure, indicating that this arm of the immune response is induced very early after infection. This is long before neutralizing antibodies are detected, providing further evidence that CTLs are assisting in containing replication. Additional animal model studies in reconstituted severe-combined immunodeficiency disease (SCID) mice also supported an important role for CTL, in that CD8+ cells mediate resistance to HIV infection **(Abstract 90)**, and that passive transfer of antibody does not modulate disease progression in these mice after experimental infection **(Abstract 101)**.

Strong immune responses to HIV thus appear to be important in containing the virus, and emerging data raise the important question as to whether genetic polymorphisms may in fact modulate the specific immune responses generated in response to HIV-1 infection (Symposium 9). There now seems to be consensus that the V62I mutation in the chemokine receptor CCR2 is associated with a decreased rate of disease progression only in seroconverting cohorts, and not in studies of seroprevalent infection **(Abstract S3, S45a)**. This is likely due to the slight effect that is conferred, such that it can only be observed when cohorts are closely matched for time of infection. Moore presented an important new genetic analysis that may help to explain the observation that a CCR2 polymorphism is associated with reduced disease progression in seroconverters. This has been a curious finding because CCR2 is not a major co-receptor for viral entry. Moore described a genetic polymorphism in the CCR5 promoter region, which is 100% linked with the V62I mutation in CCR2 as shown by a novel molecular beacon technology **(Abstract S3)**. This particular mutation in the promoter region may affect CCR5 expression, thus accounting for its observed effects on disease progression. These data provide further support for the development of therapies designed to block viral entry via the chemokine receptors.

Studies of persons at opposite ends of the progression spectrum are now beginning to shed light on the role of virus-specific immune responses in disease progression. In a plenary session, it was reported that virus-specific helper cell responses directed at the viral p24 protein are associated with the control of viremia in long-term non-progressors who have not been treated with antiviral therapy **(Abstract L4)**. This finding was confirmed by Autran (personal communication) with data generated from a cohort of non-progressing persons in Paris, France. New data presented at the Conference suggest that these helper cells mediate their antiviral activity through the modulation of virus-specific CTLs, since strong helper cell responses are always associated with strong CTL responses **(Abstract L4)**. This finding is consistent with the detailed characterization of a person with rapidly progressive infection, in whom strong CTL responses were detected just after seroconversion but they rapidly waned **(Abstract 73)**. CTLs could be detected up until the time of this patient's death, which were able to recognize endogenous viruses present, indicating that emergence of immune escape was not playing an important role in disease progression. However, these CTLs did not expand in vivo, and the CTL response did not diversify over time. These data suggest the presence of an activation block in vivo. Analysis of virus-specific helper cell responses in this person revealed a dramatic lack of detectable responses, providing a potential explanation for the lack of in vivo activation. Another potential explanation is the report that circulating CD8+ lymphocytes downmodulate the CD3 zeta chain **(Abstract 105)**. Since the zeta chain expression is increased following exposure to inter-

leukin-2 (IL-2), it is possible that a deficiency of this cytokine contributes to impaired CTL function in vivo.

In addition to specific immune dysfunction leading to disease progression, it is also possible that viral attenuation may contribute to differences in disease course. One possible mutation affecting the pathogenicity of HIV was reported in the SIV model by Hoxie **(Abstract 520).** All SIV and HIV isolates reported to date contain a highly conserved tyrosine in the cytoplasmic tail of the transmembrane protein gp41 at position 721, which is part of an endocytosis signal for membrane glycoproteins. Substitution of the Tyr by Ile or deletion of the Tyr resulted in markedly attenuated disease and lower viral load in macaques experimentally infected with SIV. Whether the attenuated pathogenicity is due to an immune response is currently under investigation.

## Persistence of Viral Reservoirs During Therapy

One of the most important advances in understanding HIV-1 pathogenesis and the possibility of virus eradication was the identification in 1997 of persistent viral reservoirs. A number of studies presented at the conference confirm and extend these findings. Siliciano who was the first to report on these reservoirs of post-integration latency, presented extended data on persons who received potent antiretroviral therapy for periods of up to 30 months **(Abstract S15a).** Eighteen of 22 persons evaluated had detectable infectious virus that was isolated from resting CD4+ lymphocytes; in the other 4 individuals there were insufficient cells isolated to complete the study. Of note, some of the isolated viruses were syncytium-inducing phenotypes. Importantly, the size of the latent reservoir was found to be of the same order of magnitude for persons treated for 2 years with potent antiretroviral therapy, persons treated for just 2 months, and persons who did not receive therapy. In preliminary longitudinal studies there was no evidence of a progressive decay in the size of the reservoir, indicating that it has some intrinsic stability.

Other investigators also examined the residual reservoir during potent antiretroviral therapy. Wong presented data showing viral transcriptional activity in CD4+ cells despite prolonged antiretroviral therapy, and persistence of recoverable infectious virus **(Abstract S19).** Ho **(Abstract S16)** reported that the isolation rate for infectious virus decreased from 43% at 18 months to 28% at 22 months. Although this difference was not statistically significant, aggregate data suggested a progressive decline in the size of the cellular reservoir. He also examined proviral DNA, and preliminary evaluation suggested a steady decay with a half-life of 110 to 120 days. He also reported that multiply-spliced RNA was not detected, only unspliced RNA was, which he speculated could be produced by defective viruses or be due to trapped virions. The residual pool after 15 months of effective therapy was calculated to be a mean of 30,000 cells (range 4000-100,000 cells), and thus Ho's estimate of pool size is approximately tenfold lower than that reported by Siliciano. There was much variability in the decay rate calculation, but at best this seemed to be a half-life of 3 months. If one assumes the worst-case scenario, with a pool size of 100,000 and a decay rate of 18 months, then it would take more than 20 years to reduce the body burden to less than one infected cell.

An assessment of the cellular location of the persistent virus infection was provided by Bucy **(Abstract S17).** In situ studies revealed that 85% to 100% of the residual pool of RNA-positive cells are T cells, a finding supported by Shacker **(Abstract 523).** This remains the case even after prolonged potent antiretroviral therapy: although this led to an increase in the relative percentage of macrophages in lymph nodes, the cells harboring viral RNA were still predominately T cells. In a separate session, Bucy posited that much of the increase in CD4+ cell number seen early after potent antiretroviral therapy may be due to redistribution **(Abstract 519).** He suggested that immune activation was leading to increased sequestration of virus in lymph nodes. He presented data from lymph nodes obtained from ACTG 328 in which persons were treated with potent antiretroviral therapy. The total number of cells in lymph nodes was found to decrease with treatment, and staining with antibodies to adhesion molecules markedly decreased. This was interpreted to be consistent with a decrease in lymph node sequestration due to a decrease in adhesion markers on CD4+ cells, which in turn was due to decreased antigen exposure following institution of therapy. Further studies seem warranted to address this issue. One such study by Hellerstein **(Abstract 273)** suggests that increases in T-cells following institution of therapy may be due to increased rates of T-cell proliferation.

## Effects of Antiretroviral Therapy on Immune Function

A major issue related to antiretroviral therapy is whether this therapy alone will lead to enhanced virus-specific immune responses. At last year's Conference there was considerable excitement due to data presented by Autran, since published in *Science,* that potent antiretroviral therapy led to an increase in naive CD4+ cells. This offered hope that infected persons might generate enhanced immunity to HIV-1 following prolonged therapy, but numerous studies indicate that this

is not the case, at least over the 1 to 2 years of follow-up thus far. One possibility is that persons have just not been treated long enough to expect immune reconstitution, particularly given the slow kinetics of immune reconstitution following intensive chemotherapy **(Abstract L7).**

Prolonged therapy with potent antiretroviral therapy has been associated with some measurable changes in immune function. CD4+ and CD8+ cell numbers increase rapidly in the first few weeks of therapy, with T-cell receptor analysis suggesting early redistribution followed by de novo repopulating with naive cells **(Abstract 20).** In terms of the virus-specific immune responses, different groups reported that prolonged therapy resulted in diminished CTL responses as might be expected with the decrease in antigen to drive this response **(Abstracts 19, 522).**

However, potent antiretroviral therapy in the earliest stages of infection does clearly result in the generation of vigorous virus-specific CD4+ helper cell responses directed at the viral p24 protein. It was reported at the conference that 11 of 11 persons who were treated in the pre-seroconversion stage of infection all developed vigorous p24-specific helper cell responses, and a portion of these persons also developed gp160-specific responses **(Abstract L4).** These responses were persistent at up to one year of follow-up, despite the fact that viral loads had been persistently below the limits of detection by sensitive RNA assays. The magnitude and breadth of these responses were reported to be analogous to those seen in persons in whom viremia is controlled in the absence of antiretroviral therapy. However, these persons treated in the earliest stages of infection were reported to still have abnormal CD4+ and CD8 profiles, such as decreased IL-2 receptor expression, which persisted with therapy **(Abstract 587).**

The above studies of early intervention raise the question as to whether persons treated in the earliest stages of infection develop a mature immune response that is able to control the virus. There was one report at the conference that suggests this may be the case. Lori reported on a cohort of patients treated by Jessen in Berlin, who were given a combination of didanosine, hydroxyurea and a protease inhibition prior to seroconversion **(Abstract LB11).** All of these persons developed undetectable viral loads on therapy, and remain well at a year or more of follow-up. One individual stopped therapy after 6 months and maintains a viral load below the limits of detection one year later. Replication competent virus could be recovered from his resting CD4+ cells, at levels comparable to those seen in other treated persons, but he has not become viremic **(Abstract S15a).** Whether this person is maintaining an undetectable load due to immune control is a vital question that needs to be answered. This report also has to be viewed in the context of another report that a person treated during the acute retroviral syndrome developed a recurrent symptomatic retroviral syndrome after stopping therapy at six months **(Abstract 588).** ■

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