HIV Pathogenesis

HIV pathogenesis was discussed at the Los Angeles and Atlanta courses by H. Clifford Lane, MD, from the National Institutes of Health, Bethesda, Maryland.

Recent studies in viral dynamics, host immune response, and the regenerative ability of the immune system have yielded a substantial amount of information on pathogenesis of HIV infection. Studies of HIV replication and survival dynamics, detailed investigation of the architecture of lymphoid tissue in infection, and studies of CD4+ lymphocyte dynamics have demonstrated that HIV infection is a dynamic process of continuous viral replication (see accompanying article). Disparate lines of investigation have merged in the discovery of coreceptors to HIV and research has characterized a progressive decrease in the size and diversity of the CD4+ lymphocyte pool.

Susceptibility to Infection

The identification of factors that may influence susceptibility to infection upon exposure is the subject of much ongoing research. Building on the early work of Gallo et al on chemokines as anti-HIV factors and of Berger et al on a coreceptor with CD4 that mediated fusion, fusin was identified as a coreceptor for viral entry for the T-cell-tropic (syncytium inducing [SI]) isolates of HIV and of the β-chemokine receptor CCR5 as the coreceptor for the primary isolates of the macrophage-tropic (non-SI) phenotype isolates of HIV. In a study by Berger et al, CD4+ cells transfected with fusin are easily infected with the T-cell-tropic isolates of HIV-1 (LAV, HTLV-IIIB and RF), however, these cells lines could not be infected with macrophage-tropic isolates. Similarly, if CD4+ cells are transfected with CCR5, they can be easily infected with macrophage-tropic strains but not T-cell-tropic strains. The chemokine receptors are 7-transmembrane, G-protein coupled receptors. CCR5 is the physiological receptor for the cysteine-cysteine (CC) linked β-chemokines, RANTES, MIP-1α, and MIP-1β. Fusin is the receptor to SDF-1, a CXC chemokine (see Figure 1).

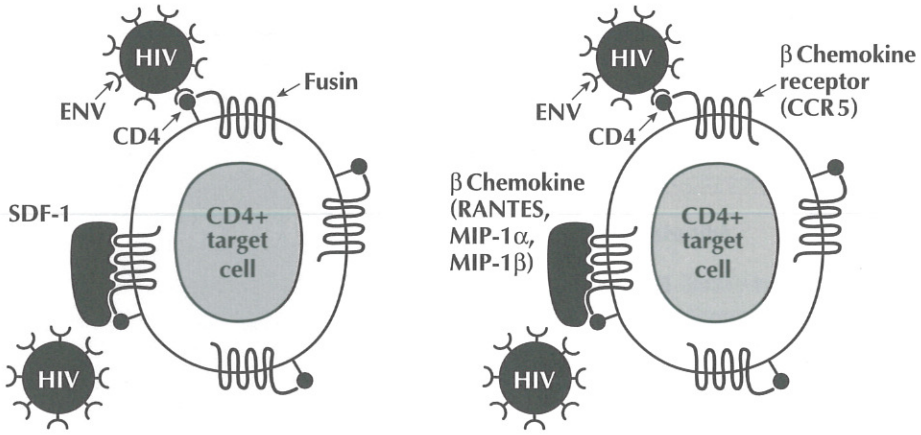

Figure 1. Fusin and CKR5 Act as Coreceptors for HIV. *Schematic drawing of HIV entry into the cell. Both fusin and CD4 are required for infection with T-cell-tropic isolates; CD4 and CKR5 or CCR5 are required for infection with a macrophage-tropic isolate.*

Since macrophage-tropic isolates are the more prevalent isolates in patients, it may be possible to use a congener of the ligand as a therapeutic agent.

The identification of coreceptors in part explains why some people, despite multiple high-risk behaviors, do not become infected with HIV. Genotypic analyses identified that some of these high-risk uninfected individuals are homozygous for deleted alleles of the CCR5 gene. The frequency of this mutation, a 32 base-pair deletion, is estimated at 11% in the Caucasian population and 1.7% in the African-American population. The homozygous genotype appears to confer resistance to HIV-1 infection. In a cohort of 1343 HIV-positive and 612 HIV-negative patients, none of the HIV-positive patients were homozygous for the Δ32 CCR5 mutation compared with 3% of the HIV-negative patients.

The heterozygous genotype does not appear to have an effect on resistance to infection; several studies have shown a consistent, but minor difference in the rate of disease progression between patients with the heterozygous Δ32 mutation and those with no mutation.

Disease Progression

The strength of the immune response to acute HIV infection appears to have a long-term impact on the course of the disease. Those patients who are able to control the virus well, as evidenced by low plasma HIV RNA levels and diverse T-cell responses, advance to clinical disease much more slowly than do those with high plasma HIV RNA levels (eg, >100,000 copies/mL) and a more restricted immune response. Nevertheless, the majority of patients eventually exhibit disease progression. A small proportion of patients—estimated by Dr Lane at probably less than 5%—do not appear to progress. "Long-term nonprogressors" have been the focus of much recent study that has attempted to characterize the immune effector mechanisms of such an apparently potent and enduring response to infection.

Much of the work done in the area of nonprogression has been descriptive and has thus far failed to adequately characterize the mechanisms underlying the phenomenon. In general, those persons categorized as long-term nonprogressors have lower levels of plasma HIV RNA and broader T-cell immune responses, ie, more CD8+ T-cell clones, than do persons who progress more rapidly. In fact, long-term nonprogressors do not constitute a discrete subset of patients; Dr Lane maintained that it is more likely that "nonprogression" is part of a continuum of responses ranging from very rapid to very slow progression.

He cited data from a study in Multicenter AIDS Cohort Study (MACS) patients that showed that those who maintained relatively stable CD4+ cell counts over the first several years of HIV disease still exhibited a characteristic decline in counts in subsequent years. In that study, a cohort of 56 patients who had been identified as long-term nonprogressors on the basis of follow-up over the first 7 years, during which they exhibited a mean CD4+ lymphocyte count increase of 18 cells/μL per year, were found to have a mean decrease of 67 cells/μL per year over the following 5 years—a rate of decline comparable to that observed in patients exhibiting a more typical infection course.

CD4+ Lymphocyte Dynamics

Studies of CD4+ lymphocyte dynamics in HIV infection have shown that the immune system is in a state of constant turnover far greater than under normal conditions. This phenomenon is readily demonstrated by the rapid increases in CD4+ cell counts following initiation of effective antiretroviral therapy and by measuring the fractions of CD4+ cells that are in the S phase (actively preparing to divide) at any given time.

Despite the increased production of CD4+ cells during HIV infection, there is a steady decline in the number of CD4+ cells over time. Along with this quantitative change, there are qualitative changes that have profound implications for treatment. Studies of changes in "naive" and "memory" CD4+ lymphocyte populations, analyses of the survival and distribution of genetically marked CD4+ lymphocytes, and analyses of specificity-mapping of the CD4+ lymphocyte receptor repertoire all support the conclusions that (1) elements of the T-cell repertoire are lost during progressive infection, and (2) increases in cell counts observed during treatment represent expansion of the remaining elements of the repertoire rather than addition of new or reacquisition of lost elements.

Naive and Memory Cell Dynamics

Antigen specificity of CD4+ lymphocytes is conferred by expression of α/β heterodimers on the cell surface—the T-cell receptors. CD4+ lymphocytes can be phenotypically characterized as "naive" or "memory" on the basis of CD45R isoform expression: those cells that have a high molecular-weight isoform (CD45RA) are termed naive, while those with a low molecular-weight isoform (CD45RO) are referred to as memory cells. Naive CD4+ T lymphocytes have a long half-life (>10 years), do not exhibit effector functions, and express L-selectin, an adhesion molecule that facilitates binding to the lymph node high endothelial venules. The memory T cells have a shorter half-life (1 year), exhibit effector functions, and express adhesion molecules that facilitate binding to tissues (LFA-1,3 and α4, α5, α6, and β1 integrins).

In initial studies, loss of ability to respond to recall antigen suggested that memory cells were selectively lost.

When T cells exit the thymus, they all bear the high molecular-weight isoform. Through selection processes in the thymus, each cell is "programmed" to respond to a specific potential antigen. Thus, although each cell has a unique specificity, the total cell population produced represents a possible response to an enormous number of different potential antigens. As the cells encounter the antigen for which they are specific (eg, in early life), the CD45 gene undergoes differential splicing in such a way that the low molecular-weight isoform comes out on the cell surface, with clonal expansion of the memory cell. In any individual, the total T-cell population comprises naive cells and memory cells, with the character of the overall system gradually reflecting the specific antigenic environment of that individual. These phenotypes, however, are not stable: naive cells can become memory cells after they encounter their specific antigen, and memory cells can revert to naive cells if they do not encounter antigen.

In initial studies in AIDS patients, a loss of ability to respond to recall antigen was observed, suggesting that memory cells were selectively lost. However, it subsequently has been demonstrated that the proportion of naive CD4+ cells declines as overall CD4+ counts decline (Figure 2); it has been postulated that only memory cells remain by the end stages of HIV infection. Dr Lane noted that this phenomenon is similar to what is observed in normal aging, suggesting that HIV infection might be likened to an accelerated immune senescence.

With the use of the CD45 marker, characteristics of the CD4+ cells produced

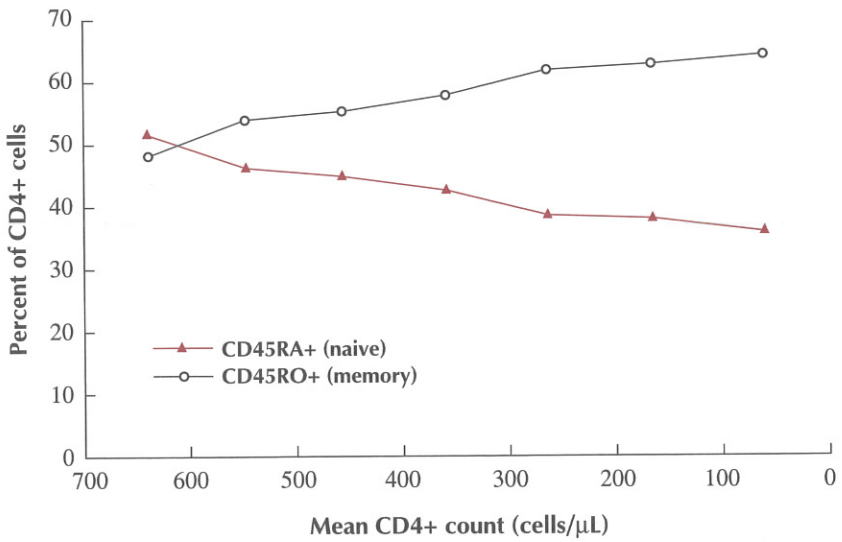

Figure 2. Changes in Naive and Memory CD4+ Cells During Progressive HIV Infection. *Data from 40 patients followed for several years showing relative proportions of naive and memory cells during progressive HIV infection.* Adapted from *Nature Med.* May 1997.

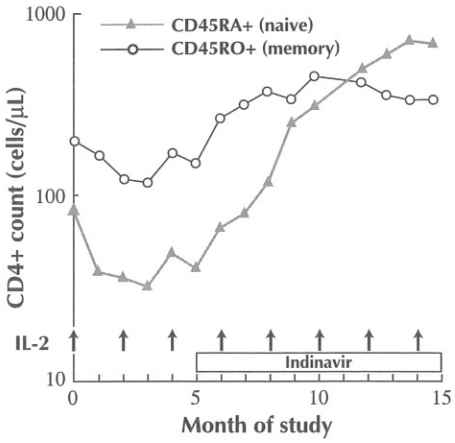

Figure 3. Increases in Naive and Memory CD4+ T Lymphocytes During Therapy with IL-2 and Indinavir, Despite Involution of the Thymus. *Changes in naive and memory CD4+ cell counts during indinavir treatment at month 14 of therapy. Both naive and memory cell populations increased. A CT of the thymus at 14 months of therapy showed that the increases were not accompanied by thymic hyperplasia, however.*

during treatment-related increases in cell counts have been examined. Patients who have both naive and memory cells when treatment is initiated exhibit increases in both cell types, whereas those who have lost naive cells exhibit increases only in memory cells despite the fact that the magnitudes of overall increases in CD4+ counts can be identical. The finding that some patients exhibit an increase in naive-cell populations suggested that new cells may be entering the system from the thymus, and thus that the system might be able to regain elements of the repertoire deleted through quantitative loss.

However, a number of findings suggest that in fact this is not the case. High-resolution CT scanning of the thymus during treatment-associated increases in CD4+ cell counts has shown increases in both naive- and memory-cell populations despite involution of the thymus. In one example presented by Dr Lane, an increase in CD4+ cell counts from approximately 50/μL to more than 500/μL was not accompanied by thymic hyperplasia or other evidence that the cells originated from the thymus (Figure 3). Labeling of existing cells with a genetic marker has shown that the proportion of marked cells remains constant throughout the expansion of the population, indicating that the increase in cell numbers can be explained by expansion of existing circulating cells, not entry of new cells from the thymus.

Polymerase Chain Reaction (PCR) Studies of T-cell Receptor Families

Other data demonstrating that loss of elements of the T-cell repertoire occurs during HIV infection come from PCR studies of T-cell receptor repertoires. A number of different subsets of T cells are produced by rearrangement of the T-cell receptor gene after stem cells enter the thymus. Some of these subsets can be recognized by distinctive T-cell receptor variable region β (V_β) chains. A total of 24 different types of V_β chains has been identified, each of which gives rise to T-cell receptors of 8 different sizes, producing a total of 192 different T-cell receptor families. Selective PCR amplification of the V_β chains allows mapping of the distribution of the different T-cell receptor types. Figure 4 shows the results of such studies in syngeneic twins discordant for HIV infection. Such results indicate that HIV infection is associated with a severe disruption of CD4+ lymphocyte repertoire. This disruption does not appear to be reversed, at least over the short term, by effective antiretroviral treatment. Figure 5 shows the distribution of receptor families for 3 V_β chains before and after treatment with a protease inhibitor and interleukin-2, which resulted in an increase in CD4+ cell counts from 238/μL to 1102/μL.

The determinants of the quality of the CD4+ lymphocyte pool in the context of HIV infection can be understood schematically (see Figure 6). Cells leave the pool both through death as part of the natural remodeling of the immune system and through HIV-induced death. Cells can enter the pool by stem-cell differentiation and processing in the thymus in early life or by somatic cell division. In adults, regardless of whether HIV infection is present, the entry of new cells appears to play little, if any, role; the division of cells already existing in the pool accounts for all replenishment of cells lost through natural or other death. Thus, it appears that if diversity within the existing pool is lost in the adult, it is not likely to be replaced, at least during the short term. According to Dr Lane, the T cells of the immune system can be viewed as the tiles in a game of Scrabble. In the normal aging process, a memory pool is generated of the letters that are commonly needed; the crucial part of the immune system resides in the memory pool. Some of the naive pool is retained, analogous to the letters that are not

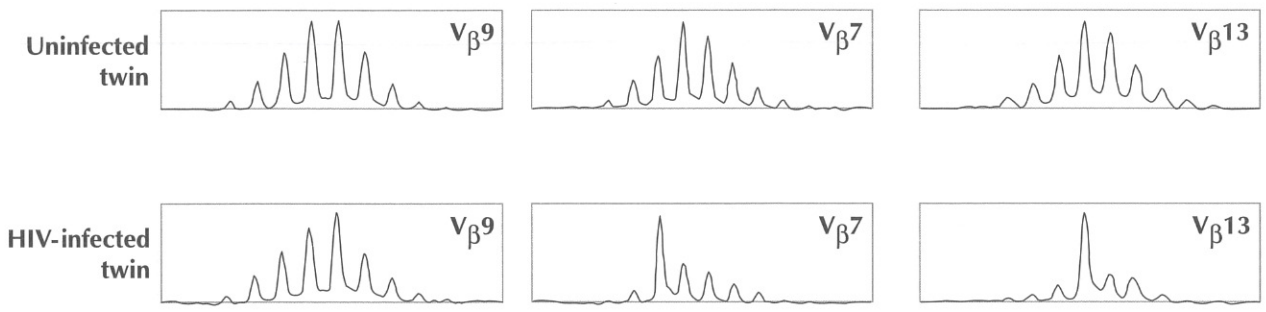

Figure 4. HIV Infection Results in a Severe Disruption in the CD4+ T-Cell Repertoire. *Comparison of distributions of T-cell-receptor sizes for three T-cell-receptor variable region β chains (V_β) in syngeneic twins discordant for HIV infection. The peaks represent amounts of T-cell receptors of different sizes within the given V_β chain determined by PCR. Dramatic disruptions in T-cell repertoire can be observed in $V_\beta 7$ and $V_\beta 13$ in the infected twin.*

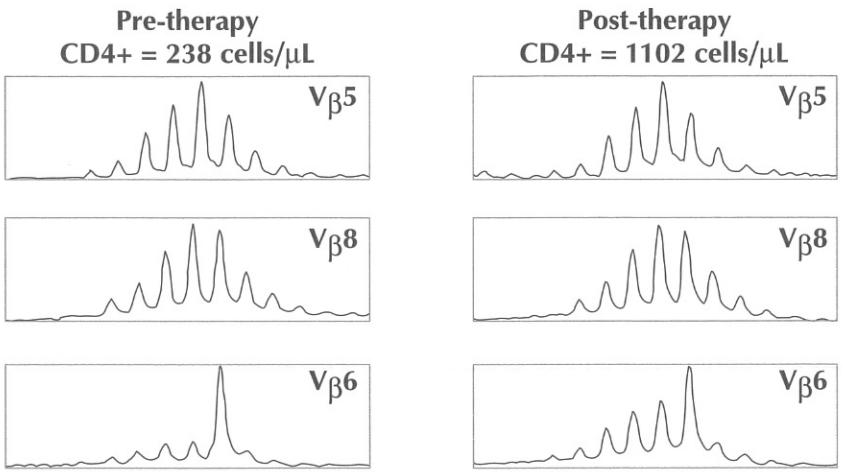

Figure 5. Disruption of the CD4+ T-Cell Repertoire Is Not Reversed by Treatment With Protease Inhibitor + IL-2. *T-cell-receptor repertoires for three variable region β chains (V_β) in an HIV-infected patient before and after treatment with a protease inhibitor and interleukin-2, which resulted in an increase in CD4+ cell count from 238 cells/μL to 1102 cells/μL. Despite the increase in CD4+ count, no change is observed in receptor repertoires for $V_\beta5$ or $V_\beta8$ chains. For $V_\beta6$, the tallest peak under normal conditions would be that two peaks to the left of the tallest one in this patient both prior to and after therapy. Although some change in receptor repertoire is observed, the skewing of the distribution observed before therapy persists after treatment.*

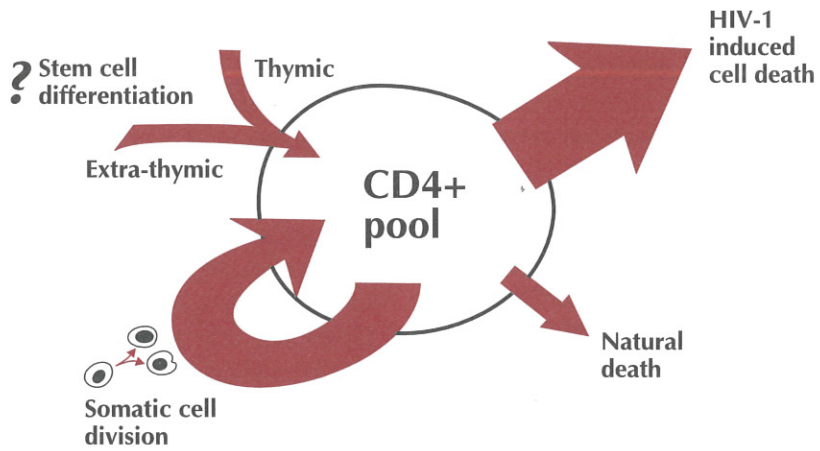

Figure 6. The CD4+ Lymphocyte Pool. *The CD4+ lymphocyte pool is depleted by natural and HIV-induced cell death; in adults, the pool is replenished by somatic cell division.*

used as often. As HIV progresses, there are fewer letters and fewer different letters. As Dr Lane noted, it is still possible to communicate with these fewer letters, but far more difficult.

Summary

Current understanding of viral and immune system dynamics can be summarized as follows: (1) HIV infection is characterized by ongoing viral replication that leads to progressive depletion of CD4+ lymphocytes with preferential loss of "naive" cells. (2) This viral replication is driven by the number of productively infected cells and is associated with an increased turnover of CD4+ lymphocyte. (3) As the CD4+ lymphocyte pool is quantitatively reduced, there is a progressive and irreversible loss in immunologic diversity. Dr Lane emphasized that these data all point to the importance of early therapeutic intervention in patients with HIV infection.

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Suggested Readings

Chou CC, Gudeman V, O'Rourke S, et al. Phenotypically defined memory CD4+ cells are not selectively decreased in chronic HIV disease. *J Acquir Immune Defic Syndr.* 1994;7:665-675.

Cocchi F, DeVico AL, Garzino-Demo A, et al. Identification of RANTES, MIP-1α and MIP-1β as the major HIV-suppressive factors produced by CD8+ T cells. *Science.* 1995;270:1811-1815.

Embretson J, Zupancic M, Ribas JL, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature.* 1993;362:359-362.

Graziosi C, Pantaleo G, Gantt KR, et al. Lack of evidence for the dichotomy of TH1 and TH2 predominance in HIV-infected individuals. *Science.* 1994;265:248-252.

Roederer M, Dubs JG, Anderson MT, et al. CD8 naive T-cell counts decrease progressively in HIV-infected adults. *J Clin Invest.* 1995;95:2061-2066.

Rowland-Jones S, Sutton J, Ariyoshi K, et al. HIV-specific cytotoxic T cells in HIV-exposed but uninfected Gambian women. *Nat Med.* 1995;1:59-64.