A complex array of multiphasic and multifactorial immunopathogenic mechanisms are involved in the establishment and progression of human immunodeficiency virus (HIV) disease. After primary infection, acute viremia occurs with wide dissemination of HIV. During this early viremic phase, the virus is trapped within the processes of follicular dendritic cells in the germinal centers of lymphoid tissue. Also, during this phase of primary infection, some patients show major expansions of certain subsets of CD8+ T cells that are identified by the expression of a particular variable region of the beta chain of the T-cell receptor. These expansions are manifestations of responses to HIV that may be important in controlling the progression of HIV infection. In addition, inappropriate immune activation and elevated secretion of certain proinflammatory cytokines occur during HIV infection; these cytokines play a role in the regulation of HIV expression in the tissues. Infection of progenitor cells in bone marrow and the thymus contribute to the lack of regeneration of immunocompetent cells. Dendritic cells are involved in the initiation and propagation of HIV infection in CD4+ T cells. In studies of long-term nonprogressors—persons who have stable CD4+ T-cell counts and no HIV disease progression despite years of HIV infection—preserved lymph node architecture, low viral burden, and viral expression were found.


Dr. Anthony S. Fauci (National Institutes of Health [NIH], Bethesda, Maryland): The immunopathogenic mechanisms of infection with the human immunodeficiency virus (HIV) are multifaceted and multiphasic (1). Figure 1 shows the typical prolonged course of HIV infection. The complex nature of HIV disease involves various overlapping features, including persistence of viral replication, aberrant and persistent immune activation, cytokine secretion and dysregulation, and, ultimately, progressive immunologic deterrioration (1, 2).

Soon after HIV enters the body, it is widely disseminated, predominantly to lymphoid tissues (1-4). The burst of virus replication early in the course of HIV disease is partially, but not completely, contained by an appropriate immune response (5-7), together with trapping of virions in the lymphoid tissue (see below). The onset of a robust immune response leads to a marked downregulation of virus in the blood (7, 8). However, with rare exceptions, virus is not completely eliminated from the body, and a state of chronic, persistent viral replication ensues. In almost all other viral infections of humans, virus either kills the host within a short period (this is relatively rare), is completely eliminated from the body (this is the case with most viral infections), or enters a state of microbiological latency (this often occurs with herpes simplex virus infections). This transition by HIV from acute to chronic infection with persistent replication of virus (see below) is unique among viral infections in humans.

We have previously shown that the lymphoid tissue is the major reservoir for and site of persistent viral replication; this is true even early in the course of infection, during the period of clinical latency when the CD4+ T-cell count is only moderately decreased (3). With the availability of sensitive assays for plasma viremia, it became clear that plasma viremia could also be measured at every stage of HIV infection, including the early asymptomatic stage (9). Most recently, it has been shown (10, 11) that virus is present at high levels in the plasma and rapidly turns over, particularly in advanced-stage disease. In this setting of persistent viral replication, progressive deterioration of immune function usually occurs, ultimately resulting in profound immunosuppression and clinically apparent disease (3, 5, 6). The link between the persistent replication of HIV and chronic activation of the immune system is critical to the pathogenic events seen in HIV disease (1, 12, 13).

In the early stage of HIV infection, the lymph nodes of persons with progressing HIV disease are activated and hyperplastic, and many virions are trapped in the germinal centers of lymph nodes in an extracellular manner on follicular dendritic cells (3, 4). This occurs when production of virus by individual cells within lymphoid tissue is low (3, 14, 15). Virus continues to be trapped by follicular dendritic cells in the germinal centers of the lymph nodes, initiating continuous immune stimulation (16) and...
constant exposure to possible infection of CD4+ T cells that reside in or are migrating through the lymph nodes (1, 2, 16). In this regard, recent studies (17, 18) have shown that the HIV that is trapped on the follicular dendritic cells is infectious for CD4+ T cells, even though the virions are coated with neutralizing antibodies. Thus, the mechanisms operable in an appropriate immune response to HIV, particularly activation of the immune system, are paradoxically the same mechanisms that propagate HIV infection and lead to the ultimate destruction of lymphoid tissue and to profound immunosuppression (1).

Cytokine secretion is closely linked with the phenomenon of generalized cellular activation. Since the mid-1980s, our laboratory has studied the role of cytokines in the pathogenesis of HIV disease (19). Cells communicate with each other through the secretion of cytokines as part of normal immunoregulatory homeostatic mechanisms (20). During HIV infection, cytokines are hyperexpressed and, in some cases, dysregulated. Constitutive expression of cytokines was examined in the HIV-infected lymph node, where virus-infected cells reside, to determine their potential physiologic relevance. The constitutive and induced expression of various cytokines was assayed by polymerase chain reaction (PCR) (21). At early, intermediate, or advanced stages of disease, it was found that interleukin-6, tumor necrosis factor-α, interferon-γ, and interleukin-10 were overexpressed in the lymph nodes of HIV-infected persons compared with the lymph nodes of persons with other diseases. In contrast, interleukin-2 and interleukin-4 were rarely secreted constitutively at any stage of HIV disease, despite a state of persistent immune activation.

Previous studies of chronically infected monocyte and T-cell lines (22–24) showed that cytokines such as interleukin-1β, interleukin-6, granulocyte–macrophage colony-stimulating factor, and tumor necrosis factor-α can upregulate HIV expression. These observations assume potential physiologic relevance in light of the fact that several proinflammatory cytokines capable of inducing HIV expression are chronically overexpressed in the lymphoid tissue of HIV-infected persons (21). We have recently shown that a tightly controlled autocrine loop of endogenous cytokine control of HIV expression exists (25). In this regard, inhibitors of cytokine expression can markedly downregulate the expression of HIV in an in vitro infection model. Hence, the expression of HIV in vivo is probably at least partly modulated by the endogenous cytokine network that is generally responsible for maintaining the homeostasis of the immune system.

Virologic and Immunologic Events Associated with Primary HIV Infection

Dr. Giuseppe Pantaleo (NIH): A substantial proportion (50% to 70%) of persons with primary HIV infection have a clinical syndrome of variable severity (5, 6, 8). The symptoms associated with this syndrome are nonspecific and may include fever, sore throat, skin rash, lymphadenopathy, splenomegaly, myalgia, arthritis, and, less often, meningitis. The lack of specificity and the variable severity of the clinical syndrome may explain, at least in part, why most HIV-infected persons generally do not report the symptoms of primary HIV infection to the physician.

However, delineation of the immunologic and virologic events associated with primary infection is important for several reasons. First, infection is established and virus is systemically disseminated during primary HIV infection. Second, during this period, the initial encounter between HIV and the
immune system of the host occurs, and an HIV-specific immune response is generated. Third, although both cellular and humoral immune responses are detected early in primary infection, these HIV-specific immune responses fail to eliminate HIV completely (see above). This suggests that the immune response may be inadequate or that certain mechanisms of viral escape from the immune response may be operative.

Cell-mediated and humoral immune responses specific to HIV have been detected early in primary HIV infection (5-7, 26, 27). The contribution of these immune responses to the dramatic downregulation of viral replication during primary infection has been debated. It is likely that both cell-mediated and humoral immune responses are important in the initial downregulation of HIV replication. The cell-mediated immune response consists predominantly of HIV-specific cytotoxic T lymphocytes and is critical in the elimination of virus-expressing cells; thus, it results in decreased virus production (26, 28). The humoral immune response, composed of antibodies against different HIV proteins, may substantially contribute to the downregulation of viremia through the formation of immune complexes composed of virus particles, immunoglobulin, and complement (C') that may be trapped in the reticulo-endothelial system (16, 28). The appearance of trapped virus in the follicular dendritic cell network of germinal centers in lymph nodes coincides with an increase in the levels of C' binding antibodies. In contrast, neutralizing antibodies are detected only several months after seroconversion. Therefore, the downregulation of viremia during the transition from the acute to the chronic phase of HIV infection may result from the combined action of both cellular and humoral immune responses.

To better characterize the cell-mediated immune response during primary HIV infection, we analyzed the T-cell receptor repertoire in peripheral blood mononuclear cells (27). Both CD4+ and CD8+ T cells can be further subdivided on the basis of “families” of cells that are identified by a particular variable region of the β chain of the T-cell receptor (Vβ). The entire spectrum of Vβ families, which number 24, are referred to as the “Vβ repertoire” of T cells. The Vβ repertoire has been analyzed on peripheral blood mononuclear cell samples collected at different time points after the onset of symptoms by combining a semiquantitative polymerase chain reaction (PCR) assay and cytofluorometry. The analysis, done in 20 persons with primary HIV infection, showed three predominant patterns of perturbations of the Vβ repertoire: major expansion in a single Vβ, moderate expansions in more than one Vβ, and no expansions or minimal expansions in one or more Vβ families.

We then determined the cell subset involved in these expansions and the antigen specificity and function of the expanded Vβ cell subsets. Patient 1, who is representative of the group that showed a major expansion in a single Vβ family, has been an ideal patient in whom to address these issues. Results from analyses of Patient 1 are shown in Figure 2. Peripheral blood mononuclear cell samples were collected at different time points (days 16, 20, 34, and 136 after the onset of symptoms), and the Vβ repertoire was analyzed by using semiquantitative PCR. This analysis showed high expression of one Vβ family, Vβ19 (corresponding to Vβ17 in the new nomenclature). Sixteen days after the onset of symptoms, Vβ19 represented about 40% of the total circulating T lymphocytes, whereas all other Vβ families were expressed at low levels (Figure 2). The percentage of Vβ19 progressively decreased over time (4% at day 136; Figure 2). The results
were determined by using two-color cytofluorometry in which an antibody against Vβ19 was used (data not shown).

The cell subsets involved in these expansions were determined by using two-color cytofluorometry of Vβ19 compared with CD4 and CD8 antigens. The number of CD4⁺ T lymphocytes remained unchanged over time, whereas the expansion of Vβ19⁺ cells was shown to be predominantly within CD8⁺ T cells (data not shown). Furthermore, cytofluorometry showed that 20 days after the onset of symptoms, about two thirds of all CD8⁺ T cells were activated as indicated by the expression of HLA-DR antigen; more than 75% of these activated CD8⁺ T cells belonged to the Vβ19⁺ cell subset. This suggests that these cells could potentially be involved in an antigen-specific immune response against HIV (data not shown). This hypothesis was further supported by the finding that the kinetics of Vβ19 expansion coincided with those of viral replication during primary HIV infection. Sequence analysis of several recombinant clones of Vβ19 obtained from peripheral blood mononuclear cells showed that the expansion of CD8⁺ T cells was oligoclonal in nature. This further indicated that these expanded CD8⁺ Vβ19⁺ cell subsets contained precursors of HIV-specific cytotoxic T lymphocytes and that these expansions were probably driven by HIV.

These expansions of Vβ subsets may have clinical and biological significance. Preliminary data indicate that different patterns of Vβ expansions are associated with different clinical outcomes: Major expansions in a single Vβ are associated with rapid clinical progression, whereas moderate, minimal, or absent Vβ expansions are associated with a stable and more favorable clinical outcome. This pattern of immune response is probably not unique to HIV infection but may be generated in various viral infections and may not have been recognized previously because of the limited availability of biological specimens collected during the acute phase of viral infections. Elucidation of the biological significance of this type of immune response may provide important insights into the protective as well as the potentially pathogenic mechanisms involved in the immune response to HIV.

Dr. Sharilyn Stanley (NIH): A striking feature of HIV infection is the typically persistent decline in the peripheral blood CD4⁺ T lymphocyte count throughout the course of HIV disease (1, 20). Although numerous factors undoubtedly contribute to this loss, the lack of ability to completely regenerate or repopulate these cells may be due to a failure of the bone marrow, the thymic progenitor cells, or the thymic and lymphoid tissue stromal environment that is critical in the generation of immunocompetent cells. In this regard, HIV-infected persons have a high incidence of cytopenias and other hematologic abnormalities (29), and hematopoiesis has been shown to be depressed. It has been shown that CD34⁺ bone marrow cells, a population of cells that includes both the pluripotent stem cell and the committed myeloid progenitor cell, cannot be infected with HIV in vitro (30).

To address the question of in vivo progenitor-cell involvement in HIV infection, CD34⁺ cells that had been enriched to 99% purity from the bone marrow of HIV-infected Zairian and American patients were examined for the presence of culturable HIV or HIV that could be detected by DNA PCR. In each group of patients, a subset of persons was found to have HIV infection in this purified CD34⁺ progenitor-cell fraction to a level that could not be explained by the approximately 4% contamination with other cells (31). In certain patients, HIV could be isolated more efficiently and to a higher titer from CD34⁺ bone marrow cells in vitro than from the remaining CD34⁻ bone marrow cells; viral burden, as measured by quantitative DNA PCR, was higher in the progenitor-cell population in these patients than in the CD34-depleted bone marrow mononuclear cells. In the Zairian patients, no correlation among stage of disease, hematologic variables, and infection of progenitor cells could be established because of numerous confounding factors such as malaria and sickle cell anemia. However, among the U.S. patients, only persons with markedly reduced total CD4⁺ T-cell counts (<50 cells/μL) had infection in the progenitor-cell population, suggesting that infection of these cells is a late event in these patients.

Thus, it is clear that bone marrow progenitor cells identified by the presence of the CD34 antigen on their surfaces can be infected with HIV in vitro and are infected with HIV in vivo in a subset of persons. Although in vitro infection of these cells is ultimately cytotoxic, the fate of the cells infected in vivo, including their hematopoietic capacity and their ability to serve as a reservoir for chronic virus production in the bone marrow, are unknown. Furthermore, the contribution of this in vivo infection to the clinically observed hematologic abnormalities is unclear. It seems likely that several factors, such as abnormal cytokine production in the bone marrow and infection of stromal or other bone marrow cells, contribute to the overall hematologic defects in various persons; infection of bone marrow progenitor cells may play an important role only in

Precursor Cell Infection and Immunopathogenesis of HIV Disease

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late-stage disease, when patients often develop varying degrees of pancytopenia.

In addition to the bone marrow abnormalities observed in these persons, several studies have shown marked abnormalities in the thymuses of HIV-infected adults, children, and neonates (32). These abnormalities typically include thymocyte depletion, premature atrophy of the thymus, and disruption of the thymic stromal network. Because it is difficult to obtain thymic tissue from patients, most of these studies have been done on autopsy material. To study in greater depth the role of the thymus in HIV infection, a model of human thymopoiesis, the severe combined immunodeficient (SCID)-hu mouse, was used. This model consists of a SCID mouse into which specimens of human fetal thymic tissue (providing the stroma) and liver tissue (providing the progenitor cells that seed the stroma) are implanted under the renal capsule of the SCID (severe combined immunodeficient) mouse and allowed to mature over 3 to 4 months (original magnification × 15).

A. Uninfected, normal-appearing thymus with distinct lobes, well-defined corticomedullary junctions, and Hassall corpuscles. B. HIV-infected thymus. A primary isolate of HIV was injected intrathymically, and the tissue was harvested 3 weeks later. Note the marked thymocyte depletion, fibrosis, and infiltration with adipose tissue (original magnification × 15). C. Electron microscopic image of HIV-infected thymus showed marked dropout of thymocytes, leaving behind the network of interdigitating thymic epithelial cells (original magnification × 2300). D. Destruction of thymic epithelial cells. The thymocytes in this area of an HIV-infected thymus appear healthy, but the thymic epithelial cells are degenerating, appearing to undergo a toxic insult with resultant cell death. No HIV is visible (original magnification × 6000).

Using the SCID-hu model, we and others (34–36) have shown that this tissue can be infected with HIV. Injection of virus into the thymic tissue resulted in a spreading cytopathic infection, depletion of thymocytes, and disruption of the thymic stromal network (Figure 3B). In situ hybridization for HIV RNA showed diffuse signals throughout the thymus, indicating the presence of many infected cells in both cortex and medulla, and electron microscopy showed that most of these infected cells were thymocytes that were extremely permissive for infection; multiple virions were often seen budding from a single cell (data not shown). By using DNA and RNA PCR analysis of thymocytes that had been teased from the tissues and sorted into CD4+CD8+ double-positive and CD4 or CD8 single-positive populations, it was shown (34) that all populations of cells are infected with HIV; the kinetics of infection differed between the different cell types. CD4+ cells were infected first, followed by CD8+ and CD4+CD8+ thymocytes (34).

Infection of thymocytes was cytopathic; this is shown most impressively by electron microscopic images of areas of thymus in which lacunae were created by the dropout of thymocytes, leaving the interconnected network of thymic epithelial cell processes (Figure 3C). Perhaps more importantly, the thymic stromal environment was severely disrupted as a result of this infection. As shown in Figure 3D, certain areas of the thymus showed intact thymocytes but degenerating thymic epithelial cells and loss of the critical intimate contact between the thymocytes and thymic epithelial cells. By combining HIV in situ hybridization with immunohistochemical stains for antigens specific for thymic epithelial cells, productive infection of these thymic epithelial cells with HIV could be shown (data not shown).

In summary, HIV infects not only mature CD4+ T cells but also bone marrow progenitor cells, developing thymocytes, and thymic stromal cells, associated with marked disruption of the thymic microenvironment. The importance of these findings lies principally in their implications for immune reconstitution. Although antiretroviral therapy is a cornerstone of treatment for HIV-infected persons, it has become increasingly apparent that therapeutic strategies targeted to the regeneration of a normal immune system are also needed. It is currently not known whether lymphoid tissues, such as the thymus or lymph node, can regenerate after viral replication has been adequately controlled, but main-
Dendritic Cells in the Pathogenesis of HIV Disease

Dr. Drew Weissman (NIH): Dendritic cells are a population of extremely potent antigen-presenting cells derived from the bone marrow and present in almost every tissue in the body; these cells are vital in the initiation of T-cell responses, particularly responses to new antigens (37). Dendritic cells function by taking up antigens and processing them into peptides that are associated with surface major histocompatibility complex proteins, the complex that interacts with the lymphocyte antigen receptor. The cells then migrate to lymphoid organs and activate T cells in the paracortical regions by presenting the major histocompatibility complex-bound antigen to the T cells. The role of dendritic cells in HIV disease is controversial. In certain studies (38–40), these cells have been found to be dysfunctional, depleted, infected in vivo, and susceptible to infection in vitro; findings from other studies contradict these results (41, 42). Additionally, HIV-pulsed dendritic cells have been found to infect activated CD4⁺ T cells (43), and conjugates of dendritic cells and T cells have been isolated from skin sections and found to be easily infected with HIV in vitro (44). We believe that the contradictory findings about dendritic cell infection, dysfunction, and depletion can be explained by the existence of three populations of cells with dendritic morphology that are present in peripheral blood; only one of these populations is susceptible to infection with HIV in vitro.

To show this, dendritic cells were isolated from peripheral blood by culturing peripheral blood mononuclear cells in vitro and successively depleting T, B, natural killer, and monocytic cells until a morphologically pure population of cells with dendritic structure was obtained (38–42). Similar methods were used to isolate precursors of dendritic cells, except that no in vitro culturing step was used before purification (45, 46). These precursors develop into dendritic cells when allowed to mature in culture. Mature dendritic cells purified by standard techniques, including in vitro culture, were analyzed for expression of CD83, an antigen previously shown to be present on dendritic cells in peripheral blood (47), HLA-DR, CD32 (receptor for the Fc region of the IgG molecule [FcγR] type II), and CD64 (FcγR type I). Two populations of cells were identified, one that was strongly positive for HLA-DR and CD83, and a second, expressing lower HLA-DR levels, that was negative for CD83 and had low FcγR I and II levels (48). Under light and electron microscopy, these two populations of cells showed similar dendritic structures (Figure 4). The function of these two populations of cells was tested in an autologous mixed lymphocyte reaction that measured the ability of antigen-presenting cells to activate autologous T cells. The CD83-positive cells gave substantial stimulation (as much as a 10-fold increase over control conditions), whereas the CD83-negative–FcγR-positive cells stimulated only slightly (less than a two-fold increase over control conditions) (48); this was similar to the degree of stimulation provided by macrophages.

Dendritic cell precursors isolated by standard methods without in vitro culture (45, 46) were found to be negative for CD83 but, when allowed to mature in culture, gained CD83 positivity as well as the immunostimulatory properties and morphologic
Figure 5. Human immunodeficiency virus (HIV)-pulsed CD83+ dendritic cells can bind virus to their surfaces and transmit virus to unstimulated, autologous CD4+ T cells. The CD83+ population of cells with dendritic structure were purified by flow cytometry using HLA-DR brightness. Monocytes were purified by adherence to plastic for 24 hours. B cells and CD4+ T cells were isolated with CD19 and CD4 magnetic beads (Dynal, Lake Success, New York) and were detached from the beads as per the manufacturer's instructions. Cells were pulsed with HIV at various concentrations for 1.5 hours at 37 °C and then washed three times. The HIV-pulsed cells were added to CD4+ T cells (2 x 10^6/well) and followed for released reverse transcriptase activity. 

Top. CD4+ T cells and HIV-pulsed cells were mixed at a ratio of 1:10 and were followed for infection. Dendritic cells were pulsed with HIV at a multiplicity of infection of 0.01 (open square), 0.001 (open diamond), and 0.0001 (open circle). Monocytes (open triangle) and B cells (closed circle) were pulsed with HIV at a multiplicity of infection of 0.01. Bottom. Cells were pulsed with HIV at an multiplicity of infection of 0.01 and were added in decreasing numbers to 2 x 10^6 CD4+ T cells. Peak reverse transcriptase activity of the infection is shown. RT CPM = reverse transcriptase counts per minute.

appearance of mature dendritic cells (48). In addition to the CD83-negative dendritic-cell precursors that developed into CD83-positive dendritic cells in culture, CD83-positive dendritic cells were isolated directly from peripheral blood mononuclear cells with no in vitro culture using positive selection with CD83- or HLA-DR-specific antibodies. When these two populations of dendritic cells were analyzed in an autologous mixed lymphocyte reaction, dendritic cells derived from precursors were less potent stimulators than were mature dendritic cells isolated directly from peripheral blood mononuclear cells. This suggests that the accumulation of antigens in vivo gave the mature dendritic cells isolated directly from peripheral blood mononuclear cells a superior ability to stimulate autologous CD4+ T cells (48).

Thus, three populations of cells with either dendritic structure or the ability to develop dendritic structure are present in peripheral blood. Two populations appear to be similar cells at different stages of maturation, whereas the CD83-negative–FcγR-positive cells have the same structure as dendritic cells but are functionally more similar to monocytes. All three populations express CD4 at low levels, although the CD83-positive cells and dendritic-cell precursors lose CD4 expression in culture (48). On exposure to HIV, only the FcγR-positive cells become infected as measured by released reverse transcriptase activity or DNA PCR analysis for HIV gag DNA (48).

The CD83-positive mature and precursor dendritic cells were not susceptible to HIV infection, but they still appear to play an important role in HIV pathogenesis. When these CD83-positive dendritic cells were pulsed with HIV, extensively washed, and then viewed using transmission electron microscopy, numerous virions were found attached to the cell surfaces. If the cells were left in culture for 24 hours after virus pulsing before examination with transmission electron microscopy, virions were still present on the surface (49). The HIV-pulsed dendritic cells, when added to unstimulated, autologous CD4+ T cells, could induce infection of the T cells (Figure 5). Dendritic cells, whether pulsed with a low multiplicity of HIV infection (0.0001) (Figure 5, top) or added in small numbers (1 HIV-pulsed dendritic cell per 180 CD4+ T cells; Figure 5, bottom), could still induce a productive infection in CD4+ T cells in the absence of exogenous stimuli. B cells and monocytes pulsed with HIV could not infect autologous CD4+ T cells (Figure 5).

One function of dendritic cells is to migrate through the body, bind and process antigens, and activate T cells. It has recently been shown (50) that dendritic cells are the first cells to appear at sites of inflammation in mucous membranes. Given these findings, a model of the initiation of HIV infection is that HIV enters through a defect or site of inflammation in a mucous membrane and is bound by
dendritic or Langerhans cells. Dendritic cells then carry HIV to a lymphoid organ and migrate to the paracortical region, which is rich in CD4+ T cells. These CD4+ T cells are activated by the dendritic cells and are exposed to bound HIV, which leads to their productive infection and to subsequent wide dissemination of virus. Dendritic cells probably also interact with CD8+ T cells in lymphoid organs and initiate an immune response that partially but not completely controls HIV replication (3, 7, 14).

Long-Term Nonprogressors

Dr. Fauci: Fewer than 5% of HIV-infected persons show no indication of HIV disease progression and have stable CD4+ T-cell counts despite years of HIV infection; these persons are called long-term nonprogressors (Figure 6). This is in contrast to the typical HIV-infected person in whom the number of CD4+ T cells progressively declines over time. The duration of clinical latency varies, but progression to the acquired immunodeficiency syndrome typically occurs after a mean of approximately 10 years (51, 52). We studied 15 long-term nonprogressors whose dates of seroconversion ranged from 1980 to 1987; all have been infected with HIV for between 8 and 15 years (53). The blood and lymph nodes of these persons were examined for viral burden, viral expression, and integrity of lymphoid tissue architecture.

We had previously shown a consistent correlation between destruction of lymph nodes and progression of HIV disease in patients at various stages of disease progression (3). However, despite the presence of follicular hyperplasia and germinal center formation in the lymph nodes of some long-term nonprogressors, the integrity of the architecture and stromal environment was preserved. Other long-term nonprogressors had little activation and minimal germinal center formation. Lymph nodes in the long-term nonprogressors were found to be less hyperplastic than the lymph nodes in progressors. Morphometric analysis of the proportion of lymphoid tissue occupied by germinal centers can be used to measure the state of activation of the lymph node. This proportion was found to be significantly greater in progressors who are in the early stage of disease than in persons not infected with HIV or in long-term nonprogressors. In general, lymph nodes of long-term nonprogressors maintained their normal architecture (53).

In one case, lymph node biopsy specimens taken 9 years apart showed remarkable preservation of lymph node architecture despite persistent HIV infection during the intervening period. In contrast, progressors examined after 3 to 5 years of HIV infection had clearly disrupted lymph node architecture. In progressors, HIV disease progression correlates with a decrease in the ability of lymph nodes to trap virus and an increase in the number of individual lymph node and peripheral blood mononuclear cells infected with HIV.

In addition to the morphologic differences observed in the lymph nodes, the level of trapped virions detected by in situ hybridization and by electron microscopy was, in general, much lower in the lymph nodes of long-term nonprogressors than in the lymph nodes of persons whose disease progressed. Expression of virus in individual cells was
also much lower in the lymphoid tissue of long-term nonprogressors than in the lymphoid tissue of progressors.

Comparative analysis by DNA PCR showed that the mean viral burden in peripheral blood mononuclear cells of the progressors was \( 4 \times 10^6 \) copies per cell compared with the long-term nonprogressors, whose mean viral burden was \( 279 \times 10^6 \) copies per cell. Lymph node mononuclear cell viral burden was \( 9000 \times 10^6 \) copies per cell in progressors and \( 1000 \times 10^6 \) copies per cell in long-term nonprogressors. In addition, RNA PCR examination of viral replication showed striking differences between the two groups; levels of viral replication were much lower in the mononuclear cells of long-term nonprogressors than in the mononuclear cells of progressors. Quantitative competitive PCR analysis showed the expected high levels of plasma viremia in progressors. Most long-term nonprogressors had generally low but variable levels of viremia similar to those of persons who were in the early, clinically latent stages of HIV disease.

It should be pointed out that in our cohort (53), we could isolate replication competent virus from the mononuclear cells isolated from lymph nodes of most persons, even though levels of viral burden were low. Similarly, other investigators (54) have also reported low levels of viral burden, whereas it was extremely difficult for them to isolate virus from peripheral blood mononuclear cells. Furthermore, one report described a defective virus in one of five long-term nonprogressors studied (55). Both brisk cell–mediated and humoral immune responses have been seen in long-term nonprogressors (53, 54). Therefore, on the basis of these observations, it is likely that these persons represent a heterogeneous group whose state of long-term nonprogressive disease probably results from robust immune responses against HIV, a poorly replicative virus, or both. In any event, the phenomenon of long-term nonprogression should serve as an excellent model to dissect out the complex mechanisms of HIV pathogenesis. An understanding of these mechanisms is critical for the design of strategies for therapeutic intervention and vaccine development.

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