Human Immunodeficiency Virus Pathogenesis: Insights from Studies of Lymphoid Cells and Tissues

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Although plasma virus load is invaluable for monitoring human immunodeficiency virus (HIV) infection, key pathogenesis events and most viral replication take place in lymphoid tissues. Decreases in virus load associated with therapy occur in plasma and tissues, but persistent latent infection and ongoing viral replication are evident. Many unanswered questions remain regarding mechanisms of HIV-associated lymphocyte depletion, but partial CD4⁺ cell reconstitution after therapy likely reflects retrafficking from inflamed tissues, increased thymic or peripheral production, and decreased destruction. Rapid establishment of latent infection and the follicular dendritic cell–associated viral pool within lymphoid tissues suggest that only early intervention could substantially alter the natural history of HIV. If therapy is started prior to seroconversion, some individuals retain potent HIV-specific cellular immune responsiveness that is suggestive of delayed progression. Although complete virus eradication appears out of reach at present, more attention is being directed toward the prospect of boosting HIV-specific immune responses to effect another type of “clinical cure”: immune-mediated virus suppression in the absence of therapy.

The ability to quantify HIV genetic material in plasma (virus load) has provided a clinical assay with practical applications [1] and an experimental tool for studying pathogenesis [2, 3]. In many cases, highly active antiretroviral therapy (HAART) results in a reduction of plasma virus load to less than the current assay detection thresholds. Because an “undetectable” virus load that occurs during therapy does not reflect a clinical cure, novel approaches are necessary to determine the nature of persistent immunodeficiency in this setting.

In parallel with these developments, a different set of questions has evolved regarding obstacles on the path to long-term control over viral replication: Which early immunologic events are critical determinants for the course of chronic disease? What is the mechanism of HIV-associated CD4⁺ depletion, and what is the source and potential extent of the “partial immune reconstitution” of CD4⁺ cells after HAART? What are the reservoirs for persistent infection in the face of potent plasma virus load suppression? What role will immune-based strategies play in improving long-term outcomes? This review focuses on recent insights from studies involving lymphoid cells and tissues that shed light on HIV disease mechanisms and provide new perspectives on these unresolved questions.

CLINICAL COURSE OF PROMINENT LYMPHOID TISSUE

Generalized lymphadenopathy, tonsillar enlargement, and splenomegaly were prominent features in the ear-
liest clinical descriptions of acute HIV infection [4], and French investigators initially referred to the causative agent of AIDS as “lymphadenopathy-associated virus” [5]. Lymphadenopathy is described in >75% of reported cases of acute HIV syndrome; it is the second-most-common reported feature after fever [6].

Although there is often a gradual decline in lymphadenopathy after seroconversion, enlarged lymph nodes may persist throughout the course of disease. Enlarged lymph nodes within the salivary glands can result in salivary duct obstruction and the formation of large lymphoepithelial cysts [7, 8]. Previously, generalized adenopathy was believed to be associated with disease progression and served as a clinical marker for HIV staging [9], but this is an outmoded concept, because there is no evidence that chronic adenopathy has prognostic implications. In fact, in late-stage HIV disease, the architecture of lymph nodes is completely disrupted, germinal centers involute, and adenopathy may become less prominent [10]. Precipitous or asymmetric lymph node enlargement in later stages of disease often suggests an opportunistic infection or neoplastic condition rather than HIV disease progression alone. In early or advanced HIV infection, substantial intrathoracic, intra-abdominal, or retroperitoneal adenopathy should also prompt an evaluation for other infectious or neoplastic complications.

Splenomegaly is also very common among patients with early, asymptomatic HIV infection (prevalence, 23%, as determined by means of physical examination, and 66%, as determined by means of ultrasound). Like adenopathy, uncomplicated chronic splenomegaly in this setting does not appear to correlate with specific clinical events or to predict more rapid disease progression [11].

**LYMPHOID TISSUES IN TRANSMISSION AND EARLY INFECTION**

Expansion of lymph nodes in patients with acute infection corresponds with proliferation of HIV-specific lymphocytes and follicular hyperplasia; however, inflammatory cell recruitment from the circulation is probably largely responsible. Virus likely spreads to regional lymphoid tissues within hours of mucosal acquisition (figure 1). In primate simian immunodeficiency virus (SIV) models, virus replication is detectable in iliac lymph nodes within a few days of vaginal inoculation [12] and numerous infected cells are present in tissues in <2 weeks [13]. Detectable viremia likely follows lymph node involvement; it typically occurs in humans within 1–2 weeks of genital acquisition [6, 14].

Individual HIV isolates may use different pathways to reach lymphoid tissues. The affinities of HIV strains for distinct host receptors influence cellular tropism and, therefore, impact on the patterns of HIV tissue involvement. Previously, the only known receptor for HIV was CD4, which normally mediates interactions between T helper cells and major histocompatibility complex class II molecules on antigen-presenting cells. Recent discoveries, however, reveal HIV entry into cells also requires one of the chemokine receptors (CCR5 and CXCR4) [15–17]. HIV transmission frequently involves virus strains that use CCR5 (R5 viruses, previously designated “macrophage-tropic”) [18]; a specific CCR5 mutation confers relative protection from infection [19]. Although viruses that bind CXCR4 (X4 or “T-cell tropic” viruses that induce syncytium formation in cell cultures) may predominate the late-stage disease virus pool [20], there is a selective advantage in the transmission of R5 over X4 variants in the setting of bloodborne, sexual, and maternal or fetal HIV exposures [21, 22].

Following mucosal exposure, HIV or SIV may first come into contact with monocyte/macrophage cells [12, 23]. Particularly noteworthy are Langerhans’ cells, which are potent T cell–activating interdigitating dendritic cells found in the lamina propria [24, 25] that express CD4 and CCR5 [26]. HIV-infected tonsil and adenoid tissues contain dendritic cells that are capable of transferring HIV infection to CD4 cells [27], which relates to transmission via oral sexual contact and may be analogous to the pathogenesis of genital transmission. Gen-
hilial and rectal mucosa dendritic cells express a receptor that enables transfer of HIV infection to CD4+ cells without necessitating virus entry into the mononuclear cells [28].

Langerhans’ cells that bear HIV particles may be carried from genital mucosa to lymph nodes via afferent lymphatics (figure 1). Although the interdigitating dendritic cells in skin are a different type of cell than are follicular dendritic cells (FDC) that are found in lymph nodes (figure 2), there is evidence from murine models that certain mucosal monocytes are capable of migrating to lymph nodes where they mature into potent T cell–activating, antigen-presenting cells [29, 30]. Activated T helper cells can then facilitate humoral and cellular immune responses against the invading pathogen. HIV-specific T cells interact with antigen-specific B cells on the border of lymphoid follicles in the cortex, and B cell proliferation results in follicular hyperplasia. This interaction results in HIV-specific antibody responses. Once activated in this process, however, T cells are much more susceptible to HIV infection than are resting T cells [31].

This model, which involves transfer of HIV-1 from monocytes to T helper cells, may not be essential to HIV-1 pathogenesis, because CD4+ T cells that express CCR5 are also present at mucosal surfaces during inflammatory disease [30]. This is potentially pertinent to the increased risk of sexual HIV transmission in the presence of ulcerative genital infections [32]. Once T cells become infected by this direct route, HIV dissemination via the bloodstream would quickly follow without the need for monocyte intermediaries. When SIV-infected primates were euthanized on selected days after the vaginal inoculation of a dual-tropic virus strain, ~10% of infected lymphoid tissue cells were CD68+ (monocyte/macrophage lineage), but the majority were CD4+ lymphocytes [13]. Studies of human lymph nodes in cases of established HIV infection suggest that the majority of infected cells are lymphocytes [33], but monocyte/macrophages in tissue may harbor virus when plasma viremia is relatively low [34], and they may increase virus production during opportunistic infections [35].

Although most HIV studies have focused on blood and lymph node compartments, the majority of lymphoid cells are contained in gut-associated lymphoid tissue. Because there are higher concentrations of activated CD4+ T cells in the gut than there are in peripheral nodes, this tissue may be a major target during acute HIV infection. In the rhesus macaque, a decrease in the ratio of CD4+ cells to total T cells occurs near the intestinal epithelium within days of acute SIV infection, before similar shifts are observed in peripheral lymph nodes or the spleen [36]. The same phenomenon may occur in the human gut during early HIV infection [37].

Changes in lymph node architecture and virus distribution in lymphoid tissue during the prolonged course of disease could reflect changes in viral tropism. The transition from R5 viruses to X4 or dual-tropic viruses during the course of years in some patients appears to herald more rapidly progressive disease [20], but what drives this evolution in cellular tropism remains unclear [38–40]. Ex vivo lymphoid tissue models suggest that R5 virus causes less cytopathicity than does X4 virus [41, 42]. Because CCR5+ cells make up a minority of total lymph node cells, an alternative view is that loss of these cells is simply not as disruptive to lymph node architecture [43, 44].

Figure 2. Changes in lymphoid architecture during the course of HIV infection and treatment. Lymph node enlargement is a common manifestation of acute HIV infection. Note follicular dendritic cells (FDC) networks where antigen-presenting cells interact with naïve T cells (inset; viral particles/proteins represented by white triangles) and high concentrations of virus concentrated around germinal centers (represented by gray-white “clouds”). In cases of advanced disease, the architecture tends to involute, with fewer discrete follicles, loss of the FDC network, and more scattered distribution of infected cells (white circles). Successful virus suppression during therapy results in a partial reversal of this disorganization.

EARLY DETERMINANTS OF VIRAL PERSISTENCE AND STEADY STATE VIRUS LOAD

Lymphoid tissue biopsies obtained from animal models and humans with acute infection reveal infected “activated” and “resting” CD4+ T cells [13]. The latter cells may have been infected while “activated” and then returned to a “resting” state. HIV-infected resting T cells, while not actively contributing to viremia, may represent the major challenge to curative therapy. Inhibitors of viral enzymes (reverse transcriptase and protease) have no effect on a cell with integrated but quiescent viral genetic material. The rapid establishment of a latently HIV-infected cell pool, persisting long after initiation of suppressive antiretroviral therapy, has been well documented [45–47]. The life span of latently infected cells may be quite long [48, 49].
because they are also not conspicuous targets for immune surveillance.

It has been estimated that T cells in the circulating blood represent only 1%–2% of the total body pool, whereas the vast majority of potential HIV target cells are localized to tissue. Although SIV-infected primate models demonstrate that occasional infected cells can be found in virtually every organ system in subjects with late-stage disease, T cells in lymphoid tissues apparently remain the predominant contributors to the total body burden of viral replication [33, 50]. Patterns of infected cell distribution in nodes and the spleen suggest localized propagation of HIV takes place within lymphoid tissues [33, 51].

Increasing evidence points to the greater importance of HIV-specific cellular immune responses, relative to humoral responses, in determining the course of early events in HIV disease. Because T helper cells, which are the orchestrators of adaptive immune responses, are the principal targets for HIV, an infected host may rapidly lose the ability to mount potent and durable HIV-specific cellular immune responses. HIV-infected individuals generally do not maintain HIV-specific CD4+ responses detectable by conventional methods [52]. Rare individuals who maintain low virus levels without therapy and who have delayed clinical progression tend to possess unusually strong in vitro HIV-specific CD4+ lymphoproliferative responses [52, 53]. When aggressive antiviral therapy is instituted very early, before seroconversion, selected individuals retain potent HIV-specific CD4+ responsiveness analogous to the situation in “long-term nonprogressors” [52]. Preserving adequate T cell help may be critical in the promotion or stabilization of clonal expansion of effector cells, particularly antigen-specific CD8+ cytotoxic T cells (CTL) that are known to limit dissemination in other viral infections.

Exploratory clinical trials that have evaluated the effects of infusing HIV-specific cytotoxic T lymphocytes (CTL) in HIV-infected subjects suggest that the interactions between HIV-infected cells and HIV-specific CTL occur predominantly within lymph nodes [54]. Therefore, the key immunologic events in HIV infection—the complex interplay of viruses infecting T cells, priming T cells for antiviral responses, and the targeted lysis of infected cells via CTLs—all are centered in lymphoid tissues.

**VIRAL DYNAMICS AND LYMPHOID TISSUES**

A quantitative technique, which is based on in situ hybridization for HIV RNA and computerized image analysis, has been developed to study the distribution and dynamics of HIV-infected cells in tissues. Diffuse signal in germinal centers is interpreted as virus in the network of extracellular dendrites surrounding FDC, whereas a discretely localized signal denotes intracellular infection [55]. This experimental approach, as well as others, demonstrates that the majority of viral signal in lymphoid tissues during early disease is present on the surfaces of FDC (figures 2 and 3), compared with a relatively small number of productively infected tissue cells.

PCR assays for HIV RNA reveal high levels of virus in blood at all stages of untreated disease [56]. Furthermore, the large FDC-associated viral pool appears to be fully established extremely early—within weeks of initial infection—rather than developing gradually [57]. The increasing level of free virus in plasma during the course of untreated disease likely reflects the progeny from an increasing number of infected lymphoid tissue cells [58] rather than the transition of viral replication from tissues to blood.

Although some investigations suggest slower virologic clearance from lymph nodes than from plasma during therapy [59, 60], it is now generally accepted that highly potent combination therapy suppresses virus levels in lymphoid tissues to a similar degree as that observed in plasma [61, 62]. In fact, the frequency of productively infected tissue cells falls with an initial half-life of ~1 day [63]. When computerized image analysis was applied to serial paraffin-fixed tonsil biopsies obtained from patients who were commencing therapy, not only were productively infected cells noted to decrease dramatically, but FDC-associated HIV RNA also decreased >1000-fold [63]. After therapy, rare positive cells were detected in tissues, but they appeared to contain relatively few copies of viral RNA. Another study attempted to analyze virus distribution and tissue architecture in more advanced patients, before and several weeks after initiating HAART [58]. Cervical lymph nodes were excised intact with minimal local tissue damage and “snap frozen” with liquid
LYMPHOID CELLULAR DYNAMICS IN PATIENTS WITH HIV INFECTION

One approach to the study of HIV-associated cellular dynamics is to evaluate the myriad toxic effects of the virus on CD4⁺ lymphocytes in vitro, including direct cytopathicity; CTL-mediated lysis of infected cells; indirect mechanisms, such as triggering of apoptosis by viral components (envelope glycoproteins or TAT); and interference with maturation of T cells from stem cells (figure 4). The challenge for future research is not merely to document whether these mechanisms exist in vitro, but to quantify relative contributions of each mechanism to HIV immunopathogenesis in different clinical settings.

Another approach to the problem is to develop accurate, reproducible methods to estimate lymphocyte “turnover” rates in HIV-infected patients, compared with uninfected controls. CD4⁺ T cell depletion typically occurs very gradually, leading to increased risk for opportunistic infections and neoplasms only after many years. It has been difficult to discern whether HIV-induced CD4⁺ T cell depletion is predominately related
to increased destruction (which might be secondary to direct cytopathicity or spontaneous apoptosis, for example) or to decreased production of lymphocytes. Establishment of the circulating T cell pool begins when pluripotent lymphocyte precursors produced in fetal bone marrow migrate to the thymus to undergo maturation. The key to this process is the rearrangement of T cell–receptor genes so that lymphocytes develop the ability to recognize specific foreign antigens, and distinguish them from self-antigens, before migrating to secondary lymphoid organs, including lymph nodes, the spleen, and gut-associated lymphoid tissue [71].

The role of the spleen in the pathogenesis of HIV remains poorly understood. HIV-infected patients who undergo splenectomy (because of immune-mediated thrombocytopenia, for example) typically have an abrupt increase in the absolute number of circulating CD4+ T cells [72, 73]. This likely reflects the release of a large number of CD4+ cells from splenic sequestration [72] rather than renewed production of T cells. Recommendations have been made to monitor the percentage of total lymphocytes that are CD4+ as a clinical benchmark in this setting because the absolute CD4+ count may be misleading after splenectomy [73]. Small, preliminary reports suggest the possibility that splenectomy may improve the course of HIV disease beyond merely elevating the absolute CD4+ cell count [74, 75]; however, larger studies are needed to confirm this observation.

The “tap and drain” hypothesis of HIV pathogenesis derived from an assumption that ongoing CD4+ cell losses over the prolonged course of HIV infection are nearly balanced on a daily basis by production of new uninfected CD4+ cells [2]. If viral cytopathic effects were concentrated on mature, activated T cells, then a major research focus should be the capacity to maintain substantial circulating CD4+ cell numbers over many years in the face of rapid HIV turnover. If the only source of adult T cell replenishment were proliferation of the differentiated peripheral T cell pool, then this would result in an inability to develop efficient immune responses to newly encountered pathogens (and also an inability to replace HIV-specific lymphocytes, which are apparently lost during initial HIV infection).

T cells are generally understood to be long-lived cells that are not constantly replenished like other blood cells [76–79]. Thymic epithelial tissue gradually shrinks with age, whereas the perivascular space, containing adipose tissue and inflammatory cells that migrate in from the periphery, increases during adulthood (figure 4) [80]. There is a corresponding age-dependent decline in the capacity for proliferation of naïve T cells following myeloablative chemotherapy [81], which supports the hypothesis that most T cells in an older adult represent original fetal thymus cells or daughter cells derived from clonal proliferation of these cells.

CT scans suggest compensatory thymic hypertrophy in response to depletion of CD4+ cells during the course of HIV disease in some adults [82]; however, autopsy materials from adults with AIDS have not always shown evidence of significant thymopoiesis, regardless of the thymic CT appearance [83]. Furthermore, myasthenia patients who undergo thymectomy before becoming HIV infected or early in the course of infection manifest gradual CD4+ cell depletion typical of HIV-infected subjects without thymectomy and are not precluded from favorable CD4+ count responses to antiretroviral therapy [83].

The length of telomeres, DNA segments on the ends of chromosomes, inversely correlates with the number of previous cell proliferation cycles in a cell line. Cellular turnover estimates based on telomere length suggest the CD4+ cell pool is not turning over rapidly in HIV-infected patients [84, 85]. Other surrogate assays for heightened cellular activation and proliferation, using isotope-labeled glucose uptake [86] or the nuclear antigen Ki67 [87, 88], however, arrive at the opposite conclusion. Cellular proliferation assays based on bromodeoxy-uridine lymphocyte labeling suggest that SIV-infected macaques have heightened CD4+ cell proliferation compared with uninfected controls [89, 90]. The majority of increased CD4+ cell proliferation may be in the “memory” pools of CD4+, and especially CD8+, populations [91]. This “turnover” could reflect clonal proliferation of preexisting T cells rather than production of “new” lymphocytes as a homeostatic response to depletion.

A novel assay for excisional DNA products of T cell receptor rearrangement may distinguish between peripheral expansion of existing naïve T cells and thymic production of new cells [92]. Theoretically, T cell–receptor excision circles (TRECs) are present preferentially in cells recently emigrated from the thymus and then progressively dilute out as cells undergo cycles of clonal proliferation. Predictably, adults have an age-related decrease in recent thymic emigrants. HIV-infected adults have a greater deficit in residual thymic function by this assay. Although the mean number of excisional DNA by-products is lower in HIV-infected adults than in age-matched controls, there is considerable overlap in pooled results, which suggests that limited thymic regenerative capacity is not the universal cause of CD4+ depletion in patients with AIDS [93].

**CHANGES IN LYMPHOID TISSUES AFTER THERAPY**

T helper lymphoproliferative responses to specific antigens are significantly improved in many cases following a few months of effective virus suppression [94–96]. The levels of immune activation markers (such as β2–microglobulin and neopterin) and proinflammatory cytokines (such as tumor necrosis factor–α, interferon γ, interleukin (IL)-1, IL-6, macrophage inflammatory protein–1 α) decrease in blood and tissues as the
The susceptibility of lymphocytes to virally mediated apoptosis decreases during therapy as well [100], which may correlate with improved CD4+ T cell numbers and decreased disease progression [101]. Lymphoid tissue architecture returns toward normal following potent therapy, with more organized lymphoid follicles and FDC networks (figure 2) [102]. These immunologic improvements are reflected by decreasing numbers of opportunistic infections [103], the potential to safely interrupt prophylactic antibiotics for certain infections [104, 105], and, most important, decreased AIDS-related mortality [106, 107].

The initial increase in cell counts that occurs when patients begin antiretroviral therapy consists of all lymphocyte populations (B cells, CD4+ T cells, CD8+ T cells), not just CD4+ cells. The majority of the increase in the CD4+ cell fraction represents “memory” cells and not “naive” cells, which suggests that initial improvement is due to redistribution of cells from tissues [108]. The anti-inflammatory effects of the suppression of virus load are associated with decreased expression of adhesion molecules (VCAM and ICAM) that normally mediate homing of lymphocytes to inflamed tissues [98]. It is possible that the increase in blood lymphocytes is commensurate with the decrease in total lymphocyte populations in lymph node specimens after therapy.

Studies of mycobacterial infections, which also involve sequestration of T cells at inflamed tissue sites, provide interesting parallels [109–111]. Patients with active tuberculosis may have “anergy” to mycobacterial antigen skin testing, whereas cells with tuberculosis-specific lymphoproliferative responses can be detected simultaneously in inflamed tissues. After antibiotic treatment and decreased inflammation in infected tissues, pathogen-specific lymphocytes in local tissue sites decrease simultaneously with the reversal of systemic “anergy.”

Improved immune responses to preexisting pathogens may also bring about heightened clinical manifestations of infection soon after initiation of HAART. Patients with advanced immunosuppression may develop localized adenitis due to Mycobacterium avium complex infection soon after commencing HAART [103, 112, 113]. Examination of biopsy specimens of infected lymphoid tissue reveal organized granulomas and purulent drainage from the site, which is quite different from what is observed during the unchecked bacteremia typically associated with Mycobacterium avium complex infection in patients with AIDS. Presumably, these patients had subclinical systemic infections that manifested as inflammatory localized masses only after the resurgence in circulating T cell numbers and function on HAART. Similarly, some HIV-infected patients with tuberculosis who start to receive HAART have paradoxical expansion of inflammatory masses [114–116], whereas those with a history of cytomegalovirus retinitis may develop inflammatory vitreitis [103, 117, 118]. High fevers and localized inflammatory reactions in this special setting may respond to corticosteroid therapy [114]. Therefore, although the decrease in HIV-induced immune activation releases sequestered lymphocytes from lymphoid tissue back into the circulation, the improvement in cellular immune responses may also allow localized inflammatory manifestations to develop where there was virtually no immune response to an opportunistic pathogen before.

**TREATMENT AND LYMPHOCYTE “TURNOVER RATES”**

After several months of therapy, many patients have a gradual return of T cells with “naive” markers, which suggests much more than just an incidental redistribution of cells. It appears that residual thymic (or extrathymic) productivity contributes to a gradual return of “naive” T cells in blood [94] and in lymphoid tissues [119] after therapy. It is unclear to what degree the detection of new “naive” T cells is actually due to the transition of “memory” cells back to resting status [77, 79, 120]. Studies that have attempted to resolve whether a therapeutic increase in lymphocyte counts represents clonal proliferation of peripheral cells versus thymic production of cells have provided conflicting conclusions [79, 121, 122].

After virus suppression occurs during HAART, there is a return to supranormal levels of recent thymic emigrants detectable in blood and lymphoid tissues [92], which suggests that HIV-infected adults maintain some capacity to produce “new” T cells with novel receptor specificities. Use of TREC signals as an estimate of turnover, however, suggests significant variability from patient to patient. Though HIV-infected individuals who had normal range values for recent thymic emigrants before therapy may have no change after therapy, some patients with a baseline deficit demonstrate a modest increase while receiving therapy [93]. Furthermore, mathematical modeling suggests the key factor in determining TREC frequency could be the rate of naïve T cell proliferation, rather than changes in thymic output [91]. In other words, the assumption that TREC decrease at a nearly constant rate may be flawed. High levels of HIV antigen may drive ongoing cellular proliferation, resulting in a relative decrease in TREC frequency secondary to a dilutional effect (TRECs are not passed on to each daughter cell), regardless of the status of thymic output. Conversely, the relative increase in the frequency of TREC-containing cells after therapy may reflect the decrease in antigen-driven cellular proliferation rather than a reversal of thymic impairment.

In the setting of untreated HIV infection, the half-life of T cells, as determined by deuterated glucose labeling, is diminished without compensatory increases in production [123]. Soon after therapy is started, regenerative production rates gradually increase [123]. There is evidence from experiments...
that have involved fetal thymus organ cultures that HIV-1 can interfere with T cell production at the level of progenitor cells, and that, even among adults, HAART administration can improve the capacity to renew this activity [124]. At the same time, however, the much more dynamic transitional proliferation driven by immune activation may be decreased in the presence of therapy [51, 91]. Naïve T cells that become activated or that are caught in the transitional state of becoming effector cells also express the Ki67 marker, which may have previously resulted in erroneous assertions about overall turnover of the T cell population rather than short-term fluctuations in immune activation [51].

Beyond 12 months of therapeutic virus suppression, the “turnover rate” and the absolute production rate have returned toward normal, determined by use of the radiolabeled glucose uptake assay [123]. In general, “naïve” T cells have a longer half-life (in the range of 6 months) than do “memory” cells (~1–2 months). The latter half-life estimate, however, is complicated by the heterogeneity of the “memory” cell population, which contains a wide spectrum of cells from effector cells recently exposed to antigen (soon to undergo activation-induced cell death) to long-lived resting memory cells. There is also a heterogeneous response from patient to patient, and this appears to be at least partially explained by the thymic capacity. That is, patients who have a substantial thymic shadow seen on radiographic imaging have a larger number of “naïve” T cells, and this preponderance of “naïve” cells, in turn, is associated with a slower turnover of the overall circulating T cell population [123]. This observation is supported by other recent studies that have suggested that children may have a substantial increase in both TREC and “naïve” T cells while receiving therapy [125, 126], and that, among adults, those with larger CT thymic shadows have a tendency to demonstrate more abrupt and significant “naïve” T cell increases while receiving therapy [127].

Therefore, the effects of HIV on cellular dynamics are a complex interplay of increased destruction and interference with production. The interpretation of cellular dynamics is further complicated by heterogeneity both at the intercellular and interpatient levels. Any reconstitution of the naïve T cell pool that occurs while the patient is receiving HAART alone is likely to be gradual, limited in diversity, and potentially skewed relative to the preinfection T cell repertoire.

**FUTURE TREATMENT STRATEGIES**

Where do these immunologic insights lead us in terms of future treatment strategies? One hypothesis proposes immune activation strategies may “flush out” latently infected cell pools. Patients with established HIV infection who receive HAART plus cycles of IL-2, which is a T cell growth factor and immunoactivating cytokine, have significantly fewer detectable HIV-infected blood and lymph node cells than do those patients who receive HAART alone [128]. Nonetheless, interrupting HAART in a small number of patients who were treated with adjunctive IL-2 still resulted in virus rebound [129]. Although the possibility remains that virus resurgence could be delayed compared with control subjects when comparisons are made in larger clinical trials, concern has been raised that activation events may replenish the latent cell pool rather than purge it [130].

It remains plausible that another type of “clinical cure” can be approached without necessarily resulting in absolute clearance of the latently infected cell pool. Recent reports demonstrate that intermittent therapeutic interruptions may restimulate effective HIV-specific immune responses, at least in the setting of very early infection, to the degree that immediate virus rebound that occurs while the patient is not receiving therapy may not be observed [131–133]. Studies are underway to investigate the impact of immune-based strategies—cytokines, vaccinations, and scheduled treatment interruptions—on virus rebound kinetics when therapy is discontinued. What is necessary is a better understanding of a viral antigen threshold that might generate and sustain efficient HIV-specific immune responses without causing irreversible damage in the process. A further challenge is that this elusive threshold may differ for each individual case on the basis of reciprocal relationships between different viral isolates and host immunologic factors.

**CONCLUSIONS**

Improvements in HIV therapy have benefited patients directly while also serving as a research probe in studies of viral replication and lymphocyte dynamics. Tissue sites of viral replication represent the driving force behind the total body viral burden. Lymphoid tissue analysis provides a perspective on patterns of viral distribution and cellular interaction not revealed with blood assays alone. Unique opportunities to study the critical first encounters between the virus and the immune system during acute infection may be particularly instructive for designing future therapeutic strategies. The evaluation of concurrently obtained blood and tissue samples will likely continue to be an important analytical approach to deciphering the complexities of HIV pathogenesis.

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