

T CELL DYNAMICS IN HIV-1 INFECTION*

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■ **Abstract** In the absence of antiretroviral treatment, HIV-1 establishes a chronic, progressive infection of the human immune system that invariably, over the course of years, leads to its destruction and fatal immunodeficiency. Paradoxically, while viral replication is extensive throughout the course of infection, deterioration of conventional measures of immunity is slow, including the characteristic loss of CD4⁺ T cells that is thought to play a key role in the development of immunodeficiency. This conundrum suggests that CD4⁺ T cell-directed viral cytopathicity alone cannot explain the course of disease. Indeed, recent advances now indicate that HIV-1 pathogenesis is likely to result from a complex interplay between the virus and the immune system, particularly the mechanisms responsible for T cell homeostasis and regeneration. We review these data and present a model of HIV-1 pathogenesis in which the protracted loss of CD4⁺ T cells results from early viral destruction of selected memory T cell populations, followed by a combination of profound increases in overall memory T cell turnover, damage to the thymus and other lymphoid tissues, and physiological limitations in peripheral CD4⁺ T cell renewal.

INTRODUCTION

The hallmark of HIV-1 infection is the progressive depletion of CD4⁺ T cells. Yet the extent and nature of this depletion, and the mechanisms by which it arises, remain highly controversial (1–3). HIV-1 infection also induces profound qualitative changes in CD4⁺ T cells, and in most other elements of the immune system too, yet the mechanisms responsible for immunodeficiency are still not well characterized. No viral infection in humans, either acute resolving or chronic persistent, whatever the viral load, is known to cause such profound and inevitable CD4⁺ T cell loss, except HIV-1. Even HTLV-I, which exhibits the same fastidious tropism

for CD4⁺ T cells and renders them targets for HTLV-specific CD8⁺ T cells, results in CD4⁺ T cell lymphocytosis rather than lymphopenia (4). HTLV, however, is not cytopathic, whereas HIV-1 is well known to infect and kill primary CD4⁺ T cells (5). Yet, as we discuss below, cytopathicity alone is unlikely to provide a satisfying explanation for the course of the disease. A more complete explanation would undoubtedly highlight T cell dynamics as a central factor in HIV-1 pathogenesis, affecting virtually all aspects of the infection, including (a) viral dynamics, via the regulation of viral target densities; (b) the development, maintenance and effectiveness of HIV-1-specific cellular immunity; and (c) the mechanisms that maintain the integrity of the naive and memory T cell pools.

What singular feature underlies the dynamics of T cells and virus at the microscopic level that sets this infection apart from other viral infections? Is it the unique combination of the destructive potential of the virus with the intimate, multifaceted relationship between T cell activation and virus replication? Introduction of virus into the host results at first in explosive activation and replication. The dynamics of both T cells and virus then appear to stabilize, but the initial events that establish the chronic infection leave a profound and long-lasting impact on the immune system that may set the stage for the subsequent slow progression of the disease.

This review examines HIV-1 infection from the perspective of lymphocyte dynamics, linking recent empirical and conceptual advances in HIV-1 biology with new insights into T cell dynamics to provide an immunological view of the pathogenesis of this deadly infection. Notably, most studies of such dynamics have been performed only in peripheral blood during the chronic stage of HIV-1 infection. Therefore, we attempt to deconstruct HIV-1 disease into its acute and chronic phases, which manifest markedly distinctive viral and lymphocyte dynamics. We address the contributions of the virus itself, T cell activation, T cell reconstitution, and target cell availability in the shaping of these dynamics during each phase. We highlight the major role of persistent immune activation during the chronic phase, but also suggest that profound memory CD4⁺ T cell destruction occurring during the acute phase may have a crucial impact on the subsequent course of the infection.

THE PHASES OF HIV INFECTION

The course of untreated HIV-1 infection in the majority of individuals is illustrated in Figure 1 (see color insert). It begins with an acute symptomatic illness, lasting only a few weeks, which is associated with a high viremia, a sharp drop in peripheral blood CD4⁺ T cell counts (6–14), establishment of a reservoir of latently infected CD4⁺ T cells (15–18), and development of an HIV-1-specific immune response (19–22). This is followed by a 100- to 1000-fold fall in viral load, a partial rise in peripheral blood CD4⁺ T cell counts and then a generally asymptomatic phase of chronic infection, lasting on average 10 years, which is marked by slowly falling peripheral blood CD4⁺ T cell counts and slowly rising viremia. As peripheral

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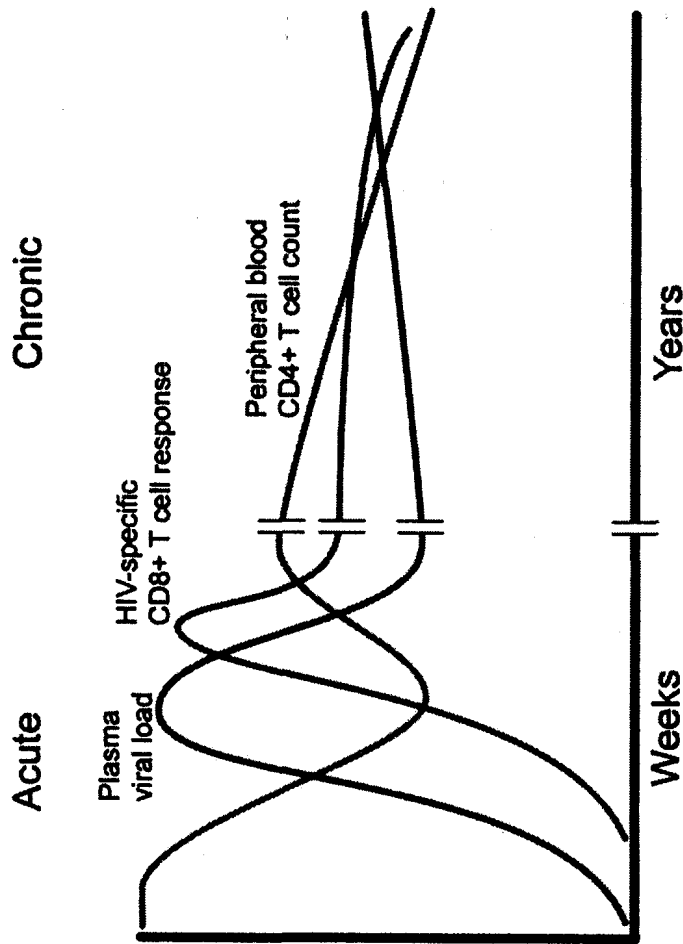


Figure 1 The natural history of typical HIV-1 infection depicting changes in plasma HIV-1 viral load, peripheral blood CD4⁺ T cell count, and HIV-1-specific CD8⁺ T cell response.

blood CD4⁺ T cells decline to less than 200 cells/ μ l, when the total number of CD4⁺ T cells in the body has been reduced by at least half (23), the opportunistic tumors and infections that characterize AIDS beset the individual. This often occurs concomitantly with a precipitous rise in viremia and a crash in peripheral blood CD4⁺ T cell counts (24). Although these phases are very different in terms of their viral and T cell dynamics, they are obviously interdependent in many of their pathogenic mechanisms, and one cannot be considered in the absence of the other.

THE ACUTE PHASE OF INFECTION

Establishment of the Infection

Exposure to HIV-1 is primarily through the mucosal route, either gastrointestinal or reproductive, which is thought to result in initial local replication of the virus within target cells of the mucosal tissue. The establishment of infection is dependent on the target cells' expression of CD4 and a chemokine receptor (CCR) (25). Although a variety of CCRs can serve as coreceptors *in vitro*, CCR5 and CXCR4 are likely to be the major receptors used by HIV-1 *in vivo*, with CCR5 almost always being the initial target coreceptor for naturally transmitted virus (25–30). Although HIV-1 usage of CXCR4 develops over time in many individuals, significantly expanding the target cell repertoire of the virus and accelerating disease progression (31–33), R5-tropic strains predominate also in chronically HIV-1-infected patients (34) and cause CD4⁺ T cell depletion.

Little is known about the primary pathophysiological events of lymphocyte and viral dynamics in acute, as opposed to chronic, HIV-1 infection in humans. Thus, we may turn to recent studies that have shed light on the earliest events in SIV infection of rhesus macaque monkeys. Whereas the kinetics of viral replication and disease progression differ somewhat in these two systems, and indeed in different viral strains within each system, the fundamental pattern remains the same (12, 35). CXCR4 conversion is rare in most commonly studied SIV strains but is also not a prerequisite for peripheral CD4⁺ T cell depletion and progression to AIDS (28, 36, 37). After intravenous, intrarectal, or oral inoculation of SIV, it appears that the major, and possibly the earliest, cellular substrates for the initial burst in viral replication are CD4⁺ T cells in the lamina propria of the mucosal tissues, rather than nonlymphocyte targets such as dendritic cells (38–44). Similarly, 2–3 days after intravaginal inoculation of SIV, the majority of productively infected cells are CD4⁺ T cells in the lamina propria of the endocervix (45). This is not altogether surprising, considering that viral replication in dendritic cells is considerably less efficient than in CD4⁺ T cells and is actually blocked in mature dendritic cells (46).

However, whereas dendritic cells may not be the initial target for viral replication, they may act as local facilitators for productive infection. The binding and internalization of intact virions via DC-SIGN enhances infection *in trans* of CD4⁺ T cells (47–49). In transmission of SIV across the vaginal epithelium, which is considerably thicker than the friable rectal and cervical epithelia, the picture may be

different (50). Intraepithelial Langerhans cells can become productively infected with SIV at a low level by 18 h postinoculation, after which they may mature and migrate directly to local lymph nodes where the virus can be propagated to resident CD4⁺ T cells (51).

The Tissue Distribution of Target Cells Directs Viral Replication

After establishment of infection in the mucosa, SIV is rapidly disseminated over the next 2 weeks, infecting increasing numbers of CD4⁺ T cells in both local and distant lymphoid tissue including lymph node, thymus, spleen, and mucosal tracts (41, 42, 45, 52). There is also evidence from lymph node biopsies from small cohorts of acutely infected humans that T cells are the major target for and source of HIV-1 (45, 53), resulting in the early establishment of a pool of latently infected CD4⁺ T cells (15). Indeed, *in situ* hybridization for SIV and HIV-1 RNA in combination with quantitative image analysis shows that the frequencies of infected T cells in lymph node and mucosal tissue are considerably higher than during the asymptomatic phase (23, 44, 45, 53). Furthermore, HIV-1 can accumulate to very high levels in the pool of lymph node follicular dendritic cells during acute infection (54) and may become a major reservoir of infectious HIV-1 in the late stages of infection (23, 55–59).

Direct tracking of HIV-1 viral genotypes in local microenvironments indicates that cell to cell transmission of virus is a local phenomenon, wherein an infected cell releases virus that efficiently infects only nearby targets (60). This implies that propagation of infection depends upon local cell interaction and local target cell densities, rather than on the overall number of targets in the body (61). During acute infection the mucosal tissues presumably offer, in effect, a continuum of target cells through which the virus rapidly propagates and multiplies. The target cells for this explosive dissemination are, as discussed above, predominantly CCR5⁺ CD4⁺ T cells, a memory T cell subset that is infrequent in peripheral blood, lymph node, and spleen but that accounts for almost all CD4⁺ T cells in other tissues including the mucosal surfaces of the intestinal, respiratory, and reproductive tracts (40, 62–67). Figure 2 shows the distribution of CCR5 expression on peripheral blood T cells and lung lavage T cells, as an example of a mucosal site, in a healthy rhesus macaque (L.J. Picker, unpublished observation). CXCR4 levels tend to be low on mucosal tissue memory CD4⁺ T cells and much higher on memory CD4⁺ T cells in peripheral blood, lymph node, spleen, naive CD4⁺ T cells, and thymocytes (40, 63, 65, 66, 68).

Mucosal CCR5⁺ T cells qualify as targets for virus replication also by virtue of their activation phenotype. Many of these cells express surface antigens such as CD69 and HLA-DR (40, 43, 65) that have also been defined on peripheral blood T cells as markers of T cell receptor (TCR)-mediated activation, a cellular state generally thought to be a prerequisite for high-level lentiviral replication (69–71). However, the ubiquity of CCR5 expression on extra-lymphoid T cells suggests that

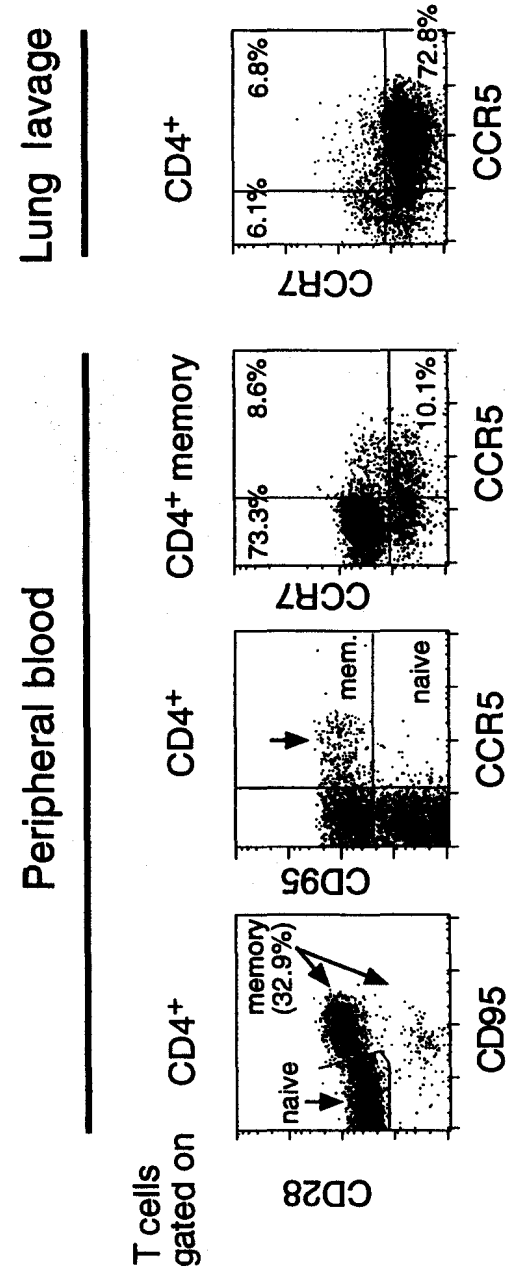


Figure 2 CCR5 is preferentially expressed by mucosal memory T cells. The phenotypic profiles of CD4⁺ T cells from peripheral blood and bronchoalveolar lavage specimens from a healthy rhesus macaque are shown (5000 events gated on total CD4⁺ T cells or CD4⁺ CD95^{hi} memory T cells). Note that peripheral blood contains both naive (CD95^{lo}) and memory (CD95^{hi}) CD4⁺ T cell subsets (106), the latter including a majority of the CD28⁺/CCR7⁺ “central” (lymph node homing) memory subset and a minority of CD28⁻/CCR7⁻ “effector” (extra-lymphoid tissue homing) memory subset. Whereas both central and effector memory T cells in blood can express CCR5, the frequency of this expression is about fivefold higher in the latter population (56% vs. 11%). In keeping with this, lung T cells are essentially 100% memory, the vast majority (~80%) lacking CCR7 and expressing CCR5.

it may be a general phenotype of tissue-infiltrating T cells (65, 72, 73), which often express so-called activation markers induced by environmental signals unrelated to suprathreshold TCR signaling. Indeed, the mucosal environment is rich in inflammatory cytokines, some of which activate T cells (74) and promote the infection and propagation of HIV-1 in resting CD4⁺ T cells (75, 76). Furthermore, HIV-1 gene products themselves such as Nef, Tat, Vpr and even Env induce TCR-independent T cell activation programs and/or viral transcription (77–85). Thus, it appears that even in the absence of full antigen-induced TCR-mediated activation, the phenotype and environment of mucosal CD4⁺ T cells makes them excellent substrates for HIV-1 infection. Together, the effects of antigen- and cytokine-mediated stimulation, as well as of HIV-1 proteins with the innate ability to activate T cells, will result in efficient production and propagation of the virus from infected cells to adjacent CCR5⁺ CD4⁺ T cells.

One final point to bear in mind in considering the impact of acute HIV-1 infection is that the gastrointestinal tract may be the largest lymphoid organ in the body. Indeed, it has been estimated that it contains, at steady state, at least 60% of the total body T cell load (86, 87). Furthermore, it has been shown that, after infectious antigenic challenge in mice, the epithelia of the gastrointestinal and respiratory tracts are the major site for the sequestration of memory CD4⁺ and CD8⁺ T cells (88–90). Thus, a large fraction of CD4⁺ T cells in the body reside in the mucosal tissues, express CCR5, and are prime targets for R5-tropic HIV-1 infection and replication.

The Dynamic Consequences of Being Targeted

The abundance of mucosal substrates for viral replication accounts for a profound impact of the virus on the immune system early after infection. In macaques infected with SIV, intravenously or via a mucosal route, there is a rapid and profound loss of intestinal CD4⁺ T cells, such that their numbers are almost entirely depleted by 3 weeks after infection (38, 39, 41, 42). Furthermore, because this loss is specific to CCR5⁺ CD4⁺ T cells, which are underrepresented in peripheral lymphoid organs compared with extra-lymphoid tissues, mucosal CD4⁺ T cell depletion is far greater in proportion than that seen in peripheral blood, lymph node, or spleen, and occurs sooner (39–41). However, those CD4⁺ T cells in peripheral lymphoid organs that do express CCR5 are indeed selectively depleted during acute infection (40). Interestingly, studies of lymph nodes in acutely SIV-infected macaques and early HIV-1 infection in humans have shown that many of the infected CD4⁺ T cells are not in an “activated state” by their lack of expression of Ki67 and HLA-DR (45). However, as discussed above, such markers may not fully reflect the actual activation state of T cells in their local environment of cytokines and viral gene products.

Figure 3 shows the depletion of CD4⁺ T cells in peripheral blood and lung lavage following intravenous infection of a rhesus macaque with the pathogenic SIV isolate SIVmac239 (L.J. Picker, unpublished observation). The analysis of pulmonary lavages following infection reveals that lung CD4⁺ T cells (which are

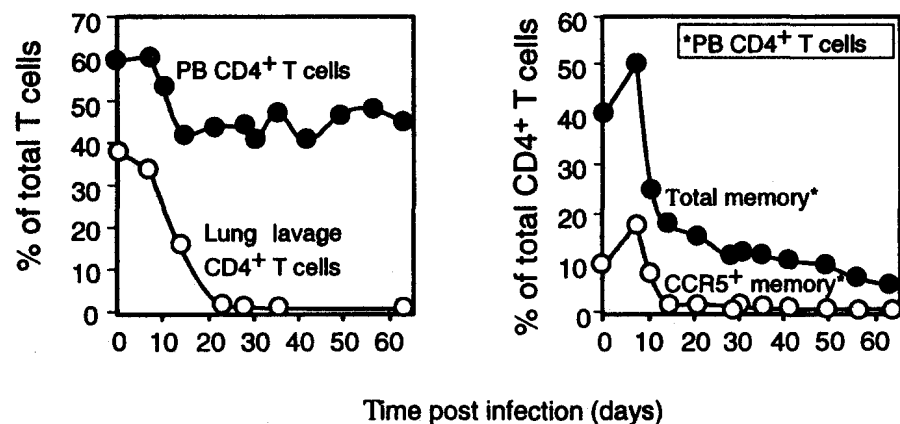


Figure 3 Loss of T cells in pathogenic CCR5-tropic SIV infection is rapid and reflects the distribution of the CD4⁺, CCR5⁺ memory T cell subset. CD4⁺ T cell population dynamics are shown following infection of the same animal shown in Figure 2 with pathogenic CCR5-tropic SIV. Note that the frequencies of CD4⁺ T cell in lung plummet in the first 3 weeks of infection, accompanied by only a very modest decline in the representation of the overall CD4⁺ T cell subset in peripheral blood (*left panel*). However, closer examination of the subset composition of the circulating CD4⁺ T cells (*right panel*) reveals a substantial decline of total memory CD4⁺ T cells and profound depletion of the CD4⁺, CCR5⁺ memory subset.

essentially all CCR5⁺) (Figure 2) are so rapidly depleted that they are almost entirely lost by day 21 of infection. By contrast, there is only a very modest decline in the representation of the overall CD4⁺ T cell subset in peripheral blood (Figure 3, *left panel*). However, closer examination of the subset composition of the circulating CD4⁺ T cells (*right panel*) reveals a profound depletion of the CD4⁺, CCR5⁺ memory subset. Thus, the primary SIV target population is systemically depleted in the first weeks of infection, and despite increased T cell proliferation (see below), these populations are not reconstituted. This confirms previous studies showing that regardless of the systemic or mucosal route of inoculation with SIV there is rapid and massive mucosal CD4⁺ T cell depletion in both gastrointestinal and respiratory tracts well before significant depletion occurs in lymph node and peripheral blood (41).

That the dynamics of CD4⁺ T cell depletion are determined by coreceptor usage of the virus has been strikingly illustrated by the use of chimeric viruses (SHIVs) consisting of an SIV backbone with an HIV-1 envelope. SHIVs have been constructed with envelopes that are exclusive in their usage of either CCR5 or CXCR4. As expected from infection with natural SIV, intravaginal R5-tropic SHIV infection results in rapid and profound depletion of intestinal CD4⁺ T cells, with little effect on those in peripheral blood and lymph node (91–93). X4-tropic SHIV infection, however, results in a similarly rapid and profound depletion of

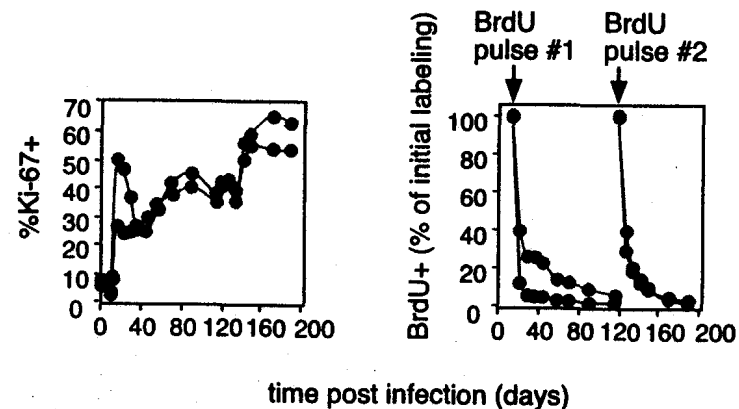
CD4⁺ T cells but affects mainly those in peripheral blood and lymph node, while leaving intestinal CD4⁺ T cell populations intact (91). Furthermore, the target cell population of X4-tropic SHIV is significantly expanded to include naive CD4⁺ T cells and thymocytes (94). The effect of HIV-1 coreceptor usage on target cell depletion has also been examined in vitro with lymph node (95, 96) and thymus (97) tissue-culture explants and in SCID-hu chimeric mice (mice implanted with human fetal thymus and liver) (98–102). Lymph node CD4⁺ T cells and thymocytes in these models are profoundly depleted by X4-tropic HIV-1 strains but only mildly depleted by R5-tropic HIV-1 strains.

The Dynamics of Depletion and Activation

Taken together, these data indicate that R5-tropic virus infection results in a systemic depletion of CD4⁺ memory T cells that is out of proportion to the decline in peripheral blood CD4⁺ T cell counts and is characterized by a particularly striking decline in the CCR5⁺ CD4⁺ memory T cell subset. This suggests that CD4⁺ T cell loss is essentially biphasic, with a rapid, massive but somewhat concealed depletion of preexistent T cell targets during acute infection, followed by the well-known slow decline of the remaining CD4⁺ T cells that characterizes chronic infection. Of the several hypotheses raised to explain CD4⁺ T cell loss in HIV-1 infection, those that invoke impaired production or chronic activation-induced cell death may have validity for the chronic phase of the infection but would clearly operate too slowly to account for the rapid kinetics of this depletion in acute infection. The almost complete elimination of mucosal CCR5⁺ CD4⁺ T cells within 2–3 weeks of infection suggests that SIV removes cells that would not have ordinarily died and may also remove cells that are mobilized to replace them. It is possible to examine the proliferation and turnover of the relevant T cell subsets in acute SIV infection by flow cytometric analysis of Ki67 expression and BrdU uptake and decay (103–105). Cellular BrdU incorporation after in vivo pulsing allows determination of the fraction of cells in S-phase during the pulse and provides a measure of their survival and proliferation. Expression of the cell cycle-associated nuclear antigen Ki67 is associated with cells that have predominantly undergone DNA synthesis in the past 3–4 days (106).

Figure 4 (see color insert) shows that after an initial slight decrease, by day 14 after infection with SIV there is a marked and sustained increase in the fraction of CD4⁺ and CD8⁺ memory T cells expressing Ki67 (L.J. Picker, unpublished observation). Subsequently, the frequency of proliferating CD4⁺ and CD8⁺ memory T cells are closely matched and slowly increase with time. Rhesus CMV infection elicits an analogous proliferative burst, but in this situation the virus is controlled and proliferation returns to baseline by day 56 postinfection. In both acute SIV and RhCMV infection, BrdU incorporation is correspondingly high in CD4⁺ and CD8⁺ memory subsets. However, although the decay kinetics of BrdU label (owing to death and proliferative dilution) are similar within the CD8⁺ and CD4⁺ subsets in RhCMV infection and the CD8⁺ subset in SIV infection, essentially

A. SIV(mac239) Infection



B. Rhesus CMV Primary Infection

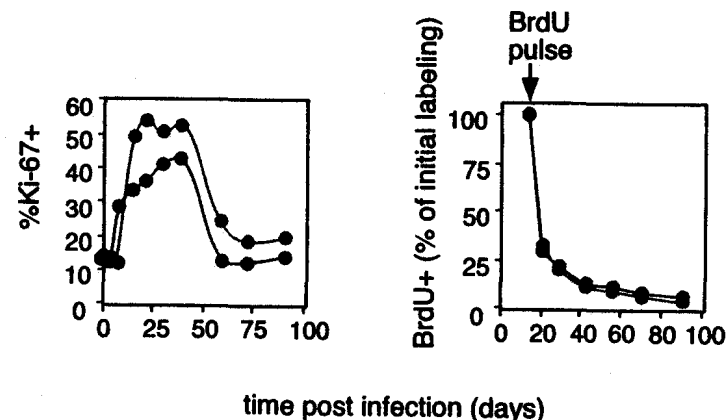


Figure 4 Memory T cell dynamics in progressive SIV and nonprogressive CMV infection. CD4⁺ and CD8⁺ memory T cells were followed for their expression of the proliferation marker Ki-67 or their incorporation of the thymidine analogue BrdU by multiparameter flow cytometry after primary infection with pathogenic SIV (A) or rhesus CMV (B). BrdU was given in single daily IV injections on postinfection days 10–13 (A and B) and days 115–118 (A only). CD4⁺ memory T cells are shown in blue, CD8⁺ memory T cells in red.

all circulating CD4⁺ BrdU-labeled memory T cells have disappeared from the SIV-infected macaque within one week.

This disproportionate loss of proliferating CD4⁺ memory T cells in acute SIV infection—over and above that expected for a given level of proliferation—is consistent with excess cell death, which is likely to be due to direct or indirect viral cytopathicity (5, 107–111) and CD8⁺ T cell-mediated destruction of infected CD4⁺ T cells (112). Additionally, in both CMV and SIV-infected macaques there was a marked increase in the number of peripheral blood and lymph node CCR5⁺ proliferating CD4⁺ T cells, but significantly, in SIV infection such CCR5⁺ expression is observed in the setting of progressive depletion of preexistent CCR5⁺ memory T cells (L.J. Picker, unpublished observation). Thus, the proliferative generation of new CCR5⁺ CD4⁺ memory T cells may constantly provide new targets for viral replication in the postacute phase.

Interestingly, it has been shown that in acute or very early HIV-1 infection, although peripheral blood CCR5⁺ CD4⁺ T cell proliferation increases dramatically, their total number remains in the normal range. In striking contrast, in acute EBV infection, proliferating CCR5⁺ CD4⁺ T cells accumulate to very high levels (113), which suggests that CCR5⁺ CD4⁺ T cells are rapidly removed in primary HIV-1 infection. Thus, although much of the evidence for profound and rapid depletion of CCR5⁺ CD4⁺ T cells during acute infection presented above is derived from studies in SIV infection of macaques, it is likely that a similar series of events occurs in HIV-1 infection of humans. Indeed, although studies have not been performed in the most acute stages of HIV-1 infection in humans, it is clear that a profound and rapid loss of intestinal CD4⁺ T cells occurs in early infection and that this loss is maintained at later stages (114–116).

T Cell Activation Alters T Cell Trafficking

The movement of T cells between blood, lymphoid, and extralymphoid tissues is directed by the interplay of homing receptors and their endothelial ligands, adhesion molecule pairs, and cytokines, chemokines, and their receptors, all of which are profoundly altered in the setting of immune activation, with marked differences between naive and memory T cell subsets (64, 117–119). Thus, in HIV-1/SIV infection both the specific activation of T cells by the virus and the generalized immune activation, which are evident very early in infection (Figure 4), necessarily affect the lymphocyte homing and recirculation patterns. In conjunction with the greater expansion of CD8⁺ T cells in lymphoid tissue, such changes in T cell distribution could account in part for the observation of decreased and falling peripheral blood CD4⁺ T-cell counts, CD8⁺ lymphocytosis, and inversion of the CD4/CD8 ratio in peripheral blood both in the acute and chronic phases of infection (23, 120–123). In fact, the early rise in peripheral blood CD4⁺ T cell counts with highly active antiretroviral therapy (HAART) is generally thought to be due to a reversal of this activation state, leading to normalization of lymphocyte distribution (123–129). Indeed, dramatic antigen- and cytokine-mediated changes

in T cell trafficking and sequestration no doubt occur (23, 130–134), and it has been reported that the decline in peripheral blood CD4⁺ T cell numbers overestimates the actual CD4⁺ T cell loss in some lymphoid tissue (135). While these considerations and evidence indicate the inappropriateness of the customary extrapolation from peripheral blood cell counts to total-body T cell numbers, the evidence discussed above from early SIV and HIV-1 infection suggests that peripheral blood CD4⁺ T cell counts actually underestimate the total-body depletion of CD4⁺ T cells during acute infection.

T Cell Renewal After Profound Depletion

To understand the consequences of the rapid and profound depletion of CD4⁺ T cells described above, we digress briefly from acute HIV-1 infection to establish a framework for understanding the situation in which the immune system finds itself as viral loads diminish and the infection enters its chronic phase. The human immune system seems to have an impressive ability to reconstitute itself after profound depletion, through both thymic-dependent and thymic-independent (antigen-driven peripheral expansion) pathways (136–140). After all, one of the most successful treatments for leukemia/lymphoma involves myeloablative chemotherapy, often with total body irradiation, followed by the intravenous infusion of hematopoietic stem cells and mature T cells. Reconstitution is often robust, such that many of the complications arise from graft-versus-host disease or recurrence of the malignancy, rather than immunodeficiency.

However, if we take a closer look at the reconstitution of T cell numbers in the first 2 years after chemotherapy-induced depletion, a consistent theme becomes apparent: Whereas reconstitution of CD8⁺ T cell numbers is rapid and occurs by peripheral expansion, recovery of CD4⁺ T cell numbers is limited and delayed (141, 142) and is constrained by the age-dependent decline in thymopoiesis. There is compelling evidence that successful CD4⁺ T cell reconstitution after chemotherapy, or under HAART in HIV-1 infection, is determined to a large degree by thymic output (136, 140, 143–151). In contrast, CD8⁺ T cell reconstitution is dependent neither on age (152), nor on thymic output and can reach prechemotherapy baseline levels by 3 months posttherapy, a time when CD4⁺ T cell numbers average only 35% of pretherapy levels (153, 154). Furthermore, expanded populations of memory CD4⁺ T cells may subsequently decrease in number owing to an increased susceptibility to apoptosis (154). Even after autologous peripheral blood stem cell transplantation in children and adults, mature CD4⁺ T cells in the graft do not contribute substantially to CD4⁺ T cell reconstitution, and CD4⁺ T cell lymphopenia is prolonged (155, 156) and correlates with increasing age (156). When CD4⁺ T cells are specifically depleted with monoclonal antibodies in the treatment of multiple sclerosis and rheumatoid arthritis, their depletion is both profound and sustained (157, 158), with no effect on the numbers of circulating CD8⁺ T cells (157). Thus, the CD4⁺ and CD8⁺ T cell regenerative pathways appear to be distinct and, unlike CD8⁺ T cells, reconstitution of memory CD4⁺ T cell numbers

after profound depletion is dependent on the reconstitution of naive $CD4^+$ T cell numbers, which in turn depends on thymic output.

Indeed, such data from human studies confirm what we are beginning to understand of the homeostatic mechanisms that maintain naive and memory T cell numbers in mice after lymphopenia and during antigenic stimulation. First, in lymphopenic states it appears that reconstitution and maintenance of the naive T cell pool depends on continued thymic output (159, 160) and that naive and memory T cells can supply the resting memory pool by peptide/MHC-dependent expansion (159–166). Second, antigen-driven memory $CD4^+$ T cell expansion is limited, in contrast to the extensive expansion seen with $CD8^+$ T cells (167–172). Third, whereas a significant proportion of activated expanded $CD8^+$ T cells may “rest down” to resupply the resting memory $CD8^+$ T cell pool, the fates of $CD4^+$ T cells are very different after activation, and fewer seem to survive (170, 172–177). Indeed $CD4^+$ T cells appear to have an intrinsically lower capacity for survival (165). Finally, maintenance and homeostatic expansion of the naive $CD4^+$, but not $CD8^+$, T cell pool is critically dependent on the presence of normal secondary lymphoid tissue (178). Taken together, these data suggest there is a substantial difference in the homeostasis and regenerative capacity of the $CD4^+$ and $CD8^+$ memory T cell pools, and that maintenance of the memory $CD4^+$ T cell pool may be critically dependent on input from the naive $CD4^+$ T cell pool.

Figure 5 (see color insert) illustrates the dynamics of normal T cell homeostasis. The main principles, all based on experimental evidence, are (a) More $CD4^+$ than $CD8^+$ naive T cells exit the thymus; (b) turnover within the naive T cell pools is minimal; (c) naive T cells may be activated to enter the memory T cell pools, where they may remain activated or return to a resting memory state; (d) the expansion of the activated $CD8^+$ T cell pool is much greater than that of the activated $CD4^+$ T cell pool; (e) many more activated $CD8^+$ T cells reenter the resting memory T cell pool than activated $CD4^+$ T cells; and (f) the vast majority of activated T cells die, far more so for activated $CD4^+$ T cells than activated $CD8^+$ T cells. In a healthy individual over the course of a lifetime sufficient $CD4^+$ and $CD8^+$ T cell numbers are maintained to ensure immune competence. However, the proportions of the various pools change dramatically and differentially with age: Naive T cells decrease with respect to memory T cells, and $CD4^+$ T cells decrease with respect to $CD8^+$ T cells (179–186).

Thus, as the brief period of acute HIV-1 infection wanes, the immune system is faced with two problems, both caused by the virus and both particular to $CD4^+$ T cells. The first is to reconstitute a profoundly depleted memory $CD4^+$ T cell pool in the face of age-attenuated thymic output and ineffective peripheral expansion—renewal mechanisms that are wanting, irrespective of ongoing viral replication. The second problem, discussed in the next section, is that ongoing viral replication aggravates those renewal limitations by impairing thymic output, disrupting lymph node architecture, and inducing $CD4^+$ T cell activation that serves only to propagate the virus while placing additional homeostatic strain on maintenance of the “resting” naive and memory $CD4^+$ T cell pools.

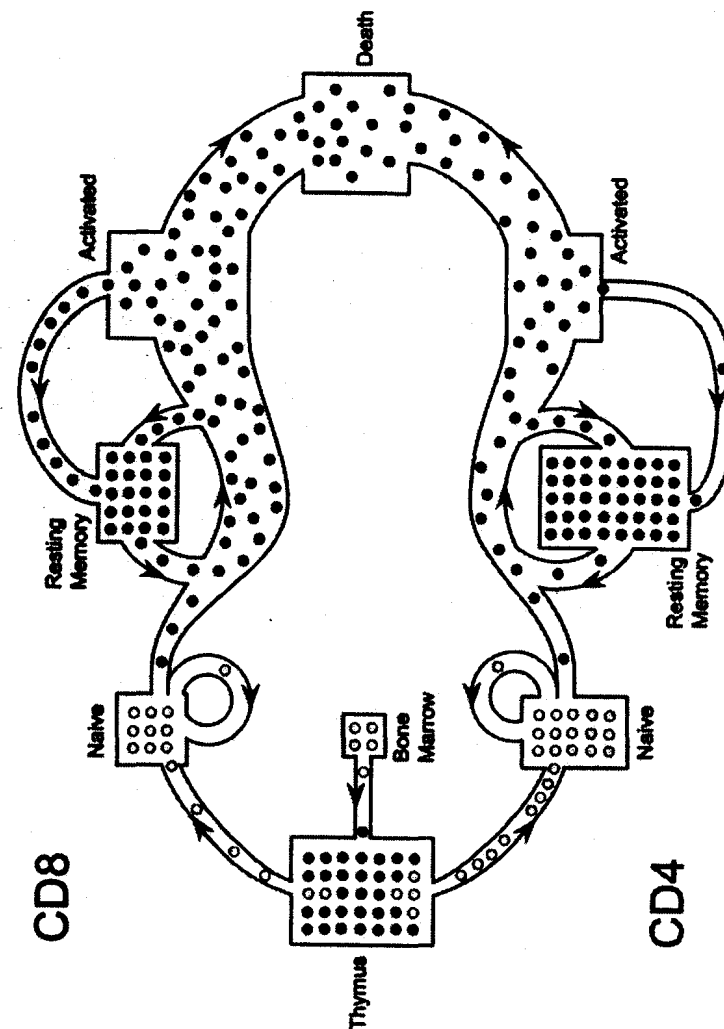


Figure 5 Diagrammatic representation of T cell dynamics in a healthy individual. Resting cells along the bone marrow to naive T cell axis are shown in yellow. Activated T cells (due to any stimulus) are shown in green. Resting memory T cells are shown in blue. Dying cells are shown in black. The arrows depict movement of cells between the pools. The sizes of the boxes depicting the pools and the number of cells therein represent the relative magnitude of those cellular pools, but they are not to scale. The “activated” box would include so-called effector memory cells—those elaborating effector functions. The peripheral T cells shown comprise those in lymphoid and extra-lymphoid tissues.

THE CHRONIC PHASE OF INFECTION

Chronic Activation and T Cell Death

Whereas acute HIV-1 infection is marked by rapidly increasing (and then decreasing) viral load and rapid CD4⁺ T cell depletion, the chronic phase of the infection manifests markedly different viral and T cell dynamics. Plasma viral loads are orders of magnitude lower but then rise slowly; peripheral blood CD4⁺ T cell counts often recover partially from their nadir during acute infection but then fall slowly over a period of, on average, 10 years before the onset of AIDS (14). However, even though things look quieter in this phase, it is actually characterized by a high level of activation of both CD4⁺ and CD8⁺ T cells. Thus, chronic infection is a state of chronic immune activation (187). Indeed, the chronic activation that results from persistent viral replication may be a better predictor of disease progression than the plasma viral load itself (188–191).

Multiple studies of T cell turnover have resulted in various, often contradictory, interpretations of the T cell dynamics in chronic HIV-1 infection. Generally, these studies have shown a state of “high turnover” affecting all T cells, both CD4⁺ and CD8⁺ (129, 192–197). High turnover may imply simply moderately increased rates of division and death for most slowly dividing cells or activation of a small fraction of cells at any given time and their subsequent rapid expansion followed by death. The latter concept of high turnover involves varying degrees of increased T cell activation, expansion, movement through the naive to memory to effector axis, and ultimately, activation-induced cell death. Usually, however, only average turnover rates are observed. Studies of SIV-infected rhesus macaques have shown increased rates of *in vivo* incorporation and loss of BrdU in all T cell populations, with memory T cells being affected far more than naive (193, 195). Studies using Ki67 expression as a marker of cell proliferation have indicated that CD4⁺ and CD8⁺ T cell turnover increases in both naive and memory subsets during HIV-1 infection (129, 198, 199). Yet another study has shown that proliferation within the naive CD4⁺ T cell compartment is not increased in chronic HIV-1 infection (200). Quantitative image analysis of lymph nodes from HIV-1-infected individuals has revealed CD4⁺ T cell depletion in an environment of increased T cell proliferation and apoptosis (201). Finally, the measurement of T cell half-lives using *in vivo* labeling with stable deuterium isotope has shown that HIV-1 infection causes a decrease in memory (but not naive) CD4⁺ and CD8⁺ T cell half-life (192, 194).

Notably, these studies have also shown that the number of proliferating and dying CD4⁺ and CD8⁺ peripheral blood T cells decreases rapidly with HAART (129, 192, 196–198), and other studies have measured a similar decrease in the number of T cells expressing markers of activation in both peripheral blood and lymph node (123–125, 128, 201–205). The interpretation of these data in terms of cause and effect has been the subject of considerable controversy: Does the virus cause massive CD4⁺ T cell death for which the immune system attempts to compensate with an impressive homeostatic proliferative response, or does the

virus cause massive T cell activation and proliferation, with death being the natural immunological consequence?

First, it is unlikely that the virus directly causes massive death of CD4⁺ T cells in chronic infection, because the degree of their productive infection appears to be very low in both peripheral blood and lymph node, with estimates ranging from ~0.01–1% (18, 23, 57, 206–210). Second, HIV-1 preferentially infects expanding populations of activated CD4⁺ T cells (211), the majority of which are already destined to die rapidly after proliferation and elaboration of effector function (212). Third, CD8⁺ T cell death occurs at the same rate as that of CD4⁺ T cells in kinetic models, yet their numbers are not significantly depleted until late in the course of infection (192, 194–196). *In vivo* BrdU pulse-labeling in HIV-1-infected individuals has shown no significant differences between CD4⁺ and CD8⁺ T cells in their high proliferation and death rates, and when viral replication is suppressed with HAART, T cell death rates do not change, implying that the death of recently divided cells is independent of HIV-1 (196). This effect can also be observed in SIV-infected macaques. Figure 2 shows that whereas decay of BrdU-labeled memory CD4⁺ T cells is more rapid than their CD8⁺ counterparts in acute infection, the decay rates of these two populations overlap during the plateau phase of chronic infection. The high rates of T cell death in chronic infection therefore seem to be the consequence, rather than the cause, of T cell activation and expansion. Thus, it is more likely that high lymphocyte turnover rates in HIV-1-infected individuals are caused by T cell activation, either virus-specific or owing to nonspecific “bystander” activation (3, 196, 211, 213). Notably, the predominant mechanism for CD4⁺ cell death, during the quasi-steady state dynamics of chronic infection, differs completely from that during acute SIV infection in macaques discussed above where the dynamics are entirely different.

Chronic Activation and T Cell Depletion

If chronic immune activation accounts for the high death rates of both CD4⁺ and CD8⁺ T cells, these high rates per se do not explain what causes CD4⁺ T cell depletion. However, evidence has been accumulating in support of the concept that chronic activation can effect T cell depletion (3, 187, 206, 214). In HIV-1 infection the degree of chronic activation may be the best predictor of disease progression (188–191). SIV-infected sooty mangabeys and African green monkeys—natural hosts of this virus—develop high viral loads but neither profound CD4⁺ T cell depletion nor progressive disease (215–218), even though the virus is cytopathic (216, 218–220). The striking finding in SIV-infected sooty mangabeys that might explain this is that there is no generalized increase in T cell activation (218). Furthermore, HIV-2 infection is associated with lower viral loads and a slower decline in CD4⁺ T cell counts than in HIV-1 infection, but when CD4⁺ depletion does reach similar levels, equivalent levels of immune activation are found in individuals infected with either virus (221). Consistent with this concept is the finding of substantially decreased levels of immune activation in individuals whose

viremia is not controlled by HAART but who nevertheless maintain increasing CD4⁺ T cell counts (205). It has been proposed that the decisive events that result in CD4⁺ T cell depletion are exerted through various mechanisms on the populations of resting T cells—both memory and naive (3, 23). These mechanisms include (a) attrition of the memory T cell pools by repeated immune activation events, (b) broad activation of naive T cells to enter the memory T cell pools, (c) reduced steady-state numbers of resting T cells owing to recurrent subthreshold stimulation, and (d) impaired supply of naive T cells from lymphopoietic sources and destruction of stromal elements involved in peripheral homeostatic functions owing to chronic infection and activation.

These mechanisms involve direct, destructive effects of the virus as well as non-destructive effects. Obviously, CD4⁺ T cells are far more vulnerable than CD8⁺ cells to the directly destructive effects. However, as discussed above, the proliferative responses to antigen and the regenerative capacities of the CD4⁺ and CD8⁺ memory T cell pools differ markedly, by physiological design, which should result in differential susceptibility of the two subsets to the other detrimental effects of chronic immune activation. The greater clonal expansion of CD8⁺ cells than CD4⁺ cells is associated with larger steady-state numbers of activated cells, which may explain in part the increases in total CD8⁺ T-cell numbers but not CD4⁺ numbers in the chronic phase (3). In addition, the unremitting rounds of memory T cell expansion and death would place a greater strain on maintenance of the resting CD4⁺ memory T cell pool because of its inherently greater dependency on the differentiation of activated naive cells. Moreover, although persistent homeostatic and antigen/inflammation-driven flow of naive T cells into the memory T cell pools, in the context of age-attenuated thymic output (185), would “drain” both the CD4⁺ and CD8⁺ naive T cell pools (222), the pressure may be greater on the naive CD4⁺ T cell pool because of the higher dependency of the CD4⁺ memory pool on input from the naive compartment. It is not surprising, therefore, that naive T cells play a major role in the long-term immune reconstitution of individuals on HAART (124–126), and that overall CD4⁺ T cell reconstitution may depend to a large degree on naive CD4⁺ T cell reconstitution via the thymus (143–149, 152, 223).

Infection and Inflammation: The Effects of HIV-1 on the Thymus, Bone Marrow and Lymph Nodes

We have so far discussed the effects of HIV-1 infection on peripheral T cell pools; however, the primary supply route for naive T cells, before they settle in the lymph node, is the bone marrow–thymus axis, which is also a target for HIV-1-mediated suppression. Whereas mature T cells in the periphery seem able to adjust their proliferation and death rates to maintain homeostasis of numbers during normal ageing, it is unclear whether this is the case under the strain of HIV infection. It is also unclear how the thymus responds to depletion of the peripheral T cell compartment.

Naive CD4⁺ T cell increases following chemotherapy in children are associated with enlargement of the thymus above baseline—a phenomenon termed “thymic rebound” (224). Increased plasma levels of interleukin-7 (IL-7) in the context of lymphopenia owing to genetic abnormalities or chemotherapy (225, 226) or HIV-1-infection (226, 227) have been suggested to indicate an IL-7-mediated homeostatic response to augment both thymic and peripheral pathways of T cell renewal (227). However, it is more likely that plasma IL-7 levels simply reflect the dynamics of binding of secreted IL-7 to T cells expressing IL-7 receptors: The fewer circulating T cells, the more free IL-7 (225). Indeed, the mouse thymus varies neither the export rate nor the composition of emigrant thymocytes in response to changes in either the size or composition of the peripheral T cell compartment (228–230). Evidence from mice suggests that thymocytes are exported at a fixed rate of ~1–2% of total thymocytes per day, implying that the absolute amount of emigrants is determined by thymic mass (228). This is supported by the positive correlation between thymic size and the rate of naive CD4⁺ T cell reconstitution after HAART for HIV-1 infection in humans (143–149). Clearly, the thymus functions in adults and can contribute substantially to immune reconstitution (150, 231), a contribution that is crucial for producing a broad T cell receptor repertoire (136, 140, 151). However, age takes a considerable toll on thymic output (185, 186). Thus, it makes sense that the age-associated decrease in thymic size and output (185, 186) would, even in the absence of any inhibitory effects of the virus, render it increasingly difficult for the thymus to keep up with the constant drain on the peripheral naive T cell pool in chronic HIV-1 infection engendered by the processes described above (23).

It also makes sense that the significance of inhibition of thymic output by HIV-1 depends on age. The reliance upon thymic output for the generation of an immune-competent memory T cell pool, both in terms of size and T cell receptor (TCR) diversity, is greater at younger ages when the memory pool is smaller. Thus, the consequences of thymic inhibition would be large in children but almost negligible in old age. It is generally accepted, through a considerable body of evidence, that HIV-1 infection adversely affects the thymus in both children and adults (186, 232, 233). Clinical studies have shown that the thymuses of HIV-1-infected children and adults undergo abnormal morphological changes including thymocyte depletion and advanced involution (186, 234–237). Thymic dysfunction has been associated with early progression of disease in perinatally infected infants (238–240). SIV infection in rhesus macaques causes similar changes (241, 242). Studies using reaggregate thymic cultures, thymic organ cultures, and SCID-hu mice have shown that thymocytes at almost all stages of maturation are targets for HIV-1 infection (97–102, 243–248). Finally, the observation that peripheral blood TREC levels are decreased in untreated chronically HIV-1-infected children and adults is thought to reflect, at least in part, diminished thymic output (1, 147, 185, 200, 249–254).

The effects of in vitro infection depend to a large degree on coreceptor tropism, as CD4 and CXCR4 are expressed on nearly all thymocytes, whereas CCR5 is expressed only at low levels on mature thymocytes (68, 255, 256). X4-tropic strains

are highly cytopathic in vitro and rapidly deplete thymocytes, whereas R5 strains cause less thymocyte depletion but result in stromal cell abnormalities (100, 255). A recent study suggested that children infected with X4-tropic HIV-1 had lower thymic output than those infected with R5-tropic virus (257). However, analysis of coreceptor usage of thymic primary HIV-1 isolates from neonates has shown that X4- and R5-tropic isolates were both present and equally cytopathic for thymocytes (258).

Either way, the thymus may act as a fertile ground for the replication of HIV-1, particularly X4-tropic viruses, owing to the high number of proliferating thymocytes (259) and the local production of cytokines such as IL-7 (248, 258, 260). Indeed this may explain the higher set-point viral loads in children and the observations that HIV-1-infected children often progress to AIDS more rapidly than adults (238, 261–263). In addition, it has been shown in the SCID-hu mouse model that HIV-1-infected thymocytes may survive to be exported into the periphery, where the virus remains latent until T-cell receptor stimulation, indicating that the thymus might also be a source of latent HIV-1 in humans (264).

It has long been known that HIV-1 infection may also inhibit the production of hematopoietic lineages other than CD4⁺ T cells, by directly or indirectly suppressing the maturation of hematopoietic progenitor cells (265, 266). Indeed, HIV-1-infected individuals are often pancytopenic. Bone marrow architecture and cellularity is usually abnormal (267). However, the inhibitory effect of HIV-1 does not seem to involve direct infection of progenitor cells. The evidence clearly suggests that the progenitor populations are intact and largely uninfected and that the growth and differentiation of the few infected cells is unaffected. It is rather the stromal auxiliary cells of the bone marrow that are persistently infected and dysfunctional (268–270). Thus, as the memory, naive, and thymic compartments are progressively exhausted in chronic HIV-1 infection, the supportive functions of the hematopoietic stromal tissues fail as well and the primary source of all lymphocytes founders.

The ongoing cycles of viral replication and chronic immune activation are also likely to be the cause of observed pathological changes in lymph node architecture in HIV-1 infected individuals (23, 271–273). As HIV-1 infection progresses, the lymph node CD4⁺ T cell population becomes depleted with, eventually, loss of all recognizable anatomic structures. This niche must preserve its structural integrity to ensure (a) proper function of the homeostatic mechanisms that maintain the naive CD4⁺ T cell pool (119, 178, 274–276), (b) productive interactions between T cells and antigen-presenting cells that are involved in generating and maintaining the memory CD4⁺ T cell pool, and (c) that cytokine signals reach target cells not only within their own microenvironment, but in other microenvironments within lymphoid tissue as well (64, 277–280). Importantly, studies of mice have shown that CD4⁺ T cell homeostasis is far more dependent on the presence of lymph node than CD8⁺ T cell homeostasis (178). Thus, over time, damage by the virus to the supply routes and anatomical niches that maintain the resting CD4⁺ T cell compartments act together with the homeostatic strains imposed by chronic

immune activation to exacerbate further the progressive net loss in CD4⁺ T cell numbers.

What Makes HIV Infection Unique?

Why does HIV-1 infection differ from all other infections? Other chronic viral infections in humans do not result in a substantial depletion of CD4⁺ T cell numbers and certainly do not cause AIDS (281–284). Whereas chronic parasitic infections may cause chronic high-level T cell activation, inversion of CD4⁺/CD8⁺ T cell ratios, and reduction of naive T cell numbers in peripheral blood, they do not result in profound CD4⁺ T cell loss unless there is co-infection with HIV-1 (285, 286). When addressing the roles of chronic activation and the limited capacity for renewal of CD4⁺ T cells in disease progression, one should not overlook the fact that HIV-1 has additionally an exquisite predilection for infecting CD4⁺ T cells, is cytopathic, and targets infected cells for killing by HIV-1-specific CD8⁺ T cells. The consequences of this were starkly illustrated in the preceding section on acute infection, which suggested that an HIV-1-infected individual enters the chronic phase of the disease with an already profoundly depleted total CD4⁺ T cell compartment, with the memory T cells of the mucosa mainly affected. Indeed, in SIV and HIV-1 infection the depletion of mucosal CD4⁺ T cells persists throughout the course of the disease (39, 114–116). Such profound depletion would place an added strain on CD4⁺ T cell renewal mechanisms, as the lymphopenia would impose an even greater homeostatic pressure on naive CD4⁺ T cells to enter and maintain the memory CD4⁺ T cell compartment (159–162). Thus, HIV-1 infection differs from other chronic infections in that, in addition to attrition of the resting memory and naive T cell pools caused by persistent immune activation, such activation is coupled to a state of severe memory CD4⁺ T cell lymphopenia almost from the outset.

Given the destructive potential of the virus so clearly evident during the acute phase, it seems ironic that in the chronic phase of HIV-1 infection it is so difficult to pinpoint and evaluate the consequences of infection by the virus on its well-known target cells. Clearly, HIV-1 can also cause the lytic death of CD4⁺ T cells during the chronic phase. Yet even though a small number of the rapidly proliferating, activated CD4⁺ T cells identified in turnover studies is likely to be the major source of virus production, the consequence of this on the size of that T cell pool is moot, because the vast majority is destined to die anyway as a direct result of such activation (3). However, those cells that contribute to the maintenance of the resting memory CD4⁺ T cell pool—the small proportion of activated naive and memory CD4⁺ T cells that survive to enter or reenter the resting memory CD4⁺ T cell compartment—are also subject to HIV-1-mediated killing. In particular, studies show that naive T cells that have been activated are exquisitely sensitive to HIV-1 infection (210) and that cytokine signals in the absence of full TCR-mediated activation are sufficient for HIV-1 infection of resting naive and memory CD4⁺ T cells in vitro and in vivo (76, 287, 288). Thus, in the midst of the homeostatic

upheaval and CD4⁺ T cell depletion caused by chronic activation, the virus itself slowly chips away at those cells attempting to sustain the resting CD4⁺ T cell pool.

T Cell Dynamics During the Chronic Phase: The Overall Picture

Whereas the dissemination of HIV-1 in acute infection is explosive and suggests rapid propagation of virus between many available target cells (23, 45), observations of HIV-1 viral genotypes in local microenvironments (60, 289), and other studies (210), support a concept of "proximal immune activation and virus transmission" (61). The implications of this proximal activation are that during much of the chronic phase, when viral loads are low, efficient transmission of the virus from cell to cell is largely limited to local immune activation bursts in lymphoid tissue that may be provoked by antigenic and inflammatory stimuli. Given that HIV-1 infection itself induces immune activation, it has been proposed that immune activation is the engine driving viral replication (3). Thus, the virus constantly generates its own targets. As most of these target cells are, by nature, short-lived and "expendable," their massive subsequent death does not immediately affect, in the chronic phase, the crucial cellular resources of the immune system, namely, the naive and resting memory T cell pools. However, we have described how chronic immune activation may slowly but progressively drain these pools. In addition, the virus directly interferes destructively with the supply routes of naive and memory T cells and destroys the organs that maintain them. All of these events affect CD4⁺ T cells more than CD8⁺ T cells, owing both to physiological differences between the two subsets and the early established lymphopenia affecting the first, hence their preferential loss. Under such conditions the immune system eventually collapses.

The scheme shown in Figure 6 (see color insert) summarizes T cell dynamics during the chronic phase of HIV-1 infection. The main principles depicted are, (a) there are pathological changes in bone marrow, thymus, and lymph node architecture, and there is a decrease in thymic output; (b) the memory CD4⁺ T cell pool is already decreased in size after the acute phase; (c) the CD4⁺ and CD8⁺ naive and resting memory T cell pools become chronically activated; (d) increased T cell activation results in increased T cell death; (e) the expansion of the activated CD8⁺ T cell pool is much greater than that of the activated CD4⁺ T cell pool; (f) more activated CD8⁺ T cells than activated CD4⁺ T cells re-enter the resting memory T cell pool; (g) the vast majority of activated T cells die, more so for activated CD4⁺ T cells than activated CD8⁺ T cells; (h) the main source of virus is the activated CD4⁺ T cell pool; (i) the majority of infected activated CD4⁺ T cells are physiologically destined to die simply owing to their activation; and (j) a fraction of the already small proportion of infected CD4⁺ T cells destined to enter or reenter the resting memory CD4⁺ T cell pool will fail to contribute to that pool.

In essence, the chronic phase of HIV-1 infection is a quasi-stable steady state, a slowly decaying unstable equilibrium between T cell activation, death, and

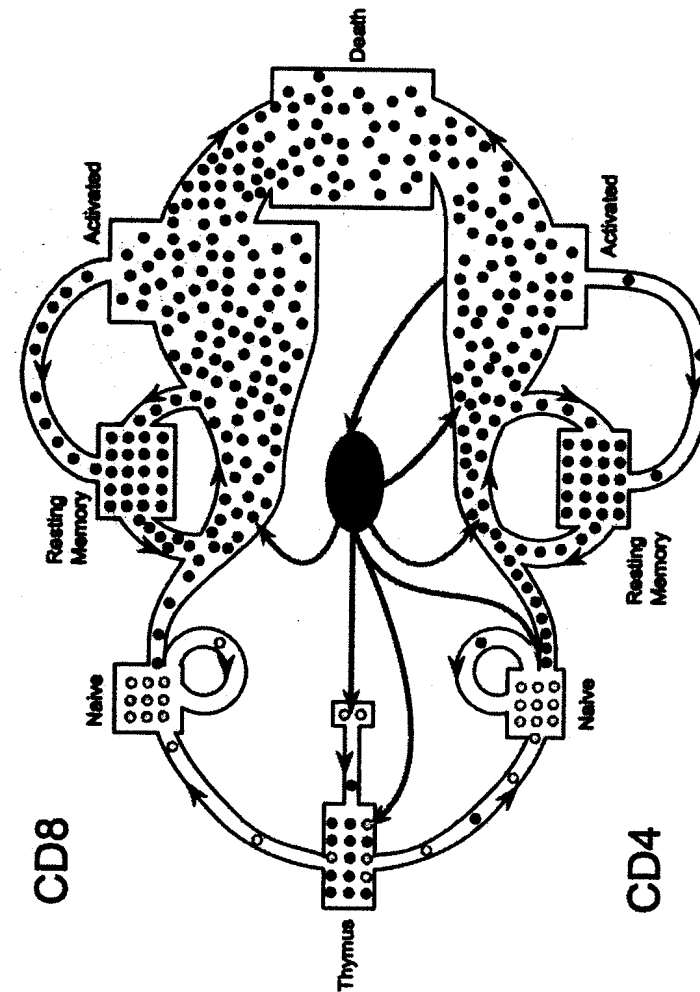


Figure 6 Diagrammatic representation of T cell dynamics in an individual in the chronic phase of HIV-1 infection. Resting cells along the bone marrow to naive T cell axis are shown in yellow. Activated T cells (due to any stimulus) are shown in green. Resting memory T cells are shown in blue. Dying cells are shown in black. HIV-1-infected T cells are shown in red and are considered to have a reduced life-span. The peripheral T cells shown comprise those in lymphoid and extra-lymphoid tissues. The red arrows depict direct and indirect negative effects of HIV-1 on T cell production and/or survival, including destruction of lymphoid organ architecture. The green arrows depict HIV-specific and "bystander" HIV-1-induced T cell activation.

renewal and production and removal of virus. Exponential spread of the virus by self-perpetuating rounds of activation and infection is kept in check by the transient and local nature of viral bursts, by the short life-span of most virus-producing cells, and by physiological constraints on the accumulation of the longer-lived infected memory T cells (61). Eventually the equilibrium breaks down completely as progressive immune degradation ultimately results in clinical immunodeficiency manifested by the development of the opportunistic infections and associated malignancies that characterize overt AIDS (14, 24). Loss of HIV-1-specific immunity might play a role in the acceleration of the viral replication that can be observed in late disease (290, 291). Furthermore, the mutability and genetic flexibility of HIV-1 and SIV allow these viruses to respond to selection pressure, adapt to changing host conditions, and evolve into viral variants with an expanded repertoire of target cells and enhanced pathogenicity (26–28, 100, 292, 293) or diminished susceptibility to HIV-specific T cell immune responses (112).

HIV-1-Specific T Cell Immune Responses: Part of the Solution but Also Part of the Problem

Despite a rapid fall in HIV-1 load after acute infection, this quasi-stable steady state often takes months to become established, usually preceded by a period of strong viral load fluctuation. The factors involved in this initial partial control of viral replication remain controversial. Indeed, it has been suggested that the main determinant of the decrease in viral load is the rapidly diminishing availability of activated CD4⁺ T cell substrates for infection (294). The profound depletion that has now been observed in the mucosal tissues, as we have described, is largely consistent with this interpretation.

However, a significant body of evidence suggests that the immune response is actively involved in viral control. There is early circumstantial evidence in humans showing a temporal correlation between the initial drop in viremia and the appearance of HIV-1-specific CD8⁺ T cells but not neutralizing antibodies (19, 20). Several studies have also shown a direct association between class I HLA types and rates of HIV-1 disease progression (295–299). In acute SIV infection of rhesus macaques, the emergence of SIV-specific CD8⁺ T cells coincides with clearance of virus (52, 300), and transient depletion of CD8⁺ T cells in chronically SIV-infected rhesus macaques leads to a rapid increase in viral replication (301–303). Finally, viral sequence changes allowing escape from virus-specific CD8⁺ T cells develop very rapidly in acute infection in humans (304) and monkeys (305–307), indicating that these responses exert sufficient antiviral pressure to drive the outgrowth of minor viral species. The interplay between newly generated T cell responses and rapid viral escape that occurs during and after acute infection may be partially responsible for the period of viral load fluctuation that precedes the establishment of a viral set point.

Much of the T cell activation in HIV-1 infection is likely to be due to virus-specific responses, as evidenced by the high frequency of circulating

HIV-1-specific T cells (particularly CD8⁺ T cells) throughout the course of infection before the onset of AIDS (21, 22, 308–313). However, although strong and broad HIV-1-specific responses that are clearly able to reduce viral loads are elicited early in infection, they fail to prevent disease progression. Studies that show absence of correlation or positive correlation between viral load and the overall frequency or breadth of circulating HIV-1-specific CD8⁺ T cells (308–310, 314, 315) suggest that HIV-1-specific CD8⁺ T cells respond to the virus, but these studies fail to indicate the effectiveness of this response.

Recruitment of naive HIV-1-specific CD4⁺ T-cells, along with HIV-1-specific CD8⁺ T cells, into infected lymphoid sites may affect the rate of viral clearance (316) but may also provide cellular substrates for viral replication (289). Indeed, rapidly expanding CD4⁺ T cells, in transition from naive to fully activated phenotype, are exquisitely susceptible to productive HIV-1 infection (210). Thus, the initial antigen-specific activation of HIV-1-specific T cells may augment viral replication, which will in turn stimulate more HIV-1-specific T cells, leading to further activation and expansion in a positive feedback loop (61). With this in mind, we have confirmed that HIV-1-specific CD4⁺ T cells are preferentially infected by HIV-1 at all stages of disease (210). Indeed, we might speculate that it is the HIV-1-specific response itself that provides both the initial thrust and continued momentum for the maintenance of chronic activation.

The other side of the coin is that recurring cycles of activation and infection may result in the progressive loss of HIV-specific CD4⁺ T cell responses that begins during acute infection and continues throughout its course (21, 22, 311, 317), although circulating HIV-1-specific CD4⁺ T cells are clearly present in the chronic phase (21, 308, 312). In addition to the actual reduction in the specific CD4⁺ T cell population, the postactivation state of many of the remaining HIV-1-specific CD4 cells and their continued stimulation during a state of high viremia may be associated with a transient inability to proliferate in response to activation signals (312), as occurs in other acute or chronic viral infections (318–324).

A similar form of unresponsiveness is apparently also exhibited by many of the HIV-1-specific CD8⁺ T cells. Indeed, these exhibit many so-called defects. Peripheral blood HIV-1-specific CD8⁺ T cells are highly sensitive to Fas-induced apoptosis (291), have low expression of perforin (325), may not produce cytokines in response to antigen (326, 327), often have TCRs of low functional avidity (328) and inappropriate signaling (327), exhibit skewed maturation profiles (329–331), and are often unable to proliferate in response to antigen (J. Brenchley, N. Karandikar, M. Betts, D. Ambrozak, B. Hill, L. Crotty, J. Casazza, J. Kuruppu, S. Migueles, M. Connors, M. Roederer, D. Douek, R. Koup, submitted manuscript). However, CD8⁺ T cells with these phenotypes are often the dominant subset at particular stages of protective immune responses against other viruses such as CMV and EBV (332–334), and may also arise in other situations of chronic antigenic stimulation (120, 335–337). As such, these “defective” HIV-1-specific CD8⁺ T cells, trafficking between blood and lymphoid tissue, are likely to reflect the consequences of chronic antigen stimulation rather than being the cause of inadequate control of

viral replication (334). The viral epitope escape mutants that develop rapidly during acute infection (304–306), particularly from epitopes that elicited high-avidity CD8⁺ T cells (307), could still maintain high frequencies of those HIV-1-specific CD8⁺ T cells, the majority of which may be impaired in their ability to clear the predominant circulating viruses (338).

Thus CD4⁺ and CD8⁺ HIV-1-specific T cells make a valiant and partially successful attempt to control virus production and halt the disease. However, from the earliest stages of the infection, by driving CD4⁺ and CD8⁺ T cell activation, by providing substrates for viral replication, and by unwittingly being targeted for preferential infection, the HIV-1-specific response becomes part of the problem as well as part of the solution. With the emergence of escape mutants, a self-destructive situation develops in which the virus may persistently activate the immune system at little cost to itself but with the benefit that the activation increases virus production.

CONCLUSION

We began this review by asking what sets HIV-1 infection apart from other viral infections and suggesting that the answer rests in understanding the underlying dynamics of T cells and virus. The data we have reviewed do not provide definitive answers but offer promising clues. New observations suggest that considerable damage is caused to the immune system during the acute phase of the infection, resulting in a selective but substantial lymphopenia. Several other observations reveal a unique strategy in which HIV-1 induces immune activation to generate replaceable target cells in order to sustain its replication. Most of the target cells are short-lived and “expendable,” by physiological design. Nevertheless, as we have described, chronic activation can strain homeostasis of the naive and resting memory T cell pools indirectly in a number of ways and, when coupled to the combined impact of ongoing, low-level destructive events mediated by virus and of the “historic” lymphopenia resulting from acute infection, this strain leads to the progressive depletion of the more vulnerable CD4⁺ T cell pools. We do not think destroying the immune system is part of this strategy, but rather that AIDS is a consequence of incomplete adaptation of the virus to its relatively new host. Better understanding of the causes and effects underlying the unstable dynamics that we call disease progression will undoubtedly contribute to our ability to intervene and treat this unique infection.

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