T cell depletion in HIV-1 infection: how CD4+ T cells go out of stock

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HIV-1 infection is characterized by a gradual loss of CD4+ T cells and progressive immune deficiency that leads to opportunistic infections, otherwise rare malignancies and ultimately death. Extensive research over the past two decades has increased our insight into the pathogenic mechanisms underlying these features of HIV-1 infection. Here, we will give a brief overview of the most recent findings and present a model that fits most of the relevant aspects of HIV-1 infection as known. We hypothesize that HIV-1 infection depletes T cell supplies (which are not replaced because of low and static thymic function) by direct infection and killing of cells and through hyperactivation of the immune system.

For about a decade, HIV-1 infection was considered to be static, with a long, initially latent, phase. However, from 1990 onwards, this picture has gradually changed[1,2]. The discovery of many T cells dying from apoptosis[3] and the detection of relatively high viral loads in blood and tissues in asymptomatic individuals[4,5] confirmed the persistently active nature of HIV-1 infection. Early studies also clearly showed a strong component of immune activation, which seemed to increase with duration of HIV-1 infection[6,7].

HIV-1 and T cell turnover

Kinetic studies of HIV-1 viral load and T cell dynamics were finally possible after the introduction of highly active antiretroviral therapy (HAART). The general view of HIV-1 infection was permanently changed in 1995 by demonstrations that during clinical latency, the virus is continuously replicating with a high and rapid turnover rate[8,9]. By analogy, it was concluded from the observed rise in peripheral blood CD4+ T cell numbers during the first four weeks of HAART that T cell production is highly increased during clinical latency in response to massive destruction of CD4+ T cells by the virus. Consequently, development of AIDS (acquired immunodeficiency syndrome) was thought to be caused by exhaustion of an immune system unable to maintain this high rate of T cell production.

To experimentally test this hypothesis, the replicative history of CD4+ and CD8+ T cells in HIV-1–infected individuals was determined by measuring T cell telomere length. As telomeres of CD4+ T cells were not significantly shorter in HIV-1–infected patients compared to healthy individuals, exhaustion of T cell proliferation (due to prolonged high turnover) as a cause of depletion of CD4+ T cell pool seemed less likely[10,11]. Subsequent studies showed that the initial lymphocyte repopulation also consisted of CD8+ T cells[12]. Now it is generally believed that redistribution of previously sequestered, memory lymphocytes from lymphoid tissues to the circulation, rather than *de novo* production of T cells, accounts for most of the reappearance of T lymphocytes in the blood during the first weeks of HAART[12,13]. Although it does not seem to lead to exhaustion of the replicative capacity of T cells, it was subsequently shown that HIV-1 infection induces a two- to threefold increase in CD4+ T cell turnover[14–18]. T cell division rates are highest in the CD8+ T cell pool[10,15–17] and are also increased in B cells and NK cells[19]. Apoptosis rates are highest in the CD8+ T cell fraction[3]. Thus, HIV-1 infection leads to a sustained increase in lymphocyte turnover that is not limited to the CD4+ T cell pool.

HIV-1 is assumed to preferentially infect and kill dividing cells, which could obscure cell division measurements (before they can be accounted for, cells are lost). Homeostatic increases in cell proliferation would thereby be 'masked', at least in part, in untreated HIV-1 infection[19]. If this were true, one would anticipate an increase in cell division rates when masking is removed, as in the case of effective HAART. In fact, the opposite occurs: HAART leads to an immediate reduction in CD4+ and CD8+ T cell division rates, as measured by expression of the Ki-67 antigen[17] and *in vivo* cell labeling studies using deuterated glucose[20], despite severe T cell depletion. This indicates that in HIV-1 infection, increased cell division and death rates are mainly a reflection of persistent immune activation rather than a homeostatic response to T cell depletion[17].

Interference with renewal

Once it became apparent that it might not be exhaustion of highly increased T cell turnover that leads to loss of CD4+ T cells, research focused on the possibility of interference of HIV-1 with T cell renewal as a cause of T cell depletion[21]. In HIV-1 infection, the capacity of bone marrow–derived progenitor cells to develop into mature naïve T cells is affected, as was shown by culturing CD34+ cells on murine fetal thymi[22]. Studies of thymic function, as measured by chest computed tomography of thymic tissue, showed that residual thymic function slows HIV-1 progression by maintaining adequate numbers of naïve T cells[23,24]. Patients with abundant thymic tissue show a faster improvement of naïve T cell numbers during HAART[25].

More direct measurement of thymic function was achieved by using a polymerase chain reaction method that identifies recent thymic emigrants by detecting circular excision products, formed during T cell receptor rearrangements in the thymus[26]. The number of T cell receptor excision circles (TRECs), as measured in periph-

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eral blood mononuclear cells or purified CD4+ and CD8+ T cells, was thought to correlate with thymic function[27]. In most HIV-1–infected patients, TRECs are significantly decreased and recovered on commencement of HAART[26,28]. In HIV-1–infected children, thymic function is also suppressed, according to TREC content[28,29], and recovery depends on the efficacy of HAART[29]. TRECs were also found to have prognostic value, such that individuals with low numbers of TRECs progress to AIDS at a faster pace[30]. Although not all infected patients with low CD4+ T cell numbers or AIDS have low TREC numbers, these data were taken as evidence that thymic dysfunction plays a role in HIV-1 pathogenesis. However, because TRECs do not replicate during mitosis, they are diluted during cell division[31,32]. In fact, various factors besides thymic production may determine TREC content of the T cell population, even when measured in purified naïve T cells. These may include cell division and cell death, priming of naïve T cells to become memory cells, reversion of memory cells to cells with a naïve phenotype, and intracellular degradation of TRECs (**Fig. 1**).

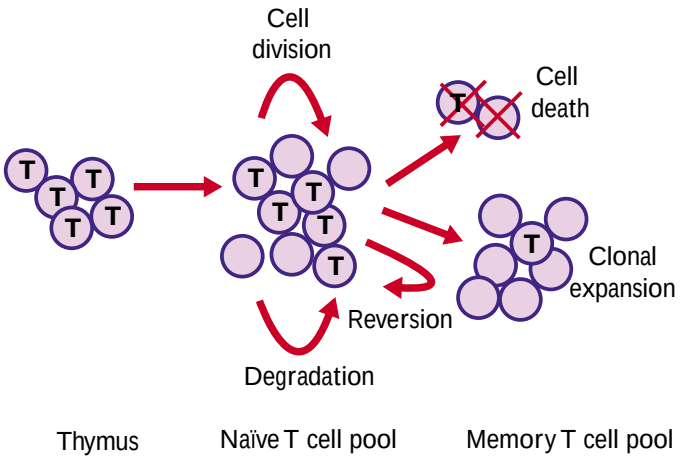

Figure 1. Factors that determine TREC content of the naïve T cell population. TREC content is decreased by cell division, intracellular degradation of TRECs and/or reversion of memory cells to a naïve phenotype. A rise in cell death or priming rate increases TREC content of the naïve T cell pool, as the remaining naïve cells are younger on average. Thymic output of naïve T cells adds to TREC content. Naïve T cell division was identified as the most important factor for decreasing TREC content[33]. Circle with T: TREC-containing cell; empty circle: non-TREC-containing cell; circle with small T: degrading TREC.

To interpret TREC data, a mathematical model that identified naïve T cell division as the most important factor to affect TREC content of the naïve T cell population was developed[33]. In HIV-1–infected and healthy individuals, TREC content correlates with the proportion of dividing naïve cells. This indicates that chronic immune stimulation, and not thymic dysfunction, best explains the loss of TRECs as observed in HIV-1 infection[33]. Our conclusion is supported by the fact that HIV-1–negative Ethiopian individuals, who have a persistently activated immune system[34] reminiscent of what is observed in HIV-1–infected Dutch subjects, have even lower TREC amounts than the Dutch patients tested in our study[33]. Although interference of HIV-1 with thymic output could contribute to CD4+ T cell depletion, measurement of TRECs does not provide direct experimental evidence for thymic impairment.

Effects of chronic immune activation

How may HIV-1 infection lead to loss of T cells? HIV-1 infects CD4+ T cells but direct infection and killing of these cells can only partly account for HIV-1–associated lymphocyte depletion. The actual number of productively infected cells is estimated to be relatively low, in the order of 5×10^7 to 5×10^8 CD4+ T cells[35,36], whereas the human body contains an average of 2.5×10^{11} CD4+ T cells[37]. Direct infection of CD4+ T cells does not explain the loss of naïve CD8+ T cells that parallels the decline in naïve CD4+ T cells during asymptomatic HIV-1 infection[38–40].

More important in this respect may be the response of the immune system. HIV-1 activates the immune system persistently because of high and continuous virion production and possibly by viral gene products such as Nef[41]. This is reflected by increased expression of various leukocyte activation markers[42], production of pro-inflammatory cytokines and a rise in cell proliferation and death rates[3,17]. Proliferating T cells that are activated by their cognate antigen, by immunostimulatory cytokines, or both, are bound to die after several rounds of division[43]. *In vivo* labeling with deuterated glucose in HIV-1–infected humans and with 5-bromodeoxyuridine in simian immunodeficiency virus (SIV)-infected macaques showed rapid incorporation and loss of label in CD4+ and CD8+ T cells, mainly of the memory phenotype[19,20,44,45]. This can be interpreted to largely reflect clonal expansion and contraction of activated memory T cell populations[46]. Thus, everyday for many years, T cells are activated to a higher extent than in healthy individuals, increasing the fraction of T cells prone to apoptosis. Normally, such activation, proliferation and death presumably result in a net increase in cell numbers due to the generation of more than one new memory cell per each activated cell. However, this may not be the case for the generalized, chronic immune activation associated with HIV-1 infection. This activity may actually cause suppression of the regenerative proliferation of the remaining bulk of 'resting' cells[47]. Interestingly, not only is the pool of dividing memory cells increased, but naïve cell division is also enhanced to some extent[17]. Continuous hyperactivation of naïve T cells, whether antigen-specific, induced by cytokines[48] or by viral gene products[41], may lead to accelerated consumption of naïve T cells through apoptosis of activated naïve T cells or differentiation towards a memory phenotype.

The importance of persistent immune activation in HIV-1 pathogenesis is underscored by the observations that disease progression is associated with immune activation[7], and expression of activation markers on CD8+ T cells has stronger prognostic value for development of AIDS than do HIV-1 viral load or low CD4+ T cell numbers[49]. Rapid loss of CD4+ T cells and progression to AIDS has been associated with a particular HLA type (A1, B8, DR3)[6]. Individuals with this haplotype are considered "immunologically hyperactive" and are at relatively high risk for autoimmune diseases[6]. In agreement with this idea, infection with HIV-2 is associated with lower levels of immune activation, which may explain the slower decline in CD4+ T cell numbers as compared with HIV-1 infection[50,51]. Finally, normal T cell division rates were reported in SIV-infected sooty mangabeys that harbor high SIV viral loads but do not develop disease. This is in contrast to SIV-infected macaques, which have a persistently activated immune system (possibly reflecting proper presentation of the virus or absence of natural adaptation to SIV) and do progress to AIDS[52]. Other conditions during which the immune

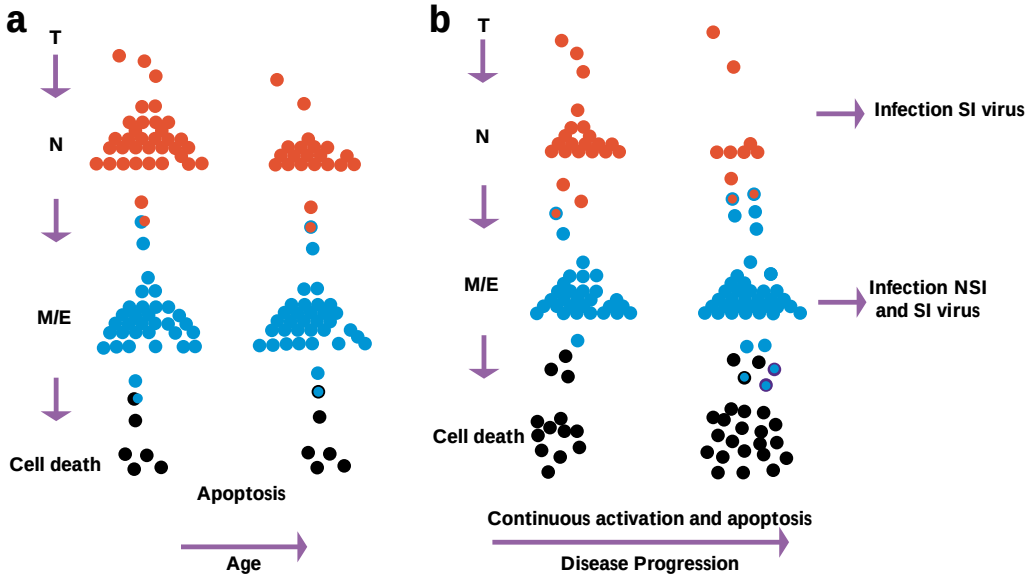

Figure 2. T cell depletion by persistent immune activation. (**a**) In healthy adults, thymic output of naïve T cells is low and constant. With age, the naïve T cell pool is reduced, because, on encountering their cognate antigen, naïve T cells are primed, acquire a memory phenotype and ultimately die by apoptosis. This physiologic loss of T cells is slow because bursts of immune activation are relieved with periods of relative rest. (**b**) In HIV-1 infection, an identical process occurs but at a faster pace because of the continuous attendance of pathogens. Various factors may contribute to the effect of continuous immune activation. In early stage of infection, patients are infected with NSI variants, which only infect memory T cells. In later stages, viral evolution towards an SI phenotype could increase loss of both naïve and memory T cells by infecting and killing naïve T cells. T: thymic output; N: naïve T cells; M/E: memory/effector T cells; NSI: nonsyncytium-inducing; SI: syncytium-inducing.

system is chronically activated may, in similar ways but to different extents, induce T cell depletion. For example, non–HIV-1–infected Ethiopians with persistently activated immune systems show a significant loss of peripheral naïve $CD4^+$ and naïve $CD8^+$ T cells[34]. Thus, increased immune activation and subsequent increased consumption of naïve T cells may play a role in $CD4^+$ T cell depletion and may be causal in AIDS development.

Exhaustion of lymphocyte stock?

Given the elevated consumption of naïve T cells, the question of what role the thymus could have in HIV-1 pathogenesis comes to the forefront. HIV-1 may infect thymic stroma and thymocytes and could thereby reduce thymic output[53,54]. The impact of this may be relatively small because in adults, thymic output is already low and estimated to be in the order of 10^8 thymic emigrants per day. It is now increasingly recognized that the thymus may not be an organ capable of adaptation to higher demands. Repopulation of T cells in cancer patients who underwent bone marrow transplantation or chemotherapy, or in multiple sclerosis patients treated with T cell–depleting doses of monoclonal antibodies, is slow and not always complete[55,56]. This is generally attributed to the age-related involution of thymic tissue after puberty, which may reflect an evolutionary process[57].

According to this hypothesis, *in utero* and during the first years of life, the thymus produces enough naïve T lymphocytes to enable the immune system to fight a lifelong battle against various pathogens. After establishing the T cell pool after puberty, the thymus involutes as "the organism economizes on maintenance"[57]. Indeed, thymectomy in adults does not lead to severe immunodeficiency because of the longevity of naïve T cells, whereas congenital thymic anomalies, such as with complete or incomplete DiGeorge Syndrome, are associated with severe loss of immune function[58]. In the past, when people had a maximal life expectancy of 40 years, the stock of lymphocytes provided early in life did suffice. However, in two to three generations, life expectancy in industrialized countries doubled. The physiologic age-related decline in naïve T cell numbers may reflect emptying of this stock by gradual consumption of naïve T cells with virtually no replenishment. Thus, long-lasting overconsumption of naïve supplies through persistent immune activation, such as observed during HIV-1 infection (but also in other conditions of continuous immune activation), will lead to accelerated depletion of the $CD4^+$ and $CD8^+$ T cell stock[21] (**Fig. 2a,b**).

Finally, thymic output may only depend on age and not on homeostatic demand, a concept that is not widely shared[54]. In our opinion, the rapid recovery of naïve T cells frequently observed in HIV-infected children during HAART[59–61] does not reflect a homeostatic adaptation[29]. Rather, it is compatible with continuous thymic output to match age-related requirements[62,63]. If true, then thymic volume that correlates with thymic output in adults on HAART[24,25] reflects residual function of this organ that may be genetically determined, rather than being a sign of thymic rebound.

Unique aspects of HIV-1 infection

Although Ethiopians not infected with HIV-1 do show signs of persistent immune activation and have lower numbers of naïve cells compared with healthy individuals from the developed world, they do not develop AIDS-like symptoms. Hence, there are at least some aspects of HIV infection that are critical for progression to disease.

In general, primary HIV-1 infection starts with non-syncytium-inducing (NSI) HIV-1 variants that use CCR5 as a coreceptor and are therefore capable of infecting macrophages and $CD4^+$ T cells of the memory phenotype[64]. In half of the cases, syncytium-inducing (SI) HIV-1 variants evolve that are capable of infecting not only memory T lymphocytes but also naïve $CD4^+$ T cells[65] and thymocytes[66,67] through the CXCR4 coreceptor. SI HIV-1 infection of naïve $CD4^+$ T cells correlates with loss of $CD4^+$ T cells[65]. Direct infection and killing of naïve T cells, and possibly of T cell precursors, may result in a poor prognosis for individuals infected with SI variants[65,68]. In addition, through these phenotypic changes and with antigenic variation, the virus will activate a larger variety of antigen-specific T cells, resulting in higher levels of immune activation.

Lack of $CD4^+$ T cell depletion and disease progression in long-term survivors could be attributed to low immune activation, given the correlations between disease progression and immune activation. Our hypothesis would predict that in HIV-1–infected individuals that virologically fail antiretroviral therapy but do show improvement of

T cell numbers ('discordant responders'), persistent or recurrent viral replication does not lead to increased activation of the immune system. Indeed, *in vivo* labeling of dividing cells with deuterated glucose in these patients showed normal T cell turnover (M. Hellerstein, personal communication).

Finally, if accelerated depletion of the T cell store through persistent immune activation could indeed lead to severe immunodeficiency in HIV-1 infection, then why do other virus infections not result in CD4$^+$ T cell depletion and what is different about HIV-1 in this regard? Most chronic infections are characterized by clinically manifested reactivations and long periods of true latency during which the virus is dormant. In acute HIV-1 infection, HIV-specific T helper cell functions may be irreversibly impaired because HIV-1 preferentially infects and kills activated CD4$^+$ T cells[69]. In addition, HIV-1–infected CD4$^+$ T cells may be directly killed by cytotoxic T lymphocytes. Because of insufficient CD4$^+$ T cell help[70] and impaired CD8$^+$ T cell responses[71], a true latent stage will never be reached[72] and persistent viral replication will continuously activate the immune system. The preference to kill activated CD4$^+$ T cells applies to all antigen-specific activated T cells, not only to those directed against HIV-1. Consequently, deletions in the T cell repertoire ensue over time. These deletions are not easily replaced by T cell renewal mechanisms or during HAART[73,74] and may predispose patients to opportunistic infections.

Conclusion

Although our understanding of the pathogenesis of AIDS is still incomplete, significant new insights have been obtained over the past five years. In that respect, it is critical to understand the gradual depletion of the CD4$^+$ T cell pool. Through both immune activation and virus infection, continuing high-level virus replication, because of failing or incomplete immune control, may be a key feature for the erosion of the naïve T cell pool. The inability of the thymus to efficiently compensate for even a relatively small loss of naïve T cells may be the second key feature in AIDS pathogenesis[75]. Although infection of the thymus and consequent impairment of its function may accelerate the disease process, it may not be critical for HIV-1 pathogenesis[21,76]. Taken together, it may not be exhaustion of homeostatic responses, but rather thymic homeostatic inability along with gradual wasting of T cell supplies through hyperactivation of the immune system that lead to T lymphocyte depletion in HIV-1 infection.

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