T cell turnover and thymic function in HIV-1 infection
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13 Epilogue
In this thesis, studies on the effects of HIV-1 on T cell turnover, thymic function and T cell depletion are described. In October 2000, we published a paper that integrated all available observations from several laboratories and our own to come to a new model that may explain HIV-1 related T cell loss (chapter 12). Below, this model is extended by putting it into the context of the most recent findings (until April 2002).

T cell turnover during T lymphocyte depletion: push or pull

Longitudinal analysis of peripheral blood T cell division rates using Ki67 has demonstrated that the several-fold increased T cell turnover that involves CD4+ and CD8+ T cells and characterizes untreated HIV-1 infection, rapidly declined during antiretroviral treatment, despite still very low peripheral blood CD4+ T cell numbers (chapter 3). Recently, these findings were confirmed using more advanced techniques. In these studies, T cell turnover was measured in vivo using deuterated glucose or BrdU, showing that in HIV-1 infection, CD4+ and CD8+ T cell turnover was several-fold increased and normalized after the introduction of antiretroviral therapy (1:2). These data suggested that elevated T cell turnover in HIV-1 infection was driven by chronic immune stimulation (push) rather than by CD4+ T cell depletion (pull) (chapter 3). Indeed, when we measured T cell division rates in oncology patients who had received a stem cell transplantation, we found that increased T cell proliferation after transplantation was mainly related to infectious complications and Graft versus Host Disease (GvHD), but not to CD4+ or CD8+ T cell depletion itself. In other words, stem cell transplant recipients without inflammatory complications showed normal T cell proliferation rates despite very low numbers of T cells (chapter 11). Thus, increased T cell division in lymphopenic patients should not be taken to reflect homeostatic or regenerative responses to T cell depletion. In fact, in vivo labeling studies in untreated HIV-1 infected individuals demonstrated rapid loss of labeled cells after infusion of label was stopped (1:2). It therefore remains to be determined to what extent elevated T cell division rates in patients with low T cell numbers actually contribute to long-term recovery of T cell numbers.

In this context, it is important to distinguish regenerative from activation-induced T cell proliferation (3:4). Regenerative proliferation reflects low-level proliferation of quiescent T cells, estimated to be once every 3 years for naive T cells and once every 263 days for memory T cells, that most likely serves to maintain the T cell pool (5:6). Activation-induced T cell proliferation on the other hand is much faster, leading to rapid expansion of antigen-specific clones followed by contraction, such that most activated T cells die after several rounds of division, mainly via apoptosis. Recently, Mohri et al suggested that in HIV-1 infection, increased T cell turnover is associated with increased CD4+ T cell death rates that lead to CD4+ T cell depletion (1). Even though these authors did acknowledge that with deuterated glucose administration only a subset of T cells
is labeled, they applied the estimated death rates equally to all CD4⁺ T cells. However, elevated T cell turnover in untreated HIV-1 infection has been shown to reflect virus-driven immune activation that involves part of the CD4⁺ T cell pool only and, importantly, applies also to CD8⁺ T cells (chapter 3). As pointed out recently by Grossman et al., increased activation-induced death rates by themselves do not necessarily lead to a net decline in T cell numbers, when death of these T cells is preceded by expansion (7). Thus, increased T cell turnover and death does not immediately explain the gradual CD4⁺ T cell decline that characterizes HIV-1 infection.

Nevertheless, elevated T cell activation does seem to play a role in HIV-1 and SIV pathogenesis. For long it has been recognized that activation marker expression by CD8⁺ T cells is predictive of HIV-1 disease progression (8-15). Using cryopreserved lymphocytes from participants of the Amsterdam Cohort Studies on HIV-1 infection and AIDS, we have confirmed and extended these previous findings showing that persistently increased levels of immune activation in the CD8⁺ and in the CD4⁺ T cell pool were associated with progression to AIDS. Interestingly, also pre-seroconversion levels of immune activation were predictive for disease outcome (chapter 5).

The importance of increased T cell activation in HIV-1 pathogenesis was recently further confirmed by studies in SIV-infected sooty mangabeys (Cercocebus atys) (16). SIV prevalence is high in monkeys that live in the wild (estimated prevalence at least 20%, but up to 90% in sexually active adult monkeys (17)). These natural hosts, of which sooty mangabeys are one example, maintain normal levels of CD4⁺ T cells despite persistent high levels of plasma SIV RNA. In contrast, rhesus macaque monkeys who are not a natural host to SIV infection experience progressive CD4⁺ T cell decline and progression to AIDS when, in the experimental setting, infected with SIV (reviewed in (18)). Feinberg and colleagues recently showed that SIV infection in sooty mangabeys does not evoke a cytotoxic T-lymphocyte (CTL) response and leads to only minimally increased levels of activated CD4⁺ and CD8⁺ T cells in parallel with a minor reduction in CD4⁺ T cell numbers. These data suggest that sooty mangabeys ‘ignore’ the presence of SIV, which seems a property of the host as the same virus strain did induce high levels of immune activation and declining numbers of CD4⁺ T cells in rhesus macaques, without evidence for adaptation of the virus (16).

Taken together, even though increased T cell turnover by itself can not explain HIV-1 related T cell loss, persistent immune activation does play a role in HIV-1 and SIV pathogenesis. In chapter 12, we have presented a model where continuous, HIV-1 related hyperactivation of the immune system was hypothesized to induce CD4⁺ T cell decline through gradual consumption of T cells. Crucial to this hypothesis is the assumption that T cells that are lost are not adequately replaced.

Thymic function
Replacement of T cells through de novo T cell production depends on bone marrow derived progenitor T cells that migrate to the thymus, where they mature into naive T cells. T cell progenitor function was shown to be suppressed in untreated HIV-1 infected patients (19). In addition, HIV-1 infection may lead to thymic dysfunction through direct infection and killing of thymocytes, and by affecting the function of thymic stromal tissue (chapter 6 and references therein). Until 1998 only indirect methods were available to measure thymic function, such as analysis of the number of phenotypic naive T cells in blood and lymphoid tissues, or estimation of thymic size by CT or MRI scans. At the end of 1998, Douek et al introduced a new, more direct approach to quantitatively estimate thymic function (20). This technique is based on the detection of T cell receptor excision circles (TRECs), by-products of the T cell receptor gene rearrangement processes that characterize T cell maturation in the thymus. Because of low TRECs content, several groups have concluded that thymic output is reduced in HIV-1 infection (20-24). However, TRECs are episomal circles that are diluted upon cell division. We have demonstrated that HIV-1 related reduction in TRECs content (the average number of TRECs copies per microgram DNA) is not related to thymic dysfunction, but is best explained by increased naive T cell division levels (chapter 7). In addition, by applying mathematical modeling to TRECs data, we have suggested that the accelerated loss of naive CD4+ T cells that characterizes the emergence of syncytium inducing (SI), CXCR4 using (X4) HIV-1 variants is mainly related to increased killing of mature naive T cells, rather than to abrogation of thymic output (chapter 10). These findings do not exclude an effect of HIV-1 on thymic function, but they demonstrate that TRECs content or TRECs numbers cannot be used as a measure of thymic output. In fact, because of the longevity of naive T cells, complete abrogation of thymic function, for example because of HIV-1 infection or thymectomy will only have an effect on TRECs content in the presence of cell division (chapter 7). Unfortunately, this has erroneously been interpreted as if an increase in cell division would be necessary to lower TRECs content (22;25). Recently, it was suggested that in HIV-1 infection, CD8 T cell TRECs content was significantly affected by cell division, whereas CD4 T cell TRECs content was only affected by thymic dysfunction (25). This conclusion was based on the assumption that CD4+ T cell division in HIV-1 infection is only 1-2 fold increased in contrast to a 7-8 fold increase in CD8+ T cell division. In chapter 3 we have however shown that the HIV-1 induced increase in the proportion of dividing CD4+ and CD8+ T cells is in the same order of magnitude.

These results may seem contradictory to previous results showing that HIV-1 and in particular SI / X4 variants may be harmful to the thymus (chapter 6), but in fact they are not. Naive T cells that are produced by the thymus are generally long-lived (26;27). For example, 5 years after thymectomy, CD4+ T cell numbers were not significantly lower in thymectomized compared to control individuals (28;29), but it should be noted that extensive detailed analyses of naive and memory CD4+ and CD8+ T cell numbers after thymectomy are lacking thusfar. Given the
longevity of naive T cells, complete abrogation of thymic output will only slowly affect the naive T cell pool and additional peripheral events such as immune activation are in fact required to significantly reduce peripheral blood naive T cell and TREC numbers within 5 to 10 years, the average time to AIDS diagnosis. Indeed, in thymectomized SIV-infected rhesus macaques, naive CD4$^+$ and CD8$^+$ T cell decline was not faster compared to non-thymectomized SIV-infected control animals (30). These data confirm anecdotal observations in HIV-1 infected individuals that had been thymectomized prior to HIV-1 infection (31), and suggest that thymic dysfunction by itself does not significantly contribute to SIV / HIV-1 related CD4$^+$ T cell decline. Taken together, although immediate and complete abrogation of thymic function as a result of SIV / HIV-1 infection can not be excluded, thymic dysfunction alone can not explain the naive T cell loss or TREC decline that characterizes pathogenic SIV and HIV-1 infection because it would by itself not induce such relatively rapid effects.

Thymic function declines with age, and it has frequently been suggested that the effects of thymic dysfunction on HIV-1 pathogenesis, if any, may be more pronounced in pediatric HIV-1 infection. Similarly, immune reconstitution in HIV-1 infected children on HAART is thought to be significantly faster than in adults, because of a more functional thymus. Combining analyses of TREC content, TREC number, peripheral T cell proliferation and naive T cell numbers in a cohort of HIV-1 infected children, we did however not find conclusive evidence for decreased thymic output during untreated HIV-1 infection (chapter 9). Interestingly, during HAART, naive T cell numbers recovered only partially in patients older than two years. This suggests that even in children, who are considered to have abundant thymic tissue, thymic output could not be significantly increased to overcome naive T cell depletion (chapter 9 and reference (32)). This is in line with previous observations in pediatric (33) and adult (chapter 11) oncology patients who received a bone-marrow transplantation, and suggests that thymic output is constant, albeit at higher levels in children, and does not depend on peripheral blood T cell numbers.

Establishment and maintenance of the T cell pool

To understand CD4$^+$ T cell depletion during HIV-1 infection, it may be important to better understand how the T cell pool is formed during fetal life and childhood, and how it is maintained thereafter. Unfortunately, not much is known, except that thymic size is highest in infants and declines with age, in parallel with a gradual decline in peripheral blood T cell numbers (34:35). Recovery of naive T cell and TREC numbers after iatrogenic T cell depletion suggested low but significant thymic output in adults (chapter 11 and references therein). Nonetheless, the contribution of the thymus to maintenance of the T cell pool is considered to be small, as adult thymectomy does not result in a rapid decline in peripheral blood T cell numbers (28:29). In contrast, the thymus does seem to be important for
establishment of the T cell pool during childhood. Congenital thymic defects such as Complete DiGeorge Syndrome are not compatible with life, and thymectomy before the age of 1 year resulted 4-5 years later in significantly lower numbers of T cells compared to thymectomy performed at older ages (29). Thus, establishment of the naive T cell pool may occur mainly \textit{in utero} and during early life, with subsequent thymic involution.

Formation of the memory compartment seems to occur especially during childhood, when \textit{de novo} antigen-exposure is high, which may be largely subclinical. When studying a group of 29 healthy children of different ages, we found high proportions of Ki67$^+$ naive T cells in infants that declined with increasing age (chapter 9). This may reflect antigen-specific T cell activation resulting in proliferation and differentiation of naive T cells, a subset of which survives to become memory T cells. Because it has been suggested that recent thymic emigrants are Ki67$^+$ (36), finding increased proportions of Ki67$^+$ naive T cells in the peripheral blood could simply relate to high thymic output in infants. However, Ki67 expression was also increased in memory CD4$^+$ T cells (chapter 9), suggesting activation induced up-regulation of this protein. These observations are compatible with reports of rapid telomere shortening during childhood that may divulge periods of accelerated T cell division (37;38). During early childhood, until the age of two, telomere lengths of granulocytes and of naive CD4$^+$ and naive CD8$^+$ T cells declined with similar rates, after which telomere shortening slowed down considerably (38). This suggested increased cell division of the common hematopoietic progenitors of both cell types. Telomere lengths of memory T cells shortened at a faster pace, however, suggesting repeated periods of antigenic stimulation that only slowed down after the age of four (38). A schematic outline of the formation of the T cell pool is shown in Figure 1.

Maintenance of the naive and memory T cell compartments is thought to depend on low-level regenerative proliferation. However, this preservation is not complete, because with increasing age the total number of T cells, including the number of naive CD4$^+$ and naive CD8$^+$ T cells, declines (35;39). Taking the longevity of naive T cells into consideration, this age-dependent naive T cell decline may not simply reflect declining thymic output, but most likely is related to continuous recruitment of naive T cells to the memory compartment. As the vestigial thymus can not compensate for this slow but continuous antigen-driven loss of T cells from the naive T cell pool, peripheral blood naive T cell numbers decline with age.

T cell depletion in HIV-1 infection

We propose that in HIV-1 infection, similar mechanisms as those that determine age-related T cell decline are involved but at a higher pace. Continuous high plasma HIV-1 RNA levels are associated with high levels of immune activation, that may lead to continuous rather than occasional recruitment of naive CD4$^+$ and
Figure 1. Establishment of the T cell pool. (a) Naive T cell numbers that are initially high decline rapidly with increasing age, whereas the number of memory T cells remains stable. Data are derived from chapters 3 and 9. It should be noted that T cell numbers are depicted per microliter blood. Given the rapid increase in total body mass and blood volume during childhood, total body numbers of naive T cells decline much slower or may even temporarily increase, in parallel with an increase in total body number of memory T cells. (b) In infants, thymic output of naive T cells is high, in addition to de novo antigen (Ag) exposure that leads to formation of the memory compartment. With increasing age, thymic size declines and through repeated antigen exposure, naive T cells are recruited to the memory compartment. In adults, migration of naive T cells to the memory pool leads to a gradual decline in naive T cell numbers as thymic output is too low to compensate for the loss of naive T cells. T: thymus; N: naive T cell pool; M: memory T cell pool.

CD8⁺ T cells to the memory compartment. T cells that are thereby lost from the naive compartment are not replaced, as the thymus is not capable to increase its output because of HIV-1 induced dysfunction, and even more likely, because of limited cellular output of the thymus (chapters 9, 11 and 12). Indeed, having low numbers of CD4⁺ T cells before HIV-1 seroconversion was associated with more rapid progression to AIDS, which suggests accelerated emptying of a T cell stock that was already low in some patients (chapter 5).

Using an empirical mathematical model for BrdU-labeling kinetics, Kovacs et al recently distinguished in both the CD4⁺ and in the CD8⁺ T cell pool two dynamically distinct populations of T cells: one rapidly and one slowly proliferating subset (2). It should be noted that in vivo labeling of dividing cells with BrdU has some disadvantages compared to labeling with deuterated glucose, because labeled cells instead of labeled DNA strands are detected. Proliferation after cessation of label infusion will in case of BrdU initially result in expansion of the proportion of labeled cells followed by loss of label, that does not distinguish between dilution or death of labeled cells (40). Nevertheless, decay kinetics of deuterated glucose and BrdU-labeled cells followed similar patterns (1:2).
validating the recognition of rapidly and slowly dividing subpopulations by Kovacs et al (2). Taking the presence of T cell subsets with different turnover rates into consideration, HIV-1 related depletion of naive CD4+ and naive CD8+ T cells (41) and the gradual decline in total CD4+ T cell numbers may best be explained by continuously elevated levels of immune activation that drive increased numbers of slowly replicating, mostly naive CD4+ and CD8+ T cells to the rapidly proliferating part of the memory compartment. Due to the differential survival kinetics of activated memory CD4+ and CD8+ T cells (42), persistent immune activation may lead to a gradual loss of memory CD4+ T cells, but expansion of the CD8+ T cell pool, as observed in HIV-1 infection. In addition, direct infection and killing of naive and memory CD4+ T cells by X4 HIV-1 variants may accelerate this process (chapter 10). In Figure 2, a schematic representation of this model is shown. According to the model, continuous hyperactivation of the immune system in the absence of HIV-1 infection could lead to naive T cell decline. Indeed, it was recently demonstrated in mice that long-term hyperactivation of the immune system, through constitutive expression of the co-stimulatory molecule CD70, was associated with progressive CD4+ and CD8+ T cell decline and development of lethal immune deficiency reminiscent of AIDS (43). In addition, HIV-negative Ethiopians, who typically have a persistently activated immune system, probably because of increased domestic exposure to pathogens, had significantly lower numbers of CD4+ T cells compared to healthy Dutch individuals (44). This was related to reduced numbers of naive and CD27+ memory CD4+ T cells, and in addition to a significant loss of naive CD8+ T cells. Due to expansion of the memory and effector CD8+ T cell compartment, total CD8+ T cell numbers were not lower than in HIV-1 Dutch subjects (44). It is however not known whether continuous immune hyperactivation in Ethiopians leads to the development of lethal immune deficiencies, because due to recurrent episodes of famine, civil war and the AIDS epidemic, life expectancy in Ethiopia is very low (in 1998, life expectancy at birth was only 41 years compared to 78 years for Dutch individuals (45)).

Clinical implications

If indeed chronic immune activation depletes CD4+ T cells, immunosuppressive drugs might deserve a place in the treatment of HIV-1 infected patients, in addition to or as an alternative for anti-retroviral therapy. Only limited data are available thusfar. Treatment of chronically HIV-1 infected patients with mycophenolic acid in combination with long-term HAART has shown to reduce T cell activation, but the 24-week follow-up period was too short to detect significant differences in T cell numbers (46). In a more recent pilot study, patients were treated with cyclosporin A (CsA) in combination with HAART during acute HIV-1 infection (47). Peripheral blood CD4+ T cell numbers in CsA + HAART patients were twice as high compared to HAART-alone treated patients as
Figure 2. The role of increased T cell turnover in HIV-1 induced T cell depletion. (a) In uninfected individuals, there is continuous, low-level T cell proliferation that allows maintenance of the T cell pool (regenerative proliferation, RP). At any given timepoint, only a small subset of T cells (approximately 1%, chapter 3) is involved in this type of T cell proliferation. (b) In case of HIV-1 infection, an increase in the proportion of dividing T cells is observed. This involves activation-induced T cell proliferation (A-IP) that is related to high levels of plasma HIV-1 RNA levels (chapter 3). Activated CD4+ and CD8+ T cells die (D) because of activation induced cell death or apoptosis, CD4+ T cells to higher extend than CD8+ T cells because of different survival kinetics. In addition, a small subset of CD4+ T cells dies through direct infection and killing of these cells by HIV-1. According to Mohri et al., HIV-1 related CD4+ T cell decline is caused by increased CD4+ T cell death rates, which was assumed to be related to direct effects of the virus. However, measurements of T cell turnover using deuterated glucose labelling yield estimations of proliferation and death rates mainly for the rapidly proliferating T cell compartment (A-IP) that should not be applied to the entire T cell pool. Increased death rates in the high turnover compartment do not deplete T lymphocytes, as these cells die only after they have divided several times. (c) More important to HIV-1 pathogenesis may be therefore the continuous recruitment (R) of non- or slowly dividing T cells to the rapidly dividing compartment (chapter 12, and references). When cells that are lost from the quiescent part of the T cell pool are not replaced, total numbers of T cells gradually decline.

early as 7 days after initiation of treatment and this difference remained for at least 64 weeks despite discontinuation of CsA after 8 weeks. Interestingly, in an early study conducted before HAART became available, 1 year of prednisolone treatment alone in chronically infected patients also induced significant changes in peripheral blood CD4+ T cell numbers. This occurred in parallel with a decline in immune activation levels, despite stable, high levels of plasma HIV-1 RNA (48). Changes in CD4+ T cell numbers in these patients were also most pronounced early after start of treatment, confirming the importance of immune activation-related T cell redistribution in recovery of T cell numbers (chapter 4 and reference 49). Thus, suppression of immune activation may prevent loss of CD4+ T cells from the peripheral blood, however, its effect on long-term maintenance of total body CD4+ T cell numbers remains to be determined. Detailed longitudinal analyses of patients on HAART who despite virologic failure experience immunologic improvement may be helpful in this respect.

Furthermore, if HIV-1 infection indeed accelerates aging of the immune system and early emptying of T cell stocks, early initiation of antiretroviral treatment may be important to prevent T cell loss that seems at least partially irreversible. Indeed, while true long-term follow-up studies on T cell restoration during HAART are lacking, available data suggest that following initial recovery of
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Peripheral blood T cell numbers a plateau is reached, after which no further growth is observed (50-52).

Concluding remarks

HIV-1 infection is characterized by continuously elevated levels of immune activation that is now increasingly recognized to be pivotal in HIV-1 pathogenesis. Continuous immune hyperactivation apparently results in recruitment of naive T cells to the memory pool. The inability of the thymus to replace lost naive T cells seems the second critical parameter in AIDS pathogenesis. Whether HIV-1 has a severe effect on thymic function and whether such an affect would be critical in AIDS pathogenesis remains to be elucidated. Development of new tools to measure thymic function is therefore required. One of the most intriguing questions for future research is how some species such as sooty mangabeys ignore the presence of a virus that induces continuously high levels of immune activation ultimately leading to AIDS in others.

References

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