T cell turnover and thymic function in HIV-1 infection
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6 Introduction:

Tilted balance of T cell renewal in HIV-1 infection

Mette D. Hazenberg, Dawn R. Clark and Frank Miedema
Abstract

The cause of CD4⁺ T lymphocyte depletion in HIV-1 infection is still not understood. Over the past years, attention has been focused on the hypothesis of highly increased T cell turnover with subsequent exhaustion of the immune system. However, the rate of T cell destruction was reported to be relatively low and the modest increase in lymphocyte production to compensate for this loss is not likely to be able to exhaust the immune system. Therefore, other mechanisms must be involved in the pathogenesis of lymphocyte depletion in HIV-1 infection. Interference of HIV with the renewal capacity of the immune system provides another explanation for the observed gradual decline in CD4⁺ T cell numbers. It has been postulated that the regenerative capacity of the immune system is intrinsically slow and cannot be significantly increased. Moreover, HIV-1 was shown to be able to infect bone marrow and thymic tissues, thereby impairing de novo production of naive T cells. The net result of a modestly increased T cell loss and a severely impaired capacity of the immune system to compensate for this deprivation is a gradual decline in T cell numbers. We here argue that in HIV-1 infection the balance between lymphocyte production and destruction is disturbed, ultimately leading to severe immunodepletion and development of AIDS.
Introduction

One of the hallmarks of HIV infection is a change in T lymphocyte subset composition and function, which ultimately leads to severe immunodeficiency and progression to AIDS. Although usually this feature is referred to as HIV-induced decline in CD4+ T cell number, this could suggest a steady decline in numbers. However, the picture is more complex and much more dynamic (Figure 1). Both the CD4+ and CD8+ naive T cell compartment begin to decline shortly after infection, whereas the CD4+ memory population initially expands due to peripheral expansion but is then progressively depleted. Total numbers of CD8+ T cells increase over time, due to a massive expansion of the CD8+CD45RO+ memory subset. This subset only starts to decline shortly preceding AIDS diagnosis (1-3). The exact cause of the changes in the different CD4+ T cell compartments is not understood. For CD4+ T cell depletion in general, several hypotheses have been postulated, such as virus-related killing, activation-induced apoptosis, high turnover-induced exhaustion and interference with renewal mechanisms (4-14).

Most notably the high turnover model, proposed first by Ho and Wei in 1995 (13:14), received a lot of attention. Their application of mathematical modelling to viral load reduction and raise of CD4+ T cell numbers after treatment with anti-retroviral drugs has changed our view on HIV pathogenesis dramatically. It was shown that HIV infection is a highly dynamic process with much more virus turnover than was anticipated. Furthermore, based on the observation that CD4+ T cell numbers in the peripheral blood increase rapidly after initiation of potent anti-retroviral therapy, these investigators suggested that in HIV infection loss of cells is compensated for by an increased lymphocyte production. It was believed that cell production during untreated HIV infection could be raised enormously, even up to 70-fold, which would ultimately lead to exhaustion of the immune system with subsequent CD4+ T cell decline and progression to AIDS. Although this seemed a
very attractive and plausible explanation for CD4\(^+\) T cell loss, data have accumulated showing that in HIV infection turnover is only modestly increased (15–22). Loss of CD4\(^+\) T cells in HIV infection may involve, directly and indirectly, activation induced apoptosis and virus mediated killing of infected lymphocytes. Surprisingly, the number of productively infected T cells was shown to be very low, in the order of \(10^7\) to \(10^8\) cells (23:24), and in lymphoid tissue only a two-fold increase in CD4\(^+\) T cell apoptosis was reported (25). These results indicate that the daily loss of CD4\(^+\) T cells is only modestly augmented in HIV infection. Since the rate of CD4\(^+\) T cell destruction is relatively low, the limited increase in lymphocyte production to compensate for the loss is not likely to be able to exhaust the immune system. We and others have thus proposed that alternative mechanisms must be involved in HIV mediated T cell decline (26:27). Mounting evidence points towards interference of HIV with T cell renewal mechanisms as a cause for T lymphocyte depletion. If de novo production of T cells is disturbed by viral interference with bone marrow cells and stromal thymic tissue and thymocytes, then even a modest limited virus-mediated loss of CD4\(^+\) T lymphocytes cannot be compensated for by an increased production of new cells. This could then by itself result in a net decline of naive T cell numbers. Here, we will review what is known about normal T cell renewal, the influence of HIV infection on T cell production and we will discuss the relevance of these findings with regard to HIV pathogenesis and therapeutic strategies.

**T cell development**

Animal and human studies have revealed that post-natal CD4\(^+\) and CD8\(^+\) T cell generation involves two different pathways: 1. thymus-dependent development of new T lymphocytes from a progenitor source and 2. thymus-independent proliferation of mature cells in the peripheral blood and lymphoid tissues (reviewed in (28)). In this section, the relative importance of each pathway in repopulating the immune system after depletion will be discussed. In patients who received T cell depleting doses of chemotherapy or anti-CD4 monoclonal antibodies, regeneration rates of lymphocytes were evaluated. New T cells that evolve from the thymus enter the periphery as naive, unprimed cells. In adults, restoration to a normal naive population took at least more than one year, whereas in children, CD4\(^+\) T cell depletion was restored within six months (29–32). Furthermore, reconstitution of the T lymphocyte compartment in adult individuals undergoing bone marrow transplantation was reported to be biphasic. Initial immune restoration was dominated by an expansion of memory-type cells (33–35). Expansion of pre-existing T cells appeared to be antigen induced, and its quality is determined by the diversity of the remaining T cell pool. Indeed, the TCR repertoire of these expanded, primed subsets was shown to be limited. Then,
in parallel with the reappearance of naive CD4⁺ lymphocytes—at the earliest after six months—the T cell repertoire diversified (36).

It has been suggested that damage of thymic stroma, inflicted by chemotherapy, could hamper thymic function and therefore be the cause of the reported delay in immune recovery. However, restoration rates observed in multiple sclerosis and rheumatoid arthritis patients who received T cell depleting doses of monoclonal anti-CD4 antibodies were not different from those observed in oncology patients (30-32).

Taken together, these results indicate that T lymphocyte reconstitution does depend on both mechanisms, however, with increasing age peripheral expansion dominates, possibly to compensate for an age-related decline not only in thymic function, but also in CD34⁺ progenitor cell developmental capacity (37). Moreover, the very slow reconstitution rates of naive T cells even after severe immunodepletion has lead to the suggestion that in adults, immune regenerative function is always near maximum capacity and cannot be substantially increased (25).

**T cell renewal in HIV infection**

Naive CD4⁺ and CD8⁺ T cells start to decline shortly after HIV infection, long before the development of immunodeficiency and AIDS related symptoms. Two factors are essential in the generation of newly produced naive T cells: the presence of bone marrow derived CD34⁺ progenitor cells capable to develop into thymocytes and an efficiently functioning thymus in which these thymocytes can mature. In the following sections, we will discuss the influence of HIV infection on both issues.

**Bone marrow and progenitor cells**

Several hematological abnormalities, above all the frequently observed cytopenias, have led to the suggestion that bone marrow dysfunction could be involved in HIV pathogenesis (38). Indeed, evidence is accumulating pointing towards HIV-induced suppression of bone marrow progenitor cell capacity.

Viral replication is detectable in the bone marrow, probably through infection of stromal cells (39:40). Hematopoietic progenitor cells cannot be infected directly, however, in HIV infection their function has been reported to be diminished which can be reversed by anti-gp160 or anti-gp120 antibodies in vitro or anti-retroviral therapy in vivo (39:41-44). Suppression of progenitor function could be caused by a virus-induced diminished potential of bone marrow stromal cells to respond to regulatory cytokines, or through endogenously produced cytokines such as TGF-1β that exert negative growth signals (40:44). Recently, Vignoli et al reported a gp120 and TGF-1β mediated impairment of telomerase activity in progenitor cells, and they suggested that this could diminish the replicative potential of these cells (45).
The ability of CD34+ progenitor cells to develop into thymocytes can be evaluated by applying the fetal thymic organ culture (FTOC) system. In this assay, CD34+ progenitor cells, harvested from the peripheral blood, are cultured on murine fetal thymi. It was shown that in HIV-1 infected individuals, the capacity to develop T lymphocytes in FTOC is significantly impaired, even in patients with normal CD4+ T cell numbers (46). Moreover, longitudinal studies from our laboratory revealed an early loss of T cell development capacity in patients who progressed to AIDS, whereas long-term non-progressors (LTNP) still retained significant capacity after 8 years of infection (47). In conclusion, in untreated HIV infection, progenitor function is suppressed, possibly as a consequence of infection of bone marrow stromal cells and cytokine abnormalities resulting from immune activation.

**Thymocytes**

The observed improvement in T cell developmental capacity following antiretroviral therapy, as has been measured in FTOC, correlated with a raise in naive CD4+ T cell numbers. However, in some individuals, CD4+ T cell counts did not improve, despite normal progenitor capacity. This observation could be explained by an impairment of the other parameter pivotal in T cell development, thymic function (47).

In a recent study, thymic tissue of individuals who died of complications related to HIV infection was analysed at autopsy. In all thymi, inflammatory infiltrates were found that surrounded lymphodepleted thymic epithelium (48). These results reflect the notion that HIV- mediated reduction in thymic function could be due to virus- induced changes in thymocytes, their environment, or both (49).

In severe combined immunodeficiency mice grafted with human tissue (SCID-hu model), HIV infection induced histopathological changes in thymic tissues that were reminiscent of those observed in infected human thymus (9:50:51). Thymocytes and thymic stromal cells were reported to be directly infected in these animals (9:51). As a consequence, thymocyte loss was shown to be due to direct virus mediated killing of immature progenitors and to induction of apoptosis (52).

These results are compatible with observations made *in vivo*. Analysis of thymic tissue in an asymptomatic HIV infected infant also revealed direct infection of thymocytes and stromal tissue. A depletion of double positive CD3+CD4+CD8+ and single positive CD3+CD4+CD8- thymocytes was observed, in parallel with a proportional raise in CD8+ thymocytes (53). It should be noted that this proportional increase does not exclude a decline in absolute numbers of these CD8+ cells.

Preferential infection of either thymic stromal cells or thymocytes could depend on the type of virus isolate a patient is infected with. In SCID-hu mice, infection with the highly cytopathic, syncytium inducing variant NL4-3 resulted in a preferential depletion of CD3+CD4+CD8+ thymocytes, whereas infection with the less cytopathic Ba-L strain caused infection of stromal cells, initially without any obvious immunological consequences, but in some cases ultimately leading to
Thymocyte depletion (54). Viral entry of phenotypic different strains could be readily explained by co-receptor distribution on thymocyte subsets (55). In humans, phenotypic switch of HIV-1 from the non syncytium inducing (NSI) variant towards the syncytium inducing (SI) variant is associated with a more rapid decline in CD4+ T cell numbers and progression to AIDS diagnosis (56). NSI viruses use the CCR5 receptor as a co-receptor, whereas SI viral strains are CXCR4-tropic. The majority of thymocytes express CXCR4, whereas a small, more mature subset bears the CCR5 co-receptor (55). Taking into account the above described results obtained with the SCID-hu model, it can be imagined that SI variants have the capacity to infect maturing thymocytes, thereby selectively depleting double positive and CD4+ immature T cells and inducing a more rapid T cell decline and progression to AIDS diagnosis.

Direct determination of thymic output in humans has been hampered by the absence of a true marker to identify peripheral blood cells that where recently produced in the thymus and subsequently released into the periphery, the so-called recent thymic emigrants. Therefore, thymic function has only been addressed indirectly so far. Very recently, a more direct method has been reported that is based on the detection of stable circular excision products that are formed during rearrangement of T cell receptor genes (T Cell Receptor Excisional Circles, or TRECs). αβ-TCR bearing lymphocytes are exclusively generated in the thymus, during which process the δ-locus is excised and forms a stable circle in the cell nucleus (57). PCR based measurement of TRECs allows for assessment of thymic function. By applying this technique, it was shown that indeed thymic function declines with age, however, even in late adulthood, measurable thymic output is maintained. In untreated HIV infected individuals, a decrease in thymic function was observed, whereas anti-retroviral therapy led to a sustained increase in thymic output. From this it was concluded that impairment of thymic output is involved in CD4+ T cell depletion, and that the adult thymus contributes to immune

### Table 1. The influence of HIV-1 infection on bone marrow and thymus

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<thead>
<tr>
<th>Bone Marrow</th>
<th>Thymus</th>
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<td><strong>Stroma</strong></td>
<td><strong>Progenitors</strong></td>
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<td>Viral replication detectable (39:40)</td>
<td>TGF-1β / gp160 / gp120 mediated impairment of</td>
</tr>
<tr>
<td>Disturbed cytokine secretion? (40:44)</td>
<td>- developmental capacity(39:41-44,47)</td>
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<tr>
<td>Disturbed response to cytokines? (40:44)</td>
<td>- telomerase activity(45)</td>
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reconstitution following anti-retroviral therapy (58). Together, these studies indicate that maintenance of a normal T cell population requires functional thymic tissue, even in adults, and that HIV-1 both directly, by infecting thymocytes, as well as indirectly, by infecting and thereby negatively influencing thymic stroma function, interferes with T cell development. Moreover, differences in pathogenicity between NSI and SI variants could be explained by their biological properties.

**Improvement of T cell renewal after introduction of anti-retroviral therapy**

Treatment of HIV infected individuals with highly active anti-retroviral therapy (HAART) resulted in an impressive decline of the viral load, concomitant with a raise in CD4\(^+\) T lymphocytes. This recovery of CD4\(^+\) T cell numbers is characterized by a steep increase in the number of memory cells, reaching a plateau phase within a month, which is accompanied by a slow but steady increase in the number of naive cells. However, complete immune recovery may require years, if reached at all (59:60). Studying the patterns of immune reconstitution will provide us with additional insight into the influence of HIV-1 on T cell renewal mechanisms.

It has been argued that during chronic HIV infection, as a result of the inflammatory state, lymphocytes are trapped in lymphoid tissues (61–63). Using mathematical modelling, we demonstrated that the observed increase in CD4\(^+\) memory cells after initiation of therapy could be explained by redistribution of these memory cells from lymphoid tissues into the peripheral blood (59). Very recently it was shown that in SIV infected, asymptomatic macaques, changes in CD4\(^+\) T cell distribution had more impact on peripheral blood CD4\(^+\) lymphocyte numbers than cell depletion (64). In humans, HIV infection of resting T cells resulted in enhanced homing of these cells into lymphoid tissues via upregulation of the lymph node homing receptor CD62L (65). In conclusion, as a cause of chronic HIV infection, T cells are trapped in lymphoid tissues, only to be released upon significant lowering of the viral load. The latter accounts for the initial raise in CD4\(^+\) memory cells seen after induction of therapy.

Restoration of naive cells is probably more complex, since several parameters are involved in this process. Evaluation of CD34\(^+\) progenitor cell function in FTOC following HAART revealed that reduction of viral load leads to improved capacity of these cells to develop into mature thymocytes. Moreover, this improvement was in most, but not all, patients correlated with CD4\(^+\) T cell increase (47). Thymic output, as measured by TRECs, improved as well (58), indicating that both progenitor capacity as well as thymic function are important factors in immune reconstitution. The observation that a number of patients did show increased progenitor capacity without an improvement in CD4\(^+\) T cell counts suggests that progenitor and thymic function are two entities in HIV pathogenesis.
In adults, peripheral expansion of pre-existing lymphocytes might also be involved in therapy-induced immune reconstitution. However, total body numbers of dividing CD4$^+$ and CD8$^+$ cells declined in individuals receiving HAART (19:25) (M.D. Hazenberg, manuscript in preparation), thereby excluding a prominent role for peripheral expansion in this setting.

Since thymic function is thought to be age-dependent, it would be very interesting to study restoration of naive T cells in HIV infected children. Few reports have been published, and they described conflicting results (66:67) (N.G. Pakker, unpublished results). This could partly be due to the limited number of infants that were studied. It is frequently hypothesized that in children, immune reconstitution would be faster after initiation of HAART. However, paradoxically, a more active thymus could also be more susceptible to HIV infection, which would require more time to recover from HIV induced damage. Furthermore, destruction of the in utero or post-natally infected thymus could be devastating and even irreversible.

Taken together, immune restoration following therapy is biphasic and accounted for by different parameters. This is illustrated by the biphasic resolution of TCR repertoire disruptions. In HIV infection, CD4$^+$ T cell decline is accompanied by a decrease in TCR repertoire diversity (68). Initiation of HAART induced a transient increase in repertoire perturbation, reflecting redistribution of oligoclonal naive and memory cells from the lymphoid tissues into the peripheral blood. One year later, TCR diversity had improved in some patients, but did not reach normal levels (69:70). As has been outlined above, de novo production of naive cells is age-dependent and slow in adults, reflected by the sustained repertoire disturbances even after 12 months of therapy.

In conclusion, at least two major factors account for the raise in CD4$^+$ T cells following HAART: redistribution of T cells from lymphoid tissue, and de novo production of naive lymphocytes, the capacity of which improves following reduction of viral load.

Discussion

In conclusion, HIV interferes with several parameters important in T cell renewal. Direct infection of bone marrow stromal cells might hamper normal progenitor development, whereas the intrinsic capacity of progenitor cells to develop into mature thymocytes is impaired as well. This could lead to a reduced number of capable progenitor cells entering the thymus, thereby reducing thymic output. Moreover, thymic stromal tissue as well as immature and mature CD4$^+$ thymocytes get infected during the course of HIV infection. Individual immunological consequences of thymic infection — depletion of defined subsets — might depend on the type of virus involved.

We here argue that interference of HIV with renewal mechanisms of the immune system could be the cause of the initial slow but later progressive CD4$^+$ T cell depletion and subsequent development of immunodeficiency. If indeed the adult
immune system is not able to quicken its pace, then a modestly increased daily loss of T cells, together with a reduced capacity to produce new T lymphocytes, will result in a net loss of lymphocytes that is enough to account for the observed CD4⁺ T cell decline.

HAART has beneficial effects on both progenitor cell capacity and thymic function, correlating with immune restoration. This has several implications. First, the importance of de novo T cell production in immune restoration should not be underestimated, despite the age-related decline in thymic function. Second, prevention of possibly irreversible damage to an organ that because of age already has a diminished functionality should have priority in the management of HIV infection. As a consequence, early treatment seems even more imperative. In the clinical setting, attention should therefore be focused on early identification of HIV infected individuals. Since acute HIV infection is presented with an enormous variety of symptoms, it might implicate introducing HIV tests as a routine procedure in patients with unexplained clinical symptoms. Furthermore, research should focus on therapies aimed at improving renewal capacity, to the benefit of not only HIV infected individuals, but lymphopenic oncology patients as well.

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