The Thymus During HIV Disease: Role in Pathogenesis and in Immune Recovery

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Abstract: The thymus is the primary lymphoid organ supplying new lymphocytes to the periphery. Clinical and morphologic studies of HIV-infected children and adults indicate that the thymus is affected by HIV. Thymic dysfunction and thymic involution occur during HIV disease and have been associated with rapid progression in infants infected perinatally with HIV. *In vitro* information of thymic organ culture, thymic epithelial cell culture, the SCID-hu mouse system and SHIV infection of primates have supported HIV-induced thymic damage. The mechanisms underlying this could be many, including direct thymocyte killing by the virus, apoptosis, or disruption of thymic stromal architecture. T cell receptor excision circles (TREC) have been developed as a marker of new thymic emigrants. Decreases in TREC concentrations have been found in both HIV-infected pediatric and adult patients. Mathematical models have suggested that thymic infection in children is more severe than in adults, particularly during infection with strains that use CXCR4 as coreceptor. Recent evidence suggests that thymic recovery may be achieved in some patients as a result of potent antiretroviral therapy. Extensive thymic damage may, however, hamper immune reconstitution, particularly in pediatric patients. This review attempts to summarize evidence for thymic involvement during HIV infection in children and in adults, the role of thymic infection in disease progression, and the contribution of the thymus to immune restoration following potent antiviral therapy. Immunologic interventions aiming at restoring thymic function in AIDS patients are also reviewed.

Keywords: Thymus, HIV Infection, HAART, Pediatric Patients, Mathematical Model

INTRODUCTION

It is well recognized that thymopoiesis, the basic process of production of mature naïve T lymphocytes populating the lymphoid system, is most active during the earlier parts of life [29]. However, the role and importance of the thymus in maintenance of immune homeostasis in the adult have been somewhat controversial. Following the discovery of T-cell receptor excision circles (TREC), the continued importance of thymic function in later life was demonstrated [25]. Several investigators have reported that HIV-induced thymic dysfunction could influence the rate of disease progression to AIDS, suggesting a crucial role of impaired thymopoiesis in HIV pathogenesis [15, 51]. The role and contribution of thymopoiesis to immune recovery following highly active antiretroviral therapy (HAART) is currently being deciphered. This review addresses evidence for thymic involvement in HIV disease, the impact of HIV-induced impairment of thymic function on progression to AIDS, and the potential for thymic regeneration following antiviral therapy. The roles of other immune therapies in aiding thymic restoration in HIV disease are also addressed.

THYMIC FUNCTION IN HEALTH

The human thymus is the primary lymphoid organ supplying new T lymphocytes to the periphery. These newly exported T lymphocytes are referred to as recent thymic emigrants (RTEs). RTEs help establish and maintain the peripheral T cell pool, as well as provide T cell repertoire diversity necessary for potential immune responses to a vast number of antigens throughout life (Fig. (1)) [29].

Functional thymic tissue shows a continuous involution from the first year until the end of life [79]. Consequently, the numbers of thymocytes and RTEs gradually decline during aging [29]. Therefore, in adults, most newly generated T cells are derived from peripheral T cell proliferation. Furthermore, children tend to have faster T cell reconstitution than adults after cancer therapy and stem-cell transplantation [24, 52]. However, recent advances in characterizing thymic function suggest that the adult thymus is still actively engaged in thymopoiesis and exports new T cells to the periphery till at least 60 years of age [36, 67].

MARKERS OF THYMIC FUNCTION

Exact quantification and characterization of RTEs in humans is not yet possible since no direct phenotypic markers have been discovered for RTEs [87]. Thymic size has been used as a marker of thymic function. Several thoracic imaging approaches have been developed, including the most widely used mediastinal CT scan. However, most of these approaches have their technical limitations [16, 18, 57]. Concurrent profound reduction in both CD4+ and CD8+ T cell counts, as well as CD5+ B cell counts, have been proposed as an immunophenotypic profile indicative of

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thymic dysfunction, based on observations in patients with congenital DiGeorge anomaly who have thymic absence or hypoplasia [49, 51]. However, measurement of CD5+ marker on B cells is not widely available, and the profile is of greater prognostic significance in children. Naïve T cell subsets, thought to originate in the thymus, have also been suggested as a marker of thymic output. However, markers reflecting most accurately newly produced naïve T cells have been a matter of debate [30, 65].

T cell receptor excision circles (TRECs) were developed as a marker of recent thymic emigrants in humans [25, 92]. TRECs are generated within thymocytes as episomal DNA during T cell receptor gene rearrangements. They are exported from the thymus to the periphery within T cells. TRECs are stable, and their concentration is diluted out after each cell division [87]. Therefore, TREC concentration in the periphery is a function of the number of RTEs that emigrate from the thymus to the periphery.

Studies have shown that TREC concentration declines with aging [25, 92]. However, controversy exists as to whether TREC concentration can be used to represent RTE level [31, 88]. TREC concentration is not only affected by thymic output, but also influenced by peripheral T cell turnover events including T cell division and death. Experimentally it is not yet possible to quantitatively differentiate effects of thymic output and T cell turnover on TREC concentration. Mathematical model analyses suggest that thymic involution primarily induces an age-dependent decline in TREC concentration within both CD4+ and CD8+ T cell populations. Therefore, TREC is a good marker of RTE levels in healthy people [88].

THYMIC FUNCTION DURING HIV-1 INFECTION

HIV-1 disease is characterized by a gradual decline in circulating CD4+ T cells as infection progresses over an average span of ten years, leading to death due mostly to opportunistic infections [22]. Several hypotheses have been proposed to explain HIV-1 mediated depletion of CD4+ T cells, including accelerated destruction of CD4+ T cells, altered circulation of CD4+ T cells, and impaired production of new T cells from the thymus [2, 39, 56, 89].

The thymus is the primary lymphoid organ that provides a specialized microenvironment for thymocyte development [29]. Thymocytes can be infected by HIV-1 in experimental models [81], suggesting that this mechanism contributes to CD4+ T cell depletion and accelerates disease progression. Clinical and experimental evidence suggests that the thymus is a critical target organ for HIV-1 infection [25, 51]. Since thymocytes are progenitors of peripheral T lymphocytes, it is expected that progeny will not be produced if progenitor cells are destroyed or nonfunctional.

CLINICAL EVIDENCE

Clinical studies of HIV-1 infected children and adults indicate that thymic function is impaired during infection with HIV-1 [9, 13, 81]. Examination of human thymic specimens has revealed abnormal morphological changes including thymitis, disruption of stromal architecture with loss of Hassal's corpuscles, thymocyte depletion and enhanced involution [27, 68]. These changes are associated with detection of HIV-1 replication within thymocytes [68, 81]. Numbers of CD4+ and CD8+ naive T cells have been

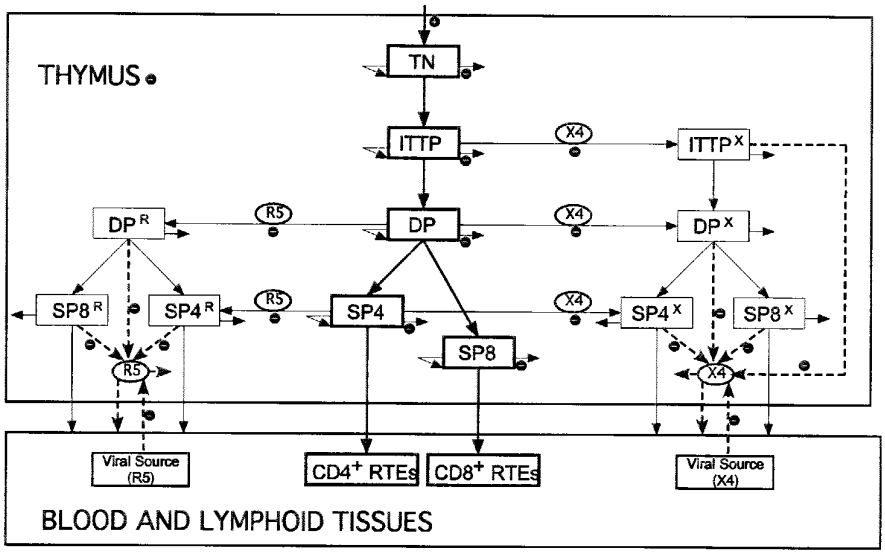

Fig. (1). The model of human thymopoiesis, thymic infection with HIV-1 R5 or X4 strains, and thymic function reconstitution during HAART. Rectangles represent cell populations and ellipses represent viral populations. Cell dynamics are illustrated using solid lines and viral dynamics are illustrated using dashed lines. Thymocyte subsets include triple negative (TN), intrathymic T progenitor (ITTP), double positive (DP), CD4 single positive (SP4) and CD8 single positive (SP8) cells. SP4 and SP8 cells emigrate to the periphery as RTEs [88]. R5 or X4 strains flow in from blood into the thymus and infect thymocyte subsets. Infected thymocytes further differentiate and generate new virions that emigrate to the periphery [89]. The effects of HAART are represented by plus or minus signs on cell and viral dynamics. Plus signs suggest an increase while minus signs suggest a decrease. HAART decreases viral influx from blood into the thymus, increases cell numbers in the thymus, and decreases the enhanced death of uninfected thymocytes. If HAART drugs can penetrate into the thymus, thymic drug efficacy is represented by a decrease in viral infection and production rates [90](reproduced here with permission).

observed to decrease as disease progresses [31]. Thymic volume correlates negatively with HIV-1 viral load [86]. In addition, TREC concentrations also decrease as a function of HIV-1 disease progression in both HIV-1 infected pediatric and adult patients [25, 92].

HIV-1 infection in children is characterized by higher viral load and higher levels of immune activation [50]. Pediatric thymic dysfunction and early thymic involution have been postulated to account for the rapid disease progression observed in a subgroup of infants infected perinatally with HIV-1 [51, 58, 59]. A peripheral blood immunophenotypic profile of rapid and profound decreases in the counts of both CD4+ and CD8+ T lymphocytes and CD5+ B lymphocytes was observed in this subgroup of infants. Several hypotheses have been proposed to explain this phenomenon, including X4 strain infection, host susceptibility to specific strains, and host immune status [51, 90]. The timing of vertical transmission of HIV-1 may also be relevant since prenatal infection would have a more severe impact on thymic function than intrapartum infection with the same viral strain [47, 51, 90]. Pediatric thymic infection with HIV-1 induces more severe effects on *de novo* T cell generation from the thymus as compared with adults, since the thymus is the primary source of newly generated T lymphocytes in children.

EXPERIMENTAL EVIDENCE

Since it is difficult to study the thymus in HIV-1 infected humans, several model systems have been developed, including thymic organ culture [7], thymic epithelial cell culture [61], SCID-hu Thy-Liv mouse model [8], monkey model infected with SIV or SHIV [48, 75], and FIV infection of felines [21]. Infection and destruction of thymocytes and detection of HIV-1 are realized in both thymic organ culture and thymic epithelial cell culture [7, 51a, 61]. HIV-1 infection in SCID-hu Thy-Liv mice is associated with rapid depletion of thymocytes of different maturational stages and an inversion of the CD4+/CD8+ ratio [8]. Decreased TREC concentrations are observed together with morphological alterations within the thymus of SIV infected macaques [3, 75]. FIV infection in cats is characterized by rapid and progressive CD4+ T-cell loss, lymphoid depletion, and severe thymic atrophy [21].

MECHANISMS OF HIV-INDUCED THYMIC DAMAGE

Various mechanisms have been proposed for HIV-induced thymic damage through study of these model systems. First, viral infection may directly infect thymocytes of different maturational stages [35, 77] or indirectly induce the loss of thymocytes through apoptosis [8, 81]. Second, HIV-1 infection of stromal cells including epithelial cells, dendritic cells, and macrophages impairs their essential function in regulating normal thymopoiesis [9, 13, 46]. Third, different HIV-1 strains may induce different infection scenarios within the thymus [89].

The entry of HIV into target cells depends on both receptor CD4 molecules and co-receptor, either CCR5 (CC chemokine receptor 5) or CXCR4 (C-X-C motif receptor 4). The effects of thymic infection with HIV-1 depend to a large degree on co-receptor tropism, as CD4, the primary receptor, is expressed on nearly all thymocytes. CXCR4 is highly expressed in immature intrathymic T progenitor (ITTP) cells and then down-regulated in subsequently more mature thymocyte stages, whereas CCR5 is expressed only at low levels on mature thymocytes [4, 82, 91]. Studies using SCID-hu Thy-Liv mice and human thymocyte tissue cultures have shown that CXCR4-utilizing X4 isolates of HIV-1 destroy thymocytes rapidly in various stages of maturation and cause a severe decrease in all thymocyte subpopulations. By contrast, CCR5-utilizing R5 isolates infect stromal cells and more mature thymocytes, resulting in thymocyte depletion after much longer periods of time [5, 85, 91]. These distinct infection patterns of R5 and X4 strains are consistent with the relative CCR5 and CXCR4 co-receptor surface expression on thymocytes in various stages of maturation. It is thus likely that thymopoiesis is particularly vulnerable to X4 strains. A recent study suggests that children infected with X4 HIV-1 have lower thymic output than those infected with R5 virus [17]. Thus, tropism of HIV-1 can affect the capacity of the thymus to produce new T lymphocytes (Fig. (**1**)).

HIV-1 strains of different virulence may also explain different infection scenarios in the thymus. One study has shown that thymus-derived HIV-1 strains have higher affinity for thymocytes as compared with other strains isolated from other compartments [12]. Clinical strains from pediatric HIV-1 patients with different patterns of disease progression have differing abilities to infect thymic epithelial cells, suggesting that different HIV-1 strains appear to employ different mechanisms by which they affect thymic components [46].

Mathematical models have been developed to capture reduced cell production by the thymus due to HIV-1 infection and study subsequent effects on peripheral T cell patterns [40, 89]. These models have indicated that thymic infection in children is more severe than in adults, particularly during infection with X4 strains. This outcome is likely due to both a higher viral load and a more active thymus in pediatric patients. Mathematical model analyses have revealed that a decrease in thymic output is the major factor contributing to the decline in TREC concentration within CD4+ T cells, while both increased peripheral T cell division and decreased thymic output induce the decline in TREC concentration within CD8+ T cells [88]. Therefore TREC concentration may be a good measurement for CD4+ RTEs but not for CD8+ RTEs during early HIV-1 infection (first three years of infection).

The thymus is essential in healthy children and adults to providing newly-generated T cells to the periphery. However, its role becomes complicated during the course of HIV-1 infection. On one hand, the thymus may still be able to export RTEs even in adults to help re-establish T cell function in the periphery. SHIV infection in some macaques did not lead to declines of CD4+ T cell counts despite high viral loads, suggesting that the thymus still contributed to peripheral T cell generation [48]. On the other hand, the thymus exports infected cells as well as new virus to the periphery [10], accelerating peripheral infection.

EFFECTS OF HAART ON THYMIC FUNCTION

One of the most critical challenges in treating HIV disease is to develop effective strategies to reconstitute a functional immune system and recover the peripheral T cell pool in HIV-1 infected patients. HAART, the most widely used treatment strategy for HIV-1 infection, has been demonstrated to be effective in substantially reducing viremia and aiding in the recovery of CD4+ T cells for some HIV-1 infected individuals [72]. Antiretroviral regimens have also shown great efficacy in reducing transmission of HIV-1 from mother to infants [44, 45].

The peripheral T cell pool damaged by HIV-1 infection can be regenerated by production of new T cells either from the thymus or from peripheral T cell proliferation or both. T cells generated from the thymic-dependent pathway are more effective in restoring immune responses to neoantigens. Biphasic kinetics of CD4 repopulation following HAART were initially described, including an early phase of cellular redistribution from lymph nodes, and a later phase characterized by increases in naïve T cells associated with new cell production [64]. Available evidence points to recovery of some antigen-specific responses after HAART, even though HIV-specific help may not be restored [70].

Recent data on thymic function during HIV-1 treatment in both children and adults support the view that the thymus acts as an important part of HAART-related immune reconstitution. A number of signs signal the recovery of thymopoiesis after treatment of some individuals with effective HAART. A significant increase in absolute CD4+ T cell counts has been observed after HAART and most new CD4+ T cells carry a naive cell marker [6, 23, 28]. Emergence of naïve CD8+ T cells and rapid correction of TCR diversity in naïve CD8+ T cells occurs after HAART, suggesting export of new T cells from the thymus [43]. Children have earlier and greater increases in naive T cell numbers, probably due to a more active thymus [26]. An increased thymic size is observed by computed tomography after HAART [69]; one study suggested this thymic enlargement represents active thymopoiesis [53]. TREC increases after HAART supporting an active role of the thymus in reconstitution of the immune system [23]. Increases in naive T cell counts, in thymic size and in TREC concentrations have also been observed in parallel with substantial improvement of antigen-specific responses and broader immunological repertoires [14, 19, 20, 42, 43, 63, 69, 74, 86]. On the other hand, poor CD4+ restoration after suppression of HIV-1 replication may reflect lower thymic function since these patients, whose CD4+ response to HAART is poor, are generally older than CD4+ responders, with minimal thymic tissue and lower TREC level [76, 83]. Enhanced viral pathogenicity in the thymus could at least partially explain immunological failure after antiretroviral therapy.

Mathematical modeling has suggested that suppressing viral load in peripheral blood and improving inherent thymic function are both necessary for reconstitution of RTE levels [90]. However, recovery of RTE levels during more virulent X4 strain infection also depends on high drug efficacy within the thymus (Fig. (**1**)). The model also predicts that protease inhibitors have high levels of efficacy directly suppressing viral replication within the thymus, while reverse transcriptase inhibitors are not as effective [90].

Alternative mechanisms may also contribute to early T cell reconstitution during HAART. HIV-1 infected thymectomized patients have an initial rise of both naive and memory T cells after HAART, suggesting redistribution and peripheral expansion of T cells as a contributing mechanism [30]. Decreased activation-induced apoptosis leads to increased survival of circulating T cells [6], and increased input of hemopoietic progenitor cells from bone marrow into the thymus enhances the output of RTEs to the periphery [6, 34].

Several studies have suggested that Nef may be the viral region responsible for cellular depletion of the infected thymus [80]. The regions of Nef that affect T-cell development were recently characterized, pointing to target sites in Nef that may therapeutically aid in restoration of thymopoiesis [80].

OTHER IMMUNE RESTORATION THERAPIES IN HIV DISEASE

Aside from HAART, several other immune-based therapies have been studied in order to restore immune function during HIV-1 infection. These include infusion of growth hormone, cytokines such as IL-2 and IL-7, and thymic transplantation.

Growth hormone was used in one study of HIV-1 infected adults [60]. Increased thymic tissue and circulating naïve T cells were seen in all growth hormone recipients. This might suggest that growth hormone has a role as an adjunctive therapy of HIV-infected adults since *de novo* T cell production may be inducible in these patients. The safety profile of this agent must be carefully weighed against its potential benefit, however.

Administration of IL-2 in conjunction with HAART has been used to induce immune reconstitution in HIV-1 infected patients [1, 11, 38, 66]. HAART supplementation with IL-2 was observed to lead to rapid increases in CD4+ T cells and to be well tolerated by HIV-1 patients [1, 11, 66]. One recent study has shown that elevated CD4+ T cell levels were accompanied by TREC decreases in HIV-1 patients on HAART supplemented with IL-2 [66]. This indicated that CD4+ T cell maintenance or reconstitution during initial treatment with HAART supplemented with IL-2 is largely due to thymus-independent mechanisms.

IL-7 secreted by thymic stromal cells plays a role in thymocyte development. Administration of IL-7 to young mice has induced both increased thymopoiesis and peripheral T cell proliferation [73]. *In vivo* and *in vitro* experiments have shown that exogenous IL-7 increased the TREC concentration in infant thymus, suggesting that use of IL-7 might enhance *de novo* naive T-cell generation in HIV-1 patients [62]. While some interest has been generated within the research community regarding the beneficial effects of IL-7, it should be kept in mind that IL-7 has also been associated with an increase in T cell and thymocyte susceptibility to HIV infection [78]. Evidence has also shown that IL-7 secreted by thymic stromal cells positively

regulated CXCR4 expression favoring X4 viral replication within the thymus [71].

Therapeutic vaccines that enhance replication of HIV-specific lymphocytes are another approach that has been tested with contradictory results. Two studies suggested beneficial effects on suppressing viral load, improving CD4+ T cell counts and HIV-specific immunity [37, 84], while another failed to note such effects [66]. Such patients had no significant increase in TREC levels [66], suggesting that the thymus played a negligible role in reconstitution of T cell function during immunogen treatment.

Thymic transplantation may be the only option available for immune reconstitution of patients who have suffered irreversible thymic damage as a result of HIV infection. Thymic transplantation has been performed in adults with HIV-1 infection as well as in infants with DiGeorge anomaly [33, 54, 55]. Whereas successful immune restoration has been achieved in some infants with DiGeorge anomaly [54], no such success was shown in patients with AIDS [55]. Several factors may account for this, including the observation that most early attempts at thymic transplantation of AIDS patients were performed in the face of continued unchecked viral proliferation (pre-HAART era), as well as the employment of different techniques. Progress has been made both with regard to controlling viral replication with potent antiviral regimens, as well as optimizing *ex vivo* maintenance and maturation of thymic tissue [32, 33]. Related approaches in the future might involve the use of CD34+ stem cells on thymic stromal monolayers (since stem cells are not susceptible to HIV) or autologous genetically modified to be HIV-1 resistant T-cell progenitors [41, 70]. Thymic transplantation, done under viral suppression, may have the potential to aid in immune reconstitution of AIDS patients with marked thymic damage.

CONCLUSIONS

Several lines of evidence have now convincingly demonstrated that the thymus is affected by HIV via several different mechanisms, leading to reduced naive T cell production. This contributes to CD4+ lymphocyte depletion and disease progression, both in adult, as well as, most importantly, in pediatric AIDS patients. While HAART may lead to a level of immune reconstitution, not all patients experience immune recovery, particularly those ones most severely affected by HIV.

Strategies aimed at increasing thymopoiesis in HIV-1 infected patients will help improve immune reconstitution in HIV-1 infection. This is more important in pediatric patients whose thymus play a more significant role in contributing new T cells to the peripheral T cell pool. Only when thymic function is reconstituted can we expect that normal T cell levels and a fully functional and diverse immune repertoire will be restored.

ABBREVIATIONS

RTEs = Recent thymic emigrants

TRECs = T cell receptor excision circles

HAART = Highly active anti-retroviral therapy

TN cells = CD3-CD4-CD8- triple negative cells

ITTP cells = CD3-CD4+CD8- intrathymic T progenitor cells

DP cells = CD3+CD4+CD8+ double positive cells

SP4 cells = CD3+CD4+CD8- single positive cells

SP8 cells = CD3+CD4-CD8+ single positive cells

CCR5 = CC chemokine receptor 5

CxCR4 = (C-X-C motif) receptor 4

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