STATUS OF THE THYMUS IN ADULT HIV-1 INFECTION: IMPLICATIONS FOR IMMUNE RECOVERY

Evidence of continued thymic production of naive T lymphocytes and potential implications for immune reconstitution in HIV-1-infected individuals were discussed by Richard A. Koup, MD.

Potent antiretroviral therapy results in persistent and sustained increases in CD4+ T cells. The initial increase observed with institution of treatment consists of memory T cells, as indicated by the finding that the expanded T-cell population reflects the skewed T-cell repertoire present in individuals prior to therapy. After approximately 6 to 12 months, increases in naive CD4+ T cells are observed, occurring in both peripheral blood and lymphoid tissue (Figure 1). Although peripheral expansion of existing naive cells is likely, new findings indicate that increases in naive CD4+ T cells include cells recently produced by the thymus. Whereas expansion of existing cell populations cannot replace elements of the T-cell repertoire lost through HIV-1-associated depletion, continued thymic output may be expected to produce cells reflecting the full repertoire present prior to HIV-1 infection. These findings raise the possibility of immune reconstitution in patients with prolonged suppression of viral load.

THYMIC FUNCTION

The thymus is the major source of T-cell production in the body. In the thymus, bone marrow precursor cells with germline T-cell receptors undergo rearrangement of the T-cell receptor genes, alterations in surface phenotype (Figure 2), and proliferation. In cells that become T-cell receptor alpha/beta T cells, initial rearrangement of the beta gene triggers rearrangement of the alpha gene, preventing the possibility of use of the delta chain within the alpha gene. During this process,

Figure 1. *Increase in CD4+ cell count with reduction in viral load on potent antiretroviral therapy showing late development of increase in naive CD4+ cells. Plasma HIV-1-RNA level is indicated with triangles. Red squares indicate overall CD4+ cell level, with circles showing memory CD4+ cell level and black squares showing naive CD4+ cell level. Adapted from Li TS, et al. Long-lasting recovery in CD4 T-cell function and viral-load reduction after highly active antiretroviral therapy in advanced HIV-1 disease. Lancet. 1998;351:1682–1686. Copyright 1998 by The Lancet Ltd.*

cells express both CD4 and CD8 surface antigens; differentiation of the cells into distinct CD4+ and CD8+ lineages occurs subsequent to and by processes independent of the T-cell receptor-gene rearrangement. This differentiation occurs just prior to release of the mature naive T cells into the peripheral circulation from the thymic medulla. It is estimated that 95% to 99% of all T-cell receptor rearrangements fail to recognize self-major histocompatibility complex and are eliminated by apoptosis in the thymus. Nevertheless, the remaining naive CD4+ and CD8+ T cells have T-cell receptor specificities reflecting a highly diverse repertoire estimated at 10^{16} different specificities.

The naive phenotype of the cells emigrating from the thymus consists of the expression of the low-molecular-weight isoform of CD45 (CD45RA) and L-selectin (CD62L), and this phenotype can be maintained by circulating cells for many years. Upon exposure to their cognate antigen in secondary lymphoid tissue, the cells are stimulated to differentiate and proliferate, and acquire the memory T-cell phenotype characterized by expression of CD45RO. Although some memory cells may bear CD45RA, they do not express CD62L, with coexpression of CD45RA and CD62L being specific for the naive cell population.

The thymus is almost fully developed at birth. After an increase in weight over the first 6 months of life, it remains stable in size and exhibits a slight decrease in weight over the course of the individual's
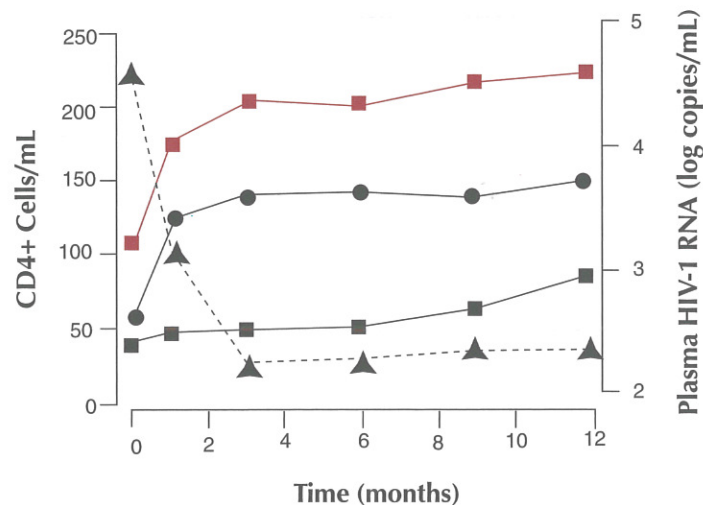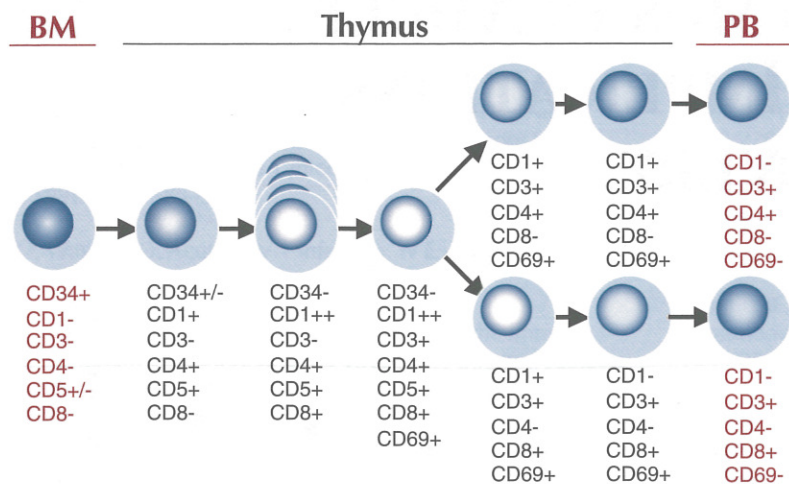
BM **Thymus** **PB**

CD34+	CD34+/-	CD34-	CD34-
CD1-	CD1+	CD1++	CD1++
CD3-	CD3-	CD3-	CD3+
CD4-	CD4+	CD4+	CD4+
CD5+/-	CD5+	CD5+	CD5+
CD8-	CD8-	CD8+	CD8+
			CD69+

CD1+ CD3+ CD4+ CD8- CD69+ (upper path)
CD1+ CD3+ CD4+ CD8- CD69+
CD1- CD3+ CD4+ CD8- CD69-

CD1+ CD3+ CD4- CD8+ CD69+ (lower path)
CD1- CD3+ CD4- CD8+ CD69+
CD1- CD3+ CD4- CD8+ CD69-

Figure 2. Changes in phenotype with maturation from bone marrow (BM) precursors to mature naive T cells released from the thymus into the peripheral blood (PB).

life. However, the thymus undergoes progressive pathologic changes (fairly linear from the first year through the fourth decade) characterized by replacement of perivascular spaces with adipose tissue and extreme loss of the cortical and medullary tissue responsible for production of new T cells. Although there is great interindividual variability in amounts of remaining thymic tissue by age, an average of approximately 70% to 80% of cortical and medullary tissue is lost by age 30, with very little functional thymic tissue remaining by late life (Figure 3). It was thus believed that the thymus was largely inactive in producing new T cells in mature individuals. However, it has been found that remaining cortical and medullary tissue in the aging thymus is histologically normal, and that phenotypes of the expected T-cell intermediates are present, with evidence of ongoing cell proliferation and T-cell receptor gene rearrangement. Thus, thymic tissue in adults appears to be functional, with the quantitative reduction in naive T-cell output (estimated at 10^9 in infancy to 6–7 x 10^7 by age 20) not implying a qualitative reduction.

CD4+ CELL CHANGES UNDER POTENT ANTIRETROVIRAL THERAPY

As noted, the importance of determining whether the thymus continues to function

in producing new T cells in later life resides in the possibility of a global recovery of immune function. T cells emigrating from the thymus are programmed to detect a single antigen on a single pathogen. Expansion of existing naive cells would not serve to replace clones with defined specificities deleted through viral cytopathic effects and host cytolytic response, whereas thymic production could replenish the full complement of specificities. Evidence that some of the increase in naive CD4+ T cells is derived from peripheral expansion

of existing cells comes from studies indicating increases in naive cell number in thymectomized patients receiving potent antiretroviral therapy. However, recent findings indicating the thymus as a source of some of this increase include studies showing that presence of CT-detected thymic tissue is associated with a greater CD4+ T-cell recovery in patients on potent antiretroviral therapy and studies in SCID-Hu mice showing recovery of function of implanted thymic tissue in HIV-infected animals treated with potent antiretroviral therapy. More direct evidence that cells constituting the later potent antiretroviral therapy-related increase in naive cell populations are derived from the thymus comes from recent studies utilizing a novel assay to identify recent thymic emigrants.

T-cell receptor-gene rearrangement in the thymus results in production of episomal DNA fragments, termed T-cell receptor rearrangement excision circles (TRECs), that can be used as markers of recent thymic emigration. These episomes are stable but do not replicate during mitosis, and are thus diluted with each round of cell division. The presence of TREC in naive T cells constitutes a surrogate marker of recent emigration since the episome(s) may also be present in cells that had emigrated at some time in the past but failed to replicate substantially. The assay exploiting the presence of
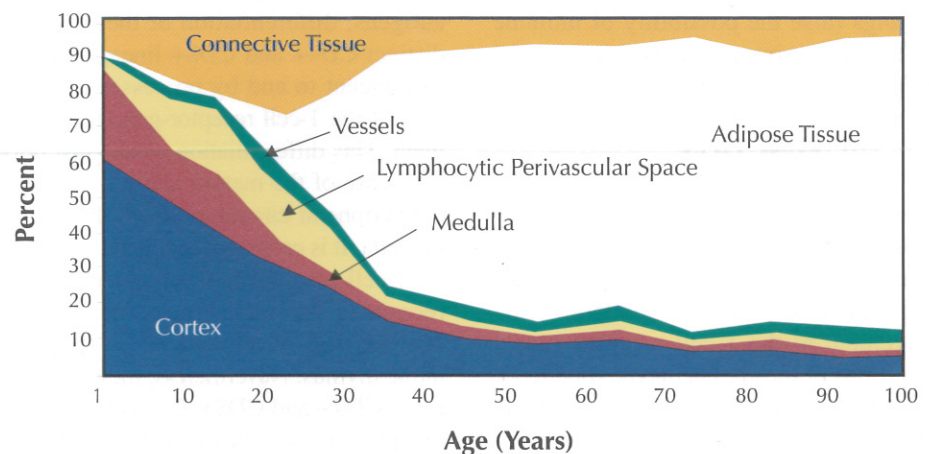

Figure 3. Age-related changes of relative volumes (%) in tissue compartments of the thymus. Adapted from Steinmann GG, et al. Scand J Immunol. 1985;22:563–575.
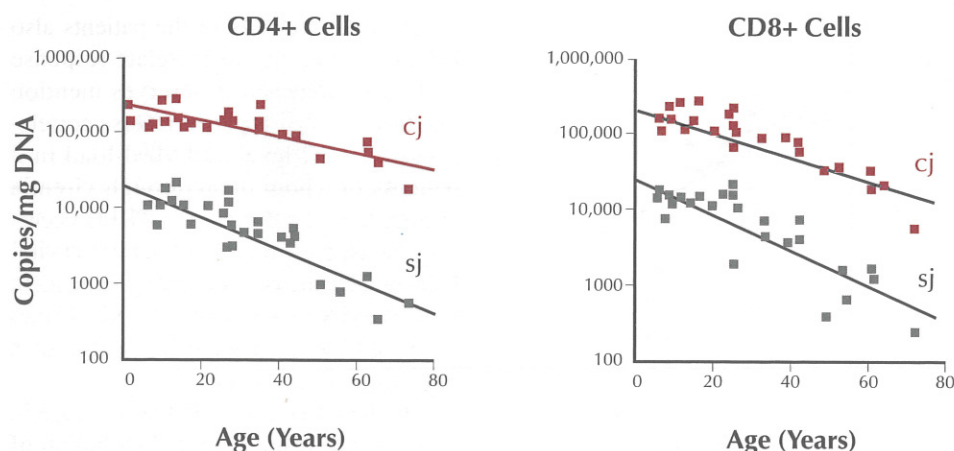
CD4+ Cells

CD8+ Cells

Figure 4. *Age-related changes in levels of cj and sj TREC in peripheral blood CD4+ and CD8+ cells. Adapted with permission from Douek DC, et al.* Nature. *1998;396:690–695.*

TREC is based on the recognition that the delta locus within the alpha gene of the alpha/beta T-cell receptor T cells is eliminated during maturation in the thymus; the elimination of the delta locus results in excision circles containing a signal joint (sj) and a coding joint (cj), both of which can be detected through polymerase chain reaction amplification. The sj and cj TREC are detected in phenotypically naive alpha/beta T cells (CD45RA+, CD45RO-) and not in memory T cells (presumably as a result of proliferation accompanying the conversion from naive to memory phenotype), B cells, or gamma/delta T cells. Use of the cj/sj TREC assay in peripheral blood from individuals ranging from birth to more than 70 years of age has shown that TREC levels decline with increasing age (Figure 4), as predicted from understanding of age-related changes in thymic output. In the context of a functioning thymus, the population of naive cells containing TREC represents a dynamic pool, with TREC levels being reduced by dilution through proliferation of these cells into subsequent generations of naive cells or proliferation and conversion into memory cells and variably replenished by different interindividual rates of thymic production of new naive cells. As shown in Figure 5, studies in normal and thymectomized individuals show that age-associated TREC levels are lower in the latter, with an approximately 1 log difference being observed at earlier ages and the difference decreasing with progressive age. These findings indicate that continued thymic output is required to maintain the persistent low level of TREC observed in adults.

In order to interpret findings using the TREC assay in HIV-1-infected individuals, additional disease-specific effects on naive cell levels need to be considered. HIV-1 would be expected to decrease TREC levels in the peripheral naive T-cell pool via infection and death of the cells and via dilution through stimulation of proliferation of these cells. In addition, a number of studies have identified effects of HIV-1 within the thymus. For example, there have been multiple reports of destruction of thymic architecture and limited thymopoietic potential in patients with later-stage HIV-1 disease, compared with a relatively low frequency of productively infected cells and relatively preserved histology in early infection. In addition, infection and killing of thymocytes bearing CD4 or other inhibition of thymocytes is likely to occur. Studies in the SCID-Hu mouse model have indicated that HIV-1 infects and depletes thymocytes double-positive for CD4 and CD8 molecules, and studies in the rhesus monkey/simian immunodeficiency virus model have indicated that infection results in inhibition of thymic precursors. Use of
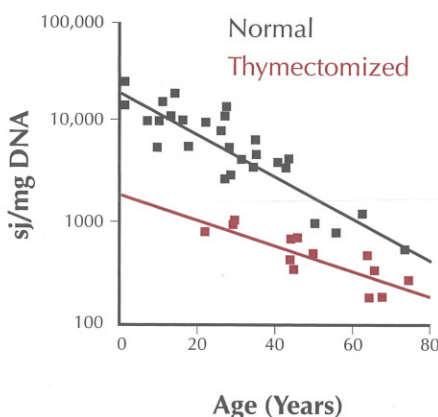

Figure 5. *TREC levels (sj) in CD4+ cells in normal and thymectomized individuals according to age. Adapted with permission from Douek DC, et al.* Nature. *1998; 396:690–695.*

Remaining cortical and medullary tissue in the aging thymus is histologically normal, with evidence of ongoing cell proliferation and T-cell receptor gene rearrangement

the TREC assay in HIV-1-infected individuals has demonstrated lower levels of TREC in the peripheral naive T-cell pool than in uninfected individuals (Figure 6). The relative contributions of viral effects in the thymus and viral effects in the peripheral pool remain unclear.

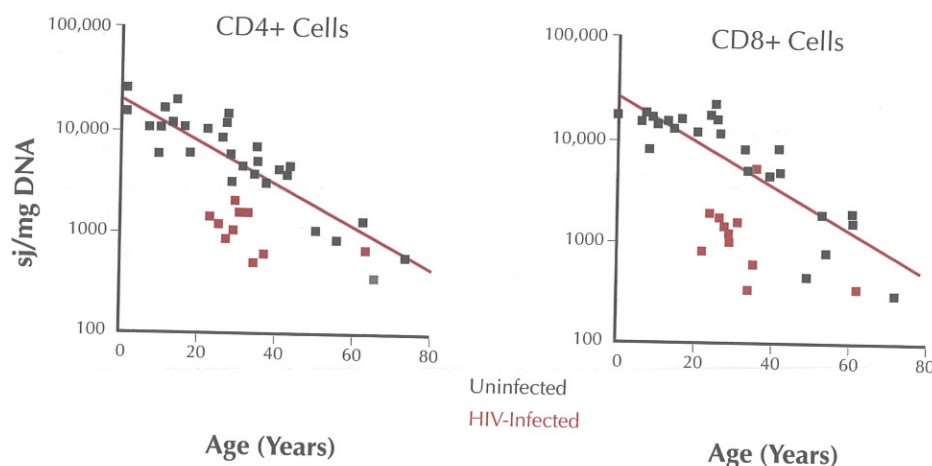To investigate whether recent thymic emigrants contributed to the reconstitution

Figure 6. *TREC levels (sj) in CD4+ and CD8+ cells in uninfected individuals and patients with HIV-1 infection by age. Adapted with permission from Douek DC, et al. Nature. 1998;396:690–695.*

range of the majority of the patients also limiting the ability to correlate response with age. However, it deserves mention that there was an apparent inverse correlation of TREC level and viral load in 2 patients in whom breakthrough viremia occurred, with a decrease in TREC occurring coincident with increased plasma viral load, and an increase occurring coincident with the decrease in plasma viral load produced by subsequent institution of a new effective regimen.

It should be noted that an increase in TREC could result from redistribution of naive T cells from a sequestered site, such as lymphoid tissue. However, quantification of TREC in lymph nodes of patients actually indicates a decrease in TREC levels (Figure 8) compared with uninfected controls, suggesting the absence of such a

of CD4+ T-cell populations in patients on potent antiretroviral therapy, TREC was quantified in peripheral blood CD4+ cells isolated from 10 patients at time points up to 9 months after initiation of treatment. TREC increases were observed in all but 1 of these patients after initiation of treatment and suppression of plasma viral load (Figure 7). Increases generally occurred within 4 to 16 weeks after treatment initiation, with 2 patients exhibiting large sustained increases to normal TREC levels. The sole patient in whom an increase was not observed was a 63-year-old individual who dropped out of the study early (all other patients were between the ages of 22 and 36 years); it is possible that an increase in TREC would have occurred with continued follow-up in this patient, or that the patient's thymus was not sufficiently functional to allow an increase to be detected. These increases in TREC were observed in the context of a general lack of significant increase in naive T-cell number; as reported, however, this increase characteristically is reported at 6 to 12 months after the beginning of therapy. The variability in the magnitude and kinetics of the TREC increases observed may reflect normal variability of thymic size and baseline TREC among individuals; the small number of patients studied did not allow for detection of a correlation of changes with pretreatment viral load or CD4+ cell count, with the age
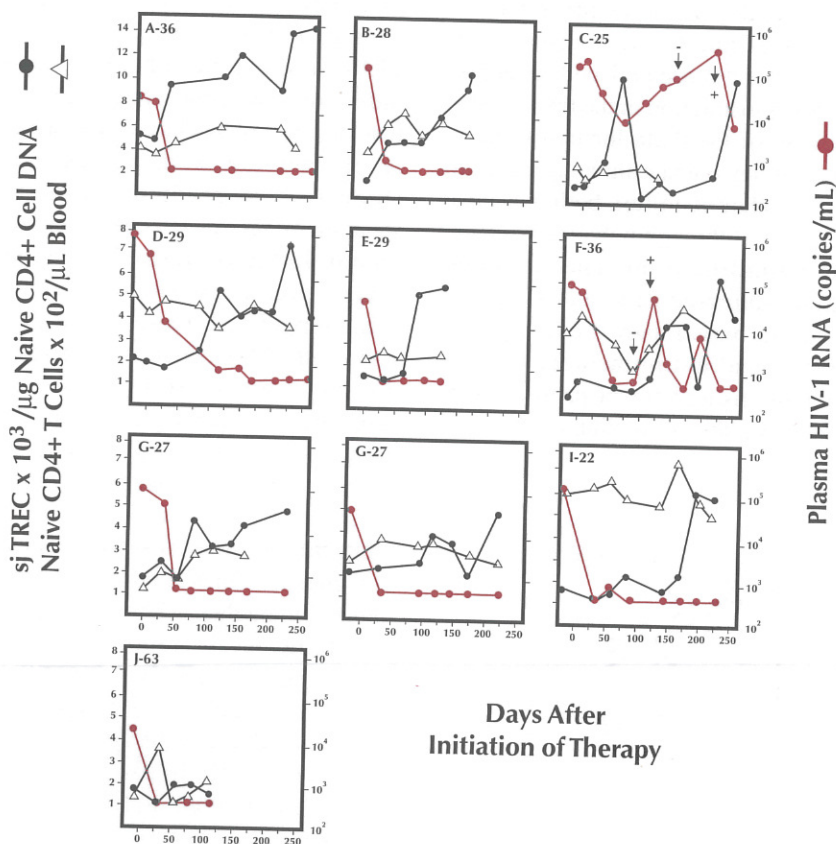

Figure 7. *Changes in TREC level (sj), naive CD4+ T-cell count, and plasma HIV-1 RNA level in 10 patients after initiation of potent antiretroviral therapy. TREC levels increased in all patients except a 63-year-old individual who discontinued the study early (J-63). Two patients with breakthrough viremia (C-25, F-36) exhibited decreased TREC in association with increased viral load and increased TREC in association with subsequent suppression of viral load upon switching therapy. Adapted with permission from Douek DC, et al. Nature. 1998;396:690–695.*
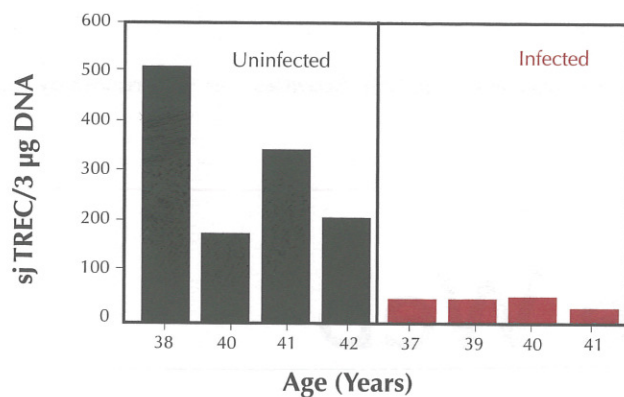
Figure 8. Comparison of TREC levels (sj) in lymph nodes of uninfected individuals and HIV-1-infected individuals of similar age. Adapted with permission from Douek DC, et al. Nature. 1998;396:690–695.

reservoir. Thus, overall, these early findings indicate that the thymus is active in HIV-1-infected patients aged 20 to 40 years, and that thymopoiesis is contributing to the naive T-cell recovery observed in patients taking potent antiretrovirals.

CONCLUSIONS

Studies of TREC in adult thymectomy patients have shown that the thymus is functional, albeit at reduced capacity, late in life. The reduced levels of TREC observed in HIV-1 infection indicate that the virus adversely affects thymic output or causes dilution of TREC within recent thymic emigrants, or both. The finding that TREC increases after initiation of potent antiretroviral therapy indicates that the thymus is functioning and producing new naive T cells and thus that some of the increase in naive CD4+ T cells observed in patients during potent antiretroviral therapy is derived from this source. These findings, however, do not refute that peripheral expansion of naive T cells is also operative in maintaining CD4+ T-cell homeostasis.

Together, these data raise the possibility that prolonged viral suppression in the presence of a functioning thymus could result in replacement of clones of naive CD4+ T cells that had been deleted by HIV-1, which could eventually lead to complete reconstitution of a virus-ravaged immune system. Although available short-term data do not show any significant return of HIV-1-specific CD4+ T-cell activity in patients initiating potent antiretroviral therapy during chronic infection, there are some indications of improvement, and it is unclear what will occur with longer-term follow-up during continued viral suppression. Other recent data have indicated that recovery of proliferative responses to antigens and repair of lymph node architecture can be observed later in the course of potent therapy, indicating a recovery of overall immune function. It currently is unclear what the time frame of reconstitution of immune function might be, and how this process is affected by age and by individual variations in thymic function. As in the case of prospects for restoring HIV-1-specific immune function by combining potent antiretrovirals with immunotherapy, the demonstration of a functioning thymus in adult patients raises the possibility of employing interventions to enhance thymic output to hasten immune reconstitution.

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