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Immune Reconstitution in Lymphoid Tissue Following Potent Antiretroviral Therapy

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Abstract

During untreated HIV-1 infection, a chronic state of immune activation and inflammation develops at the lymphoid tissue sites of viral replication. The early effect of potent combination drug therapy is a reduction in peripheral viral burden and a reduction in the production of inflammatory and type 1 cytokines. Further along in treatment there are trends toward normalization in the frequencies of $CD8^+$ T-cells, $CD4^+$ $CD45RA^+$ cells, as well as $CD4^+$ $CD45R0^+$ cells. Finally, the $CD1a^+$ dendritic cell network is re-established and germinal centers are reformed. Although this restoration of the lymphoid dynamic form is coupled to a reconstitution of peripheral blood T-cell function in vitro and by skin testing, sterilizing immunity to HIV-1 does not develop. Furthermore there is no heightened development of cytotoxic $CD8^+$ T-cell function at the site of HIV-1 latency. This is evidenced by a massive recrudescence of HIV-1 viral replication within lymphoid tissue when therapy is stopped. The development of supplemental therapies, which reconstitute anti-HIV-1 immunity, will be required. Specific defects in anti-HIV-1 activity which occur in lymphoid tissue during infection include a downregulation of perforin expression by cytotoxic T-cells, the down regulation of the TCR signal transducing chain CD3ζ, and inadequate $CD4^+$ T-cell help within the tissue compartment of immune regeneration.

Introduction

The treatment of HIV-1 infection with combinations of nucleoside analogs, protease inhibitors, and reverse transcriptase inhibitors has substantially reduced the incidence of clinical opportunistic infections and neoplastic disorders in patients

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The Human Thymus: A New Perspective on Thymic Function, Aging, and HIV Infection

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Abstract

Shortly after birth, the human thymus begins a life long process of involution, whereby the net size of the thymus is not altered but the organ is replaced by adipose tissue. As a result, it has long been believed that thymic involution is indicative of a nonfunctional organ. Recently, however, with the use of computed tomography analysis and innovative molecular approaches that measure T-cell receptor circles, indicative of recent thymic emigrants, doubt has been placed on that dogma. The thymus appears to be active in thymopoiesis throughout the adult life, albeit inversely correlated with age. Being faced with diseases that deplete T-cells such as the acquired immunodeficiency syndrome (AIDS), this recent finding has the potential to exploit novel approaches that enhance thymic output as a mecha-

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The Human Thymus: A New Perspective on Thymic Function, Aging, and HIV Infection

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nism to reconstitute the immune system. In this review, we will revisit the role of T-cells in immunity, the relationship between thymic function and age, and closely examine the impact of HIV-mediated thymic dysregulation on thymopoiesis.

The Role of the Thymus in Immunity

The human thymus, a small organ located above the heart, provides a specialized microenvironment for the maturation, education, and selection of developing T-lymphocytes. However, the presence of mature T-cells in athymic individuals suggests that extrathymic sites of T-cell maturation may exist such as in the spleen, liver, bone marrow, and gut.[1-3] Nonetheless, the contribution of these sites is secondary to that of the thymus.[4]

The thymus is divided into two bilaterally symmetrical lobes that are further subdivided into lobules by invagination of a thin, fibrous connective tissue capsule (Figure 1A and 1D). The capsule surrounds the thymus and carries blood supply through vessels that extend to the center of the thymus. The area surrounding the vessels is the perivascular space, which is composed of lymphocytes, granulocytes, macrophages, mast cells, and adipose cells. The perivascular space functions as a delivery pathway and site of exchange between the stroma and the periphery.[5]

Each of the numerous lobules of the thymus differentiates into two distinct regions, the outer thymic cortex and the inner thymic medulla (Figures 1A and 1D). Bone marrow-derived progenitor cells enter the thymus and migrate deeper into the cortex as they mature (Figure 1C).

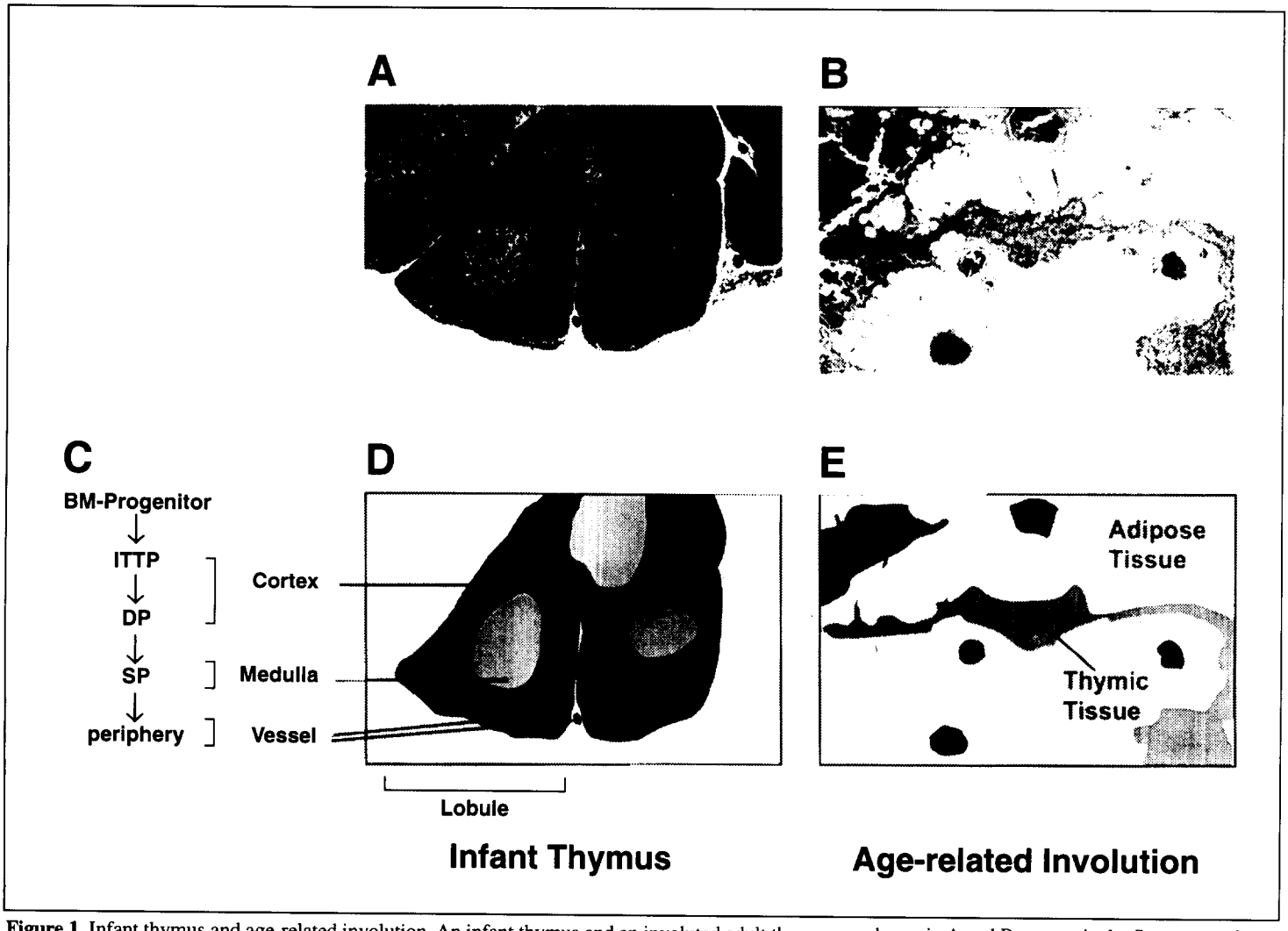

Figure 1. Infant thymus and age-related involution. An infant thymus and an involuted adult thymus are shown in A and B, respectively. Structures of interest from each thymus are schematically highlighted in D and E. Stages of T-cell development are illustrated in C, where bone marrow (BM)-derived progenitors enter the thymic cortex and undergo a process of maturation and selection. Intrathymic T-cell percursor (ITTP) migrate through the cortex and give rise to $CD4^+CD8^+$ double positive (DP) T-cells which, in the medulla, become either $CD4^+$ or $CD8^+$ single positive (SP) T-cells that exit the thymus through blood vessels (V) and into the periphery as recent thymic emigrants. Histology photos of the infant and adult thymus were kindly provided by Dr. Jerome Loew at the Department of Pathology at Rush Presbyterian St. Luke's Medical Center, Chicago, IL.

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The cortex consists of densely populated immature thymocytes, epithelial cells, and scattered macrophages, while the inner medulla contains the mature thymocytes, epithelial cells, dendritic cells, and Hassall's corpuscles. As the developing thymocytes move through the cortex and into the medulla, they receive signals for proliferation, receptor gene rearrangement, selection, and maturation.

Thymic events involved in T-cell development are schematically represented in Figure 2. Population of the thymus begins during fetal gestation when bone marrow-derived progenitor cells expressing the CD34 molecule migrate to the subcapsular epithelium and enter the thymus. Once in the subcapsular zone of the thymus, the progenitor cells interact with thymic stromal cells and are exposed to growth factors such as interleukin-7 (IL-7) and stem cell growth factor. These events induce the progenitors to proliferate and express the CD2 and CD7 cell surface markers. Expression of these two molecules identifies the developing progenitors as cells committed to the T-cell lineage. Since these early thymocytes still lack the CD4 and CD8 molecules characteristic of mature T-cells, they are referred to as "double negative (DN)" thymocytes.

Expression of the adhesion molecule CD44 on the thymocytes and movement of the cells into the thymic cortex characterize the next stage of development. At this stage, all genes encoding the T-cell receptor are still in their germline configuration. As T-cell development proceeds, expression of the CD25 molecule (α chain of the IL-2 receptor) is upregulated and CD44 is downregulated. At this stage ($CD44^{lo}CD25^{+}$), the rearrangement of the T-cell receptor (TCR) β-chain genes begins. Once the TCR β-chain genes are successfully rearranged, expression of CD25 is downregulated. If the β-chain gene rearrangement is unsuccessful, development of the thymocyte will be blocked at the $CD44^{lo}CD25^{+}$ stage.

The successfully rearranged β-chain then pairs with a surrogate α-chain (pTα), forming a complex which will be delivered to the cell surface and expressed along with the CD3 molecules. Expression of this β-chain/pTα complex induces a signal that arrests further rearrangement of the β-chain genes, resulting in allelic exclusion at the β-chain locus. These thymocytes are also triggered to proliferate, express the CD4 and CD8 cell surface molecules, and begin the rearrangement of the α-chain genes. The developing $CD4^{+}CD8^{+}$ thymocytes are now referred to as "double positive (DP)" cells.

During thymocyte proliferation, the enzymes responsible for gene rearrangement are suppressed. Once proliferation is complete, these enzymes become active again and initiate TCR α-chain gene rearrangement. Because the genes encoding the α-chain are not restricted by the allelic exclusion observed for the β-chain genes, cells expressing an α-chain can continue to rearrange the α-chain genes until positive selection occurs.[6] The result

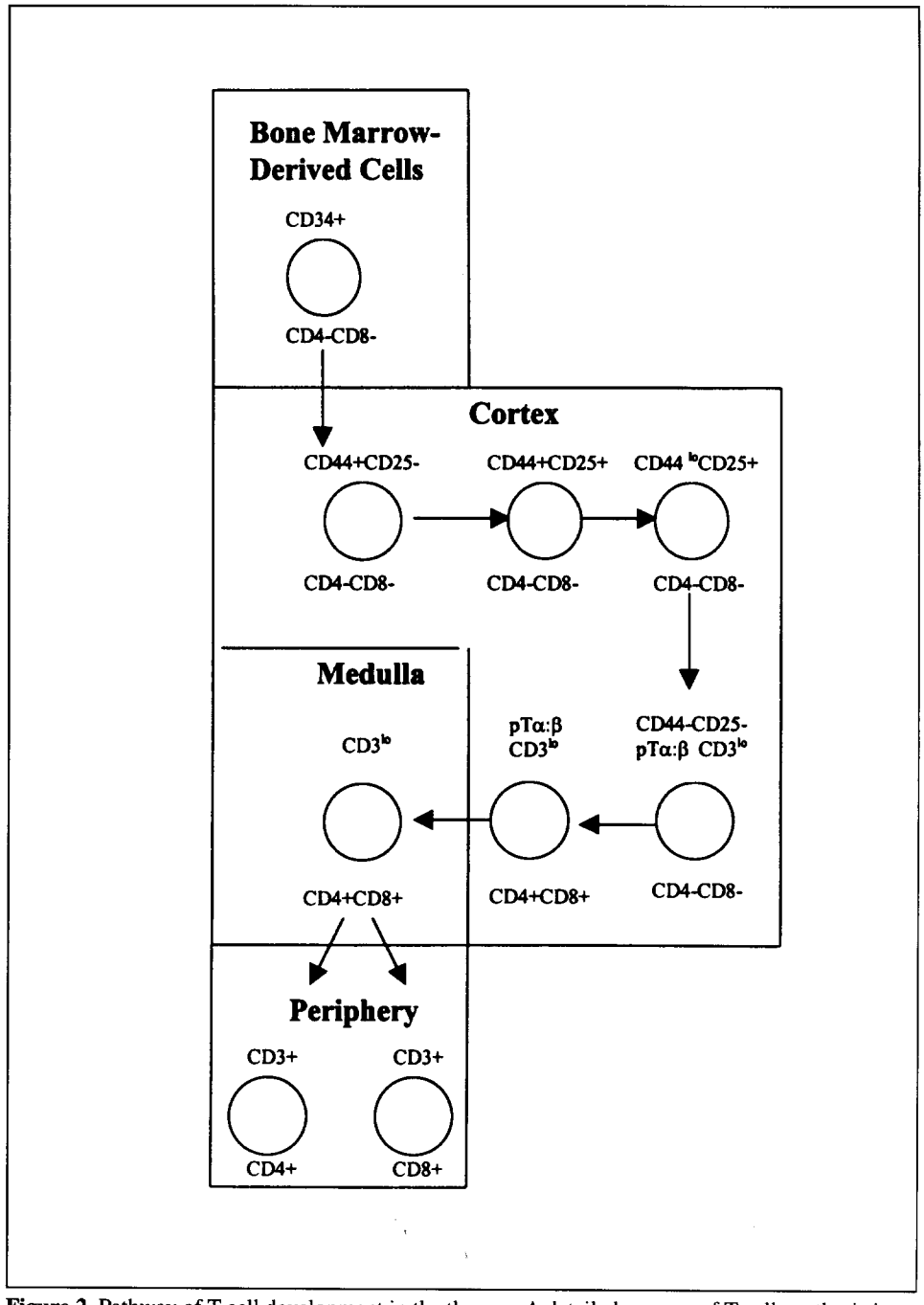

Figure 2. Pathway of T-cell development in the thymus. A detailed process of T-cell synthesis in the thymus is illustrated. Cell surface expression of CD44, CD25, CD3, CD4, and CD8 are designated where appropriate; pTα:ß refers to the formation of a chimeric precursor chain for the αß TCR.

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of this process is low level expression of the αβ T-cell receptor with the CD3 molecules on the surface of DP thymocytes.

Developing thymocytes continue to move through the thymic cortex where they undergo a further selection process that defines the TCR repertoire. While in the cortex, DP cells come into close contact with thymic cortical epithelial cells expressing major histocompatibility complex (MHC) molecules. Developing T-cells that sufficiently recognize self-peptide/MHC complexes are positively selected and migrate to the medulla. In the medulla, the thymocytes mature and increase the surface expression of TCR and CD3 molecules. Expression of either CD4 or CD8 will be downregulated in the medulla, depending on whether the TCR engaged MHC class I or class II proteins.

Almost 95% of developing thymocytes do not migrate out of the thymus. Thymocytes that express the TCR but do not sufficiently recognize self-MHC complexes fail to be positively selected and die in the thymus via apoptosis. Those thymocytes that recognize the self-MHC or self-peptide complexes with high affinity are eliminated, as they could be autoreactive if released into the periphery. This process is called "negative selection" and results either in anergy or death of the cell, also via apoptosis.

Cells that survive the process of development and selection in the thymic stroma emerge from the thymus as either $CD4^+$ or $CD8^+$ single positive cells with high level expression of αβ TCR and CD3 molecules. After completing these stages of differentiation, the thymocytes migrate to the periphery and are referred to as recent thymic emigrants (RTE) representing a vast TCR repertoire.

Thymopoiesis and Age

The thymus is fully developed at birth, with absolute growth continuing only through the first year of life.[7] After birth, the process of thymic involution begins, which may be regulated by a number of gonadal and thymic hormones.[8] The human thymus remains relatively the same size for up to 80 years, but over this period of time, the organ undergoes an almost complete lipomatous atrophy. In fact, in individuals older than 50 years, the fibrous capsule of the thymus predominantly surrounds adipose tissue.

As seen in Figure 1A, a newborn's thymus is fully developed and functional, consisting mainly of active sites of thymopoiesis (the thymic epithelium). A clear distinction between the outer cortex and the inner medulla are also clearly observed. However, examining the thymus of an older individual (>65) illustrates predominately perivascular space (adipose tissue) that surrounds areas of true thymic tissue (Figure 1B). Additionally, a clear distinction between the cortex and the medulla cannot be easily made.

The process of involution, as marked by thymic atrophy and architectural changes, occurs in stages that begin with an enlargement of the perivascular space. The connective tissue septae dividing the lobules begin to disappear and are gradually replaced by single fat cells and adipose tissue. Further development of adipose tissue underneath the capsule separates the capsule from any remaining true thymic tissue.[9] The medulla is eventually lost but islands of thymic epithelium remain.[5]

Four phases have been established to characterize the level of thymic involution.[5] Phase I, seen in newborns to 10 years of age, is characterized by a progressive decline in total lymphatic tissue, equivalent to a decline of five percent of lymphatic tissue per year. Phase II, seen in 10- to 25-year-old individuals, is characterized by a decrease in epithelial space that is correlated with a maximal increase in perivascular space. Phase III, seen in 25- to 40-year-old individuals, is demonstrated by a decline in both perivascular space and thymic epithelium accompanied by an increase of fatty atrophy at an estimated rate of five percent per year. Finally, phase IV, seen in individuals 40 years and older, is characterized by a slow involution of the remaining lymphatic tissue at a rate of 0.1% per year.[5,9] End stage involution is characterized by lipomatous atrophy.

It has long been believed that age-related thymic involution is accompanied by a non-functional thymus.[9-11] However, recently a number of investigators have challenged that hypothesis by demonstrating that thymopoiesis still continues through out life albeit inversely correlated with age.[12-15] Thymic samples from human donors up to 49 years old undergoing cardiac surgery still contained intrathymic T-cell precursors and mature thymocytes, suggesting that the adult thymus is still able to sustain T-cell differentiation.[12] Additionally, computed tomography (CT) studies have indicated that abundant thymic tissue is still present despite age-related thymic involution.[11] Innovative molecular approaches, including the measurement of T-cell receptor excision circles, have also shed some light on thymic function and age as well as on the impact of HIV drug therapy on thymopoiesis.[13-15]

T-cell Receptor Excision Circles (TREC) as Indicators of Thymopoiesis

Unlike the avian model,[16] there are no phenotypic markers that distinguish between recent thymic emigrants and the rest of the peripheral T-cell pool. However, during T-cell receptor rearrangement within the thymus, DNA excision product is generated. This product is episomal DNA referred to as a T-cell receptor excision circle (TREC). These circles vary in size depending on the recombination event. They do not undergo mitosis and thus are diluted with each successive round of cell division.[17] Therefore, TREC can function as a marker for the replicative history of a cell population and are indicative of recent thymic function.

The process of TREC generation is illustrated in Figure 3. The diversity in the TCR repertoire is a consequence of *V (D) J* TCR gene recombination. The TCR chains (α, β, γ, δ) consist of gene segments known as variable (V), diversity (D), and joining (J) that are rearranged to form a distinct V (D) J gene. β and δ chains undergo D region rearrangement while the α and γ chains do not. The process of TCR gene rearrangement is mediated by recognition signal sequences (RSS) adjacent to each V, D, and J segment (Figure 3A). The RSS consists of either heptamer or nonamer sequences separated by 12 or 23 base pair (bp) spacer sequences. Recombination-activating gene products (RAG-1 and RAG-2) join gene segments that are linked by 12 bp spacers with gene segments that are linked by 23 bp spacers (12/23 rule) or vice versa. This rearrangement process causes the coding end to form a functional TCR in the chromosome and the signal ends to be excised to generate an extra-

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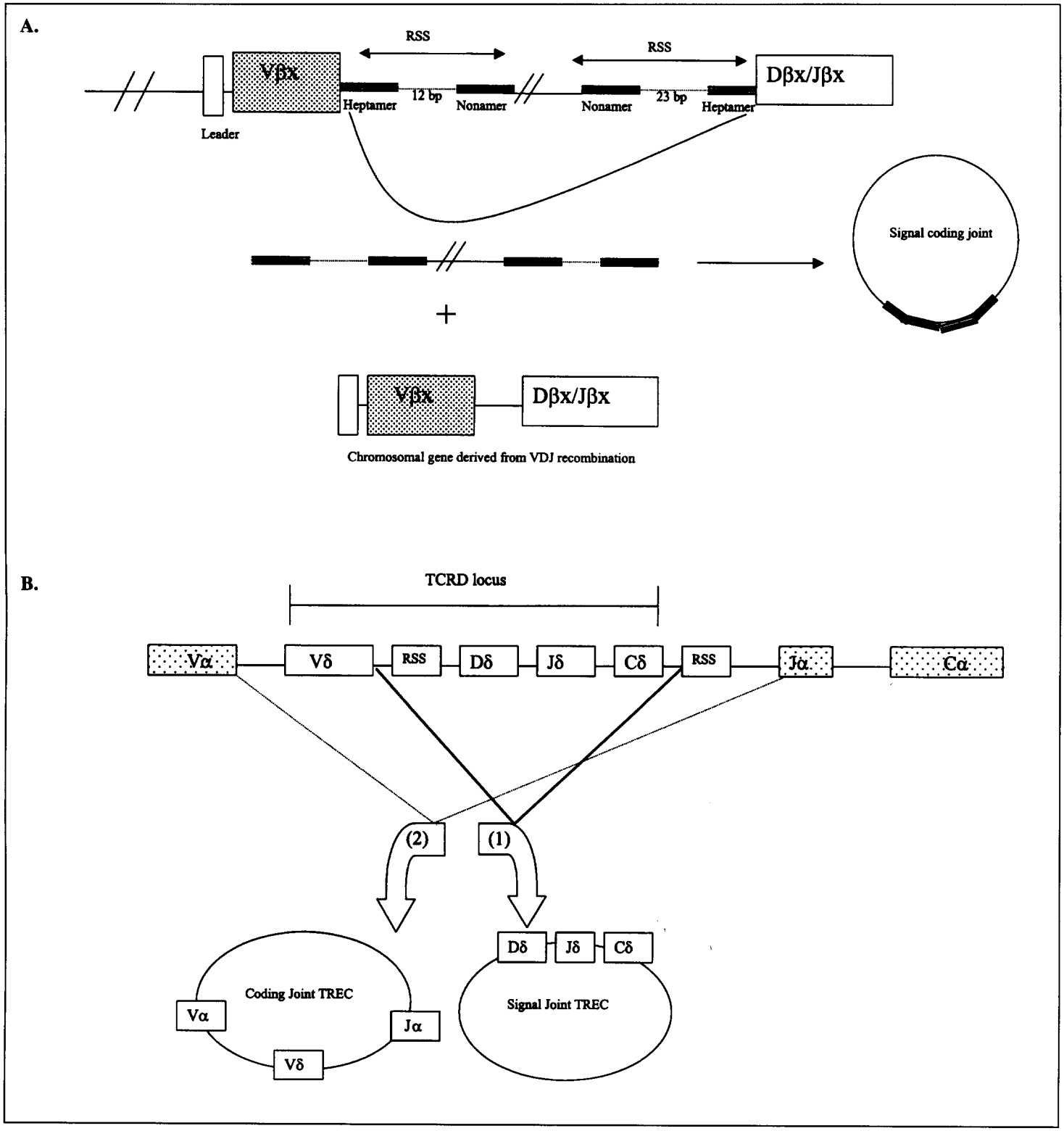

Figure 3. Mechanism of TCRß (A) and TCRα (B) TREC generation. A. In TCRß TREC generation, DJ recombination must occur prior to V recombination. A recombination signal sequence (RSS) flanks each gene to be recombined. RSS consists of a heptamer, a spacer sequence that is 12 or 23 bp in length, and a nonamer sequence. Recombination activating enzymes recognize RSS and cleave the DNA only between gene segments possessing 12 or 23 bp spacer sequence (the 12/23 rule). Cleaved sites form an extrachromosomal DNA circle, referred to as T-cell receptor excision circle sequences and the intervening gene sequence of the spliced DNA. Removal of TREC allows for the formation of a recombined chromosomal gene encoding a specific VDJ TCR. B. Common to all TCRα germline DNA is an intervening sequence for the δ gene. In order to generate a VJ recombination event, the intervening δ gene is excised, in the same mechanism described for the ß chain TREC, to initially generate a signal TREC (Dδ, Jδ, Cδ). A second TREC is then formed corresponding to the coding joint (Vα, Vδ, and Jα).

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chromosomal/episomal circular DNA fragment known as TREC.

The human TCRβ chain gene consists of more than fifty V segments, two D segments, thirteen J segments, and two constant (C) segments. TCRα chain consists of approximately forty eight V segments, seventy J segments, and one C segment. Given that TCRβ chain rearrangement occurs prior to TCRα recombination, the TCRβ TREC signals may be diluted more than that of the α TRECs, as a consequence of cell division within the thymus.[17] Additionally, within the α chain locus lies the δ chain genes that must be excised prior to α chain recombination.

The use of the TREC assay, thus far, appears to be the best biomarker to measure thymopoiesis despite several shortcomings. TREC analysis cannot distinguish between recent thymic emigrants generated from the thymus or from extrathymic/secondary sources of de novo T-cells. Given that TRECs are detected in very low numbers in the peripheral blood of athymic individuals suggests that the thymus is the major source of de novo T-cells.[13] Additionally, TRECs may still be detected in naive cells that are not recent thymic emigrants or in cells that received TREC through mitosis. Nonetheless, since no phenotypic markers exist to specifically identify recent thymic emigrants, TREC analysis is the best marker to date of thymopoiesis.

A quantitative competitive (QC)-PCR assay was recently developed by Douek and colleagues[13] based on the signal joint and coding joint TRECs (Figure 3B) generated from TCR-α gene recombination. They showed that although TRECs are inversely correlated with age, thymopoiesis is still active up to age 50, despite the long-held dogma that the thymus is nonfunctional in aged individuals. Lewin et al.[14] and Zhang et al.,[15] using real time PCR and a molecular beacon assay, also demonstrated active thymopoiesis well past the age of expected thymic involution. Sekaly and colleagues measured TRECs generated from $V\beta_1D\beta_1$, $V\beta_{17}D\beta_1$, and $V\beta_{5.1}D\beta_1$ in a limited PCR dilution assay to also demonstrate thymopoiesis despite thymic involution.[18]

Chemokine Co-receptor Expression on Thymic Cells During Development and Differentiation

The expression of particular chemokine receptors render cells susceptible to infection by particular strains of HIV-1. Several members of the chemokine receptor family (CXCR4, CCR1, CCR2a/b, CCR3, CCR4, and CCR5) have been identified which function as co-receptors for HIV-1 infection. The most important of which are CXCR4 and CCR5. CXCR4 allows infection by syncytium-inducing (SI) T-cell-tropic HIV isolates while expression of CCR5 allows for infection of non-syncysium inducing (NSI) macrophage-tropic isolates. Dual tropic viral strains can use either CCR5 or CXCR4 for infection. During T-cell development and maturation in the thymus, the expression of CXCR4 and CCR5 are differentially modulated (Table 1). In the cortex, triple negative (TN, $CD3^-CD4^-CD8^-$) and intrathymic T-cell precursors (ITTP, $CD3^-CD4^+CD8^-$) express high levels of CXCR4 but undetectable levels of CCR5, as determined by surface staining of cells. As the thymocytes mature into double positive cells (DP, $CD3^+CD4^+CD8^+$), the level of CXCR4 expression is reduced but is still detected and CCR5 is expressed at low levels. However, single positive (SP) $CD4^+$ cells loose both CXCR4 and CCR5 expression while SP $CD8^+$ cells retain low levels of both CXCR4 and CCR5.[19,20] Thus, the differential regulation of chemokine co-receptors during T-cell development and differentiation renders a cell susceptible to either NSI or SI HIV infection.

HIV Infection of the Thymus and HIV-mediated Thymic Dysregulation

Although HIV preferentially infects $CD4^+$ memory T-cells, there is convincing evidence for the infection of the thymus itself, resulting in an additional mechanism for the depletion of $CD4^+$ T-cells and defective thymopoiesis. In vitro studies,[21-26] animal models (SCID-Hu,[27-30] non-human primate[31,32]), and pathological evidence from human thymic tissue all demonstrate that HIV does indeed infect the thymus. However, the mechanism of virus entry to the thymus, which is surrounded by vascular endothelium serving as a protective barrier, is less clear. Immunocytochemistry and in situ hybridization of thymic tissue from AIDS patients have illustrated HIV proteins and mRNA in the endothelial stalk[33,38] and the loss of both the structural and functional integrity of thymic epithelial cells.[36] These changes possibly may cause the collapse of the endothelial barrier, allowing for the transmission of HIV from the periphery to the thymus.

In the thymus, a number of cells susceptible to HIV infection exist including

TABLE 1. CHEMOKINE CO-RECEPTOR EXPRESSION DURING T-CELL DEVELOPMENT[a]

Cell phenotype	CXCR4	CCR5
Thymus		
$CD3^-CD4^-CD8^-$	+++	–
$CD3^-CD4+CD8^-$	+++	–
$CD3^+CD4+CD8^+$	++	+
$CD3^+CD4+CD8^-$	–	–
$CD3^+CD4^-CD8^+$	+	+
Periphery		
$CD4^+$ or $CD8^+$ naive	+++	–
$CD4^+$ or $CD8^+$ memory	+	+
$CD8^+$ naive	+++	–
$CD8^+$ memory	+	+

[a] +++, ++, and – denote the level of chemokine co-receptor expression, corresponding to high, medium, low, or no expression, respectively.

Values are based on data from references 19 and 20.

TN, ITTP, DP, SP, early bone marrow progenitors, stromal cells, and endothelial cells.[21,24,26,38,39] In vitro studies have demonstrated, mostly through detection of integrated HIV DNA, that intrathymic precursors of the T-cell lineage ($CD4^+$ $CD8^+$, $CD4^+CD8^-$, and $CD7^+CD3^-CD4^{hi}$-$CD8^-$) are targets for HIV-1 infection.[40] Primary immature thymic lymphocytes ($CD4^+CD8^+$, $CD4^+CD8^-$, or $CD3^-CD4^-$ $CD8^-$) have also been shown to be susceptible to HIV (HTLV-IIIB and LAI strains) infection.[23,26,40] Productive infection of HIV is specifically induced by the interaction of thymocytes with autologous thymic epithelial cells (TEC).[41] The mechanism appears to be through TEC induction of proinflammatory cytokines that upregulate NF-κB, which in turn upregulate HIV infection within the thymocytes.[41-43] Thymic stromal cells, specifically dendritic cells, are also infected by HIV and may transmit the virus to other cells.[44]

To determine the effect of HIV on human thymic tissue, various animal model systems have been used. The SCID-Hu model is a small animal model designed to support thymic and hematopoietic differentiation in vivo.[27,28,45] Human fetal liver and human fetal thymus fragments are co-implanted into mice homozygous for the severe combined immune deficiency (SCID) defect, resulting in the development of a conjoint functional human organ that supports normal development of competent human thymocytes.[28] HIV infection of thymus/liver (Thy/Liv) implants results in severe and preferential depletion of immature ($CD4^+CD8^+$) human thymocytes,[28,45] defects in the thymic microenvironment,[45] and defective thymopoiesis.[30] The depletion of human thymocytes, predominately in the cortex, is via apoptosis, however, and only ten percent of these cells undergoing apoptosis are infected,[39] indicating that an HIV-independent cytopathic pathway exists.

Infection of macaques with simian immunodeficiency virus (SIV) provides another model for the study of HIV-induced disease in humans. Thymic infection of juvenile macaques with pathogenic clones of SIV resulted in increased apoptosis in thymic cells and depletion of thymic progenitors.[32] Immunohistochemical analysis has provided direct evidence of viral DNA and RNA in SIV-infected cells. Similar depletion of $CD4^+$ cells has been observed following infection of pig-tailed macaques with virulent strains of the chimeric simian/human immunodeficiency virus (SHIV).[31]

Analysis of human thymic tissue from HIV-infected infants, children, and adults demonstrated HIV RNA and proteins in the thymus, providing direct in vivo evidence for thymic infection.[22,33,46] HIV infection of the thymus not only leads to severe depletion of thymocyte subpopulations ($CD3^+CD4^+CD8^+$ and $CD3^+CD4^+CD8^-$), contributing to decreased thymic output,[33,35,46] but also causes a number of physiological abnormalities including thymus involution even among infants with AIDS,[33,35,46] infiltration of the thymus with mature plasma cells, reduction in the number of thymic epithelial cells, degenerative changes in intrathymic blood vessels, and depletion of Hassall's corpuscles in most cases but if present these structures are calcified.[33]

Immune Reconstitution and the Thymus in HIV-infected Patients

Lessons drawn from the AIDS Clinical Trial Group (ACTG), evaluating the efficacy of highly active antiretroviral therapy (HAART), have shown that while HAART is successful in decreasing viral load, this reduction is not accompanied by the complete normalization of the immune response.[47,48] Nonetheless, sustained increases in CD4 cell numbers were observed which may be from redistribution of T-cells from lymphoid organs to the periphery, clonal expansion, or new T-cell synthesis. Recent studies have extended support to the latter. The adult thymus, despite involution, continues to contribute to the generation of de novo/naive T-cells, as measured by TRECs and immunostaining of pockets of thymopoiesis.[13,15,18,49] HIV infection, however, appears to reduce thymopoiesis but at least in one study this reduction was reversed by suppression of virus replication by HAART.[13] To the contrary, Lewin and colleagues[14] also demonstrated increases in thymopoiesis following HAART but only in individuals that had lower levels of thymopoiesis prior to HAART. Their data indicate that thymopoiesis may not be affected in all HIV patients but when it is it can still be normalized by viral suppression.[14] In either case, these data suggest that in some patients, the level of thymopoiesis may be enhanced after potent suppression of HIV replication.

Concluding Remarks

The finding that the adult thymus can still contribute to new T-cells in the periphery, that HIV can effect thymopoiesis in some individuals, and that potent suppression of HIV replication can return thymic output to levels comparable to that seen in age-matched groups, all stress the need to design future therapeutic approaches that can exploit the capacity of the adult thymus to repopulate the periphery with functional naive T-cells. These strategies are of great benefit to AIDS patients, DiGeorge athymic syndrome patients, and cancer patients following intensive chemotherapy. Some of these strategies include thymic transplants, whereby a fetal thymus is transplanted into the arm (Richard Hong, University of Vermont, personal communication) or thigh[50,51] of immunodeficient patients. Thymus transplant was successfully applied to a few DiGeorge athymic syndrome patients, as determined by restoration of T-cell function in the periphery and expansion of the TCR repertoire.[50,51] Thymic transplants were also applied to 15 AIDS patients with 8 of 15 demonstrating transient increases in only the CD8 T-cell compartment.[52] This study was conducted prior to potent antiretroviral drugs that can suppress HIV replication. These disappointing results may be due to reinfection and destruction of de novo T-cells, suggesting that, in the case of HIV, thymic transplants must be accompanied by either potent anti-HIV drugs or that the new T-cells should be genetically modified to resist HIV infection, a prerequisite that is far from being a successful common clinical practice. Alternatively, cytokine therapy may enhance the pool of naive T-cells.[53-55] IL-7 has been shown to induce the proliferation of naive cells without inducing their switch into memory cells, as indicated by the expression of the CD45RO cell surface marker.[53] Collectively, for the first time, it is theoretically possible to target strategies that can enhance thymic output in the adult patient in an effort to reconsti-

tute the immune system of not only AIDS patients but also in any pathology that depletes the T-cell pool.

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Human Herpesvirus 6 (HHV-6): Could it Play A Role in the Etiopathogenesis of Multiple Sclerosis (MS)?

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The etiology of multiple sclerosis (MS), a demyelinating disease involving the human central nervous system (CNS), remains unknown. Available information suggests that it is multifactorial and implicates inherited susceptibility, an autoimmune component, and environmental factors which may be infectious in nature.[1] Although specific viruses are known to cause a number of chronic neurological diseases,[2] evidence from epidemiology, serology, and virology studies which would implicate a role for viruses in the etiopathogenesis of MS is less exacting.[3-5] Additional support for a viral etiology does, however, come from experimental animal models of virus induced CNS demyelination.[2,6] These studies demonstrate that different families of viruses are capable of inducing demyelination and raise the possibility that different viruses could play a role in MS rather then a specific "MS virus." The models also suggest that causation is multifactorial. The induction of CNS demyelination in many of these models requires the interaction of a specific virus with a specific immune repertoire determined by the host's genetic make-up. The actual mechanisms mediating the interaction can vary from one system to the next and may include "hit and run" as in the case of acute disseminated encephalomyelitis,[6] "molecular mimicry" where homologous peptide sequences of various viruses and CNS antigens such as encephalitogenic regions of myelin basic protein may be shared,[7-9] and "epitope spreading" where immune responses develop because of de novo priming of self-reactive T-cells to sequestered autoantigens released secondary to virus-specific T-cell-mediated demyelination.[10]

While human herpesvirus 6 (HHV-6) has only recently been implicated in the etiopathogenesis of MS, the idea that members of the herpesviridae family of viruses may play a role in this disease is not new. Herpes simplex virus (HSV) was isolated from the brain of a MS patient by Gudnadottir et al.[11] in 1964 and from the cerebrospinal fluid (CSF) of a second MS patient by Bergstrom et al.[12] Furthermore, Fraser et al.,[13] using Southern blot hybridization, were able to identify HSV DNA in the CNS of a number of MS patients. The interpretation of these results was made difficult however by the fact that HSV DNA was also found in the CNS of controls without MS.[13-17] Recently, Sanders et al.[18,19] have extended these studies by using polymerase chain reaction (PCR) and Southern blot hybridization techniques. They also found HSV DNA to be present in the CNS of both MS patients and controls but found viral DNA to be more likely associated with active rather then inactive plaques. Although these associations are very intriguing, they are not proof that HSV is responsible for the induction of CNS demyelination in MS patients. Even if it were to be shown that HSV is capable of inducing CNS demyelination, the presence of viral DNA in the CNS of both MS patients and con-

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