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Human T Cell Reconstitution in DiGeorge Syndrome and HIV-1 Infection

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Abstract

The thymus is essential for proper development and maintenance of a broad T cell repertoire capable of recognizing a wide-range of foreign antigens. Recent advances in multicolor flow cytometry, non-invasive imaging techniques, and molecular assessments of thymic function have enabled a more comprehensive characterization of human thymic output in clinical settings than in the past. These techniques have been particularly valuable in monitoring human T cells after therapeutic thymic grafting for complete DiGeorge syndrome and during HIV-1 infection and AIDS. By defining the degree and mechanisms of T cell reconstitution in these settings, clinical investigators and primary caregivers have been able to better diagnosis, treat and care for individuals with congenital or acquired immune deficiencies associated with loss of thymic function.

Keywords

Thymus; Thymopoiesis; DiGeorge Syndrome; Anti-retroviral Drugs; HIV/AIDS

1. Introduction

The thymus is a specialized tissue in the anterior mediastinum that is critical for the development and maintenance of an effective peripheral T cell repertoire. In healthy individuals, the thymus is most active in early life, with *de novo* production of T cells gradually declining with increasing age [1,2]. A broad repertoire of T cell receptors (TCRs) with as many as 10¹³ potential TCR specificities is vital to the ability to detect a wide-range of foreign antigens. This potential for diversity is due to various T cell receptor complexes created by combining distinct variable (V), diversity (D), and joining (J) gene segments with TCR constant regions for either TCR β or TCR δ chains, and distinct V and J gene segments with TCR α or TCR γ chains [3]. During the TCR rearrangement process, segments are recombined in a tightly

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regulated process mediated by recombinae activating gene (RAG-1) and RAG-2 to maximize diversity of the resultant heterodimeric TCR α/β or TCR γ/δ surface proteins.

Upon expression of the mature TCR complex, T cells undergo an education process made up of both positive and negative selection in the thymus. Thymocytes that fail to bind to peptide-MHC complexes in the thymic cortex die by neglect, whereas binding to the cortical epithelial cells leads to survival (positive selection) [4]. This interaction also marks the differentiation of developing thymocytes to single positive cells (i. e. cells successfully binding to MHC class I differentiate to CD8+ single positive T cells, and those binding to MHC class II differentiate to CD4+ single positive T cells). Mature single positive CD4+ or CD8+ T cells then migrate to the thymic medulla, the site of negative selection [5].

The TCR diversity generated by gene rearrangement and nucleotide additions can generate self-reactive T cells, the majority of which are removed from the repertoire during development within the thymus by a process known as ‘negative selection’ [3]. Expression of self-antigens in the thymic stroma is necessary and sufficient for deletion of strongly self-reactive T cells. Self-antigens for negative selection are provided by medullary thymic epithelial cells and by thymic dendritic cells, both of which capture and present self peptides to MHC class I and II restricted thymocytes [3]. TCRs with strong reactivity to these self-antigen/MHC complexes are deleted by apoptosis. This highly orchestrated thymic education process results in a pool of thymic emigrants that are reactive against foreign antigens but generally tolerant to self-antigens.

A congenital or acquired reduction or absence of thymopoiesis results in attenuated immune function and significant morbidity and mortality [6]. Diminished or decreased thymic output is observed in infants born with DiGeorge syndrome (congenital thymic hypoplasia or aplasia), in patients undergoing chemotherapy for cancer, bone marrow transplantation, and in patients with HIV-1 infection [1]. Significant advances have been recently made in developing therapeutic strategies for congenital and acquired T cell deficiency. Critical to these research efforts has been the ability to effectively monitor thymic output and determine the impact of thymic function on reconstitution of peripheral T cell homeostasis. This review will focus on new methodologies to monitor thymic output and TCR diversity of antigen recognition, on T cell reconstitution in patients undergoing therapeutic thymus grafting for DiGeorge syndrome, and on assessment of thymus function during the course of HIV-1 infection and treatment.

2. Measurement of thymic output and TCR diversity

Assessment of thymic output and breadth of the T cell repertoire has been hampered by thymic location in the anterior mediastinum and occasionally the neck. Until recently, assessment of thymic output was limited to determination of peripheral T cell levels – an imprecise measurement given the ability of the peripheral pool to regenerate in the absence of thymic function [1,7]. Thus, assessment of postnatal thymic function requires methods of assessing both the quantitative contribution of new thymic emigrants to the peripheral T cell pool, and the quantitative contribution of thymus output to the diversity of the peripheral T cell repertoire.

Thymic function and peripheral T cell reconstitution in humans can be measured by a variety of methods (Table 1). Chest computed tomography (CT), positron emission tomography (PET) imaging and direct immunofluorescence coupled with flow cytometry are routine, non-invasive assays that can be performed in most hospitals and clinical laboratories. Non-contrasted chest CT images, with contiguous 5 mm thoracic sections from sternal notch to xiphoid, can be used to define a thymic index score, according to McCune et al. (0 – no soft tissue, with the thymus entirely replaced by fat; 1 – minimal soft tissue, barely recognizable; 2 – minimal soft tissue, more obvious; 3 – moderate soft tissue; 4 – moderate soft tissue of greater extent, almost mass

like; and 5 – mass-like appearance of concern for hyperplasia or thymoma) [8]. CT alone can aid the investigator/clinician as to the overall size of the thymus; however, it does not provide full insight into the thymopoietic activity of the tissue.

PET is a useful non-invasive imaging technique used to evaluate thymus metabolic activity. Uptake of 2-(18)Fluoro-2-deoxy-D-glucose (FDG) can be clearly seen in normal thymopoietic thymus tissue from subjects 2–13 years of age, consistent with active thymopoiesis [9]. Thymic uptake of FDG has also been observed in healthy adults from 18–29 years of age deemed to have a healthy thymus by CT scan and an absence of thymus-related disease symptoms or thymic mass on CT [10]. This study also found a correlation between degree of FDG uptake and attenuation of the thymus seen in CT scans, supporting the notion that thymic uptake is related to the degree of fatty infiltration and decrease of thymopoiesis. It has been shown that PET provides information about the rate of thymic metabolism of glucose, but this can be affected by a number of factors (e. g. patient body fat content, weight, and serum glucose levels). It is therefore important to incorporate CT or MRI imaging of the thymus to aid PET scans for differentiating between normal and benign thymic uptake from malignancy [11].

Immunophenotyping and multicolor flow cytometry have provided powerful tools to assess naïve and memory T cell pools and therefore to assess mechanisms of T cell reconstitution [12–14]. A number of T cell surface antigens have been developed as markers of recent thymic emigrants (RTE) in chickens, rodents, and man. Kong et al. described the chT1 monoclonal antibody-defined surface marker on chicken T cells that marks RTE that have been produced by the thymus within the previous three weeks [15]. However, to date no human or murine chT1 homologues have been found; thus, multiple T cell surface antigens are needed to identify human or mouse RTEs.

Expression of the CD45RA high molecular weight isoform of the tyrosine phosphatase CD45 and CD62L (L-selectin) have been most useful in humans to measure RTE [16]. However, CD45RA+, CD62L+ is not an absolute marker profile of naïve T cells, since data show that after antigen-driven conversion from naïve to memory T cells (as manifested by T cell switching CD45 isoforms from CD45RA+, to CD45RO+), CD45RO+ memory T cells that do not undergo apoptosis can revert back to CD45RA+, but remain functionally memory T cells [17]. These data suggest that the CD45RA population contains memory cell revertants and does not provide an accurate assessment of RTE levels. An important additional point is that CD62L is shed from cells following a freeze/thaw cycle *in vitro*. Because of the practical need to use cryopreserved cells in clinical studies, this observation as well makes CD62L (L-selectin) an undesirable marker for monitoring T cell reconstitution. Studies by Steffens et al. have suggested the use of CD45RA+/CD45RO–, CD45RA+/CD62L+, and CD45RO–/CD27+/CD95 (low) phenotypes for monitoring T cell immune reconstitution [18]. Others have suggested the additional use of CD103 and CD11a as markers of naïve CD8+ T cells [13]. Although there is no clear consensus as to the best phenotypic assay for naïve T cells, the most commonly used phenotype for freshly isolated whole blood is CD45RA+/CD45RO–/CD62L+. Similarly, CD45RO+/CCR7–/CD62L+/- is frequently used as a phenotype to identify effector memory T cells, and CD45RO+/CCR7+/CD62L+ is frequently used to identify central memory T cells [19]. The field of multicolor flow cytometry has been developed in recent years, with dye chemistry and instrumentation now capable of detecting 18+ colors per sample [20]. Thus, surface phenotypes can now be coupled with intracellular cytokine analysis to combine surface phenotype with cell functional analysis to determine both T cell subset types and functional capability [20]. The concept of polyfunctional T cells has arisen, with functional memory T cells being those that secrete multiple cytokines [21].

Other less commonly used methods to identify levels of RTE, such as *in vivo* labeling of deoxyribose purines with a stable isotope, deuterium labeled (deuterated) glucose (D-glucose)

followed by FACS sorting of cell subsets and mass-spectrometry analysis of T cell DNA synthesis, have been useful to probe human thymic biology and T cell homeostasis in research settings, but this technology is restricted to clinical research facilities with mass-spectrometry expertise [22].

2.1. Monitoring thymic function at the molecular level

Imaging, immunophenotyping and *in vivo* labeling assays are useful for monitoring thymopoiesis; however, understanding the specialized gene rearrangements that occur during thymic T cell maturation has enabled the assessment of the quality of thymic output and peripheral T cell repertoire diversity. In 1998 Douek et al. applied the observation that a specific circle of excised genomic *TCRD* DNA is produced as a byproduct of human *TCRA* locus gene rearrangement to create the signal joint TCR excision circle (sjTREC) assay to measure thymic output (Figure 1) [7]. The sjTREC assay is performed using real-time quantitative polymerase chain reaction (RT-qPCR) to quantify the excised episomal DNA. At this step of TCR rearrangement, thymocytes have reached the CD4+/CD8+ double positive thymocyte stage. Following successful positive selection each *de novo* generated T cell that emerges from the thymus will carry sjTRECs until peripheral expansion dilutes them out among their clonal progeny. TRECs are also referred to as sj- α -TRECs since they are a byproduct of *TCRA* rearrangement.

sjTREC content can be affected by peripheral division of long-lived naïve T cells, intracellular degradation of sjTREC molecules, and cell death events [23–27]. These confounding factors have led to the emergence of additional assays detecting other distinct TCR gene rearrangement events to quantify thymus production of T cells. [24,25,28]. As the excised byproduct of the *TCRB* gene rearrangement is produced prior to intrathymic proliferation (between the late TN and early DP stage of differentiation), the frequency of these D β J β TRECs (β -TRECS) is inversely proportional to the divisional history of these cells. The ratio of sjTREC to D β J β TREC (sj/ β -TREC) molecules directly measures intrathymic proliferation and thus a more accurate of thymic output [28,29]. Use of the sj/ β -TREC ratio is particularly important in situations where immunological dysregulation or chronic stimulation of the immune system occurs, such as HIV-1 infection [30,31].

The ability of T cells to recognize a vast array of antigens is due to the expression of diverse T cell receptors generated from the rearrangement of the *TCRA* and *TCRB* genes during thymopoiesis. Thus, the repertoire of TCRs is also an important indicator of successful immune reconstitution. Each T cell is able to recognize its cognate MHC-peptide complex via surface expression of its TCR. The complementary determining region 3 (CDR3), the most diverse region of the TCR, is generated by the recombination of the variable, diversity, joining and constant gene segments of the TCR α and β chains, and forms the structural loops involved in the binding of the TCR to the MHC-peptide complex [32,33].

Immunoscope or “spectratyping” is a molecular technique for analyzing diversity in the TCR repertoire produced by the re-arrangements of the variable region genes [34,35] (Figure 2). This technology is based on PCR methods to generate and amplify template cDNA flanking the desired TCR family CDR3 region. Immunoscope analysis measures changes in T cell receptor diversity via assay of CDR3 lengths from V β TCRs (can also be done for V α) [36]. Each generated peak corresponds to discrete CDR3 lengths (Figure 2). A naïve population of T cells generates a Gaussian distribution of peaks; variations in the peak profile result from clonal expansions or deletions of T cells using a given CDR3 length [37,38]. The estimation of CDR3 length distributions is the best tool available for use in clinical settings for monitoring T cell receptor diversity, and is valuable for analyses of immune reconstitution following thymus or bone marrow transplantation and following antiretroviral (ARV) treatment of HIV-1 infection [17].

Kepler and He have recently reported a statistical tool to facilitate analysis of spectratype peak data based on the Kullback-Leibler divergence (DKL) [39]. The DKL measures the divergence of two probability functions; in the case of measurement of CDR3 lengths, DKL measures the divergence of each spectratype profile (i.e. per V β family) against a corresponding reference profile. The higher the DKL, the greater the divergence from the normal profile and the more limited the distribution of peaks, thus indicating a skewed or more oligoclonal T cell repertoire. A web-accessible tool, SpA, has been created for the management, visualization and statistical analysis of T cell receptor spectratype data [40].

3. T Cell Reconstitution in Clinical Settings

The aforementioned methods for measuring the extent of T cell production have proved to be powerful tools to assess patients following immune-restorative therapy. Here we discuss in detail two T cell deficiencies: an intrinsic defect in T cell production due to congenital absence of the thymus in DiGeorge syndrome, and an extrinsic agent, HIV-1, that induces apoptosis in peripheral T cells, and causes inflammation in the thymus resulting in decreased thymopoiesis. Therapeutic goals in both diseases include restoration of thymus function and peripheral cellular immunity.

3.1. Therapeutic thymic transplantation for complete DiGeorge syndrome

DiGeorge syndrome is a congenital disorder of multiple etiologies in which developmental defects occur in organs derived from the third and fourth pharyngeal pouches and the intervening pharyngeal arch [41–44]. The resulting defects affect the heart, parathyroid, and thymus. The clinical presentation is heterogeneous because the severity of defects can be heterogeneous among affected organs. [45]. In this review, athymic patients with DiGeorge syndrome are said to have “complete” DiGeorge syndrome. In the series at Duke University reported by Markert et al. [46], approximately half of these infants were hemizygous for chromosome 22q11, approximately 25% had CHARGE (coloboma, heart defect, choanal atresia, growth or mental retardation, genital hypoplasia, ear anomalies or deafness), and approximately 15% were infants of diabetic mothers. The remaining infants had no known genetic or predisposing conditions.

Complete DiGeorge syndrome has two clinical presentations. “Typical” complete DiGeorge syndrome patients have very few peripheral blood T cells and do not have a rash at birth. “Atypical” complete DiGeorge syndrome patients develop oligoclonal T cells in the blood associated with rash and lymphadenopathy [47]. The oligoclonal T cells in atypical patients can reject transplants, thus necessitating administration of immunosuppression prior to transplantation. The current immunosuppressive regimen at Duke University involves pre- and post-transplantation cyclosporine and pre-transplantation rabbit anti-thymocyte globulin (ATG) [46]. It is important to be able to diagnose both forms of complete DiGeorge syndrome in order to provide the appropriate therapy to restore T cell function. Only infants who are athymic are candidates for thymus transplantation.

In the absence of thymic transplantation, patients born with total absence of the thymus (complete DiGeorge syndrome) usually die in the first two years of life [48]. Thymus transplantation was initially attempted in the 1960s with mixed results [49]. At that time, diagnostic tools were not available to distinguish infants with severe combined immunodeficiency (who have both T and B cell deficiencies) from those with complete DiGeorge syndrome (who have selective absence of T cells). Methods were also not available in the 1960s to determine if a child with DiGeorge syndrome had partial or complete T cell deficiency. Patients with partial DiGeorge syndrome, although having small thymuses and low T cell numbers, have sufficient T cells to reject allogeneic thymic transplants. The development of new assays, techniques, and reagents outlined in Table 1 rekindled interest in thymus grafting

in the 1990s as a viable therapy for complete DiGeorge syndrome. Postnatal cultured allogeneic thymus tissue has been transplanted by Markert and colleagues at Duke University into athymic infants under an Investigational New Drug (IND) application with the Food and Drug Administration for complete DiGeorge syndrome [47,50,51].

In a series of 44 infants with complete DiGeorge syndrome transplanted with thymus tissue, 32 recipients survived, and all survivors over 1 year have developed T cell function [46]. Ongoing investigations in this series of patients include functional studies of thymus allograft output, assessment of B and T cell function, and evaluation of the quality of the T cell repertoire over time after transplantation. The patients continue to be monitored for adverse events, in particular autoimmune disease, after thymus transplantation.

3.2. Thymus transplant methodology and mechanisms of T cell development in DiGeorge syndrome

Thymus tissue used for transplantation is discarded tissue obtained from infants under 9 months of age at the time of heart surgery [51–53]. No thymus is removed specifically for transplantation; it is removed to access the cardiac surgical site. Informed consent is obtained from the parents of the donor infant. The thymus is sliced and held in culture 2–3 weeks until the safety testing for infectious diseases of the donor and the mother of the thymus donor is complete. Examples of the histology of the thymus tissue on day of harvest are shown in Figure 3. After release for transplantation, the tissue slices are inserted into the quadriceps muscle of the DiGeorge recipient in an open procedure in the operating room. Capillaries from the muscle grow into the tissue which two months later on biopsy shows ongoing thymopoiesis of host thymocytes in the donor thymic epithelium. Examples of the histology of the grafted thymus tissue in the post transplantation biopsy are shown in Figure 4.

In infants with typical complete DiGeorge syndrome, T cells begin to appear in the blood three to four months after transplantation. In all patients tested, the T cells that appear in the circulation after thymus transplantation are derived from the recipient. Thus, the recipient bone marrow stem cells migrate to the donor thymus stromal (i.e. graft) and develop into mature recipient T cells [53].

As mentioned, T cell development via both positive and negative selection occurs in the thymus [54]. T cell positive selection is thought to take place on cortical thymic epithelial cells. The T cells that successfully survive this process consider “self” to be the major histocompatibility complex (MHC) found on the cortical epithelium. In the transplant model, the donor thymic MHC appears to be irrelevant in selecting T cells that will play a role in defending against infections in the periphery. One possible mechanism for positive selection on recipient MHC is suggested by murine experiments that show that positive education can occur on the thymocytes themselves [55,56]. Since T cell immune responses restricted to self (recipient) MHC, are found in thymus transplanted and engrafted patients, we speculate that T cells with self MHC-restriction develop using recipient T cells or other recipient accessory cells such as macrophages and dendritic cells. The mediators of thymic negative selection are thought to be antigen presenting cells that migrate to the thymus from the bone marrow. This same mechanism likely works after thymus transplantation [53]. As noted below, T cells developing after thymus transplantation do respond to infections in the context of “self” MHC and do not cause graft versus host disease.

3.3. Results of thymus transplantation and immune recovery in DiGeorge syndrome

Infants with complete DiGeorge anomaly are not expected to survive without therapy because of their lack of T cells. Since 1997, fifty-four infants with complete DiGeorge syndrome have been enrolled in thymus transplantation protocols at Duke University and forty-four of these

infants underwent thymus transplantation. The Kaplan-Meier survival curve of the 44 infants with complete DiGeorge anomaly who received thymus transplants is shown in Figure 5 [46]. Thirty-two have survived and follow up has been from six months to thirteen years. All deaths to date have occurred within the first year after transplantation and most have been in the first few months, prior to development of T cells. Deaths have been mainly secondary to infection, or due to underlying congenital anomalies, such as cardiac, that resulted in infants being ventilator dependent. The most common infections leading to death were cytomegalovirus and respiratory syncytial virus (RSV) [52].

Ten of the 54 subjects did not receive thymus transplantation [46]. Four died at Duke prior to the date of transplantation, three from infection and one from sudden respiratory arrest. Two additional patients were withdrawn from the protocol by the investigator because they were clinically unstable for transplantation. Two patients with multiple congenital abnormalities were withdrawn from the study prior to transplantation by their parents shortly after enrollment and died. Another infant was withdrawn because of poor clinical condition and is surviving; another is awaiting transplantation.

Most children with typical complete DiGeorge syndrome are transplanted without immunosuppressive therapy as they have very low T cell numbers and no evidence of graft versus host disease (such as from an unirradiated blood transfusion). In these children, T cells develop at three to four months after transplantation [46,52]. Patients who do receive immunosuppression develop T cells approximately four to five months after transplantation [46,50]. An example of typical immune reconstitution after thymus transplantation is shown in Figure 6. Not shown are this patient's normal T cell proliferative response to tetanus toxoid and CD3 stimulation and normal T cell receptor beta chain variable segment repertoire (determined by spectratype/immunoscope) [50]. B cell function in this subject improved after the development of T cells.

Patients have been followed after transplantation for the incidence of adverse events. As expected for patients who develop good T and B cell function, most patients after transplantation are able to clear infections such as RSV that can be life-threatening prior to transplantation. The subgroup of children with choanal atresia and abnormal sinus or ear anatomy remains susceptible to recurrent sinus and ear infections. These infections do not appear to be related to abnormalities in immune function, but instead to abnormal upper airway anatomy. The most common adverse event after thymus transplantation has been the development of autoimmune disease, particularly immune cytopenias and thyroid disease. Five patients have developed manageable autoimmune cytopenias [46], while six patients have developed autoimmune thyroid disease with high thyroid-stimulating hormone and low thyroxine levels [46].

In summary, thymus transplantation has led to restoration of T and B cell function and survival in approximately 75% of infants with complete DiGeorge syndrome. T and B cell function, thymus function, and the incidence of autoimmune disease continue to be followed. It is hoped that this therapy may be applied to patients with other conditions in which lack of thymic function leads to morbidity and mortality.

3.4. Thymic function in HIV-1 infection

The course of HIV-1 infection can be separated into three stages; primary infection where patients exhibit a high virus titer initially controlled by CD8+ cytotoxic T lymphocytes (CTL) and antibodies, followed by an asymptomatic period of varying length where the plasma virus load reaches a plateau. As HIV-1 disease progresses, CD4+ T cell loss continues, immune dysregulation occurs, viral loads increase, and opportunistic infections result, signaling the progression to clinical AIDS [57].

A principal target of HIV-1 is CD4⁺ T helper lymphocytes, which are important in controlling the extent of HIV-1 infection in both the acute and chronic phases of the disease [58–62]. High HIV-1 specific CD4⁺ responses have been observed in long-term non-progressing subjects and these cells, along with CD8⁺ CTLs, appear to contribute to the lack of seroconversion in highly-exposed but uninfected persons [63–65]. Death of CD4⁺ T cells is from multiple mechanisms, including direct effect from the virus itself and by killing of uninfected T cells by CD8⁺ CTLs. Uninfected cells are likely killed by activation-induced cell death or the release of viral or host apoptotic proteins by nearby infected cells [66,67]. In addition to death of CD4⁺ cells, a decreased production of T cells by the thymus also occurs in HIV-infected individuals [68–70]. Soon after HIV-1 infection, the thymic perivascular space expands due to lymphocyte infiltration, and as disease progresses the thymus becomes atrophic at an accelerated rate compared to that which occurs with normal aging processes [1]. HIV-1 induced morphologic changes in the thymus include increased perivascular space infiltrates of CD3⁺ CD8⁺ T cells, calcified Hassall's bodies, and condensation of thymic epithelium with large areas devoid of thymopoiesis (Figure 7) [17]. These changes are associated with a decline in peripheral T cell sjTREC and sj/βTREC levels [1,17,28,71,72]. In early and late HIV-1 infection, HIV-1 infected cells are found in both the thymic perivascular space and within the true thymic epithelial space [17].

Studies from our group have demonstrated that the peripheral T cell pool retains sjTREC⁺, naïve T cells at a level similar to age-matched, uninfected controls shortly after infection with HIV-1 (18–72 days after the onset of symptoms) [73]. Studies by Douek and colleagues examined a more advanced cohort of infected individuals (90 to 120 days after the onset of symptoms) and demonstrated a significant loss of the peripheral naïve T cell pool by three months [71]. These reports confirm that HIV-1 infection inhibits thymic function, and that thymic output is robust only during the first three months of infection. Additional evidence of thymus function during HIV-1 infection comes from chest CT scans in HIV-1 infected patients. McCune et al. found significant amounts of thymic tissue in about 50% of HIV-1 infected subjects, and the amount of tissue corresponded to levels of CD4⁺/CD45RA⁺ naïve phenotype T cells [8]. Even in patients exhibiting profound lymphopenia and progression to AIDS, small areas of active thymopoiesis were observed by CT and by analysis of thymic tissue [8,17]. These data are interesting in that recent studies in acute SIV infection and in patients with early HIV-1 infection demonstrated that 80% of CD4 cell loss occurs in the first 20 days of HIV-1 infection [74–78]. Thus, either the thymus is protected from such effects or our assays of thymopoiesis are not sufficiently sensitive to see loss of thymocytes in acute HIV-1 infection.

Taken together, these data indicate that early in infection the thymus continues to seed the periphery with new cells to some degree soon after acute HIV infection and retains some, though diminished, thymopoietic capacity in later stages of the disease.

An additional observation in our study of primary HIV-1 infection was that CD4⁺ CD25⁺ T cells increased over time after onset of primary HIV-1 symptoms [73]. CD4⁺ T cells that constitutively express the IL-2 receptor γ chain, or CD25, primarily are a population of regulatory T cells produced by the thymus [79–82]. These cells are potent down-regulators of immune responses, and have been shown to be activated in HIV-1 and CMV infections [81, 82]. Kinter et al. demonstrated increased T regulatory cell activity in patients with lower HIV-1 viral loads, suggesting a beneficial effect of T regulatory cells in limiting T cell activation – thus limiting viral replication [82]. CD4⁺ CD25⁺ T regulatory cell numbers increase soon after HIV-1 transmission in acute HIV-1 infection [73], and T cell responses to HIV-1 antigen can be suppressed by T regulatory cell activity, thus may limit host response to HIV-1 [81,82].

3.5. Anti-retroviral treatment and thymic function in HIV-1 infection

The use of anti-retroviral therapy (ARV) in many HIV-1+ patients improves peripheral CD4+ T cell counts via multiple mechanisms, including thymopoiesis, redistribution from lymphoid organs, and proliferation of peripheral T cells [59,70,83–89]. Recent studies have shown that in HIV-1+ chronically infected patients with lymphopenia, ARV therapy resulted in increased thymic volume, thymopoiesis and peripheral CD4+ T cell counts [17,70,90,91]. Thus, thymic function is improved in patients receiving anti-retroviral drugs [70,90].

To directly address the impact of ARV on thymus function in HIV-infected individuals, we studied thymus biopsies and peripheral blood T cells of two HIV-1-seropositive patients being treated with ARV [70]. These two patients had prolonged CD4+ T lymphopenia, had low plasma viral RNA, and then developed thymic enlargement soon after ARV treatment. A composite CT scan, CD4/CD8 thymocyte phenotype plot and histologic section from a patient thymic biopsy is shown in Figure 8A. This patient was a 30 year old male diagnosed with HIV in 1994. He was on ARV for six months during 1998, had a CD4 count of $\sim 960/\text{mm}^3$ and developed an enlarged thymus. At the time of his thymus biopsy he had normal peripheral blood sjTREC levels and numbers of naïve phenotype T cells. Thymocytes isolated from the biopsy displayed a high frequency of CD4/CD8 double positive cells. Together these studies indicated that the enlarged thymus in patients on ARV can in some cases reflect robust thymopoiesis capable of maintaining an age-appropriate peripheral naïve T cell compartment [70].

In contrast, an enlarged thymus in HIV-1 infection may not always signify enhanced thymopoiesis. A composite CT scan, CD4/CD8 thymocyte phenotype plot and histologic tissue section of an enlarged thymus biopsy of a such a patient is shown in Figure 8B. This individual was a 34 year old male diagnosed in 1990 with HIV. He took intermittent ARVs from 1991–2000. In 2001 his enlarged thymus was biopsied to rule out malignancy. At the time of biopsy he was off ARV, had a CD4 count of $272/\text{mm}^3$ and had a plasma viral load of 75,698 copies/mL. Peripheral blood sjTREC and naïve phenotype cell numbers were significantly reduced compared to age-matched uninfected controls. As shown in Figure 8B he had an enlarged thymic shadow on CT, but on histologic analysis, there was very little active thymopoiesis as indicated by the presence of few CD4/CD8 double positive thymocytes and empty thymic epithelium [70]. Thus in this patient the thymic enlargement was due to infiltration of the thymic perivascular space with peripheral inflammatory cells and not due to thymopoiesis. Although CT scans can be very useful as a non-invasive tool to assess thymic size, thymic shadow does not always correlate with thymopoiesis nor export of naïve T cells to the periphery [17]. The addition of other measures such as peripheral phenotype and sjTREC levels aid the assessment of *in vivo* thymus function.

Hardy and colleagues used PET imaging of the thymus in an HIV-1-infected subject to determine if thymus activity (FDG uptake) correlated with restoration of peripheral T cells after ARV [90], and reported a positive correlation between increased FDG uptake and regeneration of the T cell compartment. They observed increased numbers of total CD4 T cell and naïve (CD45RA+/CD26L+) CD4 T cells, and reported controlled viremia and increased RTE by sjTREC analysis. Patients not responding to ARV served as controls for the PET imaging and, as predicted, did not have increased FDG uptake [90]. This study provides additional evidence of thymic reconstitution after ARV in HIV-1 infection using non-invasive PET imaging.

Together, these data indicate that ARV can regenerate the thymus in select patients. It is unclear which of these factors are responsible for these inter-individual differences. Some data have shown that CD4+ T cell recovery in ARV-treated patients is not predicted by pre-treatment CD4+ T cell counts [92]. However, when ARV is begun after a decline in CD4+ T cells,

immune restoration can be suboptimal and some patients are at an increased risk of death and opportunistic infections despite ARV-induced improvement in CD4+ T cell counts [93]. In addition to the time between infection and therapeutic intervention, host factors also contribute to the ability of ARV to enhance thymus function. The production of naïve T cells via thymopoiesis after ARV is better in both children and adults with greater thymus size before ARV [94–96]. The effects of ARV in people 55 years or older compared to subjects 35 years or younger showed that while ARV reduced viral load in both groups, mean CD4 counts increased more dramatically in the younger subjects [97]. This age-associated effect of ARV on CD4+ T cell levels was also found in a study of 80 HIV-1-positive patients, which demonstrated that while all patients showed dramatic reductions in viral load after initiation of ARV after one year of therapy, average CD4 counts were two times higher in younger patients than in older patients [98]. Taken together, these studies highlight the importance of understanding age-associated immunological changes, particularly in thymic function, in order to develop strategies to optimize HIV-1 treatment in older patients [99].

Early rises of CD4+ T cells in peripheral blood during ARV therapy likely are due to redistribution of T cells to the peripheral circulation from tissues rather than from newly produced T cells from the thymus and a decrease in activation state of sequestered T cells in lymphoid tissues [100–102]. This phenomenon was demonstrated in our study of an ARV-treated HIV-1+ patients previously thymectomized for myasthenia gravis [17]. In one subject we observed an initial rise of CD4+ T cells that reflected an increase in peripheral CD4+/CD45RA+/CD62L+ naïve phenotype T cells as well as CD4+/CD45RO+ memory phenotype T cells [17]. Since the patient had been totally thymectomized 8 years prior to onset of ARV, and the peripheral sjTREC levels were very low at the time of initial CD4+ T cell rise (first 3 months on ARV), it was concluded that the CD4+/CD45RA+/CD62L+ phenotype T cells were redistributed revertants from CD45RO+ T cells [17].

Recent studies indicate that supplementation of ARV with cytokines or hormones could be used as a strategy to enhance T cell production [6]. Growth hormone (GH) has pleiotrophic functions including enhancement of thymopoiesis [103]. In children infected with HIV-1 receiving ARVs, those with abnormally low levels of growth hormone had lower CD4 counts, thymic volume, and naïve CD4+ and CD8+ T cells compared to children with normal GH levels, indicating an interaction between GH levels and ARV effectiveness [104]. Indeed, treating HIV-1+ adults with GH increased thymic mass and the level of naïve CD4+ cells [105].

In addition to growth hormone, two recent studies demonstrated that interleukin-2 (IL-2) therapy in addition to ARVs induced a greater increase in CD4+ cells than ARV treatment alone [106,107]. IL-2 did not enhance the response to vaccinations, and the increase in CD4+ cells was accompanied by a decrease in sjTREC [107,108]. These data indicate that while IL-2 in addition to ARVs can increase CD4+ levels in the periphery, CD4+ T cell increase is mainly via thymus-independent mechanisms, and does not protect patients from opportunistic infections. Additionally, the use of IL-2 combination therapy in children (with presumably higher thymic function) was not effective at increasing CD4+ T cell counts and resulted in toxic side effects, making the utility of IL-2 treatment questionable [109]. Additional studies combining ARV and thymostimulatory biologicals such as IL-7, keratinocyte growth factor (KGF), and thymic stromal lymphopoietin (TSLP) may be warranted, and ideally should employ assays to determine the extent, breadth, and source of T cell immune reconstitution.

4. Conclusions

New molecular, imaging, and analytical tools for immunological monitoring of *de novo*, polyclonal T lymphocytes have been instrumental in developing new diagnostic tests of *in*

vivo thymic function and in developing therapeutic strategies for congenital and acquired T cell deficiencies that have improved patient outcomes. Thymus transplantation in infants with complete DiGeorge syndrome can restore T and B lymphocyte function and can improve survival rates for these children who otherwise would not survive. Anti-retroviral treatment in many HIV-1+ patients is associated with enhanced thymopoiesis and normalization of peripheral CD4+ T cell levels. Continued work is needed to understand the cellular and molecular processes involved in thymic reconstitution to improve postnatal thymic function in diseases of limited T cell production.

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References

- Haynes BF, Markert ML, Sempowski GD, Patel DD, Hale LP. The role of the thymus in immune reconstitution in aging, bone marrow transplantation, and HIV-1 infection. *Annual Review of Immunology* 2000;18:529–60.
- Haynes BF, Sempowski GD, Wells AF, Hale LP. The human thymus during aging. *Immunologic Research* 2000;22:253–61. [PubMed: 11339360]
- Parham, P. *The Immune System*. New York, NY: Garland Publishing/Elsevier Science Ltd; 2000.
- Miosge L, Zamoyska R. Signalling in T-cell development: is it all location, location, location? *Curr Opin Immunol* 2007;19:194–9. [PubMed: 17306519]
- Takahama Y. Journey through the thymus: stromal guides for T-cell development and selection. *Nat Rev Immunol* 2006;6:127–35. [PubMed: 16491137]
- van den Brink MR, Alpdogan O, Boyd RL. Strategies to enhance T-cell reconstitution in immunocompromised patients. *Nat Rev Immunol* 2004;4:856–67. [PubMed: 15516965]
- Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF, et al. Changes in thymic function with age and during the treatment of HIV infection. *Nature* 1998;396:690–5. [PubMed: 9872319]
- McCune JM, Loftus R, Schmidt DK, Carroll P, Webster D, Swor-Yim LB, et al. High prevalence of thymic tissue in adults with human immunodeficiency virus-1 infection. *J Clin Invest* 1998;101:2301–8. [PubMed: 9616201]
- Patel P, Alibazoglu H, Ali A, Fordham E, LaMonica G. Normal Thymic Uptake of FDG on PET Imaging. *Clinical Nuclear Medicine* 1996;21:772–5. [PubMed: 8896924]
- Nakahara T, Fujii H, Ide M, Nishiumi N, Takahashi W, Yasuda S, et al. FDG uptake in the morphologically normal thymus: comparison of FDG positron emission tomography and CT. *Br J Radiol* 2001;74:821–4. [PubMed: 11560830]
- Ferdinand B, Gupta P, Kramer EL. Spectrum of thymic uptake at 18F-FDG PET. *Radiographics* 2004;24:1611–6. [PubMed: 15537971]
- De Rosa SC, Herzenberg LA, Herzenberg LA, Roederer M. 11-color, 13-parameter flow cytometry: identification of human naive T cells by phenotype, function, and T-cell receptor diversity. *Nat Med* 2001;7:245–8. [PubMed: 11175858]
- McFarland RD, Douek DC, Koup RA, Picker LJ. Identification of a human recent thymic emigrant phenotype. *Proceedings of the National Academy of Sciences of the United States of America* 2000;97:4215–20. [PubMed: 10737767]
- Mackall CL, Granger L, Sheard MA, Cepeda R, Gress RE. T-cell regeneration after bone marrow transplantation: differential CD45 isoform expression on thymic-derived versus thymic-independent progeny. *Blood* 1993;82:2585–94. [PubMed: 7691265]

15. Kong F, Chen CH, Cooper MD. Thymic function can be accurately monitored by the level of recent T cell emigrants in the circulation. *Immunity* 1998;8:97–104. [PubMed: 9462515]
16. Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Buck D, Terstappen LW. Control of lymphocyte recirculation in man. I. Differential regulation of the peripheral lymph node homing receptor L-selectin on T cells during the virgin to memory cell transition. *J Immunol* 1993;150:1105–21. [PubMed: 7678616]
17. Haynes BF, Hale LP, Weinhold KJ, Patel DD, Liao HX, Bressler PB, et al. Analysis of the adult thymus in reconstitution of T lymphocytes in HIV-1 infection. *Journal of Clinical Investigation* 1999;103:453–60. [PubMed: 10021452]
18. Steffens CM, Al-Harthi L, Shott S, Yorgev R, Landay A. Evaluation of thymopoiesis using T cell receptor excision circles (TRECs): differential correlation between adult and pediatric TRECs and naive phenotypes. *Clin Immunol* 2000;97:95–101. [PubMed: 11027449]
19. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol* 2004;22:745–63. [PubMed: 15032595]
20. Kern F, LiPira G, Gratama JW, Manca F, Roederer M. Measuring Ag-specific immune responses: understanding immunopathogenesis and improving diagnostics in infectious disease, autoimmunity and cancer. *Trends Immunol* 2005;26:477–84. [PubMed: 16039158]
21. Casazza JP, Betts MR, Price DA, Precopio ML, Ruff LE, Brenchley JM, et al. Acquisition of direct antiviral effector functions by CMV-specific CD4+ T lymphocytes with cellular maturation. *J Exp Med* 2006;203:2865–77. [PubMed: 17158960]
22. Kovacs JA, Lempicki RA, Sidorov IA, Adelsberger JW, Sereti I, Sachau W, et al. Induction of prolonged survival of CD4+ T lymphocytes by intermittent IL-2 therapy in HIV-infected patients. *J Clin Invest* 2005;115:2139–48. [PubMed: 16025158]
23. Douek D. Thymic output and HIV infection: on the right TREC. *Immunity* 2004;21:744–5. [PubMed: 15589163]
24. Hazenberg MD, Borghans JA, de Boer RJ, Miedema F. Thymic output: a bad TREC record. *Nat Immunol* 2003;4:97–9. [PubMed: 12555089]
25. Hazenberg MD, Verschuren MC, Hamann D, Miedema F, van Dongen JJ. T cell receptor excision circles as markers for recent thymic emigrants: basic aspects, technical approach, and guidelines for interpretation. *J Mol Med* 2001;79:631–40. [PubMed: 11715066]
26. Ye P, Kirschner DE. Measuring emigration of human thymocytes by T-cell receptor excision circles. *Crit Rev Immunol* 2002;22:483–97. [PubMed: 12803323]
27. Ye P, Kirschner DE. Reevaluation of T cell receptor excision circles as a measure of human recent thymic emigrants. *J Immunol* 2002;168:4968–79. [PubMed: 11994448]
28. Dion ML, Poulin JF, Bordi R, Sylvestre M, Corsini R, Kettaf N, et al. HIV infection rapidly induces and maintains a substantial suppression of thymocyte proliferation. *Immunity* 2004;21:757–68. [PubMed: 15589165]
29. Almeida AR, Borghans JA, Freitas AA. T cell homeostasis: thymus regeneration and peripheral T cell restoration in mice with a reduced fraction of competent precursors. *J Exp Med* 2001;194:591–9. [PubMed: 11535628]
30. Dion ML, Bordi R, Zeidan J, Asaad R, Boulassel MR, Routy JP, et al. Slow disease progression and robust therapy-mediated CD4+ T-cell recovery are associated with efficient thymopoiesis during HIV-1 infection. *Blood*. 2006
31. Douek DC, Picker LJ, Koup RA. T cell dynamics in HIV-1 infection. *Annual Review of Immunology* 2003;21:265–304.
32. Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. *Nature* 1988;334:395–402. [PubMed: 3043226]
33. Rosenberg WM, Moss PA, Bell JJ. Variation in human T cell receptor V beta and J beta repertoire: analysis using anchor polymerase chain reaction. *Eur J Immunol* 1992;22:541–9. [PubMed: 1311263]
34. Ria F, van den Elzen P, Madakamutil LT, Miller JE, Maverakis E, Sercarz EE. Molecular characterization of the T cell repertoire using immunoscope analysis and its possible implementation in clinical practice. *Curr Mol Med* 2001;1:297–304. [PubMed: 11899078]
35. Pannetier C, Cochet M, Darche S, Casrouge A, Zoller M, Kourilsky P. The sizes of the CDR3 hypervariable regions of the murine T-cell receptor beta chains vary as a function of the recombined

- germ-line segments. *Proceedings of the National Academy of Sciences of the United States of America* 1993;90:4319–23. [PubMed: 8483950]
36. Han M, Harrison L, Kehn P, Stevenson K, Currier J, Robinson MA. Invariant or highly conserved TCR alpha are expressed on double-negative (CD3+CD4–CD8–) and CD8+ T cells. *J Immunol* 1999;163:301–11. [PubMed: 10384129]
 37. Pannetier C, Even J, Kourilsky P. T-cell repertoire diversity and clonal expansions in normal and clinical samples. *Immunol Today* 1995;16:176–81. [PubMed: 7734044]
 38. Garcia AM, Fadel SA, Cao S, Sarzotti M. T cell immunity in neonates. *Immunologic Research* 2000;22:177–90. [PubMed: 11339354]
 39. Kepler TB, He M, Tomfohr JK, Devlin BH, Sarzotti M, Markert ML. Statistical analysis of antigen receptor spectratype data. *Bioinformatics* 2005;21:3394–400. [PubMed: 15955781]
 40. He M, Tomfohr JK, Devlin BH, Sarzotti M, Markert ML, Kepler TB. SpA: web-accessible spectratype analysis: data management, statistical analysis and visualization. *Bioinformatics* 2005;21:3697–9. [PubMed: 16051675]
 41. Conley ME, Beckwith JB, Mancier JF, Tenckhoff L. The spectrum of the DiGeorge syndrome. *J Pediatr* 1979;94:883–90. [PubMed: 448529]
 42. Barrett DJ, Ammann AJ, Wara DW, Cowan MJ, Fisher TJ, Stiehm ER. Clinical and immunologic spectrum of the DiGeorge syndrome. *J Clin Lab Immunol* 1981;6:1–6. [PubMed: 6973633]
 43. Thomas RA, Landing BH, Wells TR. Embryologic and other developmental considerations of thirty-eight possible variants of the DiGeorge anomaly. *Am J Med Genet Suppl* 1987;3:43–66. [PubMed: 3130877]
 44. Muller W, Peter HH, Wilken M, Juppner H, Kallfelz HC, Krohn HP, et al. The DiGeorge syndrome. I. Clinical evaluation and course of partial and complete forms of the syndrome. *Eur J Pediatr* 1988;147:496–502. [PubMed: 3044796]
 45. Ryan AK, Goodship JA, Wilson DI, Philip N, Levy A, Seidel H, et al. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *J Med Genet* 1997;34:798–804. [PubMed: 9350810]
 46. Markert ML, Devlin BH, Alexieff MJ, Li J, McCarthy EA, Gupton SE, et al. Review of 54 patients with complete DiGeorge anomaly enrolled in protocols for thymus transplantation: outcome of 44 consecutive transplants. *Blood*. 2007
 47. Markert ML, Alexieff MJ, Li J, Sarzotti M, Ozaki DA, Devlin BH, et al. Complete DiGeorge syndrome: development of rash, lymphadenopathy, and oligoclonal T cells in 5 cases. *Journal of Allergy & Clinical Immunology* 2004;113:734–41. [PubMed: 15100681]
 48. Markert ML, Hummell DS, Rosenblatt HM, Schiff SE, Harville TO, Williams LW, et al. Complete DiGeorge syndrome: persistence of profound immunodeficiency. *Journal of Pediatrics* 1998;132:15–21. [PubMed: 9469994]
 49. Hong R. Reconstitution of T-cell deficiency by thymic hormone or thymus transplantation therapy. *Clin Immunol Immunopathol* 1986;40:136–41. [PubMed: 3521966]
 50. Markert ML, Alexieff MJ, Li J, Sarzotti M, Ozaki DA, Devlin BH, et al. Postnatal thymus transplantation with immunosuppression as treatment for DiGeorge syndrome. *Blood* 2004;104:2574–81. [PubMed: 15100156]
 51. Markert ML, Boeck A, Hale LP, Kloster AL, McLaughlin TM, Batchvarova MN, et al. Transplantation of thymus tissue in complete DiGeorge syndrome [see comment]. *New England Journal of Medicine* 1999;341:1180–9. [PubMed: 10523153]
 52. Markert ML, Sarzotti M, Ozaki DA, Sempowski GD, Rhein ME, Hale LP, et al. Thymus transplantation in complete DiGeorge syndrome: immunologic and safety evaluations in 12 patients. *Blood* 2003;102:1121–30. [PubMed: 12702512]
 53. Markert ML, Kostyu DD, Ward FE, McLaughlin TM, Watson TJ, Buckley RH, et al. Successful formation of a chimeric human thymus allograft following transplantation of cultured postnatal human thymus. *Journal of Immunology* 1997;158:998–1005.
 54. von Boehmer H, Aifantis I, Gounari F, Azogui O, Haughn L, Apostolou I, et al. Thymic selection revisited: how essential is it? *Immunol Rev* 2003;191:62–78. [PubMed: 12614352]

55. Choi EY, Jung KC, Park HJ, Chung DH, Song JS, Yang SD, et al. Thymocyte-thymocyte interaction for efficient positive selection and maturation of CD4 T cells. *Immunity* 2005;23:387–96. [PubMed: 16226504]
56. Li W, Kim MG, Gourley TS, McCarthy BP, Sant'Angelo DB, Chang CH. An alternate pathway for CD4 T cell development: thymocyte-expressed MHC class II selects a distinct T cell population. *Immunity* 2005;23:375–86. [PubMed: 16226503]
57. Sleasman JW, Goodenow MM. 13. HIV-1 infection. *J Allergy Clin Immunol* 2003;111:S582–92. [PubMed: 12592304]
58. Hay C, Rosenberg E. Immunologic response to HIV. *AIDS Clin Care* 1998;10:1–3. [PubMed: 11365074]
59. Rosenberg YJ, Anderson AO, Pabst R. HIV-induced decline in blood CD4/CD8 ratios: viral killing or altered lymphocyte trafficking? *Immunol Today* 1998;19:10–7. [PubMed: 9465482]
60. Rosenberg ES, Walker BD. HIV type 1-specific helper T cells: a critical host defense. *AIDS Res Hum Retroviruses* 1998;14 (Suppl 2):S143–7. [PubMed: 9672231]
61. Walker RE, Carter CS, Muul L, Natarajan V, Herpin BR, Leitman SF, et al. Peripheral expansion of pre-existing mature T cells is an important means of CD4+ T-cell regeneration HIV-infected adults. *Nature Medicine* 1998;4:852–6.
62. Walker BD, Rosenberg ES, Hay CM, Basgoz N, Yang OO. Immune control of HIV-1 replication. *Adv Exp Med Biol* 1998;452:159–67. [PubMed: 9889969]
63. Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Kalams SA, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* 1997;278:1447–50. [PubMed: 9367954]
64. Kaul R, Rowland-Jones SL, Kimani J, Fowke K, Dong T, Kiama P, et al. New insights into HIV-1 specific cytotoxic T-lymphocyte responses in exposed, persistently seronegative Kenyan sex workers. *Immunol Lett* 2001;79:3–13. [PubMed: 11595284]
65. Fowke KR, Kaul R, Rosenthal KL, Oyugi J, Kimani J, Rutherford WJ, et al. HIV-1-specific cellular immune responses among HIV-1-resistant sex workers. *Immunol Cell Biol* 2000;78:586–95. [PubMed: 11114968]
66. Alimonti JB, Ball TB, Fowke KR. Mechanisms of CD4+ T lymphocyte cell death in human immunodeficiency virus infection and AIDS. *J Gen Virol* 2003;84:1649–61. [PubMed: 12810858]
67. Finkel TH, Tudor-Williams G, Banda NK, Cotton MF, Curiel T, Monks C, et al. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. *Nat Med* 1995;1:129–34. [PubMed: 7585008]
68. McCune JM. Thymic function in HIV-1 disease. *Semin Immunol* 1997;9:397–404. [PubMed: 9405269]
69. Teixeira L, Valdez H, McCune JM, Koup RA, Badley AD, Hellerstein MK, et al. Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic function. *Aids* 2001;15:1749–56. [PubMed: 11579235]
70. Markert ML, Alvarez-McLeod AP, Sempowski GD, Hale LP, Horvatinovich JM, Weinhold KJ, et al. Thymopoiesis in HIV-infected adults after highly active antiretroviral therapy. *AIDS Research & Human Retroviruses* 2001;17:1635–43. [PubMed: 11779351]
71. Douek DC, Betts MR, Hill BJ, Little SJ, Lempicki R, Metcalf JA, et al. Evidence for increased T cell turnover and decreased thymic output in HIV infection. *Journal of Immunology* 2001;167:6663–8.
72. Haynes BF. HIV infection and the dynamic interplay between the thymus and the peripheral T cell pool. *Clinical Immunology* 1999;92:3–5. [PubMed: 10413647]
73. Sempowski GD, Hicks CB, Eron JJ, Bartlett JA, Hale LP, Ferrari G, et al. Naive T cells are maintained in the periphery during the first 3 months of acute HIV-1 infection: implications for analysis of thymus function. *J Clin Immunol* 2005;25:462–72. [PubMed: 16160915]
74. Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. *Nature* 2005;434:1093–7. [PubMed: 15793563]
75. Veasey JS, Metcalfe NB, Houston DC. A reassessment of the effect of body mass upon flight speed and predation risk in birds. *Anim Behav* 1998;56:883–9. [PubMed: 9790699]

76. Guadalupe M, Reay E, Sankaran S, Prindiville T, Flamm J, McNeil A, et al. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. *J Virol* 2003;77:11708–17. [PubMed: 14557656]
77. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* 2004;200:749–59. [PubMed: 15365096]
78. Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. *J Exp Med* 2004;200:761–70. [PubMed: 15365095]
79. Shevach EM. Certified professionals: CD4(+)CD25(+) suppressor T cells. *Journal of Experimental Medicine* 2001;193:F41–6. [PubMed: 11390442]
80. Suvas S, Kumaraguru U, Pack CD, Lee S, Rouse BT. CD4+CD25+ T cells regulate virus-specific primary and memory CD8+ T cell responses. *Journal of Experimental Medicine* 2003;198:889–901. [PubMed: 12975455]
81. Aandahl EM, Michaelsson J, Moretto WJ, Hecht FM, Nixon DF. Human CD4+ CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. *Journal of Virology* 2004;78:2454–9. [PubMed: 14963140]
82. Kinter AL, Hennessey M, Bell A, Kern S, Lin Y, Daucher M, et al. CD25(+)CD4(+) regulatory T cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4(+) and CD8(+) HIV-specific T cell immune responses in vitro and are associated with favorable clinical markers of disease status. [see comment]. *Journal of Experimental Medicine* 2004;200:331–43. [PubMed: 15280419]
83. Autran B, Carcelain G, Li TS, Blanc C, Mathez D, Tubiana R, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. *Science* 1997;277:112–6. [PubMed: 9204894]
84. Connors M, Kovacs JA, Krevat S, Gea-Banacloche JC, Sneller MC, Flanigan M, et al. HIV infection induces changes in CD4+ T-cell phenotype and depletions within the CD4+ T-cell repertoire that are not immediately restored by antiviral or immune-based therapies. *Nat Med* 1997;3:533–40. [PubMed: 9142122]
85. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 1995;373:123–6. [PubMed: 7816094]
86. Lange CG, Lederman MM. Immune reconstitution with antiretroviral therapies in chronic HIV-1 infection. *J Antimicrob Chemother* 2003;51:1–4. [PubMed: 12493779]
87. Wolthers KC, Schuitemaker H, Miedema F. Rapid CD4+ T-cell turnover in HIV-1 infection: a paradigm revisited. *Immunol Today* 1998;19:44–8. [PubMed: 9465488]
88. Zhang L, Lewin SR, Markowitz M, Lin HH, Skulsky E, Karanickolas R, et al. Measuring recent thymic emigrants in blood of normal and HIV-1-infected individuals before and after effective therapy. *Journal of Experimental Medicine* 1999;190:725–32. [PubMed: 10477556]
89. Hazenberg MD, Otto SA, Cohen Stuart JW, Verschuren MC, Borleffs JC, Boucher CA, et al. Increased cell division but not thymic dysfunction rapidly affects the T-cell receptor excision circle content of the naive T cell population in HIV-1 infection. *Nat Med* 2000;6:1036–42. [PubMed: 10973325]
90. Hardy G, Worrell S, Hayes P, Barnett CM, Glass D, Pido-Lopez J, et al. Evidence of thymic reconstitution after highly active antiretroviral therapy in HIV-1 infection. *HIV Med* 2004;5:67–73. [PubMed: 15012644]
91. Wong JK, Gunthard HF, Havlir DV, Zhang ZQ, Haase AT, Ignacio CC, et al. Reduction of HIV-1 in blood and lymph nodes following potent antiretroviral therapy and the virologic correlates of treatment failure. *Proceedings of the National Academy of Sciences of the United States of America* 1997;94:12574–9. [PubMed: 9356491]
92. Hunt PW, Deeks SG, Rodriguez B, Valdez H, Shade SB, Abrams DI, et al. Continued CD4 cell count increases in HIV-infected adults experiencing 4 years of viral suppression on antiretroviral therapy. *Aids* 2003;17:1907–15. [PubMed: 12960823]

93. Egger M, May M, Chene G, Phillips AN, Ledergerber B, Dabis F, et al. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet* 2002;360:119–29. [PubMed: 12126821]
94. Clerici M, Saresella M, Trabattoni D, Ferrante P, Vanzulli A, Vigano A. Thymic volume predicts long-term immune reconstitution in HIV-infected children treated with highly active antiretroviral therapy. *Aids* 2002;16:2219–21. [PubMed: 12409744]
95. Kolte L, Dreves AM, Ersboll AK, Strandberg C, Jeppesen DL, Nielsen JO, et al. Association between larger thymic size and higher thymic output in human immunodeficiency virus-infected patients receiving highly active antiretroviral therapy. *J Infect Dis* 2002;185:1578–85. [PubMed: 12023763]
96. Vigano A, Vella S, Saresella M, Vanzulli A, Bricalli D, Di Fabio S, et al. Early immune reconstitution after potent antiretroviral therapy in HIV- infected children correlates with the increase in thymus volume. *Aids* 2000;14:251–61. [PubMed: 10716501]
97. Manfredi R, Chiodo F. A case-control study of virological and immunological effects of highly active antiretroviral therapy in HIV-infected patients with advanced age. *Aids* 2000;14:1475–7. [PubMed: 10930176]
98. Bidwell Goetz M, Morreale AP, Rhew DC, Berman S, Ing M, Eldridge D, et al. Effect of highly active antiretroviral therapy on outcomes in Veterans Affairs Medical Centers. *Aids* 2001;15:530–2. [PubMed: 11242153]
99. Gruver AL, Hudson LL, Sempowski GD. Immunosenescence of ageing. *J Pathol* 2007;211:144–56. [PubMed: 17200946]
100. Pakker NG, Notermans DW, de Boer RJ, Roos MT, de Wolf F, Hill A, et al. Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation. *Nature Medicine* 1998;4:208–14.
101. Wolthers KC, Bea G, Wisman A, Otto SA, de Roda Husman AM, Schaft N, et al. T cell telomere length in HIV-1 infection: no evidence for increased CD4+ T cell turnover. *Science* 1996;274:1543–7. [PubMed: 8929418]
102. Bucy RP, Hockett RD, Derdeyn CA, Saag MS, Squires K, Sillers M, et al. Initial increase in blood CD4(+) lymphocytes after HIV antiretroviral therapy reflects redistribution from lymphoid tissues. *Journal of Clinical Investigation* 1999;103:1391–8. [PubMed: 10330421]
103. Savino W, Postel-Vinay MC, Smaniotto S, Dardenne M. The thymus gland: a target organ for growth hormone. *Scand J Immunol* 2002;55:442–52. [PubMed: 11975755]
104. Vigano A, Saresella M, Trabattoni D, Giacomet V, di Natale B, Merlo M, et al. Growth hormone in T-lymphocyte thymic and postthymic development: a study in HIV-infected children. *J Pediatr* 2004;145:542–8. [PubMed: 15480382]
105. Napolitano LA, Lo JC, Gotway MB, Mulligan K, Barbour JD, Schmidt D, et al. Increased thymic mass and circulating naive CD4 T cells in HIV-1- infected adults treated with growth hormone. *Aids* 2002;16:1103–11. [PubMed: 12004268]
106. Levy Y, Durier C, Krzysiek R, Rabian C, Capitant C, Lascaux AS, et al. Effects of interleukin-2 therapy combined with highly active antiretroviral therapy on immune restoration in HIV-1 infection: a randomized controlled trial. *Aids* 2003;17:343–51. [PubMed: 12556688]
107. Valdez H, Mitsuyasu R, Landay A, Sevin AD, Chan ES, Spritzler J, et al. Interleukin-2 Increases CD4+ lymphocyte numbers but does not enhance responses to immunization: results of A5046s. *J Infect Dis* 2003;187:320–5. [PubMed: 12552459]
108. Pido-Lopez J, Burton C, Hardy G, Pires A, Sullivan A, Gazzard B, et al. Thymic output during initial highly active antiretroviral therapy (HAART) and during HAART supplementation with interleukin 2 and/or with HIV type 1 immunogen (Remune). *AIDS Res Hum Retroviruses* 2003;19:103–9. [PubMed: 12639245]
109. Starr SE, McFarland EJ, Muresan P, Fenton T, Pitt J, Douglas SD, et al. Phase I/II trial of intravenous recombinant interleukin-2 in HIV-infected children. *Aids* 2003;17:2181–9. [PubMed: 14523275]
110. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol* 2003;112:973–80. [PubMed: 14610491]

111. Hellerstein M, Hanley MB, Cesar D, Siler S, Papageorgopoulos C, Wieder E, et al. Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. *Nature Medicine* 1999;5:83–9.
112. Macallan DC, Fullerton CA, Neese RA, Haddock K, Park SS, Hellerstein MK. Measurement of cell proliferation by labeling of DNA with stable isotope-labeled glucose: studies in vitro, in animals, and in humans. *Proc Natl Acad Sci U S A* 1998;95:708–13. [PubMed: 9435257]

Fig. 1. Generation of Signal Joint (sj) TRECs During *TCRA* Rearrangement. Simplified representation of the *TCRD* locus flanked by portions of the *TCRA* locus. Rearrangement of the *TCRA* gene forms a single TREC containing a unique sj sequence. (Adapted and used with permission from reference [1]).

Fig. 2. Schematic outline of Human TCRβ spectratyping (immunoscope) to determine T cell repertoire diversity.

Fig. 3.
An allograft used for thymus transplantation. A) H&E 40x on day of harvest, B) H&E 40x on day of transplantation 21 days later, C) cytokeratin (AE1/AE3) 40x on day of harvest, D) cytokeratin 40x on day of transplantation.

Fig. 4.

Biopsy of thymus allograft at 2.5 months after transplantation. A) H&E 10x, B) cytokeratin (AE1/AE3) 40x, C) CD3 40x, D) Ki-67 40x. A collection of lymphocytes is seen in the muscle. At higher power, the graft shows lacy cytokeratin with CD3+ T cells many of which are positive for Ki-67, a cortical thymocyte marker.

Fig. 5.

Kaplan Meier Survival curve of infants with complete DiGeorge anomaly. Survival of 32 infants out of 44 transplanted with thymus tissue is shown.

Fig. 6. T cell development after thymus transplantation in a typical patient. A. T cell subsets, B. Naïve T cells, C. PHA response. In A, the 10th percentiles for CD3, CD4, and CD8 numbers for children aged 2–6 years are shown as horizontal lines [110]. In B the 10th percentiles for naïve CD4 and naïve CD8 T cells for children aged 2–6 years are indicated by the lines [110]. In C the geometric mean plus and minus one standard deviation of the adult control responses are shown.

Fig. 7.
In situ hybridization for HIV-1 RNA in end-stage thymus tissue. Present in the black box are HIV-1 infected cells in the perivascular space (P) and the thymic epithelium (E). Keratin positive (brown) empty epithelium indicates a loss of thymopoiesis (40x). Histology photographs generously provided by Dr. Laura P. Hale (Duke University Department of Pathology).

Fig. 8.

Thymus function in biopsy tissue from HIV-1 infected donors with (A) or without ARV (B). Shown are chest CT scans, CD4/CD8 FACS plots of isolated thymocytes and H&E stained tissue sections of the intact thymus biopsy. Thymic perivascular space (P) and epithelium (E) are referenced (10x). Histology photographs generously provided by Dr. Laura P. Hale (Duke University Department of Pathology) [70].

Table 1

Assays used to measure thymic function and T cell reconstitution

Method	Comments	Reference(s)
Chest computed tomography (CT)	Indicative of thymic volume but not output	[8]
Positron emission tomography (PET)	Activity of thymus measured by uptake of 2- (18)-fluoro-2-deoxy-D- glucose	[9,90]
Deuterated-glucose infusion FACS/stable isotope/mass spec	T cell kinetics are calculated based on the precursor:product relationship Safe for use in humans	[69,111,112]
sjTREC / DJ β TREC	Molecular measure of RTEs	[7,28]
Surface Phenotype CD62L+/CD45RA+/CD45RO-	Flow cytometry-based phenotyping of cell surface markers. CD62L (L-selectin) is shed with cryopreservation of PBMCs	[1,12,16]
Intracellular Phenotype Ki67+ (mib1a)	Marker of proliferation, often performed in tandem with TREC	[71,73]