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Multiparameter evaluation of human thymic function: interpretations and caveats

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Abstract

After the provision of highly active antiretroviral therapy (HAART), the level of circulating CD4+ T cells increases in many adults infected with the human immunodeficiency virus, type 1 (HIV). To study factors involved in immune reconstitution, we have measured thymic abundance by CT scans, circulating naive-phenotype CD4+ T cells by flow cytometry, and T cell receptor (TCR) rearrangement excision circles (TRECs) by quantitative PCR in 40 virologically suppressed, HIV-infected adults and 33 age-matched, HIV-uninfected controls. In HIV-uninfected subjects, naive T cell numbers, thymic abundance, and the frequency of circulating naive CD4+ T cells bearing TRECs decreased with age, as expected. When corrected for this relationship with age, naive T cell numbers correlated significantly with naive T cell TREC frequencies. Virologically suppressed HIV-infected subjects had higher TREC frequencies, and subjects over the age of 39 were more likely to have abundant thymus compared to age-matched, HIV-uninfected adults. Nevertheless, all HIV-infected subjects had reduced absolute numbers of naive T cells, irrespective of thymic size, age, or TREC frequencies. These data illustrate the complex relationship between these measures of thymic size and function and underscore the need to develop more definitive measures of thymic function in the future.
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Introduction

Introduction of highly active antiretroviral therapy (HAART) in HIV-infected subjects leads to recovery of CD4+ T cell numbers in the blood. It has been shown that the early weeks after initiation of HAART are characterized by a rapid reappearance in the blood of CD4+ T cells that were presumably trapped in lymphoid tissues during untreated HIV infection [1,2]. After that rapid reappearance, CD4+ T cell recovery is characterized by a slow but steady increase in naive-phenotype CD4+ T cell numbers. Several mechanisms may contribute to the reconstitution of peripheral blood CD4+ T cell numbers. Apart from redistribution of cells from parenchymal lymphoid spaces, expansion of the CD4+ T cell pool through peripheral proliferation could play a role as could de novo production of naive T cells by the thymus. However, the relative contribution of each of these mechanisms to T cell reconstitution remains to be determined.

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A number of indirect approaches have been used to estimate the contribution of the thymus to T cell recovery in HIV-infected subjects on HAART. Most studies have evaluated the proportions of phenotypically defined naive T cells, measuring the expression of CD45RA, CD62L, and/or CD27 [1,2]. Other groups have visualized thymic morphology by computed tomography (CT) scans [3,4] or analyzed the replicative history of cell populations by measuring T cell telomere lengths [5,6]. Finally, quantification of the fraction of peripheral blood cells bearing T cell receptor (TCR) rearrangement excision circles (TREC) molecules has been used as a way to assess thymic function independently of cell surface phenotype [7,8]. The interpretation of each of these measures is associated with significant caveats: parenchymal thymic tissue identified by CT scans may or may not represent true thymic tissue; CD4+ T cells with a naive phenotype and/or bearing a TREC may not have arisen recently from the thymus; and the content of TRECs within naive phenotype CD4+ cells may be influenced by a variety of parameters (e.g., cellular division, redistribution, and changes in cell survival) other than thymic output [8,9]. As is always the case in human studies of this type, it is difficult to relate measurements obtained from peripheral blood samples to those that might exist within tissue spaces.

In this study, we present results from HIV-uninfected and virologically suppressed HIV-infected subjects using all three measures of thymic size and function. We reasoned that such an analysis would provide more information about the concordance of these measures with one another and help to better define situations in which one or all might be informative. We observed that, when corrected for age, naive T cell numbers correlated with naive CD4+ T cell TREC content in HIV-uninfected subjects. Virologically suppressed HIV-infected subjects had a higher TREC content in sort-purified naive-phenotype CD4+ T cells but lower naive T cell numbers than age-matched, HIV-uninfected controls. When all HIV-infected subjects were considered together, and when corrected for age, thymic size did not correlate with naive T cell number or with naive T cell TREC frequencies. Associations between TREC frequencies and naive T cell numbers were found to be dependent on age, the duration of infection, the time on anti-retroviral therapy (ART), and CD4 nadir. These observations lead to non-exclusive interpretations about the meaning of these measures and underscore the need for less ambiguous tools to quantify thymic function in humans.

Methods

Subjects and samples

All HIV-infected (n = 40) and HIV-uninfected (n = 33) subjects who underwent radiographic CT scans and/or blood draws for cell sorting were consented according to approved University of California at San Francisco Committee for Human Research (UCSF/CHR) protocols. Peripheral blood mononuclear cells (PBMCs) from other subjects were obtained in an approved anonymous manner from the San Francisco General Hospital (SFGH) Clinical Laboratory. All samples were evaluated for complete blood counts through the SFGH Clinical Lab. Characteristics of the HIV-infected cohort are summarized in Table 1.

Radiographic measurement of thymic tissue

All subjects consented for cell sorting also had computer tomographs (CTs) done through the SFGH Radiology Department and read at least twice in an anonymous manner by at least one qualified radiologist. Thymic indices (TI) were determined according to previously published guidelines [10].

Flow cytometric phenotyping and cell sorting

All blood was processed within 12 h, and typically within 2 h, of phlebotomy. For sorting CD4+ T cell subsets, CD4+ T Rosette-Sep (Stem Cell Technology, Vancouver, Canada) was used in combination with standard Ficoll preparations per the manufacturer's instructions. Cells were then labeled with monoclonal antibodies to CD3, CD4, CD45RA, CD62L (Becton Dickinson, San Jose, CA, USA), and CD45RO (Coulter-Immunotech). T cell subset percentages were determined by FACS analysis directly on fresh whole blood as well as on post-Ficoll preparations. Naive

Table 1
Characteristics of HIV-infected and HIV-uninfected subjects

	HIV positive		HIV negative		P value
Age (years)	44 [29-65]	n = 40	40 [29-76]	n = 33	ns
CD4 nadir (cells/mcL)	215 [7-840]	n = 35	na	na	na
Months on HAART	20.5 [5-160]	n = 32	na	na	na
CD4 (cells/mcL)	534 [44-1346]	n = 37	717 [308-1146]	n = 27	0.054
Naïve CD4 (cells/mcL)	178 [6-719]	n = 37	377 [119-890]	n = 27	<0.001
TREC (per 10^6 naïve $CD4^+$T cells)	26300 [971–610000]	n = 35	2700 [626–200000]	n = 13	0.002
Delta CD4 on ART	354 [−6–885]	n = 32	na	na	na

Depicted are median values [and ranges]. na: not applicable; ns: not significant (P > 0.05).

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