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Heightened T-cell proliferation without an elevation of CD4$^+$ T cell spontaneous apoptosis in AIDS patients

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Abstract T lymphocyte turnover has been studied extensively in HIV infection. The dynamic characteristics of various subsets of T cells in antiretroviral-naive, HIV-1-infected individuals, however, have not been well defined. Here, we performed a cross-sectional study using peripheral blood T cells from 39 antiretroviral-naive, chronically HIV-infected patients, as well as 16 healthy, HIV-negative controls. T-cell subset turnover rates were measured by Ki-67 antigen staining; levels of spontaneous apoptosis and activation in T-cell subsets were also determined by flow cytometry. Surprisingly, with disease progression, the level of T-cell spontaneous apoptosis did not increase significantly, despite a heightened rate of T-cell subset turnover and increased expression of the CD38 activation marker. These data refute the idea that increased T cell turnover is merely a homeostatic process in response to CD4 T cell loss during HIV disease progression, and suggest that future mechanistic studies may be needed for a comprehensive understanding of T-cell dynamics during HIV infection. Such understanding may help to develop new strategies for the immune modulation of clinical disease.

Introduction

HIV-1 infection is marked by a progressive depletion of CD4$^+$ T cells. Both memory and naive T cells are lost with disease progression, but there is a greater depletion of naive T cells than of memory T cells [1,2]. Increased T cell turnover appears to be one of the major mechanisms responsible for the CD4$^+$ T cell loss during HIV-1 infection [3–5]. The proportion of dividing CD4$^+$ T cells is increased 3–5-fold in HIV-infected subjects compared with healthy control individuals, and the proportion of dividing CD8$^+$ T cells is 5–8-fold greater in HIV-infected subjects than in uninfected controls [3,6,7]. Despite this increased proliferation, the number of CD4$^+$ T cells does not increase significantly during the course of HIV infection. On the contrary, elevated loss of naive T cells eventual leads to the development of AIDS. Some previous studies have suggested that the heightened T cell turnover in HIV-1-infected individuals is mainly a homeostatic reflective response to CD4$^+$ T cell depletion [8,9]. Consequently, there is an exhaustion of lymphocyte production, and the development of AIDS. Other studies, however, have suggested that the increased T cell

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turnover is caused by virus-specific T cell activation, or nonspecific "bystander" activation [10–13]. The exact relationships between T cell turnover, immune activation, and $CD4^+$ T cell depletion remain undefined. Furthermore, recent studies have suggested roles for various subsets of CD4 T cells in HIV disease progression [45–48]. Therefore, a detailed analysis of the dynamics of T cell subpopulations will be important to understanding the mechanisms relating to $CD4^+$ T cell depletion, and HIV disease progression.

$CD4^+$ T cell apoptosis has been proposed as a major mechanism responsible for T cell depletion in patients infected with HIV-1 since 1991 [14,15]. Additionally, it was generally accepted that there is a correlation between the magnitude of T cell apoptosis and HIV disease progression [16–18]. Notably, in most previous studies, the level of T cell apoptosis was detected in PBMC after *in vitro* cell culture. However, the *in vitro* cell culture models do not faithfully represent T cell apoptosis *in vivo*. Whether $CD4^+$ T cell apoptosis increases in parallel to HIV disease progression in individuals with untreated chronic HIV-1 infection is not known. Much less is known about whether various T lymphocyte subsets with distinct functions [19,20] will follow similar or different courses of expansion and depletion.

In the current study, we undertook a detailed examination of the relationship between T-cell turnover, apoptosis, and immune activation in a cohort of antiretroviral-naive, chronically HIV-1-infected patients; with a special focus on T cell subpopulation dynamics, in order to better understand the contribution of these various parameters to HIV disease progression. Our results show that the level of T cell apoptosis did not significantly increase in AIDS patients, despite marked increases in the rates of total T cell and T cell subset turnover. These data provide further insight into determinants of HIV disease progression, and such insight may be helpful to the development of new strategies for the immune modulation of clinical disease.

Methods

Subjects

Thirty-nine HIV-1-infected adult patients (cared for by Department of Infectious Diseases, Beijing You'an Hospital, Capital Medical University) and 16 age-matched healthy adults were enrolled in the study. None of the HIV-infected individuals had seroconverted in the 12 months preceding enrollment, and none had received antiretroviral treatment. The majority of these HIV-infected individuals were paid blood donors who had become infected through illegal blood collection between 1994 and 1995. Clinical details of the study subjects are shown in Table 1. All samples were collected with the approval of The Beijing You'an Hospital Research Ethics Committee, and written informed consent was obtained from each subject.

The HIV-infected patients were stratified into three groups according to their $CD4^+$ T cell count (Table 1), which is one of the most important criteria for the initiation of antiretroviral treatment according to the Chinese Ministry of Health's guidelines; treatment is indicated for all patients with a CD4 count <200 cells/mm^3 (who are considered to be AIDS patients). However, a major controversy exists as to whether the CD4 threshold for starting therapy should be set at >350cells/mm^3. The United States Department of Health and Human Services guidelines endorse offering highly active antiretroviral therapy (HAART) to asymptomatic patients with CD4 counts between 200–350 cells/mm^3. The International AIDS Society-USA guidelines recommend that asymptomatic patients with a CD4 count of 200–350 cells/mm^3 be considered for HAART. Thus, it is important to study the difference in T cell dynamics in patient groups with CD4 counts <200, 200–350, and >350.

Samples

Venous blood samples were collected in EDTA-containing tubes and processed within 4 h. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation on a Ficoll Hypaque density gradient (Pharmacia, Uppsala, Sweden) and used immediately after separation.

Monoclonal antibodies

The monoclonal antibodies (mAbs) CD4-PerCP, CD8-PerCP, CD45RO-PE, CD27-APC, CD38-FITC were purchased from BD Bioscience (San Diego, CA). Ki67-FITC was purchased from BD PharMingen (San Diego, CA). Annexin V was purchased from BioVision (Mountain View, CA).

Flow cytometry

Based on CD45RO and CD27 expression, $CD4^+$ T cells were subdivided into naive, central memory and effect memory T cells (T_N, T_{CM} and T_{EM}, respectively). $CD8^+$ T cells were

Table 1 Clinical characteristics of the study population

	HIV-infected subjects			Healthy control ($n=16$)
	<200 ($n=10$)	200–350 ($n=18$)	>350 ($n=11$)	
Age (years)	42 (25–60)	44 (31–55)	44 (30–54)	42 (29–55)
Gender (M/F)	6/4	11/7	6/5	10/6
$CD4^+$ count/mm^3	81 ± 53	294 ± 45	423 ± 62	775 ± 168
Median viral load (range) (copies/ml)	99,800 (6000–320,000)	72,216 (3000–420,000)	86,262 (12,000–430,000)	NA

M = male; F = female;
NA = none available;
All HIV-infected individuals were antiretroviral naïve.