

## Contribution of CD4+, CD8+CD28+, and CD8+CD28- T cells to CD3+ lymphocyte homeostasis during the natural course of HIV-1 infection.

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### Research Article

The relationship between the number of circulating CD4+ T cells and the presence of particular CD8+ T cell subsets was analyzed by flow cytometry on PBL from asymptomatic HIV-1-infected patients whose specimens were collected every 2 mo for a total period of 32 mo. Only slight variations were detected in the absolute number of lymphocytes and percentage of CD3+ lymphocytes, whereas both CD4+ and CD8+ T cell subsets showed wide intrapatient variation. Variations in the number of CD8+CD28+ cells paralleled those of the CD4+ T cell subset in each patient tested, while the presence of CD8+CD28- T cells correlated inversely with CD4+ and CD8+CD28+ T cells. These data show that changes in the number of circulating CD4+-and CD8+CD28+ T cells are strongly related to the presence of CD8+CD28- T cells in these patients. Insight into the significance of CD8+CD28- T cell expansion will allow us to understand the mechanisms and significance of the HIV-1- driven change in CD4+CD8+ T cell homeostasis and the basic immunopathology of HIV disease.

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# Contribution of CD4<sup>+</sup>, CD8<sup>+</sup>CD28<sup>+</sup>, and CD8<sup>+</sup>CD28<sup>-</sup> T cells to CD3<sup>+</sup> Lymphocyte Homeostasis during the Natural Course of HIV-1 Infection

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## Abstract

The relationship between the number of circulating CD4<sup>+</sup> T cells and the presence of particular CD8<sup>+</sup> T cell subsets was analyzed by flow cytometry on PBL from asymptomatic HIV-1-infected patients whose specimens were collected every 2 mo for a total period of 32 mo. Only slight variations were detected in the absolute number of lymphocytes and percentage of CD3<sup>+</sup> lymphocytes, whereas both CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets showed wide inpatient variation. Variations in the number of CD8<sup>+</sup>CD28<sup>+</sup> cells paralleled those of the CD4<sup>+</sup> T cell subset in each patient tested, while the presence of CD8<sup>+</sup>CD28<sup>-</sup> T cells correlated inversely with CD4<sup>+</sup> and CD8<sup>+</sup>CD28<sup>+</sup> T cells. These data show that changes in the number of circulating CD4<sup>+</sup>—and CD8<sup>+</sup>CD28<sup>+</sup>—T cells are strongly related to the presence of CD8<sup>+</sup>CD28<sup>-</sup> T cells in these patients. Insight into the significance of CD8<sup>+</sup>CD28<sup>-</sup> T cell expansion will allow us to understand the mechanisms and significance of the HIV-1-driven change in CD4<sup>+</sup>/CD8<sup>+</sup> T cell homeostasis and the basic immunopathology of HIV disease. (*J. Clin. Invest.* 1998. 101:137–144.) Key words: HIV • CD4 • CD28 • flow cytometry • T cell subsets

## Introduction

The two major subsets of circulating mature T lymphocytes, which express the CD4 and the CD8 cell surface markers, respectively, can be divided further according to biological properties, expression of surface markers, and ability to produce soluble factors such as cytokines and chemokines (1, 2). The genetic pattern of inheritance of the ratio between circulating CD4<sup>+</sup> and CD8<sup>+</sup> T cells was studied recently in a population of healthy donors and in randomly selected families; complex segregation analysis showed that a major recessive gene with a polygenic component together with random environmental factors control the CD4<sup>+</sup>/CD8<sup>+</sup> T cell ratio in humans (3). Although under genetic control, the CD4<sup>+</sup>/CD8<sup>+</sup> ratio undergoes profound alterations during many clinical situations character-

ized by chronic immune responses such as allograft rejection, graft-versus-host disease, and hemophilia (4, 5), or by the presence of different viral infections such as cytomegalovirus (CMV),<sup>1</sup> EBV, and influenza virus (6–8). The CD4<sup>+</sup>/CD8<sup>+</sup> T cell ratio is evaluated routinely in HIV-1-seropositive (HIV<sup>+</sup>) patients, who undergo a progressive loss of CD4<sup>+</sup> T cells and an increase in CD8<sup>+</sup> T cells. In an 8-yr follow-up of HIV<sup>+</sup> individuals, Margolick et al. (9) showed that during the asymptomatic stage of infection, a constant number of T cells is normally maintained without regard to CD4<sup>+</sup> or CD8<sup>+</sup> phenotype, termed blind T cell homeostasis. This phenomenon has also been observed in bone marrow transplant recipients (10). Since the increase in CD8<sup>+</sup> T cells in HIV<sup>+</sup> patients is sustained by different subsets expressing surface markers such as CD38, CD69, and HLA-DR (11–13), or lacking the CD28 surface marker (14–16), we asked whether a particular subset might participate in blind T cell homeostasis or instead might be related to the presence of circulating CD4<sup>+</sup> T cells during the natural course of HIV-1 infection. We analyzed CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cell subsets in 10 asymptomatic HIV<sup>+</sup> individuals whose blood samples were collected every 2 mo for a total of 32 mo. Our data show clearly that in the CD8<sup>+</sup> T cell subsets evaluated, variations in the number of CD28<sup>+</sup> T cells matched variations in the number of CD4<sup>+</sup> T cells in each patient tested. Moreover, CD3<sup>+</sup> T cell homeostasis was maintained by the continuous production of CD8<sup>+</sup>CD28<sup>-</sup> T cells. These findings raise the possibility that HIV-1-associated changes in the number of circulating CD4<sup>+</sup>, CD8<sup>+</sup>CD28<sup>+</sup>, and CD8<sup>+</sup>CD28<sup>-</sup> T cells are not random but strictly regulated, independent of their activation (CD38<sup>+</sup>, CD69<sup>+</sup>, and HLA-DR<sup>+</sup>) status.

## Methods

**Patients and clinical evaluation.** Ten HIV<sup>+</sup> patients, two females (M.N. and P.K.) and eight males (A.S., B.R., C.S., T.F., CH.M., V.G., CH.S., and S.L.) all with a past history of intravenous drug abuse, were selected at the Infectious Diseases Unit of Lovere from a cohort of 112 HIV<sup>+</sup> subjects. The inclusion criteria were positive HIV-1 ELISA, positive HIV-1 Western blot analysis, and negative hepatitis B virus, hepatitis C virus, HIV-2, and human T cell lymphotropic virus-1/2 ELISA. All patients were asymptomatic (stage A) according to the 1993 classification system of the Centers for Disease Control (17), and exhibited > 20% of all lymphocytes and  $\geq 400$  CD4<sup>+</sup> T cells/mm<sup>3</sup> in peripheral blood. None of the patients received antiviral, antibiotic, or antimycotic treatment for the period of the study. Five patients stopped drug use 1–5 yr before enrollment, and five patients were receiving methadone substitutive therapy. Blood samples were obtained from each patient every 2 mo for 32 mo. Control subjects were 50 age- and gender-matched healthy volunteers and 24

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1. Abbreviations used in this paper: CMV, cytomegalovirus; IEL, intestinal intraepithelial lymphocytes; TCR, T cell receptor.

Table I. Laboratory Findings for Subjects Studied

Study group	Lymphocytes <i>absolute number/mm<sup>3</sup></i>	Proportion of lymphocytes stained for			
		CD3 %	CD4 %	CD8 %	CD4/CD8 %
Healthy volunteers ( <i>n</i> = 50)	2680±384	79.8±6.2	47.2±6.7	27.2±5.6	1.5±0.6
HIV <sup>-</sup> subjects* ( <i>n</i> = 16)	2819±761	74.0±5.1	47.0±2.5	27.6±5.4	1.7±0.4
HIV <sup>-</sup> subjects <sup>‡</sup> ( <i>n</i> = 8)	2637±480	73.2±6.9	45.8±4.7	27.4±3.9	1.6±0.2
HIV <sup>+</sup> subjects ( <i>n</i> = 10)	2430±675	83.1±4.7	30.0±8.3	53.6±10.1	0.6±0.3

Values are mean±SD. \*Subjects with a past history of drug use. <sup>‡</sup>Subjects with a past history of drug use and currently under methadone substitutive therapy.

HIV-1-seronegative (HIV<sup>-</sup>) individuals who stated that they had been drug addicts until 6 mo–2 yr before entry in the study, 8 of whom were now receiving methadone substitutive therapy. The HIV<sup>-</sup> individuals tested negative for hepatitis B and C virus, HIV-2, and human T cell lymphotropic virus-1/2 infection. Unlike the HIV<sup>+</sup> patients and healthy volunteers, the HIV<sup>-</sup> individuals were monitored for T cell subsets every 2–8 mo, with their consent.

**Blood processing and flow cytometry.** Whole blood was obtained from overnight fasting patients and controls between 7:00 and 8:00 a.m., drawn in heparin-containing tubes, and processed for lymphocyte count and subset evaluation after no more than 3 h from blood collection. The total number of lymphocytes was determined with a cell counter (Cell-Dyn 1700; Abbott Laboratories, North Chicago, IL). Lymphocyte subsets were evaluated on whole blood using a panel of mAbs (Becton Dickinson, San Jose, CA): FITC- or phycoerythrin-conjugated Leu 4 (CD3; pan T), Leu 3 (CD4; helper/inducer T cells), Leu 2 (CD8; suppressor/cytotoxic T cells), Leu 28 (CD28; T cell subset), Leu 17 (CD38; natural killer cells and activated T cells), Leu 23 (CD69; natural killer, B, and activated T cells), anti-HLA-DR (activated cells), and peridinin-chlorophyll protein-conjugated Leu 4 and Leu 2. Two- and three-color phenotypic characterization of lymphocytes were performed as described (14). Briefly, 100 µl of heparinized blood was incubated for 30 min on ice with appropriate amounts of mAbs. Cells were then lysed with buffer (FACS<sup>®</sup> lysing solution; Becton Dickinson) and analyzed by flow cytometry (FAC-Scan; Becton Dickinson). The lymphocyte gate was set using the log fluorescence of a two-color panel of mAbs (Leucogate [CD45, panleukocytes, and CD14, monocytes, macrophages, and some granulocytes]; Becton Dickinson) and linear 90-degree side scatter. Live gat-

ing was used to collect 10,000 events within the lymphocyte gate, defined as CD45<sup>bright</sup> with low side scatter (18). Data were analyzed using Consort 32 and LYSYS II software.

**Statistics.** Linear correlation coefficients were determined between the percentage or absolute number of CD4<sup>+</sup> T cells and the percentage or absolute number of CD8<sup>+</sup> T lymphocytes expressing other surface antigens, namely CD28, CD38, CD69, and HLA-DR. A regression line was calculated by the least squares method, and correlation was defined as *P* > 0.5. Student's *t* test was used to determine significance of differences.

## Results

**Lymphocyte count and percentage of CD4<sup>+</sup> T cells.** The laboratory findings for the individuals studied are summarized in Table I. No significant difference was observed between control groups for lymphocyte absolute count or percentage of CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> T cell subsets. Compared with controls, HIV<sup>+</sup> patients generally exhibited a CD4<sup>+</sup> T cell decline, a CD8<sup>+</sup> T cell lymphocytosis, and a decreased CD4<sup>+</sup>/CD8<sup>+</sup> ratio. Table II lists the baseline lymphocyte count and percentage of T cell subsets for each HIV<sup>+</sup> patient. In Table III, longitudinal observation of patients revealed slight variations in the absolute number of lymphocytes (mean percentage variation from baseline value, -1.67%, range -9.2–8.5%) and in the percentage of CD3<sup>+</sup> (3.36%, range -3.7–7.8%) and CD8<sup>+</sup> T cells (3.98%, range -6.6–9.5%), whereas CD4<sup>+</sup> (-8.38%, range

Table II. Baseline Evaluation of Lymphocyte Subsets in HIV-1-infected Patients

	Patient									
	A.S.	M.N.	B.R.	P.K.	C.S.	T.F.	CH.M.	V.G.	CH.S.	S.L.
Lymphocyte count (cells/mm <sup>3</sup> )	3100	1400	2500	1400	2800	2600	3500	2500	2800	1700
Lymphocyte subsets (%)										
CD3 <sup>+</sup>	80.1	88.2	73.5	85.7	86.2	83.9	78.1	88.0	88.3	82.4
CD4 <sup>+</sup> *	16.1	31.4	37.8	28.5	28.8	29.3	43.5	41.6	18.8	29.6
CD8 <sup>+</sup> *	63.4	54.9	37.0	60.5	61.5	55.5	36.5	49.3	69.3	52.4
CD4 <sup>+</sup> CD8 <sup>+</sup> *	0.2	0.6	1.0	0.5	0.5	0.5	1.2	0.8	0.3	0.6
CD8 <sup>+</sup> CD28 <sup>+</sup> **	47.1	30.2	82.3	25.3	63.6	51.4	58.2	58.6	19.2	54.3
CD8 <sup>+</sup> CD38 <sup>+</sup> **	57.3	42.7	23.7	50.3	52.9	32.7	29.3	39.6	58.8	38.6
CD8 <sup>+</sup> CD69 <sup>+</sup> **	22.5	18.6	11.0	20.4	27.5	21.1	8.8	24.9	21.7	25.0
CD8 <sup>+</sup> HLA-DR <sup>+</sup> **‡	47.1	26.5	11.1	46.2	42.1	36.9	15.6	28.1	28.5	30.8

\*Lymphocytes within the CD3<sup>+</sup> T cell subset. <sup>‡</sup>Lymphocytes within the CD8<sup>+</sup> T cell subset.

Table III. Percent Variation from Baseline of T Cell Subsets in HIV-1-infected Patients

	Patient									
	A.S.	M.N.	B.R.	P.K.	C.S.	T.F.	CH.M.	V.G.	CH.S.	S.L.
Lymphocyte count (cells/mm <sup>3</sup> )	1.6±8.9	-6.1±8.7	8.5±8.9	-4.6±6.4	-6.9±4.1	1.3±6.2	-9.2±7.2	7.8±3.3	-0.3±5.3	-8.8±7.6
Lymphocyte subset (%)										
CD3 <sup>+</sup>	-3.7±3.8	3.8±3.7	5.5±6.2	4.1±3.5	7.8±4.3	2.2±1.6	1.2±3.8	4.2±3.2	4.2±3.4	4.3±5.1
CD4 <sup>+</sup> *	-26.9±14.1	0.1±17.5	1.7±7.1	16.2±12.8	-22.5±13.6	-30.7±12.3	5.4±8.1	-27.6±10.4	17.4±25.1	-16.1±5.8
CD8 <sup>+</sup> **	9.5±6.5	9.1±6.4	2.1±8.2	-0.8±5.7	3.8±3.3	9.3±9.8	2.1±6.8	8.7±6.1	-6.6±8.4	2.7±5.3
CD8 <sup>+</sup> CD28 <sup>+</sup> ‡	-6.1±8.8	17.1±15.2	1.0±3.9	7.3±23.9	-57.2±12.9	-17.8±16.9	3.4±14.3	-23.0±8.4	35.5±36.6	-6.2±5.8
CD8 <sup>+</sup> CD38 <sup>+</sup> ‡	-2.7±9.6	-13.8±12.2	7.9±18.4	1.2±11.3	3.2±8.7	-25.9±28.3	-5.3±12.9	-5.9±7.1	11.5±12.5	1.8±10.6
CD8 <sup>+</sup> CD69 <sup>+</sup> ‡	-27.7±21.3	-22.0±28.2	7.9±42.8	-11.8±18.8	1.1±21.8	1.4±31.6	-73.3±28.3	19.6±22.6	-26.9±22.7	20.8±29.5
CD8 <sup>+</sup> HLA-DR <sup>+</sup> ‡	-9.5±1.4	-25.4±20.4	4.5±22.2	3.5±11.6	-3.5±16.5	-6.5±26.0	-16.7±20.9	17.8±16.8	-37.1±24.4	8.3±21.0

Values are mean±SD. \*Lymphocytes within the CD3<sup>+</sup> T cell subset. †Lymphocytes within the CD8<sup>+</sup> T cell subset.

-30.7-17.4%), CD8<sup>+</sup>CD28<sup>+</sup> (-8.02%, range -57.2-35.5%), CD8<sup>+</sup>CD38<sup>+</sup> (-3.44%, range -25.9-11.5%), CD8<sup>+</sup>CD69<sup>+</sup> (-9.11%, range -73.3-20.8%), and CD8<sup>+</sup>HLA-DR<sup>+</sup> (-6.46%, range -37.1-17.8%) T cells showed wide inpatient varia-

tion during the period of observation. The percentage of CD4<sup>+</sup> T cells declined progressively in 2 of the 10 patients (V.G. and A.S.), and by the end of the study, their CD4<sup>+</sup> T cell population had decreased by 54% (to 420 cells/mm<sup>3</sup>) and 70% (to 266

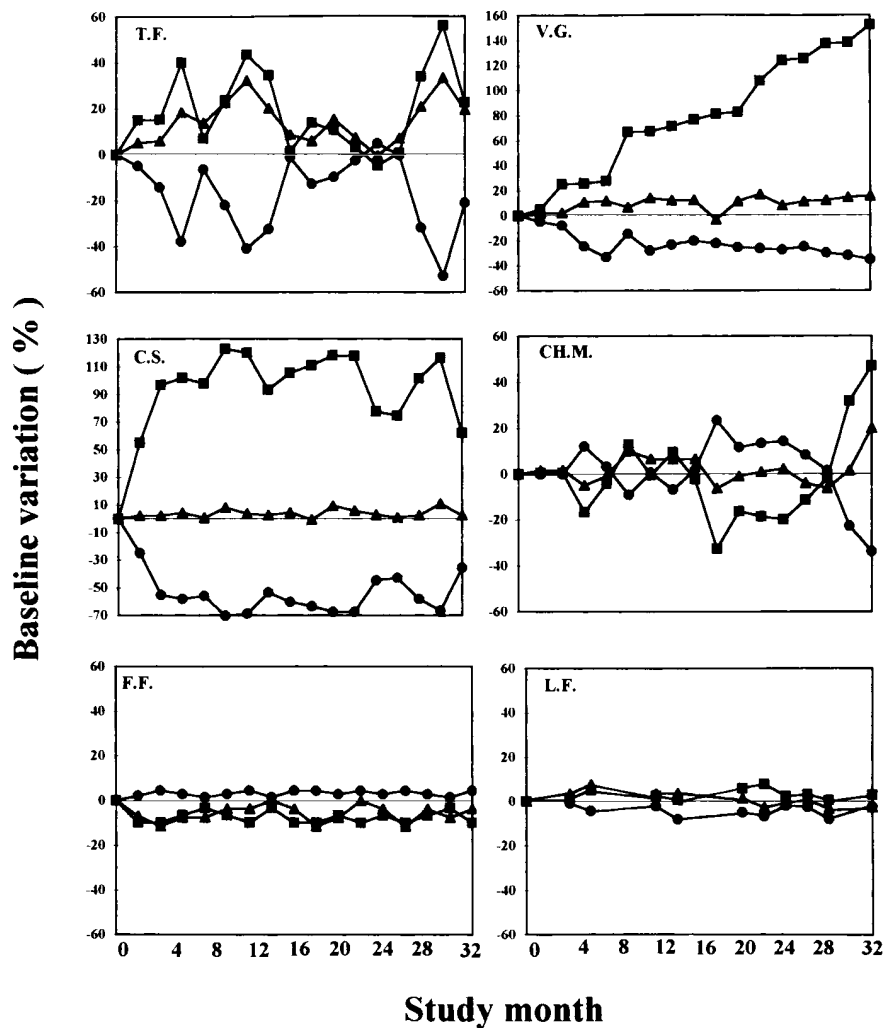


Figure 1. Variation from the baseline percentage of CD8<sup>+</sup> T cell subsets. Longitudinal evaluation of CD8<sup>+</sup> (▲), CD8<sup>+</sup>CD28<sup>+</sup> (●), and CD8<sup>+</sup>CD28<sup>-</sup> (■) T cell percentage in HIV<sup>+</sup> patients (T.F., V.G., C.S., and CH.M.) and in control subjects. F.F. is a healthy volunteer and L.F. is an HIV<sup>-</sup> individual under methadone substitutive therapy. The percentage of CD8<sup>+</sup>CD28<sup>+</sup> and CD8<sup>+</sup>CD28<sup>-</sup> T cells was calculated within the CD8<sup>+</sup> T cell subset.

Table IV. Correlation between Percentage of CD4<sup>+</sup> Cells and Percentage of Different CD8<sup>+</sup> T Cell Subsets in HIV-1-infected Patients

Patient	Correlation (P),* CD4 <sup>+</sup> T cells versus				
	CD8 <sup>+</sup> §§	CD8 <sup>+</sup> /CD28 <sup>+</sup> §§	CD8 <sup>+</sup> /CD38 <sup>+</sup> §§	CD8 <sup>+</sup> /HLA-DR <sup>+</sup> §§	
V.G.	-0.56	0.67	-0.30	-0.31	0.50
A.S.	-0.63	0.74	-0.66	-0.18	-0.25
M.N.	-0.92	0.89	-0.80	-0.22	-0.64
P.K.	-0.79	0.82	-0.64	-0.67	-0.63
S.L.	-0.45	0.66	-0.21	-0.19	-0.24
T.F.	-0.92	0.92	-0.74	-0.47	-0.76
B.R.	-0.44	0.75	-0.22	-0.10	-0.07
C.S.	-0.58	0.82	-0.15	-0.11	-0.42
CH.S.	-0.93	0.94	-0.91	-0.17	-0.65
CH.M.	-0.58	0.72	-0.60	-0.35	-0.73

\*Defined as  $P > \pm 0.5$ . §Lymphocytes within the CD3<sup>+</sup> T cell subset. §§Lymphocytes within the CD8<sup>+</sup> T cell subset.

cells/mm<sup>3</sup>), respectively. A.S. developed esophageal candidosis at 24 mo of observation and received specific antimycotic therapies, whereas V.G. has remained asymptomatic. The other patients exhibited more or less pronounced fluctuations in percentage of CD4<sup>+</sup> T cells over time.

Correlation between percentage of CD4<sup>+</sup> T cells and percentage of different CD8<sup>+</sup> T cell subsets. Statistical evaluation of the data collected over time from each patient revealed a significant positive correlation between percentage of CD4<sup>+</sup> and CD8<sup>+</sup>CD28<sup>+</sup> T cells in all of the subjects. A significant negative correlation between CD4<sup>+</sup> and CD8<sup>+</sup> T cells was detected in 8 of 10 patients, between CD4<sup>+</sup> and CD8<sup>+</sup>/CD38<sup>+</sup> or CD8<sup>+</sup>/HLA-DR<sup>+</sup> T cells in 6 of 10 patients, and between CD4<sup>+</sup> and CD8<sup>+</sup>/CD69<sup>+</sup> T cells in only 1 of 10 patients (Table IV).

Percentage of CD8<sup>+</sup>, CD8<sup>+</sup>/CD28<sup>+</sup>, and CD8<sup>+</sup>CD28<sup>-</sup> T cells. As shown in Fig. 1, the percentage of CD8<sup>+</sup> T cells varied during the natural course of HIV-1 infection with more or less pronounced fluctuations, depending on the patient. Patients with low variations in percentage of CD8<sup>+</sup> T cells over time manifested changes in percentage of CD8<sup>+</sup>CD28<sup>+</sup> and CD8<sup>+</sup>CD28<sup>-</sup> T cells. In patients with wider CD8<sup>+</sup> T cell variation, a similar trend between CD8<sup>+</sup> and CD8<sup>+</sup>CD28<sup>-</sup> T cells was observed. Comparison of the expression pattern of CD4<sup>+</sup> and CD8<sup>+</sup>/CD28<sup>+</sup> T cells throughout the observation period revealed a striking correspondence in percentage (Fig. 2) and absolute number (Fig. 3) of these subsets in each patient. Healthy volunteers and HIV<sup>-</sup> individuals did not show variations over time in percentage of CD4<sup>+</sup>, CD8<sup>+</sup>, CD8<sup>+</sup>CD28<sup>+</sup>, or CD8<sup>+</sup>CD28<sup>-</sup> T cells, as shown representatively in Figs. 1, 2, and 3.

Involvement of CD8<sup>+</sup>CD28<sup>-</sup> T cells in maintenance of con-

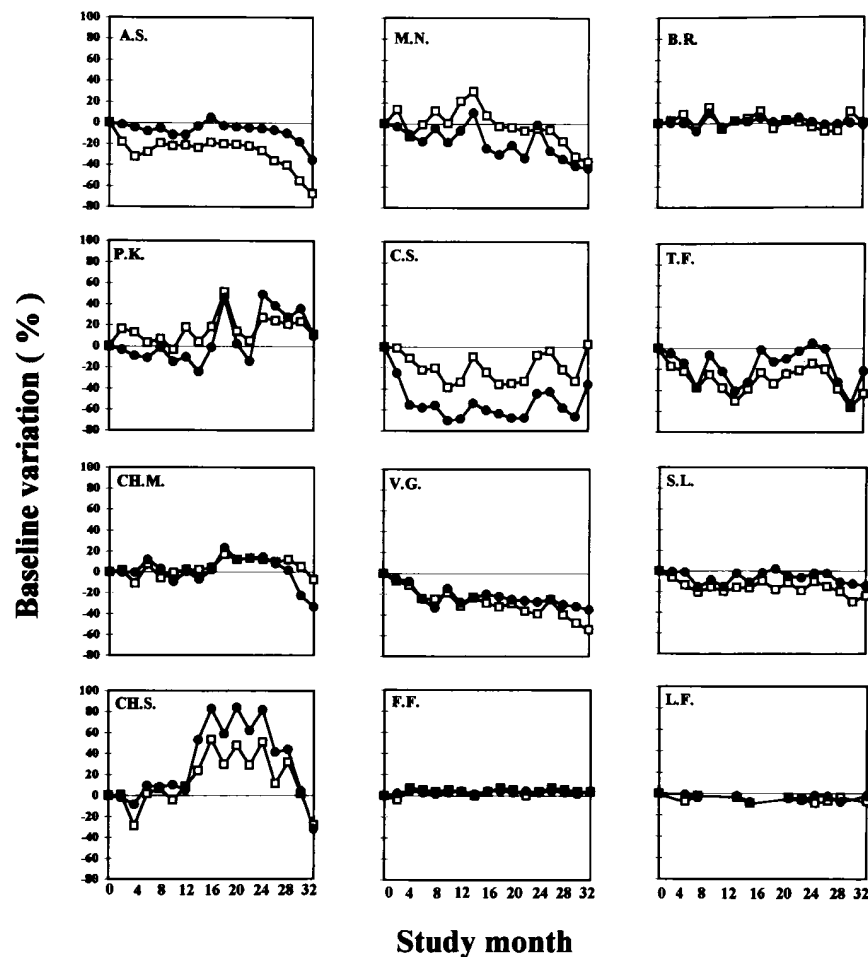


Figure 2. Variation from the baseline percentage of CD4<sup>+</sup> and CD8<sup>+</sup>CD28<sup>+</sup> T cells. Longitudinal evaluation of CD4<sup>+</sup> (□) and CD8<sup>+</sup>CD28<sup>+</sup> (●) T cells in all patients in the study and in representative control subjects (F.F. and L.F.). The percentage of CD8<sup>+</sup>CD28<sup>+</sup> T cells was calculated within the CD8<sup>+</sup> T cell subset.

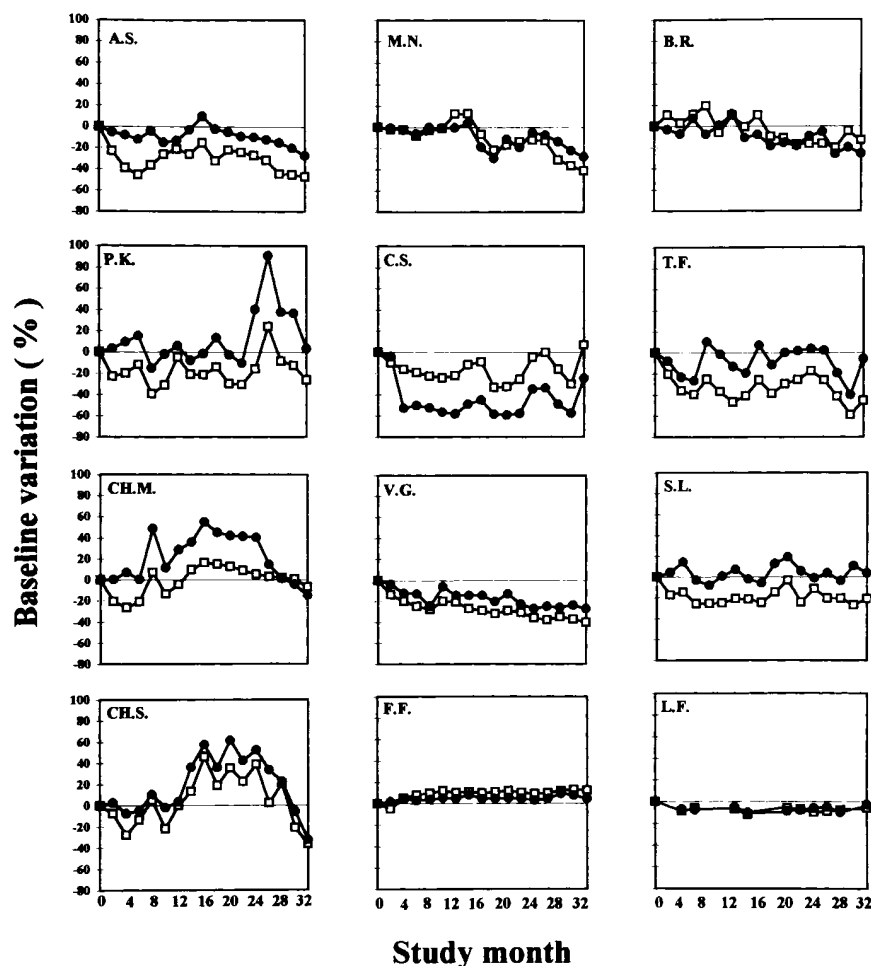


Figure 3. Variation from the baseline absolute number of CD4<sup>+</sup> and CD8<sup>+</sup>CD28<sup>+</sup> T cells. Longitudinal evaluation of CD4<sup>+</sup> (□) and CD8<sup>+</sup>CD28<sup>+</sup> (●) T cells in all patients in the study and in representative control subjects (F.F. and L.F.).

stant CD3<sup>+</sup> lymphocyte numbers. As shown representatively in Fig. 4, positive or negative variations in percentage of CD4<sup>+</sup> T cells were followed by similar variations in percentage of the CD8<sup>+</sup>CD28<sup>+</sup> T cell subset and the CD8<sup>+</sup>CD28<sup>-</sup> T cells contributed exclusively to maintain the homeostasis of CD3<sup>+</sup> lymphocytes over time. 2 of the 10 HIV<sup>+</sup> patients (A.S. and V.G.) showed a continuous decline in number of CD4<sup>+</sup> and CD8<sup>+</sup>CD28<sup>+</sup> T cells, despite a stable percentage of CD3<sup>+</sup> lymphocytes. In these patients, the decline in percentage of both CD4<sup>+</sup> and CD8<sup>+</sup>CD28<sup>+</sup> T cells was concomitant with an increase in percentage of CD8<sup>+</sup>CD28<sup>-</sup> T cells, thus maintaining CD3<sup>+</sup> lymphocytes homeostasis (Fig. 5).

## Discussion

A homeostatic mechanism that maintains normal levels of CD3<sup>+</sup> T cells in HIV<sup>+</sup> patients irrespective of CD4<sup>+</sup> or CD8<sup>+</sup> T cell levels has been described recently (9), refocusing efforts to understand the homeostatic processes that maintain T cell populations (19). Our data on the phenotype of T cells in samples of HIV<sup>+</sup> stage A patients indicated a stability over time in total lymphocyte cell count and in percentage of CD3<sup>+</sup> T cells, independent of percentage of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Our analyses, aimed at defining a relationship between the number of circulating CD4<sup>+</sup> T cells and the presence in blood of particular CD8<sup>+</sup> T cell subsets, evidenced no correlation between

percentage of CD4<sup>+</sup> and CD8<sup>+</sup> T cells expressing the surface molecule CD38, CD69, or HLA-DR. By contrast, variations in the number of CD4<sup>+</sup> T cells matched closely variations in the number of CD8<sup>+</sup>CD28<sup>+</sup> T cells in all HIV<sup>+</sup> patients tested. Moreover, an unusual CD8<sup>+</sup> T cell phenotype lacking surface expression of the CD28 marker (CD3<sup>+</sup>CD8<sup>+</sup>CD28<sup>-</sup>), which increases in HIV<sup>+</sup> patients at different stages of the disease (14–16), was inversely correlated with percentage of CD4<sup>+</sup> and CD8<sup>+</sup>CD28<sup>+</sup> T cells in all patients tested, sustaining CD8<sup>+</sup> lymphocytosis and contributing to CD3<sup>+</sup> T cell homeostasis by replenishing the number of lymphocytes to the patient's baseline values.

The CD4<sup>+</sup> and CD8<sup>+</sup> T cell subset composition evidenced in HIV<sup>+</sup> patients has also been reported for other viral infections. The CD8<sup>+</sup>CD28<sup>-</sup> T cell subset expands concomitant with a decline in the number of CD4<sup>+</sup> and CD8<sup>+</sup>CD28<sup>+</sup> T cells in CMV (20), EBV (21), and varicella zoster virus (21) infections. These uncommon CD8<sup>+</sup>CD28<sup>-</sup> T cells are elicited by the presence of viruses, and mediate virus-specific cytolytic activity (13, 22–25). These data on the T cell phenotype for CMV, EBV, and varicella zoster virus infections were derived exclusively from cross-sectional studies, whereas we observed in HIV<sup>+</sup> asymptomatic patients a mutual control between CD8<sup>+</sup>CD28<sup>-</sup> on the one hand and CD4<sup>+</sup> and CD8<sup>+</sup>CD28<sup>+</sup> T cells on the other. Moreover, the number of CD8<sup>+</sup>CD28<sup>-</sup> T cells has been shown to increase progressively from the second

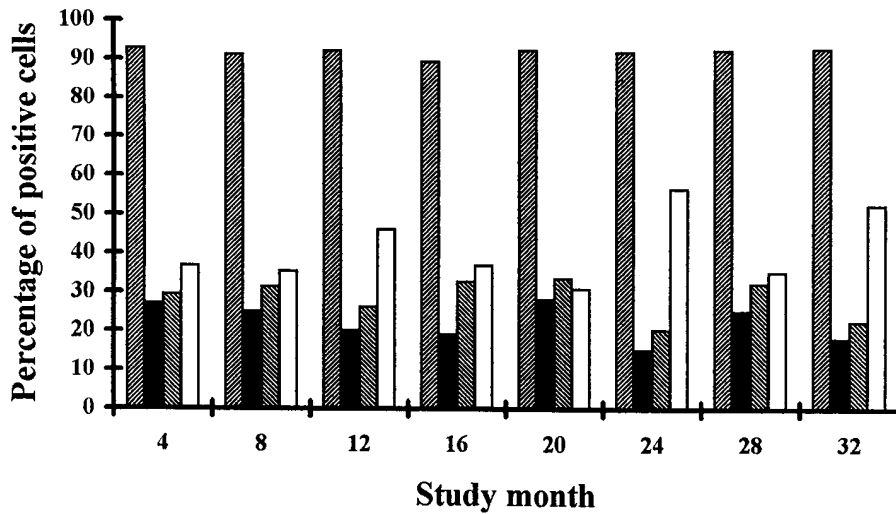


Figure 4. Relative contribution of CD4<sup>+</sup>, CD8<sup>+</sup>CD28<sup>+</sup>, and CD8<sup>+</sup>CD28<sup>-</sup> T cells to CD3<sup>+</sup> lymphocyte homeostasis. Longitudinal evaluation of CD3<sup>+</sup> (▨), CD4<sup>+</sup> (■), CD8<sup>+</sup>CD28<sup>+</sup> (▩), and CD8<sup>+</sup>CD28<sup>-</sup> (□) T cell percentage in a representative patient (T.F.).

to the last decade of life in healthy individuals (26, 27), bearing markers of armed effector cells and exerting a high anti-CD3–redirected cytotoxic activity (28). However, no information is available on the CD4<sup>+</sup> T cells in such healthy individuals. CD8<sup>+</sup>CD28<sup>-</sup> T cells are antigenically and functionally distinct from CD8<sup>+</sup>CD28<sup>+</sup> lymphocytes (28), and resemble a predominant

subset of intestinal intraepithelial lymphocytes (IELs) (26). Neither IELs nor CD8<sup>+</sup>CD28<sup>-</sup> T cells proliferate in vitro in response to T cell receptor (TCR)/CD3 stimuli (28, 29), possibly depending on the availability of integrins expressed uniquely by intestinal antigen-presenting cells (30). Both cell subsets are frequently oligoclonal (27, 31–33), primarily CD8<sup>+</sup> TCR α/β

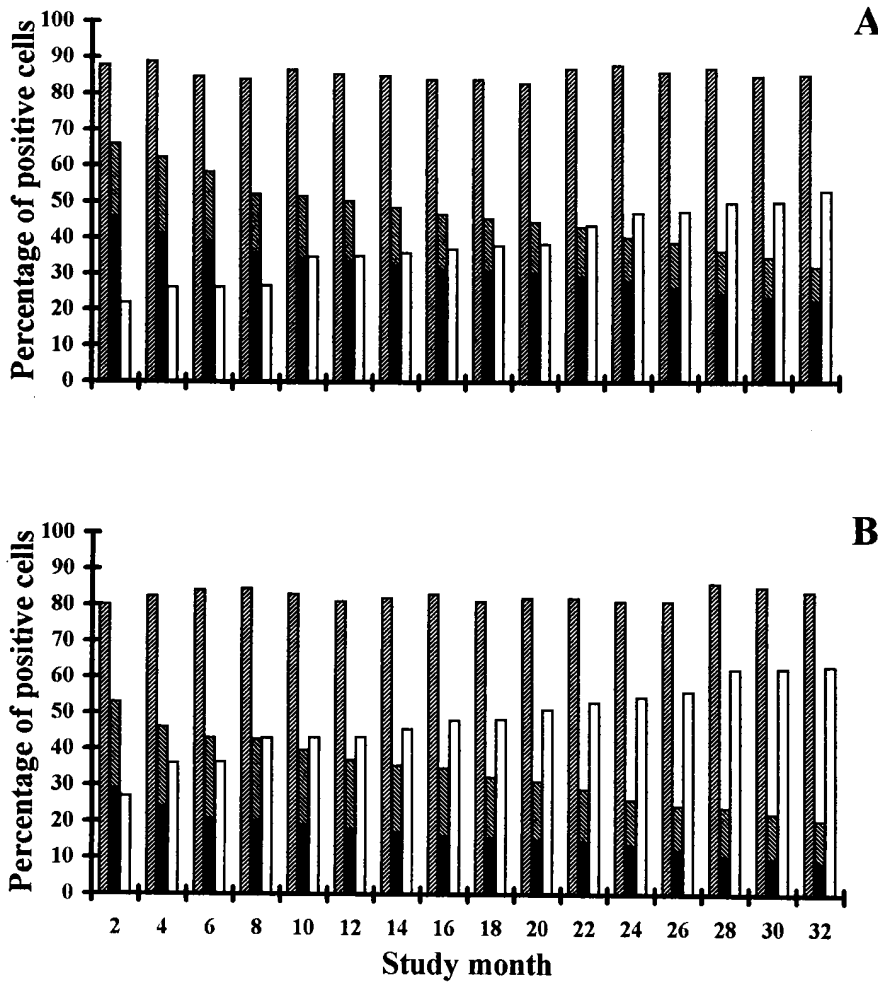


Figure 5. Relative contribution of CD4<sup>+</sup>, CD8<sup>+</sup>CD28<sup>+</sup>, and CD8<sup>+</sup>CD28<sup>-</sup> T cells to CD3<sup>+</sup> lymphocyte homeostasis. Longitudinal evaluation of CD3<sup>+</sup> (▨), CD4<sup>+</sup> (■), CD8<sup>+</sup>CD28<sup>+</sup> (▩), and CD8<sup>+</sup>CD28<sup>-</sup> (□) T cell percentage in patients A.S. and V.G.

expressors (27, 28), and express CD8 $\alpha\alpha$  homodimers rather than the more common CD8 $\alpha\beta$  heterodimers (28, 31). Thus, it is possible that CD8<sup>+</sup>CD28<sup>-</sup> T cells derive from the IEL population. Such a possibility is consistent with the absence of a thymus in elderly individuals, and with the recent findings that T lymphocytes, mostly TCR  $\alpha/\beta$  and CD8 $\alpha\alpha$  homodimers, can be formed in the gut and mesenteric lymph nodes in the absence of thymus (34, 35), and that germinal centers in peripheral lymphoid tissue appear to play a thymus-like role in selecting appropriate antigen-responsive cells and deleting autoreactive T lymphocytes (36).

The presence of high numbers of CD8<sup>+</sup>CD28<sup>-</sup> T cells after various viral infections or after years of repeated immune stimulation raises the possibility that a change in the representative blood CD8<sup>+</sup> T cell population is a general defense mechanism arising from activation of cell-mediated immunity. Note that the largest expansion of CD8<sup>+</sup>CD28<sup>-</sup> T cells was found in healthy centenarians (26), who probably benefit from a well-equipped immune system (37, 38), and that CMV-infected patients without CD8<sup>+</sup>CD28<sup>-</sup>-sustained lymphocytosis develop severe disease (39, 40). However, the possibility that CD8<sup>+</sup>CD28<sup>-</sup> T cells may be only filler cells that arise due to the lack of CD8<sup>+</sup>CD28<sup>+</sup> T cells during chronic viral infection cannot be ruled out.

Our data show that changes in the number of circulating CD4<sup>+</sup>, CD8<sup>+</sup>CD28<sup>+</sup>, and CD8<sup>+</sup>CD28<sup>-</sup> T cells are not random events in asymptomatic HIV<sup>+</sup> patients, but instead are mutually regulated. Since a decline in percentage of CD4<sup>+</sup> T cells concomitant with increased numbers of CD8<sup>+</sup>CD28<sup>-</sup> T cells was also observed in viral infections other than HIV-1 (20, 21), i.e. EBV, which does not replicate in CD4<sup>+</sup> T cells (41, 42), the decline in number of CD4<sup>+</sup> T cells might be dependent on the increase in the CD8<sup>+</sup>CD28<sup>-</sup> T cell phenotype.

The finding of close relationships among CD8<sup>+</sup>CD28<sup>-</sup>, CD8<sup>+</sup>CD28<sup>+</sup>, and CD4<sup>+</sup> T cells in HIV<sup>+</sup> patients might explain the effects of HIV-1 replication on the number of peripheral blood CD4<sup>+</sup> T cells. Indeed, the mechanism of CD4<sup>+</sup>—and CD8<sup>+</sup>CD28<sup>+</sup>—T cell decline may also be explained by a CD8<sup>+</sup>CD28<sup>-</sup> T cell-dependent killing or redistribution into different organs and tissues. Thus, the increase in CD4<sup>+</sup> T cell number observed soon after antiretroviral therapy (43, 44) might reflect not only inhibition of HIV-1-dependent CD4<sup>+</sup> T cell killing, but also a lack of the HIV-1-related antigenic stimuli that drive CD8<sup>+</sup>CD28<sup>-</sup> T cells in the blood, in turn determining the decline in CD4<sup>+</sup> T cell number. Such HIV-1-related stimuli may act at the gut level, where the virus has been detected constantly (45–47). Analysis of the CD4<sup>+</sup> and CD8<sup>+</sup> T cell phenotypes in HIV<sup>+</sup> patients soon after antiretroviral therapies should provide insights into the mechanisms governing HIV-1-mediated T cell composition in the blood.

In conclusion, HIV-1-derived factors play a role in the triggering of a novel and persistent change in the CD4<sup>+</sup> and CD8<sup>+</sup> T cell compartments beginning in the asymptomatic stages of viral infection. Determination of the mechanisms and significance of the HIV-1-driven change in CD4<sup>+</sup>/CD8<sup>+</sup> T cell homeostasis will help us understand the basic immunopathology of HIV disease.

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