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Research Article

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Frequency and Cytokine Profile of HPRT Mutant T Cells in HIV-infected and Healthy Donors: Implications for T Cell Proliferation in HIV Disease

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Abstract

It has been postulated that HIV-infected patients undergo an active production of virus and CD4⁺ T cell destruction from the early stages of the disease, and that an extensive postthymic expansion of CD4⁺ T cells prevents a precipitous decline in CD4⁺ T cell number. Based on the rebound of the CD4⁺ T cell number observed in patients undergoing antiretroviral therapy with protease inhibitors, it has been calculated that, on average, 5% of T cells are replaced every day in HIV-infected patients. To obtain an independent estimate of the recycling rate of T cells in the patients, we measured the frequency of cells carrying a loss-of-function mutation at the hypoxanthine guanine phosphoribosyl transferase (*hprt*) locus. Assuming a recycling rate of 5%/d, an accumulation of 2.6 mutations/10⁶/yr over the physiological accumulation was predicted. Indeed, we observed an elevated frequency of HPRT mutants in the CD4⁺ T cells of most patients with < 300 CD4⁺ T cells/mm³ of blood and in the CD8⁺ T cells of most patients with < 200 CD4⁺ T cells/mm³, consistent with an elevated and protracted increased division rate in both subsets. However, in earlier stages of the disease the mutant frequency in both CD4⁺ and CD8⁺ T cells was lower than in healthy controls. The cytokine production profile of most HPRT mutant CD4⁺ T cell clones from both healthy and HIV-infected patients was typical of T helper cells type 2 (high IL-4 and IL-10, low IFN- γ), whereas the cytokine production pattern of wild-type clones was heterogeneous. The cytokine profile of CD8⁺ clones was indistinguishable between HPRT mutants and wild type. Our data provide evidence of increased CD4⁺ and CD8⁺ T cell recycling in the HIV-infected patients. (*J. Clin. Invest.* 1997; 99:663–668.) Key words: HIV infection • T cell turnover • *hprt* locus mutation • cytokines • T cell clones

Introduction

Infection with HIV type 1 leads to a continuous decline in the number of circulating CD4⁺ T lymphocytes during the average period of 8–10 yr between the acute infection and AIDS (1). The pathogenesis of HIV type 1 infection is closely linked to

the replication of the virus in vivo, and it is now clear that even the early asymptomatic stages of the disease are characterized by very active viral replication, especially in lymphoreticular tissues (2). Because HIV type 1 directly targets CD4⁺ T cells, at least some of the decrease in CD4⁺ T cell number is presumably due to the direct viral cytopathic effect on the infected cells. However, in addition to direct viral infection, immunological mechanisms of destruction of CD4⁺ T cells have been postulated, including induction of T cell anergy and apoptosis or destruction by inflammatory or immune cells (1, 3). Destruction of the anatomical architecture of lymphoid organs, the thymus in pediatric AIDS, and lymphoreticular tissues in all patients, might also contribute to the decrease in T cell number (1). It has also been postulated that an altered reactivity and ability of CD4⁺ T cells to proliferate in patients may eventually lead to their decrease in number. Indeed, a deficient ability of T cells from HIV⁺ patients to proliferate in response to recall antigens, alloantigens, or mitogens is observed at early stages of the disease, when the patients are asymptomatic and CD4⁺ T cell numbers are still within normal range (4, 5). This inability of T cells to proliferate in vitro may not be due uniquely to an intrinsic defect of T cells, but also to a deficiency in the antigen-presenting cells (APC)¹ to which T cells are exposed in vivo and in vitro.

Numerous observations indicating activation of T cells at all phases of HIV disease challenge the notion that the destruction of a proportion of CD4⁺ T cells in latent HIV infection is accompanied by a lack of reactivity and proliferation of T cells, resulting in decreased production and eventual decline of CD4⁺ T cells. Indeed, during the clinical latency period of HIV infection, there is a massive infection of CD4⁺ T cells and macrophages in lymph nodes, and a gradual disappearance of T cells with naive phenotype (CD45 RA⁺, RO⁻), which are replaced largely by cells with a memory phenotype (CD45 RA⁻, RO⁺) (6, 7). This suggests either a continuous proliferation in response to antigenic stimulation or an active postthymic T cell proliferation directed to maintain the CD4⁺ T cell number (7–9). Based on the rebound in CD4⁺ T cell number in response to anti-viral therapy with protease inhibitors, a rapid CD4⁺ T cell turnover has been demonstrated in HIV patients, with an average replacement of 5%/d (10–12). However, the interpretation of these data has been challenged based on the possibility that most of the rebound CD4⁺ T cell number reflects increased recirculation from lymphoid tissues (13), thus overestimating the putative increased turnover of CD4⁺ T cells in HIV-infected patients.

A CD4⁺ cell turnover of 5% per day represents a dramatic decrease in CD4⁺ T cell life span in patients compared with

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healthy donors. Although some of the destroyed cells may be replaced through thymic production of newly generated T cells, it is likely that in adult HIV-infected patients the CD4⁺ T cell replenishment is dependent mostly on postthymic T cell expansion. Thus, to prevent a precipitous drop in the number of CD4⁺ T cells, an increase in the average division rate of CD4⁺ T cells to more than 19 divisions/yr is necessary to maintain a 5% turnover rate. One experimental approach to evaluate the number of divisions in vivo in the lymphocyte population is to analyze the frequency of somatic mutations accumulating in these cells (14). Of various possible loci that are subject to mutation and could be used for analysis, the X-linked hypoxanthine guanine phosphoribosyl transferase (*hprt*) locus represents an ideal choice for analysis of mutation in lymphocytes (14). Loss-of-function mutations in the *hprt* locus can be readily quantitated by evaluating the growth of clonal T cells in the presence of 6-thioguanine, which blocks the growth of the cells with a functional HPRT gene product (14, 15). The frequency of HPRT mutant T cells in peripheral blood increases linearly with age (from 0.5 to 10 mutants/10⁶ T cells) reflecting the normal T cell turnover and regeneration, with an increase of approximately one mutant/10⁶ T cells every 5 yr (15). This increase, assuming no negative or positive selection for HPRT mutant cells, corresponds to a mutation rate of 8.4×10^{-7} per cell division (15), based on an average T cell half-life of 3 yr (0.26 divisions/yr). A negative selection for HPRT mutant cells probably plays a major role in T cell development in the bone marrow and thymus due to an increased requirement for the purine salvage pathway in these microenvironments, but most likely only a minimal role in postthymic T cell expansion, such as that mostly responsible for T cell replenishment in adults (16). In several autoimmune diseases, e.g., systemic *lupus erythematosus*, most patients show a dramatically increased number of HPRT mutant T cells, due to the chronic stimulation and proliferation of the autoreactive T cell subsets (17, 18), indicating that the mutant T cells can accumulate in the patients without evidence of a significant negative selection to limit the expansion of the mutant clones. In this study, we tested whether the postulated increased CD4⁺ T cell turnover in HIV(+) patients is reflected in an increased frequency of HPRT mutant T cells. If the T cell replacement in HIV(+) patients is on average 5% (corresponding to 19 divisions/T cell/yr), an increase of HPRT mutants of ~ 2.6 mutants/10⁶ CD4 T cells/yr was expected.

Methods

Reagents. Phytohemagglutinin (PHA) was purchased from GIBCO BRL (Gaithersburg, MD). 12-O-tetradecanoyl-phorbol-13-acetate (TPA) was from Sigma Chemical Co. (St. Louis, MO). Anti-CD3, anti-CD4, and anti-CD8 mAb produced from cells were obtained from American Type Culture Collection (Rockville, MD). Human recombinant IL-2 (rIL-2) was a gift of Dr. T. Taguchi (Osaka University, Osaka, Japan).

Subjects. Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Hypaque gradient separation from peripheral blood of healthy volunteers and HIV type 1-infected individuals, recruited from the Immunodeficiency Program of the Hospital of the University of Pennsylvania. CD4⁺ lymphocyte counts were obtained on the same day that the patients' blood was obtained for use in this study. Patients were categorized according to the Centers for Disease Control criteria on the basis of CD4⁺ lymphocyte number and HIV-related symptomatology and complications (19).

Generation of CD4⁺ and CD8⁺ T cell clones. T cell clones were generated from PBMC by limiting dilution (20, 21). To determine clonal efficiency in nonselective medium and to generate wild-type clones, 1.0 and 0.5 PBMC/well were seeded in 96-well round bottom tissue culture plates in 200 μ l of RPMI-1640 medium with 10% FCS in the presence of 5 μ g/ml PHA, γ -irradiated (50 Gy) allogeneic PBMC (3×10^4 cells/well) and RPMI-8866 B lymphoblastoid cells (10⁴ cells/well). To expand HPRT mutants, 2 $\times 10^4$ PBMC/wells were seeded in 96-well round bottom tissue culture plates in 200 μ l RPMI-1640 medium with 10% FCS and 6-thioguanine (2 amino-mercaptopurine, 10⁻⁵ M; Sigma Chemical Co.), in the presence of 5 μ g/ml PHA, γ -irradiated allogeneic PBMC (10⁴/well) and RPMI-8866 cells (10⁴/well). After 40 h, IL-2 (50 U/ml, final concentration) was added to all cultures by replenishing half of the medium. After 1 wk, irradiated PBMC (5×10^3 /well) and RPMI-8866 (10⁴/well) as feeder cells were added again; cells were maintained in culture with IL-2 and fed and split as required with the original selective or nonselective medium maintained through the culture period. Cloning efficiency was calculated for CD4⁺ or CD8⁺ by Poisson distribution as described in references 20-22; only wells containing a sufficient T cell number to allow determination of both surface phenotype and cytokine profile were considered positive. The proportion of CD4⁺ and CD8⁺ T cells in the plated PBMC was taken into account in calculating the number of cells from either subset plated per well. The mutant frequency (mF) of T cells was determined as the ratio of the cloning efficiency in selective and nonselective medium (15, 23); 95% confidence limits were calculated using Poisson distribution tables.

Surface phenotype of T cells. The phenotype of PBMC and T cell clones was examined by immunofluorescence (flow cytometry) using FITC-conjugated anti-CD3, anti-CD4, and anti-CD8 mAbs as described in reference 21.

Induction of cytokine production by T cell clones. To induce cytokine production, T cell clones were resuspended in complete medium to a concentration of $\sim 10^6$ /ml and stimulated for 24 h with soluble anti-CD3 mAb (OKT3, ascites 1:10,000) and TPA (10 ng/ml). Cell free culture supernatants were collected and stored in aliquots at -70°C until used.

Quantitation of IFN- γ , IL-4, and IL-10. IFN- γ was quantitated in T cell clone supernatants by radioimmunoassay (RIA) using mAbs B133.1 and B133.5 (24); IL-4 was quantitated by RIA using antibodies 4F2 and 5A4 (25), gift of Dr. Lucien Aarden (Central Laboratory of the Netherlands Red Cross, Amsterdam, The Netherlands); IL-10 was quantitated by RIA using antibodies JE53, 9D7, and 12G8, gift of Dr. Anne O'Garra (DNAX, Palo Alto, CA). Recombinant human IFN- γ , IL-4, and IL-10 were used as reference standards.

Results

18 HIV(+) patients were analyzed [CD4⁺ T cells/mm³ ranging from 660 to 0, age (mean \pm SE) 34.7 \pm 1.3 yr (24-44 range), 14 males and 4 females] and compared with 10 healthy HIV(-) controls [age 35.4 \pm 3.3 yr (24-57 range), 2 males and 8 females]. The cloning efficiency in nonselective medium was comparable in the two groups [HIV(+) donors: CD3⁺ clones, 0.65 \pm 0.04; CD4⁺ clones, 0.52 \pm 0.05; CD8⁺ clones, 0.66 \pm 0.05; HIV(-) donors: CD3⁺ clones, 0.59 \pm 0.07; CD4⁺ clones, 0.65 \pm 0.08; CD8⁺ clones, 0.37 \pm 0.10]. No obvious change in cloning efficiency was observed with disease progression, except for a lower efficiency in CD4⁺ T cells in the four patients with < 100 CD4⁺ T cells/mm³. The frequency in HIV(-) control donors of HPRT mutant cells expanded in the 6-thioguanine was similar to that previously reported (15, 23) for this age range [CD3⁺ cells, 9.9 \pm 1.1 mutants/10⁶ cells; CD4⁺ cells, 11.9 \pm 1.6; CD8⁺ cells, 7.4 \pm 2.3]. As shown in Fig. 1, the mutant frequency in patients with elevated numbers of CD4⁺ T cells is similar to or lower than that of healthy donors; however, at

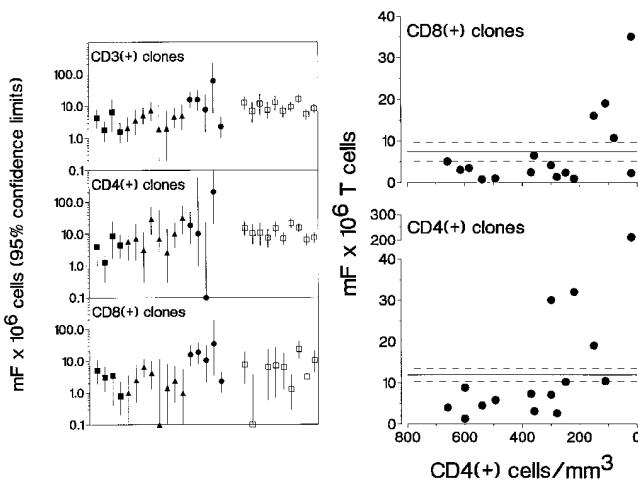


Figure 1. HPRT mutant frequency (mF) in T cells from HIV-infected and healthy donors. The left panel shows the determined HPRT mutant frequency and 95% confidence limits in total $CD3^+$ T cells and in $CD4^+$ and $CD8^+$ T cell subsets in HIV-infected patients (■, > 500 $CD4^+$ T cells/ mm^3 ; ▲, 200–500; ●, < 200) and in healthy blood donors (□). The right panel shows the mutant frequency in $CD4^+$ and $CD8^+$ T cells from HIV-infected patients plotted against the number of $CD4^+$ T cells/ mm^3 of blood at the time samples were obtained. The zero values from the patients from which no HPRT mutant clones were isolated are not reported in the right panel. The continuous and broken horizontal lines indicate the average frequency ± 1 S.E. of HPRT mutant T cells in healthy donors.

later stages of the disease, with low $CD4^+$ T cell numbers, an increase in mutant frequency was observed in most patients. An increased mutant frequency in $CD4^+$ cells was observed in most patients with < 300 $CD4^+$ cells/ mm^3 (comparison of mutant frequency between patients with more or < 300 $CD4^+$ T cells, $t = 1.84$, $P = 0.044$) and in $CD8^+$ cells in patients with < 200 $CD4^+$ cells/ mm^3 ($t = 3.8$, $P = 0.001$). Regression analysis shows that for both $CD4^+$ and $CD8^+$ T cell clones, the mutant frequency was inversely proportional to the number of $CD4^+$ T cells ($CD4^+$ clones: $F = 5.5$, $P = 0.035$; $CD8^+$ clones, $F = 5.98$, $P = 0.028$). Because of the low number of $CD4^+$ T cells available for analysis, mutant $CD4^+$ T cell clones were isolated only in one out of three patients with < 100 $CD4^+$ T cells/ mm^3 .

The pattern of cytokine production was analyzed in clones expanded for 4–5 wk and stimulated for 24 h with soluble anti-CD3 antibodies and phorbol diester; Fig. 2 shows production of cytokines from individual $CD4^+$ clones from healthy patients, and Fig. 3 shows average cytokine production for all clones. The wild-type $CD4^+$ clones expanded in the absence of 6-thioguanine produced heterogeneous levels of IFN- γ , IL-4, and IL-10, without clearly identifiable Th1 or Th2 patterns. The wild-type $CD4^+$ clones from HIV(+) patients produced similar levels of IFN- γ and IL-4 and approximately twofold higher levels of IL-10 ($P = 0.0024$, Student's t -test) than those from healthy donors. The HPRT mutant $CD4^+$ clones from healthy donors expanded in the presence of 6-thioguanine produced significantly less IFN- γ ($P = 0.0009$) and more IL-4 ($P < 0.0001$) and IL-10 ($P < 0.0001$) than the wild-type $CD4^+$ clones. Similarly, HPRT mutant clones from HIV(+) patients produced significantly less IFN- γ ($P = 0.0152$) and more IL-4

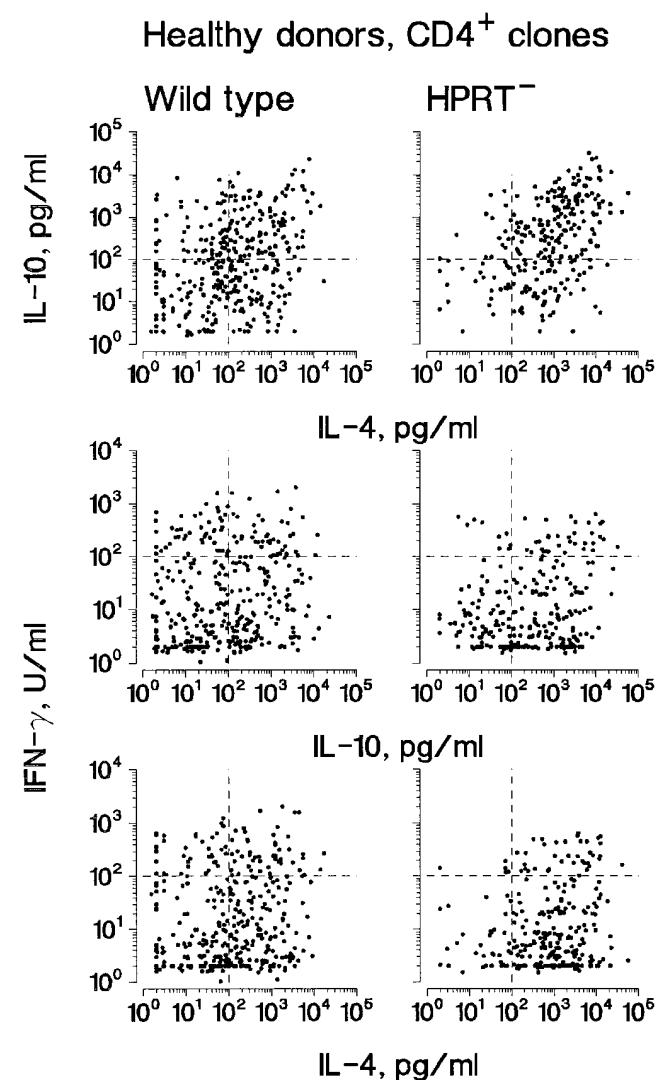


Figure 2. Cytokine production of wild-type or HPRT mutant $CD4^+$ T cell clones from HIV-negative healthy blood donors. T cell clones were expanded for four to five weeks; production of IFN- γ , IL-4 and IL-10 was determined by radioimmunoassay in the cell free supernatant fluid of the clones stimulated for 24 h with soluble anti-CD3 antibodies and phorbol diester. Each dot represents an individual clone; clones from 10 different blood donors are shown.

($P < 0.0001$) than wild-type clones. Thus, the majority of HPRT mutant $CD4^+$ clones, both from healthy controls and patients produced low IFN- γ and high IL-4 and IL-10, a cytokine pattern typical of Th2 cells.

The $CD8^+$ T cell clones from the HIV(+) patient produced higher levels of IFN- γ ($P < 0.0001$) than those derived from the healthy donors (Fig. 3). This increase in average production of IFN- γ was due to an increase in the proportion of IFN- γ -producing $CD8^+$ clones derived from healthy donors (not shown), suggesting that these results may reflect the presence of the peripheral blood of HIV(+) patients of a higher proportion of activated/memory $CD8^+$ cells primed for high production of IFN- γ . Unlike $CD4^+$ T cells, most $CD8^+$ clones produced IFN- γ and only low levels of the Th2-type cytokines IL-4 and IL-10; the cytokine profile of HPRT mutant $CD8^+$ T cell

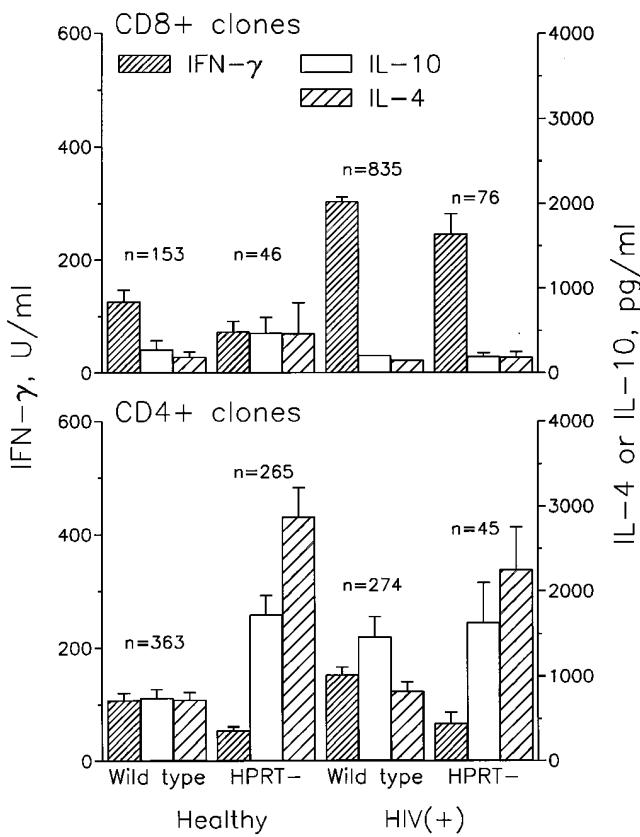


Figure 3. Cytokine production by CD4⁺ and CD8⁺ T cell clones (wild-type or HPRT mutant) derived from HIV-infected patients or healthy donors. The columns and error bars indicate the average cytokine production + S.E. by the indicated number of clones, derived from a total of 18 HIV-infected patients and 10 healthy blood donors.

clones from both healthy donors and HIV(+) patients did not differ significantly from that of wild-type clones.

Discussion

An evident increase in HPRT mutant frequency, well above that of healthy controls, was observed for CD4⁺ T cells in most patients with < 300 CD4⁺ T cells/mm³ and for CD8⁺ T cells in patients with < 200 CD4⁺ T cells/mm³. Unfortunately, the lack of a sufficient number of CD4⁺ T cells available for analysis prevented determination of whether the mutant frequency increased further in the terminal stages of the disease [< 100 CD4⁺ T cells/mm³]. Although the precise date of infection is not known for all the patients studied, the available information on some of the patients suggests that the increased mutant frequency is a function of CD4⁺ cell number rather than duration of the disease. These data support an increased division rate of CD4⁺ T cells in HIV disease. However, the HPRT mutant T cell frequency in the HIV(+) patients until the number of CD4⁺ reached levels < 300/mm³ was not higher than in healthy donors of equivalent age, but actually lower, although many of these patients have been diagnosed or are otherwise known to have been infected with HIV for more than five years, thus predicting a theoretical average accumulation of HPRT mutant T cells at least 13 mutants/10⁶ cells higher

than that of healthy controls. The observed lower mutant frequency than in healthy controls might reflect the absence of any marked increase in T cell recycling in the early phases of the disease and/or negative selection of HPRT mutant cells in the patients. A negative selection in vitro due to the presence of infectious HIV in the cultures is unlikely, because experiments in which HPRT mutant frequency was determined in a mixture of PBMC from HIV⁺ and HIV⁻ donors, no inhibitory effect on the growth of the mutant clones was observed (not shown). Because HIV preferentially replicates in and destroys activated/dividing T cells, and because the HPRT mutation frequency is a function of T cell divisions, one could argue that the mutant cells are preferentially destroyed by the virus infection. However, because there is no a priori reason to assume that the virus may preferentially infect the mutant cells compared to other proliferating T cells, the relative accumulation of mutations in the surviving cells should not be affected by the continuous elimination and replenishment of T cells during the infection. Another possible explanation may be suggested by the Th2-type cytokine profile of the mutant cells which suggests that the mutation tends to accumulate in a memory T cell subset with particular characteristics. The typical Th2 phenotype of the mutant CD4⁺ T cells was an unexpected finding, suggesting that, on average, memory Th2 cells undergo a much higher number of cell divisions than other Th subsets. This could be a general characteristic of Th2 memory cells or reflect the chronic stimulation of Th2 cells by environmental allergens, unlike the rarer occurrence of stimulation of Th1 cells by infection or other antigens inducing this Th subset. Interestingly, this shift to a Th2 phenotype in the mutant CD4⁺ T cell clones was observed in all healthy and HIV(+) donors tested, indicating that it was not a characteristic limited to a few atopic donors. Because Th2-type CD4⁺ clones have been described to be more permissive than the Th1-type clones for HIV replication (26), it is possible that the CD4⁺ T cell subset containing the HPRT mutant cells is more susceptible to HIV infection and thus that the accumulation of HPRT mutant clones is negatively regulated in infected individuals. This possibility could explain why the number of mutant cells is not increased, but rather decreased in the patients at an early stage of the disease. Unlike CD4⁺ T cells, no different cytokine production pattern was identified between wild-type and HPRT mutant CD8⁺ T cell clones.

The increase in mutant frequency in CD8⁺ cells during somewhat more advanced stages of disease than those in which increased frequency was observed in CD4⁺ T cells suggests that T cell turnover is also increased in CD8⁺ T cells at a late stage of disease. The increased CD8⁺ T cell turnover may represent a compensatory mechanism to a bystander destruction of CD8⁺ T cells, which were indeed depleted in most patients with high CD8⁺ T cell mutant frequency, and/or a reactive expansion of CD8⁺ T cells in response to the HIV infection or to opportunistic infections (27). Analysis of the possible antigen specificity, clonality, and functions of the CD4⁺ and CD8⁺ HPRT mutant clones will help to discriminate between these alternatives.

Although our study identified a marked difference in the cytokine production pattern between wild-type and HPRT mutant clones of both healthy and HIV(+) patients, relatively small differences were observed between T cell clones from controls and patients. A statistically significant increase of only approximately twofold was observed for IL-10 production in

Table I. Frequency of HPRT Mutant CD4⁺ and CD8⁺ T Cell Clones In Healthy Donors and HIV-infected Patients

Donors	Age	Sex	CDC	CD4 no.	CD8 no.	Therapy	CD4 clones			CD8 clones				
							Wild type		HPRT(-)	Wild type		HPRT(-)		
							No. clones	CE	No. clones	MF × 10 ⁻⁶	No. clones	CE	No. clones	MF × 10 ⁻⁶
D16	24	F	HIV(-)	N.D.	N.D.	N.A.	22	0.71	20	7.3	4	0.22	3	6.3
D22	24	M	HIV(-)	N.D.	N.D.	N.A.	40	0.69	51	15.9	6	0.22	11	23.6
D14	25	F	HIV(-)	N.D.	N.D.	N.A.	22	0.23	11	7.8	5	0.05	2	6.4
D13	30	F	HIV(-)	N.D.	N.D.	N.A.	10	0.99	7	11.0	0	N.D.	1	N.D.
D15	33	F	HIV(-)	N.D.	N.D.	N.A.	11	0.36	15	15.0	3	0.18	2	7.2
D23	35	M	HIV(-)	N.D.	N.D.	N.A.	45	0.76	23	6.7	20	0.63	5	3.2
D12	40	F	HIV(-)	N.D.	N.D.	N.A.	30	0.81	9	10.5	11	0.55	0	N.D.
D11	43	F	HIV(-)	N.D.	N.D.	N.A.	12	0.85	15	15.3	7	0.80	4	7.6
D24	43	F	HIV(-)	N.D.	N.D.	N.A.	40	0.70	30	7.8	7	0.17	7	10.6
D17	57	M	HIV(-)	N.D.	N.D.	N.A.	28	0.42	34	21.7	37	0.89	3	1.3
P16	27	M	1A	600	1,020	AZT/PRINH	44	0.29	3	1.3	37	0.24	6	3.1
P19	29	F	1A	600	840		24	0.73	3	8.8	23	0.58	1	3.5
P25	36	F	1A	540	1,770	AZT	30	0.60	6	4.5	82	0.63	3	0.8
P26	40	M	1A	660	1,180	AZT	32	0.40	4	4.0	38	0.70	6	5.1
P11	37	M	2A	370	1,470		13	0.41	3	7.3	37	0.59	3	2.5
P17	36	M	2A	300	600	AZT/PRINH	6	0.14	5	30.0	38	0.55	5	4.2
P18	24	M	2A	300	1,230	DDI	10	0.55	1	7.1	29	0.92	0	N.D.
P21	28	M	2A	359	733	AZT	31	0.52	2	3.1	83	0.84	11	6.5
P27	41	M	2A	280	710	AZT/DDI	25	0.68	1	2.6	38	0.45	1	1.4
P22	31	M	2B	494	1,635	AZT/PRINH	15	0.74	4	5.8	41	0.73	2	1.0
P28	44	M	2B	250	720	D4T	29	0.61	6	10.2	51	0.71	3	2.4
P14	39	F	2C	220	800	AZT	7	0.73	5	32.0	44	0.73	1	1.0
P13	31	M	3A	110	320	AZT	5	0.52	1	10.4	29	1.00	8	19.0
P15	39	M	3B	150	790	D4T	26	0.62	4	19.0	64	0.35	9	16.0
P12	40	F	3C	20	370	D4T	1	0.26	1	211.0	6	0.26	1	35.0
P20	30	M	3C	20	880		2	0.24	0	N.D.	121	0.82	8	2.3
P23	39	M	3C	80	390	AZT/DDI	3	0.31	0	N.D.	121	0.82	3	10.7
P24	34	M	3C	0	70	D4T	1	1.00	0	N.D.	20	0.95	0	N.D.

PBMC from 10 healthy donors (D) and 18 HIV-infected patients (P) were cloned under selective or nonselective conditions, as indicated in the text. The patients are listed according to the CDC classification. N.D., not determined; N.A., not applicable; PRINH, protease inhibitors; CE, cloning efficiency; MF, mutant frequency.

CD4⁺ clones from the patients compared to clones from healthy controls as well as an increase in IFN- γ production in CD8⁺ T cell clones, possibly reflecting an increased proportion of activated/memory CD8⁺ T cells in peripheral blood (7). Our findings are consistent with those of other studies using polyclonal stimulation of total peripheral blood T cells (26) and may not reflect the cytokine profile of antigen-specific clones in the patients.

Overall, our analysis of HPRT mutant T cell accumulation in HIV(+) patients supports the previous conclusion (11, 12), based on the analysis of the T cell number rebound after anti-viral therapy, that there is an increased recycling (faster division rate) in the CD4⁺ T cells of the patients and shows that, at least at late stages of the disease, an increased recycling also takes place in CD8⁺ T cells. The increased accumulation of mutant T cells is observed at late rather than earlier stages of disease and depends more on the CD4⁺ T cell deficiency rather than on duration of disease and infection. The fact that an increased mutant frequency is observed only at a late stage of the disease may rest in the time needed for accumulation of the mutants, particularly given the possibility that a negative

selective pressure is effective on the T cell subsets that comprise the mutant T cells in infected patients. However, the rate of CD4⁺ T cell expansion in the group of patients with low CD4⁺ T cell number may be higher than in the patients with high counts and could outpace any negative selection that may take place. Indeed, the combined present observation of the correlation of high mutant frequency with low CD4⁺ number and the previous observation of an increased rate of CD4⁺ T cells recovery in protease inhibitor-treated patients with low number of circulating CD4⁺ cells (12) suggest that an acceleration of CD4⁺ T cell turnover occurs in advanced disease.

Assay of the T cell mutant frequency in a large number of patients and a correlation with virus load will help to determine whether in some patients increased T cell proliferation maintains an elevated T cell number in the presence of active virus replication. However, the fact that the accumulation of HPRT mutant T cells, even when associated with a major loss in CD4⁺ T cell number, is not as extensive as that observed in the case of chronic T cell stimulation in autoimmune diseases (17, 23), suggests that the failure to control the homeostasis of CD4⁺ cell number in the patients may not be due completely

to exhaustion of the ability to replenish their number with continuous postthymic T cell expansion, but possibly also due to a defect in the ability of T cells to expand. The failure to produce a sufficient number of T cells may depend on destruction of adult thymus remnants and lymphoreticular tissues, alteration in the cytokine milieu necessary for extrathymic T cell expansion, and/or altered antigen-presenting cell functions. To use the analogy proposed by Ho et al. (12), the sink empties not only because the drain is open (CD4⁺ T cell destruction), but possibly also because the tap (lymphocyte production) is damaged and is inadequate to maintain the homeostatic level. Thus, it is important to continue the study of these mechanisms of defective T cell homeostasis in the patient and to determine whether therapeutic approaches to both improve lymphocyte regeneration and to block lymphocyte destruction are appropriate.

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