Expansion of CD57 and CD62L$^-$CD45RA$^+$ CD8 T lymphocytes correlates with reduced viral plasma RNA after primary HIV infection

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Objective: CD8 T cells, expressing cell surface molecules distinct from those on resting and naive T cells, are increased in HIV infection. The association of increased CD38 and human leukocyte antigen DR (HLA-DR) CD8 T cells with poor prognosis has suggested that activated CD8 T cells may aggravate HIV infection. We examined whether other immunological parameters might influence the viral setpoint.

Design: Peripheral T cells from nine untreated patients, obtained after primary HIV infection when plasma HIV had stabilized, were examined for proteins expressed in activated versus resting, memory versus naive, and cytolytic versus non-cytolytic T cells.

Methods: The proportion of CD8 T cells that stain for CD38 and HLA-DR, CD28 and CD57 was compared with plasma viraemia and CD4 cell count. These parameters were also compared with the proportion of CD4 and CD8 T cells that express CD62L and CD45RA, present on naive cells and down-modulated in memory cells. Internal staining for the cytotoxic protein granzyme A was also examined.

Results: An increase in CD38 and CD38 HLA-DR CD8 T cells correlated with increased plasma viral RNA ($P < 0.00002$, $P < 0.03$, respectively). An increase in CD8 T cells expressing granzyme A was associated with lower CD4 cell counts ($P < 0.04$). However, the expansion of CD57 and CD62L$^-$CD45RA$^+$ CD8 T cells was associated with a lower viral setpoint ($P < 0.01$, $P < 0.02$, respectively).

Conclusion: Phenotypically defined activated CD8 T cells may have different functions in HIV infection. Activated CD8 T cells that are CD57 or CD62L$^-$CD45RA$^+$ may be beneficial, because their expansion in untreated patients correlates with a reduced viral setpoint after primary infection.

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Introduction

The plasma viral setpoint after primary HIV infection (PHI) varies over many orders of magnitude and is an important prognostic predictor of the time to develop AIDS and to death [1]. Little is known about what determines the viral setpoint. Viral infectivity and replicative capacity, the route and dose of infection, the history of intercurrent and past infections, the level of immune activation, and genetic variations in the immune response and susceptibility to viral infection at the cellular level are probable contributory factors. The initial control of PHI viraemia is temporally related to the development of an HIV-specific CD8 cytotoxic T cell response [2,3]. Therefore factors that enhance the magnitude and quality of antiviral cytotoxic T lymphocytes (CTL) are likely to induce a lower setpoint. However, bystander activation of non-specific T cells during the primary infection may actually increase viral production because activated cells replicate HIV more efficiently [4,5].

Because of the intimate relationship between viral replication and immune activation, it has been difficult to tease apart the relative roles of the virus and the immune response in determining the clinical course. Both viral and immune parameters are predictive of clinical course. A high proportion of activated CD8 T cells that express CD38 and human leukocyte antigen DR (HLA-DR) activation markers has as much value in predicting poor prognosis as a low CD4 count or high plasma viral RNA [6–8]. The poor prognosis associated with the expansion of $CD38^+DR^+$ CD8 T cells supports a potentially deleterious role of activated CD8 T cells in disease pathogenesis. On the other hand, in untreated patients an increased frequency of tetramer-staining HIV-specific CD8 T cells correlates with lower plasma viral burden, suggesting that antiviral CTL are protective [9].

In most healthy adults, the majority of circulating CD8 T cells are resting T cells, which express the CD28 co-stimulatory receptor, but not cell surface markers, such as HLA-DR, CD25, CD38, CD57, and CD69, upregulated after CD8 T cell activation. Moreover, most circulating T cells from healthy donors stain for both phenotypic markers CD62L and CD45RA associated with naive T cells. However, in HIV infection there are substantial expansions of cells expressing various combinations of activation and memory cell surface markers [6,10–18].

Differences between subsets of cells in the CD8 T cell compartment are, however, more complex than those between activated and resting and between naive and memory subsets [19]. Phenotypically distinct subsets probably vary in their functional capacities to proliferate, traffic, activate or suppress viral production and other immune cells, induce cytolysis and secrete particular cytokines. However, not much is known about what differences in stimulation lead to one phenotypic variant rather than another, nor about what functional differences characterize the distinct phenotypic subsets.

In this study, the immune function markers of blood lymphocytes from nine individuals who had not received any antiviral therapy were examined within 14 months of PHI. The time of sampling was at least 1 month after primary infection when the viral load had stabilized around the setpoint [20,21]. The plasma HIV RNA of the group varied from 2000 to over 3 million viral RNA copies/ml and the CD4 cell counts between 182 and 1414 cells/mm^3. Strong correlations were found between the viral load and the percentage of CD8 T cells expressing various activation markers.

Materials and methods

Subjects

Eligible patients were required to have been infected with HIV within 15 months of the study, to have received no antiretroviral drug treatment and to have at least two independent consecutive assays positive for the presence of plasma HIV-1 RNA (Table 1). All subjects were homosexual men. All subjects but one had symptomatic acute viral syndrome and history supporting PHI; all but two were known to have been HIV-seronegative within the previous 2 years. The one subject without known acute viral syndrome was HIV-seronegative one year earlier. Three or more measurements of plasma HIV RNA were obtained for each subject in the month before sampling to verify that

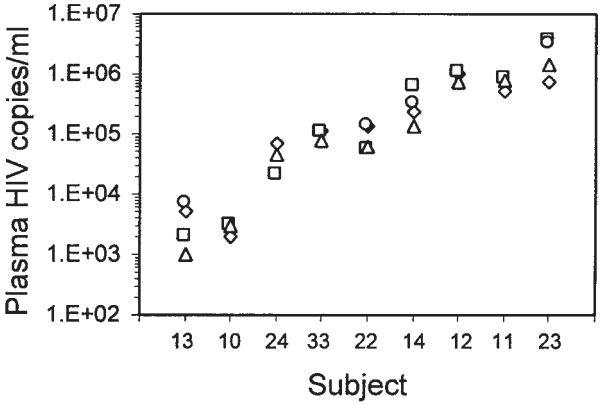

Fig. 1. Each of the subjects in this study had reached stable plasma viral levels at the time of the study. Three or four consecutive viral measurements were obtained for each patient in the month before the sample was drawn for this study. Values are depicted by ◇, □, △, and ○, in order of sampling.

Table 1. Clinical characteristics of subjects.

Subject	Age	Months post-infection	Acute viral syndrome	Plasma HIV RNA copies/ml	CD4 cell count (cells/mm^3)	CD8 cell count (cells/mm^3)
10	29	14	Fever, headache, myalgia	2000	902	794
11	34	1–2	Fever, exanthem, pharyngitis	824 000	302	2169
12	42	2–3	Fever, pharyngitis	1 064 000	494	1801
13	30	2–3	Pharyngitis	2000	1139	1269
14	30	1	Fever, exanthem, pharyngitis	609 000	182	275
22	23	1–2	Fever, pharyngitis	132 000	496	735
23	17	1–2	Fever, pharyngitis, lymphadenopathy	3 219 000	257	681
24	38	2–3	Pharyngitis, exanthem	46 000	1390	2687
33	40	7–8	Not known (seronegative 1 year earlier)	111 000	1414	2487

each patient's viral load was stable (Fig. 1). The principles outlined in the Declaration of Helsinki 'Recommendations guiding physicians in biomedical research involving human subjects (1990)' were followed, and all patients gave signed informed consent. This study was approved by the institutional Human Investigation Review Committee. Plasma viraemia was measured by branch-DNA assay (Chiron, Emeryville, CA, USA). CD4 and CD8 T lymphocyte counts were performed by routine clinical laboratory testing.

Flow cytometry

For external staining, peripheral blood mononuclear cells (PBMC) ($2–10 \times 10^5$/tube), freshly isolated by Ficoll-Hypaque density centrifugation from heparinized blood, were suspended in 100 µl fluorescence-activated cell sorter (FACS) buffer (phosphate-buffered saline (PBS) with 2% fetal calf serum (FCS)) before adding 4 µl each of CD28-fluorescein-isothiocyanate (FITC) (mAb CD28.2, Immunotech, Westbrook, ME, USA) and CD8-Cy5 (mAb B9.11, Immunotech); CD57-FITC (mAb NC1, Immunotech) and CD8-Cy5; CD45RA-FITC (mAb ALB11, Immunotech), 1 : 10 dilution of CD62L-phycoerythrin (PE) (mAb SCFI28T17G6, Coulter, Hialeah, FL, USA) and either CD8-Cy5 or CD4-Cy5 (mAb 13B8.2, Immunotech); HLA-DR-FITC (mAb 357, Immunotech), CD38-PE (mAb T16, Immunotech) and CD8-Cy5 or IgG-FITC, -PE and -Cy5 conjugated isotype-matched controls (Immunotech). After incubation for 15 min at room temperature (RT), cells were washed and fixed in 2% formaldehyde in PBS. For granzyme A (granA) staining, PBMC were suspended in 50 µl HBSS and permeabilized using the Caltag Laboratories (Burlingame CA, USA) Fix and Perm Kit according to the manufacturer's protocol. Fixed cells were incubated for 15 min at RT with 2 µl murine IgG1 isotype-matched control antibody (Coulter) or with a 1 : 5 dilution of granA CB9 mAb culture supernatant [22]. After washing with 5 ml HBSS, cells were stained with 2 µl PE-conjugated F(ab')$_2$ goat anti-mouse Ig (DAKO, Carpenteria, CA, USA). Cells were then washed twice with 5 ml HBSS buffer and resuspended in 50 µl HBSS before adding 2 µl CD4-Cy5 (Pharmingen, San Diego, CA, USA) and 2 µl CD8-FITC (Immunotech), 4 µl CD20-FITC (Becton–Dickinson, Mountain View, CA, USA) and 2 µl CD3-Cy5 (Immunotech) or IgG-FITC and -Cy5 conjugated controls. The samples were incubated for 15 min at 4°C, washed with 5 ml HBSS and fixed with 2% formaldehyde before analysis. Flow cytometry analysis was performed on a tightly gated lymphocyte population using FACscalibur (Becton–Dickinson). Gates were defined by requiring that fewer than 1% of the isotype control antibody-stained cells were positive.

Cytokine production by flow cytometry

Cryopreserved PBMC (3×10^6) were activated in a 24-well plate with 10 ng/ml anti-CD3 mAb (12F6) and 1 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma, St Lewis, MO, USA). Brefeldin A (Sigma) (10 µM/ml) was added at the beginning of culture to block cytokine secretion [23]. After overnight incubation, the cells were harvested and stained externally for Cy-5-CD4 and FITC-CD8 as above. The samples were washed, resuspended in 150 µl HBSS, split in three and internally stained with 5 µl PE-IFN-γ mAb (25723.11), PE-IL-2 mAb (5334.2), or PE-MsIgG1 isotype control antibody (IC002P) (R&D Systems, Minneapolis, MN, USA) using the Caltag Fix and Perm Kit. Cells resuspended in FACS buffer with formalin were analysed using the FACscalibur flow cytometer.

Statistical analysis

The correlation between quantitative variables was evaluated by the Pearson coefficient of correlation. The logarithmic transformation of viral plasma RNA values was used in the analysis. The statistical significance of correlations was evaluated by two-sided Student's *t*-test; a P value of less than 0.05 was considered significant.

Results

Phenotypic analysis was performed on the CD4 and CD8 T cells in the blood of nine patients who had recovered from PHI. Eight of the nine had had symptoms characteristic of the acute viral syndrome of HIV infection, which together with exposure history, enabled clear-cut identification of the time of infection.

The CD4 cell count and plasma HIV RNA level, which varied over 3 logs, did not correlate with the expected time since infection, which averaged 3.8 ± 4.0 months (Table 1). Each of the subjects had achieved stable plasma viral levels, as determined by three or more consecutive measurements of plasma HIV RNA obtained over 1 month (Fig. 1). The wide variation of HIV RNA and CD4 cell counts in this small sample allowed us to determine whether the state of T cell activation could be related to plasma viraemia. As anticipated, the CD4 cell count and CD4 : CD8 cell ratio correlated with plasma viraemia ($P < 0.03$ and $P < 0.004$, respectively), with lower counts associated with a higher viral setpoint (Table 2).

Correlation of $CD38^+DR^+$ CD8 T cell expansion with high plasma HIV RNA after primary HIV infection

High proportions of $CD38^+DR^+$ CD8 T cells and high levels of plasma HIV RNA have been associated with a poor prognosis [6–8]. These findings were confirmed in this acute infection cohort by a strong positive association of $CD38^+DR^+$ and CD38 CD8 T cells with plasma HIV level ($P < 0.03$ and $P < 0.00002$, respectively) (Table 2). The HLA-DR and CD38 defined subsets did not correlate significantly with CD4 cell counts although there was a trend towards significant correlation of the percentage of CD38 CD8 T cells with lower CD4 cell counts ($r = -0.63$, $P < 0.07$). Because CD38 expression is a continuous variable and the amount of CD38 expression has also been shown to have prognostic significance, the relationship between CD38 mean fluorescence intensity (MFI) and the clinical parameters was examined. The CD38 MFI on CD8 T cells also correlated with log plasma HIV RNA ($r = 0.73$, $P < 0.03$).

Naive CD8 T cells do not express the proteins required for target cell lysis, which begin to be expressed within a few days of activation [24]. Cells were therefore stained for the expression of granA, the predominant protease in CD8 T cell cytolytic granules, which are exocytosed during cytolysis. Although the percentage of CD8 T cells staining for granA did not correlate with plasma viral RNA, it did correlate ($r = -0.69$, $P < 0.04$) with lower CD4 cell counts. Therefore this marker of CTL activation may also be associated with a poorer outcome.

Expansion of CD57 CD8 T cells is associated with lower viral setpoint

To determine whether other markers of CD8 T cell activation also correlate with increasing viraemia, we examined the relationship between plasma RNA and the expression of the co-stimulatory molecule CD28, which is down-modulated after human (but not murine) CD8 T cell receptor (TcR) engagement, and of CD57, a carbohydrate antigen expressed by a subset

Table 2. Correlation of plasma viral load and CD4 cell count after primary HIV infection with phenotypic markers of CD8 T cell activation.

	Normal value	Subject									Correlation: log HIV RNA		Correlation CD4 cell count	
		13	10	24	33	22	14	12	11	23	*r*	*P* value	*r*	*P* value
Log viral load		3.30	3.48	4.31	5.05	5.12	5.78	5.88	5.89	6.51				
CD4 cell count		1139	902	1390	1414	496	182	494	315	257	**–0.71**	**0.03**		
CD4 : CD8 ratio		1.7	1.6	1.8	0.9	1.2	0.7	0.6	0.3	0.9	**–0.85**	**0.004**	**0.69**	**0.04**
Percentage CD8 T cells that are:														
$CD38^-DR^-$	44 ± 7	48	36	32	23	29	21	13	6	7	**–0.94**	**0.0002**	0.64	0.07
$CD38^+DR^-$	48 ± 6	33	27	29	33	21	40	30	14	54	0.27	0.48	–0.14	0.72
$CD38^-DR^+$	3 ± 1	7	17	2	7	5	1	2	2	0	**–0.73**	**0.02**	0.41	0.27
$CD38^+DR^+$	4 ± 4	12	20	37	38	46	37	55	78	39	**0.72**	**0.03**	–0.49	0.18
$CD38^+$	52 ± 7	45	47	66	71	67	77	85	92	93	**0.97**	**0.00002**	–0.63	0.07
DR^+	8 ± 5	19	37	39	45	50	39	57	80	40	0.59	0.09	–0.43	0.24
$CD28^-$	25 ± 9	58	59	59	51	48	64	69	66	40	–0.11	0.78	–0.06	0.87
$CD57^+$	16 ± 6	41	36	28	22	28	27	29	13	5	**–0.83**	**0.01**	0.47	0.20
MFI CD38 on CD8 T cells		50	49	200	180	249	134	411	698	429	**0.73**	**0.03**	–0.54	0.14
Percentage $GranA^+$ T cells	30 ± 12	44	52	33	31	41	61	60	77	45	0.40	0.29	**–0.72**	**0.03**
Percentage $GranA^+$ CD8 cells	39 ± 12	70	76	56	51	71	83	83	89	60	0.21	0.59	**–0.69**	**0.04**
Percentage $GranA^+$ CD4 cells	6 ± 5	13	33	3	5	8	20	6	17	5	–0.37	0.32	–0.19	0.62

Correlations that are statistically significant are shown in bold. GranA, granzyme A; MFI, mean fluorescence intensity.

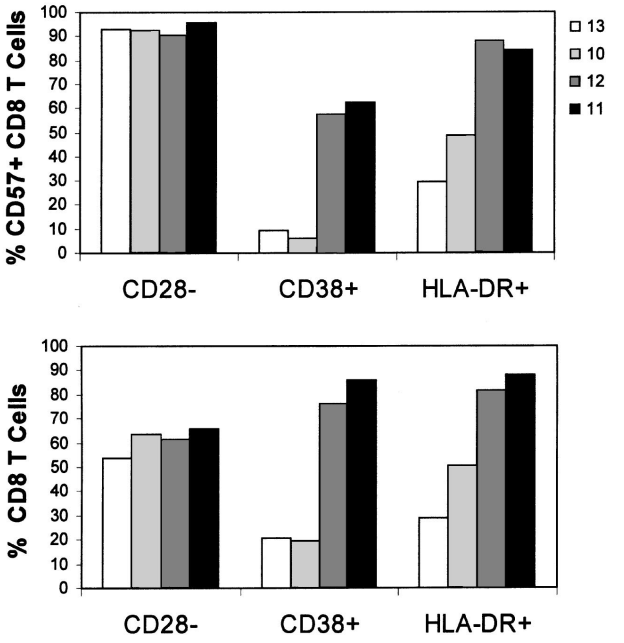

Fig. 2. Most CD57 CD8 T cells do not express CD28. The proportion of CD57 CD8 T cells that express CD38 or human leukocyte antigen DR (HLA-DR) mirrors the overall level of expression of all CD8 T cells. Results from samples from four subjects are shown in order of increasing plasma viral RNA from left to right: 13 (□), 10 (▒), 12 (▓), and 11 (■).

of activated CD8 T cells with natural killer (NK)-like activity. The subset of activated CD8 T cells which express CD57 correlated with plasma HIV RNA ($P < 0.01$) (Table 2, Fig. 2). However, unlike the CD38 populations, higher proportions of this activated subset were inversely correlated with amounts of plasma virus. The CD28 negative CD8 T cell expansion did not correlate with the clinical parameters. However, CD28 down-modulation did correlate with granA expression ($r = 0.68$, $P = 0.04$), suggesting that most CD28 negative CD8 T cells express granA and have the potential to be cytolytic.

Not surprisingly, the proportion of CD38 CD8 T cells and of CD57 CD8 T cells were inversely correlated ($r = -0.87$, $P < 0.003$). However, these two markers are not reciprocally expressed. By co-staining thawed samples from four representative subjects for CD57 with the other activation markers in this study, it was found that the expression of CD38 and DR on CD57 CD8 T cells mirrored the expression of these markers on the total pool of CD8 T cells (Fig. 2). This suggests that the induction of CD57 expression is distinct from that of HLA-DR or CD38. More than 90% of the CD57 T cells had down-modulated CD28, although the CD28 negative population includes both CD57 and CD57 negative cells.

IFN-γ is produced by CD28 negative CD8 T cells heterogeneous for CD38 and CD57 expression

Because of the correlation of CD38 and CD57 expression with plasma viraemia, we next looked at IL-2 and IFN-γ production by CD38 and CD57 CD8 T cells in thawed samples from three PHI patients with varying viral levels. In all subjects, there was little or no cytokine production without T cell stimulation. After T cell stimulation with anti-CD3 and PMA, IL-2 production by CD8 T cells was largely restricted to resting cells that express CD28 and do not express CD57 or CD38. Only 19 ± 2% of CD38 and 16 ± 8% of CD57 CD8 T cells produced IL-2 after stimulation with anti-CD3 and PMA. In contrast, IFN-γ production was largely restricted to CD28 negative CD8 T cells. In the three patients, 47.0 ± 0.8% of the CD8 T cells produced detectable IFN-γ and 93 ± 4% of these were CD28 negative. There was no obvious relationship between CD57 expression and IFN-γ production, although the majority (63 ± 7%) of the CD57 CD8 T cells were stimulated to produce IFN-γ. The percentage of IFN-γ-producing cells that express CD38 from the three donors was comparable to the proportion of CD38 expression on all CD8 T cells. Although there were insufficient cells from the PHI subjects available to test for the ability of different populations of CD8 T cells to suppress HIV production by the secretion of soluble factors, experiments with T cells from eight chronically HIV-infected donors did not suggest that viral suppression is particularly associated with the CD57 subset of CD8 T cells (data not shown).

Expansion of $CD62L^{-}CD45RA^{+}$ CD8 T cells correlates with lower viral setpoint

There was no significant correlation between plasma viral RNA or CD4 cell count with the percentage of naive $CD62L^{+}CD45RA^{+}$ circulating CD4 or CD8 T cells (Table 3). The percentage of naive CD8 T cells was substantially reduced compared with normal donors (23 ± 8 versus 49 ± 10%), consistent with recent reports of an enormous expansion of antiviral CD8 T cells during primary and chronic virus infection [25–29]. However, there was no significant difference in the percentage of naive CD4 T cells in HIV-infected donors compared with healthy controls (48 ± 11 versus 54 ± 9%), perhaps because activated and memory CD4 T cells are more susceptible to productive HIV infection and may be selectively eliminated [30]. There was a significant inverse correlation between the proportion of $CD62L^{-}CD45RA^{+}$ activated CD8 T cells and plasma viraemia ($r = -0.77$, $P < 0.02$) (Fig. 3). This subset has been identified as terminally differentiated CD8 effector CTL that contain perforin and produce IFN-γ [31].

Table 3. The proportion of naive CD62L+CD45RA+ CD4 and CD8 T cells does not correlate with clinical parameters in primary HIV infection.

	Normal value	Subject								Correlation: log HIV RNA		Correlation CD4 cell count	
	(n = 8)	13	10	24	33	22	14	12	11	23	*r*	*P* value	*r*	*P* value
Log viral load		3.30	3.48	4.31	5.05	5.12	5.78	5.88	5.89	6.51				
CD4 cell count		1139	902	1390	1414	496	182	494	315	257				
Percentage CD8 cells														
$CD62L^-CD45RA^-$	25 ± 10	42	20	26	33	37	41	53	34	15	0.07	0.86	–0.11	0.79
$CD62L^+CD45RA^-$	19 ± 7	10	17	35	28	20	25	20	51	41	0.63	0.07	–0.39	0.31
$CD62L^-CD45RA^+$	7 ± 4	24	36	9	18	16	15	16	7	7	**–0.77**	**0.02**	0.35	0.36
$CD62L^+CD45RA^+$	49 ± 10	24	27	30	21	27	20	11	7	38	–0.21	0.60	0.21	0.59
Percentage CD4 cells														
$CD62L^-CD45RA^-$	7 ± 3	18	13	5	10	15	23	11	17	18	0.31	0.41	**–0.71**	**0.03**
$CD62L^+CD45RA^-$	38 ± 7	26	33	19	42	40	30	43	44	29	0.41	0.28	–0.32	0.40
$CD62L^-CD45RA^+$	0.5 ± 0.4	2	12	2	2	1	3	1	6	1	–0.40	0.28	0.02	0.96
$CD62L^+CD45RA^+$	54 ± 9	54	43	75	46	44	44	45	33	52	–0.33	0.38	0.57	0.11

Correlations that are statistically significant are shown in bold.

Discussion

Although a relationship between the expansion of activated CD8 T cells and plasma viraemia might have been predicted given the intimate relationship between viral production and immune regulation, the close correlation in a small cohort and the fact that the expansion of some subsets of activated CD8 T cells correlates with low plasma viraemia, whereas others with high plasma viral loads, were surprising. This study is exploratory and needs to be confirmed. The correlation of $CD38^+DR^+$ CD8 T cell expansion with high viral loads after PHI supports the association of this phenotype with a poor prognosis [6–8]. The correlation of activated CD57 and $CD62L^-CD45RA^+$ CD8 T cells expansion with low viral loads suggests that these CD8 T cells may help contain HIV infection.

The protective versus the pathological role of CD8 T cells in HIV infection has been a subject of controversy. Both in-vitro and clinical studies support a protective role of antiviral CD8 T lymphocytes. CD8 T cells suppress HIV replication *in vitro* by direct cytotoxicity and the secretion of soluble factors [32–36]. PHI viraemia resolves with the appearance of HIV-specific CTL [2,3]. An increased frequency of HIV-specific CD8 T cells, detected by their binding to fluorescent HIV peptide-HLA class I complexes, correlates with lower plasma viraemia in untreated patients [9]. Moreover, antiviral CTL decline when opportunistic infections develop, and the likelihood of AIDS progression increases in patients who lack *gag*-specific CTL [37,38]. This evidence, together with the accelerated disease course in neonates with immature T cell immunity, suggests that CTL are important in controlling HIV infection [39].

However, HIV-specific CTL could hasten the decline in CD4 cells via direct lysis of infected or even uninfected cells [34,40,41]. The pathogenic role of antiviral CTL in some lymphocytic choriomeningitis virus and hepatitis B infections has led to the suggestion that HIV-specific CTL contribute to HIV immunodeficiency [42]. This concern was reinforced by the association of poor prognosis with increases in $CD38^+DR^+$ CD8 T cells [6–8].

The findings that some subsets of activated CD8 T cells correlate with better viral control after PHI, whereas others correlate with worse viral control, suggest that some activated CD8 T cells may be protective, whereas others contribute to pathogenesis. CD38 is an ectoenzyme that catalyses the synthesis and transfer of adenosine 5'-diphosphate-ribose [43] and binds to CD31 on vascular endothelial cells [44]. The ligation of CD38 promotes heterotypic adhesion and transduces signals that increase intracellular calcium and influence cellular proliferation and resistance to apoptosis. The pattern of

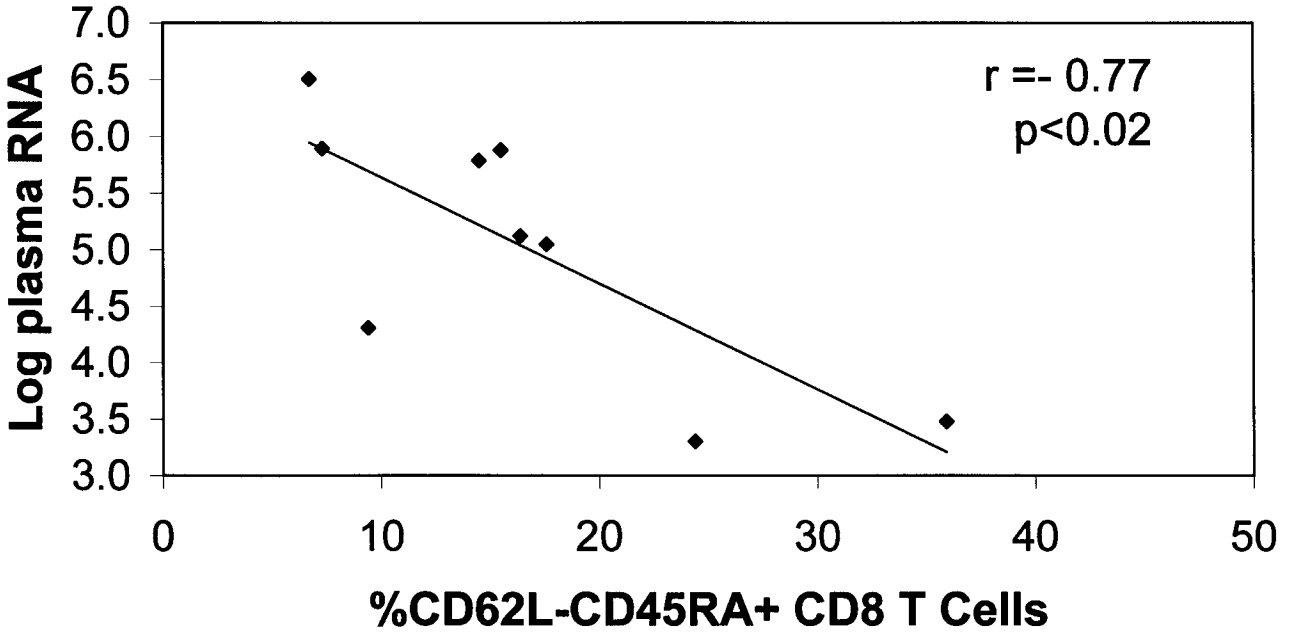

Fig. 3. The expansion of memory CD8 T cells that are CD62L negative and CD45RA positive is associated with lower plasma viraemia after primary HIV infection.

expression of CD38 in T cells is complex. CD38 is expressed on immature thymocytes, but is down-modulated during development. It is re-expressed after antigenic stimulation, but it is not known whether other stimuli, such as cytokines, can upregulate its expression, or for how long, although memory T cells are thought to be CD38 negative. However, CD38 expression has not been identified with specific T cell function. Expansion of this population might reflect the inflammatory perturbations caused by increased viral replication. In fact CD38 expression on CD8 T cells declines with highly effective antiretroviral therapy [45]. If CD38 expression is upregulated in both HIV-specific and bystander CD8 T cells by inflammatory cytokines or activated macrophages, present when viral production is poorly controlled, the mixed bag of CD38-expressing cells may include both salutary and harmful T cells. Consistent with this is the finding that CD38 expression does not correlate with IFN-γ production.

Because the increase in CD57 and $CD62L^{-}CD45RA^{+}$ CD8 T cells is associated with reduced plasma viraemia, the expansion of these subsets cannot reflect increases in viral antigen or inflammation. These subsets may have a direct antiviral role via the lysis of antiviral cells or the secretion of viral suppressor factors. Both of these phenotypically identified CD8 T cell subsets, which probably overlap significantly, have been identified as marking the subset of effector CTL because perforin-staining cells are concentrated in these subsets [31,46]. In a recent cloning experiment, it was found that five out of five HIV-specific cytotoxic T cell clones obtained from a chronically infected seropositive donor were CD28 negative and CD57 positive (Shankar, unpublished data). This suggests that viral-specific CD8 T cells may be predominantly CD57 positive in HIV-infected donors. In fact, the depletion of CD57 CD8 T cells substantially reduces HIV-specific cytotoxicity [10]. Because the subsets of cells that express CD38 and CD57 are overlapping, future studies of antiviral cytotoxicity by CD57 negative or CD38-depleted CD8 T cells need to be done. There were insufficient cells to perform these studies on this group of patients. The $CD62L^{-}CD45RA^{+}$ subset of CD8 T cells has been identified with endstage differentiated CTL that have limited proliferative capacity after TcR stimulation, and are prone to apoptose in short-term culture, and produce IFN-γ, but not IL-2 [31,47]. If protection requires antigen-specific TcR engagement, T cells bearing TcR that recognize specific HIV epitopes should be disproportionally concentrated in the $CD57^{+}CD28^{-}$ and $CD62L^{-}CD45RA^{+}$ subsets. This hypothesis can now be tested using tetramer technology [26]. Similarly, enrichment of these cells should enhance antiviral cytotoxicity.

CD57 CD8 T cells are unlikely to play a special role in the secretion of antiviral suppressor factors, other than IFN-γ. Enrichment or depletion of CD57 CD8 T cells from chronically HIV-infected donors does not have a consistent effect on soluble viral suppressor activity in transwell experiments (Shankar, unpublished data). The depletion of CD57 CD8 T cells enhances viral suppression when activated CD8 T cells are directly co-cultured with PHA-stimulated HIV-infected allogeneic CD4 T cells [48].

An alternative explanation for the correlation of CD57 expansion with viraemia derives from the identification of CD57 CD8 T cells as suppressor cells in the immunological sense [49,50]. CD57 CD8 T cells suppress the proliferation of CD4 T cells and the generation of antigen-specific CD8 CTL [51,52]. CD57 T cells might indirectly suppress viral production by suppressing CD4 T cell activation. Another possible explanation might arise from the NK activity of this subset [10]. Because an NK response may provide antiviral protection before cognate immunity develops, high levels of cells with NK activity may reduce the viraemia peak during the first weeks after infection.

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