Increased Plasma Interleukin-7 Level Correlates with Decreased CD127 and Increased CD132 Extracellular Expression on T Cell Subsets in Patients with HIV-1 Infection

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Background. Interleukin (IL)–7 levels are increased in patients with human immunodeficiency virus type 1 (HIV-1)–associated lymphopenia; however, the effects of this on IL-7 receptor (IL-7R) expression, disease progression, and immune reconstitution remain unclear.

Methods. Plasma IL-7 levels were measured, by enzyme-linked immunoassay, in patients with primary, chronic, or long-term nonprogressive HIV-1 infection (PHI, CHI, and LTNP, respectively) before and after 40–48 weeks of antiretroviral therapy (ART). Cell-surface expression and intracellular expression of the IL-7R components CD127 and CD132 were measured by flow cytometry. The effects of IL-7 and cycloheximide on IL-7R expression by peripheral blood mononuclear cells were examined in vitro.

Results. Plasma IL-7 levels were increased in both patients with PHI and those with CHI; administration of ART resulted in normalized plasma IL-7 levels in patients with PHI but not in those with CHI. Plasma IL-7 levels positively correlated with $CD4^+$ T cell immune reconstitution in patients with PHI. In vitro, exogenous IL-7 rapidly down-regulated cell-surface CD127 expression, but not CD132 expression, whereas subsequent reexpression required active protein synthesis. HIV-1 infection resulted in progressive decreases in the $CD127^+132^-$ subset and increases in the $CD127^-132^+$ subset of $CD4^+$ and $CD8^+$ T cells. Changes in $CD4^+$ T cell expression of IL-7R components were evident in patients with LTNP who lost viral control, and these changes preceded increases in plasma IL-7 levels.

Conclusions. Perturbations in the IL-7/IL-7R system were clearly associated with disease progression but did not reliably predict immune reconstitution.

Interleukin (IL)–7 is essential for T cell differentiation [1, 2]; it plays a central role in naive T cell survival [3, 4] and memory T cell genesis [5]. The IL-7 receptor (IL-7R), which consists of a specific α-chain (CD127) [6] that dimerizes to the common cytokine γ-chain (CD132) [7], triggers regulatory pathways, leading to up-regulation of the T cell survival factors Bcl-2 (reviewed in [8]) and lung-kruppel–like factor [9] and to proliferation of both naive and memory T cells [10–12]. Both CD127 and CD132 are expressed on naive and memory T cells. Disruption of the IL-7/IL-7R system leads to dramatic reductions in peripheral T cell survival [3, 4] in vitro and to severe combined immunodeficiency in vivo [13–16].

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Table 1. Baseline characteristics of patient groups.

Characteristic	Patients with PHI	Patients with CHI	P^a	Patients with LTNP-c	Patients with LTNP-loc	P^b
Age	33 (29–36)	42 (36–49)	.001	33 (31–40)	26 (23–29)	.047
Lymphocyte count, total lymphocytes/μL	2000 (1400–2350)	1200 (800–1300)	.001	...	...	...
$CD4^+$ T cell count, cells/μL	465 (396–569)	136 (41–298)	<.001	819 (621–888)	624 (553–792)	.347
$CD8^+$ T cell count, cells/μL	1107 (738–1585)	786 (536–900)	.045	1323 (844–1527)	1071 (838–1540)	.917
Viral load, 10^3 RNA copies/mL	659 (157–3005)	411 (156–798)	.204	2 (0.2–16)	57 (4–87)	.117

NOTE. Data are median (interquartile range), unless otherwise noted. CHI, chronic HIV-1 infection; LTNP-c, long-term nonprogressive HIV-1 infection with viral control and $CD4^+$ T cell counts ⩾400 cells/μL; LTNP-loc, long-term nonprogressive HIV-1 infection with $CD4^+$ T cell counts <400 cells/μL and loss of viral control; PHI, primary HIV-1 infection; ..., information unavailable.

[a] For the comparison between patients with PHI and those with CHI.

[b] For the comparison between patients with LTNP-c and those with LTNP-loc.

Circulating IL-7 levels are increased in lymphopenic conditions, including chronic HIV-1 infection (CHI), and inversely correlate with total and naive $CD4^+$ T cell counts [17–19], suggesting that IL-7 production is up-regulated to counter $CD4^+$ T cell loss. The use of animal models has shown that IL-7 drives immune reconstitution by increasing both thymic output [20, 21] and extrathymic proliferation [22].

The benefits of increased IL-7 levels remain unclear. Low IL-7 levels, despite severe lymphopenia, are associated with poor immune reconstitution [23], whereas high levels of expression of IL-7 and CD127 have been associated with successful immune reconstitution in patients with CHI after the initiation of antiretroviral therapy (ART) [24]. Although a positive correlation between baseline IL-7 level and $CD4^+$ T cell count after 20 months of ART has been reported [25], others have found that IL-7 levels do not normalize after the initiation of ART, suggesting that immune reconstitution may be limited by other factors [26]. Furthermore, recent trials in which supraphysiological doses of IL-7 were administered to simian immunodeficiency virus–infected macaques found expanded peripheral $CD4^+$ T cell populations [27, 28]. On balance, the available data suggest that IL-7 plays an important role in driving ART-mediated immune reconstitution.

Decreased sensitivity of $CD8^+$ cytotoxic T lymphocytes (CTLs) to IL-7 during CHI is associated with decreased cell-surface CD127 expression [29]. This loss of CD127 expression may result from transient down-regulation after antigen-induced T cell activation [30], which is consistent with the fact that CHI is a state of T cell hyperactivation [31]. CD127 expression on CTLs is partially restored by the administration of ART [32].

The mechanisms that regulate the expression of IL-7R components remain unclear. CD127 is down-regulated on T cells after exposure to IL-2 [33] in vitro but not in vivo [34]. IL-7 administration down-regulates CD127 in animal models [27, 35]. Conversely, CD127 is up-regulated by administration of glucocorticoids [36].

We investigated the effect of increased IL-7 levels on human T cell–surface expression of CD127 and CD132 in vitro and ex vivo. We investigated whether perturbations in the IL-7/IL-7R system during HIV-1 infection were solely a chronic disease phenomenon by studying patients with primary HIV-1 infection (PHI), CHI, and long-term nonprogressive HIV-1 infection (LTNP), both before and after the loss of viral control. We hypothesized that early dysregulation of the IL-7/IL-7R system may be related to disease progression and may limit immune reconstitution.

PATIENTS, MATERIALS, AND METHODS

Patients. Therapy-naive patients with PHI ($n = 25$) or CHI ($n = 25$) were enrolled in clinical trials of combination ART, and patients with LTNP ($n = 10$) and healthy controls ($n = 18$) were studied using cryopreserved plasma and peripheral blood mononuclear cells (PBMCs). Patients with PHI had confirmed, recent HIV-1 infection by documented seroconversion illness and incomplete Western blot or a negative HIV-1 test within the preceding 6 months [37]. Patients with CHI had been infected with HIV-1 for >6 months, were treatment naive, and had detectable viral loads on recruitment. There were no significant differences in virological or immunological responses between patients receiving different treatment regimens [38]. Patients were studied at baseline and at 40 (PHI) or 48 (CHI) weeks after the initiation of ART. Patients with PHI had a significantly lower median age and higher total lymphocyte count, $CD4^+$ T cell count, and $CD8^+$ T cell count, compared with patients with CHI (table 1). The study protocol was reviewed and approved by the institutional review board at St. Vincent's Hospital, Sydney, Australia. Informed consent was obtained from each volunteer, in accordance with guidelines of both the local institution and the US Department of Health and Human Services.

Patients with LTNP were enrolled in the Sydney LTNP cohort [39]; the inclusion criteria were HIV-1 infection for >9 years with sustained $CD4^+$ T cell counts ⩾400 cells/μL in the absence of ART. Five patients with LTNP during regular follow-up had

signs of disease progression, with $CD4^+$ T cell counts <400 cells/μL and loss of viral control (median time to progression, 11 years; LTNP-loc). These patients were matched with 5 patients with LTNP who maintained viral control and $CD4^+$ T cell counts ≥400 cells/μL during the follow-up period (LTNP-c). The time points examined were cohort entry and 1–2 years after the loss of viral control (time point 2). At time point 2, all patients with LTNP-loc had received ART. At cohort entry, patients with LTNP-c had a significantly lower median age than did patients with LTNP-loc but were matched for $CD4^+$ T cell count and viral load (table 1).

Isolation of cell populations and cell culture conditions for in vitro studies. PBMCs from healthy volunteers were separated by density centrifugation from whole blood, as described elsewhere [40]. A total of 1×10^6 PBMCs were cultured for up to 7 days at 37°C in a humidified atmosphere with 5% CO_2, in the presence of 0–10 ng/mL IL-7 (R&D Systems), in 24-well plates (Falcon; Becton Dickinson), in 1 mL of Iscove's modified Dulbecco's medium/10% pooled human serum (gift from Wayne Dyer, Australian Red Cross Blood Services). In some experiments, exogenous IL-7 was removed after 24 h, and de novo protein synthesis was inhibited using cycloheximide (50 μmol/L; Sigma).

Measurement of plasma IL-7 levels. IL-7 levels were measured, by ELISA, in cryopreserved plasma, using a commercial kit (R&D Systems) in accordance with the manufacturer's instructions; samples were run in duplicate.

Flow cytometry. $CD4^+$ T cell subsets were identified by 5- and 6-color flow cytometry using PBMCs, as described elsewhere [41], using the following monoclonal antibodies (MAbs): CD3–peridinin chlorophyll protein complex–Cy5.5, CD4–phycoerythrin (PE)–Cy7 (Becton Dickinson), CD127-PE (Beckman Coulter), CD45RO–fluorescein isothiocyanate, CD45RA–allophycocyanin (APC), and CD132-biotin (Pharmingen) with streptavidin (SA)–Cy5 (Jackson ImmunoResearch). $CD8^+$ T cells were defined as $CD3^+CD4^-$ lymphocytes. Intracellular staining was performed after cell-surface staining and incubation with FACS permeabilizing solution (Becton Dickinson), as described elsewhere [41], using the following MAbs: Ki-67–PE, Bcl-2–PE (both from Pharmingen), CD132-biotin, and SA-Cy5 or CD127-PE. After fixation, in vitro cell culture experiments were analyzed on a Coulter EPICS XL. Samples from patients were analyzed on a dual-laser LSRII (Becton Dickinson) running FACSDiva software (version 5), as described elsewhere [41].

Statistics. Differences between groups were determined using the unpaired nonparametric Mann-Whitney rank test; in longitudinal analyses, Wilcox signed rank tests were used. Correlations were determined by use of Spearman's correlation tests. All analyses were performed using StatView data analysis and presentation software (version 5.0; Abacus Concepts). $P < .05$ was considered to be statistically significant. P values were not corrected for multiple comparisons.

RESULTS

Plasma IL-7 levels in patients with PHI and those with CHI. We confirmed that patients with CHI have significantly increased plasma IL-7 levels before therapy (median, 3.07 pg/mL), compared with healthy volunteers (median, 1.25 pg/mL) ($P < .001$). In patients with CHI, plasma IL-7 levels remained significantly increased after 48 weeks of ART (median, 2.05 pg/mL; $P < .01$). Despite significantly higher $CD4^+$ T cell counts, patients with PHI also had increased plasma IL-7 levels before therapy (median, 2.35 pg/mL; $P < .01$), compared with healthy volunteers. After 40 weeks of ART, IL-7 levels in patients with PHI had normalized (median, 1.46 pg/mL; $P = .67$).

Correlation of plasma IL-7 levels with lymphocyte populations. We sought to examine whether published inverse correlations between plasma IL-7 levels and lymphocyte populations occurred in patients with PHI. Across the patient cohort, pretherapy plasma IL-7 level was significantly inversely correlated to total lymphocyte count, $CD4^+$ T cell count, and $CD8^+$ T cell count but not to viral load or age. When the patient cohort was split on the basis of PHI or CHI, these correlations occurred only in the CHI cohort (table 2).

Relationship between pretherapy plasma IL-7 levels and $CD4^+$ T cell immune reconstitution. The relationship between plasma IL-7 levels and immune reconstitution remains unclear. Pretherapy plasma IL-7 levels in patients with PHI positively correlated with the absolute number of $CD4^+$ T cells regained after 10 months of ART ($P < .05$; $\rho = 0.469$) (table 2). No statistically significant correlations between baseline

Table 2. Relationships between patient clinical data and week 0 plasma interleukin (IL)–7 levels.

Clinical data	All patients	Patients with PHI	Patients with CHI
Total lymphocyte count	−0.315[a]	−0.21	−0.648[b]
$CD4^+$ T cell count	−0.379[a]	−0.12	−0.621[b]
Naive $CD4^+$ T cell count	−0.265	0.046	−0.731[b]
$CD8^+$ T cell count	−0.32[a]	0.175	−0.538[a]
HIV-1 load	0.243	0.39	0.129
Age	0.198	0.071	0.232
Δ$CD4^+$ T cell count	0.144	0.469[a]	−0.201
ΔNaive $CD4^+$ T cell count	0.263	0.364	0.291
Week 40–48 $CD4^+$ T cell count	−0.109	0.377	−0.408
Week 40–48 naive $CD4^+$ T cell count	−0.03	0.337	−0.483

NOTE. ρ values for correlations between baseline clinical data and baseline plasma IL-7 levels are shown for all patients (with the exclusion of those with long-term nonprogressive HIV-1 infection), patients with primary HIV-1 infection (PHI), and patients with chronic HIV-1 infection (CHI). Δ, change over the study period.

[a] $P < .05$.

[b] $P < .01$.

plasma IL-7 level and either absolute $CD4^+$ T cell count or change in $CD4^+$ T cell count after therapy in patients with CHI were noted (table 2).

Differential regulation of cell-surface expression of CD127 and CD132. Preliminary investigations using whole blood revealed decreased cell-surface CD127 expression on $CD45RO^+CD4^+$ and $CD8^+$ T cells from patients with PHI and patients with CHI (data not shown). The finding of early increases in plasma IL-7 levels in combination with down-regulation of CD127 in patients with HIV-1 infection prompted us to examine more closely the relationship between IL-7 and CD127 expression in vitro. Culturing of PBMCs with IL-7 (10 ng/mL) resulted in the down-regulation of CD127, but not CD132, within 4–5 h. Before the addition of IL-7, >70% of $CD4^+$ T cells expressed CD127. After 5 and 24 h of culture with IL-7, 15% and 5%, respectively, of $CD4^+$ T cells expressed CD127. Unless IL-7 was removed, CD127 was not reexpressed after $CD4^+$ T cells had been cultured for 7 days (figure 1*A*). The extent and rapidity of CD127 down-regulation appeared to be dose dependent at 4 h, but, over the weeklong study period, it resembled a threshold effect after the addition of 10 ng/mL IL-7 (figure 1*B*). In contrast, cell-surface CD132 expression increased from 6% to 19% of $CD4^+$ T cells over 7 days in cultures with IL-7 (figure 1*A*).

Staining of permeabilized PBMCs did not reveal an intracellular localization of CD127 expression either 4 h or 7 days after the addition of IL-7 (figure 1*A*). Permeabilized PBMCs from control cultures exhibited high levels of intracellular CD132, which were not altered on incubation with IL-7 (figure 1*A*). This finding was confirmed using a directly conjugated antibody to CD132. Isotype controls and incubation with SA-APC alone were used to exclude nonspecific binding (data not shown).

De novo protein synthesis of CD127. The removal of IL-7 from PBMC cultures showed that down-regulation of CD127 was rapidly reversible. Inhibition of de novo protein synthesis

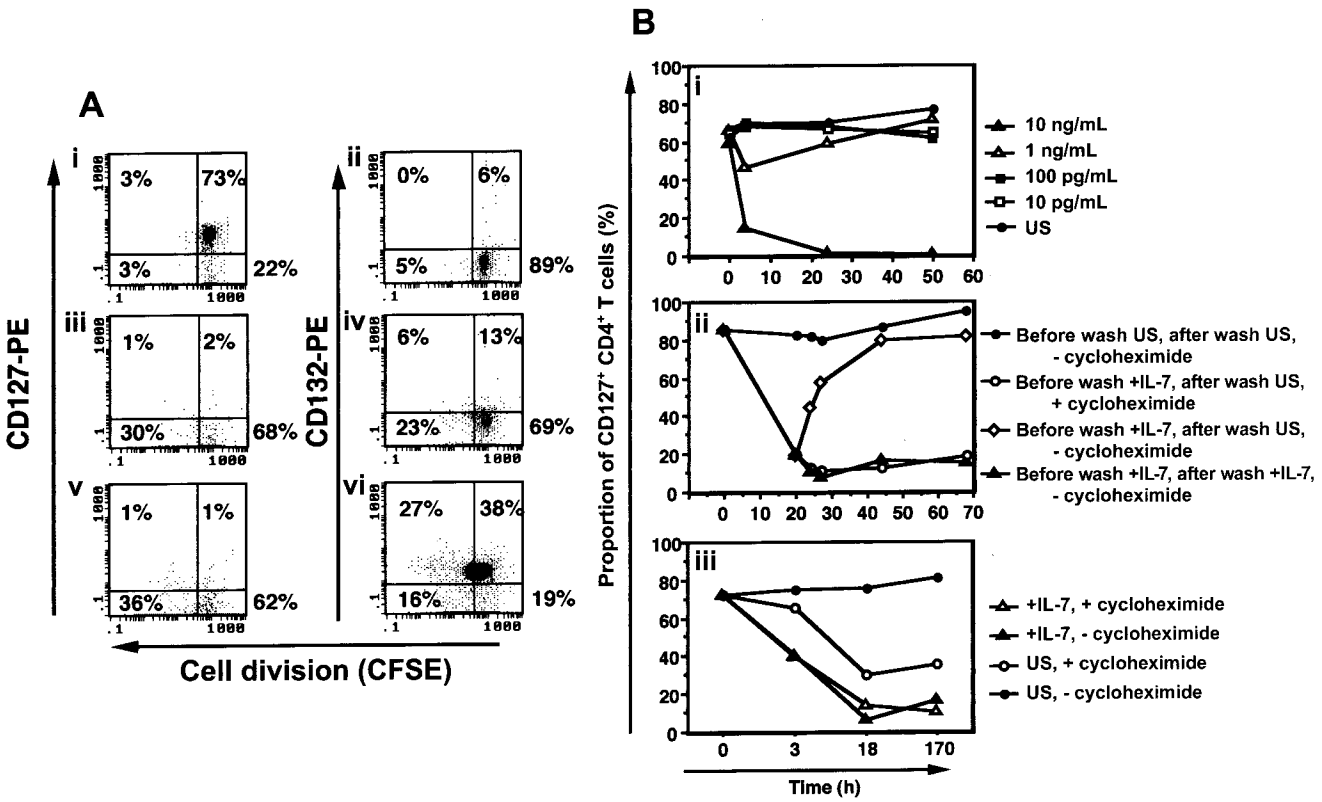

Figure 1. Regulation of interleukin (IL)–7 receptor components by IL-7 in vitro. Peripheral blood mononuclear cells from healthy volunteers were cultured in the presence of IL-7 (0–10 ng/mL) for 0–7 days, and IL-7 receptor components were detected by flow cytometry. Representative data from 2 experiments are shown. *A*, $CD4^+$ T cell expression of CD127, which is extracellular, and of CD132, which is largely intracellular. Unstimulated (US) $CD4^+$ T cells at day 0 have high levels of cell-surface expression of CD127 *(i)* and low levels of expression of CD132 *(ii)*. After day 7, with the addition of 10 ng/mL IL-7, cells had greatly reduced cell-surface CD127 expression *(iii)* and increased cell-surface CD132 expression *(iv)*. Permeabilization of cells on day 7 after incubation with IL-7 resulted in no increase in CD127 expression *(v)* and a large increase in CD132 expression *(vi)*. *B*, Demonstration that $CD4^+$ T cell–surface expression of CD127 is down-regulated by IL-7 after a threshold of 10 ng/mL is reached *(i)*. This down-regulation is rapidly reversible and requires de novo protein synthesis *(ii)*. There is evidence of CD127 turnover on the cell surface in the absence of IL-7 *(iii)*. CFSE, carboxyfluorescein diacetate succinimidyl ester; PE, phycoerythrin.

both inhibited reexpression of CD127 after IL-7 removal (figure 1*B*) and reduced the proportion of $CD127^+$ T cells (figure 1*B*).

Cell-surface expression of IL-7R components in patients with HIV-1 infection. The differential regulation of CD127 and CD132 by IL-7 in vitro prompted an examination of the association between increased plasma IL-7 levels in vivo and expression of both receptor chains on PBMCs ex vivo. Healthy volunteers had 4 distinct populations of both $CD4^+$ and $CD8^+$ T cells, on the basis of CD127 and CD132 expression (figures 2*A* and 2*B* and 3). The majority of $CD4^+$ T cells were $CD127^+132^+$ T cells (median, 52%); the second largest population was $CD127^+132^-$ T cells (median, 42%). There were minor populations of $CD127^-132^+$ and $CD127^-132^-$ $CD4^+$ T cells (3% in both cases). Similar proportions were seen in the $CD8^+$ subset: $CD8^+127^+132^+$ (38%) and $CD8^+127^+132^-$ (43%) and minor $CD8^+127^-132^+$ and $CD8^+127^-132^-$ populations (3% in both cases).

PHI was associated with a significant decrease in the percentage of $CD4^+127^+132^-$ T cells and an increase in the percentage of $CD4^+127^-132^+$ T cells. These changes were even more pronounced in patients with CHI (figure 3*A*) and were evident in both $CD45RO^-$ and $CD45RO^+$ subsets. The populations did not normalize after 40–48 weeks of ART in either group (table 3). There were no significant changes in either the $CD4^+127^+132^+$ or the $CD4^+127^-132^-$ population.

In the $CD8^+$ T cell subset, PHI was associated with decreases in the proportion of both $CD127^+132^+$ and $CD127^+132^-$ populations and an increase in the proportion of $CD127^-132^+$ T cells. Again, these changes were more pronounced in patients with CHI (figure 3*B*) and did not normalize after the administration of ART.

Permeabilization of PBMCs revealed intracellular localization of CD132, but not CD127. The mean fluorescence intensities (MFIs) of cell-surface CD127 in healthy volunteers and patients were 825 and 811, respectively. The MFIs of cell-surface CD132 in healthy volunteers and patients were 425 and 631, respectively. Permeabilization of cells resulted in no significant change in CD127 MFI but did result in a massive increase in CD132 MFI—to 4277 in healthy volunteers ($P < .05$; figure 2*C* and 2*D*) and to 6024 in patients with HIV-1 infection (data not shown).

Correlation between cell-surface expression of IL-7R components and plasma IL-7 levels. The association between plasma IL-7 levels and changes in T cell subsets were examined before therapy in healthy volunteers, patients with PHI, and patients with CHI. Plasma IL-7 levels positively correlated with the size of the $CD4^+127^-132^+$ T cell populations ($\rho = 0.422$; $P < .05$), specifically the $CD45RO^+$ subset ($\rho = 0.442$; $P < .01$). Plasma IL-7 levels inversely correlated with $CD4^+127^+132^-$ T cell counts ($\rho = -0.49$; $P < .01$) in both the $CD45RO^-$ ($\rho = -0.515$; $P < .01$) and the $CD45RO^+$ ($\rho = -0.44$; $P < .01$) subsets of these cells. There were no significant correlations with $CD4^+127^+132^+$ or $CD4^+127^-132^-$ T cell counts.

In $CD8^+$ T cells, plasma IL-7 levels inversely correlated with $CD8^+127^+132^-$ ($\rho = -0.5$; $P < .01$) and $CD8^+127^+132^+$ ($\rho = -0.395$; $P < .05$) T cell counts. Plasma IL-7 levels positively correlated with $CD8^+127^-132^+$ T cell counts ($\rho = 0.461$; $P < .01$).

Correlation between cell-surface expression of IL-7R components and HIV-1 disease progression. To further examine the relationship between these novel $CD4^+$ T cell subsets and disease progression, we examined the correlation between IL-7R expression and $CD4^+$ T cell counts before therapy. In the combined PHI and CHI cohorts, $CD4^+127^+132^-$ T cell percentage positively correlated with $CD4^+$ T cell count ($\rho = 0.428$; $P < .05$). There was a stronger inverse correlation between $CD4^+127^-132^+$ T cell percentage and $CD4^+$ T cell count ($\rho = -0.639$; $P < .0001$).

Survival and turnover of $CD127^+132^-$ T cells. The large proportion of both $CD4^+$ and $CD8^+$ $CD127^-$ T cells prompted us to define the survival and turnover characteristics of these cells. We examined the expression of antiapoptotic protein Bcl-2, a necessary component for T cell survival and a known target of IL-7, and of the cell-cycle protein Ki-67. HIV-1 infection was associated with an increase in $CD127^-$Bcl-2^- and $CD127^-$Ki-67^+ $CD4^+$ and $CD8^+$ T cells, which is consistent with the results of previous work [42] (data not shown).

Plasma IL-7 levels in patients with LTNP. Patients with LTNP with preserved $CD4^+$ T cell counts were studied in an attempt to distinguish the effect of HIV-1 infection directly, as opposed to its effect on $CD4^+$ T cell lymphopenia. To examine how IL-7/IL-7R expression related to HIV-1 progression, we selected a group of patients with LTNP, half of whom went on to lose viral control at follow-up. At cohort entry, patients with LTNP had plasma IL-7 levels that were not significantly different from those of healthy volunteers (median, 1.01 pg/mL). Plasma IL-7 levels in patients with LTNP-c did not change over the observation period. In contrast, patients with LTNP-loc displayed a significant increase in plasma IL-7 levels from cohort entry to time point 2, after the loss of viral control (median, 1.01 and 1.86 pg/mL, respectively; $P < .05$).

Perturbations in T cell expression of IL-7R in patients with LTNP before and after loss of viral control. Despite normal $CD4^+$ T cell counts, patients with LTNP-c displayed an increased proportion of $CD4^+127^-132^+$ T cells at cohort entry, compared with healthy volunteers (median, 8%; $P < .05$), suggesting that this perturbation occurs first among $CD4^+$ T cells after HIV-1 infection. Interestingly, at cohort entry, patients with LTNP-loc had increased $CD127^-132^+$ (median, 19%; $P < .01$) and decreased $CD4^+127^+132^-$ (median, 13%; $P < .01$) T cell subsets. These changes occurred in both the $CD4^+RO^-$ subset and the $CD4^+RO^+$ subset (table 3).

Both groups of patients with LTNP displayed changed ex-

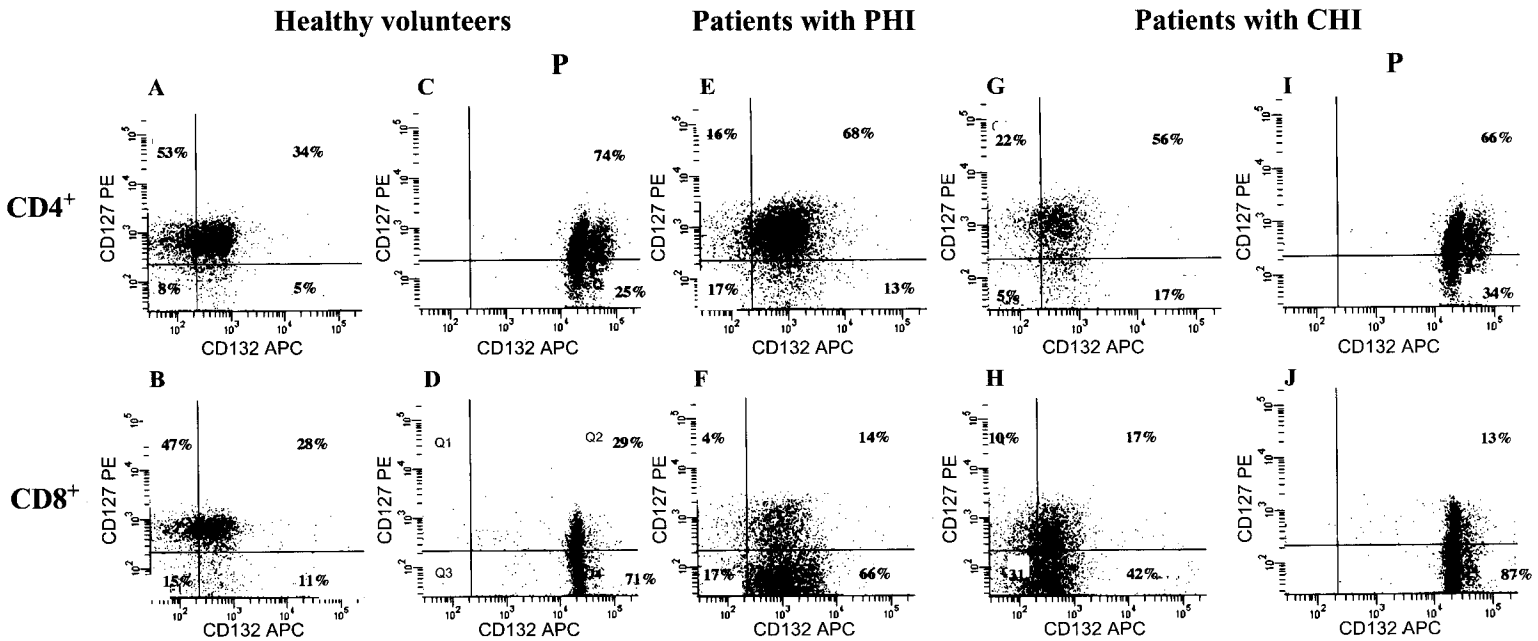

Figure 2. Cell-surface and intracellular expression of interleukin (IL)–7 receptor components in healthy and HIV-1–infected volunteers at baseline. Flow-cytometric histograms show CD127 and CD132 expression on $CD4^+$ *(A)* and $CD8^+$ *(B)* T cells from healthy volunteers. Addition of permeabilizing (P) cells resulted in increased CD132 mean fluorescence intensity in both $CD4^+$ *(C)* and $CD8^+$ *(D)* T cells from healthy volunteers. Similar increases were seen in permeabilized $CD4^+$ *(I)* and $CD8^+$ *(J)* T cells from patients with chronic HIV-1 infection (CHI). Primary HIV-1 infection (PHI) was associated with a decrease in $CD127^+132^-$ (Q1) and an increase in $CD127^-132^+$ (Q4) $CD4^+$ *(E)* and $CD8^+$ *(F)* T cells. These changes became more marked in $CD4^+$ *(G)* and $CD8^+$ *(H)* T cells from patients with CHI. Note that a large proportion of cells fall on or very near the *Y*-axis in the histograms in panels A and B. Median percentages of each population and *P* values are shown in figure 3. APC, allophycocyanin; PE, phycoerythrin.

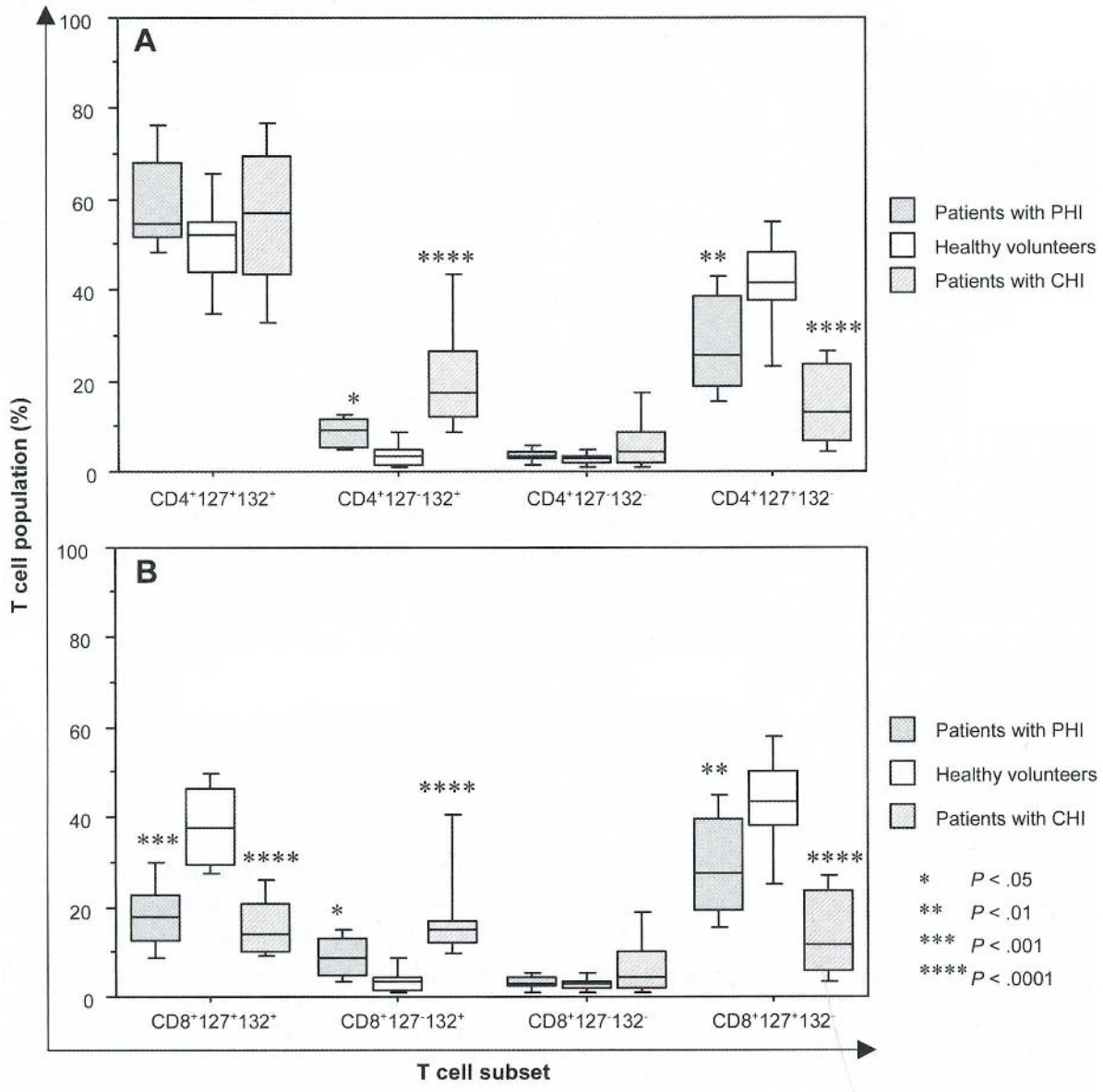

Figure 3. Extracellular expression of interleukin (IL)–7 receptor on $CD4^+$ and $CD8^+$ T cells in HIV-1–infected patients at baseline. *A,* Progressive decreases in the proportions of $CD4^+127^+132^-$ T cells in patients with primary HIV-1 infection (PHI) and patients with chronic HIV-1 infection (CHI), along with increases in the proportions of $CD4^+127^-132^+$ T cells. *B,* Progressive decreases in the proportions of $CD8^+127^+132^+$ and $CD8^+127^+132^-$ T cells in patients with PHI and patients with CHI, along with increases in the $CD8^+127^-132^+$ subset.

pression of IL-7R on $CD8^+$ T cells, compared with healthy volunteers, at cohort entry. Patients with LTNP, as a whole, displayed decreased $CD8^+127^+132^+$ (median, 16%; $P < .001$) and $CD8^+127^+132^-$ (median, 23%; $P < .05$) T cell counts and increased $CD8^+127^-132^+$ (median, 10%; $P < .001$) and $CD8^+127^-132^-$ (median, 5%; $P < .01$) T cell counts. These alterations were all significantly different at cohort entry, compared with those of healthy volunteers, indicating that perturbations in the $CD8^+$ T cell subset were independent of those in the $CD4^+$ compartment (data not shown).

DISCUSSION

Is the IL-7/IL-7R system a central T cell regulatory path that is used in lymphopenia to drive restoration of immunity? Or are increased plasma IL-7 levels merely a result of lymphopenia, which further down-regulates receptor expression, resulting in a malfunctioning system? These questions outline the central direction of current research with regard to IL-7 and require answers to confidently advance laboratory science to therapeutic use.

Table 3. $CD4^+127^-132^+$ and $CD4^+127^+132^-$ T cell populations in patients with primary HIV-1 infection (PHI), chronic HIV-1 infection (CHI), or long-term nonprogressive HIV-1 infection (LTNP).

Population, time point	$CD4^+127^-132^+$		$CD4^+127^+132^-$	
	$CD45RO^-$	$CD45RO^+$	$CD45RO^-$	$CD45RO^+$
Healthy volunteers	1	6	44	37
PHI				
Week 0	4[a]	12[b]	37[a]	21[a]
Week 40	4[a]	11[a]	35[a]	26[b]
CHI				
Week 0	9[b]	22[c]	13[c]	12[c]
Week 48	9[d]	18[c]	28[d]	23[d]
LTNP-c				
Cohort entry	8[a]	10	25	20
Time point 2	5[a]	14[a]	20	20
LTNP-loc				
Cohort entry	8[b]	20[b]	22[a]	15[a]
Time point 2	15[b]	30[b]	14[b]	8[b]

NOTE. The table depicts the median percentage of the $CD45RO^-$ (naive) or $CD45RO^+$ (memory) $CD4^+$ T cell subset. HIV-1 infection was associated with a progressive loss of naive and memory $CD4^+127^+132^-$ T cells and an increase in naive and memory $CD4^+127^-132^+$ T cells. Patients with LTNP who maintained viral control (LTNP-c) had an increase in naive and memory $CD4^+127^-132^+$ T cells but no decrease in $CD4^+127^+132^-$ T cells. Patients with LTNP who had a loss of viral control (LTNP-loc) had significant decreases in naive and memory $CD4^+127^+132^-$ T cells and increases in naive and memory $CD4^+127^-132^+$ T cells. These changes were evident at cohort entry and before viral escape had occurred.

[a] $P < .05$, compared with the corresponding $CD4^+$ T cell subset in healthy volunteers.

[b] $P < .01$, compared with the corresponding $CD4^+$ T cell subset in healthy volunteers.

[c] $P < .0001$, compared with the corresponding $CD4^+$ T cell subset in healthy volunteers.

[d] $P < .001$, compared with the corresponding $CD4^+$ T cell subset in healthy volunteers.

The present experiments were used to study the effects of exogenous IL-7 on IL-7R expression in vitro. We sought to understand how dysregulation of the IL-7/IL-7R system relates to disease progression and immune reconstitution, using a spectrum of HIV-1–infected hosts: patients with PHI, patients with CHI, and 2 groups of patients with LTNP (those who maintained viral control and those who subsequently lost viral control).

We have confirmed a previous report that plasma IL-7 levels are increased in patients with PHI [43] and have shown that, if ART is implemented, there is normalization of plasma IL-7 levels in patients with PHI but not in patients with CHI. This is most likely due to the fact that patients with PHI commenced ART with higher total and naive $CD4^+$ T cell counts and were, therefore, more likely have their T cell counts return to the normal range [44]. This reasoning is supported by the finding that patients with LTNP who had $CD4^+$ T cell counts within the normal range did not have increased plasma IL-7 levels, although disease progression and loss of viral control were associated with increased plasma IL-7 levels, compared with cohort-entry levels.

Plasma IL-7 levels inversely correlated with total and naive $CD4^+$ T cell counts in patients with CHI but not in patients with PHI. This suggests that, although IL-7 was up-regulated in patients with PHI, increased levels that inversely correlate to lymphocyte populations appear to be a phenomenon of chronic disease.

A recent study found that baseline plasma IL-7 levels positively correlate with $CD4^+$ T cell immune reconstitution over 20 months of ART [25]. However, we found that baseline plasma IL-7 level predicted ART-associated $CD4^+$ T cell immune reconstitution (between these 2 measurements there was a positive correlation over 10 months) in patients with PHI but not in patients with CHI. The difference in study design may explain the conflicting results. The present study had a larger sample size and a shorter observation period. The previous study involved a heterogenous patient group whose $CD4^+$ T cell count on average was consistent with that of patients with CHI. One possible explanation is that the positive relationship between plasma IL-7 levels and an increase in $CD4^+$ T cell counts emerges in a shorter time frame in patients with PHI and that it requires >10 months in patients with CHI.

In the examination of age as a confounding factor between our PHI cohort and our older, CHI cohort, we found no evidence indicating that baseline plasma IL-7 levels were significantly correlated with age, which is in concordance with the results of the largest cross-sectional analysis of plasma IL-7 levels [19]. There has been a report that plasma IL-7 levels inversely correlate with age in patients with late-stage HIV-1 disease for whom ART has failed [45], but the cohort in that study was very different in terms of disease stage.

We found substantial evidence indicating that there is differential regulation of cell-surface expression of the IL-7R components associated with plasma IL-7 levels both in vitro and in vivo. This regulation occurs via distinct pathways; this finding is similar to those of previous reports regarding the IL-2 receptor [46]. In vitro, IL-7 markedly down-regulated CD127 and up-regulated CD132 expression over 7 days. The sustained down-regulation of CD127 occurred after a threshold of 10 ng/mL was reached. At the highest concentration, down-regulation occurred within hours, and there was no reexpression unless the IL-7 was removed. Cell-surface expression relied on de novo protein synthesis. This finding complements work done in murine models by Park et al. [35]. Inhibition of de novo protein synthesis also decreased the proportion of $CD127^+$ T cells, suggesting a constitutive rate of CD127 turnover on the surface of quiescent cells. Inherent to this work, however, is the assumption that local IL-7 concentrations in lymphoid niches are significantly greater than levels detected in plasma, because of the propensity of IL-7 to bind to the extracellular matrix [47–49].

There is evidence of a CD127 internalization pathway [50];

however, permeabilization of both fresh cultured and thawed PBMCs resulted in no increase in detection of CD127 but did result in a large increase in detection of CD132 expression. If internalized, CD127 may be rapidly degraded or stored in an impermeable vesicle. In contrast, it is believed that CD132 mRNA is constitutively expressed [51] and stored intracellularly before translocation to the surface [52, 53]. We confirmed large intracellular stores of mature CD132 that are not depleted in patients with CHI.

Four distinct subpopulations of $CD4^+$ and $CD8^+$ T cells could be distinguished on the basis of cell-surface IL-7R component expression. HIV-1 infection was associated with a net loss of $CD127^+132^-$ T cells and an increase of the $CD127^-132^+$ subset, which correlated with absolute $CD4^+$ T cell count. These changes were evident in both the $CD45RO^-$ and the $CD45RO^+$ subsets. Additionally, HIV-1 infection was associated with a marked loss of the $CD8^+127^+132^+$ subset, illustrating that regulation of the IL-7/IL-7R system is distinct between $CD4^+$ and $CD8^+$ T cells. Loss of CD127 from the $CD8^+127^+132^+$ subset may have been due to down-regulation of CD127 during antigen-driven proliferation and may explain why, in patients with HIV-1 infection, loss of CD127 was first recognized in $CD8^+$ T cells [32]. $CD127^-$ T cells displayed decreased Bcl-2 expression and increased turnover in $CD4^+$ and $CD8^+$ populations, indicating that these cells were dividing and short-lived.

Patients with LTNP, as a whole, had altered expression of IL-7R in the $CD8^+$ subset. Increased $CD4^+127^-132^+$ cell counts were evident in patients with LTNP-c, suggesting that some changes in IL-7R expression are inevitable during HIV-1 infection. Loss of $CD4^+127^+132^-$ subsets preceded the loss of viral control in patients with LTNP-loc, illustrating that depletions in these populations may relate to disease progression. Overall, the finding that patients with LTNP have normal plasma IL-7 levels but altered receptor expression suggests that decreases in CD127 expression may initially precede an increase of circulating IL-7 during HIV-1 infection, but CD127 was then further down-regulated by its ligand.

Further work in this area should focus on the mechanism by which IL-7 modulates expression of its receptor components and the distinct functional properties of these novel $CD4^+$ and $CD8^+$ subsets. Certainly the success of IL-7 therapy as a vaccine adjuvant or promoter of immune reconstitution will rely on more information regarding whether target cells respond to IL-7 with the desired survival and proliferation signals or whether the presence of exogenous IL-7 results in further loss of receptor expression.

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