Potent Antiretroviral Therapy of Primary Human Immunodeficiency Virus Type 1 (HIV-1) Infection: Partial Normalization of T Lymphocyte Subsets and Limited Reduction of HIV-1 DNA Despite Clearance of Plasma Viremia

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Antiretroviral therapy commenced during primary human immunodeficiency virus type 1 (HIV-1) infection (PHI) may limit the extent of viral replication and prevent early loss of HIV-specific CD4 lymphocyte function. We studied the effect of current standard therapy (2 nucleoside analogues and a protease inhibitor) in 16 patients with symptomatic PHI. In the 13 patients who completed 1 year of treatment, plasma HIV RNA was <50 copies/mL and median CD4 cell counts were comparable to HIV-uninfected controls, with naive (CD45RA+CD62L+), primed (CD45RO+), and T cell receptor Vβ subsets all within normal ranges. However, HIV-1 DNA levels in treated and untreated PHI patients were similar. Furthermore, CD8 cell counts remained elevated, including activated (CD38+HLA-DR+), replicating (Ki-67+), and cytotoxic (perforin+CD28−) lymphocytes. In conclusion, early antiretroviral therapy resulted in clearance of viremia and prevented loss of crucial CD4 subsets. The persistence of HIV-1 DNA together with increased CD8 T lymphocyte turnover and activation indicate continued expression of viral antigens.

Extremely high levels of viremia are observed during primary human immunodeficiency virus type 1 (HIV-1) infection (PHI) [1, 2] and are associated with decreases in CD4 cell counts [3], involving subsets that include both naive and memory CD4 T lymphocytes [4]. Opportunistic infections in a small proportion of patients [3] and in vitro hyporesponsiveness to phytohemagglutinin, recall, and HIV-1 antigens [5–7] reflect early immunodeficiency in PHI. Furthermore, rapid disease progression is associated with both a low CD4 cell count and more-severe illness during PHI [8–10]. Therefore, therapeutic intervention during PHI may prevent the development of such deficits.

In a placebo-controlled trial of zidovudine administered in PHI [11], CD4 cell counts were increased and minor opportunistic infections were observed less frequently in the treated patients, but plasma viremia was not significantly reduced. Small uncontrolled studies of dual or triple therapy of PHI subjects have demonstrated reductions in plasma and lymph node viral load [12, 13], increases in CD4 : CD8 ratios [13, 14], and maintenance of HIV-1–specific CD4 function in vitro [7], but these therapies failed to block induction of a latent carrier state [15].

The present study describes the effect of standard combination therapy with zidovudine, lamivudine, and indinavir in 16 patients with PHI. Clearance rates of plasma HIV-1 RNA and peripheral blood HIV-1 DNA levels, phenotypic profiles of circulating CD4+ and CD8+ T lymphocytes, and anti–HIV-1 antibody levels were determined over 52 weeks of treatment and were compared with HIV-1-uninfected subjects, untreated PHI patients, and patients with established HIV-1 infection.

Materials and Methods

Subjects. Sixteen patients with a diagnosis of symptomatic PHI [3] were prospectively enrolled. Baseline characteristics and clinical and serological details of the treated PHI patients are summarized in table 1. Eight subjects were positive by HIV-1 p24 antigen EIA and negative by HIV-1 antibody EIA and Western blot; 3 subjects had positive antibody EIA but <4 bands on Western blot; and 5 subjects were positive for EIA and Western blot (≥4 bands) but had <4 bands on Western blot within the 30 days before screening. All subjects received zidovudine (200 mg 3×/day), lamivudine (150 mg 2×/day) and indinavir (800 mg 3×/day).

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This study was approved by the St. Vincent's Hospital Institutional Research Ethics Committee, and all patients gave written informed consent.

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Table 1. Characteristics of enrolled primary human immunodeficiency virus type 1 (HIV-1) patients at study entry.

Characteristic	Median (interquartile range)
Subjects	
Male	16
White	16
Age (years)	35 (30–39)
Transmission	
Homosexual activity	15
Use of injection drug	1
PHI symptom score[a]	3 (2–4)
Hospital admissions	1
Duration of illness (days)	38 (17.5–70)
Time from onset of symptoms to therapy (days)	23.5 (13–38)
Time from screening to therapy (days)	4 (3–5)
HIV-1 serology at screening	
HIV-1 p24 antigen EIA positive	11
HIV-1 antibody EIA negative	8
HIV-1 antibody EIA positive	8
HIV-1 Western blot negative	8
HIV-1 Western blot <4 bands	3
HIV-1 Western blot ⩾4 bands	5

[a] As described elsewhere [10]. PHI, primary HIV-1 infection.

Control groups comprised (1) 17 consecutive PHI patients, seen through the Sydney PHI Investigators Group and the Community HIV Research Network between 1993 and 1996, who received no antiretroviral therapy; (2) 12 consecutive asymptomatic, treatment-naive chronically HIV-infected subjects with CD4 cell counts of 275–550 cells/μL; and (3) 56 HIV-uninfected adults.

HIV serology. Coulter HIV p24 EIA (Coulter, Hialeah, FL), Abbott Axsym HIV Antibody MEIA (Abbott Laboratories, Abbott Park, IL), Wellcozyme HIV Recombinant Antibody EIA (Murex, Dartford, Kent, UK), and Diagnostic Technology HIV Western Blot (Diagnostic Technology, Singapore) were used in the diagnosis of PHI. HIV p24 quantitative antibody levels were assayed by anti-p24 HIV antibody EIA (Murex).

Viral load measurement. We measured plasma HIV RNA, using the Roche Amplicor quantitative RT-PCR kit (version 1.0; Roche, Branchburg, NJ). Specimens <400 copies/mL were analyzed by means of the UltraSensitive specimen preparation protocol, with a detection limit of 50 copies/mL.

HIV-1 DNA levels were quantified by use of a prototype assay developed at Roche Molecular Systems (Alameda, CA). In brief, cryopreserved peripheral blood mononuclear cells (PBMC) were washed twice with 1 mL of Specimen Wash Solution (Roche Molecular Systems, Somerville, NJ) with centrifugation at 16,000 *g* for 3 min. Cells were then lysed with a buffer containing proteinase K and an internal quantitation standard (QS), as described elsewhere [16]. After inactivation of the proteinase K, the samples were amplified by use of the AMPLICOR MONITOR v1.5 HIV-1 master mix (Roche) and detected by use of microwell plates coated with HIV- and QS-specific probes [16]. Total DNA was determined with Hoechst dye (Pharmacia, Uppsala, Sweden).

Flow cytometry. Monoclonal antibodies (mAbs) to the following lymphocyte cell surface proteins were used: CD3–peridinin chlorophyll protein (PerCP), CD4-fluorescein isothiocyanate (FITC), CD4-phycoerythrin (PE), CD8-FITC, CD8-PE, CD38-PE, HLA-DR-FITC, HLA-DR-PE, CD45RO-PE, CD28-PE, and CD62L(LEU-8)-FITC (Becton-Dickinson, San Jose, CA); CD28-FITC (Pharmingen, San Diego); CD4-ECD (PE–Texas Red), CD8-ECD, CD45RA(2H4)-RD1 and Ki-67-FITC (Coulter); CD38-FITC (Immunotech, Marseilles, France); CD45RO-FITC (DAKO, Carpinteria, CA); anti-human perforin-PE (Ancell, Bayport, MN); and anti–T cell receptor Vβ (TCR BV) subfamilies conjugated with FITC (Immunotech). MAbs were incubated (at the recommended concentrations) with 100 μL acid citrate dextrose–anticoagulated whole blood for 10–15 min at 25°C before lysis with Optilyse C (Immunotech) for 10 min at 25°C, followed by 1 wash with PBS. Stained cells were resuspended in 0.5 mL PBS containing 0.5% paraformaldehyde and were analyzed within 24 h of preparation.

Intracellular staining was performed on whole blood after cell surface staining, lysis, and fixation with FACS Lysing solution (Becton-Dickinson) and permeabilization with FACS Permeabilizing solution (Becton-Dickinson). Permeabilized cells were incubated with mAb and 5 μL of human gamma globulin (a kind gift from CSL, Melbourne) for 45 min at 25°C and washed once.

Samples were analyzed on a Coulter EPICS XL and compensation levels checked daily. Lymphocyte gates were set manually on forward and side scatter, and 10,000 lymphocytes were analyzed. CD3-PerCP and either CD4-ECD or CD8-ECD staining were used to set gates for analysis of 2-color FITC/PE histograms. Positively stained cells were determined by comparison with nonspecific controls. In the case of CD45RA, cells dimly stained for CD45RA were not included as CD45RA+. TCR V β (BV) distribution was determined by gating on lymphocytes and analyzing CD4-PE and BV-FITC or CD4-FITC and BV-PE combinations. Samples stained intracellularly were gated on CD3-PerCP and side scatter with an FL4 threshold. Specificity of intracellular staining was demonstrated by means of blocking with cold mAb in preliminary experiments.

Statistical analysis. Lymphocyte phenotyping results are expressed as a percentage of either CD4 or CD8 T lymphocytes or as the absolute number of positively stained cells, calculated with CD4 and CD8 cell counts. Results for each cohort are expressed as medians and interquartile ranges. The Mann-Whitney U test comparing treated PHI patients with control groups was performed by means of Statview v4.5 for Macintosh (Abacus Concepts, Berkeley, CA). The correlation between plasma viremia and activation markers was analyzed by use of the Spearman rank test. The change in levels of HIV-1 DNA in treated PHI patients between week 24 and week 52 was analyzed by Wilcoxon signed rank test. A 2-sided P value <.05 was considered statistically significant.

Results

Subjects. Three of the 16 enrolled patients did not continue treatment to 52 weeks. One patient stopped treatment intermittently because of renal calculi and withdrew from therapy by week 20, and 2 patients were lost to follow-up, both from week 8. One patient substituted stavudine for zidovudine because of drug intolerance. All treated patients remained clinically well at 52 weeks, with no HIV disease progression.

HIV-1 viral burden in peripheral blood. Plasma HIV RNA declined from 6.0 $\log_{10}$ copies/mL at baseline to <400 copies/mL within 20 weeks and was <50 copies/mL in 13/13 patients after week 36 (figure 1*A*). Plasma HIV RNA in individual pa-

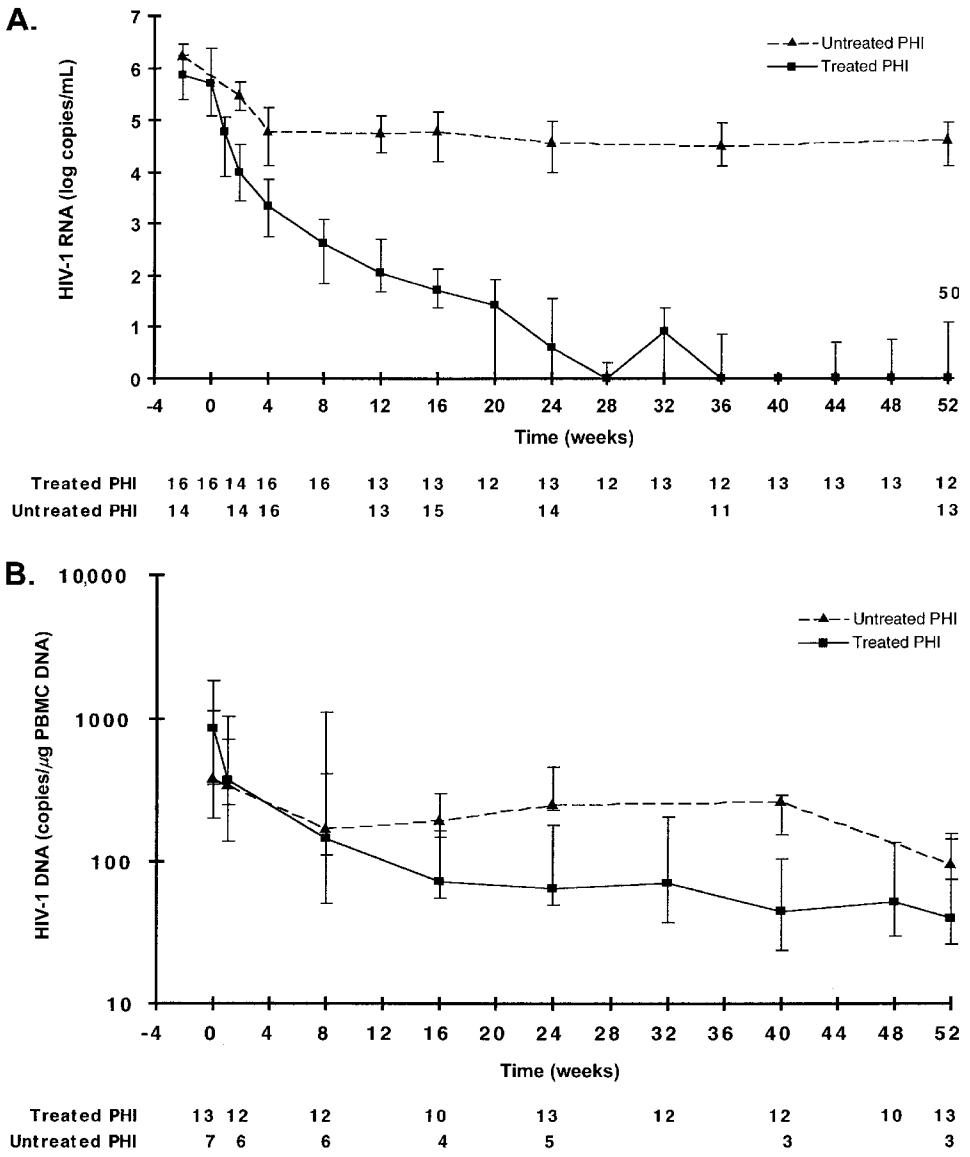

Figure 1. Human immunodeficiency virus type 1 (HIV-1) viral burden (median ± interquartile range) in peripheral blood of treated and untreated subjects with primary HIV-1 infection (PHI) over 52 weeks. Number of patients studied in each group for each time point is shown. Day 0 for treated patients represents commencement of therapy. Corresponding time point for untreated patients was assigned as day 21 after onset of symptoms (table 1). *A*, Plasma HIV-1 RNA (log copies/mL); *B,* HIV-1 DNA (copies/μg peripheral blood mononuclear cell DNA).

tients followed a biphasic decline, with a mean slope of the first phase of −0.157 (range, −0.143 to −0.192) $\log_{10}$ copies/mL/day) and a mean second phase slope of −0.024 (range, −0.019 to −0.032) $\log_{10}$ copies/mL/day). Calculated half-lives of free plasma viremia were 1.9 days and 12.5 days, respectively. HIV RNA fell much more slowly in untreated PHI patients, reaching stable levels of 4.8 $\log_{10}$ copies/mL (figure 1*A*).

All treated PHI subjects had detectable HIV-1 DNA in peripheral blood at week 52. No significant difference in the number of copies per microgram PBMC DNA was observed between treated and untreated PHI patients at baseline or at weeks 8, 24, or 52 (figure 1*B*). However, the treated PHI patients showed a greater decrease from baseline, including a significant decrease from week 24 to week 52 (medians of 65 and 31 copies/μg PBMC DNA, respectively; $P < .002$), while untreated PHI patients' HIV-1 DNA levels remained stable.

CD4 T lymphocytes. At week 52, treated PHI patients had a median CD4 cell count that was comparable to HIV-1–uninfected controls (table 2) and higher than untreated PHI patients, with differences becoming apparent after 6–12 months of therapy (figure 2*A*).

Circulating naive (CD45RA+CD62L+) CD4 T lymphocytes

Table 2. Comparison of circulating lymphocyte subset numbers (cells/μL) between treated primary human immunodeficiency virus type 1 (HIV-1) patients at 52 weeks, HIV-1-uninfected controls, untreated primary HIV-1 patients, and treatment-naive asymptomatic HIV-1–positive subjects.

	Cohort						
	Treated PHI patients (week 52) (n = 13)	HIV-negative controls (n = 56)	P[a]	Untreated PHI patients (week 52) (n = 17)	P[a]	Chronic HIV-positive patients (n = 12)	P[a]
CD4 T lymphocytes	821[b] (660–925)	823 (642–1085)	.7	525 (477–605)	<.001	381 (328–411)	<.001
CD45RA$^+$CD62L$^+$ CD4 T cells	142 (67–282)	227 (135–304)	.12	ND		70 (37–111)	.06
CD45RO$^+$ CD4 T cells	448 (356–581)	411 (323–529)	.4	ND		ND	
CD8 T lymphocytes	852 (630–1014)	430 (359–540)	<.001	864 (748–1014)	.6	1079 (685–1625)	.1
CD45RA$^+$CD62L$^+$ CD8 T cells	208[c] (147–326)	138 (104–214)	<.05	ND		ND	
CD45RA$^+$CD62L$^-$ CD8 T cells	162[c] (67–282)	60 (29–106)	<.001	ND		ND	
CD45RO$^+$ CD8 T cells	294 (210–421)	168 (130–240)	<.01	ND		ND	
CD38$^+$HLA-DR$^+$ CD8 T cells	124 (81–156)	14 (9–26)	<.001	ND		452 (230–754)	<.001
CD28− CD8 T cells	371 (308–499)	94 (63–182)	<.001	ND		745 (508–1259)	<.01

[a] Nonparametric unpaired Mann-Whitney comparison with treated primary HIV-1 (PHI) patient cohort.
[b] Numbers represent medians of cell counts (cells/μL) for each cohort, with interquartile ranges shown in parentheses.
[c] Results for 11 treated PHI patients, weeks 36–52.

rose in the treated PHI subjects in the first 2 weeks and thereafter remained steady (figure 2*A*). At week 52, there was no significant difference in the naive CD4 T lymphocyte count compared with HIV-uninfected controls (table 2). The number of naive CD4 T lymphocytes in the treated PHI patients tended to be higher than for patients with established infection (table 2).

Six treated PHI patients who commenced therapy while still HIV-1–antibody negative had significantly higher naive CD4 T lymphocyte counts at week 52 than did 4 treated PHI patients who were HIV-1–antibody positive (≤4 bands on HIV-1 Western blot) at commencement of therapy (medians, 203 and 57 cells/μL, respectively; P = .014). The median times from onset of symptoms to commencement of therapy in these 2 subgroups were 10 days and 30 days, respectively. Total CD4 cell counts at 52 weeks were not significantly different between the 2 subgroups (medians, 903 and 738 cells/μL, respectively; P = .7).

Recently activated (CD45RO+) CD4 T lymphocytes rose in the first 4 weeks and continued to increase slightly over the next 48 weeks (figure 2*B*). The final number of this primed subset in the treated PHI patients was not significantly different from HIV negative controls.

CD4 TCR BV repertoire was examined in 7 treated PHI patients and compared with 16 HIV-uninfected controls. At baseline, the only difference observed was a decrease in the proportion of BV 8.1+ CD4 lymphocytes in PHI patients compared with controls (medians, 2.2% and 4.4%, respectively; P = .013; figure 2*C*), which returned to normal at week 52 (3.6%; figure 2*C*). In contrast, significant perturbations of 2 TCR BV subfamilies were observed in CD4 lymphocytes from 6 patients with established HIV infection (data not shown).

CD8 T lymphocytes. CD8 T lymphocyte counts for treated PHI subjects were significantly elevated at week 52, compared with HIV-uninfected controls (table 2), and were not significantly different from the untreated PHI patients. The rapid decrease in CD8 cell counts in treated PHI patients over the first 4 weeks of treatment (figure 3*A*) was due to an abrupt decrease in CD45RO+ CD8 T lymphocytes (figure 3*B*). In spite of this decrease, CD45RO+ CD8 T lymphocytes in treated PHI patients were still significantly elevated at week 52, compared with HIV-uninfected controls (table 2).

In contrast, there was a continual slow increase in the CD45RA+ subset in treated PHI patients (figure 3*B*). After 36 weeks, the number of naive (CD45RA+CD62L+) CD8 T lymphocytes was increased compared with HIV-uninfected controls, but the greatest difference occurred in the resting memory [17] (CD45RA+CD62L−) CD8 T lymphocyte subset (table 2).

Figure 3*B* also shows that the number of activated (CD38+HLA-DR+) CD8 T lymphocytes in treated PHI patients decreased rapidly over the first 4 weeks of treatment but remained significantly elevated compared with HIV-negative controls at week 52 (table 2). The number of activated CD8 T lymphocytes was significantly lower than for untreated asymptomatic HIV-infected subjects (table 2). Similar results were observed for CD28− CD8 T lymphocytes (table 2).

Association between plasma HIV-1 RNA and CD8 activation. During the phase of viral load reduction, there was a linear relationship between the percentage of activated (CD38+HLA-DR+) CD8 T lymphocytes and HIV-1 plasma viral load in individual treated patients (figure 4*A*). Seven of 11 patients showed a significant correlation (Spearman's ρ, 0.71–0.96).

The correlation between levels of HIV-1 DNA and the level of activation of CD8 T lymphocytes, shown in figure 4*B,* was not quite as strong as for plasma HIV-1 RNA, with 5/12 patients showing a significant correlation (Spearman's ρ, 0.57–0.93).

Characterization of activated CD8 T lymphocytes. Turnover of activated CD8 T lymphocytes from week 52 was analyzed by intracellular staining for the nuclear antigen Ki-67 [18]. Treated PHI subjects had an 8-fold higher number of Ki-67+CD38+ CD8 T lymphocytes than did HIV-negative con-

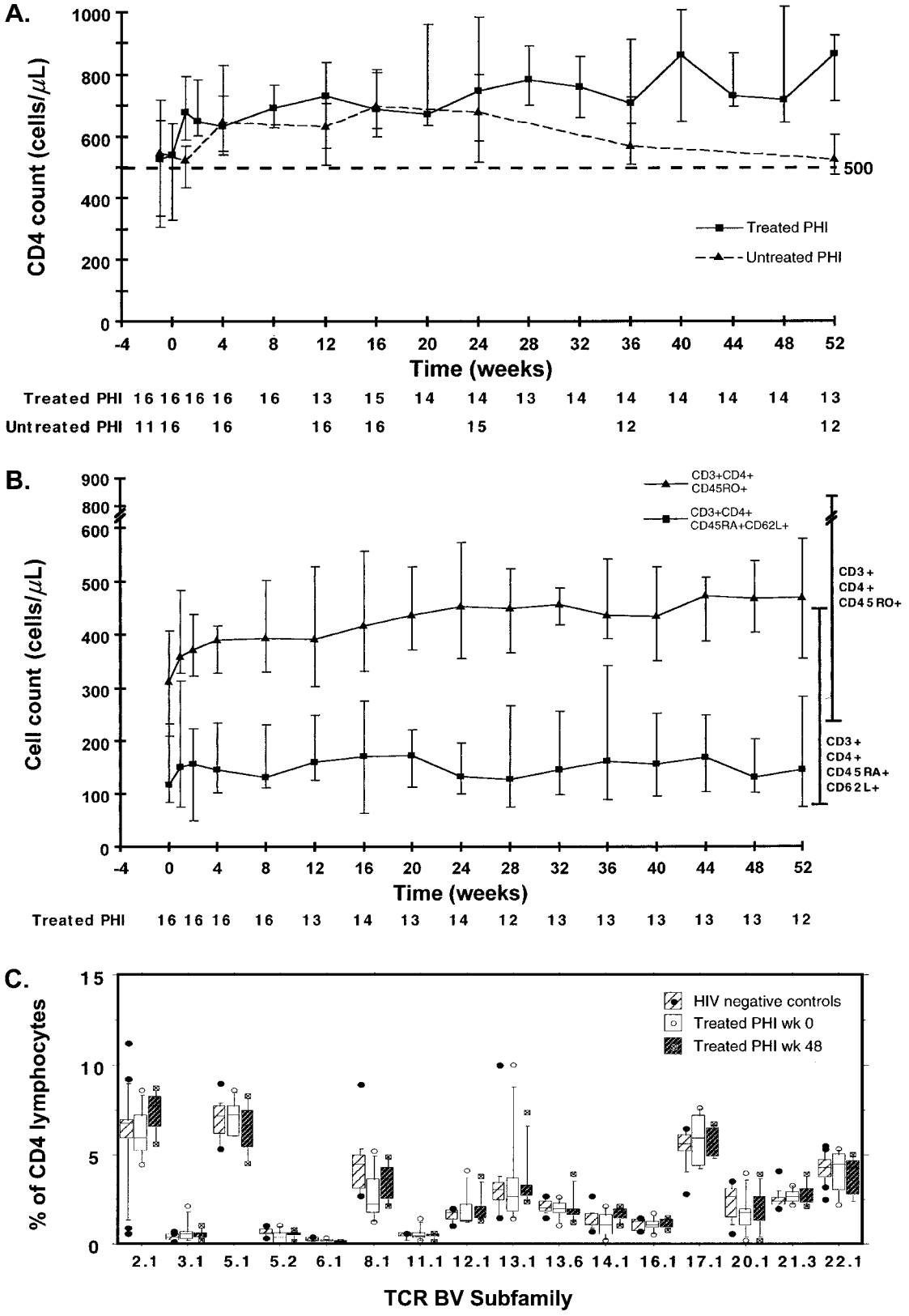

Figure 2. Numbers of circulating CD4 T lymphocytes (median ± interquartile range) over 52 weeks. Number of patients studied in each group for each time point is shown. *A,* CD4 cell counts for treated and untreated subjects with primary human immunodeficiency virus (HIV) type 1 (PHI); *B,* Naive (CD45RA+CD62L+) and primed (CD45RO+) CD4 T lymphocyte counts for treated PHI patients. Bars on the right side of the graph show the observed range for 95% of HIV-negative controls. *C,* box plots of proportion of TCR BV subfamilies as % of CD4+ lymphocytes for HIV-uninfected controls (n = 16), PHI patients (n = 7) at baseline and at week 48. Each box plot depicts the 10th, 25th, 50th, 75th, and 90th percentiles. Values above and below the 90th and 10th percentiles, respectively, are also shown.

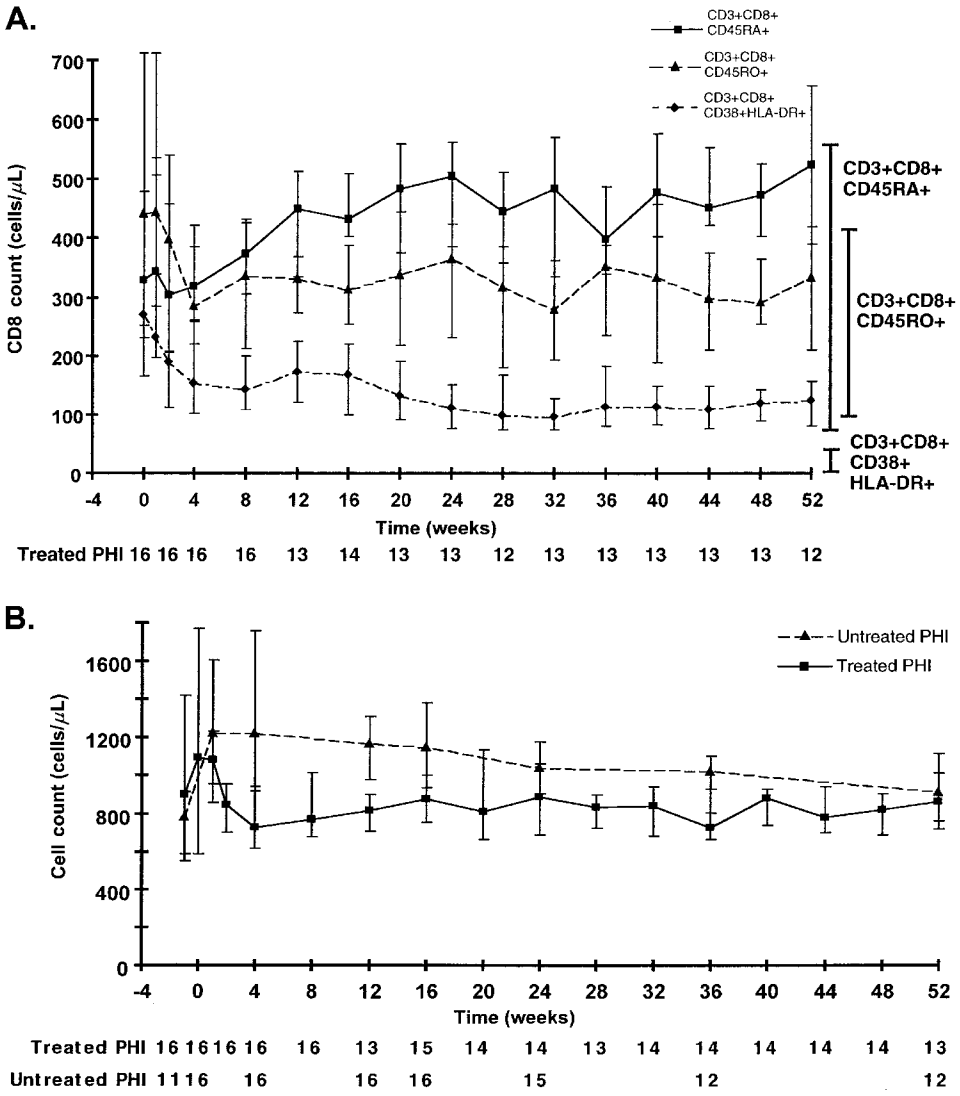

Figure 3. Numbers of circulating CD8 T lymphocytes (median ± interquartile range) over 52 weeks. Number of patients studied in each group for each time point is shown. *A,* CD8 cell counts for treated and untreated subjects with primary human immunodeficiency cirus (PHI). *B,* Naive/resting (CD45RA+), primed (CD45RO+), and activated (CD38+HLA-DR+) CD8 T lymphocyte counts for treated PHI patients. Bars on the right of the graph show the observed range for 95% of human immunodeficiency virus–negative controls.

trols (table 3). Similarly, treated PHI subjects had a 7-fold higher number of Ki-67+HLA-DR+ CD8 T lymphocytes than did HIV-negative controls. Up to 11% of CD38+ or HLA-DR+ CD8+ T lymphocytes were Ki-67+ (data not shown).

CD28− CD8 T lymphocytes at week 52 onward were investigated by intracellular staining for perforin, a molecule associated with cytotoxic activity [19]. Treated PHI subjects had a 7-fold greater number of perforin+CD28− CD8 T lymphocytes compared with HIV-uninfected controls (table 3). A median 78% of CD28− CD8 T lymphocytes were perforin+ in treated patients, and essentially all perforin+ CD8 T lymphocytes were CD28− (data not shown).

Serum anti–HIV-1 antibody concentration. Only 2/13 treated PHI subjects exhibited development of typical antibody responses to HIV-1, as determined by serial Western blots. Four treated patients exhibited low reactivity to HIV-1 p24 by both Western blot (figure 5*A*) and quantitative anti-p24 antibody EIA (figure 5*B*). All 4 patients had commenced therapy after they had detectable reactivity to p24 by Western blot. Another 3 patients showed a slight decline in their positive p24 antibody levels after 24 weeks (figure 5*B*). However, 6 treated patients showed no decline in p24 antibody levels—2 who were strongly positive and 4 who had intermediate p24 antibody levels.

Discussion

Combination therapy initiated during PHI led to sustained suppression of plasma viremia, as recently described for a group

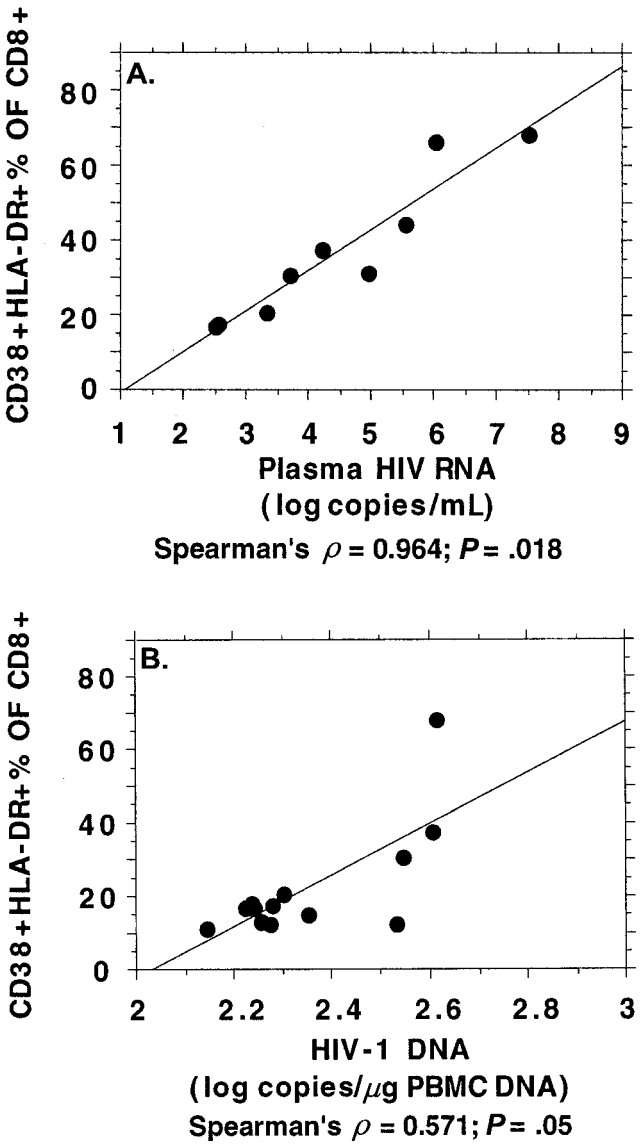

Figure 4. Linear regression plots and correlations between CD38+HLA-DR+ % of CD8 T lymphocytes, plasma human immunodeficiency virus type 1 (HIV-1) RNA (*A*), and HIV-1 DNA (*B*) for treated patient 5064.

of patients treated within 45–90 days of onset of symptoms [20]. Viral dynamics showed a biphasic decline, similar to that described for asymptomatic chronically HIV-1–infected patients on similar therapy [21]. HIV-1 DNA in PBMC, in contrast, was still detectable in treated patients after 52 weeks, at levels comparable to untreated PHI subjects, showing that treatment that was highly suppressive for plasma HIV-1 RNA had little direct effect on HIV-1 DNA burden. Whether this DNA exists in the integrated form has not been examined in the current study. A recent report [15] has demonstrated establishment of a pool of CD4 cells latently infected with replication-competent HIV-1 within the first 2 weeks of symptomatic PHI. The ability to recover infectious HIV-1 from PBMC has not so far been studied in our group of treated patients.

Early antiretroviral therapy led to a continuing rise in circulating CD4 T lymphocyte numbers, to levels indistinguishable from HIV-uninfected controls. This was in contrast to untreated PHI patients, who experienced decreasing CD4 cell counts within the first year of infection, in agreement with previous reports [22, 23]. Furthermore, analysis of CD4+ lymphocyte repertoire at the level of TCR BV subfamilies showed no significant perturbations in treated patients compared with HIV-uninfected controls. A previous study of antiretroviral therapy in chronically infected subjects reported normalization of perturbations within CD4 lymphocyte BV families, dependent on relatively sustained suppression of viral load [24]. It remains possible that, despite normal CD4 T lymphocyte counts and normal TCR BV repertoire, immune function may still be impaired.

The number of naive CD4 T lymphocytes in treated PHI subjects was within the normal range at 52 weeks, a finding not seen in patients at other stages of HIV-1 infection, receiving similar therapy [25–29]. Furthermore, the kinetics of the increase in naive CD4 T lymphocytes showed an early rise after treatment during PHI, in contrast to the slow progressive or late rises observed in treatment of chronic infection [25–29]. Interestingly, 6 treated PHI patients who commenced therapy before the appearance of serum HIV-1 antibodies recovered their naive CD4 T lymphocytes more effectively than did 4 treated PHI patients who commenced their therapy 20 days later. If confirmed with larger numbers of patients, this rapid change in the homeostasis of circulating CD4 T lymphocytes may represent an important early step in the development of immunodeficiency.

CD8 T lymphocyte counts were reduced early, as a result of treatment during PHI, but remained elevated at 52 weeks and were similar in number to those of untreated PHI patients. The CD45RO+ subset of CD8+ T lymphocytes is usually greatly increased in HIV-1 infection [30, 31], particularly in PHI [4, 32], and is associated with poor prognosis [31]—in the treatment group, the number of CD45RO+ CD8+ T lymphocytes dropped rapidly after commencement of therapy. These results also differ substantially from antiretroviral therapy of estab-

Table 3. Turnover and cytotoxic phenotype of CD8 T lymphocytes in treated primary human immunodeficiency virus type 1 (HIV-1) patients (at week 52) and HIV-1–negative controls.

T cells	Treated PHI patients (n = 6)	HIV-negative controls (n = 5)	P[a]
CD38+Ki-67+ CD8	16 (10–28)	2 (1–4)	<.01
HLA-DR+Ki-67+ CD8	14 (8–17)	2 (1.5–3)	<.01
Perforin+CD28− CD8	366 (162–407)	53 (41–112)	.02

NOTE. PHI, primary HIV-1 infection. Numbers represent medians of cell counts (cells/μL) for each cohort, with interquartile ranges shown in parentheses.

[a] Nonparametric unpaired Mann-Whitney comparison with treated PHI patient cohort.

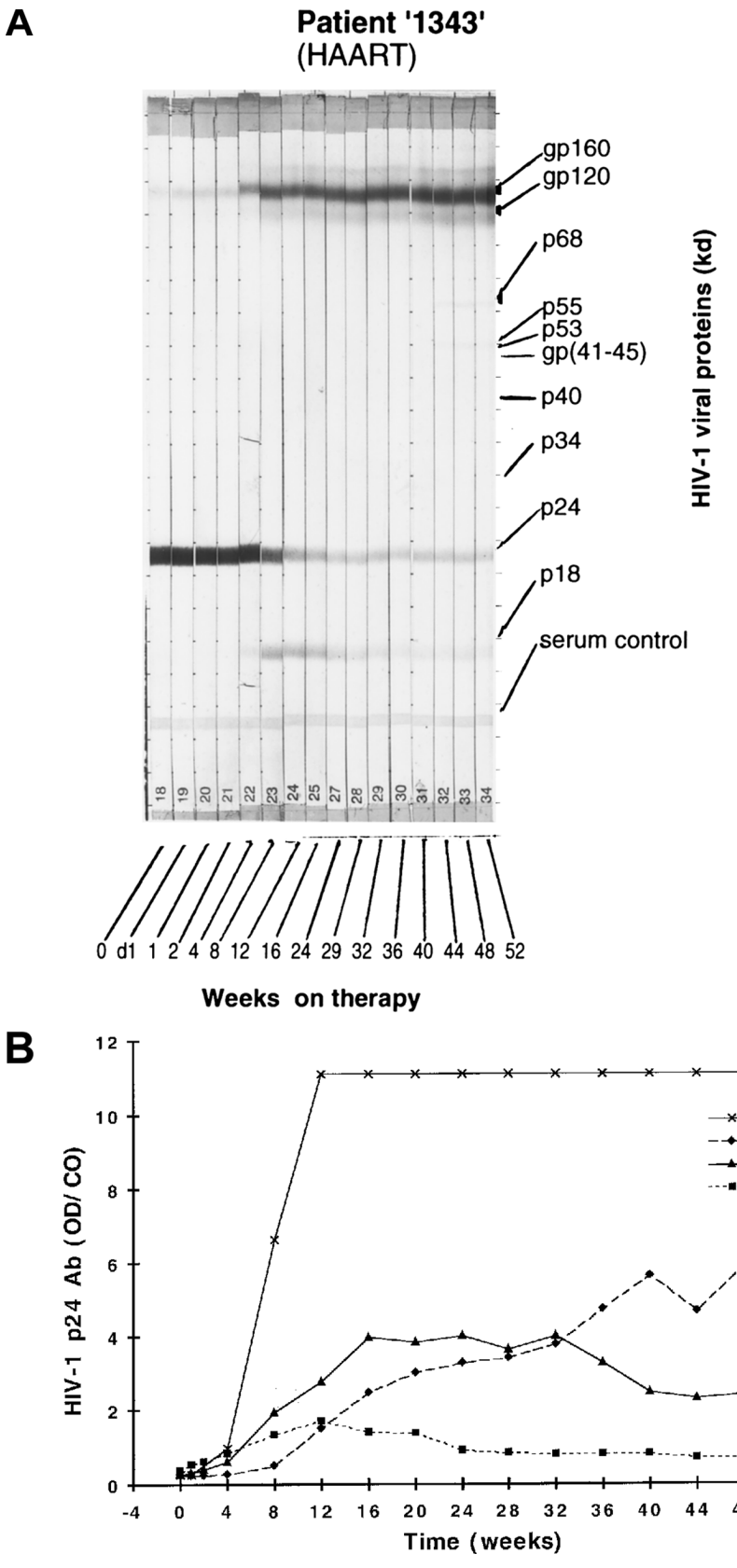

Figure 5. Effect of therapy on serum antibodies to human immunodeficiency virus type 1 (HIV-1). *A,* Serial HIV-1 Western blot profiles for 1 treated patient with primary HIV-1 (PHI), 1343, and an untreated PHI patient, AND. The results are representative for 4 treated PHI patients with low reactivity to p24 and for 4 untreated PHI patients studied. HAART, highly active antiretroviral therapy. *B,* Serum anti–HIV-1 p24 antibody levels measured by quantitative EIA. Results are shown for 1 representative treated PHI patient from subgroups exhibiting maximal response (2 patients); intermediate, nondeclining levels (4 patients); intermediate, declining levels (3 patients); and low reactivity (4 patients).

lished infection, where CD45RO+ CD8+ T lymphocytes rise initially after treatment [25], probably as a result of redistribution of pre-existing cells [29].

The number of naive CD8+ T lymphocytes was maintained at or above normal levels. Decreases in this subset have been described as an important indicator of disease progression [33]. After treatment, the number of CD8+ T lymphocytes with the phenotype of resting memory cells, CD45RA+ CD62L− [17] progressively increased, which suggests that they may have reverted from CD45RO+ to CD45RA+ cells [34]. Study of the CD45RA+ CD62L− CD8 T lymphocytes with tetramer-peptide constructs in suitable subjects may elucidate whether these cells contain resting HIV-specific memory cells [35].

Activation of CD8 T lymphocytes was significantly reduced as a result of potent antiretroviral therapy, but not to normal levels, as previously shown for established infection [25, 26, 36]. The proportion of CD8 T lymphocytes expressing CD38 and HLA-DR was clearly correlated with plasma HIV RNA concentrations, in agreement with recent studies [37–40], but was less well correlated with HIV-1 DNA burden. A significant proportion of residual CD38+ and HLA-DR+ CD8 T lymphocytes contained Ki-67 nuclear antigen, indicating recent passage through the cell cycle [18] and suggesting that the elevation of CD38+HLA-DR+ CD8 T lymphocytes is an active process. HIV-1–specific CD8 T lymphocytes have recently been shown to express CD38 [35], although the level of CD38 expression is decreased during antiretroviral treatment [41].

By comparison, in a recent study of primary Epstein-Barr virus infection, activated CD8 T lymphocytes returned to normal levels within 16 weeks [42]. Similarly, we have recently found that 3 patients, infected with an attenuated *nef*long terminal repeat mutant HIV-1 and having undetectable plasma HIV-1 RNA and stable CD4 cell counts [43], also had normal levels of activation of CD8 T lymphocytes (Zaunders, Geczy, Dyer, et al., unpublished data). Taken together, these results suggest that residual activation of CD8 T lymphocytes may represent an ongoing response to low-level HIV-1 replication that is not manifest as plasma viremia. In this regard, viral replication at greatly reduced levels has been described in lymphoid tissue of patients successfully treated with combination antiretroviral therapy [44]. Alternatively, activation may result from opportunistic infections, although this is unlikely in this treatment group, or a longer period of time may be required for full normalization.

CD28− CD8+ T lymphocytes also remained elevated after treatment; this phenotype has been reported to contain HIV-1 specific cytotoxic activity [45, 46]. Most cells within this subset contained intracellular perforin, an important effector molecule in cytotoxic T lymphocyte responses to noncytopathic viral infections [19]. Evidence has been obtained for continuing CTL responses in treated patients with sustained suppression of plasma viremia [20], and this may be similarly reflected in CD28− CD8 T cell numbers in treated PHI patients.

HIV-1 p24 antibody levels declined on therapy in approximately half of the patients, in accordance with previous reports [13, 20, 47]. A reduced humoral response may be due to loss of p24-specific CD4 T lymphocytes in PHI, as demonstrated in vitro [7], since large amounts of p24 protein, but not intact virions, remain on follicular dendritic cells in the lymph nodes of treated patients with established infection [48]. Similarly, the rapid fall in CD45RO+ CD8 T lymphocytes early in treatment also suggests interruption of the cell-mediated response.

In conclusion, standard antiretroviral therapy commenced during PHI appears to have prevented permanent loss of important subsets of CD4 T lymphocytes, particularly when treatment preceded the antibody response to HIV-1. The persistence of CD8 T lymphocyte activation, as well as the stable reservoir of HIV-1 DNA in cellular compartments, however, is consistent with the conclusion that expression of viral genes has not been totally suppressed. Further study of CD8 T lymphocytes may be useful in assessing future antiretroviral regimens.

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References

1. Clark SJ, Saag MS, Decker WD, et al. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. N Engl J Med **1991**;324:954–60.
2. Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viraemia in patients with primary human immunodeficiency virus type 1 infection. N Engl J Med **1991**;324:961–4.
3. Tindall B, Cooper DA. Primary HIV infection: host responses and intervention strategies. AIDS **1991**;5:1–14.
4. Zaunders J, Carr A, McNally L, Penny R, Cooper D. Effects of primary HIV-1 infection on subsets of CD4+ and CD8+ T lymphocytes. AIDS **1995**;9:561–6.
5. Cooper DA, Tindall B, Wilson EJ, Imrie AA, Penny R. Characterization of T lymphocyte responses during primary infection with human immunodeficiency virus. J Infect Dis **1988**;157:889–96.
6. Gaines H, von Sydow MAE, von Stedingk LV, et al. Immunological changes in primary HIV-1 infection. AIDS **1990**;4:995–9.
7. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1–specific CD4+ T cell responses associated with control of viremia. Science **1997**;278:1447–50.
8. Keet IP, Krijnen P, Koot M, et al. Predictors of rapid progression to AIDS in HIV-1 seroconverters. AIDS **1993**;7:51–7.
9. Pedersen C, Lindhardt BO, Jensen BL, et al. Clinical course of primary HIV infection: consequences for subsequent course of infection. BMJ **1989**; 299:154–7.
10. Vanhems P, Lambert J, Cooper DA, et al. Severity and prognosis of acute human immunodeficiency virus type 1 illness: a dose-response relationship. Clin Infect Dis **1998**;26:323–9.
11. Kinloch-de Loes S, Hirschel BJ, Hoen B, et al. A controlled trial of zidovudine

in primary human immunodeficiency virus infection. N Engl J Med **1995**; 333:408–13.

12. Perrin L, Rakik A, Yerly S, et al. Combined therapy with zidovudine and L-697,661 in primary HIV infection. AIDS **1996**; 10:1233–7.
13. Lafeuillade A, Poggi C, Tamalet C, Profizi N, Tourres C, Costes O. Effects of a combination of zidovudine, didanosine, and lamivudine on primary human immunodeficiency virus type 1 infection. J Infect Dis **1997**; 175: 1051–5.
14. Lisziewicz J, Jessen H, Finzi D, Siliciano RF, Lori F. HIV-1 suppression by early treatment with hydroxyurea, didanosine, and a protease inhibitor. Lancet **1998**; 352:199–200.
15. Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. Proc Natl Acad Sci USA **1998**; 95:8869–73.
16. Mulder J, McKinney N, Christopherson C, Sninsky J, Greenfield L, Kwok S. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection. J Clin Microbiol **1994**; 32:292–300.
17. Sprent J. Immunological memory. Curr Opin Immunol **1997**; 9:371–9.
18. Gerdes J, Lemke H, Baisch H, Wacker H-H, Schwab U, Stein H. Cell cycle analysis of a cell proliferation–associated human nuclear antigen defined by the monoclonal antibody Ki67. J Immunol **1984**; 133:1710–5.
19. Kagi D, Hengartner H. Different roles for cytotoxic T cells in the control of infections with cytopathic versus noncytopathic viruses. Curr Opin Immunol **1996**; 8:472–7.
20. Markowitz M, Vesanen M, Tenner-Racz K, et al. The effect of commencing combination antiretroviral therapy soon after human immunodeficiency virus type 1 infection on viral replication and antiviral immune responses. J Infect Dis **1999**; 179:527–37.
21. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science **1996**; 271:1582–6.
22. Margolick JB, Munoz A, Donnenberg AD, et al. Failure of T-cell homeostasis preceding AIDS in HIV-1 infection. Nat Med **1995**; 1:674–80.
23. Schacker TW, Hughes JP, Shea T, Coombs RW, Corey L. Biological and virologic characteristics of primary HIV infection. Ann Intern Med **1998**; 128:613–20.
24. Gorochov G, Neumann AU, Kereveur A, et al. Perturbation of CD4+ and CD8+ T-cell repertoires during progression to AIDS and regulation of the CD4+ repertoire during antiviral therapy. Nat Med **1998**; 4:215–21.
25. Kelleher AD, Carr A, Zaunders J, Cooper DA. Alterations in the immune response of human immunodeficiency virus (HIV)–infected subjects treated with an HIV-specific protease inhibitor, ritonavir. J Infect Dis **1996**; 173:321–9.
26. Autran B, Carcelain G, Li TS, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science **1997**; 277:112–6.
27. Connors M, Kovacs JA, Krevat S, et al. HIV infection induces changes in CD4+ T-cell phenotype and depletions within the CD4+ T-cell repertoire that are not immediately restored by antiviral or immune-based therapies. Nat Med **1997**; 3:533–40.
28. Lederman MM, Connick E, Landay A, et al. Immunologic responses associated with 12 weeks of combination antiretroviral therapy consisting of zidovudine, lamivudine, and ritonavir: results of AIDS Clinical Trials Group Protocol 315. J Infect Dis **1998**; 178:70–9.
29. Pakker NG, Kroon EDMB, Roos MTL, et al. Immune restoration does not invariably occur following long-term HIV-1 suppression during antiretroviral therapy. AIDS **1999**; 13:203–12.
30. Giorgi JV, Boumsell L, Autran B. Reactivity of workshop T-cell section mAb with circulating CD4+ and CD8+ T cells in HIV disease and following *in vitro* activation. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. Leukocyte Typing V. Vol. 1. Oxford: Oxford University Press, **1995**: 446–61.
31. Bofill M, Mocroft A, Lipman M, et al. Increased numbers of primed activated CD8+CD38+CD45RO+ T cells predict the decline of CD4+ T cells in HIV-1 infected patients. AIDS **1996**; 10:827–34.
32. Cossarizza A, Ortolani C, Mussini C, et al. Massive activation of immune cells with intact T cell repertoire in acute human immunodeficiency virus sydrome. J Infect Dis **1995**; 172:105–12.
33. Roederer M, Dubs JG, Anderson MT, Raju PA, Herzenberg LA, Herzenberg LA. CD8 naive T cell counts decrease progressively in HIV-infected adults. J Clin Invest **1995**; 95:2061–6.
34. Bell EB, Sparshott SM, Bunce C. CD4+ T-cell memory, CD45R subsets and the persistence of antigen—a unifying concept. Immunol Today **1998**; 19: 60–4.
35. Ogg GS, Jin X, Bonhoeffer S, et al. Quantitation of HIV-1–specific cytotoxic T lymphocytes and plasma load of viral RNA. Science **1998**; 279:2103–6.
36. Bass HZ, Hardy WD, Mitsuyasu RT, Wang YX, Cumberland W, Fahey JL. Eleven lymphoid phenotypic markers in HIV infection: selective changes induced by zidovudine treatment. J Acquir Immune Defic Syndr **1992**; 5: 890–7.
37. Bouscarat F, Levacher-Clergeot M, Dazza M-C, et al. Correlation of CD8 lymphocyte activation with cellular viremia and plasma HIV RNA levels in asymptomatic patients infected by human immunodeficiency virus type 1. AIDS Res Hum Retroviruses **1996**; 12:17–24.
38. Gray CM, Schapiro JM, Winters MA, Merigan TC. Changes in CD4+ and CD8+ T cell subsets in response to highly active antiretroviral therapy in HIV type 1-infected patients with prior protease inhibitor experience. AIDS Res Hum Retroviruses **1998**; 14:561–9.
39. Bouscarat F, Levacher M, Landman R, et al. Changes in blood CD8+ lymphocyte activation status and plasma HIV RNA levels during antiretroviral therapy. AIDS **1998**; 12:1267–73.
40. Liu Z, Cumberland WG, Hultin LE, Kaplan AH, Detels R, Giorgi JV. CD8+ T-lymphocyte activation in HIV-1 disease reflects an aspect of pathogenesis distinct from viral burden and immunodeficiency. J Acquir Immune Defic Syndr Hum Retrovirol **1998**; 18:332–40.
41. Ogg GS, Jin X, Bonhoeffer S, et al. Decay kinetics of human immunodeficiency virus–specific effector cytotoxic T lymphocytes after combination antiretroviral therapy. J Virol **1999**; 73:797–800.
42. Lynne JE, Schmid I, Matud JL, et al. Major expansions of select CD8+ subsets in acute Epstein-Barr virus infection: comparison with chronic human immunodeficiency virus disease. J Infect Dis **1998**; 177:1083–7.
43. Deacon NJ, Tsykin A, Solomon A, et al. Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. Science **1995**; 270:988–91.
44. Cavert W, Notermans DW, Staskus K, et al. Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. Science **1997**; 276: 960–4.
45. Dalod M, Fiorentino S, Delamare C, et al. Delayed virus-specific CD8+ cytotoxic T lymphocyte activity in an HIV-infected individual with high CD4+ cells counts: correlation with various parameters of disease progression. AIDS Res Hum Retroviruses **1996**; 12:497–506.
46. Fiorentino S, Dalod M, Olive D, Guillet J-G, Gomard E. Predominant involvement of CD8+CD28− lymphocytes in human immunodeficiency virus–specific cytotoxic activity. J Virol **1996**; 70:2022–6.
47. Morris L, Binley JM, Clas BA, et al. HIV-1 antigen–specific and –nonspecific B cell responses are sensitive to combination antiretroviral therapy. J Exp Med **1998**; 188:233–45.
48. Tenner-Racz K, Stellbrink HJ, van Lunzen J, et al. The unenlarged lymph nodes of HIV-1–infected, asymptomatic patients with high CD4 T cell counts are sites for virus replication and CD4 T cell proliferation. The impact of highly active antiretroviral therapy. J Exp Med **1998**; 187: 949–59.