Impact of HIV-1 infection and highly active antiretroviral therapy on the kinetics of $CD4^+$ and $CD8^+$ T cell turnover in HIV-infected patients

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To evaluate the effects of HIV infection on T cell turnover, we examined levels of DNA synthesis in lymph node and peripheral blood mononuclear cell subsets by using *ex vivo* labeling with BrdUrd. Compared with healthy controls ($n = 67$), HIV-infected patients ($n = 57$) had significant increases in the number and fraction of dividing $CD4^+$ and $CD8^+$ T cells. Higher percentages of dividing $CD4^+$ and $CD8^+$ T cells were noted in patients with the higher viral burdens. No direct correlation was noted between rates of T cell turnover and $CD4^+$ T cell counts. Marked reductions in $CD4^+$ and $CD8^+$ T cell proliferation were seen in 11/11 patients 1–12 weeks after initiation of highly active antiretroviral therapy (HAART). These reductions persisted for the length of the study (16–72 weeks). Decreases in naïve T cell proliferation correlated with increases in the levels of T cell receptor rearrangement excision circles. Division of $CD4^+$ and $CD8^+$ T cells increased dramatically in association with rapid increases in HIV-1 viral loads in 9/9 patients 5 weeks after termination of HAART and declined to pre-HAART-termination levels 8 weeks after reinitiation of therapy. These data are consistent with the hypothesis that HIV-1 infection induces a viral burden-related, global activation of the immune system, leading to increases in lymphocyte proliferation.

AIDS | proliferation | immune activation | T cell receptor rearrangement excision circles

HIV-1 infection is associated with a failure in T cell homeostasis, resulting in a gradual decline in $CD4^+$ T cell numbers. Studies examining lymphocyte turnover rates during infection with HIV or simian immunodeficiency virus have generated mixed results. Most of these discrepancies are likely the result of differences in the methods used to measure turnover rates, the use of longitudinal as opposed to cross-sectional cohorts, or the sensitivity and specificity of the assays used.

An initial study of lymphocyte turnover rates using ^{3}H-thymidine to label $CD4^+$ and $CD8^+$ T cells found increased turnover rates in patients with HIV-1 infection (1). Studies of lymphocyte turnover derived through analysis of immediate changes in $CD4^+$ T cell counts in the blood after highly active antiretroviral therapy (HAART) also led to estimates of high rates of CD4 turnover (2–4), although others have suggested that lymphocyte redistribution may be the major cause for CD4 increases immediately after HAART (5–8). Measurements of lymphocyte proliferation using Ki-67, BrdUrd, and ^{2}H-glucose have yielded varying results (9–19); however, the general consensus is that T cell proliferation is increased in HIV/simian immunodeficiency virus-infected subjects. Variations in these studies may be caused by the difference in sample numbers, cross-sectional versus longitudinal studies, patient cohort composition, and whether one is measuring the absolute or fractional level of cell division.

To gain better insight into the immunopathogenic effects of HIV-1 infection, and to attempt to resolve some of the controversy, lymphocyte turnover rates in healthy controls and patients with HIV-1 infection were determined by labeling of cells in the peripheral blood or lymph node with the thymidine analog BrdUrd. By directly measuring the kinetics of $CD4^+$ and $CD8^+$ T cell division longitudinally in cohorts of patients initiating HAART and voluntarily terminating HAART, we consistently found elevated $CD4^+$ and $CD8^+$ T cell division in patients infected with HIV-1 that directly correlated with viral load. Most striking were the findings that $CD4^+$ and $CD8^+$ division declined after treatment with HAART and rose sharply after cessation of HAART. The data presented here demonstrate a high rate of peripheral T cell turnover that is driven by HIV-1 infection.

Methods

Patients. Ninety patients with HIV-1 infection were enrolled in National Institute of Allergy and Infectious Diseases Institutional Review Board-approved protocols at the National Institutes of Health Clinical Center, and all signed informed consents. Patients were receiving combination antiretroviral chemotherapy including a protease inhibitor (HAART), except where noted. A more complete description of the cohort that included the nine patients in whom antiretroviral therapy was discontinued is provided elsewhere (16).

***Ex Vivo* BrdUrd Labeling and Flow Cytometric Methods.** The following assays were developed by using modifications of the procedure reported by Tough and Sprent (20). Lymph node suspensions (in RPMI + 10% human AB serum) or EDTA anticoagulant whole blood were incubated with 10 μM BrdUrd (Sigma) for 4 h at 37°C, 5% CO_2. Cell surface staining then was performed by using anti-human CD3 phycoerythrin PE (Becton Dickinson), or CD45RO phycoerythrin (Becton Dickinson) and anti-human CD4

Abbreviations: HAART, highly active antiretroviral therapy; TREC, T cell receptor rearrangement excision circle.

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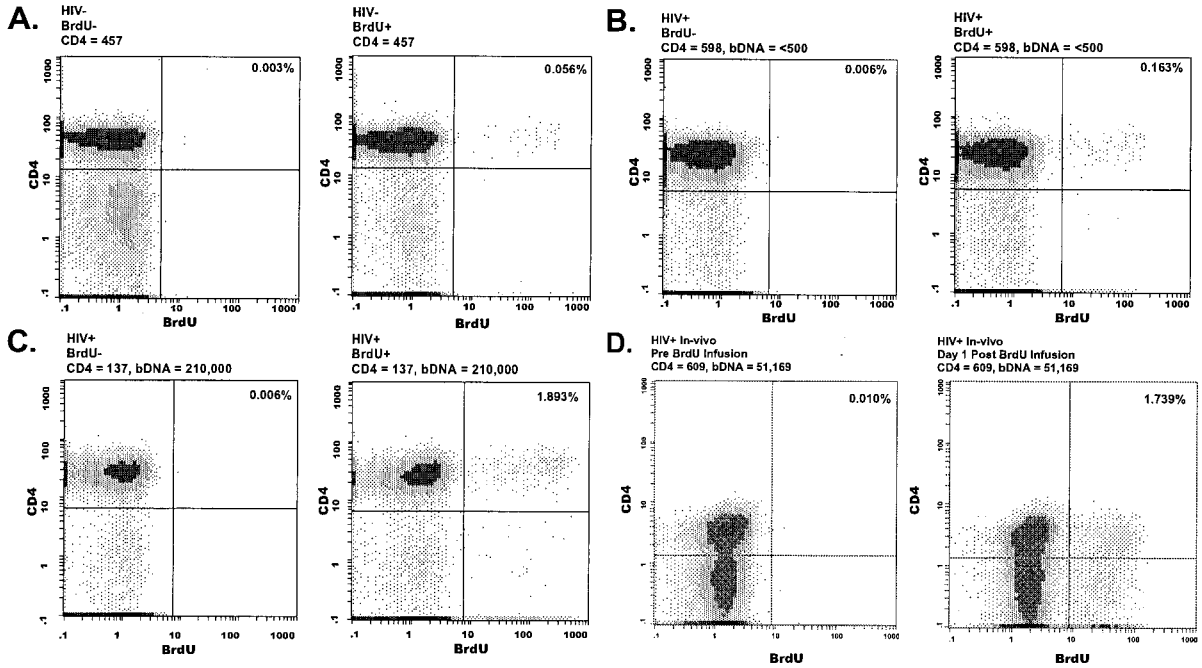

Fig. 1. Detection of BrdUrd-labeled CD4$^+$ cells. EDTA anticoagulated whole blood samples were drawn from an uninfected individual (*A*) and two patients infected with HIV-1, one with low viral load and high CD4$^+$ count (*B*) and one with high viral load, low CD4 count (*C*), and assayed for *ex vivo* BrdUrd incorporation into CD4$^+$ T cells. (*A–C*) Whole blood was incubated with (*Right*) and without (*Left*) BrdUrd for 4 h at 37°C. (*D*) BrdUrd incorporation into CD4$^+$ T cells of an individual with HIV-1 infection was determined before (*Left*) and 1 day after (*Right*) a 30-min infusion of BrdUrd (200 mg/m^2). Cell surface staining and flow cytometric analysis of *ex vivo*- and in *vivo*-labeled cells were performed by using the same procedure.

Cy-Chrome (PharMingen) or anti-human CD8 Cy-Chrome (PharMingen) antibodies. The stained cells were treated with OptiLyse C lysing solution (Immunotech, Westbrook, ME) for 10 min at room temperature, followed by incubation with 1% paraformaldehyde and 1% Tween-20 in 1× PBS for 15 min at 37°C to fix and permeablize the cells. Cellular DNA in the permeablized cells was partially digested with 100 Kunitz units of DNase-I (Boehringer Mannheim) in DNase buffer (1× PBS with 4.2 mM $MgCl_2$, pH 5) for 30 min at 37°C, then stained with anti-BrdUrd FITC (Becton Dickinson) antibody in 1× PBS containing 1% BSA and 0.5% Tween-20 for 30 min. Cells were washed twice before flow cytometric analysis. A total of 50,000–100,000 events were collected, resulting in a sensitivity of 0.01% BrdUrd$^+$ events. Intraassay and interassay variabilities were determined to be ±8.7% and ±17.6%, respectively (data not shown). Samples were analyzed in parallel with unlabeled cells (without BrdUrd) from the same individual, and this value was subtracted from the value obtained for BrdUrd-labeled cells. Data are presented as the percent of cells in the specific lymphocyte pool that are BrdUrd$^+$ or as the number of BrdUrd$^+$ T cells/μl of blood i.e., the product of the T cell blood count and the fraction of BrdUrd-labeled T cells. The words "dividing" and "proliferating" are used interchangeably in the text and refer to measurements made in the 4-h BrdUrd labeling assay above.

***In Vivo* BrdUrd Labeling.** In a preliminary set of experiments designed to examine the correlation between the *ex vivo* technique described above and *in vivo* turnover rates, thirteen patients received a 30-min infusion of BrdUrd (200 mg/m^2; NeoPharm, Bannockburn, IL). Of note, the National Cancer Institute has sponsored *in vivo* BrdUrd studies to measure cell cycle kinetics in over 4,000 patients with no significant toxicities reported (21). The plasma half-life of BrdUrd is approximately 8–11 min (data not shown). Blood samples were collected immediately before and serially after the administration of BrdUrd, and BrdUrd intensity was determined by flow cytometry using the technique described for *ex vivo* staining of peripheral blood mononuclear cells. Levels of *ex vivo* BrdUrd incorporation into T cells then were compared with peak levels of *in vivo* BrdUrd incorporation, which occurred between days 1 and 2 after BrdUrd infusion. Although readily detectable, the intensity of cells labeled *in vivo* with BrdUrd was approximately one-third of cells labeled *ex vivo* (see Fig. 1).

T Cell Receptor Rearrangement Excision Circle (TREC) Measurements. TREC levels in peripheral blood mononuclear cells were measured by quantitative competitive PCR similar to the procedure described by Douek *et al.* (22). Briefly, varying copies of a competitive internal standard DNA were added to equal aliquots of DNA from patient samples and amplified for 35 cycles. PCR products were labeled with ^{32}P-dCTP during amplification and were separated in a 6% polyacrylamide gel. The radioactivity level present in each band was quantitated as photo-stimulated luminescence (PSL) by using a Fuji Medical Systems BAS1000 phosphoimager. TREC copy numbers were determined by using a linear regression equation of the ratio of PSL for the patient sample to internal standard versus the copy number of the competitive internal standard.

The number of TRECs per million naïve T cells was determined for each peripheral blood mononuclear cell sample by the following equation: (# of TRECs/10^6 peripheral blood mononuclear cells) × (lymphocyte count + monocyte count)/([CD4 count × %CD4$^+$CD45RO$^-$ cells) + (CD8 count × %CD8$^+$CD45RO$^-$ cells]). The fraction of BrdUrd$^+$ naïve T cells was determined by the following equation: (CD4 count × %CD4$^+$CD45RO$^-$ cells × %CD4$^+$CD45RO$^-$BrdUrd$^+$ cells) + (CD8 count × %CD8$^+$CD45RO$^-$ cells × %CD8$^+$CD45RO$^-$-BrdUrd$^+$)/(CD4 count × %CD4$^+$CD45RO$^-$ cells) + (CD8 count × %CD8$^+$CD45RO$^-$ cells).

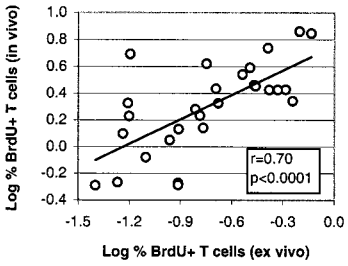

Fig. 2. *In vivo* BrdUrd incorporation in T cells correlates with *ex vivo* BrdUrd incorporation. Thirteen patients received a 30-min infusion of BrdUrd (200 mg/m^2), and levels of peak *in vivo* BrdUrd incorporation into CD4$^+$ and CD8$^+$ T cells were compared with levels obtained from *ex vivo* BrdUrd staining performed immediately before the BrdUrd infusion. The correlation is shown for the combination of CD4$^+$ ($r = 0.70$, $P = 0.005$) and CD8$^+$ ($r = 0.73$, $P = 0.003$) T cells.

HIV-1 Viral Load Measurements. Sample collection, processing, and HIV-1 quantitation (Quantiplex bDNA assay, Bayer, Norwood, MA) were performed as described (23).

Statistical Analysis. Methods for determining significance and correlation coefficients are indicated in the individual figure legends. Data were analyzed by using SIGMASTAT (Jandel, San Rafael, CA) or STATISTICA (StatSoft, Tulsa, OK) software packages.

Results

Increased T Lymphocyte Proliferation in Patients with HIV-1 Infection. Incorporation of the thymidine analog BrdUrd after 4 h of labeling was used to directly measure the relative and absolute numbers of lymphocytes progressing through S phase of the cell cycle in peripheral blood and lymph node mononuclear cells. BrdUrd incorporation into the DNA of dividing lymphocyte subsets was quantified by flow cytometry after 4 h of labeling. Fig. 1 shows representative histograms detecting BrdUrd incorporation into the DNA of dividing peripheral CD4$^+$ T cells from an uninfected individual, a patient with early HIV disease, and a patient with advanced HIV disease. Similar histograms were generated for CD8$^+$ T cells, CD45RA$^+$ and CD45RO$^+$ T subsets, and B cells (data not shown). The high sensitivity (<0.01% BrdUrd$^+$ cells) of this assay derives from analysis of large numbers of events (50,000 to 100,000), strong anti-BrdUrd staining of labeled cells, and low background binding of anti-BrdUrd antibody to unlabeled cells. As displayed in Fig. 2, the fraction of dividing CD4$^+$ and CD8$^+$ T cells determined by this method correlated well ($r = 0.70$, $P < 0.0001$) with the peak fraction of T cells labeled *in vivo* after a 30-min infusion of BrdUrd into patients with HIV infection. Overall levels of *ex vivo* BrdUrd incorporation were approximately 1/10 the levels determined *in vivo*. These findings demonstrate that *ex vivo* BrdUrd incorporation is a good reflection of relative levels of lymphocyte proliferation *in vivo*.

Table 1. Study group characteristics

Patient group	Viral load*	CD4 count†	CD8 count†
Uninfected ($n = 67$)	NA	784	429
HIV infected ($n = 57$)	54,573	339	874
Early ($n = 34$)	1,480	390	716
Intermediate ($n = 24$)	82,286	367	860
Advanced ($n = 9$)	212,033	107	1,006

Early: >200 CD4 cells/μl, <10,000 RNA copies/ml. Intermediate: >200 CD4 cells/μl >10,000, RNA copies/ml. Late: <200 CD4 cells/μl, >10,000 RNA copies/ml. NA, not applicable.
*Geometric mean; RNA copies/ml plasma.
†Arithmetic mean; cells/μl of blood.

The fraction and number of dividing T cells were determined in 67 healthy controls and 57 patients with HIV-1 infection (Fig. 3). Characteristics of the study groups are shown in Table 1. The mean fraction of dividing CD4$^+$ and CD8$^+$ cells was approximately 3- to 4-fold higher in patients with HIV-1 infection than controls. A less dramatic, but significant ($P = 0.018$), increase in the total number of BrdUrd-labeled CD4$^+$ T cells/μl of blood was seen for patients with HIV-1 infection compared with controls. This less dramatic increase in the number of dividing cells/μl of blood was the result of lower total CD4$^+$ T cell counts in the patients with HIV-1 infection compared with the controls. In contrast, there was nearly an 8-fold increase in the total number of dividing CD8$^+$ T cells/μl of blood in patients with HIV-1 infection compared with the controls ($P < 0.001$). Increases in the fraction of dividing CD4$^+$ T cells correlated with increases in the fraction of dividing CD8$^+$ T cells in patients with HIV-1 infection (data not shown; $r = 0.72$, $P < 0.001$).

CD45RO$^+$ T Cells Divide More Frequently than CD45RO$^-$ T Cells. As expected, both HIV-negative and HIV-positive donors had higher BrdUrd incorporation into the CD45RO$^+$ subsets of CD4$^+$ and CD8$^+$ T cells compared with the corresponding CD45RO$^-$ subsets (Fig. 3). The percentages of dividing CD4$^+$CD45RO$^+$ and CD8$^+$CD45RO$^+$ cells were significantly greater in cells from patients with HIV-1 infection than in healthy controls. Increased T cell proliferation also was found in the CD45RO$^-$ subset of CD4$^+$ and CD8$^+$ T cells from patients with HIV-1 infection compared with controls. Thus, changes in CD4$^+$ and CD8$^+$ T cell proliferation were reflected proportionately in both the CD45RO$^-$ and CD45RO$^+$ subsets of T cells.

Lymphocyte Proliferation Is Greater in the Lymph Node than in the Blood. To determine the degree to which measurements of peripheral blood lymphocytes reflected changes in lymphoid

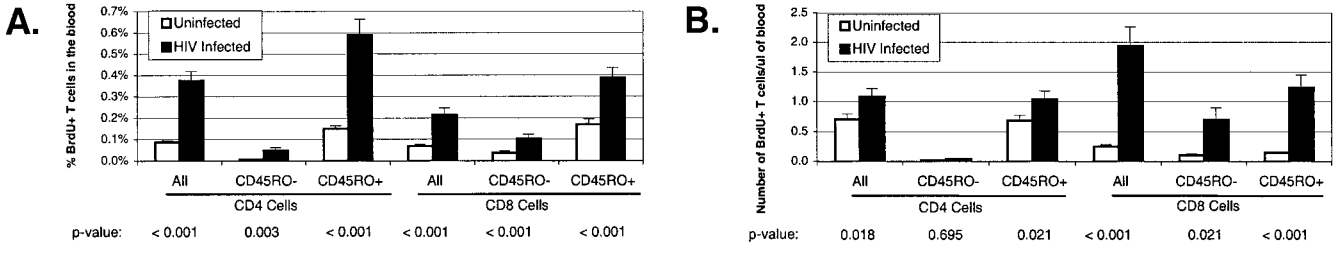

Fig. 3. The mean fraction (*A*) and number (*B*) of proliferating CD4$^+$ and CD8$^+$ T cells in patients with HIV-1 infection (closed bars) compared with healthy controls (open bars). *Ex vivo* BrdUrd incorporation was determined in CD4$^+$ or CD8$^+$ T cells in 67 uninfected controls and 57 HIV-infected individuals (see Table 1). The number of dividing CD4$^+$ and CD8$^+$ T cells/μl of blood was determined by multiplying the T cell count by the fraction of BrdUrd-marked cells. Error bar = SEM. *P* values for significance (Mann–Whitney Rank Sum Test) between healthy controls and patients with HIV-1 infection are indicated below each graph.

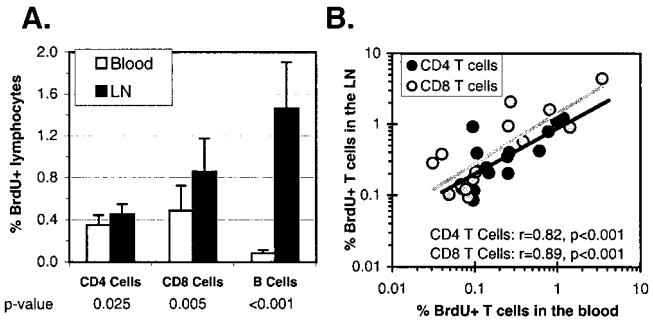

Fig. 4. $CD4^+$, $CD8^+$, and B lymphocyte proliferation rates in lymph node and peripheral blood (*A*). Correlation between rates of proliferation in peripheral blood and lymph node (*B*). The fractions of proliferating T cells in the blood and lymph nodes were compared in 15 patients with HIV-1 infection. Data for B cell division were obtained for 10 of these patients. Error bar = SEM. *P* values for significance also were determined by using Wilcoxon Signed Rank Tests.

tissues, simultaneous evaluations were performed on peripheral blood and lymph node mononuclear cells from 15 patients with HIV-1 infection. The fraction of proliferating cells was consistently higher in lymph node cells compared with peripheral blood for $CD4^+$ T cells ($P = 0.025$), $CD8^+$ T cells ($P = 0.005$), and B cells ($P < 0.001$; Fig. 4*A*). The fraction of proliferating $CD4^+$ and $CD8^+$ T cells in the peripheral blood correlated well with the fraction of dividing $CD4^+$ ($r = 0.82$, $P < 0.001$) and $CD8^+$ ($r = 0.89$, $P < 0.001$) T cells, respectively, in the lymph node (Fig. 4*B*), suggesting that changes in peripheral blood T cell division reflect changes in lymph node T cell division.

T Cell Proliferation Is Linked to Disease Status. A higher fraction of dividing $CD4^+$ and $CD8^+$ T cells was observed in the peripheral blood of patients with higher viral burdens and lower CD4 T cell counts (Fig. 5*A*; data not shown for $CD8^+$ T cells). However, the absolute number of dividing $CD4^+$ T cells/μl in blood was similar in healthy controls and in patients with early or advanced HIV infection and increased in patients with intermediate-stage HIV infection (Fig. 5*B*). Because proliferation increases and CD4 counts fall with disease progression, these results are the expected consequence of the product of two vectors moving in opposite directions. In other words, the size of the $CD4^+$ T cell pool is continuously decreasing as the rate of $CD4^+$ T cell proliferation is continuously increasing. Thus, if one compares total CD4 T cell production for a group of patients with advanced disease to a group of patients with intermediate disease, one might conclude that T cell production was decreased as a consequence of HIV infection. Examining a fuller spectrum of disease illustrates the point that a progressive increase in turnover rate in the context of a declining pool size first leads to an increase, then a decrease, in total T cell production as HIV disease progresses (Fig. 5*B*).

T Cell Division Correlates with HIV-1 Viral Load. Higher viral loads and lower CD4 counts both were associated with increased $CD4^+$ (Fig. 6 *A* and *B*) and $CD8^+$ T cell proliferation (data not shown). After adjusting for CD4 count (Fig. 6*C*), viral load remained significantly predictive of the fraction of dividing $CD4^+$ T cells ($r = 0.62$, $P < 0.001$). However, after adjusting the data for viral load, CD4 count was no longer predictive ($r = -0.15$, $P = 0.134$; Fig. 6*D*). Thus, cell proliferation appears to be driven as a direct consequence of viral load.

Reductions in T Lymphocyte Proliferation Follow Initiation of HAART. Eleven patients initiating HAART were followed longitudinally for *ex vivo* incorporation of BrdUrd into $CD4^+$ (Fig. 7) and $CD8^+$ T cells (data not shown) for 16–72 weeks. The pre-HAART viral load was 89,332 (geometric mean), and the mean pre-HAART fraction of dividing $CD4^+$ T cells was 8.2-fold greater than that of healthy controls (data not shown). Dramatic and significant reductions in the fraction of dividing $CD4^+$ T cells were observed within 2 weeks of initiating therapy (Fig. 7 *A* and *B*). This immediate reduction was seen in 8/10 patients. Data were not obtained for one patient during this 2-week period. T cell proliferation for the other patient did not fall immediately; however, after the first 12 weeks, slower declines in proliferation were seen in all patients (Fig. 7 *A* and *B*). Significant declines in the fraction ($P < 0.001$) and number ($P = 0.002$) of dividing $CD4^+$ T cells in blood also were seen in the $CD8^+$ T cell pool (data not shown). These changes in proliferation were equally reflected in the $CD45RO^-$ and $CD45RO^+$ subsets of T cells (data not shown). The decreases in $CD4^+$ T lymphocyte division after HAART occurred in the setting of moderate rises in CD4 counts (data not shown) and sharp declines in viral load (Fig. 7 *A* and *C*). To determine whether or not these declines in T cell proliferation might be at least partially responsible for the increases in levels of TRECs that have been reported after initiation of HAART (22), levels of TRECs were measured before and 24 weeks after the initiation of HAART in nine of the 11 patients. A strong correlation ($r = -0.98$; $P = <0.001$) was noted between decreases in $CD45RO^-$ T cell turnover and increases in levels of TRECs (Fig. 7*D*). To test the stability of the regression, each data point was removed one at a time and the regression analysis was performed with the remaining eight points. In all cases, $r < -0.80$ and $P < 0.02$, suggesting that the significance of the correlation did not depend on any one data point.

Increases in T Cell Proliferation after Discontinuation of HAART. Nine patients with <50 HIV RNA copies/ml plasma for at least 1 year and who voluntarily terminated HAART were followed longitudinally for 5 weeks for changes in T cell proliferation. The average baseline proliferation levels for these treated patients before terminating HAART were not significantly different from healthy controls ($P = 0.923$; data not shown). Significant increases in the fraction of dividing $CD4^+$ (5.9-fold, $P = 0.008$)

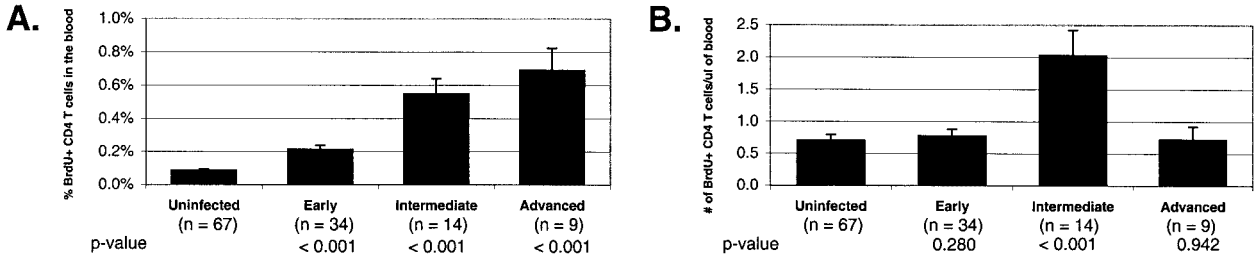

Fig. 5. Changes in the fraction (*A*) and the total number (*B*) of dividing $CD4^+$ T cells in patients with HIV-1 infection as a function of disease progression. *P* values (Mann–Whitney Rank Sum Test) relative to uninfected controls are indicated. Patient groups are defined in Table 1. Error bar = SEM.

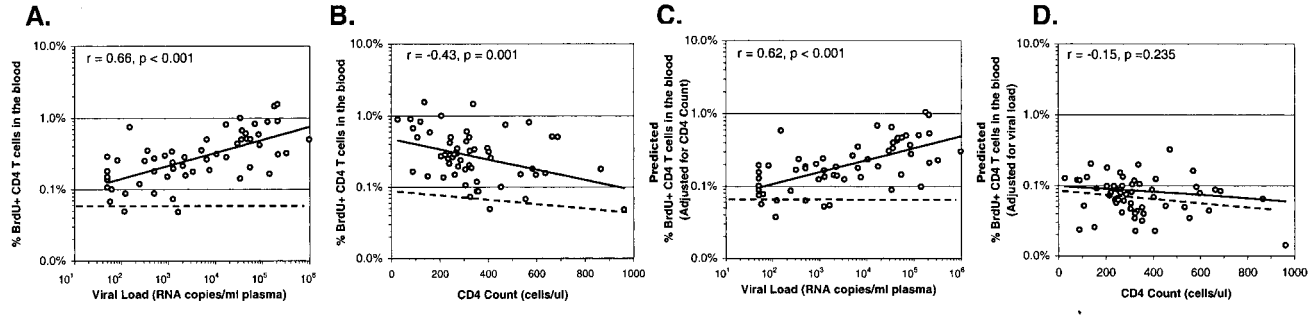

Fig. 6. Relationships between fraction of dividing $CD4^+$ T cells, viral load, and total $CD4^+$ T cell count. The fraction of dividing $CD4^+$ T cells correlates most strongly with viral load (*A*). This relationship was still maintained after normalizing CD4 count for each patient to 800 cells/μl (*C*). The fraction of dividing $CD4^+$ cells also correlated inversely with CD4 count (*B*). This relationship was lost after normalizing viral load for each patient to 0 RNA copies/ml (*D*). A multiple linear regression analysis (see *C* and *D*) was conducted to determine whether viral load and CD4 count were independently predictive of $CD4^+$ BrdUrd incorporation rates. The multiple linear regression equation was found to be: Log_{10}(% $CD4^+$BrdUrd$^+$ cells) = [Log_{10}(viral load) $\times$ 0.1652] $-$ [(CD4 Count)*0.0003] $-$ 3.0464; r = 0.67, P value (viral load) $<$ 0.001, P value (CD4 count) = 0.13. The predicted $CD4^+$ BrdUrd incorporation rate if CD4 counts were normalized to 800 cells/μl was calculated by the following equation: Log_{10}(% CD4 + BrdUrd$^+$ cells) $-$ [(800 cells/μl $-$ patient's CD4 count)*0.0003]; the predicted $CD4^+$ BrdUrd incorporation rate if viral loads were normalized to 0 RNA copies/ml was calculated by the following equation: Log_{10}(% CD4 + BrdUrd$^+$ cells) $-$ (patient's viral load)*0.1652. The dashed lines in *A* and *C* represent the average BrdUrd incorporation rate for $CD4^+$ T cells from healthy controls. The dashed lines in *B* and *D* represent the best-fit line through the healthy control data points. Correlation coefficients and *P* values were determined by using Spearman Rank Order Correlation analysis.

and $CD8^+$ (11.2-fold, $P = 0.008$) T cells and number of dividing $CD4^+$ (3.5-fold, $P = 0.004$) and $CD8^+$ (10.7-fold, $P = 0.004$) T cells were observed 5 weeks after HAART termination (Fig. 8 *A* and *B*). During the 5-week period, the mean viral load for this cohort increased from <50 to 57,703 RNA copies/ml plasma (geometric mean). Despite increased $CD4^+$ T cell proliferation, CD4 counts declined from an average of 1,155 to 806 cells/μl, indicating even a greater increase in the number of cells dying. To determine whether or not these changes might be secondary to redistribution of cells, simultaneous measurements of BrdUrd incorporation into lymph node and peripheral blood were made in two individuals before and after stopping therapy. Similar increases in proliferation were observed in the lymph node and peripheral blood, suggesting that a selective redistribution of dividing $CD4^+$ T cells to the peripheral blood was not responsible for these changes in proliferation (Fig. 8*C*).

All nine patients reinitiated HAART 5–12 weeks after cessation of HAART. Eight weeks after the reinitiation of therapy, the number (data not shown) and fraction (Fig. 8*D*) of dividing $CD4^+$ and $CD8^+$ T cells significantly declined ($P = 0.004$) to levels similar to those observed before terminating HAART. During this period, the mean viral load decreased from 90,333 to 189 RNA copies/ml plasma (geometric means).

Discussion

Recent years have witnessed considerable debate on the topic of T cell turnover in patients with HIV infection. The present study, by providing a cross-sectional comparison of patients with HIV-1 infection to healthy controls, along with a longitudinal study of patients initiating or terminating HAART, strongly supports the hypothesis that $CD4^+$ and $CD8^+$ T cell division rates are increased in the setting of HIV-1 infection and decrease after therapy. The finding that HIV RNA plasma levels correlate with levels of T cell proliferation strongly suggests that polyclonal lymphocyte activation and T cell division are driven by the level of viral replication. Blocking HIV-1 replication with HAART leads to decreased

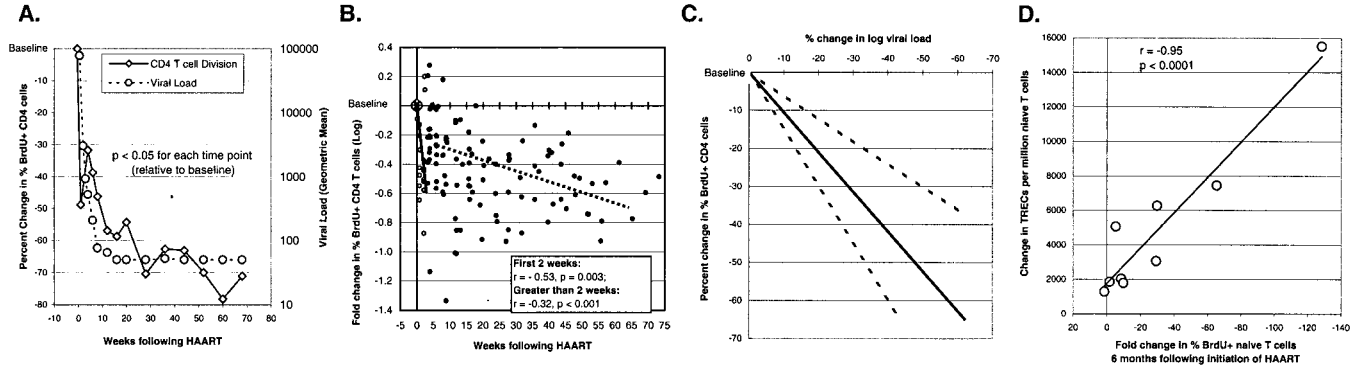

Fig. 7. The fraction of proliferating $CD4^+$T cells decline after HAART. The incorporation of BrdUrd into $CD4^+$T cells was monitored serially in 11 patients with HIV-1 infection after the initiation of HARRT. (*A*) The average percent decline from baseline measurements were determined for each of the indicated time points (◇) after initiation of HAART. Significance was determined by using the Wilcoxon Signed Rank Test. (*B*) For each patient, the fraction of dividing $CD4^+$ T cells was normalized to the baseline value (day 0), transformed to the $\log_{10}$, and plotted against time. Spearman Rank Correlation coefficients and *P* values are shown for changes in the first 2 weeks (solid line; ○) and for the weeks thereafter (dashed lines; ●) after initiation of HAART. (*C*) Declines in $CD4^+$ T cell proliferation parallel declines in viral load. Best-fit lines through the data points for percent change in percent BrdUrd$^+$ $CD4^+$ T cells versus percent change in log (RNA copies/ml plasma) were generated for each patient. The solid line represents the average change. The dotted lines define one standard deviation. (*D*) Changes in TREC levels in PBMCs (expressed in terms of TRECs per 10^6 $CD45RO^-$ T cells) from nine patients before initiation and 6 months after initiation of HAART were compared with changes in the fraction of BrdUrd$^+$ $CD45RO^-$ T cells observed during the same time period.

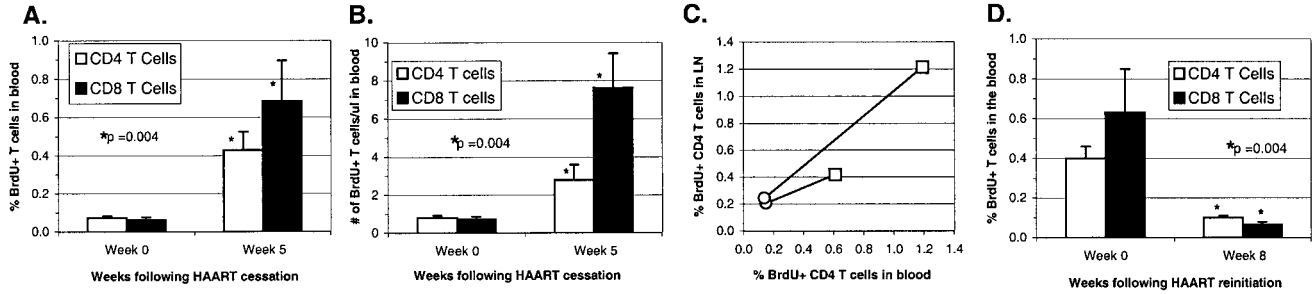

Fig. 8. Rates of BrdUrd incorporation into T cells of nine patients who had viral loads <50 RNA copies/ml plasma for longer than 1 year were determined pretermination and 5 weeks posttermination of HAART. Increases in the mean fraction (*A*) and number (*B*) of dividing $CD4^+$ and $CD8^+$ T cells were observed after cessation of HAART. (*C*) Similar increases in proliferation were observed in both the blood and lymph node in the two patients in whom both compartments were examined pretermination (○) and 5 weeks posttermination (□) of HAART. (*D*) The nine patients above each reinitiated HAART, and BrdUrd incorporation into T cells was determined pre-HAART and 8 weeks post-HAART. Error bars = SEM. *P* values were determined by using the Wilcoxon Signed Rank Test and reflect comparisons between week 0 and week 5 or 8.

immune activation, cell division, and cell death. By treating HIV-1 infection with HAART, although one sees decreases in $CD4^+$ T cell division, there is an increase in the $CD4^+$ T cell count as the rate of $CD4^+$ T cell production (although now decreased) exceeds the rate of cell death. Conversely, after cessation of HAART, although one sees increases in $CD4^+$ T cell division, T cell counts decline as cell death exceeds cell production as the viral load rebounds. It is unclear whether increased T cell proliferation during HIV-1 infection is caused by HIV antigen-specific expansion, stimulation of T cells by cross-reactive antigens, or cytokine-mediated bystander activation.

Our results are consistent with a number of studies finding 2- to 6-fold higher $CD4^+$ and $CD8^+$ T cell proliferation in subjects with HIV or simian immunodeficiency virus infection (9–13, 15–19), suggesting a global activation of the immune system caused by HIV/simian immunodeficiency virus replication. Our longitudinal studies showing declines in both fractional and absolute T cell proliferation after initiation of HAART and increases after cessation of HAART in parallel to changes in viral load clearly show that T cell proliferation is directly correlated with viral replication. These results are supported by some studies with Ki-67 (10, 17), however, other studies have data to the contrary (14, 15, 18, 19), and suggest that HIV replication blocks the ability of new $CD4^+$ T cells to regenerate. A major reason for these discrepancies may be the inherent differences between longitudinal as opposed to cross-sectional studies. Longitudinal studies, pre- and post-HAART, with fine *in vivo* labeling kinetics are required to resolve this issue.

It has been reported that levels of TRECs in peripheral T cells are lower in patients with HIV-1 infection than healthy controls and increase after initiation of HAART. These data have been interpreted as a demonstration that HIV-1 infection impairs thymic function in a reversible manner (22). However, levels of TRECs in peripheral blood T cells may not accurately reflect changes in thymic function because the combination of rates for thymic output, peripheral naive T cell proliferation, and peripheral T cell death will influence TREC levels in the blood (24). Our findings showing a strong inverse correlation between changes in naive T cell proliferation and changes in the levels of TRECs ($r^2 = 0.9$) indicate that elevated T cell proliferation in general, as opposed to impaired thymic function, may be the major reason for lower TREC levels in patients with HIV-1 infection.

Based on the data presented here, we conclude that there is an increase in the fraction of dividing $CD4^+$ and $CD8^+$ T cells in patients with HIV-1 infection and that this is the direct result of global immune activation caused by HIV-1 replication in the host. The fact that $CD4^+$ T cell counts decline during the course of HIV infection suggests that the increased destruction of $CD4^+$ T cells in the setting of HIV-1 infection is greater than this increased production. Therapies that shift the cell division rate above that of the cell death rate, e.g., IL-2, or decrease the cell death rate below that of the cell production rate, e.g., HAART, should lead to an increase in the size of the CD4 pool in patients with HIV-1 infection. The clinical benefit of this has been established for HAART and is being examined in two phase III studies of IL-2.

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