Long-term kinetics of T cell production in HIV-infected subjects treated with highly active antiretroviral therapy

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The long-term kinetics of T cell production following highly active antiretroviral therapy (HAART) were investigated in blood and lymph node in a group of HIV-infected subjects at early stage of established infection and prospectively studied for 72 wk. Before HAART, CD4 and CD8 T cell turnover was increased. However, the total number of proliferating $CD4^+$ T lymphocytes, i.e., $CD4^+Ki67^+$ T lymphocytes, was not significantly different in HIV-infected (n = 73) and HIV-negative (n = 15) subjects, whereas proliferating $CD8^+Ki67^+$ T lymphocytes were significantly higher in HIV-infected subjects. After HAART, the total body number of proliferating $CD4^+Ki67^+$ T lymphocytes increased over time and was associated with an increase of both naive and memory $CD4^+$ T cells. The maximal increase (2-fold) was observed at week 36, whereas at week 72 the number of proliferating $CD4^+$ T cells dropped to baseline levels, i.e., before HAART. The kinetics of the fraction of proliferating CD4 and CD8 T cells were significantly correlated with the changes in the total body number of these T cell subsets. These results demonstrate a direct relationship between *ex vivo* measures of T cell production and quantitative changes in total body T lymphocyte populations. This study provides advances in the delineation of the kinetics of T cell production in HIV infection in the presence and/or in the absence of HAART.

Several studies have recently demonstrated the important role of both HIV-specific CD4 helper (1) and CD8 cytotoxic (2–5) T cell responses in the control of HIV replication, and particular attention has recently been focused on the extent of immune reconstitution that can be achieved following the initiation of highly active antiretroviral therapy (HAART) (2, 3, 5–11). Initiation of HAART (12, 13) is generally associated with a rapid suppression of virus replication, an increase of the CD4 T cell count, and the recovery of a number of antigen-specific proliferative responses against a series of pathogens (6, 8, 9, 11). However, HIV-specific CD4 T cell responses are rarely recovered (9).

The quantitative increase of CD4 T cells observed after HAART has important clinical implications. Indeed, patients in whom CD4 T cell count increased above 200 cells per μl may safely interrupt prophylactic therapy against opportunistic pathogens (14–16). Therefore, it is important to delineate the kinetics of CD4 T cell recovery after HAART. Several strategies have recently been developed to monitor the production of T cells: *ex vivo* assessment of T cells that have entered into the cell cycle (17, 18) by measurement of the expression of the Ki67 nuclear antigen (19, 20); *in vivo* and/or *ex vivo* cell labeling with bromodeoxyuridine (21, 22); and direct measurement of the half-life and of the production rate of CD4 T cells *in vivo* upon i.v. administration of the stable isotope-labeled metabolite [^{2}H]glucose (23). Furthermore, two separate studies, one using the Ki67 antigen (18) and the other one using [^{2}H]glucose (23), have provided substantial evidences that CD4 T cell lymphopenia observed in HIV disease is due to both a failure to increase the production of circulating CD4 T cells and to a shortened survival time, and that CD4 T cell production is restored following initiation of HAART (18, 23).

However, long-term kinetics of T cell production following HAART have not been defined yet. In the present study, longitudinal analysis of T cell production was monitored in blood and lymph nodes in a group of 24 HIV-infected subjects treated with HAART for 72 wk. The relationships between the changes in the number of proliferating, i.e., $Ki67^+$, $CD4^+$, and $CD8^+$ T cells and the correspondent changes in T cell count in blood and percentage in lymph nodes, and in total body number of $CD4^+$ and $CD8^+$ T cells have been analyzed.

Methods

Study Populations. Fifteen HIV-negative subjects (mean age 49 years) with a mean blood CD4 count of 856/μl and 73 HIV-infected subjects (mean age 35.3 years) with a mean blood CD4 count of 685/μl were studied for T cell production. HIV-negative subjects underwent vascular and general surgery, and lymph nodes were collected at the time of the surgical intervention. HIV-infected subjects were enrolled in two therapeutic clinical trials involving combination therapy with an inhibitor of reverse transcriptase (Abacavir, Glaxo Wellcome) and different protease inhibitors [Amprenavir (Glaxo Wellcome), Nelfinavir (Agouron Pharmaceuticals, La Jolla, CA), and Saquinavir (Roche, Gipf-Oberfrick, Switzerland) soft gel capsules]. The mean time for HIV infection was 4.1 $\pm$ 3.4 years. The inclusion criteria for these trials were: (*i*) no previous history of antiviral therapy; (*ii*) CD4 cell count >250/μl; and (*iii*) plasma viremia >5,000 HIV RNA copies per ml. Twenty-four subjects completed 72 wk of HAART with Abacavir plus Amprenavir. According to the protocol guidelines, patients underwent excisional lymph node biopsy at baseline and at week 72 (end of study) and ultrasound-guided lymph node aspirations (24) at weeks 24, 36, and 48. These studies were performed under approval of the Institutional Review Board of the Centre Hospitalier Universitaire Vaudois.

Isolation of Mononuclear Cells from Blood and Lymph Nodes. Blood and lymph node mononuclear cells were isolated as previously described (18).

Abbreviation: HAART, highly active antiretroviral therapy.

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Flow Cytometry. Flow cytometry analysis was performed on freshly isolated blood and lymph node mononuclear cells as previously described (18). The percentage of blood and lymph node CD4 and CD8 cells was determined by the Tritest from Becton Dickinson (anti-human CD4-fluorescein isothiocyanate/CD8-PE/CD3-PerCP) and analyzed on a FACSCalibur. The fraction of dividing T cells was assessed by the combination of surface staining followed by intracellular staining using CD4-PE and CD8-PerCP antibodies and anti-human Ki67-fluorescein isothiocyanate (Immunotech, Marseille, France).

Calculation of Fractions of Proliferating $CD4^+$ and $CD8^+$ T Lymphocytes. The total number of circulating $CD4^+$ and $CD8^+$ T lymphocytes and of the fractions of proliferating cells within these cell subsets were calculated as previously described (18). In brief, based on hematological measures, i.e., white blood cell counts and percentage of lymphocytes, the number of total circulating lymphocytes was calculated taking into account that the blood volume in an adult subject is ≈5 liters. Therefore, by multiplying the number of lymphocytes per microliter times $10^6 \times 5$, we obtained estimates of the total lymphocyte population. The total numbers of circulating $CD4^+$ and $CD8^+$ T cells and of dividing T cells, i.e., $Ki67^+CD4^+$ and $Ki67^+CD8^+$ cells, were calculated from the percentages of these cell subsets in the total lymphocyte population as determined by cytofluorometry. The total number of lymphocytes in lymph nodes was calculated by multiplying the number of circulating lymphocytes, which represent 2% (25, 26) of the total lymphocyte population, by 49, assuming that lymphocytes in lymph nodes are representative of 98% of total lymphocytes. The total numbers of $CD4^+$ and $CD8^+$ T cells and of dividing cells, i.e., $Ki67^+CD4^+$ and $Ki67^+CD8^+$ cells, were calculated from the percentages of these cell subsets in lymph nodes as assessed by cytofluorometry.

Statistical Analysis. The differences in the means of measures in HIV-infected and in HIV-negative adults were compared by the *F* test and Student's *t* test as described (27). Total cell numbers were computed by multiplying the corresponding percentages with the estimated peripheral blood lymphocyte count. Therefore, to compare the means of the total cell numbers and of the fractions of proliferating T cell subsets between groups before and following HAART, the same tests were used after taking the logarithm of the data. Correlation between changes in the output of $CD4^+$ and $CD8^+$ T cells and changes in total cell numbers over the baseline following HAART was tested by Pearson's test. A *P* value (two-tailed) of <0.05 was considered significant.

Results

Analysis of T cell Production in $CD4^+$ and $CD8^+$ T Cell Subsets in the Absence of HAART. In a previous study (18), we reported the analysis of T cell production in the $CD4^+$ and $CD8^+$ T cell subsets from blood and lymph nodes of a group of HIV-negative ($n = 8$) and a cohort of HIV-infected subjects ($n = 22$). Here, the analysis has been performed on a substantial larger number of both HIV-negative ($n = 15$) and HIV-infected subjects ($n = 73$). All HIV-infected subjects studied were naive to antiretroviral therapy and at early stages of established infection and, according to the clinical protocol, underwent excisional lymph node biopsy before the initiation of treatment. Production of blood and lymph node $CD4^+$ and $CD8^+$ T cell subsets was estimated by the analysis of the expression of the Ki67 nuclear antigen. Ki67 antigen is expressed at different levels during G_1, S, G_2, and M phases of the cell cycle (19, 20). Therefore, one cannot exclude that cycling cells with low levels of expression of Ki67 may be missed by this analysis, as suggested by Hellerstein (28). However, the findings (see below) that estimates of T cell production obtained with the Ki67 technology were comparable

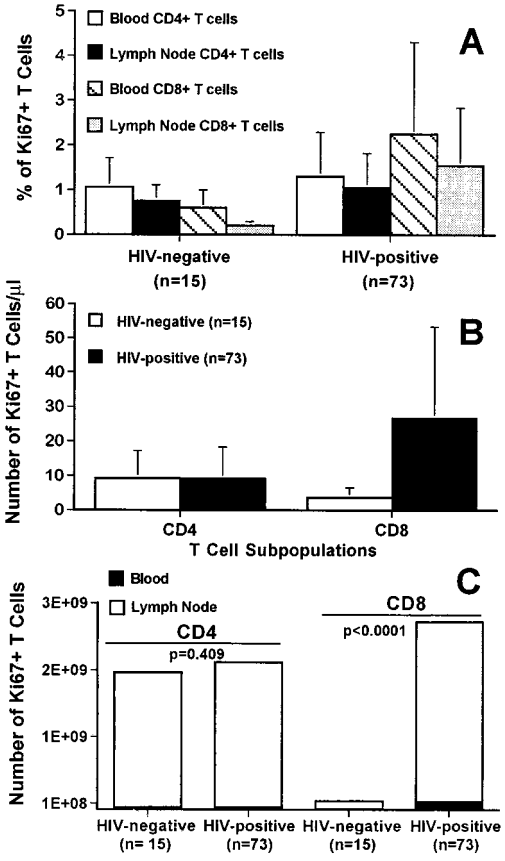

Fig. 1. Distribution of Ki67 nuclear antigen in blood and lymph node CD4 and CD8 T lymphocyte subsets. (*A*) Percentage of $Ki67^+CD4^+$ and $Ki67^+CD8^+$ T lymphocytes in blood and lymph node of HIV-negative ($n = 15$) and HIV-infected ($n = 73$) subjects before HAART. (*B*) Estimates of the number of $Ki67^+CD4^+$ and $Ki67^+CD8^+$ T lymphocytes per microliter of blood in HIV-negative and HIV-infected subjects before HAART. (*C*) Estimates of the total number (blood plus lymph node) of $Ki67^+CD4^+$ and $Ki67^+CD8^+$ T lymphocytes in HIV-negative and HIV-infected subjects. The fractions of proliferating CD4 and CD8 T lymphocytes in blood and lymph node were determined as described in *Methods,* and differences in the means between HIV-negative and HIV-positive subjects were compared by the *F* test and Student's *t* test after taking the logarithm of the data.

to those observed with the [^{2}H]glucose (23) strongly support the validity of this experimental approach.

The percentage of $CD4^+Ki67^+$ T cells (Fig. 1*A*) was slightly higher in blood as compared to lymph nodes in both HIV-negative (mean 1.06% in blood versus 0.75% in lymph node) and HIV-infected subjects (mean 1.3% in blood versus 1.05% in lymph node). Similarly, the percentage of $CD8^+Ki67^+$ T cells was higher in blood (mean 0.59% in HIV-negative and mean 2.25% in HIV-infected subjects) than in lymph nodes (mean 0.2% in HIV-negative and mean 1.54% in HIV-infected subjects). The difference in the percentage of $CD4^+Ki67^+$ T cells between HIV-negative and HIV-positive subjects was significant in lymph node ($P = 0.029$) but not in blood ($P = 0.25$), whereas the difference in the percentage of $CD8^+Ki67^+$ T cells in both blood and lymph node were highly significant ($P < 0.001$). Therefore, these results are in agreement with previous studies indicating that the relative number of proliferating $CD4^+$ and $CD8^+$ T cells are greater in HIV-infected as compared to HIV-negative subjects (17, 18, 23) and consistent with the previous hypothesis of increased T cell turnover (22).

For each subject, estimates of the number of divid-

ing/proliferating T cells per day in blood can be calculated on the basis of different hematological measurements: leukocyte and lymphocyte counts, and percentages of $CD4^+Ki67^+$ and $CD8^+Ki67^+$ T cells. These estimates also were based on the assumption that the average cell cycle in T lymphocytes has a duration of 24 h (29). The mean number of proliferating $CD4^+Ki67^+$ T cells per microliter of blood was not significantly different between HIV-infected (9.1 ± 8.8) and HIV-negative subjects (9.2 ± 8.0) (Fig. 1*B*). However, the estimates of the number of proliferating blood $CD8^+Ki67^+$ T cells revealed that the daily production of $CD8^+$ T cells was 7-fold higher in HIV-infected subjects (26.7 ± 31.6) as compared to HIV-negative subjects (3.7 ± 2.8) (Fig. 1*B*). Interestingly, the daily productions of $CD4^+$ and $CD8^+$ T cells estimated by the Ki67 strategy were very similar to those calculated by the [^{2}H]glucose technology in both HIV-negative and HIV-infected subjects (23). Indeed, the daily production of $CD4^+$ and $CD8^+$ T cells estimated by [^{2}H]glucose was 10.4 ± 6.5 and 5.9 ± 7.6 cells per μl in HIV-negative subjects and 9.0 ± 4.8 CD4 T cells per μl and 24.6 ± 13.4 CD8 T cells per μl in HIV-infected subjects (23). Studies in mice have estimated that the division time of antigen-specific CD8 T cells can be as fast as 6–8 h (30). Therefore, it is possible that the rate of T cell production in humans may be greater than previously appreciated.

Estimates of the total body number of $CD4^+$- and $CD8^+$-proliferating T cells was determined by the analysis of mononuclear cells from both blood and lymph nodes and calculated as previously described (18). Briefly, following calculation of the total number of lymphocytes, the number of proliferating/dividing $CD4^+$ and $CD8^+$ T cells were calculated from the percentage of $CD4^+Ki67^+$ and $CD8^+Ki67^+$ T cells found in lymph nodes. Because lymph nodes contain ≈50–60% of the total lymphocytes (26), it is conceivable to assume that lymph node lymphocytes are representative of the total lymphocyte population. Therefore, estimates of proliferating $Ki67^+$ T cells obtained from lymph nodes may represent a good reflection of the total body $CD4^+$ and $CD8^+$ T lymphocyte populations. The total number of proliferating $CD4^+Ki67^+$ T cells (blood plus lymph node) estimated in HIV-negative subjects (2.06×10^9) did not differ significantly ($P = 0.409$) from that found in HIV-infected subjects (2.22×10^9) (Fig. 1*C*). However, the total number of proliferating $CD8^+Ki67^+$ T cells (2.82×10^9) in HIV-infected subjects was 20-fold higher (1.42×10^8; $P < 0.0001$) than in HIV-negative subjects (Fig. 1*C*).

Changes in CD4 and CD8 T Cell Subsets in Blood and Lymph Nodes Following HAART. Changes in CD4 and CD8 T cell subsets were monitored over time in the group of HIV-infected subjects enrolled in the Abacavir/Amprenavir clinical trial and who completed the entire duration of the study (72 wk). The mean $CD4^+$ T cell count in the treatment cohort (737 CD4/μl) was significantly lower ($P = 0.001$) than the group of healthy HIV-negative subjects ($n = 49$; 967 CD4/μl) (Fig. 2*A*), which were matched for age and sex with the treatment group. Interestingly, initiation of HAART was associated with a progressive increase in the mean CD4 T cell count, and, by week 36 of therapy, the differences between the HIV-negative (967 ± 51/μl) and HIV-infected subjects (861 ± 53/μl) were no more statistically significant (Fig. 2*A*; $P = 0.16$). CD4 T cell count continued to increase in the blood of HIV-infected subjects until week 48 (935 ± 58/μl) and remained stable between week 48 and 72 (999 ± 101/μl).

As observed in blood, similar $CD4^+$ T cell changes were found in lymph nodes. Indeed, CD4 T cell percentage was significantly different ($P < 0.0001$) at time 0 between the group of HIV-negative subjects (59.2%) and the treatment cohort (37.9%) (Fig. 2*B*). Normalization of CD4 T cell percentage in the treatment group occurred by week 36 (52.5%) and at that time,

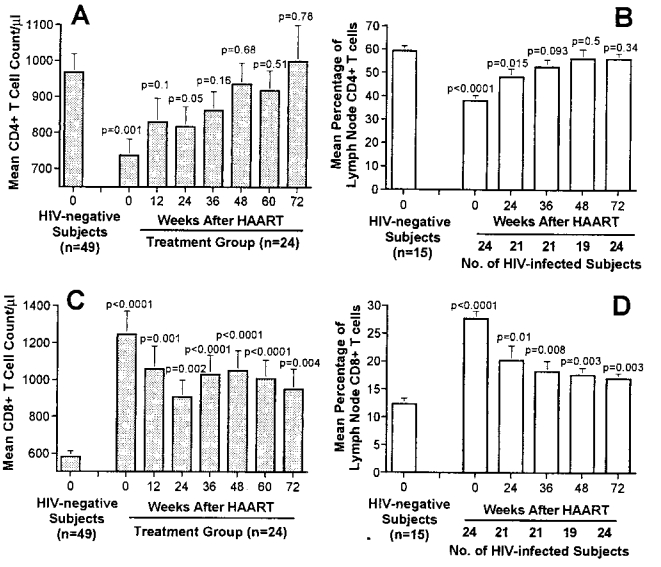

Fig. 2. Changes in CD4 and CD8 T cell counts in blood and percentage in lymph nodes following HAART. (*A*) Mean CD4 T cell counts after HAART. (*B*) Mean CD4 T cell percentage in lymph nodes after HAART. (*C*) Mean CD8 T cell counts after HAART. (*D*) Mean CD8 T cell percentage in lymph nodes after HAART. Determination of CD4 and CD8 T cell percentages was performed on mononuclear cell populations obtained by excisional lymph node biopsies at weeks 0 and 72 and by ultrasound-guided lymph node aspirates at weeks, 24, 36, and 48. The group of 49 HIV-negative subjects was matched for age and gender with the treatment group. In HIV-negative subjects, determinations of CD4 and CD8 T cell percentages were performed on mononuclear cell populations obtained by excisional lymph node biopsies. Differences in the means between HIV-negative and HIV-positive subjects were compared by the F-test and Student's *t* test.

the CD4 T cell percentage was no more significantly different ($P = 0.09$) from that in the HIV-negative group (Fig. 2*B*). Furthermore, CD4 T cell percentage remained stable between week 36 (52.5%) and week 72 (56%) (Fig. 2*B*).

Initiation of HAART had an opposite effect on the $CD8^+$ T cell subset. Indeed, HAART was associated with a substantial decrease overtime of CD8 T cell count in blood and percentage in lymph node (Fig. 2 *C* and *D*). Despite the reduction in the CD8 population over time in both blood and lymph node, the mean CD8 T cell count in blood and percentage in lymph node at week 72 remained ≈2-fold higher in HIV-infected subjects as compared to HIV-negative subjects (Fig. 2 *C* and *D*).

Changes in Naive and Memory $CD4^+$ T Cells in Blood Following HAART. The kinetics of the increase in naive and memory $CD4^+$ T cells observed in the group of 24 HIV-infected subjects following HAART were very similar in blood. Both naive and memory $CD4^+$ T cells increased of ≈50 cells per μl over baseline at week 12 (Fig. 3). No major changes were observed between week 12 and 36, whereas a substantial increase in both naive and memory $CD4^+$ T cells occurred between week 36 and 72 (Fig. 3).

Long-Term Kinetics of Proliferating $CD4^+$ and $CD8^+$ T Cells Following HAART. Estimates of the total number of proliferating $CD4^+$ and $CD8^+$ T cells were derived from the analysis of Ki67 nuclear antigen in lymphomononuclear cell populations isolated from the 24 HIV-infected subjects who completed the 72 wk of HAART. Following the initiation of treatment, a progressive increase in the total number (blood plus lymph node) of proliferating $CD4^+$ $Ki67^+$ T cells was observed over time (Fig. 4*A*). The increase peaked at week 36 (mean number of $4.12 \times$

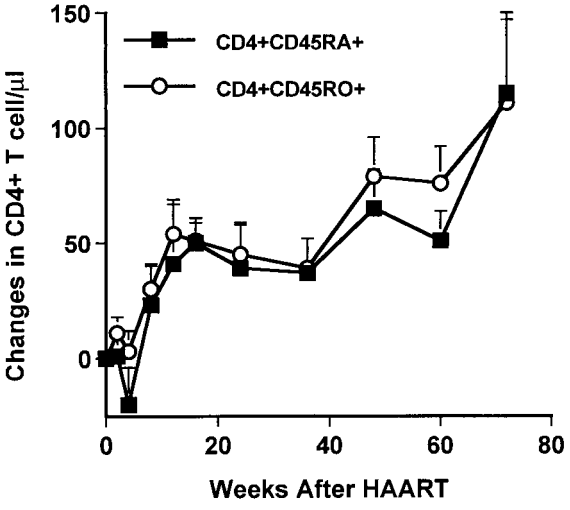

Fig. 3. Changes over base-line in naive ($CD4^+RA^+$) and memory ($CD4^+RO^+$) CD4 T cells in HIV-infected subjects treated for 72 wk with HAART.

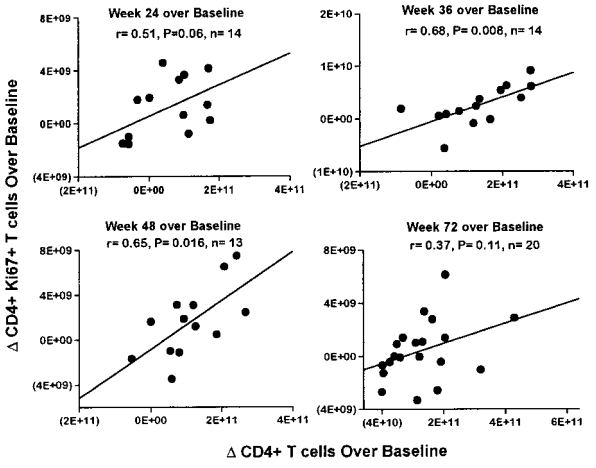

Fig. 5. Correlations between changes in the total body number of proliferating and total body number of CD4 T cells at different time points after initiation of HAART. Δ corresponds to the differences in the number of proliferating ($Ki67^+CD4^+$) T cells and of total CD4 T cells between baseline and weeks 24, 36, 48, and 72 after HAART. Correlation was tested by Pearson's test.

10^9 $CD4^+Ki67^+$ T cells) and corresponded to a 2-fold increase when compared to baseline (1.90×10^9). A progressive decline in the number of proliferating $CD4^+Ki67^+$ T cells was observed between week 36 and 72. At week 72 (Fig. 4*A*), the total number of proliferating $CD4^+Ki67^+$ T cells (2.22×10^9) went back to levels comparable to those at base-line and to HIV-negative subjects ($P = 0.64$).

Similarly, estimates of the total body number of $CD4^+$ T cells were determined. The total number of $CD4^+$ T cells progressively increased after HAART and peaked at week 48 (3.52×10^{11}) (Fig. 4*B*), which represented an increase of approximately 2-fold over baseline (2.15×10^{11}), and it declined slightly between week 48 and 72. At week 72, the total number of $CD4^+$ T cells in HIV-infected subjects (3.20×10^{11}) was similar to HIV-negative subjects (2.68×10^{11}; $P = 0.35$) (Fig. 4*B*).

With regard to the $CD8^+$ T cell subset (Fig. 4*C*), the number of proliferating $CD8^+Ki67^+$ T cells (2.11×10^9) was 15-fold greater in HIV-infected subjects as compared to HIV-negative subjects (1.42×10^8) at baseline. After initiation of HAART, a 2-fold reduction in the total number of proliferating $CD8^+Ki67^+$ T cells was already observed at week 24 (1.33×10^9), and at week 72 (3.31×10^8 $CD8^+Ki67^+$ T cells) it was only 2-fold above that observed in HIV-negative subjects (1.42×10^8). The estimated total number of $CD8^+$ T cells in HIV-infected subjects decreased of ≈50% between baseline and week 72 (Fig. 4*D*). At week 72, the total number of CD8 T cells in HIV-infected subjects (9.65×10^{10}) was approximately 2-fold higher than that in HIV-negative subjects (5.98×10^{10}; $P = 0.005$).

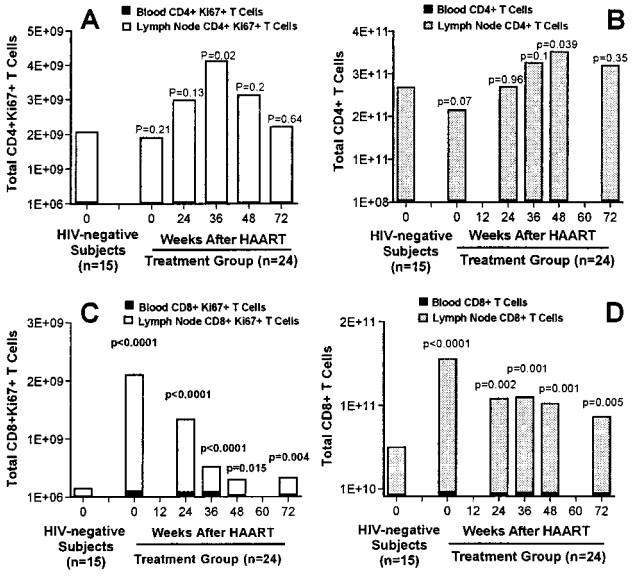

Fig. 4. Kinetics of proliferating CD4 and CD8 T cells following long-term HAART. (*A*) Changes in the total (blood plus lymph node) number of proliferating, $Ki67^+$, CD4 T cells. (*B*) Changes in the total (blood plus lymph node) number of CD4 T cells. (*C*) Changes in the total (blood plus lymph node) number of proliferating, $Ki67^+$, CD8 T cells. (*D*) Changes in the total (blood plus lymph node) number of CD8 T cells. Differences in the means between HIV-negative and HIV-positive subjects were compared by the F-test and Student's *t* test after taking the logarithm of the data.

Correlation Between the Kinetics of CD4 and CD8 T Cell Production and Changes in the Total Number of CD4 and CD8 T Cells Following HAART. To determine whether in HIV-infected subjects changes in the number of proliferating $CD4^+Ki67^+$ and $CD8^+Ki67^+$ T cells observed after treatment correlated with an increased and/or a decreased output of $CD4^+$ and $CD8^+$ T cells, the differences (delta Δ) in the total number of $Ki67^+$ $CD4^+$ and $CD8^+$ T cells between weeks 24, 36, 48, and 72 and baseline were correlated with the corresponding Δ values in the total number of CD4 and CD8 T cells. For each patient, the delta value was calculated by subtracting the mean value of T cells at either week 24, 36, 48, or 72 from the mean value of T cells at baseline. The delta from the total number of cycling T cells ($CD4^+Ki67^+$ or $CD8^+Ki67^+$) was then plotted against the delta from the total number of T cells (CD4 or CD8) observed at the same time point. As shown in Fig. 4 *A* and *B*, the maximal increases (as compared to baseline) of the number of proliferating $CD4^+Ki67^+$ T cells and of the total body $CD4^+$ T cells were respectively observed at weeks 36 and 48. Consistently with these results, significant correlation (Fig. 5) was observed between $\Delta CD4^+Ki67^+$ T cells and ΔCD4 T cells at week 24 ($r = 0.51$; $P = 0.06$), at week 36 ($r = 0.68$, $P = 0.008$), and at week 48 ($r = 0.65$, $P = 0.016$) (Fig. 5). Weeks 36 and 48 corresponded to the time points of maximal T cell production after initiation of treatment (Fig. 4*A*). The correlation between $\Delta CD4^+Ki67^+$ T cells and ΔCD4 T cells was

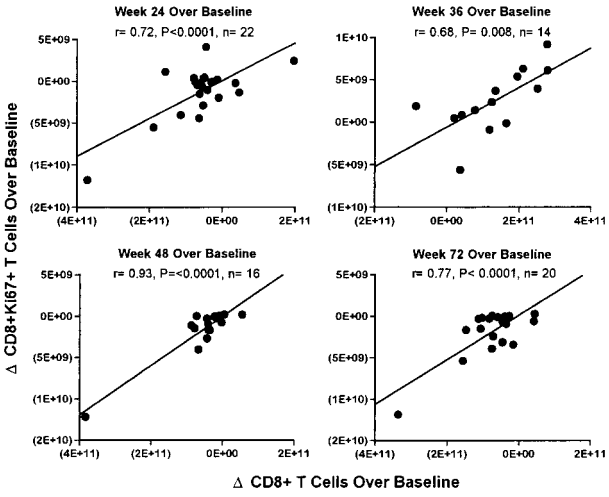

Fig. 6. Correlations between changes in the total body number of proliferating and total body number of CD8 T cells at different time points after initiation of HAART. Correlation was tested by Pearson's test.

lost at week 72 ($r = 0.37$; $P = 0.11$) (Fig. 5), which was consistent with the major drop in T cell production observed (Fig. 4*A*).

Changes in proliferating $CD8^+Ki67^+$ T cells were also highly correlated with the changes in the total number of CD8 T cells. The correlation between Δ $CD8^+Ki67^+$ T cells and Δ $CD8^+$ T cells was highly significant at all of the time points analyzed (Fig. 6); at week 24, $r = 0.72$, $P < 0.001$; at week 36, $r = 0.68$, $P = 0.008$; at week 48, $r = 0.93$, $P < 0.001$; and at week 72, $r = 77$, $P < 0.0001$.

Discussion

In the present study, the long-term kinetics of $CD4^+$ and $CD8^+$ T cells production have been investigated in blood and lymph nodes of a cohorts of 24 HIV-infected subjects who were treated with HAART for 72 wk. According to previous studies (17, 18, 23), the analysis of T cell production in blood and lymph nodes of two large cohorts of HIV-negative ($n = 15$) and HIV-infected ($n = 73$) subjects showed that the relative number of proliferating $CD4^+$ and $CD8^+$T cells was increased in HIV-infected compared to HIV-negative subjects. However, only the absolute number of proliferating $CD8^+$ and not of $CD4^+$ T cells was increased in HIV-infected subjects.

The absence of a manifest increase in $CD4^+$ T cell production may results from a preferential killing of proliferating $CD4^+$ T cell by HIV. However, kinetic studies of T cell production following 72 wk of HAART provides further support for the hypothesis that, in addition to the $CD4^+$ T cell destruction, a limited renewal of $CD4^+$ T cells might represent an important mechanism to explain the gradual depletion of $CD4^+$ T cells. The likely scenario is that in the absence of HAART and in the presence of active virus replication, the destruction of $CD4^+$ T cells cannot be compensated by an increased production because of a possible interference of the $CD4^+$ T cell production machinery (18, 23, 31, 32), at the level of the bone marrow (reduced number of progenitors) (33, 34) of the thymus (35), and of the cell cycle (36).

Inhibition of $CD4^+$ T cell production appears to be lifted by the initiation of HAART (18, 23). The longitudinal analysis of the changes in the number of proliferating $CD4^+$ T cells following HAART has helped to interpret the potential discrepancy between the increased $CD4^+$ T cell turnover and the absence of manifest increase in CD4 T cell production. In fact, if $CD4^+$ T cell production was increased before HAART, there would be no rationale and no need for the further increased in $CD4^+$ T cell production observed following suppression of virus replication and of $CD4^+$ T cell killing after HAART.

The increase in *de novo* $CD4^+$ T cell production following HAART strongly supports the hypothesis of an impaired $CD4^+$ T cell production machinery in the presence of active virus replication. Furthermore, the longitudinal analysis of the changes in the number of proliferating $CD4^+$ and $CD8^+$ T cells has provided a direct relationship between markers of T cell production such as Ki67 and the actual changes in CD4 and CD8 T cells count in blood and percentage in lymph nodes. The results obtained in the present study demonstrate that a net increase in the production of $CD4^+$ T cell remains elevated for several months after initiation of HAART. However, following the normalization and stabilization of CD4 T cell count in blood and percentage in lymph node, a decline in the number of dividing $CD4^+$ T cells was observed. Therefore, these results suggest that once the inhibition of T cell production is removed by the treatment, a fine regulation of T cell production becomes functional, which is homeostatically regulated. These results indicate that the fine regulatory mechanisms of T cell production are still functional in HIV-infected subjects at early stages of established infection. However, it is not possible to exclude that the kinetics of T cell production may differ in HIV-infected subjects at advanced stages of disease.

The changes of the total body number of $CD4^+$ and $CD8^+$ T cells after HAART are tightly correlated with the kinetics of proliferating $CD4^+Ki67^+$ and $CD8^+Ki67^+$ T cells. These results provide strong evidence that *ex vivo* measures of T cell production truly reflect quantitative changes in the total body T lymphocyte populations.

Furthermore, the increase of naive $CD4^+$ T cells paralleled that of memory $CD4^+$ T cells. Therefore, in HIV-infected subjects at early stages of established infection, the reconstitution of naive $CD4^+$ T cells occurred at the same rate as that of memory $CD4^+$ T cells. Based on these results, the hypothesis of a selective defect for the reconstitution of naive $CD4^+$ T (8) does not seem to be valid for HIV-infected subjects at early stage disease. These results are consistent with recent studies demonstrating *de novo* thymic output in HIV-infected subjects treated with HAART (37).

The long-term kinetics of the production of $CD4^+$ T cells shown in the present study may provide new insights in explaining the rapid rebound in HIV replication observed in HIV-treated subjects, especially those who were switched to simplified therapeutic regimens or in subjects who have interrupted antiretroviral therapy (38, 39). In fact, it has been demonstrated that the pool of proliferating $CD4^+Ki67^+$ T cells remains enlarged (2- to 3-fold over baseline and compared to HIV-negative subjects) for at least 12 mo after initiation of HAART. Because HIV replication is dependent on the state of activation of $CD4^+$ T cells (40, 41), the proliferating $CD4^+Ki67^+$ T cells can efficiently support virus replication. Therefore, HIV-infected subjects who have been switched to less potent antiretroviral therapeutic regimens or have interrupted HAART during the first year of treatment have a larger pool of CD4 target cells available to support efficient HIV replication. The increased target cells availability may explain why virus breakthrough occurred in the absence of any evidence of the emergence of drug-resistant viral variants in the subjects who switched to simplified antiretroviral regimens (38, 39). Based on these data, it is conceivable that therapeutic strategies aimed at the reduction of the pool of proliferating $CD4^+$ T cells may be necessary for effective control of HIV replication.

These results represent advances in the delineation of the kinetics of T cell production following antiretroviral therapy and provide insights for the development of novel therapeutic strategies that have proliferating $CD4^+$ T cells as target to achieve long-term control of virus replication.

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