The 'Triple Study': viral dynamics and immune reconstitition in HIV-1 infection during potent antiretroviral therapy
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CHAPTER V

BIPHASIC KINETICS OF PERIPHERAL BLOOD T CELLS
AFTER TRIPLE COMBINATION THERAPY IN HIV-1
INFECTION: A COMPOSITE OF REDISTRIBUTION AND
PROLIFERATION

Pakker NG, Notermans DW, de Boer RJ, Roos MTL, de Wolf F,
Hill A, Leonard JM, Danner SA, Miedema F, Schellekens PTA

Viral dynamics and immune reconstitution during potent antiretroviral therapy

The origin of CD4\(^+\) T cells reappearing in the blood following antiretroviral therapy in human immunodeficiency virus type-1 (HIV-1) infection is still controversial. Here we show, using mathematical modeling, that redistribution of T cells to the blood can explain the striking correlation between the initial CD4\(^+\) and CD8\(^+\) memory T-cell repopulation and the observation that 3 weeks after the start of treatment memory CD4\(^+\) T-cell numbers reach a plateau. The increase in CD4\(^+\) T cells following therapy most likely is a composite of initial redistribution, accompanied by a continuous slow repopulation with newly produced naive T cells.

Treatment of human immunodeficiency virus-type 1 (HIV-1)-infected individuals with potent triple combinations of antiretroviral drugs results in a dramatic decline of the viral load to 'undetectable' HIV-1 RNA levels in plasma and tissue in the majority of patients. Apart from controlling virus replication, the major goal of antiviral therapy is to achieve a degree of immune reconstitution. Although significant increases in CD4\(^+\) T-cell numbers have been observed, the mechanisms of T-lymphocyte repopulation and restoration of immune function after sustained suppression of HIV replication is still unclear. Recent studies have provided evidence for a high turnover of CD4\(^+\) T cells driven by the large amounts of virus that are produced daily, suggesting physical exhaustion of CD4\(^+\) T-cell renewal as a cause of CD4\(^+\) T-cell depletion. If so, antiviral treatment, despite very efficient control of virus replication, could have it limits as to the restoration of the immune system. In the present study we sought to address this issue and studied in detail the kinetics and characteristics of immune reconstitution after triple combination therapy.

Response on therapy

Patient recruitment and study treatment were as previously described in detail. Briefly, the study recruited 33 adults with documented HIV-1 infection and CD4\(^+\) T lymphocyte counts of at least 50 cells/\(\mu\)L, a plasma HIV RNA level of at least 30,000 copies/mL and no prior treatment with antiretrovirals. Patients were randomized to receive either immediate triple combination therapy with ritonavir [600 mg twice a day (BID)], zidovudine (ZDV) (300 mg BID) and lamivudine (3TC) (150 mg BID), or delayed triple therapy, that is, starting with ritonavir monotherapy, and 3 weeks later adding ZDV and 3TC. Baseline characteristics of patients are shown in Table 1. No significant differences in baseline values of the immunological and virological parameters were observed between the two treatment groups.

HIV-1 RNA levels in plasma decreased significantly over 36 weeks (p < 0.005) to levels below the quantification limit in a large majority of patients in both groups (Fig. 1E). During 36 weeks of follow-up, mean CD4\(^+\) T-cell counts increased significantly in both groups (p < 0.005) (Fig. 1A). At 36 weeks, the mean rise in the group receiving the triple combination therapy from the start of the study was 196 CD4\(^+\) T cells per microliter blood (s.e.m. 43 CD4\(^+\) T cells/\(\mu\)L) and in the group with delayed triple therapy a rise of 145
± 33 CD4+ T cells/μL blood was observed. For the complete period of follow-up, no significant difference was observed between the two treatment groups in either CD4+ T-cell response or HIV RNA reduction. There was an increase in CD8+ T-cell counts in the first 2 months of treatment (214 ± 67 and 400 ± 144 CD8+ T cells/μL blood for the early and delayed treatment groups, respectively), comparable with results from ritonavir monotherapy, however, the absolute number of CD8+ T cells subsequently declined toward baseline values (Fig. 1B). CD19+ B cells had a response similar to that observed for the CD4+ T cells (Fig. 1C). In parallel with the quantitative increase in T-cell numbers the in vitro proliferative response to CD3 monoclonal antibodies, expressed per 10^3 CD3+ T cells, showed a sustained improvement to approximately 50% of normal responses (Fig. 1D).

<table>
<thead>
<tr>
<th>No. of participants</th>
<th>Early treatmenta</th>
<th>Delayed treatmenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male / female</td>
<td>16/1</td>
<td>15/1</td>
</tr>
<tr>
<td>Age (years)b</td>
<td>38 (26-57)</td>
<td>39 (26-61)</td>
</tr>
<tr>
<td>CDC class at entryc</td>
<td>6 / 7 / 4</td>
<td>8 / 5 / 3</td>
</tr>
<tr>
<td>T lymphocytes (cells/μL)b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>177 (40-403)</td>
<td>134 (37-600)</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>1027 (273-1777)</td>
<td>927 (320-2277)</td>
</tr>
<tr>
<td>CD4+ /CD8+ T cell ratio</td>
<td>0.18 (0.09-0.68)</td>
<td>0.16 (0.06-0.60)</td>
</tr>
<tr>
<td>T cell proliferative response (cpm/10^3 T cells)b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3 mAb</td>
<td>34 (3-260)</td>
<td>25 (4-258)</td>
</tr>
<tr>
<td>Viral load (log copies/mL)b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma RNA levels</td>
<td>5.27 (4.59-6.36)</td>
<td>5.37 (4.50-5.92)</td>
</tr>
<tr>
<td>Phenotype</td>
<td>11/6</td>
<td>11/5</td>
</tr>
</tbody>
</table>

Table 1. Description of baseline characteristics. a The immediate triple (early treatment) started with ritonavir, ZDV and 3TC simultaneously, the delayed triple started with ritonavir and added 3 weeks later ZDV and 3TC. b Median values (5 and 95 percentile). c According the 1993 revised AIDS definition.42142

**CD4+ and CD8+ T-cell recovery rates**

As treatment groups did not differ with regard to the pattern of T-cell recovery, we combined the data from both patient groups to analyze the kinetics of reappearing naive and memory T-cell subsets. T-cell subsets expressing CD45RA and CD62L are regarded as truly naive T cells, whereas T cells expressing CD45RO and T cells that express CD45RA but lack CD62L are regarded as memory lymphocytes.9,9 For one representative, well-responding patient, flow cytometric images of CD45RA and CD62L staining

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are plotted at three different time points during follow-up (Fig. 2). The patterns for recovery of naive and memory CD4⁺ and CD8⁺ T cells in the total study population are depicted in Fig. 3, A and B.

![Graphs showing CD4⁺ T cell count, CD8⁺ T cell count, B cell count, T cell function, HIV-1 RNA load](image)

Fig. 1. Peripheral blood CD4⁺ and CD8⁺ T-cell numbers, improvement of T-cell function in vitro, and HIV-1 RNA load. The immediate triple treatment (○) started with ritonavir, ZDV and 3TC simultaneously, the delayed triple therapy (□) started with ritonavir and added ZDV and 3TC three weeks later.

A. Mean change from baseline of CD4⁺ T-lymphocyte numbers. B. Mean change from baseline of CD8⁺ T-lymphocyte numbers. C. Mean change from baseline of CD19⁺ B-lymphocyte numbers. D. Mean change from baseline of proliferative response per 10⁷ T cells after stimulation with CD3 mAb. E. Mean change from baseline of plasma HIV-1 RNA levels. Bars represent s.e.m.

Both the CD4⁺ and CD8⁺ recovery rates seem to be biphasic. For the CD4⁺ T cells the most rapid recovery occurs during the first three weeks, whereas rapid CD8⁺ T-cell recovery is observed within the first six weeks. The individual T-cell recovery rates of all patients over the entire period of follow-up, and over the rapid and slow phases separately, were estimated by linear regression (Table 2). Calculating the total-body production over the initial period, we found a daily increase of 1.3 x 10⁹ CD4⁺ T cells, in agreement with earlier findings.⁵,⁶,¹⁰ and 2.1 x 10⁹ CD8⁺ T cells. The average exponential growth rates over this period were 0.027/day and 0.008/day, for
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the CD4⁺ and CD8⁺ cells, respectively. Thus, although the total increase of CD8⁺ T cell number is larger than the recovery of CD4⁺ T cells, CD4⁺ T cells expand three times as fast on a per capita basis. Comparing recovery rates of naive and memory T cells, in both the CD4⁺ and CD8⁺ compartments, we saw that most of the early recovery is due to memory cells (Table 2, \(4/5.3 = 75\%\) and \(5.6/8.3 = 67\%\), respectively). For the CD4⁺ T cells the naive and memory exponential growth rates differ by a factor of two (0.015/day and 0.03/day, respectively). For the CD8⁺ cells they are approximately the same (0.011/day and 0.007/day, respectively). The early fast recovery of CD4⁺ T cells is similar to the estimated total CD4⁺ T-cell recovery reported in earlier studies\(^5,6\) which suggests that the latter also largely resulted from memory T-cell recovery in the blood.

During the subsequent slow recovery period, the total CD4⁺ T-cell production slowed down to \(10^8\) CD4⁺ T cells per day, which is largely explained by production of naive T cells (Table 2A). For naive CD4⁺ T cells we found no significant difference in the early and late production rates (\(p = 0.07\)), hence on average the naive CD4⁺ T-cell recovery rate over the entire period was \(5.7 \times 10^7\) cells/day. This is in agreement with preliminary

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**Fig. 2.** Representative FACScan images of one well-responding patient at baseline, week 6, and week 36 during follow-up. Depicted are CD4⁺ and CD8⁺ cells stained for CD45RA and CD62L. Percentages of cells are presented within the according quadrant, the corresponding CD4⁺ and CD8⁺ T cell counts are shown above the images.
observations in a monotherapy trial with ritonavir, which also reported slow kinetics of naive CD4\(^+\) T cells.\(^7\) In contrast, the recovery rates of the memory CD4\(^+\) T cells in the early and late periods are significantly different (p < 0.005) and drop from 10\(^9\) cells/day to virtually zero. Although the early CD8\(^+\) T-cell recovery resembles that of the CD4\(^+\) T cells, total CD8\(^+\) counts decline during the late phase. This can be explained by a diminishing antigenic stimulation once the HIV-1 viral load levels have dropped significantly. The CD8\(^+\) T-cell response will not only involve HIV-1-specific cells, but also nonspecific CD8\(^+\) T cells, activated as bystander cells stimulated by cytokines.\(^11,12\) Indeed most of the decline in CD8\(^+\) cells is caused by a drop in memory cells (Table 2B). In fact, kinetics of naive CD8\(^+\) T cells resemble the kinetics of naive CD4\(^+\) T cells, with a steady increase on the order of 10\(^8\) cells/day over the entire period. Also CD19\(^+\) B cell numbers increase with strikingly similar kinetics as memory CD4\(^+\) T cells (Fig. 1C).

Fig. 3. Kinetics of CD4\(^+\) and CD8\(^+\) naive and memory lymphocyte subpopulations. A. Pattern of (absolute) mean response on therapy in the CD4\(^+\) T-lymphocyte naive (O) and memory (□) subset and the total CD4\(^+\) T-cell count (▲). B. Pattern of (absolute) mean response on therapy in the CD8\(^+\) T-lymphocyte naive (O) and memory (□) subset and the total CD8\(^+\) T-cell count (▲). Bars represent s.e.m.

Redistribution or proliferation
There has been some debate as to the origin of the initial CD4\(^+\) T-cell repopulation.\(^13,14\) Originally it was argued that this fast repopulation reflects the pretreatment turnover of proliferating CD4\(^+\) T cells during HIV-1 infection.\(^5,6\) An important argument for such a high steady-state CD4\(^+\) T cell-production of 10\(^9\) cells/day was the observation that after treatment the
exponential growth rate of the CD4\(^+\) T cells was inversely correlated with the baseline CD4\(^+\) T cell count. Such a CD4\(^+\) T cell count density-dependent growth would account for a growth rate up to 0.08/day at low CD4\(^+\) counts.\(^3\)

We find very similar "density-dependent" results for the recovery of memory CD4\(^+\) T cells, as depicted in Fig. 4A, also suggesting growth rates up to 0.1/day at low baseline CD4\(^+\) T-cell counts, and decreasing to virtually zero at CD4\(^+\) counts over 400 cells/\(\mu\)L. However, one finds a similar relation between the exponential growth rate of CD8\(^+\) memory T cells and the baseline CD4\(^+\) count (Fig. 4B). Thus, surprisingly, the per capita CD8\(^+\) recovery is higher in patients with lower CD4\(^+\) counts who have a more impaired immune function. The original hypothesis of a density dependent proliferation leaves unexplained why the reconstitution pattern of the memory CD8\(^+\) T cells is so similar to that of the memory CD4\(^+\) T cells.

### A

<table>
<thead>
<tr>
<th>CD4 cells</th>
<th>Cells per (\mu)L blood/day</th>
<th>Total body cells/day</th>
<th>Exponential growth/day</th>
<th>Baseline cells/(\mu)L</th>
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<tr>
<td>Total</td>
<td></td>
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<tr>
<td>0-36 wk</td>
<td>0.9</td>
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<td>0-6 wk</td>
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<td>7.5x10^7</td>
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<td>304</td>
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<tr>
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<td>5.7x10^7</td>
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<td>43</td>
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<td>1.3x10^8</td>
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<td>0.000</td>
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### B

<table>
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<tr>
<th>CD8 cells</th>
<th>Cells per (\mu)L blood/day</th>
<th>Total body cells/day</th>
<th>Exponential growth/day</th>
<th>Baseline cells/(\mu)L</th>
<th>N</th>
</tr>
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<tr>
<td>Total</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0-36 wk</td>
<td>1.1</td>
<td>2.7x10^8</td>
<td>0.002</td>
<td>918</td>
<td>33</td>
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<tr>
<td>0-6 wk</td>
<td>8.3</td>
<td>2.1x10^9</td>
<td>0.008</td>
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<td>6-36 wk</td>
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<td>-2.0x10^8</td>
<td>-0.001</td>
<td>1296</td>
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<td>Naive</td>
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<td></td>
</tr>
<tr>
<td>0-36 wk</td>
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<td>7.5x10^7</td>
<td>0.001</td>
<td>88</td>
<td>33</td>
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<tr>
<td>0-6 wk</td>
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<td>3.0x10^8</td>
<td>0.011</td>
<td>90</td>
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<td>5.0x10^7</td>
<td>0.001</td>
<td>135</td>
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<tr>
<td>Memory</td>
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<td>0-36 wk</td>
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<td>7.5x10^7</td>
<td>0.001</td>
<td>752</td>
<td>33</td>
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<tr>
<td>0-6 wk</td>
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<td>1.4x10^9</td>
<td>0.007</td>
<td>757</td>
<td>32</td>
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<tr>
<td>6-36 wk</td>
<td>-0.8</td>
<td>-2.0x10^8</td>
<td>-0.001</td>
<td>910</td>
<td>25</td>
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</table>

Table 2. Recovery rates of naive and memory CD4\(^+\) and CD8\(^+\) T cells. Individual linear regression was performed on the peripheral blood T-cell count over the full 36 weeks, over the initial fast repopulation period (3 and 6 weeks for CD4\(^+\) and CD8\(^+\) T cells, respectively), and over the last period (33 and 30 weeks for CD4\(^+\) and CD8\(^+\) T cells, respectively). The slopes of
these regression lines provided the estimates for the daily rates of T-cell recovery. The average daily increase in T cells per microliter of peripheral blood is represented in column 3 as the mean of all individual slopes. If we assume 2% of the T lymphocytes to reside in the peripheral blood, and that a 70-kg individual has 5 liters of blood, we obtained the total body average rate of increase in column 4 by multiplying column 3 with $5 \times 50 \times 10^6$. We calculated an average daily exponential growth rate by performing a linear regression after taking the natural logarithm of the T-cell count (see column 5). The number of patients and the average baseline CD4$^+$ and CD8$^+$ T-cell count are shown the last two columns. Note that missing T-cell subset data for one patient resulted in a difference in baseline value between the total and the first time-period. Since the exponential growth was estimated using individual slopes, the average rates, as shown in this table, can not directly be recalculated.

This hypothesis also does not explained why CD4$^+$ T-cell repopulation is slowing down to $10^8$ cells/day during the second phase of T-cell recovery with a virtual lack of memory T-cell production. If during clinical latency large numbers of CD4$^+$ T cells are produced on a daily basis as has been hypothesised,[5,6] one would expect the same production to continue during therapy until normal CD4$^+$ T-cell levels are reached.

It has been proposed that the initial CD4$^+$ T-cell recovery results from redistribution of cells from the lymphoid tissue to the peripheral blood, rather than from de novo production.[13,14] Since 98% percent of lymphocytes resides in the lymphoid tissues, measurements in peripheral blood should be interpreted with care.[15] Indeed one could argue that with progressive HIV-1 infection, increasing viral load and decreasing CD4$^+$ T cells, a larger fraction of both CD4$^+$ and CD8$^+$ T cells will be trapped in the infected and inflamed lymphoid tissues because of increasing cytokine levels and HIV-related immune activation.[16-19] Furthermore, as memory and naive cells have different recirculation and homing pathways, differences in sequestration of naive and memory T cells are to be expected.[20] Once the viral load levels are strongly decreased by antiretroviral treatment, inflammatory responses will be reduced, cytokine levels will normalize and sequestered T cells will be released to the peripheral blood compartment. It is noteworthy that the apparently density-dependent growth indeed is also fully compatible with redistribution. As the magnitude of the memory T-cell recovery in the peripheral blood should be proportional to the total number of memory T cells in the lymphoid tissue, we calculated for each patient a “fractional memory T cell recovery” $\Delta B/B$, where $\Delta B$ is the increase in memory cells during treatment and $B$ is the baseline peripheral blood memory T-cell count. Fig. 4, C and D, depicts the fractional recovery of the CD4$^+$ and CD8$^+$ memory cells as a function of the total CD4$^+$ baseline count. Like the density-dependent growth, the fractional recovery is inversely related to the baseline counts. Calculating the fractional CD4$^+$ and CD8$^+$ memory recovery one finds a strong correlation between these two parameters (Fig. 4E, $R = 0.83$).
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Fig. 4. Individual CD4⁺ and CD8⁺ T-cell increases as a function of the baseline total CD4⁺ T-cell count. Depicted are the recovery rates after start with antiviral therapy during the first 3 weeks for CD4⁺ T cells and during the first 6 weeks for CD8⁺ T-cell numbers. CD4⁺ and CD8⁺ T-cell increases were expressed as the exponential rate of increase (A, B, column 5 in Table 2) and as the fractional increase (C, D). In addition, the relation between the fractional increase of memory CD4⁺ T cells and memory CD8⁺ T cells during the initial fast repopulation period after start with antiretroviral therapy is shown (E).

Mathematical model for immunological recovery

By mathematical modelling we explored the idea of redistribution as the first immediate response of T cells on highly active antiretroviral therapy, taking into account the two major lymphocyte compartments. We chose \( B \) and \( L \) as the total number of CD4⁺ T cells in the peripheral blood and the lymphoid tissue, respectively, and \( T \) as a "trapping factor", defined in terms of the lymphocytes that are trapped in the lymphoid tissue when \( T > 1 \). As argued above this increased retention of lymphocytes (\( T > 1 \)) is caused by virus-induced immune activation such as increased cytokine levels, which will decrease quickly following potent antiviral therapy. The total number of CD4⁺ T cells in the blood can be expressed as,

\[
B = \frac{L}{50T}
\]
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which in the absence of trapping ($T = 1$) says that 2% of the lymphocytes reside in the blood. If one argues that the rapid increase in peripheral CD4+ T cells during the first three weeks of treatment is entirely due to redistribution, that is, the loss of trapping, the difference in the peripheral blood CD4+ T cell count,

$$\Delta B = \frac{L}{50} - \frac{L}{50T} = \frac{L(T - 1)}{50T}$$

is proportional to $L$, and is inversely proportional to $T$. This can be simplified by calculating the relative increase of peripheral CD4+ T cells, that is, fractional increase, as,

$$\frac{\Delta B}{B} = \frac{L(T - 1)}{50T} \times \frac{50T}{L} = T - 1$$

According to this simple mathematical model, the fractional recovery $\Delta B/B$ in the peripheral blood is determined by the trapping factor $T$ only, reflecting the increased residence of T cells in tissue. Thus, if we assume redistribution, the model shows that fractional growth is directly related to the trapping factor $T$; the data demonstrate that there is an inverse correlation between baseline CD4+ T-cell count and increase in CD4+ T cells after therapy; from that we conclude that $T$ is apparently higher in patients with lower baseline CD4+ counts. Furthermore, trapping of CD4+ T cells in the lymphoid tissue is also significantly correlated with viral load levels at baseline ($R = 0.65$). The average fractional recovery for the CD4+ T cells was 1.2 and for the CD8+ T cells 0.6. This results in trapping factors $T$ of 2.2 and 1.6, respectively, meaning that instead of the normal 2%, in these HIV-1-infected patients only about 1% of the T cells resides in the peripheral blood. Additionally, if we assume that a 70-kg individual has 5 liters of blood, this would mean that the average CD4+ T-cell count of 120 cells/µL blood before the onset of therapy corresponds to $6 \times 10^{10}$ memory CD4+ T cells in the lymphoid tissue ($120 \times 5 \times 100 \times 10^6 = 6 \times 10^{10}$ cells). This is in agreement with Zhang et al. who studied the immunological data of CD4+ repopulation in lymphoid tissues obtained for a subpopulation of the patients that are described in the present study. We argue that after the initial recovery period following antiviral therapy, a new steady-state is approached with an approximately normal distribution of 2% of lymphocytes in the blood. This steady-state corresponds to the ceiling in the memory CD4+ T-cell number that is reached after 3 weeks and remains lower than normal, since total CD4+ T cells numbers in the body are still significantly decreased.

In summary, the biphasic CD4+ T-cell kinetics, the finding that CD4+ memory T cells quickly reach a subnormal plateau, the similarities between the CD4+ and CD8+ T-cell repopulation in the blood, the improved CD8+ recovery at low CD4+ counts and the strong correlation between the fractional CD4+ and CD8+ recovery, all find a straightforward explanation in the redistribution model, but are not explained by the proliferation model. Obviously, we cannot rule out some contribution to the initial rise in
peripheral blood CD4⁺ T-cell numbers of memory CD4⁺ T-cell production. The daily production of naive CD4⁺ and CD8⁺ T cells does contribute to long-term total T-cell repopulation and can be compatible with little changes in the Vβ repertoire and CDR3 region in the first months of antiviral therapy.²²

Altogether our results thus suggest that the initial rise in memory CD4⁺ T cells is not a reflection of high CD4⁺ T-cell production levels before treatment. During the natural course of HIV-1 infection in patients with CD4⁺ T-cell counts above 200 cells/μL, normal CD4⁺ T-cell telomere lengths [23-25] and normal frequencies of HPRT mutations [26] were found, which also argues against a markedly increased daily CD4⁺ T-cell production.

**Consequences for immune reconstitution**

Irrespective the mechanism that causes the initial steep increase in memory CD4⁺ T-cell counts after therapy, our data provide evidence that the long-term repopulation of newly produced naive CD4⁺ T cells is a much slower process than was generally anticipated.⁵,⁶ Our calculated daily production rate of naive CD4⁺ T cells of 5.7 x 10⁷ cells per day is in good agreement with the repopulation rate of naive CD4⁺ T cells in lymphoid tissues [²¹] and approaches the estimates reported after chemotherapy in adults.²⁷ These authors showed that substantial repopulation of CD4⁺ T cells is mainly caused by an increase in CD45RA⁺ T cells, and is only achieved after more than 12 months with an estimated CD4⁺ T-cell increase on the order of 10⁶ cells per day. The same is observed in patients who received bone marrow transplantation or were transplanted with purified allogenic peripheral stem cells [²⁸] and in multiple sclerosis patients that had been treated with a depleting CD4 monoclonal antibody.²⁹ Slow naive CD4⁺ T turnover is also compatible with the normal rate at which naive CD4⁺ T cells lose their telomere length by cell division.³⁰-³²

If the initial restoration of CD4⁺ T-cell numbers in the blood is mainly due to redistribution of cells previously sequestered to lymphoid and inflamed tissues, one has to reinterpret this 'repopulation' with respect to its contribution to the patient's immune reconstitution. In a recent paper Autran et al. [¹³] showed a substantial improvement of antigen-specific responses by peripheral blood lymphocytes in the first three months following antiviral combination therapy. If this is merely caused by an increase in the number of CMV- and tuberculin-reactive T cells in peripheral blood mononuclear cells (PBMCs) as a result of redistribution of memory T cells to the blood, it need not reflect a generalised immune reconstitution in the patients.

It has been shown before that a rise in CD4⁺ T cell numbers following therapy is a correlate of clinical benefit.³⁴-³⁷ Qualitative T-cell functional improvement, which is known to be of prognostic relevance in HIV-1 infection, [³⁸] has been observed in clinical trials.³⁹-⁴¹ Complete quantitative or qualitative reconstitution of the immune system may not be achieved or may take a long time to be achieved. It remains to be seen how the initial rapid distribution of memory cells, the gradual slow increase in naive T cells or the
functional improvement of T cells contribute to immune reconstitution and long-term clinical response to antiviral combination therapy. We anticipate that the latter two may be most critical for successful immune restoration following therapy.

METHODS

Lymphocyte counts and T-cell function
Patients were followed at weekly intervals between week -2 and 4 from the onset of therapy, with additional visits at day 3 and day 10, and at week 6, 8, and every four weeks thereafter. Whole blood collected in EDTA was analysed by a whole-blood lysis technique. Lymphocyte immunophenotyping for peripheral CD4+ and CD8+ T cells and CD19+ B cells was assessed by two-color immunofluorescence flow cytometry with a fluorescence-activated cell sorter (FACScan, Becton Dickinson, San Jose, CA). Proliferative response to CD3 monoclonal antibodies (mAbs) was determined in whole-blood lymphocyte culture, as previously described in detail.\(^\text{[24]}\) In short, proliferative capacity was measured by adding \(^{3}\)H-thymidine to the cultures at day 3. After a 24-h incubation, the incorporation of \(^{3}\)H-thymidine was measured as counts per minute. Proliferative capacity was expressed per 10\(^3\) CD3+ T cells using the absolute CD4+ and CD8+ T-cell count.

Naive and memory lymphocyte counts
Three-color flow cytometry was performed for supplemental T-lymphocyte subsets, expressing the surface proteins CD45RA, CD45RO and CD62L (L-selectin). A panel of mAbs to the following cell surface markers was used: fluorescein isothiocyanate (FITC)-conjugated CD62L, phycoerythrin (PE)-conjugated CD45RA and CD45RO, CD4 and CD8 PerCP. T-cell subsets expressing CD45RA and CD62L were regarded as truly naïve T cells, whereas T cells expressing CD45RO, as well as T cells that express CD45RA but lack CD62L, were regarded as memory lymphocytes. Note that the sum of memory and naïve cells is less than the absolute number of T cells because of dull stained T cells for CD45RA and CD45RO.

Viral RNA load
Plasma HIV-1 RNA levels were measured by a quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) assay (Amplicor-HIV Monitor Test, Roche Molecular Systems, Branchburg, NJ) with a mean lower limit of quantification for this study of 251 copies/mL. Plasma RNA load levels were log-transformed, and cutoff values were assigned to measures below the assay’s lower quantification limit (log\(_{10}\)251 = 2.4 log copies/mL).

Statistical analysis
Analyses were performed for the intention-to-treat population with no data exclusions. Patients withdrawn from study medication were followed until other antiretroviral treatment was started. Time-dependent analyses were
performed using a repeated measurement model (Proc mixed, SAS 6.11) including baseline values, time since the start of treatment and therapy group as covariates. Immunological and virological baseline values were calculated as the mean value of three measurements taken before treatment (week -2, -1 and 0).

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