CD4+ T Cell Kinetics and Activation in Human Immunodeficiency Virus–Infected Patients Who Remain Viremic Despite Long-Term Treatment with Protease Inhibitor–Based Therapy

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T cell dynamics were studied in human immunodeficiency virus–infected patients who continued using antiretroviral therapy despite detectable plasma viremia (RNA copies >2500 /mL). CD4+ cell fractional replacement rates, measured by the deuterated glucose technique, were lower in treated patients with detectable viremia than in untreated patients and were similar to those in patients with undetectable viremia. Cell cycle and activation markers exhibited similar trends. For any level of viremia, CD4+ cell fractional replacement rates were lower in patients with drug-resistant virus than in patients with wild-type virus, which suggests that the resistant variant was less virulent. Interruption of treatment in patients with drug-resistant viremia resulted in increased CD4+ cell activation, increased CD4+ cell turnover, and decreased CD4+ cell counts. These data indicate that partial virus suppression reduces CD4+ cell turnover and activation, thereby resulting in sustained CD4+ cell gains, and that measurements of T cell dynamics may provide an in vivo marker of viral virulence.

Combination antiretroviral therapy results in sustained increases in CD4+ T cell counts and slowing of disease progression [1–3]. Although these immunologic and clinical benefits are related to suppression of human immunodeficiency virus (HIV) replication, the degree to which suppression of plasma viremia to undetectable levels is required for durable immunologic benefit is not known [4–7]. Indeed, in patients continuing a protease inhibitor–based regimen despite detectable viremia, CD4+ T cell counts often remain elevated above the pretherapy nadirs. CD4+ T cell changes in these patients are associated with the magnitude of reduction in plasma HIV-1 RNA levels below the pretherapy steady-state level of viremia and not by the absolute level of viremia achieved [4]. These observations indicate that partial suppression of viral replication in some manner alters the long-term dynamics of CD4+ T cell production, destruction, or distribution and thereby results in a durable increase in CD4+ T cells.

This apparent “disconnect” between incomplete suppression of HIV-1 replication (“virologic failure”) on the one hand and immunologic benefit on the other has generated uncertainty regarding the clinical management of such patients. Measurement of T cell kinetics and T cell activation might be useful in this regard. Using deuterated glucose to label DNA in actively proliferating CD4+ T cells, we showed earlier that untreated HIV infection is associated with accelerated CD4+ T cell replacement (i.e., shorter T cell survival time) and limited or no compensatory increase in absolute production rate of new cells [8–10]. Durable suppression of plasma viremia to undetectable levels for 12–24 months results in longer CD4+ T cell survival and restoration of normal CD4+ T cell kinetics [9]. T cell activation shows a similar pattern, with elevation of activation during untreated HIV-1 infection and lowering of activation by durable virus suppression [11–15]. The effects on T cell kinetics and activation of continuing combination therapy, despite detectable viremia (virologic failure), have not been determined.

Interruption of therapy has proven to be a useful strategy in studying the pathogenesis of treated and untreated HIV disease. We recently investigated the virologic consequences of prolonged incomplete virus suppression (plasma HIV RNA level >2500 copies/mL) by interrupting therapy in a controlled man-
ner [7]. Recombinant viruses containing patient-derived reverse transcriptase and protease sequences demonstrated significant reductions in replicative capacity in the absence of drug, whereas discontinuation of therapy for 12 weeks resulted in the emergence of wild-type HIV-1 with increased replicative capacity. This was temporally associated with increased levels of plasma HIV-1 RNA and decreased peripheral CD4+ T cell counts. Whether reduced viral replicative capacity in vitro has any functional consequences on T cell survival, production, or activation in vivo was not addressed in our initial report.

Here, we sought to establish the immunologic basis for sustained CD4+ T cell increases in patients continuing to receive therapy despite significant levels of plasma viremia (plasma HIV RNA levels >2500 copies/mL). Two studies were done to address this question. The first study was a cross-sectional analysis of 3 groups of HIV-1–infected patients. The second study examined the effects of a 12-week treatment interruption in a group of patients with detectable viremia despite prolonged treatment with protease inhibitor therapy. In each study, deuterated glucose was used as a marker of CD4+ T cell production, to measure CD4+ T cell kinetics by labeling and quantifying replicating DNA and RNA and decreased peripheral CD4+ T cell counts. Whether re-
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newly divided T cell in the peripheral blood is balanced by the loss of a previously existing cell). In patients whose antiretroviral therapy was discontinued, CD4+ T cell counts continued to decline throughout the 12-week treatment-interruption period, including the period of the isotope-labeling study. Accordingly, we applied a nonsteady-state correction (modified Steele equation) [19] to calculate the CD4+ T cell fractional removal rate \( (k_d) \). CD4+ T cell counts were measured every week during the period 4–6 weeks before to 2 weeks after the infusion, and the interval change in the CD4+ T cell counts was measured for each subject. \( k_d \) was calculated from the measured production rate (based on label incorporation) and the rate of change in the circulating pool size (i.e., removal rate \( \text{cells/µL per day} \) = production rate \( \text{cells/µL per day} \) – change in pool size \( \text{cells/µL per day} \)) as a negative number.

We have observed no differences in T cell labeling kinetics between 24- and 48-h intravenous \(^{[2H]}\)glucose infusions (unpublished observations). The proportion of labeled T cells after pulse label was discontinuous, CD4+ T cell counts continued to decline throughout the 12-week treatment-interruption period, including the period of the isotope-labeling study. Accordingly, we applied a nonsteady-state correction (modified Steele equation) [19] to calculate the CD4+ T cell fractional removal rate \( (k_d) \). CD4+ T cell counts were measured every week during the period 4–6 weeks before to 2 weeks after the infusion, and the interval change in the CD4+ T cell counts was measured for each subject. \( k_d \) was calculated from the measured production rate (based on label incorporation) and the rate of change in the circulating pool size (i.e., removal rate \( \text{cells/µL per day} \) = production rate \( \text{cells/µL per day} \) – change in pool size \( \text{cells/µL per day} \))/mean interval pool size (cells/µL), where a decrease in pool size is expressed as a negative number.

Statistical analysis. Median values (with interquartile ranges [IQRs]) were determined for all variables. Nonparametric methods were used for univariate, comparison, and correlation tests. Differences in variables between patient groups were analyzed with the Mann-Whitney \( U \) test. All correlations were determined by use of Spearman’s rank correlation. All \( P \) values were 2-sided. Statistical analyses were done by use of SPSS software (version 8.0; SPSS).

### Results

**Cross-sectional study of 3 treatment groups.** In total, 71 patients were included in our cross-sectional study: 36 were in the virologic failure group and 18 and 17 in the virologic success and untreated groups, respectively (table 1). The pretreatment CD4+ T cell nadir was similar among the 3 groups: The virologic failure and success groups had median pretherapy CD4+ T cell counts of 142 and 215 cells/µm³, respectively, and the untreated patients had 162 cells/µm³. The median change in CD4+ T cell count between initiation of protease inhibitor therapy and study entry was 277 cells/µm³ in the virologic success group and 109 cells/µm³ in the virologic failure group. Patients in the virologic failure group had detectable viremia while receiving a protease inhibitor-containing regimen for a median of 28 continuous months preceding study entry (table 1).

Virus obtained from plasma of patients experiencing virologic failure exhibited moderate to high reductions in phenotypic drug susceptibility, as we have reported elsewhere [7]. The median decrease at study entry in susceptibility to the protease inhibitor used was 24-fold (IQR, 12–58-fold). The median viral replicative capacity was 0.12 (IQR, 0.09–0.18) for the virologic failure group and 0.62 (IQR, 0.49–0.68) for untreated patients (\( P < .001 \)).

CD4+ T cell phenotype and cell cycle markers in the 3 treatment groups. The level of CD4+ T cell activation differed significantly across the 3 treatment groups (figure 1). The median percentages of CD4+ T cells coexpressing CD38 and HLA-DR

### Table 1. Characteristics of 3 groups of human immunodeficiency virus (HIV)–1–infected adults receiving 3 different treatment regimens.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Untreated ( n = 17 )</th>
<th>Virologic failure ( n = 36 )</th>
<th>Virologic success ( n = 18 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>41.5</td>
<td>40.7</td>
<td>42.9</td>
</tr>
<tr>
<td>Pre–protease inhibitor CD4+ T cell count, cells/µm³</td>
<td>NA</td>
<td>142 (87–202)</td>
<td>215 (105–287)</td>
</tr>
<tr>
<td>Pre–protease inhibitor plasma HIV RNA, log₁₀ copies/mL</td>
<td>NA</td>
<td>4.81 (4.50–5.11)</td>
<td>4.98 (4.36–5.15)</td>
</tr>
<tr>
<td>Duration of protease inhibitor therapy, months</td>
<td>NA</td>
<td>32.1 (28.7–6.5)</td>
<td>32.9 (29.0–38.9)</td>
</tr>
<tr>
<td>Time elapsed since virologic failure, months</td>
<td>NA</td>
<td>27.8 (24.6–32.1)</td>
<td>NA</td>
</tr>
<tr>
<td>Change in CD4+ T cell count/µm³, pretherapy to study entry</td>
<td>NA</td>
<td>+109 (56–197)</td>
<td>+277 (143–356)</td>
</tr>
<tr>
<td>CD4+ T cell count/µm³ at study entry</td>
<td>162 (128–244)</td>
<td>258 (205–316)</td>
<td>478 (342–597)</td>
</tr>
<tr>
<td>CD8+ T cell count/µm³ at study entry</td>
<td>721 (620–952)</td>
<td>989 (613–1402)</td>
<td>767 (637–928)</td>
</tr>
<tr>
<td>Plasma HIV RNA levels at study entry, log₁₀ copies RNA/µL</td>
<td>5.33 (4.71–5.38)</td>
<td>4.35 (3.83–4.63)</td>
<td>1.7 (&lt;1.7)</td>
</tr>
<tr>
<td>% Naive CD4+ T cells</td>
<td>16.5 (13–25.5)</td>
<td>22.1 (14.9–33)</td>
<td>23.2 (14.4–38.8)</td>
</tr>
<tr>
<td>% Naive CD4+ T cells</td>
<td>34 (23–52)</td>
<td>56 (30–96)</td>
<td>101 (56–251)</td>
</tr>
<tr>
<td>% Memory/effector CD4+ T cells</td>
<td>83.5 (74.6–87.1)</td>
<td>78 (67–85.1)</td>
<td>76.9 (61.3–85.6)</td>
</tr>
<tr>
<td>% Memory/effector CD4+ T cell count/µm³</td>
<td>149 (92–205)</td>
<td>170 (141–256)</td>
<td>315 (222–384)</td>
</tr>
<tr>
<td>% Naive CD8+ T cells</td>
<td>11.7 (4–16)</td>
<td>8.8 (6–16.6)</td>
<td>11.2 (4.3–22.9)</td>
</tr>
<tr>
<td>% Naive CD8+ T cell count/µm³</td>
<td>59 (38–108)</td>
<td>103 (39–59)</td>
<td>83 (23–159)</td>
</tr>
<tr>
<td>% Memory/effector CD8+ T cells</td>
<td>88.3 (84–96.1)</td>
<td>91.3 (83.5–94)</td>
<td>88.7 (77.2–95.7)</td>
</tr>
<tr>
<td>Memory/effector CD8+ T cell count/µm³</td>
<td>640 (455–739)</td>
<td>833 (610–1246)</td>
<td>644 (517–866)</td>
</tr>
</tbody>
</table>

**NOTE.** Values are median (25th–75th interquartile range). Characteristics of patients in the 3 study groups at the time of the study were as follows: virologic failure group, plasma HIV RNA level >2500 copies/µL while receiving stable therapy with a protease inhibitor–based regimen; virologic success group, plasma HIV RNA level <50 copies/µL while receiving stable therapy with a protease inhibitor–based regimen; and untreated group, plasma HIV RNA level >2500 copies/µL and no antiretroviral therapy. Pretherapy, date protease inhibitor therapy was initiated (these data were obtained retrospectively through chart review); virologic failure, time of documentation of viremia (HIV RNA level >2500 copies/µL). Naive cell populations were defined as those coexpressing CD45RA and 62L, and memory/effector cell populations were defined as those with the following phenotypes: CD45RA+62L-, CD45RA–62L+, and CD45RA–62L–. NA, not applicable.
there were 13.1%, 7.6%, and 3.1% (untreated, virologic failure, and virologic success groups, respectively; \( P < .01 \) for all pairwise comparisons; figure 1).

The median percentage of CD4+ T cells expressing Ki67 antigen also varied across the 3 groups (figure 1B), with values highest among the untreated patients (8.7%), intermediate among patients with virologic failure (4.3%), and lowest in the virologic success group (2.3%). The difference between the virologic failure and success groups was not significant (\( P = .4 \)). The percentage of CD4+ T cells expressing Ki67 was significantly higher in the untreated patients than in patients in the 2 treated groups (\( P = .04 \) for untreated group vs. virologic failure group; \( P = .01 \) for untreated group vs. virologic success group).

The percentage of CD4+ T cells expressing either naive or memory/effector cell surface markers was similar across the 3 treatment groups (table 1). However, the absolute number of naive CD4+ T cells differed among the 3 groups and was highest in the virologic success group, intermediate in the virologic failure group, and lowest in the untreated group (\( P = .02 \) for virologic failure group vs. success group; \( P = .06 \) for virologic failure group vs. untreated group; \( P = .002 \) for virologic success group vs. untreated group). Similar trends were observed with the absolute number of memory/effector CD4+ T cells (\( P < .10 \) for each pairwise comparison).

**CD8+ T cell activation markers and cell cycle markers in the 3 treatment groups.** The level of CD8+ T cell activation was assessed in the 3 treatment groups. The median percentages of CD8+ T cells coexpressing CD38 and HLA-DR were 24.2%, 13.9%, and 4.2% (untreated, virologic failure, and virologic success groups, respectively; \( P < .01 \) for each pairwise comparison). The median percentage of CD8+ T cells expressing Ki67 antigen also varied across the 3 groups, with values being highest among the untreated patients (4.4%), intermediate among patients with virologic failure (3.5%), and lowest in the virologic success group (2.5%). The differences among these 3 groups were not statistically significant (\( P > .1 \) for each pairwise comparison).

**CD4+ T cell kinetics in treated patients with detectable plasma viremia.** Using the [3H]glucose/FACS/mass spectrometric technique to measure cell proliferation [8, 9], we analyzed CD4+ T cell turnover in a group of 17 patients experiencing virologic failure while receiving a protease inhibitor–based treatment regimen (median CD4+ T cell count, 295 cells/mm\(^3\); [IQR, 236–358 cells/mm\(^3\)]; median HIV-1 RNA level, 4.37 log\(_{10}\) copies/mL; [IQR, 3.89–4.93 log\(_{10}\) copies/mL]). CD4+ T cell counts in this group were 138 cells/mm\(^3\) above their pretherapy levels (IQR, 77–233 cells/mm\(^3\)). The median k of the total peripheral CD4+ T cell population was 0.010 d\(^{-1}\) (IQR, 0.008–0.014 d\(^{-1}\); table 2).

Figure 2 compares values from individual subjects with previously reported [9] \( k \) from patients who were untreated \( (n = 10); \) median CD4+ T cell count, 243 cells/mm\(^3\); plasma HIV-1 RNA level, 4.63 log\(_{10}\) copies/mL; mean CD4+ T cell count, 0.032 d\(^{-1}\); or who had achieved long-term virus suppression on a protease inhibitor–based regimen \( (n = 8); \) median CD4+ T cell count, 487 cells/mm\(^3\); CD4+ T cell count, 0.009 d\(^{-1}\)). The \( k \) of CD4+ T cells was significantly lower in the virologic failure group than in the untreated group \( (P < .001); \) however, the difference between the virologic failure and virologic success groups was not significant \( (P = .32) \). The median CD4+ T cell half-lives for the 3 groups were 68 days (virologic failure), 22 days (untreated), and 82 days (virologic success).

Similar results were observed for the kinetics of memory/effector CD4+ T cells, with significantly higher \( k \) rates in the...
Absolute CD8+ T cell production, cells/virologic failure and virologic success groups was not significant. Factor CD8+ T cells paralleled the results for CD4+ T cells (table 1). Kinetic results for CD8+ T cells and memory/effector CD8+ T cells are shown for each of 3 treatment groups of human immunodeficiency virus (HIV)–1–infected adults receiving different treatment regimens. Data for the untreated and virologic success groups have been reported elsewhere [9]. For any given level of plasma viremia, the change in absolute number of memory/effector CD4+ T cells was lower in the virologic failure group than in the untreated group (untreated group than in either treated group but no significant difference between the virologic failure and success groups (table 2).

CD8+ T cell kinetics in treated patients with detectable plasma viremia. Kinetic results for CD8+ T cells and memory/effector CD8+ T cells paralleled the results for CD4+ T cells (table 2). The k of CD8+ T cells and of memory/effector CD8+ T cells was significantly lower in the virologic failure group than in the untreated group (P < .01), whereas the difference between the virologic failure and virologic success groups was not significant.

CD4+ T cell kinetics in patients with drug-resistant versus wild-type viremia. The relationship between the level of viremia (log10 HIV RNA copies/mL) and CD4+ T cell k was evaluated (figure 2B). Plasma virus from all patients in the virologic failure group had phenotypic evidence of protease inhibitor resistance. For any given level of plasma viremia, k was lower in treated patients with drug-resistant viremia than in untreated patients. This difference was particularly evident when plasma HIV RNA levels were >4.5 log10 RNA copies/mL of plasma.

The effects of treatment interruption. Eighteen patients underwent a prospective 12-week treatment interruption. As detailed elsewhere [7], treatment interruption for 12 weeks was associated with decreased CD4+ T cell counts (median decrease, 89 cells/mm3), increased plasma HIV-1 RNA levels (median increase, 0.87 log10 copies/mL), and the emergence of HIV-1 with increased replicative capacity (median, from 0.26 to 0.63; P = .001). Although the percentage of CD4+ T cells expressing either a naive or a memory/effector phenotype did not change during treatment interruption (median, from 20.2% to 16.5% for naive CD4 T cells and from 79.8% to 83.5% for memory CD4 T cells), the absolute number of both naive and memory/effector phenotype CD4+ T cells decreased, with most of the reduction in total CD4+ T cells in the memory/effector population (median, from 36 to 31 cells/mm3 for change in absolute number of naive CD4+ T cells [P = .004] and from 162 to 100 cells/mm3 for change in absolute number of memory/effector CD4+ T cells [P = .001]). Counts of total and naive phenotype (percentage or absolute) CD8+ T cells and memory/effector phenotype CD8+ T cells (percentage or absolute) remained stable during the 12-week treatment interruption (data not shown).

During the 12-week treatment interruption, the percentage of CD4+ T cells coexpressing CD38 and HLA-DR increased significantly.

Table 2. T cell turnover in 3 groups of human immunodeficiency virus (HIV)–1–infected adults receiving different treatment regimens.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Virologic failure</th>
<th>Virologic success</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T cell fractional replacement rate, (k) (d⁻¹)</td>
<td>0.032 (0.023–0.036) (n = 10)</td>
<td>0.010 (0.008–0.014) (n = 17)</td>
<td>0.009 (0.007–0.011) (n = 8)</td>
</tr>
<tr>
<td>Absolute CD4+ T cell production, cells/µL/day</td>
<td>6.6 (5.7–8.8) (n = 10)</td>
<td>2.8 (1.9–3.3) (n = 17)</td>
<td>3.6 (2.9–5.3) (n = 8)</td>
</tr>
<tr>
<td>Memory/effector CD4+ T cell fractional replacement rate, (k) (d⁻¹)</td>
<td>0.026 (0.021–0.031) (n = 8)</td>
<td>0.012 (0.006–0.016) (n = 10)</td>
<td>0.011 (0.009–0.014) (n = 8)</td>
</tr>
<tr>
<td>CD8+ T cell fractional replacement rate, (k) (d⁻¹)</td>
<td>0.028 (0.022–0.033) (n = 8)</td>
<td>0.007 (0.005–0.01) (n = 16)</td>
<td>0.005 (0.004–0.014) (n = 7)</td>
</tr>
<tr>
<td>Absolute CD8+ T cell production, cells/µL/day</td>
<td>23 (11.4–30.1) (n = 8)</td>
<td>7 (4.7–13.6) (n = 15)</td>
<td>5.6 (3.3–9.8) (n = 7)</td>
</tr>
<tr>
<td>Memory/effector CD8+ T cell fractional replacement rate, (k) (d⁻¹)</td>
<td>0.031 (0.022–0.032) (n = 12)</td>
<td>0.011 (0.009–0.013) (n = 15)</td>
<td>0.01 (0.005–0.014) (n = 6)</td>
</tr>
</tbody>
</table>

NOTE. Values are median (interquartile range). CD4+ and CD8+ T cell kinetics are shown for treated patients experiencing virologic failure (plasma HIV RNA level >2500 copies/mL during long-term protease inhibitor treatment) and are compared with data reported earlier for untreated patients or treated patients experiencing long-term virus suppression (plasma HIV RNA level <50 copies/mL) [9]. For each parameter tested, the difference between the virologic failure group and the untreated group was significant (P < .01), whereas the difference between the virologic failure and virologic success groups was not (P > .10).

Figure 2. A, Fractional replacement rates (k) of CD4+ T cells are shown for each of 3 treatment groups of human immunodeficiency virus (HIV)–1–infected patients (untreated, virologic failure, and virologic success). Data for the untreated and virologic success groups have been reported elsewhere [9]. B, k vs. the absolute level of viremia (log10 copies/mL) for the 2 treatment groups with detectable viremia (virologic failure and untreated).
were significant \((n = 9)\) in human immunodeficiency virus (HIV)–infected patients. At the time therapy was discontinued, patients were in a state of long-term virologic failure (HIV RNA level >2500 copies/mL) while receiving a protease inhibitor–based regimen. The differences between baseline and week 12 were significant \((P = .006\) for percentage of CD4+ T cells coexpressing CD38 and HLA-DR and \(P = .01\) for fractional removal rate).

Nine subjects agreed at the time of enrollment to have repeated measurements of CD4+ T cell kinetics after the 12-week treatment interruption. After correcting for the non–steady-state decline in CD4+ T cells during the infusion study period, the fractional removal rate \((k_d)\) of peripheral CD4+ T cells after 12 weeks of treatment interruption was \(0.023 \pm 0.017 d^{-1}\) (IQR, 0.019–0.034 \(d^{-1}\)), which was significantly higher than the value obtained before treatment interruption \((0.011 d^{-1})\) [IQR, 0.01–0.018 \(d^{-1}\); \(P = .01\); figure 3B]. There was no change in \(k_d\) for CD8+ T cells (data not shown).

**Discussion**

The immunologic consequences of partial virus suppression with a protease inhibitor–based regimen have not been fully established. Here, we measured host-related immunologic factors (including proliferation, removal, and activation of circulating T lymphocytes) and their relationship to the virologic response to therapy or to its discontinuation. A number of interesting findings emerged. First, the \(k\) values of CD4+ T cells in long-term treated patients with detectable plasma viremia (plasma HIV RNA levels >2500 copies/mL) were lower than those we observed earlier in untreated patients and were similar to values found in long-term treated patients with undetectable levels of plasma viremia [9]. Second, CD4+ T cell activation and cell cycle markers also were lower in treated patients with detectable plasma viremia than in untreated patients and were similar to levels observed in treated patients with undetectable levels of plasma viremia. Third, for any given level of viremia, the CD4+ T cell \(k\)—a possible in vivo marker of virulence—was lower in treated patients with drug-resistant viremia than in untreated patients with wild-type viremia. Last, when treatment was interrupted for 12 weeks after a period of prolonged virologic failure, HIV-1 RNA levels increased, and this was associated with increased CD4+ T cell activation and an accelerated removal rate of circulating CD4+ T cells.

Collectively, these observations suggest that partial virus suppression with a protease inhibitor–based regimen results in a state of relative immunologic quiescence, in which T cell dynamics are similar but not identical to those observed in successfully treated patients. Thus, the apparent benefits of effective antiretroviral therapy for CD4+ T cell dynamics can be achieved despite the continued presence of detectable plasma viremia, at least during 2–3 years of follow-up.

The interpretation of CD4+ T cell kinetics in the context of both untreated and treated HIV infection remains controversial [10]. In our current analysis of patients with prolonged virologic failure, we assumed that patients had achieved steady state in CD4+ T cell pools. Thus, at the time of deuterated glucose labeling, the number of peripheral CD4+ T cells being produced was equal to the number of CD4+ T cells being removed, and the fraction of CD4+ T cells incorporating deuterated glucose into DNA was balanced by an equal fraction of CD4+ T cells being destroyed. Compared with the untreated patients studied earlier [9], we observed that the relative fraction of cells actively being produced and removed in the virologic failure group was low. Indeed, this low turnover state was similar to that observed earlier by our group in patients achieving long-term virus suppression (HIV RNA levels <50 copies/mL) while receiving combination therapy [9].

The sustained CD4+ T cell increases during treatment with a partially suppressive regimen might be attributed to increased CD4+ T cell production, decreased CD4+ T cell destruction (i.e., prolonged survival), or both. Our labeling data indicating that CD4+ T cells exhibit slower turnover in patients with partial virus suppression suggest prolonged T cell survival as the primary mechanism underlying the sustained CD4 cell increase.
often observed in these patients. The fraction of CD4+ T cells expressing markers of activation (i.e., CD38+ HLA-DR*) and the fraction of cells actively cycling (i.e., Ki67+) demonstrated similar trends, with these fractions being much lower during partial virus suppression than in untreated patients. Last, when compared with untreated patients, patients experiencing a partial virologic response to therapy had a CD4+ T cell population that was enriched for naive CD4+ T cells, which are known to have a prolonged survival time [9]. Each of these observations—low turnover, less activation, and greater numbers of naive cells—is consistent with a greater mean life span for peripheral CD4+ T cells.

Longer survival of peripheral CD4+ T cells may, in turn, have several explanations. First, reduced levels of viral replication and/or the emergence of a less virulent virus may lead to reduced CD4+ and CD8+ T cell activation and thus less activation-induced cell death [12, 20–23]. Second, chronic, diffuse T cell activation may suppress the normal homeostatic production of long-lived T cells by altering their activation threshold for cell division [22, 24]. Third, the emergence of a drug-resistant virus population that is less able to productively infect thymic tissue [25, 26] may allow for a greater production of long-lived naive and true memory CD4 cells. The first 2 mechanisms are supported by our observations that CD4 T cell half-life was longer in those patients with lower measures of T cell activation (i.e., virologic failure subjects vs. untreated subjects and pretreatment interruption subjects vs. posttreatment interruption subjects). The final mechanism is consistent with our recent observation that protease inhibitor–resistant viruses, compared with wild-type viruses, have attenuated replicative potential in human thymic tissue [25]. The observation that thymic output (as measured by the frequency of CD4+ T cells containing T cell receptor excision circles) predicts CD4+ T cell gains during virologic failure [27, 28], is also consistent with the latter hypothesized mechanism.

We currently cannot distinguish between these possible mechanisms, nor can we assess to what degree the immunologic benefit of partial virus suppression is due to altered pathogenicity of the drug-resistant virus or to the lower virus load present in each individual. Because plasma virus load and viral phenotype varied in parallel in both our cross-sectional and longitudinal studies, their independent causal roles cannot be distinguished. However, for any given level of viremia, T cell destruction rates were generally lower in treated patients with drug-resistant virus than in untreated patients with wild-type virus (figure 2B). This difference was particularly striking at plasma HIV RNA levels >4.5 log_{10} copies/mL. These observations, although preliminary and limited by small sample size, suggest that drug-resistant virus has altered pathogenicity that is not fully explained by reduced in vivo replication.

At present, no laboratory markers are available to characterize the in vivo virulence, or immunopathogenicity, of an HIV-infected patient’s predominant viral phenotype. Ex vivo replication capacity of a viral isolate does not necessarily reflect the in vivo consequences for T lymphocyte survival or production. Our documentation of widely different CD4+ T cell kinetics (fraction replacement and destruction rates, reflecting longer average survival) in patients with drug-resistant versus wild-type virus isolates at similar levels of plasma viremia (figure 2B), therefore, represents a potential functional marker of virulence in patients who are viremic. If confirmed in other settings, this may prove to be a useful test for research studies or for clinical management of HIV-infected individuals with viremia.

Elevated levels of CD8+ T cell activation have been associated with accelerated disease progression in untreated patients [13]. The mechanism underlying this observation has not been elucidated. In particular, it is not clear whether a high CD8+ T cell activation level drives CD4+ T cell depletion and disease progression or whether high CD8+ T cell activation is an epiphenomenon associated with other factors driving disease progression. In the current study, we observed significantly higher levels of CD8+ T cell activation and turnover (k) in untreated patients with wild-type virus than in treated patients with resistant virus, which suggests that partial virus suppression and/or emergence of a less-fit virus results in less CD8+ T cell activation and death. When therapy was discontinued, CD8+ T cell activation levels increased, although the change was not significant. CD8+ T cell fractional destruction (k_d) and the total CD8+ T cell count did not change. We cannot offer a definitive explanation for the stability in CD8+ T cell counts and dynamics after treatment interruption, but we can offer some speculations. One possibility is that the initial effect of increased viremia with a wild-type virus acts directly on CD4+ T cells, whereas the effect on CD8+ T cells is indirect (i.e., requires emergence of increased antigenic load due to opportunistic infections). The sources of CD8+ T cells may also be different from those of CD4+ T cells and may be differentially affected by increased viremia with wild-type virus.

It might be argued that plasma HIV RNA levels less than or greater than 50 copies/mm^3 represent an arbitrary distinction and that the virologic response to treatment should be considered to be a continuum. Of note, we observed differences between “complete” (i.e., plasma HIV RNA levels <50 copies/mL) and “incomplete” virologic suppression (i.e., plasma HIV RNA levels >2500 copies/mL). Patients achieving and maintaining undetectable levels of plasma viremia had greater treatment-mediated CD4+ T cell increases than those who had achieved only a partial virologic response. Furthermore, there were nonsignificant trends suggesting improved CD4+ T cell activation or dynamics (figures 1 and 2) in treated patients with undetectable plasma viremia compared with treated patients with detectable plasma viremia. Collectively, these observations are consistent with cohort data indicating that the greater the degree of virus suppression, the greater the degree of peripheral CD4+ T cell recovery [4, 29, 30]. Our observations, therefore, continue to support recommendations that durable suppression of plasma viremia to below the level of detection remains the standard goal of combination therapy.
The clinical implications of our findings remain to be established. Continuation of a drug regimen in the presence of ongoing viral replication will likely select for further drug resistance, thus limiting future options for more effective virus suppression. Presumably, unless more effective therapy is initiated, viral replication will increase over time and will be associated with increased CD4+ T cell activation, accelerated CD4+ T cell destruction, insufficient compensation by CD4+ T cell production, and decreasing peripheral CD4+ T cell counts. When or whether to switch therapy in treated patients with detectable viremia cannot be determined from our data. Nevertheless, these results are consistent with observational data that the natural history of HIV-1–mediated CD4+ T cell depletion is altered by the continued use of antiretroviral therapy, even when plasma viremia remains detectable [4, 6].

Last, the complexities inherent in labeling T cells in vivo should be recognized when interpreting our results. During any period of time, only a subpopulation of the T cell pool is actively dividing. The functional effects on CD4+ T cell pool size of any rate of cell division depends on the nature of the proliferation. Some T cells divide in response to an antigenic stimulus and are destined to die off rapidly, perhaps through activation-induced cell death [12–15, 21, 31]. Other cells may divide and differentiate into long-lived cells as part of “homeostatic” proliferation [22, 24, 31, 32]. If most cell proliferation in an individual is of the short-lived variety, the result will be a smaller pool size at steady state, compared with the same total proliferation rate in mainly long-lived cells. Thus, in our current study, a lower daily replacement fraction but larger pool size of CD4+ T cells in patients with virologic failure (vs. those with untreated HIV-1 infection) implies that the cells being produced have a longer life span. Direct testing of this kinetic explanation requires measurement of the die-away curves of labeled T cells in the 2 settings. Such measurements are now feasible [9] but were not performed in the present study.

In summary, our data indicate that failure of antiretroviral therapy to durably suppress plasma viremia to undetectable levels results in the emergence of a virus population with reduced replicative capacity in vitro, decreased levels of viral replication, and prolonged CD4+ T cell survival, compared with findings in untreated HIV-1 infection. These effects on T cell kinetics are similar but not identical to those observed with long-term complete virus suppression [9]. As a consequence, sustained increases in CD4+ T cell counts occur in patients with drug-resistant virus. active antiretroviral therapy in advanced HIV-1 disease. Lancet 1998; 351:1682–6.


