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Markers of Lymphocyte Homing Distinguish CD4 T Cell Subsets That Turn Over in Response to HIV-1 Infection in Humans

Richard L. Hengel,1* † Bonnie M. Jones,* M. Susan Kennedy,* Marjorie R. Hubbard,* and J. Steven McDougal2*

In HIV-1 infection, the abrupt rise in CD4 T cells after effective antiretroviral therapy has been viewed as a measure of HIV-1-related CD4 T cell turnover in the steady state. The early (2–4 wk) response is reportedly dominated by CD4 T cells with a memory (CD45RO) phenotype. It is controversial whether the measurement of steady-state kinetics identifies cells that otherwise would have been recruited into a short-lived, virus-producing pool or reflects lymphoid redistribution/sequestration. We performed detailed phenotypic and kinetic analysis of CD4 T cell subsets in 14 patients. Turnover occurs in memory (CD45RO) as well as naive (CD45RA) cells, if the latter are present at baseline. Most of the turnover occurs in those memory (CD45RO) and naive (CD45RA) cells that are programmed for recirculation through lymphoid organs (CD62L+ and CD44low), whereas very little turnover occurs in memory cells (CD45RO) destined for recirculation from blood to tissue (CD62L− and CD44high). Turnover occurs in both activated (CD25+ and HLA-DR+) and nonactivated populations, although it is restricted to CD38-positive cells, indicating that turnover does not measure cells that are already infected. More likely, turnover occurs in cells that replace infected cells or are on their way to becoming infected. Taken together, markers of lymphocyte trafficking better describe cell turnover related to virus replication than do naive and memory markers per se, and lymph organs, not tissue-destined cells or peripheral blood cells, appear to be the important site of virus replication and CD4 T cell turnover, destruction, and redistribution. The Journal of Immunology, 1999, 163: 3539–3548.

H uman immunodeficiency virus 1 infection results in a numerical depletion and functional impairment of CD4 T cells. In vitro, HIV-1 depletes optimally infected and activated CD4 T cells, but the mechanism (excess destruction, inadequate production, or both) of CD4 T cell depletion in vivo is unresolved. CD4 T cells mature into functionally heterogeneous subsets from a common lineage by a reasonably well-understood process of division, migration, selection, differentiation, and proliferation. However, little is known about the normal life span and turnover of CD4 T cells or how HIV-1 infection affects these parameters. The advent of triple-drug treatment regimens that result in dramatic declines in plasma viremia in most patients has provided a unique opportunity to measure viral and cellular turnover in the steady state (1, 2). Theoretically, the initial rise in CD4 T cells or specific subsets of CD4 T cells identifies those cells that otherwise would have succumbed to infection. Although theoretically valid, this interpretation can be questioned for several reasons. The initial CD4 turnover may exceed that required to produce the amount of virus observed (see Discussion). Non-CD4 lymphocytes that are not infected by HIV-1 may also rise initially (5–8). Independent and indirect measurements of CD4 life span (telomere length) do not indicate a shortened life span or accelerated mitotic rate of CD4 T cells (9). Full restoration of CD4 T cell number and function is not achieved (1, 2, 6–8, 10–12), as might be expected if CD4 T cell decline were solely due to undercompensated destruction by HIV-1. When CD4 T cell subsets are examined after initiation of therapy, there are clearly biphasic or multiphasic responses: generally an early rise in CD4 T cells of memory phenotype followed in some by a slower, more sustained response of naive cells (6–8, 10, 11, 13). Pakker et al. (6) proposed that the early memory response represents lymphocyte redistribution related to cessation of virus production and that the later rise in naive cells (as well as memory cells) reflects regeneration of the immune system; the two need not involve the same cellular subsets initially. Of course, destruction and regeneration need not involve the same subsets either. Regardless of whether this reflects steady-state destruction due to cellular infection, bystander effects, or redistribution, the early rise in CD4 T cells after antiretroviral therapy is clearly a response related to interruption of the HIV-1 life cycle.

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In this study, we focus on the early (2–4 wk) rise in CD4 T cells after potent antiretroviral therapy. Our purpose is to define CD4 T cell subsets that turn over in response to virus replication, rather than the subsets that regenerate after prolonged suppression of virus replication. The CD4 T cell subsets examined included the CD45 isoforms RA and RO, representing “naive” and “memory” cells, respectively. We examined subsets thought to further refine the phenotypic definition of these cells by distinguishing homing patterns of lymphocyte traffic (CD62L and CD44) (14–24). Activation markers that are elevated in HIV infection and are expressed in vitro and in vivo on cells actively producing virus or on cells with the potential to produce virus were also examined (CD25, IL-2 receptor alpha (CD25), and HLA-DR). CD45 isoforms helped in setting the cursors because of differences in CD45 expression in the two subsets (Fig. 1). Nevertheless, cursor placement is not arbitrary, because there are not discrete clusters of positive and negative cells; rather, there is a range of intensities that hover above the designated threshold. The inclusion of CD45RA or CD45RO mAbs helped in setting the cursors because of differences in expression in the two subsets (Fig. 1). However, the uncertain placement is arbitrary and may underestimate positivity. However, because of the standardization, a given fluorescence intensity (expression) registered consistently from bleed to bleed, and cursor placement was not changed. Therefore, any errors of under- or overestimation should be systematic. In this case of CD44 mAb, fluorescence intensity was distributed as a continuum of moderate to high intensity, and cursor placement and rationale are explained in Results.

Materials and Methods

Selection of study subjects

We selected subjects from a study of 20 patients undergoing triple drug antiretroviral therapy (a protease and two reverse transcriptase inhibitors). In the first eight patients, a protease inhibitor was added to an existing regimen of two reverse transcriptase inhibitors. The last 12 patients were naive to prior retroviral treatment. More specific details are available elsewhere (8). Informed consent was obtained under a protocol approved by the institutional review boards at Emory University and the Centers for Disease Control and Prevention. From this group of patients, we selected those subjects who had a prompt antiretroviral response, defined as a drop in viral load to undetectable levels (<1000 copies/ml). Sixteen subjects had such a response; of these, 14 had rising absolute CD4 T cell counts in the first 2–3 wk of therapy. The two other had an increase in percentage of CD4 cells but an initial, and possibly drug-related, decline in absolute CD4 cell counts due to a decline in total lymphocyte count. We did not analyze kinetics in terms of CD4 T cell percentage, because the value is influenced by reciprocal changes in other lymphocyte subsets, making interpretation difficult.

Viral load

Viral RNA levels were determined on EDTA-anticoagulated plasma by the nucleic acid sequence-based amplification technique (NASBA, Organon Teknika, Durham, NC) according to the manufacturer’s instructions. Two patients were also tested using the Roche Monitor assay (Roche Diagnostics, Mannheim, Germany). Plasma was stored at −70°C, and all specimens from each individual patient were tested in the same run.

Flow cytometry

Flow cytometry and automated complete blood counts were performed on EDTA-anticoagulated whole blood. Blood was labeled with mAbs, lysed, and fixed within 4 h of collection (36). Analysis was performed on a three-color multiparameter flow cytometer (FACScan, Becton Dickinson Immunocytometry Systems, San Jose, CA) using Cellquest (Becton Dickinson) software. We collected data on 30,000 lymphocytes using a CD45 (FITC) vs side-scatter gate. Within this gate, major lymphocyte subsets were enumerated using peridinin chlorophyll protein (PerCP)3 anti-CD3 mAb, and a third PE-labeled mAb, anti-CD4, CD8, CD16/56, or CD19 (and appropriate fluorescence-labeled isotype controls) (36). The CD45, CD3, and CD4 mAb combinations allowed us to validate the gate used for CD4 subset analysis. For CD4 T cell subset analysis, CD4-positive T cells were gated by reactivity with CD4 mAb (PerCP) and side scatter. Within this gate, CD4 T cells were analyzed for reactivity with either CD45RA or CD45RO mAbs (FITC) and a third PE-labeled mAb, anti-CD62L, CD44, CD38, CD25, or HLA-DR. When possible, data from 5000 CD4+ T cells were collected (a minimum of 2000 were required to be included in analysis). Because all tubes contained either CD45RA mAb or CD45RO mAb, which together stain all CD4 T cells and individually stain separate subsets with minimal overlap, consistency in enumeration could be evaluated both among and between tubes containing the respective mAbs. Of the 16 three-color tubes analyzed at each bleed, 11 contained either or both mAbs. All mAbs were pretreated for optimal and saturating staining, and the same lots were used for all subjects. All mAbs were from Becton Dickinson except the following: FITC-CD45RO mAb (Dako, Carpinteria, CA) and PE-CD44 mAb (Caltag Laboratories, Burlingame, CA).

Daily quality control on the flow cytometer was performed using AutoComp (Becton Dickinson), and photomultiplier tube voltages were adjusted daily to standardize fluorescence measurements by using glutaraldehyde-fixed chicken red blood cells (36). Because of the standardization, cursor placement for discriminating positive and negative cell clusters was highly consistent over the time of blood collection and did not require readjustment. Cursor placement was straightforward for most mAbs, maximizing the demarcation between positive/high and negative/low fluorescence intensity cells. Examples are shown in Fig. 1. For some markers, notably the activation markers HLA-DR and CD25, the designation of positive/negative may seem arbitrary, because there are not discrete clusters of positive and negative cells; rather, there is a range of intensities that hover above the designated threshold. The inclusion of CD45RA or CD45RO mAbs helped in setting the cursors because of differences in expression in the two subsets (Fig. 1). Therefore, any errors of under- or overestimation should be systematic. In this case of CD44 mAb, fluorescence intensity was distributed as a continuum of moderate to high intensity, and cursor placement and rationale are explained in Results.

Kinetic analysis

Data were fitted to both an exponential (first-order) and linear (zero-order) regression model. Regression analysis of viral load data included baseline value, all time points with a measurable load, and the first “undetectable” measurement. The latter was entered in the regression as 1000 copies/ml if its inclusion improved the regression r value. If not, this last value was omitted. Similarly, all ascending CD4 T cell counts up to 28 days were included in the regression. A median of 5 points (range, 4–7) was used for these regressions. No intervening data points were omitted. Statistical analysis comparing rates and regression r values for the linear and exponential models used the paired Wilcoxon signed rank test.

Results

The fit of post-steady-state, HIV-1-related CD4 T cell dynamics to kinetic models

To identify CD4 T cell subset(s) that fluctuate as a proximate effect of virus replication, steady-state analysis requires an intervention that abruptly stops virus production. An accurate measure of the initial change in CD4 cell count is used to infer the kinetics of CD4 T cell turnover in the steady state. Thus, we confine our analysis to patients with a prompt antiviral response and an initial rise in absolute CD4 T cell count. To make this distinction between the models, we analyzed CD4 T cell subset data by both linear and exponential regressions, but we present all subsequent data using linear rate determinations. Linear rate constants (cells/ml/day) are additive for cellular subsets. Exponential rate constants (1/day) require mathematical manipulation (including weighting relative subset proportions) to arrive at total CD4 rate from component subset rates. A point-to-point plot of viral load and absolute CD4 T cell count and the resultant regression lines are shown in Fig. 2.

3 Abbreviation used in this paper: PerCP, peridinin chlorophyll protein.
Turnover occurs in both CD4:CD45RA naive and CD4:CD45RO memory T cell subsets provided the subset is present at baseline.

Combined use of CD4, CD45RA, and CD45RO mAbs defines three subsets of CD4⁺ T cells: CD4⁺RA⁺RO⁻, naive; CD4⁺RA⁻RO⁺, transitional; and CD4⁺RA⁻RO⁻, memory (RA⁻RO⁻ CD4 cells are essentially nonexistent). Both CD4⁺RA⁺RO⁻ and CD4⁺RA⁻RO⁺ cells rose in response to therapy, although CD4⁺RA⁻RO⁻ increases were confined to those patients who had >40 of these cells per microliter at baseline (Fig. 3, Table II). CD4⁺RA⁺RO⁻ increases occurred regardless of baseline pretherapy levels. The transitional, RA⁺RO⁻, population did not, in general, increase. Most of the double-positive enumeration resulted from low-density RA⁺RO⁻ cells bridging the two main clusters of
RA\textsuperscript{+}RO\textsuperscript{−} and RA\textsuperscript{−}RO\textsuperscript{+} cells on 2 × 2 dot plots (Fig. 1). True transitional double-positive cells, as occur when RA cells are stimulated to become RO cells, have higher density RA and/or RO staining and are rarely found in peripheral blood, although they may comprise up to 10% of CD4 cells in lymphoid tissue (23, 37). Selective gating on higher density double-positive cells revealed that they consistently comprise <1% of the CD4 population in PBL. Although kinetic analysis on such a minor population is not really valid, there was certainly no indication that this subset responded.

FIGURE 2. Serial determinations of plasma HIV-1 RNA and CD4 T cell levels after antiretroviral therapy. Plasma HIV-1 levels (left) and CD4 T cell levels (right) are shown in point-to-point plots (top), and the regression lines of these data are shown (bottom). For plasma HIV-1 levels, exponential regressions are shown (shown is log_{10} plot; for kinetic analysis, natural log plots were used). For CD4 T cell levels, linear regressions are shown.

RA\textsuperscript{−}RO\textsuperscript{−} and RA\textsuperscript{−}RO\textsuperscript{+} cells on 2 × 2 dot plots (Fig. 1). True transitional double-positive cells, as occur when RA cells are stimulated to become RO cells, have higher density RA and/or RO staining and are rarely found in peripheral blood, although they may comprise up to 10% of CD4 cells in lymphoid tissue (23, 37). Selective gating on higher density double-positive cells revealed that they consistently comprise <1% of the CD4 population in PBL. Although kinetic analysis on such a minor population is not really valid, there was certainly no indication that this subset responded.

RA\textsuperscript{+}RO\textsuperscript{−} and RA\textsuperscript{−}RO\textsuperscript{+} cells on 2 × 2 dot plots (Fig. 1). True transitional double-positive cells, as occur when RA cells are stimulated to become RO cells, have higher density RA and/or RO staining and are rarely found in peripheral blood, although they may comprise up to 10% of CD4 cells in lymphoid tissue (23, 37). Selective gating on higher density double-positive cells revealed that they consistently comprise <1% of the CD4 population in PBL. Although kinetic analysis on such a minor population is not really valid, there was certainly no indication that this subset responded.
62L+ cells. In the RO subset, CD44low cells accounted for most of the recovery with relatively little participation of CD44high cells (Fig. 4, Table II).

CD38 has a multilineage and discontinuous pattern of cell surface expression on T cells. It is expressed on immature, early T cells and recurs with activation of mature T cells in which it has been implicated in mediating signal transduction and possibly adhesion (39–41). Like CD62L, CD38 was expressed on virtually all CD4:RA cells and subdivided the CD4:RO population into CD38-1 and CD38-2 subsets, the latter of which contribute little to CD4:RO recovery rates (Fig. 4, Table II).

Both activated and nonactivated CD4 T cells participate in CD4 T cell turnover

HLA-DR and the IL-2 receptor, CD25, are expressed upon activation and on productively infected cells, and the number of cells expressing these markers tends to be elevated in HIV infection (25–34). These two markers were expressed at higher levels in CD4:RO cells than in CD4:RA cells (Fig. 1). However, recovery rates were not confined to activation-positive or negative subsets (Fig. 5, Table II).

**Discussion**

The kinetics of viral decline fit an exponential (first-order) regression (Table I), consistent with other studies of viral dynamics (1, 25).

**FIGURE 3.** Serial determinations of CD4 T cells with naive, transitional, and memory phenotypes after antiretroviral therapy. Linear regression plots are shown for CD4 subsets CD4:CD45RA+ (left), CD4:CD45RA+RO+ (middle), and CD4:CD45RO+ (right). The mAbs used for enumeration are listed in Table II.
2) and the known replicative properties of HIV-1. For CD4 T cells, an exponential fit implies a proliferative source for cell turnover, and a linear fit implies a differentiative source. CD4 T cell kinetic measurements have generally not conformed to either an exponential fit implies a proliferative source for cell turnover, and a linear fit implies a differentiative source. CD4 T cell kinetic measurements have generally not conformed to either an exponential or a linear model of production (1, 2, 6–8, 10). We sought to capture the earliest kinetics possible by frequent measuring over a short time frame and by confining the analysis to patients with prompt antiviral responses. Nevertheless, the data do not allow distinction, although in both cases the fits (r values) were quite good (Table I). Failure to clearly distinguish exponential vs linear kinetic fits to total CD4 T cell turnover was also found with the CD4 T cell subset data. We report linear rates as a practical matter, because they are easier to add and compare in the context of total CD4 T cell subset kinetics, without implication as to how these subsets are produced. We have identified a CD4 T cell subset that does not participate in virus-related CD4 T cell turnover and several subsets that do participate. Most analyses of posttherapy CD4 T cell subset recovery have focused on RA (naïve) and RO (memory) phenotypes. In general, an early rise in RO cells may be followed later (in some patients) by a more sustained rise in RA cells (6–8, 10, 11). However, in our study and in others, RA cells did rise immediately after the onset of therapy, provided they were present at baseline (6, 8, 11, 13) (Fig. 3, Table II). Thus, the turnover or “consumption” appears to draw from what is available, regardless of RA or RO phenotype. We found that other markers related to lymphocyte homing discriminate subsets that do and do not participate in turnover better than do the RA and RO markers. CD4 T cells that express CD62L, CD38, and/or low levels of CD44 are “consumed” in the steady state, and cells that express high levels of CD44 and/or are negative for CD62L and CD38 are spared (Fig. 4, Table II). Because of high concordance in expression, three broad phenotypic classes of CD4 cells can be described. These are represented by the first, third, and fourth sets of vertical graphs in Fig. 4: CD4 RA (naïve) cells, virtually all of which express CD62L and CD38 and, by definition, are CD44low, CD4 RO (memory) cells that also express CD62L and CD38 and are CD44 low; and CD4 RO (memory) cells that do not express CD62L or CD38 and are CD44 high. The first two participate in virus-related steady-state turnover; the third does not (Fig. 4, Table II). The markers CD62L, CD38, and CD44 were identified in conjunction with RA and RO and independently of each other. In more limited simultaneous marking experiments, CD62L and CD38 are usually coexpressed and are not found on CD44 high cells. Nevertheless, such broad categorization may discount possibly important subcategories of cells that are discordant for one or more markers, the enumeration of which is highly dependent on how discriminate cursors are set for distinguishing positive and negative.

We propose that recirculation pattern rather than RA or RO phenotype dictates the differing dynamics of these three broad categories of CD4 T cells. Lymphocytes continuously recirculate from blood to tissue or secondary lymphoid organs and back to

### Table II. CD4 T cell subset baseline and turnover

<table>
<thead>
<tr>
<th>Defining mAb Combination</th>
<th>Subset</th>
<th>Baseline, cells/μl (mean ± SD)</th>
<th>Slope, cells/μl/day (mean ± SD)</th>
<th>p Value</th>
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<tr>
<td>CD4, CD3, CD45</td>
<td>CD4</td>
<td>153 ± 199</td>
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<td>CD4, CD45RA, CD45RO</td>
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<td>CD4-RO</td>
<td>90 ± 96</td>
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<td>CD4, CD45RA, CD38</td>
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<td>CD4 CD62L−</td>
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<td>CD4 CD44-high</td>
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<tr>
<td>CD4, CD38</td>
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<td>CD4 HLA DR−</td>
<td>121 ± 180</td>
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*Wilcoxon signed rank test comparison of slopes of designated CD4 T cell subsets. NS, not significant (p > 0.05).*
blood again as frequently as once or twice a day (22). Recirculation is targeted by homing receptors on lymphocytes that mediate tissue-specific, endothelial cell interactions. Lymphocyte subsets display distinct tissue-selective patterns of homing and recirculation, the most striking of which is the differential recirculation pattern of memory and naive T cells. In general, naive T cells recirculate from blood to lymph node (and other lymphoid tissues such as Peyer’s patches, tonsil, and spleen) and back to blood via efferent lymphatics (21–24, 37). This traffic is highly dependent on expression of L-selectin (CD62L) by lymphocytes and the uniquely high expression of CD62L ligand by high endothelial venules in lymph node (18, 22–24, 42). Memory cells (or subsets of them) recirculate through lymphoid organs as well, most probably the subset expressing CD62L. However, memory cells can also traffic through nonlymphoid tissue, a process in part related to concomitant sulfation and high expression of CD44 and the interaction of CD44 with hyaluronate in endothelium and extracellular matrix (15–17, 19–21, 24). The combinatorial and sequential interactions of selected homing receptors dictate the microenvironmental destination and trafficking pattern of memory cells (22, 24). Mindful that phenotypic identification of subsets that are defined functionally may be an imperfect and oversimplified association, we found that memory cells destined for recirculation through tissues do not participate to any degree in virus-related CD4 T cell turnover, whereas naive and memory populations programmed for recirculation through lymphoid organs do.

FIGURE 4. Serial determinations of CD62L, CD44, and CD38 subsets within the CD45RA (naive) and CD45RO (memory) CD4 T cell population after antiretroviral therapy. CD4:RA and CD4:RO cells were analyzed for CD62L-positive/negative subsets (top), CD44 low/high subsets (middle), and CD38-positive/negative subsets (bottom). The mAbs used for enumeration are listed in Table II.

In this context, the presumption of steady-state analysis is that the initial rise in cell count identifies cells that feed directly into a pathway of virus destruction either because they are infected or because they are about to be infected. Our data would indicate that CD4 T cells, naive and memory, that recirculate through lymph organs are the target of virus-related consumption, whereas those CD4 T cells that recirculate through tissue, presumably long-lived memory cells, seem to be relatively spared (Fig. 6). If so, it is likely that these cells are not infected in peripheral blood but become infected in lymph node, where they are ultimately destroyed. If initial infection and replication occurs in a site other than blood, several apparently paradoxical observations could be resolved. Under optimal conditions of in vitro infection and activation, all CD4 T cells, RA and RO, can be infected and depleted. Yet, in peripheral blood, infection and replication-competent virus are found preferentially in CD4 RO cells (25, 26, 29, 30, 32, 43–45). Initial infection may occur in both RA and RO CD4 T cells within lymph node, and the subsequent activation required for viral replication, release of virus, and cell death may result in RA-to-RO conversion (Fig. 6) (25, 26, 29, 30, 32, 43, 45). Surviving cells that escape death and gain egress to peripheral blood would be predominantly RO cells. Primary HIV-1 infection, replication, and release of virus at a site other than blood are compatible with the observation that infected cells are found infrequently in peripheral blood (26, 29, 46) and with the observation that new mutations that appear in plasma virus are not found until some time later in the cellular
FIGURE 5. Serial determinations of activated (HLA-DR and CD25) subsets within the CD45RA (naive) and CD45RO (memory) CD4 T cell population after antiretroviral therapy. CD4:RA and CD4:RO cells were analyzed for HLA-DR-positive/-negative subsets (top) and CD25-positive/-negative subsets (bottom). The mAbs used for enumeration are listed in Table II.

FIGURE 6. Diagram of CD4 T cell homing and HIV-1 infection. In this model, HIV-1 infection occurs in lymph node, and CD4 T cell infection is determined by trafficking pattern. CD4 T memory and naive cell trafficking is determined by differential expression of CD62L, CD44, and CD38 as indicated. Memory cells trafficking through tissue are generally spared exposure to active virus infection. Most memory and naive cells that traffic through lymph node also recirculate without becoming infected. A portion of these cells, however, are infected in situ (a process amplified by an activating microenvironment including potent HIV-1-transmitting dendritic cells). Both CD45RA and CD45RO cells are susceptible to infection. The process of cellular activation required for active virus replication results in CD45RA-to-CD45RO conversion, such that the majority of demonstrably infected cells are CD45RO positive. These cells then die or are sequestered, although a few may gain egress to peripheral blood.
In our study, mean total production of virions is 2.95 higher than that required to produce the observed amount of virus. The observed CD4 T cell turnover is 1–2 orders of magnitude faster than the observed CD4:CD45RO cells that are infected in peripheral blood. Moreover, Wang et al. (42) report that exposure to HIV-1 (without infection) enhances lymphocyte homing of CD4:CD45RO cells and promotes their apoptosis. There is no preferential turnover of CD4 T cells with activation markers, CD25 or HLA-DR, that are found on productively infected cells in vitro (25, 29–32). This would also indicate that PBL kinetics measure turnover in a cell population before rather than after it is producing virus. From this model, one would also predict that those CD4:CD45RO cells found to be infected in peripheral blood are not the CD44high subset of CD4:RO cells, and indeed this is the case. Studies that find a predominance of infection in RO cells in PBL have not found preferential infection in CD44high cells in humans (26), although in SIV infection of rhesus macaques, preferential infection of CD44high cells has been reported (47, 48).

In the previous discussion, we assume that most or all of the turnover involves cells that are or shortly will be producing virus and shortly thereafter will die or disappear. Similar considerations would apply if turnover reflects virus-related sequestration or redistribution away from peripheral blood without cell death or if turnover reflects virus-related cell death without active infection (bystander effect) (6, 42, 51–53). Others have questioned the implication that CD4 T cell turnover represents measurement of a sink of virus-related destruction based on the following: failure to conform to an exponential or linear model (6, 7, 10); apparent biphasic or multiphasic subset responses (6–8, 10, 11, 13); the fact that non-CD4 lymphocyte subsets other than CD4 T cells respond to therapy (6–8); and failure to find a shortened life span of CD4 T cells based on telomere analysis (9), although some combination of accelerated proliferation/activation coupled with random destruction could result in telomere lengths that, overall, are normal. Especially problematic for the explanation that CD4 T cell turnover measures virus-related consumption is the fact that estimates of total body CD4 T cell turnover exceed the number that would seem to be required for virus production, based on our data and those of others (1, 2). From analysis of virus decay, estimates of total-body, daily virus production can be made, and the number of infected cells required to produce this much virus can be calculated (with assumptions about virus burst size and volume of distribution of virus and virus-producing cells) (1, 2). Assuming that each infected cell produces 100–1000 virions (3, 5, 54–56) and that turnover in peripheral blood applies to all CD4 T cells in the body, the observed CD4 T cell turnover is 1–2 orders of magnitude higher than that required to produce the observed amount of virus. In our study, mean total production of virions is 2.95 × 10^9 per day. This is similar to that found in other studies and was calculated in the same way (1, 2). The number of infected cells required to produce this much virus is 2.95–29.5 × 10^6 per day (assuming 100–1000 virions per cell). The mean total-body CD4 T cell turnover observed is 512 × 10^6 per day, 17- to 170-fold greater than that required for virus production. Estimates of virus production and the required CD4 T cell turnover are based on assumptions about distribution of virus and cells and treat the lymphoid system as a sink where measured turnover in PBL is extrapolated to the entire lymphoid system (1, 2). One need only invoke a two- (or more-) compartment model of CD4 consumption, in which PBL represent a pipeline into the lymphoid tissue sink. Here, turnover within blood is the measure of total body turnover. Recalculated, the observed CD4 T cell turnover (10 × 10^6) is compatible with the turnover required for virus production (2.95–29.5 × 10^6 cells/day). The smaller estimate of CD4 T cell turnover is also consistent with more stringent measurements of total body cells containing replication-competent virus (29). Moreover, refined analytic modeling of virus decay with more detailed measurements indicates that the calculation of virus production we used with our data may underestimate virus production by as much as 15-fold (3). If so, this would also serve to rectify the observed and required measurements of cell turnover. Alternatively, the discrepancy in CD4 cell turnover relative to virus production could be resolved by postulating that the majority of CD4 cell death is a bystander phenomenon (42, 51–53).

Although our study does not resolve the issue of whether CD4 turnover represents consumption attributed to direct cytopathicity or bystander cell death or redistribution, it does focus attention on lymphocyte trafficking as an important parameter in virus-related turnover. The differential turnover of tissue-destined memory cells and lymphoid organ-destined memory and naive cells implies that infection and virus-related turnover reflects activity in lymphoid organs rather than PBL or tissue. PBL are in equilibrium and recirculate through tissue and lymphoid compartments, and a sampling of PBL is often considered (hoped) to be representative of the whole mature immune system. However, when the relevant immunologic/virologic activity is in one of the many compartments with which PBL are in equilibrium, sampling PBL can provide a glimpse of the relevant activity, but the sampling cannot be generalized without serious over- or underestimation.

References

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