Homeostasis of Naive and Memory T Cell Subpopulations in Peripheral Blood and Lymphoid Tissues in the Context of Human Immunodeficiency Virus Infection

Mostafa A. Nokta, Xiao-Dong Li, Joan Nichols, Anna Pou, David Asmuth, and Richard B. Pollard

Department of Internal Medicine, Division of Infectious Disease, University of Texas Medical Branch, Galveston

To understand the nature of naive and memory T cell depletion in human immunodeficiency virus (HIV) immunopathogenesis, their homeostasis in peripheral blood (PB) and lymph node (LN) compartments of HIV-infected patients was examined. Although the percentage of naive CD4+ cells was higher in LN than in PB mononuclear cells (LNMC and PBMC, respectively), the memory cells were higher in PBMC than in LNMC. The ratio of naive:memory CD4+ cells from PB positively correlated with that in LNs and with the absolute CD4+ cell counts and recall antigen responses, and the ratio inversely correlated with the cellular virus load from the corresponding compartment. These findings indicate that although the pattern of naive and memory cells in the LN and PB compartments appear divergent, their relationship is nonrandom and is significant. The naive:memory ratio in PB appears to reflect the lymphoid microenvironment and may potentially be useful as a surrogate marker for treatment efficacy and immune reconstitution.

The central defect in human immunodeficiency virus (HIV) disease is the infection and continuous depletion of CD4+ cells. HIV preferentially infects the memory CD4+ T cell subpopulation, eventually compromising the immune response of the infected person. Such immunocompromised individuals experience a loss of naive CD4+ and CD8+ T cells [1, 2], a restricted T cell repertoire [3], and loss of memory and/or effector function [4]. When physiologically matured, single-positive CD4+ or CD8+ lymphocytes emigrate from the thymus, they express a specific isotype of the CD45 family, the CD45RA antigen [5]. These cells are immunologically naive [6, 7]. After challenge with an antigen in the periphery, they convert into a CD45RO+ phenotype characteristic of memory T cells [8–10]. The proportion of naive cells in healthy newborns is >90% and decreases with age to ~40%–50% in adults [11, 12]. Naive CD4+ T cells are characterized more functionally by the coexpression of the CD45RA isoform plus the l-selectin (CD62L). The latter facilitates the cell’s entry in the lymph nodes (LNs) through the high endothelial venules [13, 14]. These cells do not express CD45RO and only poorly express CDlla [15, 16].

The naive and memory/effector cells differentially produce immunoregulatory cytokines that are heavily influenced by the microenvironmental conditions. Interleukin (IL)–2 is generally made by naive cells [17], which, with proper antigen presentation, can develop into effector/memory T cells that produce either interferon (IFN)–γ or IL-4 [18–22]. IFN–γ–producing cells generally retain the ability to produce IL-2. Thus, the balance between the naive and memory T cells is crucial for maintaining an efficient immune response.

In HIV infection, CD45RA+ and CD45RO+ CD4+ T cell counts decrease in different stages of disease. Adults with HIV infection initially show a selective loss of memory cells [23, 24], and as absolute CD4+ T cell counts decrease to <400/mm3, the CD45RA+ CD4+ T cells also are lost [25, 26]. However, in a more recent study, in which phenotypic markers of cellular activation among patients at different phases of HIV infection were compared, a progressive balanced loss of both naive and memory cells was observed [27, 28].

The recovery of the immune response is dependent on the regeneration of the naive and memory T cell subsets. To further understand the role of naive and memory T cells in HIV immunopathogenesis and the dynamics of their loss and regeneration, we studied the distribution of these subsets across compartments. The homeostasis of naive and memory CD4 and CD8 subpopulations obtained from peripheral blood (PB) and LN mononuclear cells (PBMC and LNMC, respectively) and their relationship to HIV virus burden across compartments were examined.

Patients and Methods

Patient population. Sixteen HIV-infected patients (14 men and 2 women) participated in the study. They had a median age of 38.5 years (range, 25–61 years), a median CD4 cell count of 443 cells/
mm$^3$ (range, 145–1456 cells/mm$^3$), and a median HIV plasma virus load of $3 \times 10^5$ copies/mL (range, 100–7.5 $\times 10^5$ copies/mL). The patients either had received no therapy or had been maintained on stable antiretrovirals for 3–6 months before sampling of LNMC and PBMC. All patients were immunologically and virologically stable (absence of perturbations induced by increasing virus load and/or the introduction of antiretrovirals). The patients underwent cervical excisional LN biopsy, and their LN cells were dissociated mechanically in suspension before being analyzed for immunophenotypic markers or extraction for cellular virus burden. The control subjects were healthy HIV-seronegative blood donors ranging in age from 25 to 55 years (median, 41 years).

**HIV burden.** Plasma was separated from EDTA-anticoagulated blood samples and was frozen for batch analysis. The number of HIV RNA copies was determined by reverse transcription–polymerase chain reaction, using standard and ultrasensitive Amplicor HIV-1 Monitor kits (Roche Diagnostic). To determine the cellular HIV burden, $10^5$ and $5 \times 10^5$ viable LNMC or PBMC were suspended in 200 $\mu$L of cell PBS. Cellular RNA from the cell suspensions was extracted, and HIV RNA was reverse transcribed and was assayed using the Roche Monitor kit.

**Flow cytometry.** Naive versus memory subsets were evaluated, as described elsewhere [28]. In brief, $20 \mu$L of anti-CD4 or anti-CD8 peridinin–chlorophyll protein and $20 \mu$L of anti-CD45RO phycoerythrin (PE)/CD45RA fluorescein isothiocyanate (FITC), CD45RA FITC/CD62L PE, or isotype-matched control antibodies (Pharmingen) were mixed with 100 $\mu$L of EDTA-treated whole blood. The cells were incubated in the dark for 30 min at 4°C. After incubation, red blood cells were lysed by adding 2 $\mu$L of 10% FACS lysing solution (Becton Dickinson) to each sample. Samples then were incubated for 10 min at room temperature, were centrifuged, and were washed once with cold PBS. After fixation in 2% paraformaldehyde, the samples were stored at 4°C until analyzed. Within 24 h of fixation, 10,000 lymphocytes were collected and were analyzed by 3-color flow cytometry (FACSORT), using CellQuest software (both from Becton Dickinson) for data analysis.

**PBMC recall antigen proliferation assay.** PBMC from study subjects were separated from acid citrate dextrose blood by standard ficoll-hypaque gradients. The cells then were incubated at a density of $1 \times 10^5$ cells in quadruplicates in 96-well microtiter plates in 0.2 mL of RPMI 1640 supplemented with 10% heat-inactivated human AB serum, t-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 $\mu$g/mL) in the presence of an optimum concentration of cytomegalovirus (CMV) antigen (1:10 dilution) or its control (BioWittaker). The cultures then were incubated for 6 days at 37°C in a 5% CO$_2$ atmosphere. Six hours before harvest, 5 $\mu$Ci/mL tritiated thymidine (specific activity, 6.7 Ci/mmol; NEN Life Science Products) was added to each well. The cells then were collected onto discs, which were washed, dried, and placed in scintillation cocktail (Scintiverse; Fisher Scientific). $[\text{H}]$-thymidine uptake was determined by use of a liquid scintillation counter (LS9800; Beckman). The recall antigen response to CMV was expressed as the stimulation index (SI; mean of CMV-treated wells divided by mean of control antigen–treated wells). The optimum concentration for CMV antigen was predetermined from preliminary kinetic experiments in PBMC from healthy control individuals at the indicated times. All assays were performed in real time.

**Statistical analysis.** Comparisons of the mean of the data points were evaluated by use of the paired Student’s $t$ test. Simple regression analysis was used to determine the linear correlations between 2 parameters. $P$ and $r$ (Pearson’s linear correlation coefficient) values were calculated by use of the Stat-100 statistical package (Biosoft).

**Results**

**CD4$^+$/CD8$^+$ profiles of LNMC and PBMC from HIV-infected patients.** The distribution of CD4$^+$ and CD8$^+$ lymphocytes in the LN and PB compartments was compared. The mean percentage of CD4 cells was significantly higher in LNMC (34% ± 4%) than in PBMC (21% ± 2%; $P < .001$). However, the mean CD8 cell percentage was much higher in the PB (57% ± 2%) than in the LNs (31% ± 2%; $P < .0001$). The CD4:CD8 ratio from both compartments was significantly different, with a mean of 1.3 in LNMC and 0.4 in PBMC. Despite this difference, linear regression analysis revealed a highly significant positive correlation for the CD4:CD8 ratios from both compartments ($r = .78; P < .005$; figure 1). These results suggest that the distribution of CD4 and CD8 T cells in the LN and PB compartments in HIV-infected patients is in equilibrium.

Tonsillar tissue from 5 HIV-1–seropositive patients was examined for the distribution of CD4$^+$ and CD8$^+$ T lymphocytes (data not shown). The mean CD4:CD8 ratio in the tonsils was 5.3 ± 0.5, which is in close agreement to the value (5.1) reported by Fleury et al. [29] in LNs of HIV–uninfected subjects. These observations suggest that the significant depletion of CD4$^+$ T cells in LNs of infected patients has occurred and that this depletion probably is balanced across hematopoietic compartments.

**Distribution of naive and memory T cell subsets in LNMC and PBMC.** The distribution of naive and memory CD4 and

![Figure 1](image-url) Linear regression analysis for the relationship of CD4:CD8 ratios in peripheral blood and lymph node (LN) compartments of human immunodeficiency virus–infected patients. PBMC, peripheral blood mononuclear cells.
Figure 2. Linear regression analysis for the relationship of naive:memory ratios of CD4$^+$ and CD8$^+$ T cells in lymph node (LN) and peripheral blood compartments of human immunodeficiency virus–infected patients. PBMC, peripheral blood mononuclear cells.

CD8 lymphocytes, phenotypically characterized as CD45RA$^+$ CD62L$^-$ and CD45RO$^+$ CD45RA$^-$, was determined. An inverse relationship between compartments was observed in HIV-infected patients. The mean percentages of naive CD4$^+$ LNMC and PBMC were 49% ± 2% and 36% ± 4%, respectively ($P < .001$). The mean percentages of memory CD4$^+$ LNMC and PBMC were 42% ± 3% and 51% ± 4%, respectively ($P < .05$). A similar observation was seen in the distribution of the naive but not the memory CD8 cells. The mean percentages of CD8$^+$ LNMC and PBMC were 45% ± 4% and 28% ± 3%, respectively ($P < .001$), and the mean percentages of memory LNMC and PBMC were 38% ± 4% and 40% ± 3%, respectively.

The percentage of memory CD4 cells from patients’ PBMC (51% ± 4%) was significantly less than that for the healthy control subjects (63% ± 4%; $P < .05$); however, the memory cell percentage in the CD8 subpopulation essentially were unchanged, 40% ± 3% and 44% ± 3%, respectively. On the other hand, the percentage of naive CD4$^+$ cells was the same in PBMC from both HIV-infected patients (36% ± 4%) and HIV-uninfected subjects (35% ± 4%). The percentage of CD8$^+$-naive cells in PBMC from HIV–infected patients (28% ± 3%) was significantly less than that for control subjects (45% ± 3%; $P < .002$).

Relationship of naive and memory T cells in LNMC and PBMC. Several immune regulatory cytokines, such as IL-2, IFN-γ, and IL-4, are produced differentially by naive and memory cells. It would seem important that the balance between both populations should be maintained for an optimum immune response. Thus, we next examined the naive:memory ratio of CD4 and CD8 cells. The naive:memory ratio increased from a mean of 0.6 ± 0.2 in CD4$^+$ controls to 0.8 ± 0.15 and 1.3 ± 0.2 in CD4$^+$ cells from PBMC and LNMC of HIV-seropositive patients, respectively.

Linear regression analysis for the relationship of the naive:memory ratio of CD4 cells in the LN and PB compartments is shown in figure 2 (upper left panel). The naive:memory ratio of CD4 T cells in the LN compartment positively correlated with that of the PB compartment ($r = .67; P < .005$). A similar positive correlation was observed for the naive:memory ratios of CD8 lymphocytes ($r = .67; P < .005$) between compartments (figure 2, upper right panel).
We further analyzed the relationship of naive:memory ratios of CD4 to those of CD8. As shown in figure 2 (lower panels), these ratios positively correlated between cells from the same compartment. These results suggest that the regulation of naive and memory CD4 subsets appears to be tightly related to that of CD8 cells.

Relationship of naive:memory CD4 ratios to cellular virus burden and absolute CD4 cell counts. To determine whether naive:memory ratios were influenced by cellular virus load, the total unspliced HIV RNA of PBMC and single-cell suspensions of LNs were measured. The LN and PB cellular virus load ranged from $0.02 \times 10^5$ to $2.4 \times 10^3$ copies/10^5 cells (median, $3 \times 10^4$ copies/10^5 cells) for the former and from $0.05 \times 10^4$ to $2.2 \times 10^3$ copies/10^5 cells (median, $0.3 \times 10^3$ copies/10^5 cells) for the latter. The naive:memory ratio inversely correlated with the cellular virus load from the same compartment, which suggests that an increase or recovery of naive cells relative to memory cells is associated with lower cellular virus load (figure 3). Furthermore, the naive:memory ratios in PB were directly proportional with the absolute CD4 cell counts (figure 4). This suggests that a higher ratio of naive:memory cells in HIV-infected patients is associated with a more intact immune response.

Relationship of naive:memory CD4 ratios to recall antigen response. The naive:memory ratio appeared to increase in patients with a more intact immune system. To test the association of naive:memory ratio to the functional ability of the immune system, we examined CMV-induced recall antigen proliferative responses of PBMC from study patients who were CMV seropositive. As shown in figure 4, the naive:memory ratio from PBMC positively correlated with the CMV SI ($r = .6; P < .05$). The CMV SI in this cohort ranged from 3 to
300, with a mean ± SD of 85 ± 32. These results indicate that an increase in the naive:memory ratio was associated with an improvement in the helper function capacity of the T cells.

**Relationship of absolute counts of naive and memory cells to naive:memory ratios and markers of disease progression.** We further examined the relationship of naive:memory ratios to the absolute number of circulating naive and memory CD4⁺ T cells. A significant positive correlation was observed between the absolute number of naive cells and the naive:memory ratio (figure 5, upper left panel); however, there was no correlation between this ratio and the absolute number of memory cells (figure 5, upper right panel). This indicates that changes in the naive:memory ratio are primarily influenced by the naive rather than the memory T cell subpopulation. The increase in the

**Figure 5.** Linear regression analysis for the relationship of absolute counts of naive and memory CD4⁺ cells to naive:memory ratios and markers of disease progression in the peripheral blood compartment of human immunodeficiency virus (HIV)-infected patients.
absolute naive and memory CD4 cell counts correlated positively with the CD4 cell counts (figure 5, lower panels) and inversely with circulating HIV plasma RNA (figure 5, middle panels). These relationships further support the relationship of the naive:memory ratio to the recovery of the immune response in HIV-infected patients.

Discussion

Our data show that the distribution pattern of T cells and their naive and memory subsets differ in the PB and LN compartments of HIV-infected patients. Despite this difference, their relationship to each other appeared to be nonrandom and in equilibrium. Moreover, the ratios of CD4 and/or CD8 naïve:memory cells in PBMC and LNMC correlated with each other. This implies that alterations in the makeup of a T cell subpopulation, due to apoptosis or regeneration, probably affect the homeostasis of the other subpopulations systematically. The relationship observed indicates that the profiles of T cells and their subpopulations in LN tissues can be inferred from the PB distributions.

The percentage of CD4+ T cells was significantly higher in LN tissue than in PB, and the CD4:CD8 ratio in most patients was <1. This might explain the higher virus burden observed in LN tissues, compared with that in PB [30–33]. The CD4:CD8 ratio of 1.3 in LN tissue was equivalent to that for treatment-naive HIV-seropositive patients and was significantly lower than that for LN tissue of HIV-seronegative control subjects, as reported elsewhere [29], and tonsillar tissue (authors’ unpublished results). The low CD4:CD8 ratio reflects the extensive reduction in CD4+ T cells in LN tissue from virus-induced cell destruction and/or impaired thymic maturation of CD4 T cells [34–36].

The cellular virus load was several-fold higher in LNMC than in PBMC, which is in agreement with previously reported studies [30–33], and inversely correlated with the naïve:memory ratio in both compartments. The association of the naïve:memory ratio to the pattern of cellular virus load and the differential susceptibility to infection of both subpopulations [4] suggests that the recovery and the regeneration of the naive and memory subsets could be affected by the amount of virus in these subsets.

An increase in naïve:memory ratio was associated with higher CD4 cell counts and lower HIV plasma and cellular RNA levels. Therefore, this ratio may reflect the immune status and, possibly, predict disease progression. The increase in the naïve:memory ratio may result from an increase in thymic production [37, 38] and/or peripheral expansion of naive cells [39] or destruction of memory cells [27, 28]. The observed increase in naïve:memory ratio is unlikely to have occurred from decreases in memory cells. This is not inconceivable, since an actual increase in the absolute number of memory cells was detected, which also correlated positively with the CD4 cell count and negatively with the plasma virus load.

Although the absolute cell count of both the naïve and memory cells increased with the increase in CD4 cell counts, it is likely that the rate of increase of naïve cells is higher than that of the memory cells, which significantly influenced the naïve:memory ratio. The fact that the naïve T cell pool can be replenished during recovery from 2 sources (the thymus and through extrathymic peripheral expansion) supports this possibility. In addition, recent reports indicate that a significant number of adult HIV-infected patients harbor thymic tissue and that the abundance of this tissue correlates with circulating numbers of naïve T cells [37, 40]. The increase in the naïve T cell pool that was associated with a reduced virus load may also have resulted from less immune activation. High levels of antigen-driven activation can lead possibly to a decrease in the naïve cell pool because of their high level of differentiation into effector and memory cells.

The naïve:memory ratios are conceivably important in maintaining the balance between the functional activities of both populations, which is needed for an efficient immune response and for suppression of HIV-1 replication. This is supported by the fact that the naïve:memory ratio correlated linearly with the recall antigen proliferative responses of the study patients. The patients with higher CD4 naïve:memory ratios showed more robust responses to CMV antigen and had higher absolute CD4 cell counts than patients with lower ratios.

In summary, CD4 T cells and their naïve and memory subpopulations in PB and LN tissue compartments were in equilibrium. PB values were reflective of events in the LN tissue. In addition, the naïve:memory ratio of T cell subsets correlated with markers of HIV disease progression and immunogenic recall antigen responses. The naïve:memory ratio in the PB appeared to reflect the LN microenvironment and may potentially be useful as a surrogate marker for treatment efficacy and immune reconstitution.

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