Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9838–9842, November 1991
Medical Sciences

Lymphoid organs function as major reservoirs for human immunodeficiency virus

(viral burden/peripheral blood/polymerase chain reaction)

GIUSEPPE PANTALEO*†, CECILIA GRAZIOSI*, LUCA BUTINI*, PHILIP A. PIZZO‡, STEVEN M. SCHNITTMAN*, DONALD P. KOTLER§, AND ANTHONY S. FAUCI*

*Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, ‡Division of Cancer Treatment, Pediatric Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and §St. Luke's–Roosevelt Hospital Center, New York, NY 10025

Communicated by Richard M. Krause, August 16, 1991

ABSTRACT **The total number of human immunodeficiency virus type 1 (HIV-1)-infected circulating CD4$^+$ T lymphocytes is considered to be a reflection of the HIV burden at any given time during the course of HIV infection. However, the low frequency of HIV-infected circulating CD4$^+$ T lymphocytes and the low level or absence of plasma viremia in the early stages of infection do not correlate with the progressive immune dysfunction characteristic of HIV infection. In this study, we have determined whether HIV-infected circulating CD4$^+$ T lymphocytes are a correct reflection of the total pool of HIV-infected CD4$^+$ T cells (i.e., HIV burden). To this end, HIV burden has been comparatively analyzed in peripheral blood and lymphoid tissues (lymph nodes, adenoids, and tonsils) from the same patients. The presence of HIV-1 DNA in mononuclear cells isolated simultaneously from peripheral blood and lymphoid tissues of the same patients was determined by polymerase chain reaction amplification. We found that the frequency of HIV-1-infected cells in unfractionated or sorted CD4$^+$ cell populations isolated from lymphoid tissues was significantly higher (0.5–1 $\log_{10}$ unit) than the frequency in peripheral blood. Comparable results were obtained in five HIV seropositive patients in the early stages of disease and in one patient with AIDS. These results demonstrate that a heavy viral load does reside in the lymphoid organs, indicating that they may function as major reservoirs for HIV. In addition, the finding of a heavy viral load in the lymphoid organs of patients in the early stages of disease may explain the progressive depletion of CD4$^+$ T lymphocytes and the immune dysfunction associated with the early stages of HIV infection.**

The most consistent and striking observation of human immunodeficiency virus (HIV) infection is a progressive depletion of CD4$^+$ T cells (1). However, the magnitude of the quantitative depletion of CD4$^+$ T cells, as well as the resulting functional immunosuppression, seems inconsistent with the low frequency of infected cells demonstrable in the peripheral blood (PB) of infected individuals. Initial examination of cells in PB and lymph nodes (LNs) of patients in advanced stages of disease revealed that viral RNA synthesis occurred in a very low number of cells (1/100,000 to 1/10,000 of total mononuclear cells) (2, 3). Recently, application of the polymerase chain reaction (PCR) amplification technique has enabled investigators to detect cells containing HIV proviral DNA but not expressing viral RNA (4, 5). PCR analyses showed that CD4$^+$ T lymphocytes were the "reservoirs" for HIV-1 in PB and that high frequencies of HIV-1-infected circulating CD4$^+$ T lymphocytes (>1/100) were generally found in patients with AIDS (5). In contrast, lower frequencies of HIV-1-infected CD4$^+$ cells (1/50,000) were associated with early stages of infection (6). On the basis of these findings, quantitative analysis of HIV-1-infected circulating CD4$^+$ T lymphocytes has been considered as a reflection of the HIV burden at any given time during the course of HIV infection (5–7). In this regard, it has been problematic to reconcile the low proportion of HIV-1-infected cells and the difficulty in detecting any virologic markers in PB in the early stages of infection (6, 8) with the invariably progressive deterioration of the immune system characteristic of HIV infection (1).

PB lymphocytes, however, represent only 2% of the total lymphocyte pool, and in several instances perturbations observed in PB may not reflect the modifications in other anatomic sites where lymphocytes may reside (9). By a quantitative study aimed at defining the levels of viral burden in PB and lymphoid tissue (LT) of the same patients, we have found that (*i*) the total body pool of HIV-1-infected CD4$^+$ T lymphocytes is significantly higher than was previously thought based on conventional studies using PB and (*ii*) LT may function as a major reservoir for HIV-1.

MATERIALS AND METHODS

Patients. The group of patients studied included three patients with Centers for Disease Control (CDC) (10) stage 3 disease, one pediatric case (P2-D2, P2-A according to CDC classification), one patient with CDC stage 2 disease, and one patient with AIDS (CDC stage 4). The biopsies and/or adenectomies were carried out for diagnostic or therapeutic indications and the studies were performed under protocols approved by institutional review boards of the National Institutes of Health and the St. Luke's–Roosevelt Institute for Health Sciences.

Monoclonal Antibodies (mAbs). Fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated mAbs that recognized CD2, CD3, CD4, CD8, or CD14 were purchased from Becton Dickinson.

Isolation of Mononuclear Cells (MCs) from PB and LT. Freshly drawn blood from the six HIV-1-seropositive patients studied was obtained at the same time that the LT biopsies were performed. MC were isolated from PB by Ficoll/Hypaque density gradient centrifugation. To isolate MC from LT, specimens were placed into 60 × 15-mm tissue culture dishes (Costar) containing RPMI 1640 plus 10% fetal bovine serum (3 ml). Tissue specimens were minced with a scalpel and the cells were teased out. The cell suspensions

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HIV, human immunodeficiency virus; MC, mononuclear cell; PB, peripheral blood; LN, lymph node; LT, lymphoid tissue; CDC, Centers for Disease Control; mAb, monoclonal antibody; LTR, long terminal repeat; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

†To whom reprint requests should be addressed at: National Institutes of Health, Building 10, Room 11B-13, Bethesda, MD 20892.

were subjected to Ficoll/Hypaque density gradient centrifugation to eliminate red blood cells. The MC preparations obtained from PB and LT were frozen and stored at −80°C until utilized.

Flow Cytofluorometric Analysis and Cell Sorting. Analysis of PBMCs and LTMCs for the distribution of several T-cell and activation markers and cell sorting were performed as described (11). Cells were stained with FITC- and PE-conjugated mAbs and two-color cytofluorometry was performed on an EPICS Profile (Coulter). To isolate $CD4^+$ versus $CD4^-$ or $CD4^+$ versus $CD8^+$ cell subsets, PBMCs and LTMCs were stained with FITC-conjugated anti-CD4 mAb alone or simultaneously with FITC-conjugated anti-CD4 mAb and PE-conjugated anti-CD8 mAb. Sorted cells were collected in sterile plastic tubes whose inside surfaces had been coated with sterile fetal bovine serum; after sorting cells were washed and immediately diluted in culture medium. Cell sorting was performed on an EPICS C (Coulter). The purity of the sorted cell populations ranged between 97.5% and 99%.

Quantitation of Serum HIV-1 p24 Antigen. Serum samples from all five patients in the early stages of disease were tested for p24 antigen with a commercial enzyme immunoassay (Abbott). p24 antigen was undetectable in all the patients.

PCR Analysis. HIV-1 proviral DNA was detected by standard PCR analysis (5, 12). Briefly, unfractionated or sorted $CD4^+$, $CD4^-$, and $CD8^+$ cell populations isolated from PB and LT were washed three times in phosphate-buffered saline and pelleted by centrifugation. Cells were then lysed and DNA was prepared for PCR. For quantitative analysis of HIV-1-infected cells in both PB and LT, either the cells were lysed and the lysate was serially diluted or the cells were first serially diluted and then lysed. Dilutions were obtained by mixing different amounts of cells or of the stock lysate (corresponding to 10^6 cells) with Jurkat (T-lymphoma line) cell lysates (corresponding to 10^5 cells) in a total volume of 50 μl. DNA corresponding to 10^5, 10^4, and 10^3 cells was amplified using primer pairs specific for *gag* (SK 145/101) and long terminal repeat (LTR) (SK 29/30) gene segments of HIV-1. Amplified products were hybridized to probes (SK 102 and SK 31 for *gag* and LTR, respectively) that had been end-labeled using [γ-^{32}P]ATP. Products of hybridization were analyzed by electrophoresis in 10% polyacrylamide gels and visualized by autoradiography after 6- and 12-hr exposures. PCR signals obtained from serial dilutions of unfractionated and sorted cell populations were compared to PCR signals from 10-fold dilutions of ACH-2 (positive control), a T-cell clone containing a single proviral copy of HIV per cell (13). As a negative control, lysis buffer and cell lysates from 10^5 Jurkat cells that were prepared as described above were run. The QH 26/27 (*HLA-DQA*) primer pair was used as control for DNA amplification. Amplified products were visualized directly in agarose gels stained with ethidium bromide.

RESULTS

Analysis of HIV-1 Burden in PB and LT by PCR Amplification. PBMCs and LTMCs obtained from six HIV-1-seropositive patients were analyzed (Table 1). In the first series of experiments equal numbers (10^5) of unfractionated MCs from PB and LT were analyzed for the presence of HIV-1 proviral DNA by PCR. The signals detected in LNMCs were significantly higher than those detected in PBMCs in all three patients studied (Fig. 1*A*). Furthermore, these signals were compared with those from 10-fold serial dilutions of the cell lysates of HIV-1-infected ACH-2 cells, a chronically infected T-cell clone containing one proviral copy per cell (13). This analysis suggested that far greater numbers (ranging from 0.5–1 $\log_{10}$ unit) of HIV-1-infected cells were present in LNs than in PB. A more accurate estimation of the frequency of HIV-1-infected cells in PB and LNs was performed by comparing 10-fold serial dilutions of cell lysates from PBMCs and LNMCs with 10-fold serial dilutions of ACH-2 (Fig. 1*B*). We determined that in patient 1 the frequency of HIV-1-infected cells was 1/10,000 in the LNs whereas the frequency was between 1/10,000 and 1/100,000 in the PB. In patient 2 the frequency of HIV-1-infected cells was 1/10,000 in the LNs and 1/100,000 in the PB (Fig. 1*B*), and in patient 3 the frequency was 1/1000 and 1/10,000 in the LNs and PB, respectively. In agreement with previous reports (14), we found that the percentage of $CD4^+$ T lymphocytes was higher in LT than in PB and that the CD4/CD8 ratios were significantly lower in PB than in LT (data not shown). Therefore, an obvious question was whether the differences in the frequency of HIV-1-infected PBMCs and LNMCs could be explained solely on the basis of the different percentages of $CD4^+$ cells in PB and LN. To exclude this possibility, in a second series of experiments we determined whether the increased frequency of HIV-1-infected cells observed in unfractionated cell populations in LN could be confirmed in purified $CD4^+$ cells, the major reservoirs for HIV-1 in PB (5). PBMCs and LNMCs were stained either with anti-CD4 mAb alone or with anti-CD4 and anti-CD8

Table 1. HIV-infected individuals evaluated for viral burden

Patient	Sex	Age, years	Transmission	CDC class	Therapy*
1	F	23	Heterosexual	3	None
2	M	28	Homosexual	3	None
3	M	32	Homosexual	3	AZT
4	F	22	Transfusion	2	None
5	F	3	Perinatal	P2-D2, P2-A	ddI
6	M	41	i.v. drug use	4	None

*AZT, 3′-azido-3′-deoxythymidine; ddI, 2′,3′-dideoxyinosine.

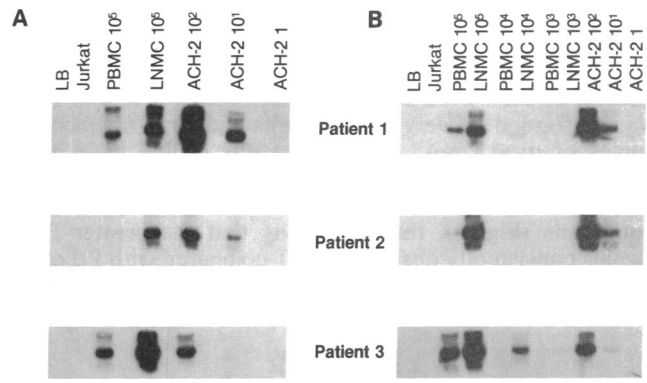

FIG. 1. (*A*) PCR amplification of HIV-1 DNA in unfractionated cell populations isolated from PB and LN of three different HIV-seropositive patients. Intensities of PCR signals in 10^5 PBMCs and LNMCs from patients 1, 2, and 3 are compared with 10-fold serial dilutions of cell lysates from ACH-2 (positive control). As a negative control, lysis buffer (LB) and cell lysates from 10^5 Jurkat cells were used. The results shown were obtained using *gag* primer pairs and probe as described in *Materials and Methods*. Similar results were obtained with LTR primer pairs and probe. The oligomer hybridization autoradiographs shown were obtained after 12-hr (patient 1) or 6-hr (patients 2 and 3) exposures. (*B*) Comparative analysis of the frequency of HIV-1-infected PBMCs and LNMCs. For these experiments, cells were lysed and then serial dilutions were performed with the stock cell lysate. Tenfold serial dilutions of cell lysates (corresponding to 10^5, 10^4, and 10^3 cells) are compared with those of ACH-2 (corresponding to 10^2, 10^1, and 1 cell). Lysis buffer (LB) and cell lysates of Jurkat (negative controls) are shown. The results shown were obtained using *gag* primer pairs and probe; similar results were obtained with LTR primer pairs and probe. The oligomer hybridization autoradiographs shown were obtained after 6-hr (patients 1 and 3) or 12-hr (patient 2) exposures.

mAbs and sorted into $CD4^+$ versus $CD4^-$ or $CD4^+$ versus $CD8^+$. Tenfold serial dilutions of cell lysates from the various cell subsets were analyzed by quantitative PCR and compared with those from ACH-2. At least 1 $\log_{10}$ difference in the frequency of HIV-1-infected $CD4^+$ T lymphocytes was detected between LN and PB (Fig. 2).

We had the opportunity to determine the HIV burden in LTs other than LNs. PCR signals detected on equal numbers (10^5) of MCs isolated from PB, LNs, adenoids (Ad), and tonsils (Ton) from the same patient (patient 5) were compared. The PCR signals detected in all LTs tested were significantly higher than those observed in PB (Fig. 3*A*). These results obtained on unfractionated MC populations were further confirmed on sorted $CD4^+$ T lymphocytes. In fact, by quantitative PCR analysis we determined that 1/100 LN or Ad $CD4^+$ T lymphocytes were infected with HIV-1, whereas 1/1000 $CD4^+$ T lymphocytes were infected in PB (Fig. 3*B*). Similar differences in the frequency of HIV-1-infected $CD4^+$ T lymphocytes between LN and PB were observed in two additional patients (data not shown). PCR signals were never detected in sorted $CD4^-$ or $CD8^+$ cell subsets from PB. In one case (patient 5), weak PCR signals were detected in $CD4^-$ or $CD8^+$ cell subsets isolated from LT (data not shown). However, in this patient the frequency of HIV-1-infected $CD4^+$ T lymphocytes in LT was 1/100, suggesting that the PCR signals detected in sorted $CD4^-$ or $CD8^+$ cells (the purity of the sorting was 98–99%) could possibly have resulted from contaminating $CD4^+$ lymphocytes.

It was previously demonstrated (5) that circulating $CD4^+$ T lymphocytes contained predominantly one copy of viral DNA per cell. Since the results of the present study indicated that a higher HIV-1 burden was present in LT than in PB, the possibility existed that the differences in HIV burden observed between LT and PB did not reflect increased numbers of HIV-1-infected cells in LT but could be explained by multiple (integrated or unintegrated) copies of viral DNA in a very few LT cells. To rule out this possibility, cells were first lysed and then PCR was performed on serial dilutions of lysates, or cells were serially diluted, then lysed, and PCR was performed. If very few LT cells had large numbers of copies of viral DNA, disparate results should have been obtained in the two experimental conditions. However, identical PCR signals were obtained under both experimental conditions (Fig. 4), thus indicating that the greater PCR signals consistently observed in LT compared with PB of all the patients studied did in fact reflect higher frequencies of HIV-1-infected cells.

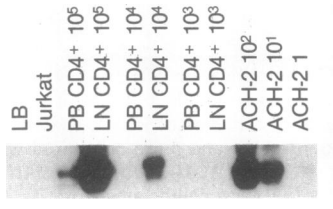

FIG. 2. Comparative analysis of the frequency of HIV-1-infected $CD4^+$ T lymphocytes isolated from PB and LN of the same patient (patient 2). PBMCs and LNMCs were stained with FITC-conjugated anti-CD4 mAb and PE-conjugated anti-CD8 mAb and sorted into $CD4^+$ and $CD8^+$ subsets. Sorted PB and LN $CD4^+$ cells were lysed and 10-fold serial dilutions of cell lysates were made. By comparative analysis with serial dilutions of cell lysates of ACH-2, one can deduce that in LN, 1/1000 $CD4^+$ T cells contained HIV-1 proviral DNA, as opposed to between 1/10,000 and 1/100,000 in PB. Lysis buffer (LB) and cell lysates of Jurkat cells (negative controls) are shown. The results shown were obtained using *gag* primer pairs and probe; similar results were obtained with LTR primer pairs and probe. The oligomer hybridization autoradiograph shown was obtained after a 12-hr exposure.

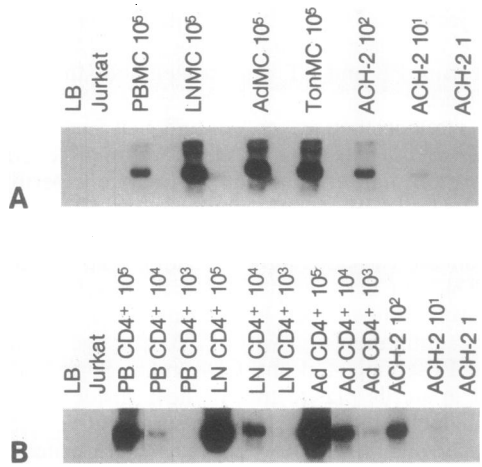

FIG. 3. (*A*) Oligomer hybridization of HIV-1 DNA in unfractionated MC isolated from PB, LN, adenoids (Ad), and tonsils (Ton) of the same patient (patient 5). Intensity of the PCR signals in 10^5 PBMCs, LNMCs, AdMCs, and TonMCs are compared. Tenfold dilutions of cell lysates of ACH-2 (positive control) and lysis buffer (LB) and cell lysates of Jurkat (negative controls) are shown. (*B*) Analysis of the frequency of $CD4^+$ T lymphocytes in PB, LN, and Ad of patient 5. Sorted $CD4^+$ T cells were obtained as described in *Materials and Methods*. Tenfold serial dilutions of PB, LN, and Ad $CD4^+$ cell lysates were compared with those of ACH-2 cell lysates. In these experiments cells were first lysed and then serial dilutions of cell lysates were performed. Negative controls [lysis buffer (LB) and cell lysate of Jurkat] are shown. The results shown were obtained using *gag* primer pairs and probe; similar results were obtained with LTR primer pairs and probe. The oligomer hybridization autoradiographs shown in *A* and *B* were obtained after 6 hr of exposure.

DISCUSSION

We have performed a quantitative comparative analysis of the viral burden in PB and LT in individual patients with HIV infection. Unfractionated or sorted $CD4^+$ T-cell populations were isolated simultaneously from PB and LT of the same patients and assayed for the presence of HIV-1 proviral DNA by quantitative PCR. In all six patients, only one of whom had advanced to AIDS, the results consistently demonstrated that the frequency of HIV-infected cells in LT was significantly higher (by 0.5–1 $\log_{10}$ unit) than in PB of the same patient.

As previously demonstrated for $CD4^+$ T cells in PB (5), $CD4^+$ T lymphocytes were the major reservoir of HIV in the LT. However, we did not formally address the presence of HIV in dendritic cells in LT (see below), whereas CD14 (monocyte/macrophage marker)-positive cells were poorly represented (1–2%) in LT (data not shown).

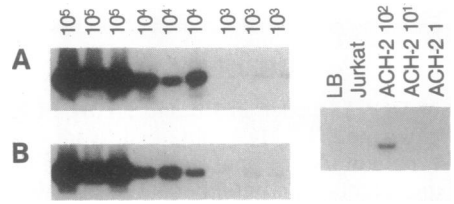

FIG. 4. LTMCs do not contain multiple (integrated or unintegrated) copies of viral DNA per cell. Adenoid MCs were serially diluted and then lysed (*A*) or were lysed before serial dilution (*B*). Each dilution of cell (*A*) or cell lysate (*B*) was run in triplicate. Positive (cell lysates of ACH-2) and negative [lysis buffer (LB) and cell lysate of Jurkat] controls are shown. The results shown were obtained using LTR primer pairs and probe; similar results were obtained with *gag* primer pairs and probe. The oligomer hybridization autoradiograph shown was obtained after 6 hr of exposure.

Given the greater intensity of PCR signals in LT compared with PB, an obvious question would be whether this represented multiple integrated or unintegrated copies of HIV in a few cells as previously shown for other tissues (15) or whether it truly represented greater numbers of infected cells. We excluded the former possibility by comparing PCR signals of dilutions of cell lysates with those of cells that had been diluted first and then lysed. It was clear from these studies that there was indeed a greater frequency of HIV-infected cells in LT than in PB.

It has been logistically difficult to obtain LT and PB from the same patients and so the group of patients in the present study is relatively small. However, within the limitations resulting from such constraints, similar results have been obtained in all the patients studied regardless of the clinical stage of the disease, the age of the patients, the retroviral therapy, or the anatomic site of the LN biopsy. In this regard cervical LNs, mesenteric LNs, tonsils, and adenoids yielded similar results.

Attempts to delineate the precise immunopathogenic mechanisms of HIV infection have been problematic for a number of reasons. Foremost among these have been the logistic constraints associated with determining the total viral burden and the degree of accuracy with which the lymphocytes in PB reflect the total body lymphoid pool. The observations of very low frequencies of HIV-infected cells (1/50,000) (6) and the relative negativity of virologic markers (6, 8) in the PB of HIV-infected individuals in the early stages of infection have made it difficult to explain the relentlessly progressive depletion of $CD4^+$ T cells resulting in the profound immunosuppression invariably observed over time in HIV-infected individuals (1). Failure to adequately address the role of LT in this process has propagated misconceptions concerning the relationship between viral burden and pathogenesis of HIV infection. The results shown in the present study demonstrate that the frequencies of HIV-1-infected $CD4^+$ T lymphocytes isolated from LT of patients in the early stages of HIV infection are comparable to those generally found in the PB of patients in the advanced stages of disease (patients with AIDS or AIDS-related complex) (5–7), indicating that a heavy burden of HIV-infected cells does reside in the lymphoid organs. Furthermore, high levels of HIV-specific mRNA have been detected in tissue sections as well as cell suspensions of LT, suggesting that active virus replication occurs in the lymphoid organs (C. Fox and A.S.F., unpublished work). In contrast, the virologic marker p24 antigen was not detected in the PB in any of the five patients with early-stage disease included in the present study (data not shown). Although p24 antigen is a less sensitive virologic marker than plasma viremia, in previous studies it has been shown that plasma viremia could be detected in a minor percentage (20%) of patients with early-stage disease (8). Therefore, these results indicate that, early in the course of HIV infection at a time when viral burden is low in the PB, lymphoid organs serve as major reservoirs for HIV. This may explain in part the progressive depletion of $CD4^+$ T lymphocytes and deterioration of immune function during the early stages of HIV infection. Not only are the LTs the major reservoirs for HIV, but the immune response to HIV as well as the spread of HIV infection likely occur predominantly within the microenvironment of the LTs.

The lymphoid organs are the anatomic sites where the generation and propagation of antigen-specific immune responses occur (16), and lymphocytes migrate from the circulation and the lymphatics into lymphoid organs, particularly LNs, during antigen-specific immune responses (16). Antigen-activated T lymphocytes (predominantly $CD4^+$ cells) move to the interface between the paracortex (T-cell zone) and the germinal center (B-cell zone) in order to provide help for the generation of antigen-specific B-cell responses (17), including humoral responses against HIV (18, 28). In this regard, redistribution or migration of lymphocytes from the circulation to other anatomic sites such as lymphoid organs is likely to occur during HIV infection. In fact, it has been observed that either $CD4^+$ or $CD8^+$ T cells and B cells were significantly reduced in the acute phase of HIV infection (19, 20). It is conceivable, although unlikely, that the precipitous decline in $CD4^+$ T cells could be explained by direct infection of these cells. However, this mechanism could not be applicable to the drop in $CD8^+$ cells and B cells, given the refractoriness of $CD8^+$ cells and B cells to HIV infection. In addition, HIV DNA was readily demonstrated in LN biopsy samples from seropositive individuals (21, 22), and HIV has been visualized in association with antigen-presenting dendritic cells (23–25). Thus, the migration of lymphocytes from the circulation and lymphatics to lymphoid organs, the state of activation of $CD4^+$ T cells, the interaction of $CD4^+$ T cells with antigen-presenting cells potentially infected with HIV, and the close cellular contact within the LT microenvironment favor the establishment of lymphoid organs not only as the major reservoir of HIV in the body but also as the predominant environment for propagation of HIV.

All of these considerations must be kept in mind when viral load is measured in PB and when studies aimed at delineating immunopathogenic mechanisms are designed using PB lymphocytes in an artificial *in vitro* environment. In this regard, by the analysis of PB it was shown recently that a precipitous decline in viral load occurred in patients after primary HIV infection (26, 27). Since we have provided evidence that PB may not always represent an accurate reflection of lymphoid organs, the observed decline in viral burden in PB may not necessarily represent a comparable decrease in viral burden in other anatomic sites. One may speculate that, in addition to the development of an effective immune response in the host (26, 27), the decline in circulating HIV-infected cells may be the result of the redistribution and migration of lymphocytes from the PB to the lymphoid organs occurring during an ongoing HIV-specific immune response. Therefore, attention should be paid to the fact that studies limited to PB do not always accurately reflect *in vivo* pathogenesis, in which LT most likely plays the major role. Studies utilizing serial biopsies of LT where feasible in HIV-infected humans, as well as studies in animal models in which careful comparisons can be made between PB and LT during the entire course of infection, should contribute greatly to our understanding of the complexities of the immunopathogenesis of HIV infection.

1. Fauci, A. S. (1988) *Science* **239**, 617–622.
2. Harper, M. E., Marselle, L. M., Gallo, R. C. & Wong-Staal, F. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 772–776.
3. Biberfeld, P., Chayt, K. J., Marselle, L. M., Biberfeld, G., Gallo, R. C. & Harper, M. E. (1986) *Am. J. Pathol.* **125**, 436–442.
4. Ou, C., Kwok, S., Mitchel, S. W., Mack, D. H., Sninsky, J. W. K., Feorino, P., Warfield, D. & Schochetman, G. (1988) *Science* **239**, 295–297.
5. Schnittman, S. M., Psallidopoulos, M. C., Lane, H. C., Thompson, M., Baseler, M., Massari, F., Fox, C. H., Salzman, N. P. & Fauci, A. S. (1989) *Science* **245**, 305–308.
6. Ho, D. D., Moudgil, T. & Alam, M. (1989) *N. Engl. J. Med.* **321**, 1621–1625.
7. Schnittman, S. M., Greenhouse, J. J., Psallidopoulos, M. C., Baseler, M., Salzman, N. P., Fauci, A. S. & Lane, H. C. (1990) *Ann. Intern. Med.* **113**, 438–443.
8. Coombs, R. W., Collier, A. C., Allain, J. P., Nikora, B., Leuter, M., Gjerset, G. F. & Corey, L. (1989) *N. Engl. J. Med.* **321**, 1626–1631.
9. Westermann, J. & Pabst, R. (1990) *Immunol. Today* **11**, 406–410.
10. Centers for Disease Control (1986) *Morbid. Mortal. Wkly. Rep.* **20**, 334–339.

11. Pantaleo, G., Koenig, S., Baseler, M., Lane, H. C. & Fauci, A. S. (1990) *J. Immunol.* **144,** 1696–1704.
12. Pantaleo, G., Butini, L., Graziosi, C., Poli, G., Greenhouse, J. J., Gallin, J. I. & Fauci, A. S. (1991) *J. Exp. Med.* **173,** 511–514.
13. Clouse, K. A., Powell, D., Washington, E., Poli, G., Strebel, K., Farrar, W., Barstad, P., Kovacs, J., Fauci, A. S. & Folks, T. M. (1989) *J. Immunol.* **142,** 431–438.
14. Biberfeld, P., Ost, A., Porwit, A., Sandstedt, B., Pallesen, G., Bottiger, B., Morfelt-Mansson, L. & Biberfeld, G. (1987) *Acta Pathol. Microbiol. Immunol. Scand.* **95,** 47–65.
15. Pang, S., Koyanagi, Y., Miles, S., Wiley, C., Vinters, H. V. & Chen, I. S. Y. (1990) *Nature (London)* **343,** 85–89.
16. Parrot, D. M. V. & Wilkinson, P. C. (1981) *Prog. Allergy* **28,** 193–284.
17. Rouse, R. V., Ledbetter, J. A. & Weissman, I. L. (1982) *J. Immunol.* **128,** 2243–2246.
18. Rieckman, P., Poli, G., Kehrl, J. H. & Fauci, A. S. (1991) *J. Exp. Med.* **173,** 1–5.
19. Cooper, D. A., Tindall, B., Wilson, E. J., Imrie, A. A. & Penny, R. (1988) *J. Infect. Dis.* **157,** 889–896.
20. Gaines, H., von Sydow, M. A. E., von Stedingk, L. K., Biberfeld, G., Bottiger, B., Hansson, L. O., Lundberg, P., Sonneborg, A. B., Wasserman, J. & Strannegard, O. O. (1990) *AIDS* **4,** 995–999.
21. Shibata, D., Brynes, R. K., Nathwani, B., Kwok, S., Sninsky, J. & Arnheim, N. (1989) *Am. J. Pathol.* **135,** 697–702.
22. Shibata, D. & Klatt, E. C. (1989) *Arch. Pathol. Lab. Med.* **113,** 1239–1244.
23. Tenner-Racz, K., Racz, P., Bofill, M., Schulz-Meyer, A., Dietrich, M., Kern, P., Weber, J., Pinching, A. J., Dimarzo-Veronese, F., Popovic, M., Klatzmann, D., Gluckman, J. C. & Jannossy, G. (1986) *Am. J. Pathol.* **123,** 9–15.
24. Ward, J. M., O'Leary, T. J., Baskin, G. B., Benveniste, R., Harris, C. A., Nara, P. L. & Rhodes, R. H. (1987) *Am. J. Pathol.* **127,** 199–205.
25. Parmentier, H. K., van Wichen, D., Sie-Go, D. M., Goudsmith, J., Borleffs, J. C. & Schuurman, H. J. (1990) *Am. J. Pathol.* **137,** 247–251.
26. Clark, S. J., Saag, M. S., Decker, W. D., Campbell-Hill, S., Roberson, J. L., Veldkamp, P. J., Kappes, J. C., Hahn, B. H. & Shaw, G. M. (1991) *N. Engl. J. Med.* **324,** 954–960.
27. Daar, E. S., Moudgil, T., Meyer, R. D. & Ho, D. D. (1991) *N. Engl. J. Med.* **324,** 961–964.
28. Fauci, A. S., Rieckman, P., Poli, G. & Kehrl, J. H. (1991) *J. Immunol.*, in press.