

# CD4 lymphocytes in the blood of HIV<sup>+</sup> individuals migrate rapidly to lymph nodes and bone marrow: support for homing theory of CD4 cell depletion

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**Abstract:** The mechanism(s) by which human immunodeficiency virus (HIV) causes depletion of CD4 lymphocytes remains unknown. Evidence has been reported for a mechanism involving HIV binding to (and signaling) resting CD4 lymphocytes in lymphoid tissues, resulting in up-regulation of lymph node homing receptors and enhanced homing after these cells enter the blood, and induction of apoptosis in many of these cells during the homing process, caused by secondary signaling through homing receptors. Supportive evidence for this as a major pathogenic mechanism requires demonstration that CD4 lymphocytes in HIV<sup>+</sup> individuals do migrate to lymph nodes at enhanced rates. Studies herein show that freshly isolated CD4 lymphocytes labeled with <sup>111</sup>Indium and intravenously re-infused back into HIV<sup>+</sup> human donors do home to peripheral lymph nodes at rates two times faster than normal. They also home at enhanced rates to iliac and vertebral bone marrow. In contrast, two hepatitis B virus-infected subjects displayed less than normal rates of blood CD4 lymphocyte migration to peripheral lymph nodes and bone marrow. Furthermore, the increased CD4 lymphocyte homing rates in HIV<sup>+</sup> subjects returned to normal levels after effective, highly active antiretroviral therapy treatment, showing that the enhanced homing correlated with active HIV replication. This is the first direct demonstration of where and how fast CD4 lymphocytes in the blood traffic to tissues in normal and HIV-infected humans. The results support the theory that the disappearance of CD4 lymphocytes from the blood of HIV<sup>+</sup> patients is a result of their enhanced migration out of the blood (homing) and dying in extravascular tissues. *J. Leukoc. Biol.* 72: 271–278; 2002.

**Key Words:** human · T lymphocytes · AIDS/HIV · cell trafficking

## INTRODUCTION

The mechanism by which human immunodeficiency virus (HIV) depletes CD4 lymphocytes has remained unclear. Stud-

ies have shown that there is not a significant increase of dying cells in the blood of HIV<sup>+</sup> patients, in comparison with uninfected subjects [1, 2], but depletion is very gradual, taking an average of 10 years, and the extent of killing may be too small to notice grossly. However, increased frequencies of dying cells are found in lymph nodes of HIV patients [3, 4]. These cells have been shown to be undergoing apoptosis and are usually not productively infected (i.e., they are not producing virus); thus, they have been termed “bystander” cells [5]. In fact, the frequencies of infected cells making significant amounts of virus at any given time in the blood and lymph nodes of HIV<sup>+</sup> patients are very low (approximately 1 in 100,000 cells on average) [6, 7]. Thus, direct killing of CD4 T cells by HIV replication could not account for much CD4 lymphocyte depletion. Furthermore, several studies demonstrated that as CD4 lymphocyte numbers decrease in the blood in early phases of the disease, the CD4 lymphocytes in lymph nodes do not disappear and often increase in number for a period of time [3, 8]. Accordingly, the CD4/CD8 ratios in lymph nodes do not invert until very late in the disease [8] in contrast to the blood. These data cannot be easily reconciled with a simple view that the disappearance of CD4 lymphocytes observed on a daily basis in the blood occurs similarly throughout the lymphoid tissues, as conjectured in certain mathematical models [9]. Using that premise, those studies speculated that a very large number of CD4 lymphocytes (~10<sup>9</sup>) are eliminated per day in HIV<sup>+</sup> individuals. Furthermore, researchers had to speculate as to a compensatory increase in production of new CD4 lymphocytes, otherwise depletion of CD4 lymphocytes would occur very rapidly, and AIDS would occur within weeks. In the last few years, studies examining the production of new CD4 lymphocytes have provided strong evidence that there is no major increase, but rather a slight decrease, in the production of new CD4 lymphocytes in HIV<sup>+</sup> subjects [10–12].

Other studies have shown that HIV has an effect on resting CD4 lymphocytes, which would be the predominant cells surrounding any productively infected lymphocytes present in

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lymphoid tissues (as 95–99% of all lymphocytes are resting) [13]. Virus has been shown to bind (and signal upon binding) and enter resting lymphocytes and to reverse transcribe partial or complete DNA proviruses. These do not integrate, however [14]. This is a type of abortive infection, with the unintegrated viral DNA having a reported half-life of 6 h to about 1 week [14, 15]. If this abortively infected cell is activated by antigen into the cell cycle within this period, the virus can complete its replication cycle and produce progeny virions. The cell is then productively infected. The ratio of the frequencies of productively infected cells (~7 per 10<sup>6</sup>) in blood and lymph nodes to abortively infected cells (approximately 7500 per 10<sup>6</sup> cells) [16] shows that greater than 99% of all HIV-infected cells in the body of an infected individual are abortively infected, resting lymphocytes. This should be expected, as most CD4 lymphocytes are resting. The binding of HIV to its cellular receptors (CD4 and chemokine receptors) signals these cells, and we showed that this results in up-regulation of L-selectin, the receptor for homing to lymph nodes [17]. This receptor stays elevated for approximately 3 days, and these cells display enhanced binding (~12-fold increase) to high endothelial venules in sections of lymph nodes and enhanced homing when injected into the blood of severe combined immunodeficiency (SCID) mice [17]. During the homing process, a large number (40–50%) of these cells are induced into apoptosis after they enter the lymph node, which appears to be a result of secondary signaling through any of several homing receptors (minimally L-selectin, CD44, CD11a) [18]. Thus, a scenario has arisen from these studies that depicts resting CD4 lymphocytes in lymphoid tissues coming into contact with HIV virions, productively infected cells, or HIV-coated, follicular dendritic cells, resulting in induction of a partially activated phenotype, including up-regulation of L-selectin and Fas. This is maintained for many days. Because of normal lymph node/blood circulation in which most lymphocytes in lymphoid tissues migrate back to the blood within 2 days [19], many of these cells will end up back in the blood at the time of maximum-induced expression of L-selectin. These cells would then home very rapidly back to lymph nodes. Following transendothelial migration and entry into the lymph nodes, approximately half of them would be induced into apoptosis, and they do not produce HIV [20].

This scenario can explain many important observations in HIV<sup>+</sup> patients. 1) As CD4 lymphocytes disappear in the blood, their numbers do not drop, and the CD4/CD8 ratios do not invert in lymph nodes until late in disease. 2) There is no increased frequency of dying cells in the blood, but there is in lymph nodes. (Note that during later stages of HIV disease, the blood CD4 cells possess a greater propensity for spontaneous or activation-induced apoptosis when cultured *in vitro* [1, 2, 21], but this may be due more to their chronic immune (antigen-mediated) activation status, which occurs in infected patients, than to direct HIV infection/replication. This propensity is not significant at early disease stages when blood CD4 cell levels are above ~300/mm<sup>3</sup>, yet more than half of the blood CD4 cells have disappeared.) 3) Cells that are dying in lymph nodes are not making HIV, and they are dying by apoptosis. 4) The early increase of CD4 cells in the blood following highly active antiretroviral therapy (HAART) treatment appears to be

because of redistribution from tissues. This would be expected if the disappearance of blood CD4 cells is mainly a result of HIV-induced, enhanced migration out of the blood [22]. 5) Steroids, which are known to down-regulate L-selectin and retard lymph node homing, have been shown to retard or stop the disappearance of CD4 cells in the blood of HIV<sup>+</sup> patients [23, 24].

More direct evidence is now needed to show whether this scenario actually occurs in HIV<sup>+</sup> patients. The studies reported herein were initiated to examine the rates of migration (homing) of blood CD4 lymphocytes *in vivo*.

## MATERIALS AND METHODS

### Human subjects

We recruited eight volunteers: three healthy, uninfected donors, three HIV<sup>+</sup> patients who were not on antiviral drug treatment yet, and two chronic hepatitis B patients. We explained the study to all eight volunteers, who read and signed International Review Board (IRB)-approved consent forms. The CD4 counts, frequencies of CD45RA, CD45RO, and CD62L+ CD4 cells, and plasma HIV load for each patient are listed in **Table 1**.

### Lymphocyte purification and radiolabeling with <sup>111</sup>In

Venous blood (75–100 ml) was drawn from the eight study volunteers into heparinized tubes. The blood was centrifuged, and some of the plasma was collected and saved for later use. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood by centrifugation through lymphocyte separation medium (Organon Teknika Corp., France) and were washed twice with Hanks' balanced salt solution (HBSS). The cell pellet was resuspended in RPMI-1640 medium (Gibco-BRL, Grand Island, NY) supplemented with 10% autologous serum at 1 × 10<sup>6</sup> cells/ml. Enriched CD4 T lymphocytes were obtained from the PBMCs by negative-panning procedures. Briefly, Petri dishes were pre-treated with 10 ml affinity-purified goat anti-mouse (GAM) immunoglobulin G (IgG; Sigma Chemical Co., St. Louis, MO) in HBSS (5 μg/ml) overnight at 4°C. The dishes were then rinsed with 10 ml HBSS containing 2% autologous plasma five times and incubated for 1 h at 4°C with 20 ml of the same solution. The PBMCs were incubated in 100 μl customized antibody cocktail (Stem Cell Technology, Vancouver, BC, Canada) for 1 h at 4°C with constant mixing, washed twice, and then placed onto the GAM-IgG-coated plates for 3 h at 4°C. The antibody cocktail contained monoclonal antibodies to CD14, CD16, CD19, and CD56 (all at 30 μg/ml) and glycoporin A (10 μg/ml). Nonadherent cells were then collected, washed, and kept in supplemented RPMI-1640 media. An aliquot of the purified cell population was analyzed by dual-color flow cytometry, and the percentage of CD4<sup>+</sup> cells (varied between 92% and 97% purity) and CD4<sup>+</sup> cells expressing CD62L, CD45RA, and CD45RO was determined.

The cells were then delivered to the Department of Nuclear Medicine (University of Texas Medical Branch, Galveston) for <sup>111</sup>In labeling and injection. The saved autologous plasma was centrifuged at 2450 RPM for 20 min to produce platelet-poor plasma. The purified CD4 lymphocytes were resuspended in 6 ml 0.9% saline, and the solution was drawn up gently into a syringe and dispensed back into the tube. This process was repeated until the

TABLE 1. CD4 Cell Counts and Viral Loads of HIV<sup>+</sup> Volunteers

Subject	CD4 lymphocytes			HIV load (RNA copies/ml plasma)	
	Cells/μl blood	RA+ (%)	RO+ (%)		
1	503	58	42	37	27,694
2	396	61	39	21	271,552
3	594	54	46	25	25,427

button of CD4 cells was completely dispersed.  $^{111}\text{In}$  oxine (1 mCi) was added drop by drop to the CD4 cells suspension, and the mixture of CD4 cells and  $^{111}\text{In}$  oxine was incubated for 30 min at room temperature. The mixture was gently agitated three to four times during the incubation period, and the CD4 cell/ $^{111}\text{In}$  oxine mixture was brought up to a volume of 15 ml with an appropriate volume of platelet-poor plasma. The suspension was centrifuged at 1400 RPM for 5 min. After spinning, the supernatant was withdrawn from the tube using a syringe and spinal needle without disturbing the CD4 cell pellet. The supernatant, which contained the unincorporated  $^{111}\text{In}$  oxine, was discarded. Platelet-poor, autologous plasma (8 ml) was added to the CD4 cell platelet, and the cells were resuspended. An aliquot was analyzed on a  $\gamma$  counter to determine the efficiency of labeling.  $^{111}\text{In}$ -labeled CD4 cells (500  $\mu\text{Ci}$ ) were dispensed into a syringe and injected intravenously (i.v.; antecubital fossa) into the original donor. Thus, each donor received the same amount of radioactivity, even though the absolute numbers of labeled CD4 cells injected differed. In this manner, the amount of radioactivity accumulating in any given body site can be directly compared between different study subjects.

## Camera and scanning

For the total body scintiphotos, the subjects were imaged, ~1, 3, and 24 h post-injection with a dual-head  $\gamma$  camera (Vertex, ADAC Laboratories, Milpitas, CA), equipped with medium-energy, general-purpose collimators. Flood correction was done with In intrinsic floods, and two 20% energy windows at 173 and 250 KeV were used. For static planar view, 600 s/frame were collected on the chest and pelvic area. The matrix size was  $256 \times 256 \times 6$ . The scan speed for the total body was 5 cm/min, and the matrix size was high-resolution, eight deep. Two experienced nuclear medicine physicians visually interpreted total body and planer scans from a computer display. Images were analyzed using the Pegasys processing terminals; irregular regions of interest (ROI) in each organ were drawn, duplicated, mirrored, and positioned on the sites of interest. Average counts in the ROIs were obtained as counts/pixel.

## RESULTS

Three normal volunteers and three HIV-infected individuals were recruited for this part of the study. The HIV<sup>+</sup> donors had been diagnosed with HIV for variable amounts of time, and none were taking antiretroviral drug therapy. Their CD4 counts ranged from 396 to 594 cells/ $\mu\text{l}$ , and viral loads ranged from 25,427 to 271,552 RNA copies per ml (Table 1). After signing IRB-approved, informed consent, they donated between 75 and 100 ml blood. Mononuclear cells were isolated from the blood, enriched for CD4 T cells by negative-selection panning (92–97% pure), and then labeled in vitro with  $^{111}\text{In}$  (1 mCi total). Following rinsing, 0.5 mCi-labeled cells were infused i.v. back into the original donors so that each subject received the same amount of radioactivity. Based on similar lymphocyte homing studies performed in mice or rats [19, 25, 26], blood T cells that express CD62L (L-selectin) have a half-life in blood of approximately 30 min, and migration rates of these cells from the blood into tissue, without any significant amount of return of these cells into the blood, can be measured within the first 3 h following injection into the venous system. Thus, our subjects were scanned with a  $\gamma$  camera, 1, 3, and 24 h after infusion, and localization of the CD4 cells was determined and quantitated.

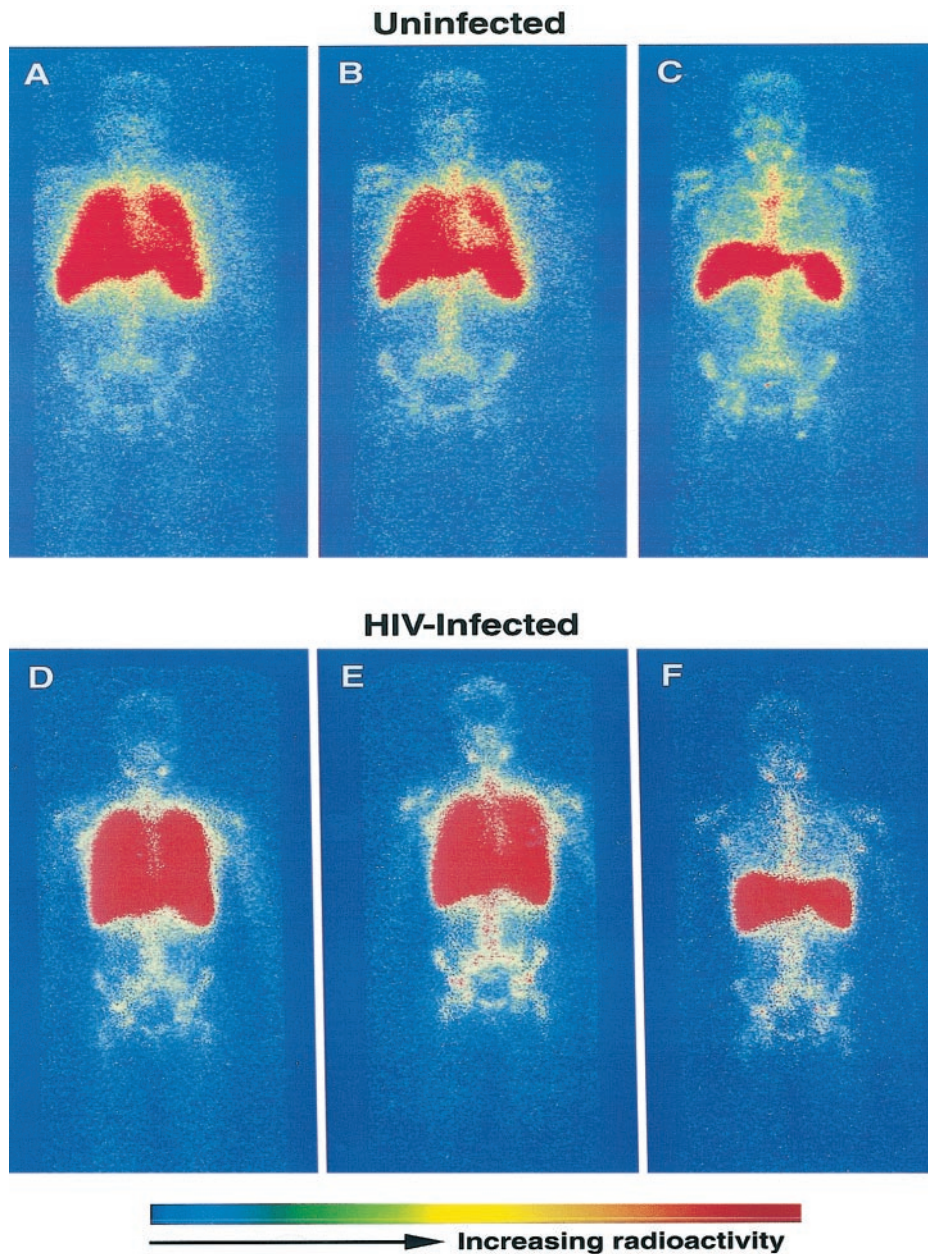
**Figure 1** shows whole-body scintiphotos of one representative uninfected and one representative HIV<sup>+</sup> volunteer at various time points post-infusion. The radioactivity detected by the  $\gamma$  camera was computer-digitized and is presented as colors (red>orange>yellow>green; blue is negative). The actual quantitative data, however, are presented in **Table 2**. The

lungs contain the greatest percentage of labeled cells at 1 and 3 h post-infusion in all subjects, but that is expected as the lung is the first major organ where the infused, labeled cells will travel, and a large percentage of them will stay in the lung for the first few hours. Another obvious finding is the increased intensity of labeled CD4 T cells in the vertebral and iliac bone marrow and cervical lymph nodes in the HIV<sup>+</sup> patient compared with the control at 1 and 3 h. This demonstrates that more CD4 T cells migrated to these areas per unit time in the HIV<sup>+</sup> subject compared with the uninfected subject. The two other HIV<sup>+</sup> subjects tested displayed similar enhanced accumulation of CD4 T cells in the bone, compared with the two other control subjects (**Fig. 2**, **Fig. 3**, Table 2). It was also obvious that most of the labeled CD4 T cells in normal subjects migrated to bone marrow, and this was almost exclusively to vertebral and iliac marrow and not marrow in the long bones in both types of subjects. Also, the axillary lymph nodes of the HIV<sup>+</sup> patient contained more labeled cells than the control subjects at 1 and 3 h post-infusion (observed best in Fig. 3), and this was a statistically significant difference ( $P<0.01$ ). By 24 h, the labeled cells appeared to have distributed homogeneously, and there were no significant differences between control and HIV<sup>+</sup> subjects at that time point.

Higher resolution of the pelvic area (Fig. 2) for radioactivity quantitation on all subjects demonstrated that there was approximately twofold more labeled CD4 lymphocytes in the bone marrow at 1 and 3 h in the HIV patients in comparison with the three controls (Table 2). This difference was statistically significant. There was also increased accumulation of CD4 cells in what appeared to be the gut (Fig. 2). High resolution of the chest and neck regions demonstrated greater CD4 T cell localization in cervical and axillary lymph nodes in all three HIV<sup>+</sup> subjects compared with the three uninfected subjects (Fig. 3). This was also determined to be about twofold higher (Table 2). There was no evidence for enhanced homing of CD4 T cells to the central nervous system or any other organs in the body.

As we compared HIV-infected to -uninfected subjects, we next examined if CD4 lymphocytes displayed enhanced migration in another viral (non-HIV) infection to ascertain whether enhanced, CD4 T cell homing is a common feature of other viral infections. It should not, as antigen activation of T cells induces shielding of L-selectin, and less-than-normal lymph node homing from the blood would be expected [26]. Two patients with chronic hepatitis B virus (HBV) infection volunteered for this part of the study. The results, shown in Table 2, demonstrate that CD4 lymphocytes in the blood of these HBV-infected subjects migrated slightly less to bone marrow and considerably less to cervical and axillary lymph nodes in comparison with control subjects, as expected. We also did not see significantly increased accumulation of CD4 cells in the liver, which was surprising. Thus, the enhanced migration to lymph nodes and bone observed in HIV<sup>+</sup> subjects appears somewhat specific for HIV infection with active viral replication, as determined by detectable virus on quantitative HIV RNA assays.

To further confirm this, we retested two of the HIV<sup>+</sup> volunteers after they had been on HAART since their first scans. The virus load of volunteer #1 at the initial test had been



**Fig. 1.** Whole body  $\gamma$  camera scinti photos localizing  $^{111}\text{In}$ -labeled CD4 lymphocytes in an uninfected and HIV-infected subject. Freshly isolated blood CD4 lymphocytes were labeled with  $^{111}\text{In}$  (500  $\mu\text{Ci}$ ) in vitro, rinsed, and then i.v. reinfused back into the original donor. The subject was then scanned by a  $\gamma$  camera at 1 (A, D), 3 (B, E), and 24 (C, F) h post-infusion. The intensity of the signals in organs corresponds to the number of radioactive lymphocytes that migrated from the blood within the indicated time points. Computer digitization of the radioactivity is presented as the following colors: red > yellow > green > blue (negative). The images presented are 600-s exposures.

27,694 copies/ml and at repeat testing after 3 months on HAART, was <400 copies/ml. Volunteer #3's virus load before HAART was 25,427 copies/ $\mu\text{l}$  and after 10 months on HAART, was 1050 copies/ $\mu\text{l}$ . **Figure 4** shows the results and demonstrates that the CD4 T cells in the blood of both volunteers were now migrating at rates similar to those of uninfected subjects. The blood CD4 counts of volunteer #1 had also gone up from 503 to 692 cells/ $\mu\text{l}$ , and those of volunteer #3 rose from 594 to 707 cells/ $\mu\text{l}$ . Thus, the enhanced homing of CD4 lymphocytes in HIV<sup>+</sup> patients appears to correlate with the presence of replicating HIV.

## DISCUSSION

As 95–99% of all blood lymphocytes are resting [13], our studies predominantly assessed where resting lymphocytes traffic from the blood in humans. Our previous studies examining migration of HIV-exposed human CD4 lymphocytes injected into the blood of SCID mice also used mostly resting T cells [17, 18]. Other studies that examined trafficking of simian immunodeficiency virus (SIV)- or HIV-infected cells using the SCID/hu mouse model used activated cells exclusively. Donze and co-workers [27] demonstrated that HIV and SIV produc-

TABLE 2. Quantitation of  $^{111}\text{In}$ -Labeled CD4-T Cells in Various Organs at 1 and 3 h Post-Infusion

Subject	Bone <sup>b</sup>	Lymph nodes <sup>a</sup>	
		Cervical	Axillary
1 h			
Uninfected: 1	1448 <sup>c</sup>	1874	1663
Uninfected: 2	756	2056	2358
Uninfected: 3	1487	1663	2603
(Ave. $\pm$ SD)	(1234 $\pm$ 414)	(1864 $\pm$ 197)	(2208 $\pm$ 488)
HIV-infected: 1	2385	3570	7322
HIV-infected: 2	3173	2528	4861
HIV-infected: 3	2265	3245	5414
(Ave. $\pm$ SD)	(2608 $\pm$ 493)*	(3114 $\pm$ 533)*	(5865 $\pm$ 1291)*
HBV-infected: 1	971 $\pm$ 257	841 $\pm$ 53	1407 $\pm$ 209
HBV-infected: 2	1000 $\pm$ 300	650 $\pm$ 38	1653 $\pm$ 300
3 h			
Uninfected: 1	1566	2632	1796
Uninfected: 2	2015	2094	1876
Uninfected: 3	2357	1877	2523
(Ave. $\pm$ SD)	(1979 $\pm$ 396)	(2201 $\pm$ 389)	(2051 $\pm$ 408)
HIV-infected: 1	4560	3930	2901
HIV-infected: 2	3331	3683	3054
HIV-infected: 3	4200	4095	4508
(Ave. $\pm$ SD)	(4030 $\pm$ 631)*	(3936 $\pm$ 256)*	(3488 $\pm$ 886)*
HBV-infected: 1	1071 $\pm$ 290	937 $\pm$ 71	1480 $\pm$ 40
HBV-infected: 2	1100 $\pm$ 350	780 $\pm$ 52	1729 $\pm$ 51

Ave., Average. <sup>a</sup> Counts in all cervical nodes were combined; same for axillary nodes. <sup>b</sup> Counts in vertebral and iliac bones were combined. <sup>c</sup> Average radioactivity as counts per 460 pixel area per 600 s. \* The differences between HIV-infected and control subjects are statistically significant ( $P < 0.01$ ).

tively infected cells (and thus presumably activated) migrated to the gut following injection into SCID mice. Bragardo and co-workers [28], using gp120-treated, cultured T cell clones injected into SCID mice, showed increased homing of these

cells into the spleen, intestine, and mesenteric lymph nodes and decreased homing into peripheral lymph nodes. The locations of viral or gp120-induced T cell migration in those studies differ somewhat from our results, but they can be explained, as activated T cells shed L-selectin and express homing receptors for other tissues [29]. We also observed some increased migration of CD4 cells to the gut in comparison with uninfected subjects, but these may be the activated CD4 cells, which should be in higher frequencies in the infected subjects. Veazey and co-workers [30] showed that CD4 cells replicating SIV in infected macaques were in highest proportion in the gut, but this is where the high proportion of activated CD4 lymphocytes resides, and the virus predominantly replicates in activated lymphocytes. Furthermore, a study of adhesion molecules on lymphocytes of HIV-infected subjects showed that the number of blood T cells expressing L-selectin was reduced in comparison with uninfected subjects, which correlated with the stages of disease [31]. One explanation for this might be that many of the cells expressing high levels of L-selectin as a result of HIV-induced signaling within the previous day or two have already left the blood and migrated into lymphoid tissues. The three HIV<sup>+</sup> subjects in this study also had slightly reduced levels of L-selectin-positive CD4 cells in their blood and slightly reduced levels of CD45R0-positive cells (Table 1). Thus, it would not be evident that these blood CD4 cells would be homing at an increased rate to lymph nodes. Studies have shown that the rate of homing from the blood into lymph nodes directly correlates with the level of L-selectin expression [32]. We have also found that HIV binding to resting CD4 cells down-regulates expression of CD43 (unpublished data), which antagonizes L-selectin function, and it is likely that the dual effects of increased L-selectin expression and reduction of CD43 lead to a cell that migrates more rapidly from the blood into the lymph nodes. Furthermore, it is possible that other

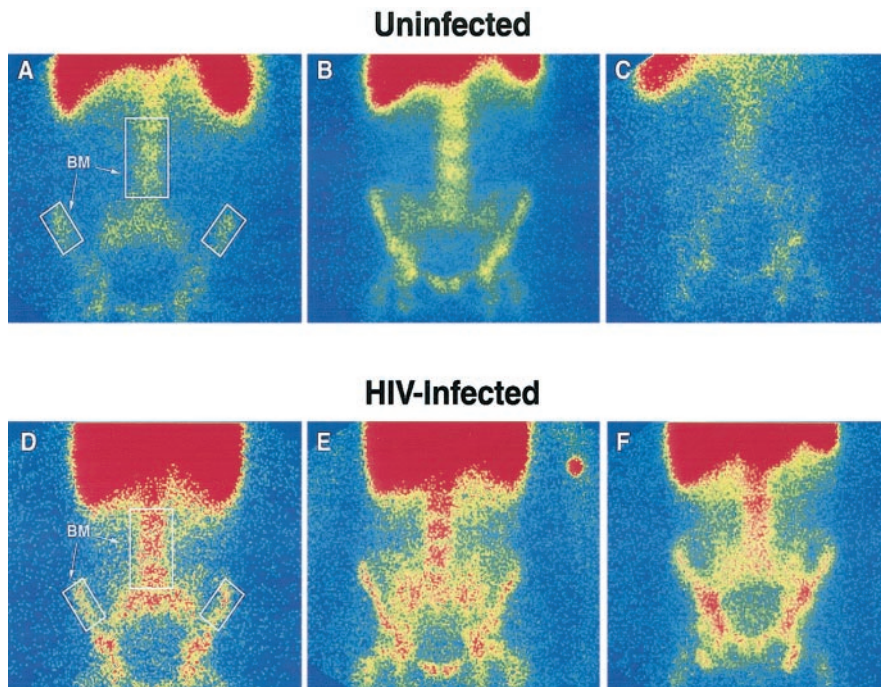
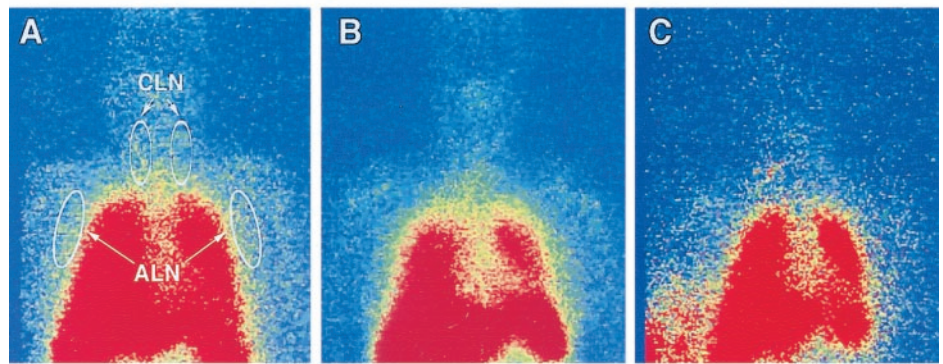


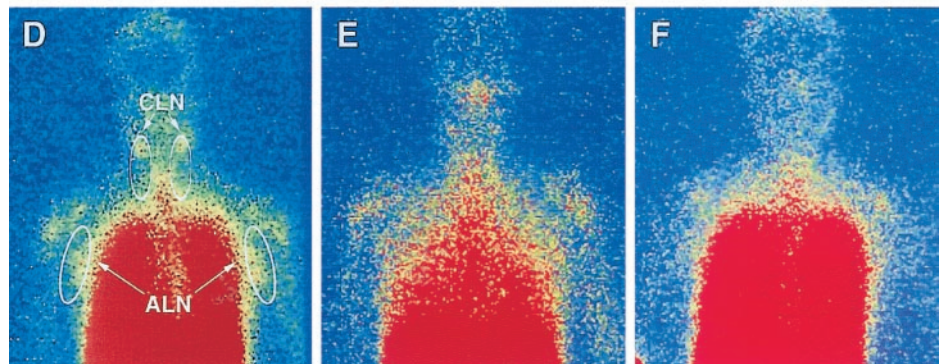
Fig. 2. Higher resolution of the hip/pelvic area of the three uninfected and three HIV-infected individuals at 3 h post-infusion. (A–C) Three uninfected subjects; (D–F) three HIV<sup>+</sup> subjects. (A, D) The rectangles indicate the areas of bone marrow (BM) that were used for quantitation (data of Table 2).

## Uninfected



**Fig. 3.** Higher resolution of the chest/neck area. (A–C) Three uninfected subjects at 3 h post-infusion; (D–F) three HIV<sup>+</sup> subjects at 3 h. (A, D) Circled areas indicate the axillary lymph nodes (ALN) and cervical lymph nodes (CLN) that were used for quantitation in the data of Table 2.

## HIV-Infected

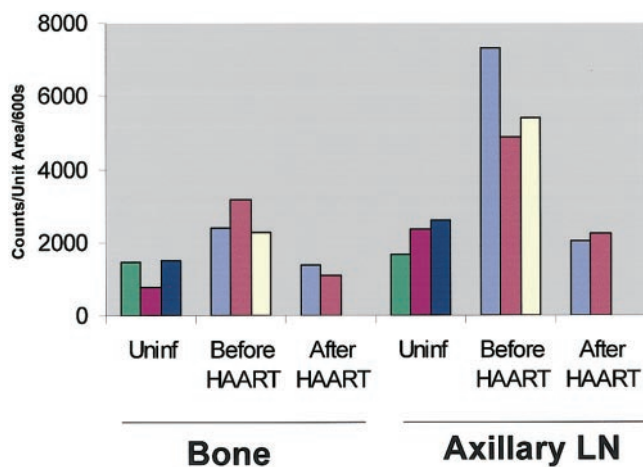


receptors involved in lymph node homing (e.g., CCR7) could be modulated during HIV infection.

Many recent studies examining the CD4 lymphocytes in HIV-positive subjects on HAART have shown that the first increase of CD4 lymphocytes is predominantly comprised of memory cells and not newly made naïve cells [22] and has been termed the “redistributive phase.” This indicated that HIV was

causing CD4 lymphocytes to be sequestered in or to migrate to nonvascular compartments. This strongly supported our earlier studies showing that HIV contact could directly induce resting CD4 lymphocytes to home to peripheral lymph nodes and the theory that the disappearance of CD4 lymphocytes from the blood was actually a result of their migration out of the blood [17, 18, 33]. The present study further supports this concept.

The situation now known to occur in HIV<sup>+</sup> individuals where their blood CD4 lymphocytes are accumulating at about twofold increased rates in peripheral lymph nodes and bone marrow is likely because of the HIV-induced signaling of resting CD4 cells, which have come into contact with HIV-producing cells, virus-coated follicular dendritic cells, or cell-free virions [17]. These effects are much more frequent on a cell-to-cell basis than the frequencies of productively infected cells (~1000-fold) [16]. Specifically, although ~1 in 100,000 CD4 cells is productively infected making significant amounts of new progeny virus, between 0.8% and 1% of the CD4 lymphocytes possess unintegrated HIV proviruses [16], and these are most likely resting cells that have come into contact with virus. It has been amply documented that when a resting CD4 lymphocyte is bound by HIV, the virus does enter (as well as signal through its cellular receptors) and reverse transcribes partial or complete provirus; however, this remains unintegrated [14]. Thus, studies quantitating the frequencies of CD4 lymphocytes possessing unintegrated HIV proviral DNA are actually also quantitating the frequencies of resting CD4 cells that have been signaled by HIV contact. Therefore, the twofold, enhanced accumulation rate of CD4 T cells in the peripheral



**Fig. 4.** Quantitation of CD4 T cell migration in two HIV<sup>+</sup> patients before and after 3–10 months of HAART treatment. The radioactive counts per 460 pixels on 600-s scans are presented for bone and axillary lymph nodes at 1 h post-infusion of each uninfected (Uninf) subject and each HIV-infected subject before HAART and two of the HIV-infected subjects after 3–10 months on HAART.

lymph nodes and axial bone marrow is presumably a result of the behavior of about 1% of the cells. A mathematical model of this HIV-induced, enhanced migration process affecting approximately 1% of the CD4 cells on a continuing basis has revealed that gradual depletion of CD4 would occur over an 8- to 10-year period and closely mimics the rate of CD4-cell depletion in AIDS patients [34].

These findings that CD4 lymphocytes in HIV<sup>+</sup> subjects migrate at enhanced rates to peripheral lymph nodes and bone marrow lend strong support to the hypothesis that a major pathogenic mechanism of HIV is its ability to signal resting CD4 cells and induce them to home to lymph nodes and die there. This hypothesis stems from previous studies, which show that HIV binding to resting CD4 lymphocytes induced increased expression of L-selectin and enhanced lymph node homing as judged following placement in the blood of SCID mice [17]. Other studies showed that many of these cells induced to home to lymph node are also induced into apoptosis via signals received during the homing process, and they do not make HIV [18]. This is identical to what is observed in HIV patients. (There is an increase of dying CD4 cells in lymph nodes, and they are not making virus; they have been termed “bystander” cells.) Thus, building on that background, it was important to show whether CD4 lymphocytes in HIV<sup>+</sup> patients are actually migrating at enhanced rates or not, and that was the reason for this study. What is happening in the bone marrow of these patients remains unknown. Perhaps, they are induced into apoptosis, as they appear to be in lymph nodes [18]. Enhanced homing of CD4 cells from the blood into peripheral lymph nodes would not occur during normal antigen-mediated activation of CD4 cells, as that induces shedding of L-selectin, and these cells migrate to other locations [32]. It was not surprising, then, when we did not observe enhanced lymph node homing of CD4 cells in the HBV-infected subjects. Thus, our finding of increased lymph node homing of blood CD4 cells in HIV-infected individuals speaks against the “global immune activation and attendant activation-induced cell death” theory of HIV depletion of CD4 cells, as it would be expected that less-than-normal homing from blood to lymph nodes would occur in that situation. Furthermore, these new data do not support one of the other major competing theories on how HIV causes depletion of CD4 cells, which states that the virus is causing reduced production of new CD4 cells. Fewer new cells means fewer naïve cells, and naïve cells rapidly migrate (home) from the blood into lymph nodes. If that theory were true, one would expect to observe less-than-normal rates of blood CD4-cell homing into lymph nodes in HIV<sup>+</sup> subjects.

Further studies testing the hypothesis that HIV causes depletion of CD4 cells via its effect on resting CD4 lymphocytes could examine whether inhibitors of lymph node homing or homing receptor induction of apoptosis would prevent depletion of CD4 lymphocytes in patients. Steroids, which down-regulate L-selectin expression on T cells, have been shown to stop CD4-cell depletion in the blood of patients [24]. However, as steroids have many effects, this correlation is not definitive. Newer compounds that are very specific for lymph node homing may prove to be informative. Also, it may be worthwhile to consider targeting these events for therapy.

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