Collagen deposition in HIV-1 infected lymphatic tissues and T cell homeostasis

Timothy W. Schacker,[1] Phuong L. Nguyen,[2] Gregory J. Beilman,[3] Steven Wolinsky,[4] Matthew Larson,[1] Cavan Reilly,[5] and Ashley T. Haase[6]

[1]Department of Medicine,
[2]Department of Laboratory Medicine, and
[3]Department of Surgery, University of Minnesota, Minneapolis, Minnesota, USA
[4]Department of Medicine, Northwestern University, Chicago, Illinois, USA
[5]Department of Biostatistics, and
[6]Department of Microbiology, University of Minnesota, Minneapolis, Minnesota, USA

Lymphatic tissues (LTs) are structurally organized to promote interaction between antigens, chemokines, growth factors, and lymphocytes to generate an immunologic response and maintain normal-sized populations of CD4+ and CD8+ T cells. Inflammation and tissue remodeling that accompany local innate and adaptive immune responses to HIV-1 replication cause damage to the LT architecture. As a result, normal populations of CD4+ and CD8+ T cells cannot be supported and antigen-lymphocyte interactions are impaired. This conclusion is supported herein following LT sampling before and during anti-HIV therapy in persons with acute, chronic, and late-stage HIV-1 infection. Among seven individuals treated with anti-retroviral therapy (ART) and four individuals deferring therapy we found evidence of significant paracortical T cell zone damage associated with deposition of collagen, the extent of which was inversely correlated with both the size of the LT CD4+ T cell population and the change in peripheral CD4+ T cell count with anti-HIV therapy. The HIV-1–associated inflammatory changes and scarring in LT both limit the ability of the tissue to support and reestablish normal-sized populations of CD4+ T cells and suggest a novel mechanism of T cell depletion that may explain the failure of ART to significantly increase CD4+ T cell populations in some HIV-1–infected persons.

Introduction

There is increasing recognition that the structure of secondary lymphatic tissue (LT) plays a critical role in immune system homeostasis (1–3). These architectural elements provide an organizing

Address correspondence to: Timothy W. Schacker, Department of Medicine/Infectious Diseases, University of Minnesota, MMC 250, 516 Delaware Street, Minneapolis, Minnesota 55455, USA.
Phone: (612) 624-9996; Fax (612) 625-4410; E-mail: schacker@lenti.med.umn.edu.
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structure that supports a unique microenvironment, or niche, necessary to support the immune functions of that tissue. The paracortical T cell zone (TZ), where 98% of CD4+ T cells in the human body normally reside (4), is one such niche. It is organized to mount and resolve an immune response through interaction between naive and memory T cells with antigen-presenting cells (APCs) and to provide a source of growth factors and cytokines. It is also a site for MHC class I, II, and self-peptide interactions that are necessary to maintain a normal-sized population of CD4+ and CD8+ T cells (5–9).

It is within this complex TZ niche that most significant events of HIV-1 pathogenesis occur. Over 99% of virions produced during the course of infection are made in activated CD4+ T cells that

reside in the TZ, and it is here that the pathological hallmark of HIV-1 infection, depletion of CD4+ T cells, is manifest and where adaptive and innate immune defenses interact in an attempt to halt viral replication (4, 10–12). As the infection progresses, the size of the TZ is diminished and the organizing structure is lost (13–15). This pathological change may limit the ability of the tissue to recover normal function with antiretroviral therapy (ART).

In studies reported here, we examined the TZ niche before and during ART with the hypothesis that the extent of CD4+ T cell depletion at baseline and the subsequent increase in CD4+ T cell count with highly active antiretroviral therapy (HAART) is related to the extent of structural damage within the TZ. This hypothesis was suggested and supported by the importance of the TZ in immune system homeostasis with well-documented failures of HAART to restore the peripheral CD4+ T cell population in some individuals, despite suppression of HIV-1 replication (16, 17). In addition we found pathological changes and abnormally small TZs in LTs collected from 21 individuals with established HIV-1 infection who had no detectable plasma viremia at a mean of 20 months after initiation of HAART and had a mean increase in peripheral CD4+ T cell count of 151 cells/mm³ (18). These findings show that current definitions of immune reconstitution that equate success with increases in the peripheral CD4+ T cell count will miss underlying structural changes in LTs that may account for the rate and extent of recovery and the variability in the peripheral CD4+ T cell response to HAART. Among the 21 patients studied, the CD4+ T cell count increase was

less than 50 cells/mm³ in six patients and the count actually decreased in four patients. In a cross-study analysis of 1,650 treatment-naive patients with a median CD4$^+$ cell count of 137 cells/mm³ (IQR, 32–310) who were enrolled in HAART treatment protocols developed by the Community Programs for Clinical Research on AIDS, 27% of 1,373 patients followed for at least 8 months had a CD4$^+$ cell count change from baseline that was less than 50 cells/mm³ (J. Neaton, personal communication). These data suggest there are intrinsic factors beyond suppression of viremia that may limit the ability for immunologic reconstitution, as has been suggested by others measuring proliferative responses of CD4$^+$ and CD8$^+$ T cells (19).

The observation that up to 25% of ART-naive persons increase peripheral CD4$^+$ T cell counts by a clinically insignificant margin upon initiation of HAART supports our hypothesis that damage to the TZ structure limits restoration of the CD4$^+$ T cell population. In the long struggle between immune defenses and HIV-1 that partially controls replication, the immune system is maintained in a state of chronic activation. In this condition, the associated inflammation could well damage the TZ so that it cannot support a normal population of T cells even though the inciting stimulus has been largely removed by HAART. To examine this possibility, we obtained LT from acute and chronically HIV-1–infected, ART-naive individuals at several timepoints before and during HAART. We demonstrate for the first time to our knowledge significant architectural damage in LT from chronic inflammation and fibrosis in the TZ that changes the organizing structure of the tissue. The amount of fibrosis is significantly associated with the size of the population of CD4$^+$ T cells in LT at the time of biopsy and the subsequent recovery of the population associated with HAART. These data provide new insight into the pathogenesis of immune depletion in HIV-1 infection and suggest that examination of LT prior to initiation of HAART may provide important information on the likelihood and extent of immune reconstitution.

These data also suggest that adjunctive anti-inflammatory therapy may improve the extent of immune recovery with HAART.

Methods

ART-naive HIV-infected individuals were recruited into a prospective study of intensive sampling of secondary LT before and during HAART. The study procedures were approved by the Human Subjects Committee of the University of Minnesota. Entry criteria were: age greater than 18, positive for HIV antibody by ELISA and Western blot, and no prior history of ART. At entry, each subject was interviewed to characterize risk factors for HIV infection, and medical records were reviewed to categorize stage and duration of infection. Clinical stage of infection was characterized as acute (proven serologically), early (within 6 months of a documented seroconversion), presymptomatic (CD4$^+$ cell count greater than 200 cells/mm³ with positive HIV$^+$ antibody test for more than 6 months), or AIDS (CD4$^+$ cell count less than 200 cells/mm³).

All study-related procedures were carried out in the University of Minnesota General Clinical Research Center (GCRC) except for the excisional inguinal lymph node biopsy, which was completed in a research clinic at the University of Minnesota with facilities for biopsy under local anesthetic. Upon admission to the GCRC, blood was drawn for the CD4$^+$ T cell count and plasma HIV-1 RNA. Immediately after the baseline biopsy was obtained, HAART was initiated. The choice of components for the HAART regimen was left to each individual and their primary care provider; however, it was required the subject be given a regimen meeting a minimal standard for HAART, defined as at least three drugs from two different classes. On the third day, 72 hours after initiation of HAART, and again at 1 month and 6 months after initiation of HAART, an additional excisional inguinal lymph node biopsy was completed for the patients on HAART. Patients choosing to deter HAART did not undergo a biopsy at 6 months. Peripheral blood was collected for assessment of peripheral CD4$^+$ cell count and plasma HIV-1 RNA at

weekly intervals during the first month and then monthly for 6 months on all patients. In addition, LT from HIV-negative individuals was obtained from the Division of Surgical Pathology at the Fairview-University Medical Center (Minneapolis, Minnesota, USA) to act as an HIV-1–negative standard. These tissues were removed during routine surgical procedures and were reviewed by the pathologist to ensure they were histologically normal before we included them as negative control tissue.

At the time of biopsy, tissues were immediately placed into fresh 10% buffered formalin for 4 hours and then processed through the clinical laboratories of the Fairview-University Medical Center for mounting into paraffin blocks. Five-micrometer sections were cut and stained with hematoxylin and eosin to examine overall morphology and characterize the size of the TZ as expanded, normal, or depleted.

Additional 5-μm sections were cut and reacted with antibodies to CD4 (clone 1F6; Novocastra Laboratories Ltd., Newcastle upon Tyne, United Kingdom) to quantify numbers of CD4$^+$ T cells per μm² using MetaMorph software (Version 4.5; Universal Imaging Corp., Downington, Pennsylvania, USA). The methods used for quantitative image analysis of the CD4$^+$ T cell population were similar to those we have used in the past (4, 10–12, 20–23). Every fifth image (at 40× magnification) was captured and imported into the MetaMorph software program. The threshold tool was used to isolate CD4$^+$ T cells and the standard area of a CD4$^+$ T cell used to determine the quantity of cells per field (expressed as cells/μm²). A total of 18 fields was analyzed for each LT sample and the mean was calculated. We have compared the mean number of CD4$^+$ T cells/μm² in a tissue sample by counting cells in every field of view to the mean number of cells per unit area in 18 representative fields/tissue and found the results to be comparable (12). This procedure was completed for each LT sample at each timepoint.

To identify collagen fibers, a 5-μm sample was cut from the baseline tissue and stained with a trichrome stain using the Masson method. A total of 18 images was obtained from the TZ of each tissue sample and imported into

Photoshop Version 6.0 (Adobe Systems Inc., Mountain View, California, USA). The color sampler tool was used to analyze representative shades and hues of the blue-stained collagen tissue. The area of the field containing collagen was selected and the background tissue was removed from the image. The image was then transported into the MetaMorph software program to determine the percent area of tissue occupied by collagen.

Results

A total of 11 individuals were enrolled in the study, with seven initiating HAART after the first biopsy and four choosing to defer HAART. The duration of infection and clinical stage of disease are summarized in Table 1. The protocol called for a total of four biopsies over 6 months among the patients choosing HAART, and three biopsies over 1 month for patients choosing to defer therapy. Four of the seven treated subjects (57%) completed all four biopsies, six of seven completed three biopsies, and one subject completed only the first two biopsies. All four untreated subjects completed their three biopsies over a period of 1 month. At baseline, the median CD4+ T cell count for all patients was 400 (range 112–905) cells/mm³, and median plasma HIV-1 RNA was 20,014 (range 10,684–484,694) copies/ml. All HAART regimens among the seven treated subjects met the definition of HAART, with six receiving two nucleoside analogues plus at least one protease inhibitor, and the seventh receiving two nucleoside analogues plus one non-nucleoside analogue. Plasma HIV-1 RNA was reduced to less than 500 copies/ml in all treated subjects by approximately 2 weeks after initiation of HAART and remained at that level for the 6-month follow-up period. The seven HAART-treated subjects had a median increase of 198 CD4+ T cells/ mm³ in peripheral blood over the 6-month follow-up period (range, 50–937 CD4+ T cells/mm³), but in three individuals the increase was only ≤ 62 cells/mm³ between baseline and the 6-month follow-up.

Histologic analysis of tissues at baseline and during follow-up. The size and character of B cell follicles and the TZ were assessed at each timepoint for all patients (Figure 1). At baseline in all LTs there was well-described and characteristic follicular hyperplasia except in tissue obtained from 1327, in whom follicles were hypoplastic and decreased in number, consistent with her diagnosis of AIDS (13–15). The TZ was normal in only two patients, 1329 and 1391, both of whom were in the very early stages of HIV-1 infection. In all other biopsies, the TZ was diminished, with a decreased population of cells and prominent vessels, consistent with loss of tissue volume.

As expected, there was no significant change in the histologic appearance of tissues at the 1-month follow-up for untreated subjects. Among the seven treated subjects, we observed only minimal improvement in B cell follicle hyperplasia or in the size and organization of the TZ. Of interest, among two of four subjects (1329 and 1391) with acute or early HIV infection and one patient with presymptomatic infection (1293), there was a decrease in the size and number of B cell follicles with a trend toward normalcy in the size and organization of the TZ. At the 6-month biopsy we noted continued and significant improvement in these same patients. However, there was no improvement in the tissues obtained 1 month after initiation of HAART from 1324, 1086, or 1327, or in the 6-month sample obtained from 1327 (1324 and 1086 did not have a biopsy at 6 months).

LT CD4+ T cell population. We used immunohistochemistry and quantitative image analysis to quantify the CD4+ T cell population before initiation of and during HAART. Five-micrometer sections were stained with anti-CD4 antibody and counted using the MetaMorph software program as previously described (4, 10–12, 20–23). Based upon previous studies we expected (and found) the number of CD4+ cells to be approximately 13.9×10^{-3} cells/μm². This is commensurate with the total body estimates of 2.2×10^{11} CD4+ T cells (4) in tissue from normal, immunocompetent adults that was obtained from the Division of Surgical Pathology of Fairview-University Medical Center (Table 2). The median baseline CD4+ T cell population for these HIV-1–infected patients in our study (both treated and untreated) was

Table 1
Demographic characteristics and change in plasma HIV-1 RNA and peripheral CD4+ T cell count as a response to HAART

PT	Mo. HIV infected	Stage	No. LT biopsies	Baseline periph. CD4+ (cells/mm³)	Baseline Plasma HIV RNA (copies/ml)	HAART regimen	CD4+ count at 6 mo. (cells/mm³)	Plasma HIV RNA at 6 mo. (copies/ml)
1329	1	Acute	4	370	484,694	ABV, 3TC, IDV, RTV	568	<50
1324	2	Early	3	494	14,696	ABV, 3TC, IDV, RTV	544	<50
1391	6	Early	4	290	24,718	AZT, 3TC, EFV	765	<50
1389	6	Early	2	824	32,173	ABV, 3TC, IDV, RTV	1,278	<50
1086	24	Presx	3	512	20,562	AZT, 3TC, IDV	574	<50
1293	>24	Presx	4	905	14,225	AZT, 3TC, IDV	1,842	<50
1327	>24	AIDS	4	112	12,046	AZT, 3TC, IDV, RTV	172	<50
1335[A]	>24	Presx	3	400	15,284	None	ND	ND
1407[A]	>24	Presx	3	372	31,922	None	ND	ND
1406[A]	>24	Presx	3	188	10,684	None	ND	ND
1408[A]	>24	Presx	3	685	20,014	None	ND	ND

[A]Patients who have chosen to not take HAART. Periph, peripheral; pt, patient; presx, presymptomatic; nd, not done.
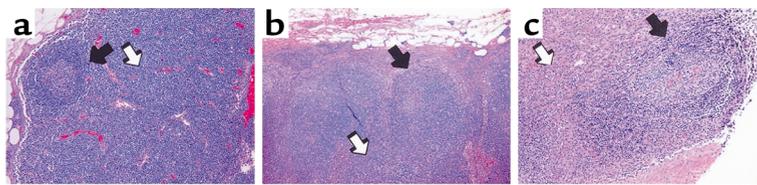
Figure 1
Histologic changes in B cell follicles and TZ characteristic of HIV-1 infection. (**a**) Section of lymph node from an HIV-negative patient. There is one B cell follicle that is normal in size and shape (black arrow). The TZ is normal in size with a normal population of cells (white arrow). (**b**) Section of a lymph node from an HIV-1–infected patient with presymptomatic HIV-1 infection. The follicles are hyperplastic and disorganized (black arrow), and the TZ is small and somewhat depleted of cells (white arrow). (**c**) Lymph node section from a patient with AIDS. The follicular space is disorganized and without structure (black arrow), and the TZ is unrecognizable with few cells (white arrow). All images are at a magnification of ×4.

6.81×10^{-4} cells/μm^2 (range, 2.41×10^{-4} to 9.78×10^{-4}, which is approximately 50% of normal (Table 2). While there was general correlation between stage of disease and the size of the LT CD4+ T cell population (the earlier the stage of infection the higher the LT CD4+ T cell count), it was not significant. We found no correlation between the baseline LT CD4+ T cell count and the baseline peripheral CD4+ T cell count or plasma HIV-1 RNA ($P = 0.5$, Wilcoxin signed rank test, Figure 2). This suggests that among HIV-1–infected persons who are not taking ART, common measures of disease severity (duration or stage of infection, peripheral CD4+ T cell count, and plasma HIV-1 RNA) do not correlate with the size of the baseline LT CD4+ T cell population.

We observed a general trend toward expansion of the LT CD4+ T cell population with HAART in all treated patients, and a significant increase in the LT CD4+ T cell population among the four treated individuals who provided LT at 6 months (two-sample paired Student t test, $P = 0.0039$) (Table 2). At baseline, the mean population size for all patients was 6.81×10^{-4} cells/μm^2 and among the treated patience, at 1 month it was 7.51×10^{-4} cells/μm^2 (range 2.75×10^{-4} to 10.30×10^{-4}) cells/μm^2 ($n = 6$), representing only a 2% increase over baseline to 54% of the normal population size. After 6 months of HAART, the median CD4+ T cell population size was 11.1×10^{-4} cells/μm^2 ($n = 4$), representing a mean 29% increase in the overall size of the LT population, with some patients

(1329, 1293, and 1391) having a near-normal CD4+ T cell population and others (1327) remaining significantly diminished.

Measurement of percent area collagen as a marker of fibrotic changes in LT. We consistently found prominent foci of scar tissue in LTs, suggesting that inflammation-associated damage might be reducing the capacity of the TZ niche to maintain cellular homeostasis. We hypothesized that the damage might correlate with the size of the CD4+ T cell population, and

tested this hypothesis by measuring the percentage of the TZ area occupied by collagen.

We used a trichrome stain to identify collagen deposition in the TZ and observed significantly greater deposition among all samples from HIV-1–infected subjects than was found in the HIV-1–negative sample (Figure 3). In the tissue obtained from the HIV-1–negative patient (Nl in Table 3), less than 1% of the TZ area consisted of collagen; however, among tissues from all HIV-1–infected persons, the values ranged from 2.2% to 19.9% (median 10.0%). To determine whether there was an association between the amount of fibrosis in the TZ and the size of the CD4+ T cell population in the TZ, we compared the percent area collagen in the TZ with the size of the CD4+ T cell population in the baseline LT biopsy specimen. We found a significant inverse relationship (Figure 4a). The greater the area occupied by collagen, the fewer CD4+ T cells were found in the LT ($R^2 = 0.72$, $P < 0.0001$, Spearman rank correlation). There was no association between percent area collagen and duration or stage of HIV-1

Table 2
Quantitative comparison of CD4+ T cells in LT and peripheral blood

| | LT CD4+ cells [A] | | | | Peripheral CD4+ cells | | | |
PT	Baseline	Day 3	Mo. 1	Mo. 6	Baseline	Day 3	Mo. 1	Mo. 6
HIV negative patient								
Nl[B]	13.9	—	—	—	—	—	—	—
Treated HIV+ patients								
Acute or early								
1329	9.78	7.10	9.96	13.0[B]	370	439	819	871
1324	6.14	5.84	7.27	—	494	339	466	544
1391	8.71	8.59	10.30	11.0[B]	234	414	436	765
1389	8.95	—	—	—	824	994	828	1278
Presymptomatic								
1086	4.45	5.72	7.81	—	512	450	236	574
1293	8.08	—	7.00	11.2[B]	905	915	1,098	1842
AIDS								
1327	2.41	—	2.75	6.63[B]	112	109	172	180
Untreated HIV+ patients								
Presymptomatic								
1335	7.44	—	7.77	—	400	468	359	ND
1407	8.5	Pend.	8.27	—	372	483	572	Pend.
1406	4.53	Pend.	4.14	—	188	173	181	Pend.
1408	5.88	8.22	6.58	—	685	744	681	Pend.

[A]The CD4+ T cell population in LT is expressed as no. cells $\times 10^{-4}/\mu m^2$. [B]The difference between baseline and 6-month LT CD4+ T cell counts is significant (two-tailed Student t test, $P = 0.0039$). N1, HIV-negative control. Pend, pending.
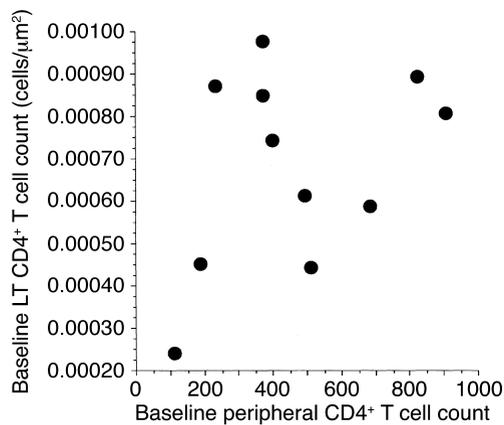
Figure 2
Comparison of peripheral versus LT CD4+ T cell population at baseline shows no significant relationship.

infection, baseline peripheral CD4+ T cell count, or plasma HIV-1 RNA, which are the markers of disease severity that are often used in the clinic.

To determine the impact of TZ fibrosis on the response to HAART and immune reconstitution, we compared the percent area collagen with the change in peripheral CD4+ T cell count over the 6-month follow-up period. We used the peripheral count for two reasons: this is the most common marker of HAART efficacy used in clinical practice, and we did not have a 6-month lymph node biopsy from all of the HAART-treated patients. Using multiple regression, we found a significant relationship between the change in the peripheral CD4+ T cell count over 6 months and the baseline percent area collagen, controlling for baseline peripheral CD4+ T cell count and baseline viral load (R^2 = 0.91, P < 0.001) (Figure 4b). This suggests that a single baseline measurement of percent area of LT occupied by collagen before initiation of HAART has predictive power to estimate the change in peripheral CD4+ T cell count, independent of baseline peripheral CD4+ T cell count or plasma HIV-1 RNA.

Discussion

We have prospectively examined the impact of chronic HIV-1 infection on the structure and function of the TZ niche to gain further insight into mechanisms of immune reconstitution and factors that might inhibit or

enhance this process. At baseline we observed significant depletion of the LT CD4+ T cell population in every patient and at all stages of HIV-1 infection. Others have made the observation that LT obtained from HIV-1–infected persons can contain a variable amount of collagen (24, 25). We show

that the amount of collagen in the TZ is significantly associated with the number of CD4+ T cells occupying that same space and the magnitude of CD4+ T cell recovery with HAART. We view this measure of collagen as a marker of damage to the structure and function of LT before therapy is begun and as a potential predictor of the change in CD4+ T cell count with HAART.

These relationships between collagen deposition in the TZ and the CD4+ T cell population before and during HAART suggest that in the state of chronic immune activation and inflammation from ongoing HIV-1 replication, there is damage and disruption to the LT microenvironment that results in the impaired recruitment, retention, and proliferation of CD4+ T cells. We would anticipate that the greatest impact would be on the subset with the naive phenotype. This is suggested by investigations in the mouse immune system showing that normal-sized populations of naive
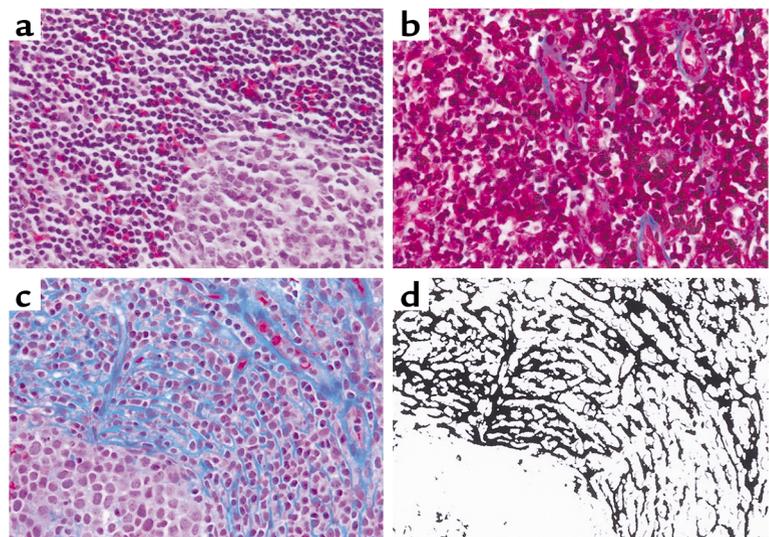
Figure 3
Quantitative analysis of collagen deposition in the TZ in HIV-1–infected persons. Tissues were stained with a modified trichrome stain using the Masson method to identify collagen fibers as blue. (**a**) HIV-negative patient. Collagen makes up 0.23% of the tissue section. (**b**) Patient 1407. Section shows collagen deposition (stained blue) in the wall of the high endothelial venule. Percent area collagen was calculated to be 2.2%. (**c**) Patient 1327, the patient with AIDS. In contrast to **b**, this section shows significant deposition of collagen in the TZ. Percent area collagen was calculated to be 18.6%. A total of 18 images from each tissue sample, imported through the Photoshop program (Adobe, Version 6.0), are displayed (magnification, ×40). (**d**) The transformed Photoshop image from **c**. All shades of blue representing collagen in **c** were averaged and displayed in black; non-collagen area was removed. This image was imported into the MetaMorph imaging program and the percent area in black was calculated.

Table 3
Percent area collagen and change in peripheral CD4^{+} cell count and plasma HIV-1 RNA over 6 months of follow-up

PT	Mo. HIV^{+}	Stage	Baseline peripheral CD4^{+} cells (cells/mm^3)	CD4^{+} at 6 mo. (cells/mm^3)	Baseline plasma HIV RNA (copies/ml)	Plasma HIV RNA at 6 mo. (copies/ml)	Percent area collagen
NI	—	—	—	—	—	—	0.23
1329	1	Acute	370	568	484,694	<50	5.4
1324	2	Early	494	544	14,696	<50	11.2
1391	6	Early	290	765	24,718	<50	2.7
1389	6	Early	824	1278	32,173	<50	6.2
1086	24	Presx	512	574	20,562	<50	12.3
1335[A]	>24	Presx	400	—	15,284	ND	13.3
1407	>24	Presx	372	—	31,922	Pend.	2.2
1406[A]	>24	Presx	188	—	10,684	Pend.	19.9
1408[A]	>24	Presx	685	—	20,014	Pend.	10.0
1293	>24	Presx	905	1842	14,225	<50	2.4
1327	>24	AIDS	112	172	12,046	<50	18.6

Patients are not receiving therapy.[A]

CD4^{+} T cells are dependent on intact LT structure (7) and our demonstration that the pool of naive CD4^{+} T cells in LT obtained from HIV-1–infected humans in the presymptomatic stage of disease is significantly depleted (4). Collectively, these data suggest a novel mechanism of CD4^{+} T cell depletion in HIV-1 infection, with viral replication in the TZ leading to inflammation and localized tissue damage such that the growth factors and overall structure are unable to support the normal resident population of cells. This is the most likely mechanism to explain collagen deposition in the TZ; however, it

is possible that another mechanism would explain this phenomenon. Whatever the mechanism, in effect, the space that is optimally organized to maintain 98% of the CD4^{+} T cell population is slowly destroyed through fibrosis and tissue remodeling.

This process of fibrosis leading to loss of tissue function is analogous to the pathogenesis of cirrhosis in chronic active hepatitis B and C infection, wherein ongoing viral replication in hepatocytes leads to a state of chronic inflammation and fibrosis. Over time, functional liver tissue is replaced by collagen, and the patient develops cirrhosis

with all of its sequelae. In HIV-1 infection, secondary LTs become gradually replaced by collagen, leading to a gradual inability to maintain a population of CD4^{+} T cells. Recognition of this process suggests new opportunities for anti–HIV-1 therapy. Presently, most therapeutic strategies focus on suppression of viral replication. However, it may be that adjunctive anti-inflammatory therapy would provide substantial benefit by limiting, and possibly reversing, the damage to the TZ. Furthermore, longitudinal studies that assess changes in the structure of the TZ after prolonged HIV-1 therapy would be required
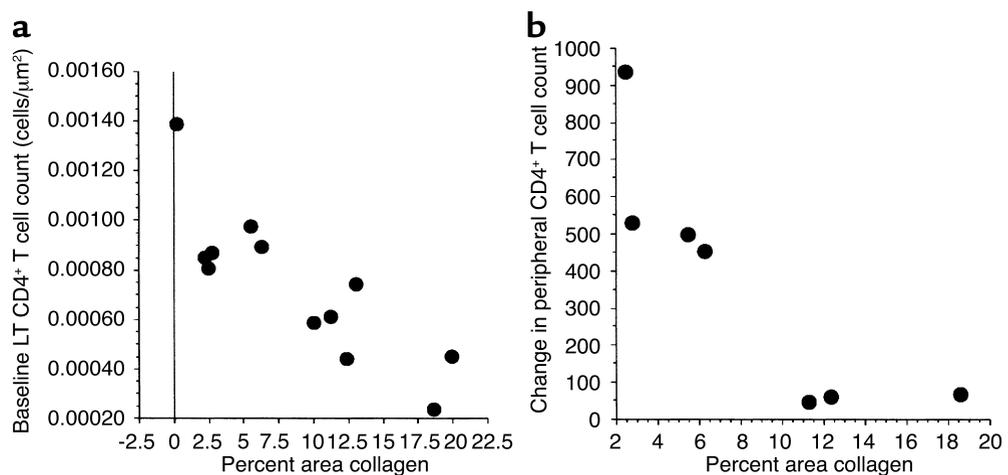
Figure 4
Relationship between the percentage of LT occupied by collagen and two parameters: baseline LT CD4^{+} T cell population and change in peripheral CD4^{+} T cell count after 6 months. (**a**) Size of the baseline LT CD4^{+} T cell population is compared with the percent area of the TZ occupied by collagen. There is a significant correlation between these values (R^2 = 0.72, P < 0.0001, Spearman rank correlation). (**b**) Change in the peripheral CD4^{+} T cell population after 6 months of HAART is compared with the percent area of the TZ occupied by collagen. There is significant correlation between these measurements (R^2 = 0.91, P < 0.001).

to determine whether the niche damage is reversible and what role anti-inflammatory therapy would have.

In summary, our data provide new insights into HIV-1 pathogenesis and offer a potential explanation for the failure of HAART to provide complete immune reconstitution in some individuals. The finding that a single measurement of collagen in LT before initiation of HAART is predictive of subsequent reestablishment of the CD4$^+$ T cell population suggests that a staging lymph node biopsy would provide useful information on when to initiate therapy. Recognition of the role of inflammation in the pathogenesis of CD4$^+$ T cell depletion suggests that studies of the impact of anti-inflammatory drugs on immune reconstitution are warranted.

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