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Lymphoid Fibrosis Occurs in Long-Term Nonprogressors and Persists With Antiretroviral Therapy but May Be Reversible With Curative Interventions

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Human immunodeficiency virus (HIV) replication causes lymphoid tissue (LT) fibrosis, which causes CD4⁺ T-cell depletion. It is unknown whether people who spontaneously control HIV replication have LT fibrosis. We measured LT fibrosis and CD4⁺ T cells in 25 HIV controllers, 10 noncontrollers, 45 HIV-positive individuals receiving therapy, and 10 HIV-negative individuals. Controllers had significant LT fibrosis and CD4⁺ T-cell depletion, similar to noncontrollers, but the so-called Berlin patient (in whom HIV infection was cured) had near normal LT. Thus, LT fibrosis occurs in all HIV-infected subjects, and current therapy does not reverse it. Reversal of fibrosis during a curative intervention suggests that ongoing low-level virus production may maintain LT fibrosis.

Keywords. HIV; fibrosis; HIV controllers.

The hallmark of human immunodeficiency virus (HIV) infection is progressive loss of CD4⁺ T cells in peripheral blood (PB) and lymphoid tissues (LTs) that is not fully restored with antiretroviral therapy (ART) [1–4]. HIV infection is also associated with chronic inflammation that is only partially normalized with ART [5, 6]. This chronic inflammatory state is maintained by several factors, including the direct effects of HIV replication [7], coinfections such as cytomegalovirus (and other herpes viruses) [8], and chronic damage to the gut mucosa that causes microbial translocation [9–11]. Acute and chronic inflammation associated with HIV infection leads to upregulation of transforming growth factor

β in the LT, where HIV replicates and stimulates local production of collagen [12–14]. Collagen deposition into LT dramatically alters the architecture and function of the LT and ultimately causes progressive loss of naive T cells. This happens because the fibroblastic reticular network that produces interleukin 7 (important for naive T-cell homing and survival) is replaced by collagen [13, 15–17]. Potent and sustained suppression of HIV replication with ART reduces immune activation, but the degree to which LT pathologies are reversed and tissue architecture is restored is unknown.

A subset of HIV-infected individuals called elite controllers have durable control of HIV in the absence of therapy, in that their plasma viral load is maintained at <50 copies/mL [18]. The response by HIV-specific mature CD8⁺ T cells is an important mechanism contributing to elite control [19–21]; however, virus replication and immune activation persist [18, 22, 23]. Clinical conditions attributable to immune activation and inflammation that are increasing in incidence among ART-treated persons (ie, coagulopathy and vascular disorders [24–26]) are also seen in elite controllers [27, 28]. We hypothesized that individuals exhibiting elite

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control of virus replication would have inflammatory damage to lymphoid structures but that the degree to which the tissue architecture was damaged would be less, given the lower burden of virus and decreased amount of immune activation, compared with persons without elite control. To test this hypothesis, we obtained a series of rectal tissues from elite controllers, HIV-infected patients requiring ART to control virus replication, HIV-infected persons who were not receiving ART, and people without HIV infection.

MATERIALS AND METHODS

Subjects

All subjects for this study were selected from SCOPE, which is a longitudinal cohort of HIV-infected and uninfected adults followed in San Francisco, California. Five unique groups were studied: (1) individuals with untreated HIV infection and HIV RNA levels of >2000 copies RNA/mL (hereafter, “noncontrollers”), (2) individuals with untreated HIV infection and low but detectable HIV RNA levels (ie, 50–2000 copies RNA/mL; hereafter, “viremic controllers”), (3) individuals with untreated HIV infection and undetectable HIV RNA levels (ie, <50 copies RNA/mL; hereafter, “elite controllers”), (4) individuals with long-term receipt of ART and undetectable viral loads (hereafter, “ART suppressed individuals”), and (5) HIV-uninfected individuals. The treated subset was categorized on the basis of their peripheral CD4⁺ T-cell count as either immunologic responders (CD4⁺ T-cell count, >500 cells/ μ L) or immunologic nonresponders (CD4⁺ T-cell count, <350 cells/ μ L). We also studied the so-called Berlin patient, who, 5 years earlier, received an allogeneic hematopoietic stem cell transplant from a donor homozygous for the CCR5- Δ 32 deletion. All subjects underwent a rectal biopsy using previously described methods [6].

The SCOPE cohort was approved by the University of California–San Francisco (UCSF) Committee on Human Research, and all subjects provided informed consent. Informed consent was obtained from subjects prior to enrollment, and human experimentation guidelines of UCSF and the University of Minnesota were followed in the conduct of clinical research.

Specimen Collection and Analysis

Rectal biopsy specimens were obtained, and specimens were divided into portions used for immunohistochemical staining and quantitative image analysis (four 3-mm biopsy specimens in each tissue block). Tissue sections were stained with Masson trichrome to identify collagen fibers, and CD4 antibody staining was used to identify CD4⁺ T cells. Quantitative image analysis was used to measure the amount of tissue fibrosis and size of the CD4⁺ T-cell population [1, 15, 16].

Statistical Analysis

Since the groups that we delineated above are not the result of randomization, we first tested for differences between our

groups for potentially important confounding variables. Not surprisingly, we found significant differences between groups in terms of viral load and PB CD4⁺ T-cell counts. We also detected differences in age and sex. We used analysis of variance for continuous variables and the Fisher exact test for categorical variables. To test for group differences in lymphoid aggregate (LA) collagen levels and LA CD4⁺ T-cell counts (both measured as a percentage of tissue area), we fit linear models with these variables as response variables, as well as age, sex, peripheral blood CD4⁺ T-cell count, HIV load, and indicators for the groups we delineated previously. We then refit these models without our group indicator variables and conducted an *F* test on the residuals from the model with and the model without the group indicator variables. We then conducted tests for differences between the HIV-positive groups and the uninfected subjects, using the test statistics from the linear model that had indicators for group differences and all identified potential confounders. To estimate the correlation between collagen and CD4⁺ T-cell levels, we used the Pearson correlation coefficient. To test for an association between these 2 variables, we used the usual test of no correlation, based on the *t* distribution. All calculations were done using the statistical computing language R, version 2.15.2.

RESULTS

Description of Cohort

Ninety subjects from the SCOPE cohort underwent rectal biopsies between November 2006 and May 2010. Eighty of these subjects were HIV-infected and 10 were uninfected. Among the HIV-infected subjects, 14 were elite controllers, 11 were viremic controllers (plasma viral load, <50–2000 copies/mL in the absence of therapy), 34 received ART and had poor recovery of the CD4⁺ T-cell count (ie, <350 cells/ μ L), and 11 had a good immunologic response to ART (PB CD4⁺ T-cell count, > 500 cells/ μ L). The demographic and clinical characteristics of the HIV-uninfected and HIV-infected groups are described in Table 1. All analyses that adjusted for clinical and demographic features were found to differ between the groups.

Elite Controllers and Individuals Who Received Long-Term Treatment Had High Levels of Collagen Deposition in Gut-Associated LT Aggregates

We used a modified trichrome stain to identify collagen fibers in rectal tissues and quantitative image analysis to determine the proportion of tissue containing collagen. After comparing HIV-uninfected individuals to the 3 HIV-infected groups in which treatment was not received (elite controllers, viremic controllers, and noncontrollers), we found that collagen deposition was increased in all HIV-infected groups, including elite controllers. Figure 1A contains a representative section of LAs from each group and shows increased levels of collagen

Table 1. Characteristics of the Study Population, by Study Group

Characteristic ^a	HIV-Uninfected (n = 10)	Elite Controller (n = 14)	Viremic Controller (n = 11)	Untreated Viremic (n = 10)	Long-Term Therapy (n = 45)
Age, y	46.1	46.1	49.8	41.7	49.9
Male sex	9 (90)	11 (79)	10 (91)	7 (70)	44 (98)
CD4 ⁺ T-cell count, cells/ μ L	913	828	512	490	362
Plasma VL, copies/mL	N/A	<75	1089	33842	<75
Plasma VL < 75 copies/mL	N/A	14 (100)	0 (0)	0 (0)	44 (98)
Time of ART-associated virologic suppression, y	N/A	0	0	0	7 ^b

Data are no. (%) of subjects or mean value.

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; VL, viral load.

^a Differences in age, sex, and CD4⁺ T-cell count were found among the 5 groups. VL and therapy differed on the basis of group assignment. Analyses controlled for these differences in characteristics.

^b Longitudinal VL data were available in 34 of 45 subjects. The mean time from VL undetectability to biopsy was 7 years.

deposition in the T-cell zone of all groups of HIV-infected subjects. Compared with HIV-uninfected controls, all HIV-infected groups had abnormally high levels of LA collagen, including elite controllers ($P = .011$; Figure 1B). There was no detectable difference in the amount of collagen between the elite controllers and the other untreated HIV-infected groups.

High Levels of Collagen Deposition Persist in Patients With Long-Term Suppression of Viremia Due to ART

In earlier studies of patients who initiated ART and were followed for 6 months, we found no change in the amount of LT fibrosis [1] and that some lymph node pathologies continued to persist for up to 2 years of ART [4]. However, we have not systematically looked at a cohort of patients treated with ART for an extended period. In rectal tissue specimens from patients who achieved virologic suppression during ART for an average of 7 years, we found no difference in collagen levels between the untreated viremic subjects and those receiving long-term therapy (mean percentage of the LA area occupied by collagen, 16.5% and 12.6%, respectively). Even among the group with good CD4⁺ T-cell recovery (ie, a CD4⁺ T-cell count of > 500 cells/ μ L) and virologic suppression during ART for a mean of 9 years, the amount of LA fibrosis was high.

Elite Controllers Have Depleted CD4⁺ T-Cell Populations in LAs

We used immunohistochemical methods to identify CD4⁺ T cells in LA and quantitative image analysis to determine the size of the CD4⁺ T-cell population (Figure 2A). LA CD4⁺ T-cell populations were significantly depleted in the HIV-infected groups, compared with the uninfected group ($P < .002$ for comparisons of the HIV-uninfected group to either elite controllers, viremic controllers, noncontrollers, or ART-suppressed groups).

Collagen Deposition in Rectal LAs Is Negatively Correlated With CD4⁺ T-Cell Population Sizes in the Same Tissue

We have previously shown that the size of the CD4⁺ T-cell population in the T-cell zone of lymph nodes negatively correlates with the amount of collagen in the same tissue [16]. We compared the size of the CD4⁺ T-cell population to collagen in the LAs of the gut-associated LT in all subjects and found a similar correlation ($r = -0.46$; $P < .0001$; Figure 3).

Reversal of Collagen Deposition and Restoration of CD4⁺ T-Cell Population Size in LAs of the Berlin Patient

We studied rectal tissues obtained from the Berlin patient, in whom HIV infection was cured after 2 bone marrow transplantations using donor marrow from an individual with the CCR5- Δ 32 mutation. The major difference between the Berlin patient and elite controllers was the absence of replicating virus and HIV-specific immune responses [29]. In the Berlin patient, we found that levels of rectal LA collagen and the size of the rectal LA CD4⁺ T-cell population were nearly identical to the median levels observed in the HIV-uninfected individuals (Table 2). We noted that a few of the HIV-infected subjects had LA collagen levels that were the same as or lower than those for the Berlin patient, highlighting the heterogeneity of the process. However, in the Berlin patient, tissues had near normal CD4⁺ T-cell populations and tissue morphological characteristics more consistent with immunologically normal individuals.

DISCUSSION

The important findings of this study include that rectal LAs in elite controllers also contained significant levels of tissue fibrosis that did not reverse, even with prolonged suppression of virus replication in the plasma. However, in the one patient cured of HIV infection, we found that collagen and CD4⁺ T-cell levels

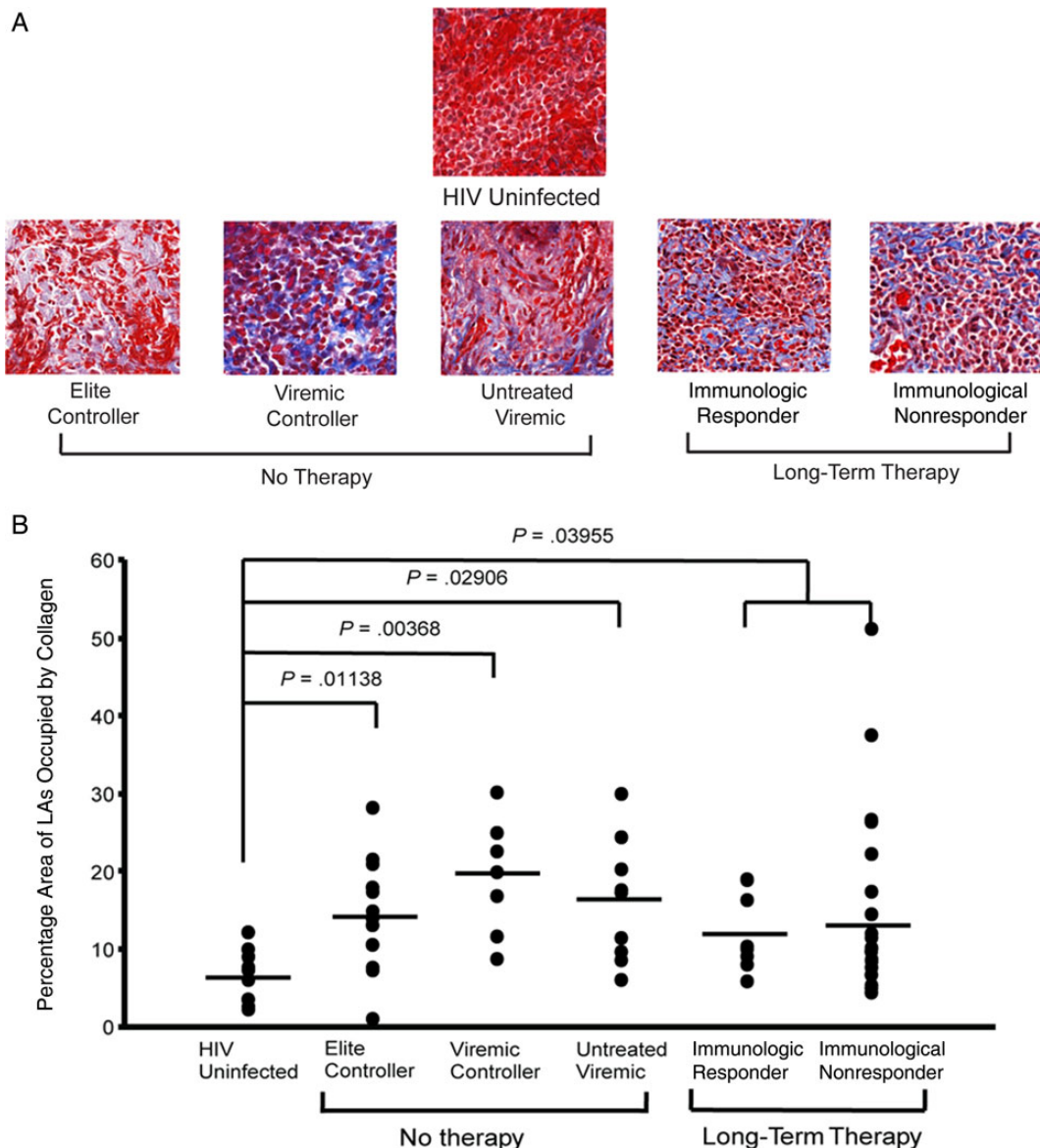


Figure 1. A, Collagen deposition in lymphoid aggregates (LAs) of the rectum. Representative sections from the LAs of human immunodeficiency virus (HIV)-uninfected individuals, elite controllers, viremic controllers, untreated viremic individuals, immunologic responders ($CD4^+$ T-cell count, >500 cells/ μ L), and immunologic nonresponders ($CD4^+$ T-cell count, >350 cells/ μ L) were subjected to trichrome blue staining to identify collagen fibers. These images show the higher levels of collagen in the infected groups, compared with the uninfected subjects. B, Quantitative comparison of collagen deposition in rectal LAs in HIV-uninfected individuals, elite controllers, viremic controllers, untreated viremic individuals, immunologic responders, and immunologic nonresponders. There were significantly higher levels of collagen deposition in all groups of HIV-infected subjects, including elite controllers, compared with HIV-uninfected individuals. The mean percentage area of LA occupied by collagen in the study groups was 7.0%, 14.5%, 19.2%, 16.5%, 11.8%, and 12.9%, respectively. No differences were detected between any of the infected groups, including between untreated viremic subjects and individuals receiving long-term therapy.

were similar to those of HIV-negative individuals. These data suggest that even small amounts of virus replication contribute to lymphoid fibrosis, which may prevent complete restoration of immune function. The fact that the Berlin patient had apparently normal levels of lymphoid fibrosis may suggest that a complete sterilizing cure may be required to fully reverse the immunopathology of HIV infection, although further studies need to be done to determine whether some part of the

conditioning regimen (ie, total body irradiation or chemotherapy) might have contributed to reversal of tissue fibrosis in this individual and/or whether other models of so-called functional cure (as in the VISCONTI cohort) are also capable of reversing lymphoid fibrosis [30]. Despite these caveats, the fact that elite controllers have levels of fibrosis that are similar to those in people without spontaneous control suggests the inflammatory reaction to even very low levels of virus replication is an important

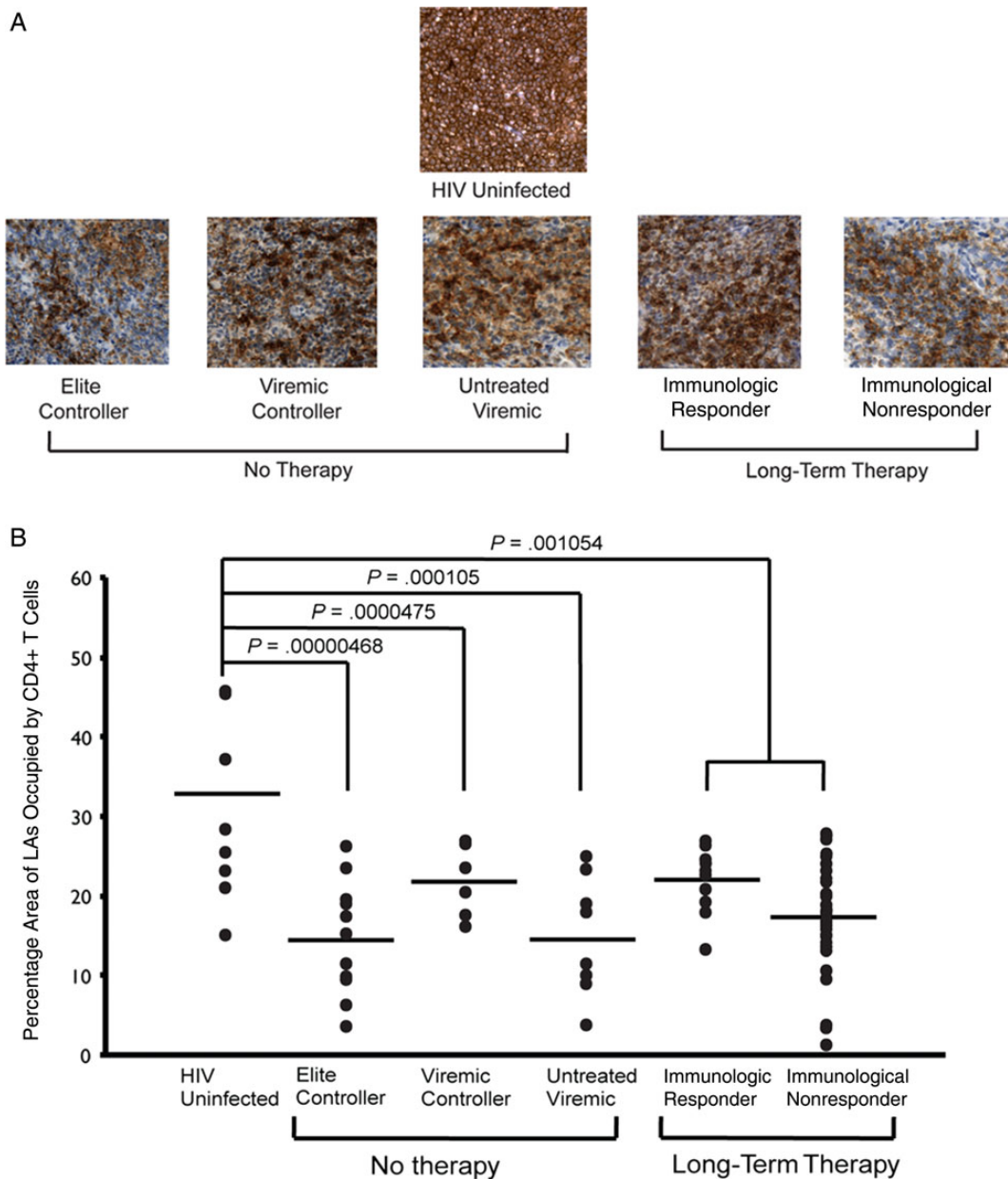


Figure 2. A, CD4⁺ T cells in rectal lymphoid aggregates (LAs). Representative sections from the LAs of human immunodeficiency virus (HIV)-uninfected individuals, elite controllers, viremic controllers, untreated viremic individuals, immunologic responders (CD4⁺ T-cell count, >500 cells/ μ L), and immunologic nonresponders (CD4⁺ T-cell count, >350 cells/ μ L) were stained with antibodies against CD4, shown in brown. Images were subjected to quantitative image analysis. B, Quantitative comparison of the size of the CD4⁺ T-cell population in rectal LAs in HIV-uninfected individuals, elite controllers, viremic controllers, untreated viremic individuals, immunologic responders, and immunologic nonresponders. There were significantly smaller CD4⁺ T-cell population sizes in HIV-infected groups, including elite controllers, compared with HIV-uninfected subjects. The mean percentage area of LAs occupied by CD4⁺ T cells in the study groups were 31.9%, 14.7%, 21.9%, 14.4%, 21.9%, and 17.2%, respectively. No differences were detected between any of the infected groups, including between untreated viremic subjects and those receiving long-term therapy.

part of the mechanism that creates and sustains fibrotic changes in LT.

Definitive conclusions about the contribution of immune activation to sustaining tissue fibrosis are limited in this study by the fact that we studied only rectal tissues using immunohistochemical methods. Lymph node and terminal ileum specimens

were not available. In addition, the number of snips available to study was variable, with an increased potential for sampling error, given the very small tissue size. Another limitation is that we did not have plasma or other markers of inflammation available for this analysis, although they would have provided limited insight, as they offer a snapshot of the inflammatory

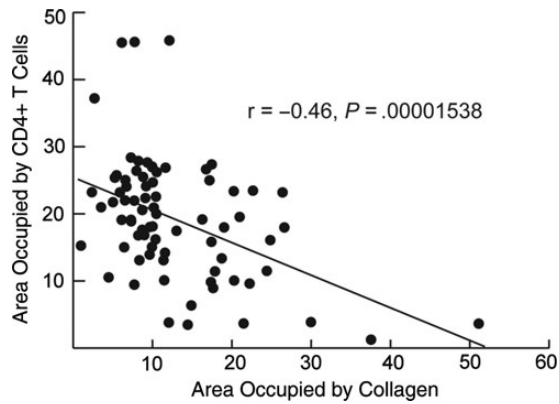


Figure 3. Relationship between the area occupied by collagen and the area occupied by CD4⁺ T cells in rectal lymphoid aggregates, including all groups of the SCOPE cohort. There was a significant and inverse relationship between the area occupied by CD4⁺ T cells and the area occupied by collagen ($r = -0.46$, $P = .00001538$).

state at a specific moment. Tissue fibrosis is a cumulative process that occurs over a relatively prolonged period. However, data from the group of HIV-infected individuals with detectable viremia who were not receiving therapy were very similar to data from previous studies we have conducted [1]. We also found the expected inverse and significant correlation between LT collagen level and CD4⁺ T cell count [16]. Thus, we have confidence that the data support our conclusions.

The finding of surprisingly advanced LT fibrosis in patients who exhibit spontaneous control of virus replication suggests that elite control is not a sufficient model for functional cure. Even under these clinical conditions (ie, elite control or virus suppression with ART), there is persistent fibrotic damage. These data are consistent with several recent studies suggesting that, despite viral control, elite controllers have abnormal systemic T-cell activation, microbial translocation [18], innate immune activation [31], and atherosclerosis [27] and that ART further reduces immune activation in elite controllers [22].

Table 2. Comparison of Collagen Levels and CD4⁺ T-Cell Population Size Among Human Immunodeficiency Virus (HIV)-Uninfected Patients, Elite Controllers, and the Berlin Patient

Variable	HIV-Uninfected Patients	Berlin Patient	Elite Controllers
Lymphoid aggregate collagen, percentage area	7.0	6.8	14.5
Lymphoid aggregate CD4 ⁺ T cells, percentage area	28.7	35.5	11.6

The areas occupied by collagen and CD4⁺ T-cell population sizes in the Berlin patient appeared to be very similar to those in HIV-uninfected subjects and unlike those in elite controllers.

On the other hand, it is important to acknowledge that ART-mediated suppression of viral replication clearly improves health, decreasing not just clinical progression to AIDS, but also non-AIDS events, including cardiovascular disease [32], even though it fails to reverse tissue fibrosis. Thus, lymphoid fibrosis cannot be the only immunologic defect driving AIDS progression and non-AIDS events in HIV infection. Systemic immune activation, which declined during suppressive ART, may also contribute to morbidity and mortality, independent of its effect on lymphoid fibrosis. Nevertheless, persistent lymphoid fibrosis likely affects complete restoration of immune function in HIV-infected individuals, and reversal of lymphoid fibrosis should probably be a goal for functional-cure strategies [2]. For example, we found that the amount of collagen in lymph nodes at the initiation of ART predicts PB CD4⁺ T-cell counts at 6 and 36 months after starting ART [2] and that collagen deposition is responsible for loss of the fibroblastic reticular cell network that leads to depletion of naive T cells [15]. We think it is likely that these persistent tissue abnormalities help explain, at least in part, the increased incidence of infections, compared with the incidence in HIV-negative individuals [33–35]; an increasing diagnosis of non-AIDS-associated malignancies [36–38]; persistently abnormal responses to vaccines [39–43]; and continued observation of early mortality [44–48]. The fact that lymphoid fibrosis appeared to be reversed in the Berlin patient may suggest that only sterilizing cure may be effective in fully reversing these abnormalities, although future studies should assess whether other models of functional cure without systemic immune activation (as reported in the VISCONTI cohort) also have preserved LT architecture.

We believe that full immune reconstitution is unlikely to occur unless these tissue pathologies are reversed and LT function restored. Some data suggests that this process can be inhibited or even reversed with antifibrotic therapy initiated with ART during acute HIV infection [49, 50], but our study suggests that even very small levels of HIV persistence in lymph nodes may be enough to continue to drive lymphoid fibrosis. Fully reversing lymphoid fibrosis will almost certainly require ART regimens that fully suppress virus replication in LT (and possibly other tissue sanctuaries that have yet to be studied) and may even require near-complete elimination of viral reservoirs. Until a sterilizing cure is possible for the majority of chronically infected patients, adjunctive therapies to limit or reverse tissue fibrosis should be studied to determine the extent to which these pathologies can be reversed.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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