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Reversibility of the pathological changes in the follicular dendritic cell network with treatment of HIV-1 infection

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ABSTRACT **Over the course of HIV-1 infection, the lymphoid follicles where the humoral immune response is generated initially increase in size and number and then progressively involute. In advanced disease, the network of the processes of follicular dendritic cells (FDCs) that serve as antigen repositories and anatomical substrate for B and T cells and antigen to interact is destroyed, contributing to the breakdown of the immune system. Because destruction of FDCs is associated with deposition of HIV-1, and much of the virus can be cleared from the network with antiretroviral therapy, we investigated the reversibility of damage. We measured the immunohistochemically stainable FDC compartment by quantitative image analysis, and we documented changes in this compartment at different stages of disease. We show that treatment, initiated even at advanced stages of HIV-1 disease, can slowly reverse pathological changes in the FDC network.**

Lymphoid follicles are the site where B cell clones with high affinity for specific antigens are selected to generate the humoral immune response. Follicular dendritic cells (FDCs) that trap and retain antigens play a critical role both in the generation of this humoral immune response and in B cell memory by creating a three-dimensional network where B and T cells and antigen can interact (1, 2). Thus, it is not surprising that the humoral immune response deteriorates in HIV-1 infection, concomitant with the destruction of the FDC network. Degeneration of the FDC network in HIV-1 infection is already evident in the presymptomatic stage of HIV-1 infection, and it is progressive, so that in late AIDS the network is often almost completely destroyed. These pathological changes are accompanied by a loss of specific immune response to HIV-1 and other pathogens (3). Although the underlying mechanisms of this pathological process are not completely understood, destruction occurs in association with the high antigenic load of HIV-1 virions in immune complexes on the surfaces of FDCs (4, 5). Because combination antiretroviral treatment can reduce the viral burden in the FDC compartment to undetectable levels (6), we were curious to see whether this reduction, when sustained, would be accompanied by a reversal in pathological changes in the lymphoid tissue (LT). In this report we quantitate the reduction in the FDC network during the course of HIV-1 infection and show that this pathological process can be reversed with potent antiretroviral treatment.

MATERIALS AND METHODS

LT Biopsies. We obtained five biopsies of palatine tonsil and one biopsy of a lymph node from normal volunteers. At the time the biopsies were obtained the volunteers did not have oral pathological conditions that would have increased antigenic stimulation over the usual level of exposure. HIV-1-infected tonsil and lymph nodes were obtained from participants in two trials of highly active antiretroviral therapy with two reverse transcriptase inhibitors and one protease inhibitor (6–9). The biopsies were fixed in a non-aldehyde-based tissue fixative (Molecular biology fixative, Streck Laboratories, Omaha, NE) for at least 24 hr and then processed and embedded in paraffin.

Immunohistochemical Staining of the FDC Network in LT. At least 20 sections of 5 μm were cut at different levels from the tissue blocks, attached to silane-treated slides, and then deparaffinized, rehydrated, immersed in 10 mM sodium citrate buffer (pH 6.0) and microwaved for 5 min. Nonspecific reactions were blocked by immersion in 0.3% H_2O_2 in methanol and then 5% nonfat milk. The sections were allowed to react overnight at 4°C with a 1:100 dilution of anti-human dendritic reticulum cell monoclonal antibody (mAb) (Dako) that recognizes CD35, the C3b/complement receptor. Although other cell types such as B cells express CD35, the expression is so much higher on FDCs that under the conditions described in this report only the FDC network was detectably stained. Sections were subsequently washed in PBS, incubated at room temperature with biotinylated anti-mouse IgG (Vector Laboratories), allowed to react with avidin–biotin complex (Vector Laboratories), and stained with H_2O_2 and diaminobenzidine or Vector Red Substrates (Vector Laboratories).

Quantitative Image Analysis (QIA) of FDC Network and Viral Load in LTs. We used QIA to assess the extent of injury in HIV-1 infection and recovery after highly active antiretroviral therapy. After the FDCs and their processes had been stained immunohistochemically, video images were captured with a charge-coupled device (CCD) camera and analyzed with the METAMORPH computer software program (Universal

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Abbreviations: FDCs, follicular dendritic cells; QIA, quantitative image analysis; LT, lymphoid tissue.
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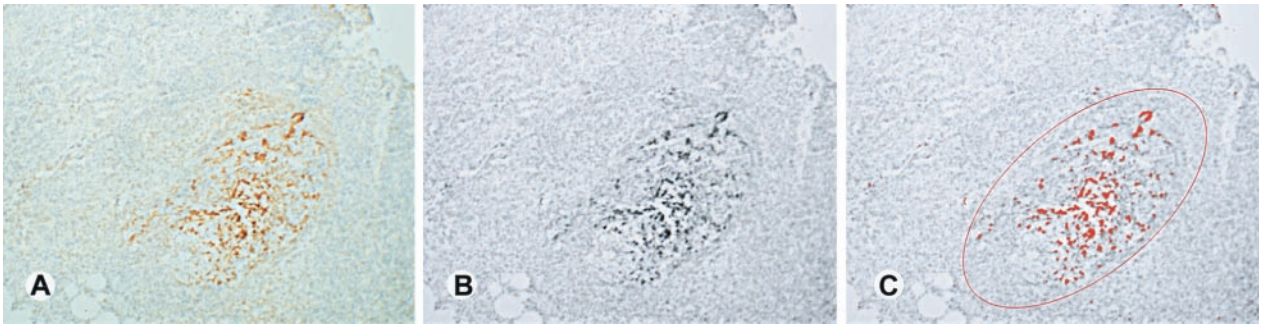

FIG. 1. QIA of the FDC network in LT. (*A* and *B*) Color and black and white video images of the FDC network in a tissue section stained immunohistochemically with antibody to CD35 (Dako) and diaminobenzidine substrate (Vector Laboratories). (*C*) The stained area of the FDC compartment in *B* is discriminated from background with the METAMORPH threshold tool and marked with a red overlay. The area of the FDC network in the field is computed automatically from the measured area of the area highlighted in red.

Imaging, West Chester, PA). This program utilizes a threshold tool to set a gray level that discriminates the stained cells and their processes from background and marks the network with a red overlay. Fig. 1 *A* and *B* illustrate in color and in black and white images of the FDC network in one follicle. Fig. 1*C* illustrates the red overlay of the thresholded image that will be measured within the region indicated by the red trace. The program measures the area of the signal above the threshold (in pixels). We designated this area divided by the total lymphoid area of the section as the FDC fraction. This fraction is a normalized global measure of the size of the FDC compartment that provides a basis for comparison of LT samples that vary in size of the sample and the number and size of primary and secondary follicles in that sample. The FDC-associated viral load was also determined by QIA of radioautographs of sections that had been hybridized to an HIV-1 specific set of riboprobes (10). Viral RNA in virions overlying FDCs generates a diffuse hybridization signal of silver grains, and the number of silver grains is proportional to the number of copies of viral RNA. Hence enumeration of these silver grains by QIA yields an estimate of viral burden in the FDC compartment.

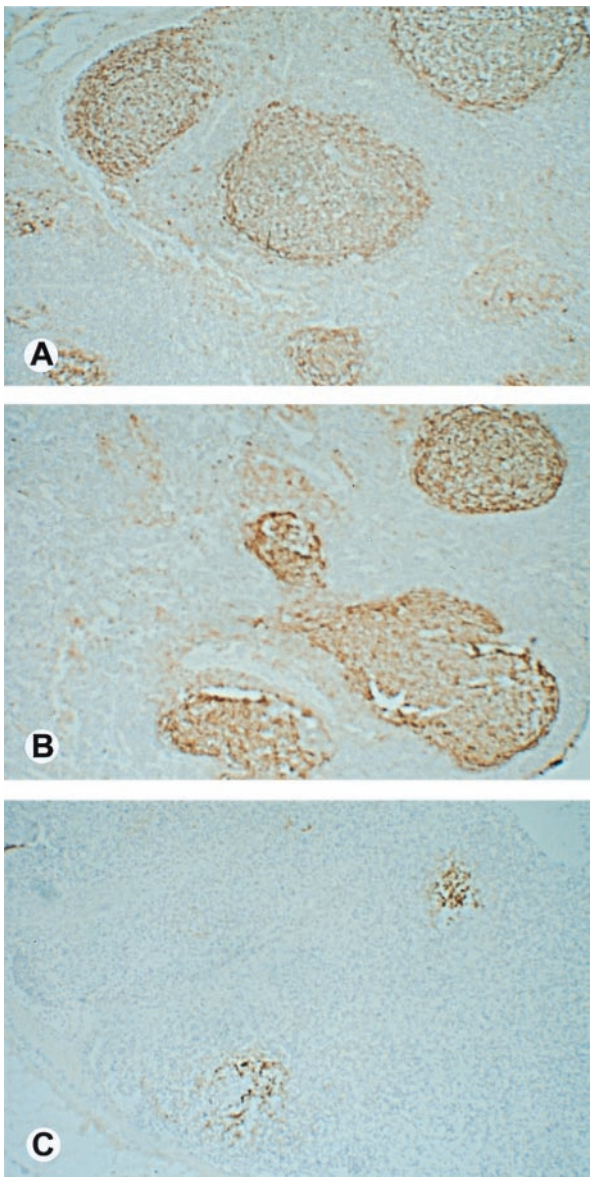

FIG. 2. Representative fields of immunohistochemically stained FDC networks in LT of a normal volunteer (*A*) and at early (*B*) and late (*C*) stages of HIV-1 infection.

RESULTS AND DISCUSSION

Quantitative Analysis of FDC Network in HIV-1 Infection. To investigate the damage to the FDC networks in prolonged

Table 1. FDC fraction and other characteristics

Status	No. of individuals	Mean blood CD4 counts per mm^3	Mean FDC viral load, no. of copies of HIV RNA per g of LT	Mean FDC fraction, %
HIV-1-negative	6	912 ± 275	—	2.4 ± 0.84
Early infection	10	391 ± 116	4.9×10^7	2.7 ± 1.1
Late infection	12	109 ± 88	1.7×10^8	0.42 ± 0.41
Study A				
Baseline	8	255 ± 170	1.6×10^8	0.83 ± 0.62
Rx 6 months	8	414 ± 197	1.2×10^5	1.5 ± 1.2
Rx 12 months	5	599 ± 340	$\leq 10^4$	2.1 ± 0.4
Study B				
Rx 12 months	4	357 ± 101	$\leq 10^4$	0.82 ± 0.68
Rx 30 months	4	422 ± 44	$\leq 10^4$	2.3 ± 0.45

FDC viral load and fraction were measured by QIA in LT obtained in cross-sectional and two longitudinal studies. The HIV-negative individuals are described in ref. 11. Participants in two studies of potent antiretroviral treatment (Rx) of HIV-1 infection are described in refs. 6, and 8. Early infection subjects included 3 individuals with the acute retroviral syndrome and 7 individuals from 29 days to 330 days after seroconversion. Late infection subjects included 12 HIV-1-infected individuals with absolute peripheral blood CD4 cell counts ranging from 10 to 220 per mm^3. Detailed information for studies A and B is given in the text.

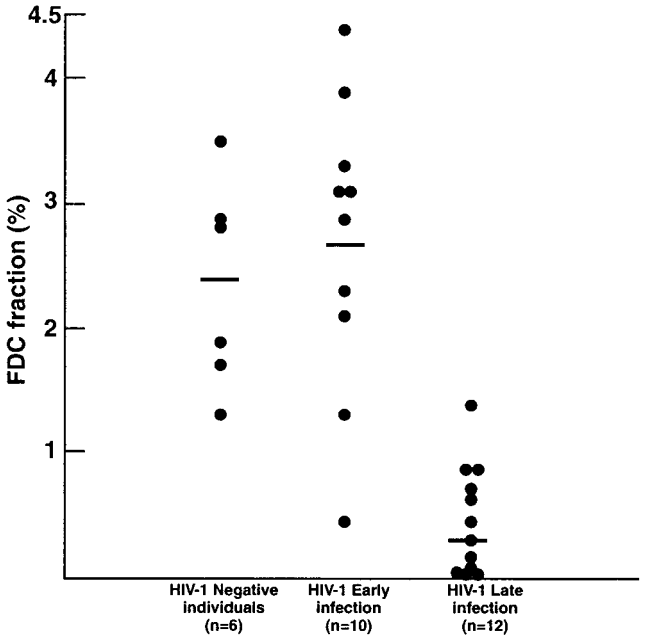

FIG. 3. Individual FDC fractions and means (—) in LT in early and late stages of HIV-1 infection and HIV-1-negative controls. These cohorts and definitions of stage of infection are described in the text.

HIV-1 infection, we measured the FDC fraction in biopsies of tonsil and lymph nodes obtained from (*i*) 6 normal volunteers; (*ii*) 10 HIV-1-infected individuals in the early stages of HIV-1 infection; and (*iii*) 12 individuals at late stages of infection. In tonsils from the normal volunteers there are mainly secondary follicles with some hyperplasia, presumably a reflection of continuous antigenic stimulation (Fig. 2*A*). In the early and presymptomatic stages of HIV-1 infection most of the follicles are hyperplastic and irregularly shaped (Fig. 2*B*) and the FDC fraction is increased over the HIV-1-uninfected controls. However, the increases in the fraction of the FDC network compared with uninfected controls were not statistically significant (Table 1, Figs. 2 and 3; $P > 0.5$, Student's t test). By the late stages of HIV-1 infection the follicles have involuted and the network is disrupted as a result of degenerative changes in the FDCs and their processes (Fig. 2*C*). In this case the reduction in the FDC network compared with uninfected individuals is highly statistically significant (Table 1, Figs. 2 and 3; $P < 0.001$).

Regeneration of the FDC Network After Treatment. We sought evidence for regeneration of damaged FDC networks in the late stages of HIV-1 infection by determining the FDC fraction before and after highly active antiretroviral therapy. We compared two groups of patients in independent studies of the impact of combination antiretroviral therapy in LT instituted at later stages of disease. In one study (designated study A), we had obtained tonsillar biopsies before treatment and 2, 22, and 168 days after treatment from eight individuals, and after 12 months from 5 of these patients. In another study (designated study B) lymph-node biopsies were obtained after 1 year of treatment and again after 2.5 years of treatment from four individuals. In both cases treatment reduced and maintained the pool of HIV-1 RNA in the FDC compartment at levels below the limits of detection of 104 copies of HIV-1 RNA per gram of LT (Table 1). HIV-1, however, had not been eliminated even after 2.5 years of treatment, as chronically and latently infected cells could still be detected (6, 8, 9).

After 6 months of treatment, the FDC fraction in tonsillar biopsies in study A had increased, with considerable variation among individuals indicated by the wide range and standard variation (Table 1, Fig. 4*A*). The increase was not statistically significant ($P > 0.3$). After 12 months of treatment, however, with further increases in the FDC, the fraction was equivalent to the HIV-1-uninfected controls and the increases were statistically significant ($P < 0.01$).

In the independent study B, the FDC fraction after 12 months of treatment again varied widely among individuals and was not significantly higher than untreated subjects' values in late infection. After 2.5 years of treatment, however, the FDC fraction was also equivalent to the HIV-1-uninfected controls and the increases were statistically significant (Table 1 and Fig. 4*B*; $P < 0.001$). In both studies, the most dramatic increases in the FDC fraction with treatment were in infected individuals with the lowest blood $CD4^+$ T cell counts and FDC fraction at baseline (Fig. 5 *A* and *B*).

By these quantitative measures the FDC network is nearly destroyed after prolonged HIV-1 infection, but this destruction is reversible. Although the kinetics of regeneration varied among individuals, the FDC network could be fully anatomically reconstituted in some HIV-1-infected individuals with sustained reduction in the FDC viral load. The mechanism and expected extent of this regenerative process are currently not clear. FDCs are probably derived from stromal cell or bone

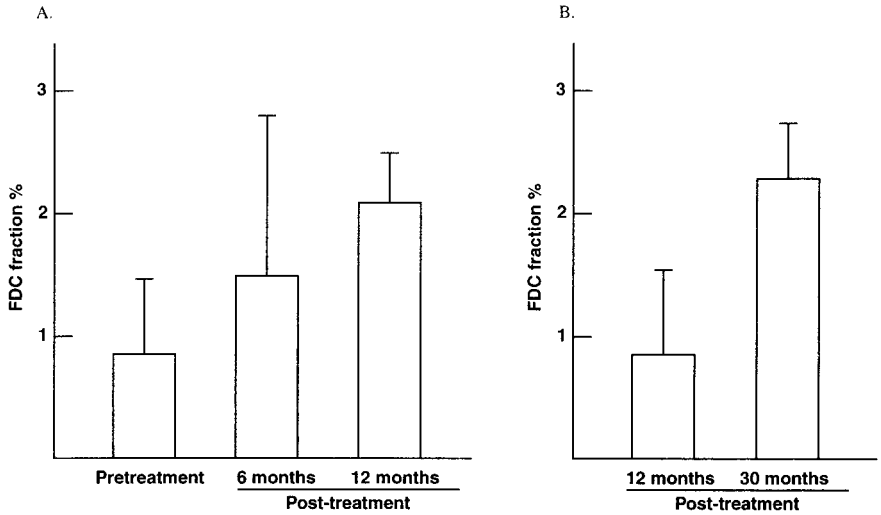

FIG. 4. Treatment-induced changes in the fraction of the FDC network in study A (*A*) and study B (*B*). These cohorts are described in the text and the corresponding changes in FDC-associated viral load are in Table 1.

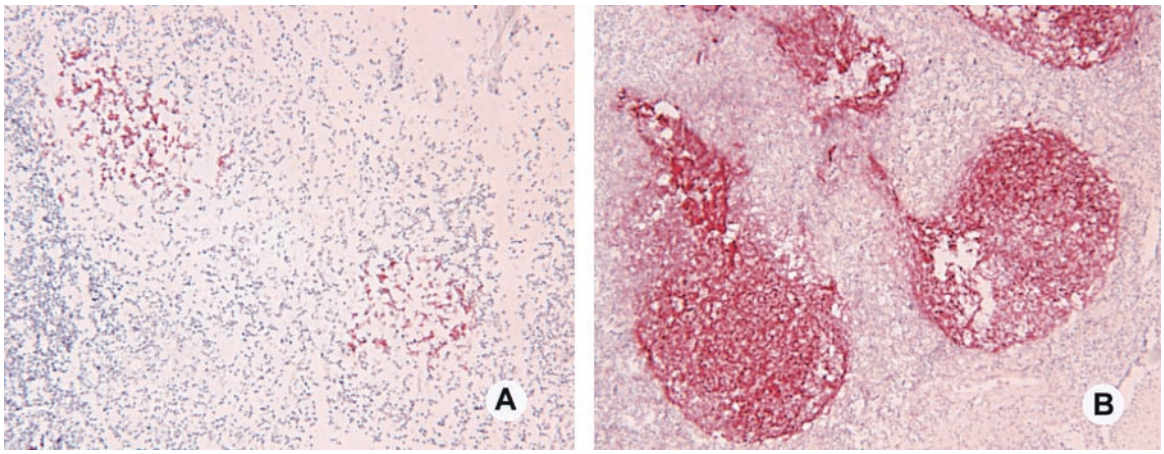

FIG. 5. An example of dramatic reconstitution of the FDC network after triple therapy. Serial tonsil biopsies from an HIV-1-infected patient before treatment (*A*) and 6 months after (*B*) treatment. Five-millimeter tissue sections were stained with CD35 mAb (Dako) and Vector Red substrate (Vector Laboratories). The absolute peripheral CD4 cell counts of this patient were 40 and 170 per mm^3 before treatment and 6 months after treatment.

marrow precursors and require B and T cells for maturation (11, 12). The previously documented slow repopulation of LT with $CD4^+$ T cells (13) and the regeneration of the FDC network in this report provide evidence that the necessary elements for reconstitution survive into the late stages of HIV-1 infection. The enhanced immunological activity of sera and recovery of B cell function from HIV-1-infected individuals after potent antiretroviral therapy suggests that the anatomical restoration of the LT microenvironment may also be associated with functional recovery.¶¶ It is encouraging that the immune system may benefit from control of viral replication and the reduction of viral load in the FDC compartment without complete eradication of virus or infected cells.

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