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LYMPHATIC TISSUE CHANGES IN AIDS AND OTHER RETROVIRUS INFECTIONS: TOOLS AND INSIGHTS

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ABSTRACT

Based on immunohistochemistry, in situ *hybridization, and electron microscopy, lymphatic tissue changes in human immunodeficiency virus (HIV) and other retroviral infections represent different stages of a dynamic process progressing from hyperplasia to atrophy. The germinal centers (GC) function early as a virus reservoir in both HIV and feline leukemia virus infection, which also produces lymphadenopathy, severe immune impairment, and death from opportunistic infections. Core proteins of HIV can be detected on the surface of follicular dendritic cells, electron microscopy reveals cell-free HIV and virus replication in the same location, and* in situ *hybridization shows that the majority of cells with mRNA of HIV can be found in germinal centers (GC). Double immunohistochemical labeling of lymphocyte populations suggests that one of the most important events in HIV lymphadenitis with explosive follicular hyperplasia is the accumulation of CD8+CD45R0+ lymphocytes in the lymph nodes. Their clustering in the vicinity of the FDC network could play a key role in disintegration of GC and lymphocyte depletion as the disease progresses.*

Immunohistochemistry, *in situ* hybridization and electron microscopy have provided tools for examining lymphatic tissue changes in HIV and other retroviral infections. The morphological patterns of these changes represent different stages of a dynamic process progressing from hyperplasia to atrophy (for review see 1). Over the past years several studies have shown that the various patterns seen in the lymph nodes (LN) reflect progressive stages in the deterioration of immunological functions of the patients (for reviews see 1,2).

Light (for reviews see 1,2) and electron microscopical (3-6) examinations have revealed that during HIV-1 infection, characteristic changes occur in the B and T cell dependent areas of lymph nodes (LNs) where important cellular interactions take place. Follicles represent a complex immunologic environment composed of B cells, macrophages, follicular dendritic cells (FDC), and T lymphocytes. Evidence that germinal centers (GC) in LNs from patients with generalized lymphadenopathy contain HIV particles was first obtained by ultrastructural analysis (3,4). In describing the presence of free virus particles in GCs, Armstrong and Horne (3) also observed budding

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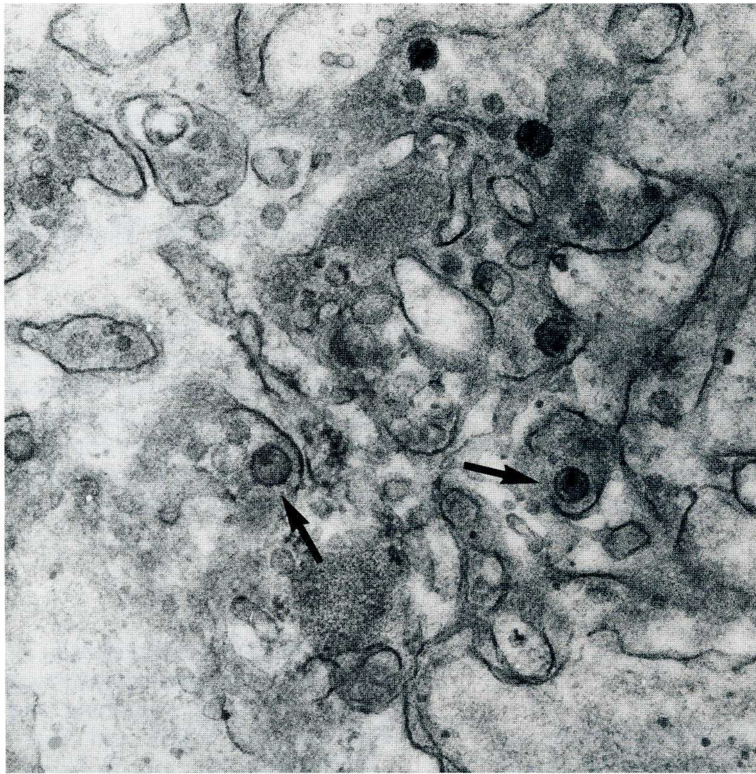

Fig. 1. HIV-1 induced lymphadenopathy. The electron micrograph shows the characteristic localization of virus particles (arrows) in the interdendritic extracellular spaces of a germinal center. Original magnification x30,000.

profiles from FDCs and suggested that viral nonlymphoid cell tropism may be a significant factor in the pathogenesis of the disease. HIV was also found to replicate in lymphocytes and macrophages inside the GCs (5). This was consistent with the results of *in situ* hybridization showing that the majority of cells with mRNA of HIV-1 can be found in GCs. Immunohistochemical examinations demonstrated that *gag* proteins of HIV can be detected on the surface of FDC (7).

The FDCs in organized lymphoid tissue are known to capture, retain and present antigens to lymphocytes (for review see 8). Apparently, HIV-1 and also some other retroviruses such as the feline leukemia virus (6), Abelson and Rauscher murine leukemia viruses (9,10) use this physiologic function of FDC to their advantage. All these virions can be trapped by FDCs. In feline leukemia virus (FeLV) infection a lymphadenopathy develops that shares many similarities with that seen in HIV-1 infection. It starts with follicular hyperplasia. Later the follicles disintegrate and the process ends with a lymphocyte depleted node. Using immunofluorescence methods, Rojko et al (11) have demonstrated that group specific antigens of FeLV are inside the GC. By electron microscopical investigations, we detected many cell free FeLV. The virions accumulate along the dendrites *(Fig. 1)*, whereas regions of GC without processes of FDC are free of virus particles. Similar to HIV-1 infection, virus replication occurs inside the GC (6). These observations underline the importance of GC in HIV infection and also some other retrovirus diseases.

In HIV-1 infection the virus gains

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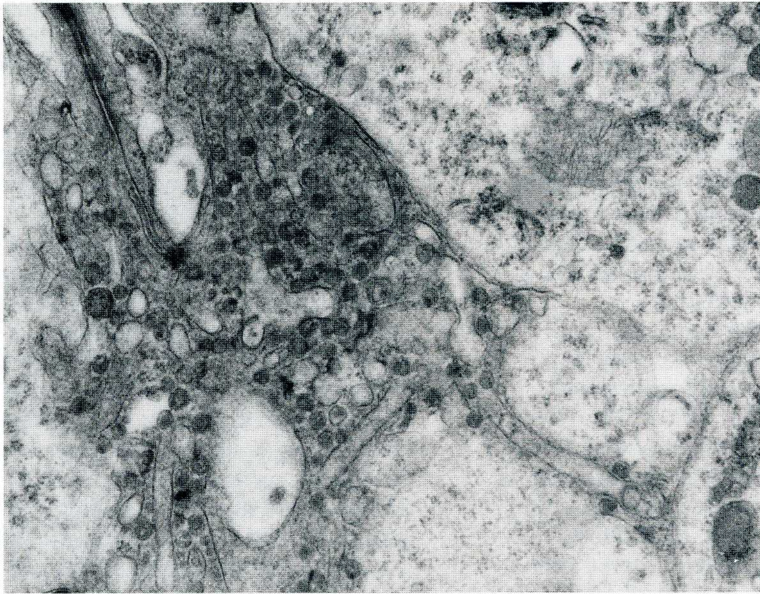

Fig. 2. Accumulation of virus particles around the dendrites of follicular dendritic cells (FDC) in an experimental infection of cats by feline leukemia virus (FeLV). Original magnification x20,000.

access to the GC in the early phase of the disease. Investigations on lymph nodes taken shortly after seroconversion demonstrate a discrete deposition of *gag* proteins (12) and the presence of a few virus particles (5). As the disease progresses, virus accumulation and continuous disintegration of GCs occur *(Fig. 2)* Fragmentation of follicles by CD8 lymphocytes, known as "folliculolysis," develops (13). At the same time, FDCs undergo degenerative changes manifested in partial or total destruction of FDC network. The cytopathic effect of HIV-1, or cytotoxic cells responsible for the loss of FDC.

Single marker immunohistochemical evaluation of LNs has shown that during the course of HIV infection in the GCs as well as the extrafollicular parenchyma there is an increase in the number of CD8 cells and a continuous drop of CD4+ lymphocyte counts (1). It has been proposed that the CD8 cell infiltration plays an important role in the loss of B follicles.

During recent years, double and triple marker immunophenotypic studies clearly demonstrate that phenotypic heterogeneity exists within both CD4 and CD8 lymphocyte populations. A number of monoclonal antibodies (MAB), including CD45RA (anti-2H4), CD45R0 (anti-UCHL1) and CD29 (anti-4B4), have been defined which subdivide the CD4 and CD8 lymphocytes into non-overlapping subpopulations (14-17). The heterogeneity detected by these subset markers are probably more closely related to the maturation state of the lymphocytes than to the stable lineages within the CD4 or CD8 cell populations (16). The antigen defined by the CD45RA (kD 220) MAB of the leukocyte common antigen (LCA) is very likely associated with the virgin T cells whereas the CD45R0 (kD 180) MAB of LCA reveals the primed T cell subset (18,19).

Functional studies demonstrate that the CD4+CD45R0+ lymphocytes are "helper/inducer" cells (14,20) and the CD4+CD45RA+ lymphocytes represent the "suppressor/inducer" cells. In addition, only the CD45R0+ cells respond to soluble antigen and could provide help for pokeweed mitogen driven immunoglobulin synthesis or a specific antibody

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response (21). Concerning the function of CD45RA and CD45R0 subsets of CD8 cells, Merkenschlager and Beverly (22) have shown that both subsets can generate allospecific cytotoxic lymphocytes (CTL) while only CD45R0+ lymphocytes 9UCHL1) generate EBV specific restricted CTL. On the basis of *in vitro* experiments Beverly et al (22) strongly suggest that CD8+CD45R0+ lymphocytes are a memory cytotoxic population.

Recent data have indicated that cytotoxic CD8+ lymphocytes play an important role in the pathogenesis of HIV infection (see for review 23). CD8+ T lymphocytes from the peripheral blood of HIV-infected patients have been shown to be capable of exhibiting virus-specific cytotoxic activity (24-29). In addition, Walker et al (24,30) have observed that CD8+ lymphocytes can control HIV-infection *in vitro* by suppressing virus replication. It has been recently demonstrated that CD8+ lymphocytes which block replication of HIV-1 or the simian immunodeficiency virus of macaques in the peripheral blood of infected hosts may be contained only within the 4B4+2H4- subpopulation of CD8+ cells, and that they must come into physical contact with their target cells (31-33).

For this reason, it is vitally important for the understanding of the progress of AIDS to follow the changes in number and tissue distribution of the putative antigen specific cytotoxic memory type (CD8+CD45R0+) T lymphocyte subset during the course of HIV infection and to accumulate more knowledge on the phenotype of CD4 subpopulations within the follicles and the paracortex.

There has been an impressive recent development, fueled by use of double marker immunophenotypic analysis demonstrating important changes in number and localization of different T cell subsets in lymphoid tissues of HIV infected patients (see for review 19). We have recently performed immunohistological double marker analysis using the CD45RA and CD45R0 MABs in combination with anti MABs detecting CD4 or CD8 molecules to acquire more information on the variation in distribution and number of these T cell subsets in control LNs and in LNs from HIV-infected persons.

Whereas in our control LN (nonspecific follicular hyperplasia) the *marginal sinus* (MS) contained about equal numbers of CD4+CD45R0+ and CD8+CD45R0+ lymphocytes, in HIV lymphadenitis CD8+CD45R0+ lymphocytes outnumbered the CD4+CD45RA+ lymphocytes. Increased numbers of CD8+CD45R0+ cells were detected also between the marginal sinus and the follicles in infected LNs. In our previous study using *in situ hybridization* we regularly found cells expressing viral RNA in these areas (18). These findings have suggested that infected cells circulating in the lymphatic vessels enter the marginal sinus and from there they go to the follicles. The presence of "cytotoxic" CD8+ lymphocytes in these areas may indicate a defense mechanism.

In the *follicles* of control LNs, the great majority of CD4+ lymphocytes belonged to the CD45R0 subset. The number of both CD8+CD45RA+ and CD8+CD45R0+ cells was minimal. In HIV lymphadenitis, the follicles were enlarged and often irregularly shaped. The mantel zone was thin. The double labeling for CD8 and CD4 in combination with CD45R0 or CD45RA demonstrated two prominent immunopathological findings. First, the great majority of CD4 and CD8 lymphocytes belonged to the CD45R0 subset. Second, they were not intermingled with each other.

The majority of the CD4 lymphocytes were localized in the light zone of the GC. In contrast, many CD8 lymphocytes were grouped in small clusters. It is of interest to note that the localization of these islets consisting of CD8+CD45R0 cells often corresponded to the focal destruction of the FDC network supporting the notion that antigen specific CD8+ lymphocytes may play a role in the destruction of the FDC network. CD8+CD45R0+ and CD4+CD45R0+ lymphocytes were also regularly found around the small blood vessels of the

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GCs.

Single marker immunohistochemistry has demonstrated that in the *paracortical zone* of LNs with nonspecific lymphadenitis, the majority of T cells are CD4-cells (see review in 1). According to our estimation, the ratio of CD4/CD8 cells in nonspecific lymphadenitis is about 3:1(1). However, there is a significant variation in the number of different lymphocyte subsets. This is understandable since the microanatomy of "normal" LNs may vary with any of several factors, where any current or previous infection, antigenic stimulation, anatomical site of the LN, age and immunological status of the patient are perhaps the most important factors.

Our double labelings have shown that in the paracortex of control LNs about two-thirds of the CD4 cells were CD45R0+ and one-third belonged to the CD45RA group. Similar to the results of Janossy et al (18,35) we also noted that lymphocytes belonging to different subsets were not simply intermingled but formed small nests consisting of T cells from the same subset. Such a distribution can indicate a clonal proliferation.

Double staining for CD45R0 and CD45RA demonstrated that the majority of T cells expressed only one of these antigens. There were only a few double-labeled T lymphocytes (1-5% of all T cells). We agree with Janossy et al (18) that these cells likely represent recently activated lymphocytes.

In the paracortex of LNs with follicular hyperplasia caused by HIV infection the ratio of CD4 lymphocytes to CD8 cells was significantly reduced (one to one) when compared with the control nodes (three to one). In several instances a reversal of this ratio (one to two) was registered. The reduced CD4/CD8 ratio in all LNs was mainly due to an increase of CD8 lymphocytes. Double marker immunohistochemistry has made it possible to further characterize these CD8 lymphocytes. About two-thirds of them were CD45R0+ and only one-third was positive for the CD45RA marker. This inversion of the CD45RA/CD45R0 subsets of CD8 lymphocytes has already been registered in early cases just after seroconversion.

These data are in agreement with our findings gained by double immunofluorescence combining CD4 or CD8 with CD45RA or CD45R0 (18,19). In the paracortex of control LNs, 68±15% CD45RA+ and 21±6% CD45R0+ cells were found within the CD8 cell population while in PGL LNs 23±11% CD45RA+ and 67±23% CD45R0+ cells were noted.

In patients with a longer clinical history, the numbers of both the CD4/CD45R0+ and CD4/CD45RA+ subpopulations were slightly decreased. Double staining for CD45R0 and CD45RA demonstrated also in the infected LNs only very few double labeled cells. In the majority of LNs, focal accumulations of CD8+CD45R0+ cells were noted which probably represented a clonal proliferation of these cells. In the lumen of the high endothelial venules CD8/CD45R0+ cells were commonly found. These findings suggest that "memory"-type CD8 lymphocytes can enter the LNs through the high endothelial venules.

In view of these observations, we believe that one of the most important events in HIV-lymphadenitis with explosive follicular hyperplasia is the accumulation of CD8+, CD45R0+ lymphocytes in the LNs. There is an increased traffic of cells with this phenotype inside the marginal sinus and their presence in high endothelial venules indicate that they probably enter the LN parenchyma through these structures. The clustering of CD8+CD45R0+ lymphocytes in the vicinity of destruction of FDC network strongly suggest that these cells could be responsible for the loss of the antigen trapping and presenting cell population of GC. Because the progress of the disease is accompanied by the disintegration of the GC and lymphocyte depletion of the LN, it is important to analyze the number and distribution of these putative antigen specific cytotoxic T cells through the whole histologic spectrum of HIV induced lymphadenopathy. Examination of

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later stages of HIV infection is in progress.

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