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ORIGINAL ARTICLE

Shigeo Mori

Demonstration of isolated follicular dendritic cells in lymphatic vessels around human immunodeficiency virus-infected lymph nodes

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Abstract Follicle lysis (FL) is a peculiar disruptive change of follicular dendritic cells (FDCs) commonly observed in the germinal centres of individuals with HIV infection. To clarify the fate of FDCs during FL, 30 HIV-infected lymph nodes were studied, mostly in the persistent generalized lymphadenopathy phase. FDCs were immunostained on paraffin sections with 1F8, a FDC-specific monoclonal antibody. In advanced FL, the FDC network was shown to be divided into cell clusters of various sizes. In the final phase of FL, these were fragmented into much smaller cell clusters and dispersed in the parafollicular area. Some of these small clusters were found in the lymphatic sinuses (6/30), and unexpectedly, even in extranodal lymphatic vessels (2/30, one being apparently located in efferent vessels). No efflux of these small FDC clusters into extranodal lymphatics was observed in 15 HIV-negative control lymph nodes. These FDC clusters located in the lymphatics may work as transporters of HIV to other lymph nodes downstream, as FDCs carry the greatest HIV load.

Key words Human immunodeficiency virus · AIDS Dendritic cell · Lymph node

Introduction

The follicular dendritic cell (FDC) is one of the major cell populations that harbours human immunodeficiency virus (HIV) in infected individuals. By examination of lymphoid organs using immunostaining and in situ hybridization, HIV viral antigens and RNA have been demonstrated in FDCs (Tenner-Racz et al. 1988; Mori et al. 1989; Fox et al. 1991). FDCs of HIV-infected patients in the persistent generalized lymphadenopathy phase occasionally lose their cytoplasmic network to form peculiar defective areas, in a process known as follicle lysis (FL, Burns et al. 1985). Areas showing early FL change have at times been described as "moth-eaten" because morphologically they mimic moth-eaten cloth. In advanced FL, the affected germinal centres are divided into small segments of cell clusters composed of FDCs and small lymphocytes. Finally, the FDCs are entirely absent in lymphoid organs at the terminal stage.

The importance of FDCs as HIV producers has been stressed recently (Embretson et al. 1993; Pantaleo et al. 1993). This has had an impact on current AIDS research by pinpointing the site where HIV accumulates and proliferates in asymptomatic HIV carries (Temin and Bolognesi 1993) although previous pathological studies had indicated that FDCs bear enormous amounts of HIV RNA and protein, and that the FDCs around areas of FL are the most heavily infected (Tenner-Racz et al. 1988; Mori et al. 1989; Fox et al. 1991). These observations collectively indicate that FL in HIV-infected patients is closely related to FDC infection.

FDCs are lost from HIV-infected lymph nodes in the terminal phase. However, the fate of HIV-infected FDCs has not been studied in detail. Electron-microscopic observation has shown occasional degeneration of FDCs in HIV-infected lymph nodes (Pantaleo et al. 1993), and this seems to have led to the idea that FDCs in the disrupting follicle are destroyed in situ. However, it remains unclear whether this form of in situ cell death implies the total loss of FDCs in FL, and whether at least a proportion of FDCs in areas of FL flow out into the lymphatics.

To address these possibilities, I studied the localization of FDCs in lymph nodes of HIV-infected patients, and have demonstrated the rare, but definite, efflux of small isolated FDC clusters into lymphatic vessels.

Materials and methods

Thirty lymph nodes obtained surgically from HIV-infected patients were used in this study. Lymph nodes bearing intact or degenerating germinal centres were selected, whereas those showing marked atrophy, corresponding to the terminal phase of the disease, were excluded. Specimens were selected from HIV-positive

S. Mori
Department of Pathology, Institute of Medical Sciences, University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108, Japan

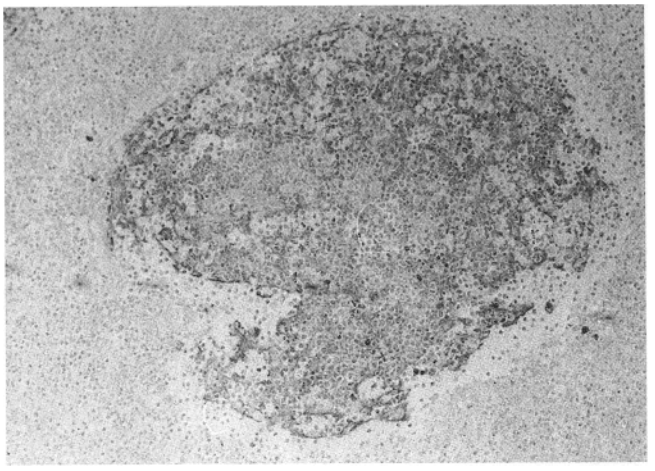

Fig. 1 Follicle lysis in the early phase. Partial defects in the follicular dendritic cell (FDC) network are evident in a germinal centre of a HIV-infected lymph node. The anti-CD21 antibody 1F8 was used as the first reagent for immunostaining, ×50

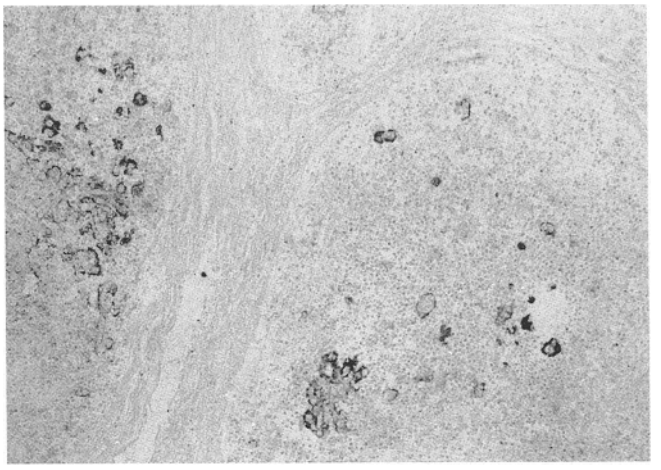

Fig. 3 Follicle lysis in the final phase (*right*). Isolated immunostained cells, corresponding to FDCs, are scattered in areas where germinal centres are present. The structure of the germinal centre on the left is fairly well-preserved, ×40

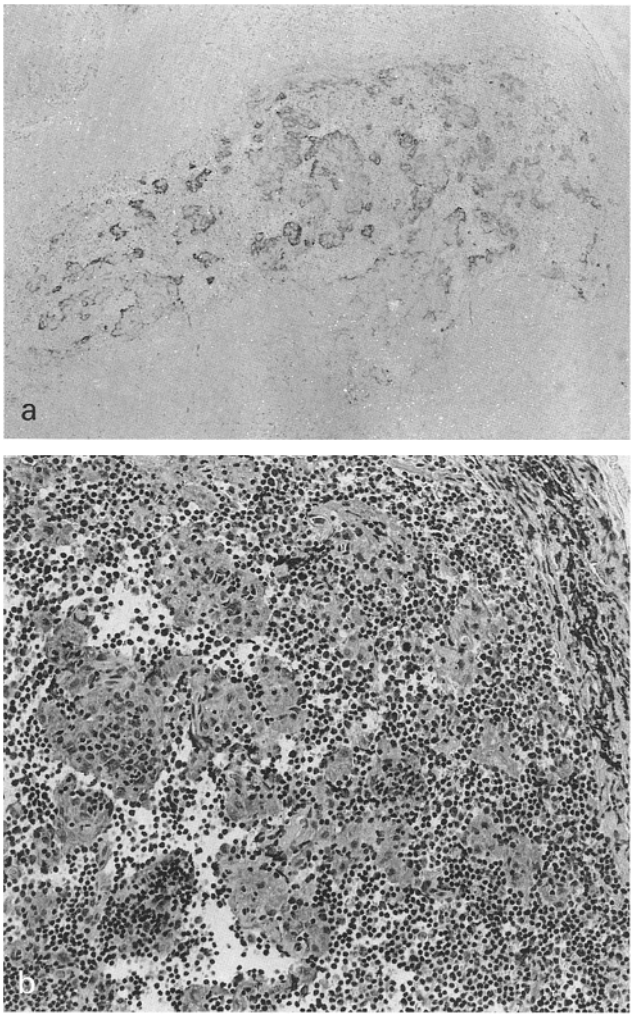

Fig. 2 Follicle lysis in the advanced phase. A germinal centre is separated into segments of various sizes, each composed of aggregates of FDCs and lymphocytes, separated by a loose sinus-like structure. (**a**) Immunostaining with 1F8 (×40). Same section counterstained with haematoxylin **b**, ×150

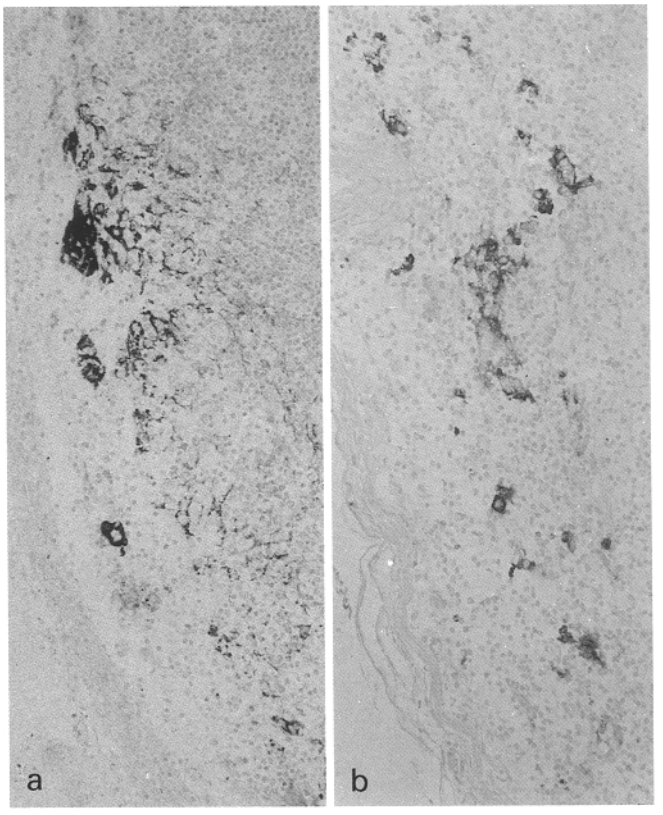

Fig. 4 Isolated FDCs in subcapsular areas. A few of them are apparently located in subcapsular sinuses, ×130. The germinal centre structure is still visible in **a**, but not in **b**

cases treated at our institute-affiliated hospital. Others were kindly supplied by Professor Marshal Kadin, Beth Israel Hospital, Harvard University, Boston, Mass., Dr. Glauco Frizzera, Armed Forces Institute of Pathology, Washington DC, and Dr. Shiro Imahori, Erie County Hospital, Buffalo, N.Y. Paraffin-embedded tissues of these specimens were cut into 3.5 μm sections and subjected to immunostaining by the labelled avidin-biotin-alkaline phosphatase method. 1F8, an anti-CD21 antibody specifically reactive with

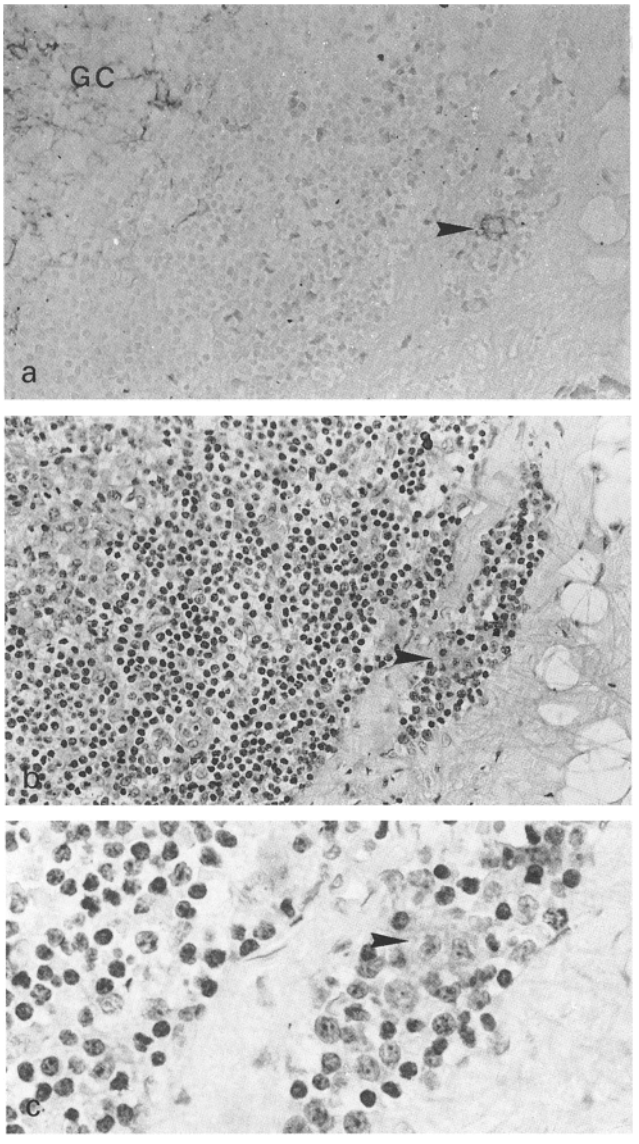

Fig. 5 An isolated 1F8-stained cell in the intracapsular lymphatics (*arrow*). GC: germinal centre. **a** Immunostaining with 1F8, ×200. **b, c** Same section counterstained with haematoxylin

FDCs on paraffin sections (Dakopatts, Denmark) was used as the first reagent for immunostaining. Its immunohistological reactivity on paraffin sections has been described previously (Pantaleo et al. 1993), as well as in the manufacturer's instructions. Immunostaining was performed according to the method of Hsu et al. (1981) with slight modification. Deparaffinized sections were first treated with 10 mg/100 ml proteinase K (Sigma, St. Louis, Mo., USA) in 0.1 M phosphate buffer, pH 7.8, for 10 min at 37° C. They were then washed with 0.1 M TRIS-HCl buffer, pH 7.4, followed by incubation with 1: 100-diluted 1F8 for 16 h at 4° C, incubated successively with appropriately diluted biotinylated anti-mouse immunoglobulin Fab fragment (Dakopatts) and alkaline phosphatase-conjugated avidin (Dakopatts), and washed with TRIS-HCl buffer between these steps. The slides were coloured with new fuchsin using a New-fuchsin staining kit (Dakopatts), for 20 min at room temperature, following the vendor's instructions. Some of the immunostained slides were counterstained with haematoxylin for detailed histological observation. As a control study, these specimens were immunostained with optimally diluted normal mouse serum (Dakopatts, 1: 200).

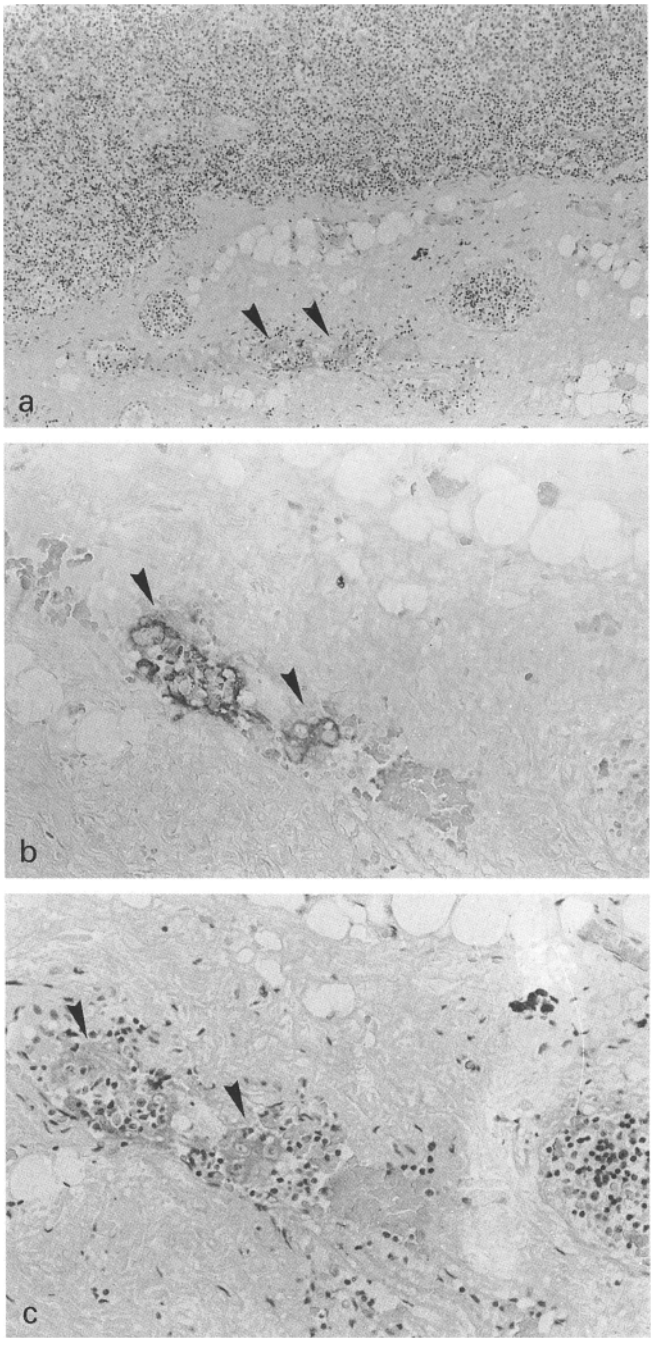

Fig. 6 1F8-positive cells forming small clusters with small lymphocytes are evident in an efferent lymphatic vessel located adjacent to the lymphatic hilum. **a** ×20; **c** ×200; counterstained after immunostaining. **b** The same slide before counterstaining, ×200

Fifteen HIV-negative lymph node biopsy samples were used as the control specimens.

Results

1F8 antibody stains FDCs exclusively in HIV-negative lymph nodes. Its specificity was again confirmed by the negative control study, in which not a single cell was

stained on HIV-negative and HIV-positive sections, using normal mouse serum as the first reagent. A large proportion of the HIV-infected lymph nodes examined showed FL in different phases. In the early phase, a partially defective FDC network, corresponding to the moth-eaten appearance, was noted (Fig. 1). In areas of advanced FL, the affected germinal centres were separated into small segments (Fig. 2), each composed of aggregates of FDCs and small lymphocytes, and separated by loose sinus-like structures lacking stromal components (Fig. 2b). In the final phase, immunostained cell clusters were much more fragmented (Figs. 3, 4), and were composed mostly of FDCs and lymphocytes, although at times each consisted of an isolated single FDC (Fig. 4). In this final phase, the clustered or isolated FDCs were scattered in parafollicular areas, as well as in areas where germinal centres were present, and occasionally in areas adjacent to subcapsular or paratrabecular sinuses. In 6 out of 30 lymph nodes, some of the isolated FDCs were observed in the marginal and peritrabecular sinuses (Fig. 4).

In two cases, large 1F8-stained cells were observed in lymphatic vessels located outside the lymph node (Figs. 5, 6). In one of these cases, the vessel was apparently the efferent vessel, as it was located adjacent to the lymphatic hilum (Fig. 5), although vessel identification was not possible in other case (Fig. 6). Morphologically, the stained cells resembled dendritic cells, and occasionally formed cell clusters composed of one to a few immunostained cells and unstained small lymphocytes, mimicking a fragmented germinal centre. However, isolated 1F8-positive cells were also noted in these lymphatics (Fig. 5). Some of the FDC clusters appeared to partially obstruct the vessels (Fig. 6). The small FDC clusters and isolated FDCs located in lymphatics were observed exclusively in lymph nodes showing advanced FL, and never in HIV-negative lymph nodes.

Discussion

The present study clearly demonstrated rare but definite efflux of solitary FDCs and small FDC clusters into lymphatic vessels of HIV-infected lymph nodes showing FL. A false positive reaction seems unlikely to have been the cause, since the antibody stained the FDC network exclusively in HIV-negative lymph nodes, as described in a previous report (Pantaleo et al. 1993) and in the vendor's instructions. Histological observation revealed that the cell clusters were composed of immunostained large dendritic cells and unstained small lymphocytes, supporting the view that the 1F8-stained cells were germinal centre-derived FDCs, thus further confirming the antibody's immunological specificity.

One possibility that cannot be discounted is that the FDCs may flow out into the lymphatic circulation, when mechanical force is applied to the lymph node. The FDC clusters located in FL lesions seem to be mobile, as they are attached very loosely to the parenchyma. In fact, on microscopic examination, these clusters appear to be "floating" in the sinus-like structures (Fig. 2). Here, the fibrous tissues that support germinal centres were not observed. Thus, with mild mechanical force, the cell clusters may be flushed out into the sinuses, and thereafter into the lymphatics. The biopsy procedure itself may produce such mechanical force, although it is also probable that such efflux could occur in the patient's daily life.

FDCs are known to disappear from lymph nodes at the end-stage of HIV infection (Tenner-Racz et al. 1987; Turner et al. 1987; Wood 1990). The mechanism of FDC disappearance has not been fully clarified. An acceptable assumption may be that the FDCs are killed in situ in the germinal centre through viral multiplication-induced cytolysis. Electron-microscopic observation has demonstrated the degenerating FDC in germinal centres, and this finding tends to substantiated the view of in situ cell death (Pantaleo 1993). However, the present result suggests another possibility that at least some FDCs may flow out into the lymphatics.

It is highly probable that the FDC clusters in lymphatics will be carried into other lymph nodes located downstream, causing the spread of HIV. T4 lymphocytes and macrophages have long been believed to be the main route of viral spread. However, these ideas were based on studies of peripheral blood, whereas investigation of the lymphatic system, of which the FDCs forms a part, have not yet been done in sufficient depth. So far, only one report has referred to HIV-bearing cells in the lymphoid circulation. Tenner-Racz et al. (1988) demonstrated viral antigen-positive "small and large cells" in sinuses of lymph nodes; it is possible that their large cells correspond to the FDCs that were demonstrated in this study in sinuses as well as in lymph vessels. Such FDCs will bear an enormous amount of HIV, like FDCs in FL lesions (Tenner-Racz et al. 1988; Mori et al. 1989; Parmentier et al. 1990; Fox et al. 1991) and these discharged FDCs will contribute to viral transport. However, this efflux of FDCs occurs in the late stage of HIV infection, and may not contribute much to the initial spread of HIV. Further detailed study of the lymphatic system should help to reveal the role of circulating FDC clusters in viral spread.

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References

Burns BF, Wood GS, Dorfman RE (1985) The varied histopathology of the homosexual men. Am J Surg Pathol 9: 287–297

Embretson J, Zupancic M, Ribas JL, Burke A, Racz P, Tenner-Racz K, Haase AT (1993) Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS . Nature 362: 359–362

Fox CH, Tenner-Racz K, Racz P, Firpo A, Pizzo PA, Fauci AS (1991) Lymphoid germinal centers are reservoirs of human

immunodeficiency virus type 1 RNA. J Infect Dis 164: 1051–1057

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29: 577–580

Mori S, Ezaki Y, Mori M, Takahashi M, Teshima M, Sagawa K (1989) Deterioration of B cell proliferation correlates with dendritic reticulum cell destruction in germinal centers of an AIDS patient. Acta Pathol Jpn 38: 1205–1214

Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, Fox CH, Orenstein JM, Kotler DP, Fauci AS (1993) HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature 362: 355–358

Parmentier HK, van Wichen D, Sie-Go DMD, Goudsmit J, Borleffs JCC, Schuurman HJC (1990) HIV-1 infection and virus production in follicular dendritic cells in lymph nodes. Am J Pathol 137: 247–251

Temin HM, Bolognesi DP (1993) Where has HIV been hiding? Nature 362: 292–293

Tenner-Racz K, Racz P, Dietrich M, Kern P, Janossy G, Veronese-Dimarzo F, Klatzman JC, Popovic M (1987) Monoclonal antibodies to human immunodeficiency virus: their relation to the patterns of lymph node changes in persistent generalized lymphadenopathy and AIDS. AIDS 1: 95–104

Tenner-Racz K, Racz P, Schmidt H, Dietrich M, Kern P, Louie A, Gartner S, Popovic M (1988) Immunohistochemical, electron microscopic and in situ hybridization evidence for the involvement of lymphatics in the spread of HIV-1. AIDS 2: 299–309

Turner RR, Levine AM, Gill PS, Parler JW, Meyer PR (1987) Progressive histopathologic abnormalities in the persistent generalized lymphadenopathy syndrome. Am J Surg Pathol 11: 625–632

Wood GS (1990) The immunohistology of lymph nodes in HIV infection: a review. Prog AIDS Pathol 2: 25–32