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Clinico-Immunopathological Alterations of Lymph Nodes from Human Immunodeficiency Virus-Infected Patients in Northern Thailand

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SUMMARY **To determine if the immunopathologic alterations of HIV-infected lymph nodes have any correlation with clinical stages in the northern Thai patients, we conducted a comparative analysis of immunopathologic features of lymph nodes between 25 HIV-infected patients from various clinical categories and 25 non-HIV individuals of reactive hyperplasia morphology of lymph node biopsies. The risk factors for HIV infection were all heterosexual. The majority of patients in clinical category A (PGL) showed a histopathologic pattern of explosive follicular hyperplasia, while category C (AIDS) patients demonstrated follicular involution and lymphocyte depletion on lymph node sections. Interestingly, weak reactivity for HIV p24 gag protein was detected within the germinal centers and scattering interfollicular lymphocytes in only 20% of the HIV-infected cases. Morphologically, the presence of MGCs was specific for HIV-infected lymph nodes. MGCs (hematoxylin & eosin stain) were found in 64% of the HIV-infected cases, which was significantly different from 4% found in control cases (p = 0.00002). By S-100 immunostaining, MGCs were demonstrated in all HIV-infected lymph node sections, while they were found in 32% of the control lymph nodes. Immunostaining with S-100 protein also revealed the appearance of syncytial ballooning and countable numbers of MGCs. High numbers of MGCs seemed to correlate with histologic and clinical changes. In conclusion, the HIV-infected patients had high numbers of MGCs or syncytia on lymph node sections in early stage and pre-AIDS conditions, which has never been reported before.**

Infection with human immunodeficiency virus (HIV) is characterized by progressive and profound deterioration of the immune system with a long period of clinical latency.[1-8] The immune system in the latent phase is largely intact, but there is smoldering, low level HIV replication, predominantly in the lymphoid tissues, which may last for several years. Patients are either asymptomatic or develop persistent generalized lymphadenopathy (PGL) which is one of the earliest manifestations of HIV infection. It is present in more than one third of the infected patient in some studies.[9,10] Actually the histopathological alteration was present in most of the HIV seropositive cases even without palpable lymphadenopathy.[11] Five to twenty percent of the patients with PGL proceed to develop full-blown acquired immunodeficiency syndrome (AIDS) after a mean observation time of 12 to 22 months.[9,12-16] In the HIV-infected patients with lymphadenopathy, two to four basic lymph node histologic patterns and the corresponding clinical picture at the time of biopsy have been well described.[15-31] It has been assumed that transformation from one histologic pattern to another occurs during the course of HIV disease and that with this histologic progression there is also a change in the clinical status of the patients.[9,16,32-34] The morphologic features, either individually or in

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combination, are not conclusive evidence of either AIDS or PGL. However, their diagnostic utility may be increased in the proper clinical setting if they are coupled with immunologic studies of lymph node tissues[15,18,19,26,32,35-38] Subtype E accounts for only 10 percent of HIV-1 strains isolated worldwide but more than 90 percent of them are present in Thailand. Approximately 95% of heterosexually acquired cases are E subtype.[39] We therefore studied the histopathology and immunocytochemistry in lymph node biopsies obtained from HIV seropositive patients presenting with lymphadenopathy and a control group without evidence of HIV infection in the Thai population.

MATERIALS AND METHODS

Subjects

Chart review allowed classification of the 50 patients examined into seropositive (25 patients) and serology not tested (25 patients) groups. All 25 patients not tested for HIV serology were selected as only a low risk group with no history of other infections or autoimmune diseases. HIV serology was tested with a commercial ELISA assay (Abbott Laboratories, Illinois, USA.) and SERODIA-HIV kit (Fujirebio Inc.,Tokyo, Japan), then western blot testing was performed in clinical categories A & B. Clinical information was obtained from hospital charts with details of clinical category, AIDS and pre-AIDS conditions including mode of acquiring the infection (Table 1). Lymph node biopsies from 50 patients were sliced into sections of 2- to 3-mm thickness and a portion was placed in 10% neutral-buffered formalin. After overnight fixation, the sections were routinely processed and embedded in paraffin.

Histopathology

A 3 μm thick section was mounted and stained with hematoxylin and eosin (H & E) for histological study, and also with Gram (Brown-Hopps), mucicarmine, periodic acid-Schiff, Warthin-Starry, Gomori methenamine silver and Ziehl-Neelson (acid-fast bacilli) to rule out other infections. Standard criteria were used for histologic diagnosis, with histological type of explosive follicular hyperplasia (EFH), follicular involution (FI), mixed EFH and FI and lymphocyte depletion (LD) according to Chadburn *et al.*[34] Lymph nodes with EFH were composed of markedly hyperplastic irregularly shaped follicles in both the cortex and medulla, and follicular involution showed small, hypocellular and frequently hyalinized follicles with hyperplastic paracortical areas and prominent intrafollicular vessels. Lymphocyte depletion was composed mainly of medullary cords and sinusoids with total absence of follicular areas and the paracortical zone. The definitions of morphologic features (Table 2) were previously described by O'Murchadha *et al.*[40] The features were described as present or absent. The relative incidence of each feature in the two groups was compared by using Fisher's exact probability test and Chi-square test with Yates' correction.[41]

Immunocytochemistry

Four-μm sections were prepared from formalin-fixed, paraffin-embedded tissue blocks and applied to positively charged slides coated with 3-amino-propylethoxysilane. The sections were dewaxed in xylene, treated with 3% hydrogen peroxide, and rinsed in PBS. Microwave antigen retrieval was performed for 10 minutes and then cooled down at room temperature for 30 minutes. The sections were then incubated with the primary antibody for 60 minutes at room temperature, then the slides were kept overnight at 4°C. After a brief wash in phosphate buffered saline, the slides were treated with the secondary link antibody for 30 minutes. The slides were then washed and incubated for another 30 minutes with streptavidin. After a final wash, the slides were stained with aminoethyl carbazole, 1.2 percent in acetate buffer containing 0.015 percent hydrogen peroxide. All monoclonal antibodies (MAb) and polyclonals were obtained from commercial sources and recognized CD 20 (MAb L-26; Dako; working dilution of 1:20), CD4 (MAb OPD4; Dako; 1:25) CD8 (MAb; Dako; 1:30), CD45RO (MAb UCHL-1; Dako; 1:20), CD3 (rabbit polyclonal; Dako; 1:50), S-100 (rabbit polyclonal; Dako; 1:200), HIV-1 p24 (MAb; Dako; 1:5), CD68 (Mab KP1; Dako; 1:30). Of the above antibodies, only p24 staining required predigestion for 9 minutes with 0.05% protease VIII (Sigma Chemical Co.) in 0.1 mol/l phosphate buffer, pH 7.6, at 37°C). Sections were counterstained with Harris's hematoxylin. Nonreactive mouse and rabbit immunoglubins were used as negative controls.

Semiquantitative studies of the number of multinucleated giant cells (MGCs) on immunostaining were carried out by counting MGCs on the entire sections and then calculating the number of MGCs per ten high power fields (HPF).

RESULTS

Patients

All available clinical information and pathological diagnosis in 25 HIV-seropositive patients are shown in Table 1. Based on the 1993 CDC revised classification system,[42] which was based here solely on clinical manifestation, since CD4 lymphocyte values were not available in this retrospective study, 11 of the 25 HIV-seropositive cases were under Category A (44%) with a presenting symptom of PGL and an equal number of 7 cases under category B (28%) and C (28%). Patients under group B presented with lymphadenopathy and conditions as stated in Table 1, while group C patients shows AIDS indicators including tuberculosis, cryptococosis, *pneumocystis carinii* and recurrent pneumonias. The HIV-infected patients comprised 12 females and 13 males with an age range from 15-66 years (a mean age of 35.2 years and a median age of 34 years). The risk factors for HIV infection in all cases were heterosexuality. Of the patients in the control group, 10 were female and 15 were male. Their age ranged from 7 to 68 years, with a mean age of 36 years and a median age of 33 years.

Histopathology

The majority of patients in clinical category A (9 of the 11 cases) showed explosive follicular hyperplasia (EFH) on lymph node sections, while all of the patients in clinical category C demonstrated follicular involution (FI) or lymphocyte depletion (LD). Lymph node histology of the patients in clinical category B included EFH, FI and mixed EFH with FI. The histopathologic alterations in 25 HIV-seropositive patients included nine (36%) of EFH, seven (28%) of mixed EFH with FI, six (24%) of FI and three (12%) of LD. The incidence of morphologic findings (hematoxylin & eosin stain) are represented in Table 2. As compared with the control group, two features significantly more common in the HIV-seropositive cases were the presence of polykaryocytes, syncy-

Table 1 HIV-Infected lymph nodes: clinicopathological data

Patients	Age (years)	Sex	*Status of HIV infection	*AIDS indicator (C) or B conditions (at the time of biopsy)	Pathology
1-3	22,35,30	F,F,F	A	-	EFH
4-6	29,34,38	M,M,M	A	-	EFH
7-9	13,21,36	M,M,F	A	-	EFH
10-11	15,46	F, F	A	-	Mixed
12	33	F	B	chronic diarrhea	Mixed
13	25	M	B	fever > 1 month	Mixed
14	50	M	B	fever > 1 month	Mixed
15	43	M	B	fever > 1 month	Mixed
16	34	M	B	trichiuriasis	Mixed
17	42	F	B	parathyroid adenoma	FI
18	35	M	B	fever > 1 month	FI
19	30	F	B	fever > 1 month	FI
20	50	M	B	chronic diarrhea	FI
21	28	F	B	chronic diarrhea	FI
22	31	M	C	PCP	FI
23	66	M	C	pneumonia, recurrent	LD
24	48	F	C	CNS, cryptococcosis	LD
25	40	M	C	pulmonary TB	LD

M = male, F = female, EFH = explosive follicular hyperplasia, Mixed = follicular hyperplasia + follicular involution, FI = follicular involution, LD = lymphocyte depletion, TB = tuberculosis, PCP = *Pneumocystis carinii* pneumonia.
*1993 CDC Classification system for HIV infection and AIDS Surveillance Case Definition for Adolescents and Adults.
A = Asymptomatic or acute or persistent generalized lymphadenopathy (PGL),
B = Symptomatic, not A or C conditions,
C = AIDS indicator conditions.

Table 2 Comparison of morphologic features between HIV and non-HIV cases

Morphologic features	HIV cases (25)		Non-HIV cases (25)		p value
	No.	Percent	No.	Percent	
Giant cells	16	64	1	4	0.00002
Mantle-zone loss	18	72	9	36	0.023
Irregular follicles	11	44	14	56	0.571
Follicle lysis	16	64	20	80	0.345
Burnt-out follicles	13	52	10	40	0.570
Monocytoid cells	12	48	8	32	0.386
Epithelioid histiocytes	8	32	7	28	1.000
Dermatopathic change	2	8	4	16	0.667
Marked plasmacytosis	4	16	1	4	0.348
Germinal center hemorrhage	8	32	9	36	1.0000

tial cells or MGCs (64% vs 4%, p = 0.00002) and mantle-zone loss (72% vs 36%, p = 0.023).

The presence of MGCs (Fig. 1) was specific for HIV infection in this study. These giant cells varied in appearance (number and location of nuclei and/or the amount of cytoplasm) but most commonly included cells with multiple nuclei clustering in the central portion of the cytoplasm. The MGCs localized in the interfollicular areas and the intrafollicular regions. The mantle-zone loss revealed a partial or complete effacement of the mantle zone in some follicles.

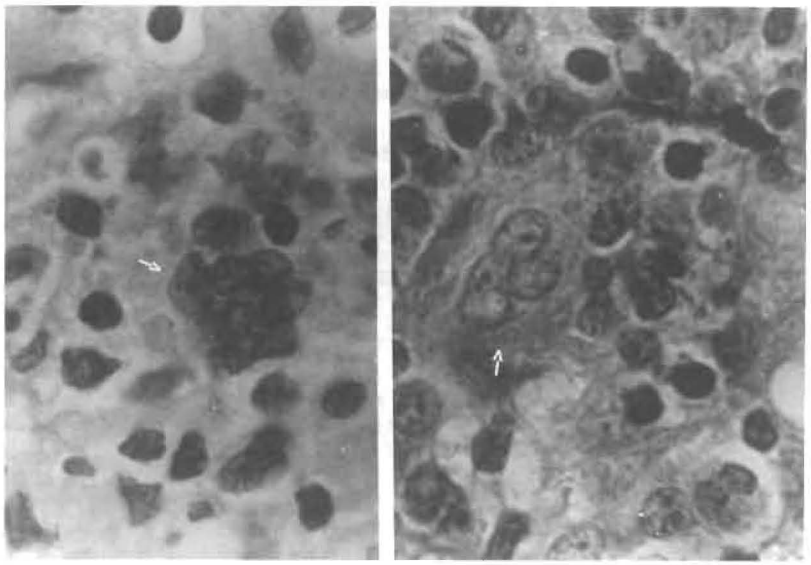

Fig. 1 Multinucleated giant cells were specific for HIV-infected lymph node. Giant cells characteristically had multiple nuclei clustered in the central portion of the cytoplasm (H & E, original x 400).

Immunohistochemistry

The immunoreactivity of polykaryocytes or MGCs with S-100 protein highlighted the number and appearance of MGCs or syncytial cells (Fig. 2) and also revealed the syncytial "ballooning" (Fig. 3) of dying MGCs. All lymph node sections from HIV-infected cases demonstrated the feature of MGCs with or without ballooning when performing S-100 protein immunostain. The number of MGCs by S-100 immunostaining varied from 3-60 cells per 10 HPF with an average number of 18 cells. Eight out of 25 cases (32%) of the control group also showed the appearance of MGCs by S-100 immunostaining, but the number of MGCs varied from 1 to 5 cells per ten HPF with the average number of 2.4 cells, which was significantly different from the HIV-infected group ($p < 0.01$). Four of six cases with histologic patterns of FI showed a strikingly high number of MGCs (30-60 cells/10 HPF) and six of nine

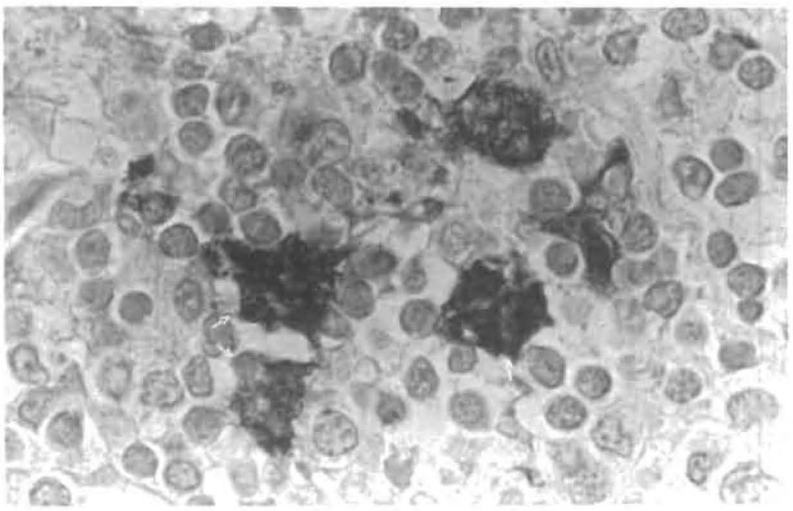

Fig. 2 Immunohistochemical reactivity of multinucleated giant cells with S-100 protein highlighted their appearance with intense cytoplasmic staining (original x 400).

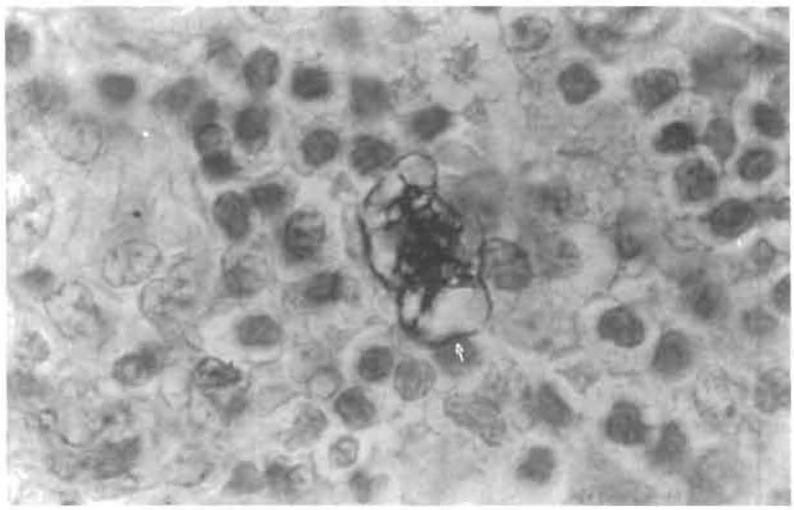

Fig. 3 Syncytium "ballooning" of dying giant cells showed vacuolated cytoplasmic staining with S-100 protein (original x 400).

cases with EFH histology showed the same number. The remaining ten cases showed a low number of MGCs (< 30 cells/10 HPF). In addition to MGCs, S-100 protein was also found within some scattering follicular dendritic cells and single interdigitating reticulum cells.

Weak HIV p24 reactivity was seen within the germinal centers, in scattering interfollicular lymphocytes in 5 of the 25 cases (20%).

CD68 (KP-1) was recognized in the MGCs, scattered macrophages and tingible body macrophages in the germinal center.

Immunophenotyping of B-cell (CD20) varied according to the histological class; being higher in the follicular type, and notably fewer in follicular involution and lymphocyte depletion types.

Immunoreactivity with T-cell markers or subsets (CD45 RO, CD3, CD4 and CD8) demonstrated a reduction or relative absence of T cell markers in the follicular involution and lymphocyte depletion patterns as compared with the follicular and mixed patterns. A predominant plasma cells infiltrate in lymphocyte depletion type showed reactivity with both kappa and lambda light chains. The MGCs showed no reactivity with either B-cell or T-cell monoclonal antibodies.

DISCUSSION

HIV-1 subtype E is predominantly spread in Thailand in heterosexual transmission groups, while in developed countries, HIV-1 subtype B is more predominant.[39] We report here the clinico-immunopathologic changes in lymph nodes from patients with different clinical staging in northern Thailand. We found that mantle-zone loss and particularly the presence of MGCs by hematoxylin & eosin staining are significantly more common in HIV-infected lymph nodes as compared to the controls (64% vs 4%). Although the presence of MGCs in lymph nodes of HIV-infected patients was previously reported,[21,22,40,43,44] but these cells were

seen in a lower percentage of cases compared with our study. Morphologically, MGCs found in lymph nodes had the nuclei clustered in the central portion and Warthin-Finkeldey-like cells in some cases and immunologically with anti S-100, the appearance of MGCs was highlighted in all seropositive cases, with the average number of 18 cells/10 HPF, which was significantly different from the control group (2.4 cells/10 HPF). This staining also revealed the feature of syncytial ballooning of dying MGCs in some cases. Fusion of infected cells with formation of syncytia (giant cells) may be a mechanism of cell death and these cells develop "ballooning" and usually die within 48 hours.[45] Interestingly, weak anti-HIV p24 core antigen reactivity was identified in only 20% of HIV-seropositive cases (negative p24 immunoreactivity was confirmed in Dr. Frankel's Lab, Walter Reed Army Institute of Research, USA.), in contrast, strong reactivity and a high percentage of positivity were reported in other studies.[46-48] This could be explained by differences in amino acid sequence in a specific epitope of p24 gag protein of E subtype.[49] In this study, high numbers of MGCs or syncytia were found in some cases of early clinical stage with EFH histology and later when disease showed progression before the advanced stage with FI changes of the lymph node. Presence of MGCs in oropharyngeal lymphoid tissues was demonstrated in the early or latent stage of HIV infection.[46-48] but in this study a high number of MGCs was not only found in early-stage but also in some pre-AIDS cases. This finding is thus the first report demonstrating syncytia on lymph node sections from pre-AIDS stage. Syncytium-inducing phenotype is an important marker for progression to AIDS, independent of CD4 cell counts and p24 antigen.[50,51] The diagnostic and prognostic values of MGCs in lymph node sections in this study are limited due to the small number of studied cases and S-100 protein is a marker of many cells, including dendritic cells. Even though we found a similar result on p55, a marker of activated dendritic cells and T-cells, we have not been able to demonstrate immunoreactivity with markers for T-cells on fused lymphocytes in MGCs on lymph node sections. Meanwhile, the other investigators reported that MGCs may represent syncytia of infected T lymphocytes and activated dendritic cells.[52] We will proceed to study more cases and also determine the extent of immuno-pathological changes and presence of HIV-1 viral RNA and proviral DNA in HIV-1 infected lymph nodes in Thailand.

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