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Clinicopathologic Study of Castleman's Disease in Korea

Castleman's disease represents an atypical lymphoproliferative disorder, infrequently associated with various immunologic abnormalities or subsequent development of malignancy such as Kaposi sarcoma, malignant lymphoma and plasmacytoma. Its clinicopathologic features depend on various etiologic factors such as Kaposi sarcoma herpesvirus (KSHV), oversecretion of IL-6, adhesion molecule and follicular dendritic cell dysplasia, etc. To investigate the relationship of Castleman's disease (CD) and the above factors, we reviewed 22 cases of CD. Four cases of KSHV positive CD were detected, all multicentric, plasma cell type, and these cases displayed prominent vascular proliferation, characteristic 'Kaposi-like lesion'. IL-6 and CD54 positive mononuclear cells were scattered in interfollicular areas of KSHV positive cases. Follicular dendritic cell hyperplasia, vascular proliferation, expression of IL-6 and CD54 did not show any significant difference between solitary vs multicentric type, and plasma cell type vs hyaline vascular type. Our study suggests that KSHV positive CD reveals unique pathologic features, and the probable relationship of KSHV and IL-6 and CD54 is discussed.

Key Words: *Giant Lymph Node Hyperplasia; Herpesvirus, Kaposi Sarcoma-Associated; Sarcoma, Kaposi; Dendritic Cells, Follicular; Interleukin-6; Intercellular Adhesion Molecule-1*

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INTRODUCTION

Castleman's disease (CD) is a rare nonneoplastic lymphoproliferative disorder of unknown etiology (1). Its morphologic recognition is based on a composite of various histologic features, but generally it is classified to two subtypes, hyaline-vascular type (HV) and plasma cell type (PC). However, clinical significance is determined by another classification that is solitary and multicentric. Clinically, solitary Castleman's disease (SCD) occurs in young people and the course is usually self-limited (1). However, multicentric Castleman's disease (MCD) faces the risk of developing malignant disease, most frequently Kaposi sarcoma, non-Hodgkin's lymphoma and Hodgkin's lymphomas (2), and occurs in elderly people. In addition, MCD tends to be accompanied by various immunologic abnormalities and systemic constitutional symptoms, suggestive of viral etiology (1). There is a strong evidence for the close association of Kaposi sarcoma and MCD in human immunodeficiency virus (HIV) infected individuals (3), and presence of novel B-lymphotropic gammaherpesvirus, Kaposi sarcoma herpesvirus (KSHV) in MCD (4). Other factors such as follicular dendritic cell dysplasia (5), abnormal adjustability to cytokines like IL-6 (6), or oversecretion of adhesion molecules (7) have been discussed as etiologic factors of CD. And it has been also considered that lots of unknown factors may be correlated. The aim of study is to find which of the above factors are related to CD's pathologic features.

MATERIALS AND METHODS

Twenty-two cases of CD diagnosed from 1989 to 1998 in Seoul National University Hospital with available paraffin tissue were included in this study. Hospital records were referred for patients' history. Immunostaining was done according to the standard labeled streptavidin method using LSAB kit (Dako, Carpinteria, CA, U.S.A.) on paraffin-embedded sections. Antibodies used and their dilution rates are listed on Table 1. Immunostaining for IL-6 (monoclonal anti-human IL-6) was restricted to MCD cases. Using polymerase chain reaction (PCR) to amplify the $KS330_{233}$ sequence of KSHV as previously described (8), we evaluated 100 ng of DNA extracted from affected lymph node sections of CD patients. Southern hybridization of the PCR amplification products with a ^{32}P end labeled probe internal to the $KS330_{233}$ sequence was performed to corroborate our results. AIDS associated KS tissue sections without MCD were tested

Table 1. Primary antibodies employed

Antibody	Source	Dilution	Main reactivity
CD21	Dako, Glostrup, Denmark	1:20	follicular dendritic cells
CD34	Immunotech, Westbrook, ME, U.S.A.	1:50	vascular endothelial cells
S-100	Dako	1:400	interdigitating reticulum cells
CD54	Pharmingen, San Diego, CA, U.S.A.	1:100	activated lymphocytes
IL-6	Genzyme, U.S.A.	1:15	activated lymphocytes

as positive controls for KSHV sequence PCR amplification. An EBV-immmortalized B cell line (B95-8) was used as negative controls. EBV detection was performed by in situ hybridization on paraffin-embedded sections with a probe specific for EBV-encoded RNA transcript (EBER-1 and EBER-2) labeled with fluorescein isothiocyanate (FITC). The detection system was applied according to the manufacturer's recommendations (Dakopatts, Glostrup, Denmark).

The above data were analyzed using Mann-Whitney U test.

RESULTS

Clinical profiles

Patient profiles are summarized in Table 2. The total number of cases was 22, and patients' ages ranged from 17 to 64 yr (median age: 36 yr, and 28 in SCD, 43 in MCD), and they were slightly younger in SCD cases. In SCD, eight cases were HV types, and only two were PC types. But in MCD, the proportion was reversed with 3 HV and 9 PC types. Constitutional symptoms like fever, weight loss and hematologic or immunologic abnormalities were accompanied in 9 cases (3 cases of SCD and 6 cases of MCD). One case (case No. 22) was associated with POEMS (polyneuropathy, organomegaly, endocrinopathy, M protein and skin changes), and had already been reported (9, 10). Two cases (case No. 12, 17) were associated with renal amyloidosis and had been reported also (11). One case (case No. 8) was linked with juvenile rheumatoid arthritis. There were no cases with HIV positivity or subsequent development of malignancy. Treatment was surgical excision and one patient (case No. 16) underwent immunosuppressive therapy. All patients were alive during the follow-up period, and recurrence was observed in one pateint (case No. 7).

Table 2. Clinical profiles of 22 Castleman's disease patients

Patient	Sex/Age (yr)	Clinical type	Histologic type	Site	Clinical manifestation
1	F/51	SCD	PC	mediastinum, abdomen	abdominal pain, anemia
2	M/26	SCD	PC	mediastinum	routine radiographs
3	M/22	SCD	HV	inguinal	DM, wt. loss, pph. neuropathy
4	F/41	SCD	HV	mediastinum	routine radiographs
5	F/23	SCD	HV	intrapulmonary	routine radiographs
6	F/17	SCD	HV	retroperitoneum	routine radiographs
7	F/29	SCD	HV	pulmonary	routine radiographs
8	F/19	SCD	HV	axilla	arthralgia, anemia, hepatosplenomegaly
9	M/35	SCD	HV	retroperitoneum	indigestion
10	M/17	SCD	HV	abdomen	routine radiographs
11	F/38	MCD	HV	mediastinum	routine radiographs
12	F/46	MCD	PC	mediastinum, abdomen	anemia, renal amyloidosis
13	F/43	MCD	PC	axilla, neck	fever, ascites
14	M/61	MCD	PC	neck	fever, wt. loss
15	F/36	MCD	HV	abdomen, neck	general weakness, ascites, wt. loss
16	F/64	MCD	PC	neck, axilla	fever, pancytopenia
17	M/45	MCD	PC	mediastinum, abdomen	renal amyloidosis, lymphadenopathy
18	M/59	MCD	PC	neck, axilla	multiple lymphadenopathy
19	F/19	MCD	HV	retroperitoneum	multiple lymphadenopathy
20	M/31	MCD	PC	neck	wt. loss
21	M/23	MCD	PC	neck, mediastinum	fever, wt. loss
22	F/44	MCD	PC	neck, mediastinum	POEMS syndrome

SCD, solitary Castleman's disease; MCD, multicentric Castleman's disease; HV, hyaline-vascular type; PC, plasma cell type; pph. neuropathy, peripheral neuropathy; wt. loss, weight loss; POEMS, polyneuropathy, organomegaly, endocrinopathy, M protein and skin changes

Table 3. Detection of KSHV and EBV and Immunostaining of CD21, S100, IL-6, CD54 & CD34

Case No.	CD21	S-100	CD34	IL-6	CD54	KSHV	EBV
1	0	0	0	ND	+/−	N	N
2	3	1	1	ND	−	N	N
3	0	1	1	ND	−	N	N
4	2	1	0	ND	+/−	N	N
5	0	1	0	ND	−	N	N
6	0	0	1	ND	+/−	N	N
7	0	2	2	ND	−	N	N
8	1	3	0	ND	+/−	N	N
9	0	1	1	ND	+/−	N	N
10	3	1	1	ND	−	N	N
11	3	0	1	+	+/−	N	N
12	0	0	1	+	+/−	N	N
13	0	2	2	+/−	−	N	N
14	0	2	2	−	+	P	N
15	0	0	0	−	+/−	N	N
16	1	3	2	+	+	P	P
17	0	0	1	−	+/−	N	N
18	2	0	0	−	+/−	N	N
19	1	0	0	+	−	N	N
20	2	0	0	−	+/−	N	N
21	2	2	2	ND	+/−	P	N
22	2	1	2	ND	+	P	N

ND, not done; −, no positive cells; +/−, few cells positive; +, a few cells positive; N, negative; P, positive

Detection of KSHV and EBV

Four cases out of 22 were KSHV positive, one of them (case No. 16) was also EBV positive (Table 3, Fig. 1). All KSHV positive cases were MCD ($p<0.05$), PC type ($p<0.05$), and showed characteristic florid interfollicular vascular proliferation ($p<0.05$), forming 'Kaposi-like lesions' (Fig. 2). Development of true vascular neoplasm was not found.

Immunostaining

Follicular dendritic cell hyperplasia demonstrated by CD21 immunostaining was graded as follows: grade 0 with few positive cells, grade 1 with positive cells occupying less than 50% of germinal center area, grade 2 with 50 to 75% of germinal center area, and grade 3 with diffuse pattern (Table 3, Fig. 3). S-100 positive interdigitating reticulum cell proliferation was graded in the same manner. The degree of FDC or interdigitating reticulum cell proliferation in the two groups classified either by SCD and MCD, and HV and PC was nearly the same (Table 4). Dysplastic FDCs were not found. CD 34 was exclusively positive in endothelial cells, and vascular proliferation marked by CD34 immunostaining was estimated according to three gradings: 0 (sparse), 1 (focal), 2 (marked). There were no differences between SCD and MCD or between HV and PC type ($p>0.05$, Table 4). However, all KSHV positive cases revealed

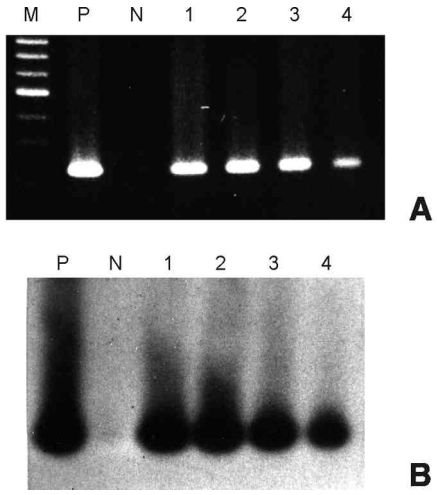

Fig. 1. Electrophoresis of polymerase chain reaction products from patients with Castleman's disease (**A**) and Southern hybridization (**B**). Lane M, 100-bp molecular weight ladder; lane P, positive control; lane N, negative control; lane 1, case No. 14; lane 2, case No. 16; lane 3, case No. 21; lane 4, case No. 22

grade 2 lesions. CD54 (Intercellular cell adhesion molecule-1, ICAM-1) expression was not found in germinal center, and occasional CD54 positive mononuclear cells were scattered in interfollicular areas of MCD, especially KSHV positive cases ($p<0.05$, Fig. 4, Table 4). IL-6 immunostaining showed the same pattern as the results of CD54 (Fig. 4), and those cells were thought to be the same ones as CD54 positive cells.

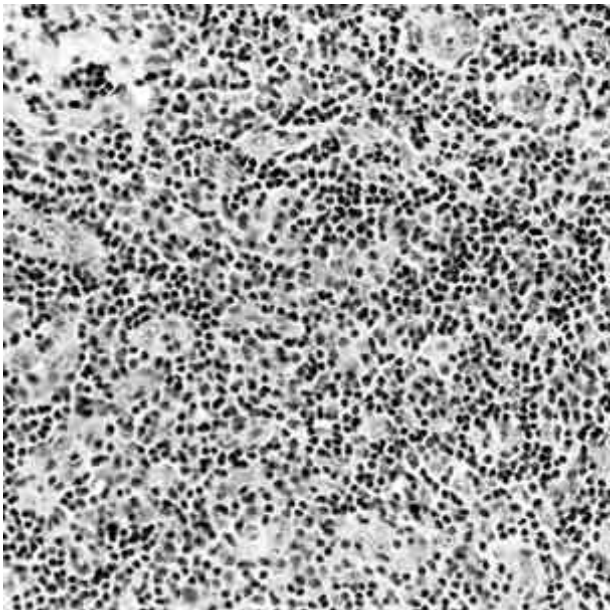

Fig. 2. Kaposi sarcoma herpesvirus positive multicentric Castleman's disease. Florid vascular proliferation with plasma cell infiltration is characteristic (H&E, ×40).

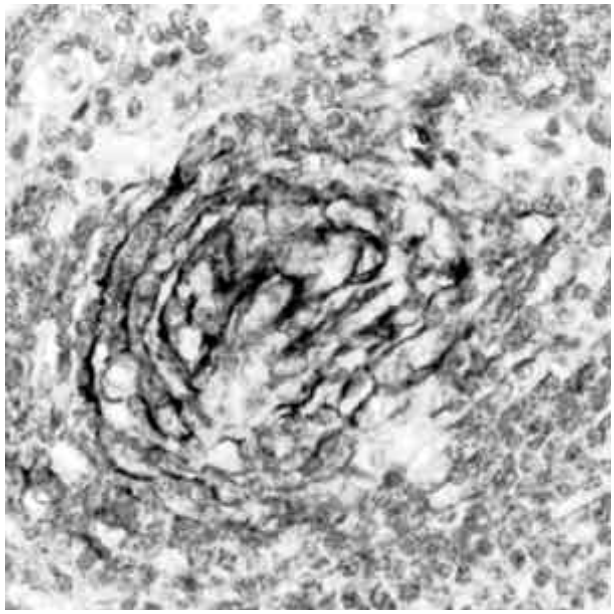

Fig. 3. Positive immunoreactivity to CD21 is restricted to germinal centers (×200).

Table 4. Comparison of various parameters between each group of CD

Features	Group A		Group B		Group C	
	KSHV (+)	KSHV (−)	SCD	MCD	HV	PC
No. of cases	4	18	10	12	11	11
Mean age (yr)	50	33	28	43	27	41
Male : Female	2 : 2	7 : 11	4 : 6	5 : 7	3 : 8	6 : 5
SCD : MCD	0 : 4	10 : 8			8 : 3	2 : 9
HV : PC	0 : 4	11 : 4	8 : 2	3 : 9		
CD21	NS		NS		NS	
S100	NS		NS		NS	
CD34	high ($p<0.05$)	low	NS		NS	
CD54	high ($p<0.05$)	low	NS		NS	

CD, Castleman's disease; SCD, solitary Castleman's disease; MCD, multicentric Castleman's disease; HV, hyaline vascular type; PC, plasma cell type; NS, not significant

DISCUSSION

CD is a relatively rare non-neoplastic lymphoid disorder of unknown etiology. In Korea, Kim et al. reported seven cases of collective study from 1981 to 1992 (12), and sporadic case reports were done (9-11). CD represents various clinical and morphological features, whose differences are also associated with various etiologic factors. The identification of MCD is based on a combination of clinicopathologic criteria: 1) multiple peripheral lymphadenopathy or multiorgan involvement; 2) an idiopathic nature; 3) histologically plasma cell type (1). However, according to the chronological stage of CD, MCD of HV or mixed type is also present because MCD is classified into proliferative phase, accumulative phase, or burned-out phase, mimicking hyaline vascular type (1). In our present study, clinical and histological features according to CD classification were agreeable overall to previous reports, and some disagreements could be explained by time sequence. To explain the various manifestation of CD, several biologic agents, cytokines or histologic markers were investigated. IL-6 was reported to be increased in serum of PC- type CD patients, and in bone marrow and lymph nodes of these patients, IL-6 induces plasma cell infiltration and increses serum gammaglobulin, so it was suggested that the variety of clinicopathologic features in CD would depend on the capacity of activated B cells to produce IL-6 (6). Although

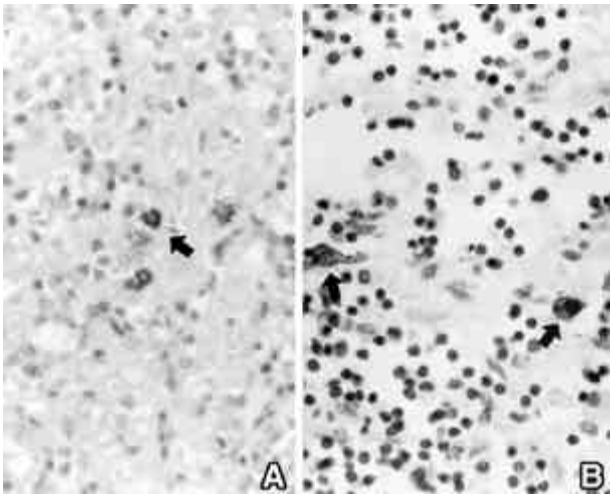

Fig. 4. Immunostaining for IL-6 (A) and CD54 (B). A few positive cells are scattered in interfollicular areas (arrow) (×200).

limited to a few restricted cases, we confirmed that IL-6 was not expressed in FDC, plasma cells or germinal center cells but in only a few interfollicular mononuclear cells. The above finding suggest that IL-6 may not significantly affect CD's pathologic features or development of disease, but the limitation of immunostaining sensitivity of the paraffin embedded tissue must be taken into account. The same explanation is also applied to CD54. Ruco et al. observed ICAM-1 and ELAM-1 expression in hyperplastic or dysplastic FDC and some germinal center cells in cryostat sections of lymph nodes from patients with CD (7). Ishiyama et al. observed IL-6 mRNA expression in interfollicular cells but not in plasma cells via in situ hybridization in MCD lymph nodes, and they revealed spontaneous IL-6 production in culture supernatants of peripheral blood monocyte in the same patients, who showed immune dysregulation (13). Both CD54 and IL-6 positive cells in our study may be interfollicular plasmacytoid monocytes as Ishiyama has mentioned, suggesting that IL-6 is expressed in interfollicular monocytes of MCD patients and induces plasma cell proliferation and consequently clinical manifestations. The monocytes already infected by KSHV should be able to induce IL-6 secretion, or under some immune dysfunction status KSHV could be infected secondarily.

Follicular dendritic cell dysplasia is observed in some cases of HV CD and can progress to FDC tumor in some reports (5). We did not note dysplastic FDC or FDC tumor in our 22 cases. However, we must consider the extreme rarity of FDC tumor in interpreting the relationship of FDC dysplasia and CD. The degree of FDC proliferation is not significantly different between SCD and MCD or between HV and PC, therefore it is likely to be a non-type specific process.

KSHV positive MCD is relatively common in Western countries (4). The most distinctive characteristics associated with KSHV are plasma cell and vascular proliferation. The former may be connected with subsequent development of B-cell lymphoma and plasma cell dyscrasia, and the latter with vascular tumors like Kaposi sarcoma. Viral IL-6 may be transcribed in KSHV infected bone marrow dendritic cells (macrophages) of multiple myeloma or monoclonal gammopathy, perpetuating the growth of plasma cells. Thus, KSHV could play an oncogenic role in these disorders (14). The assumptive mechanism of developing vascular neoplasm in MCD is two. 1) activated lymphocytes or dysplastic FDCs could produce an angiogenetic factor (7, 15), 2) KSHV is concurrently associated with MCD and Kaposi sarcoma. In our present study, the percentage of KSHV positivity is relatively low (18% in CD, 33% in MCD) compared with Western countries, so its causitive role in MCD may be explained partly. However, the above figure is significant considering the extreme low positivity of HIV and Kaposi sarcoma in Korea. It is difficult to define clinicopathologic features of KSHV positive MCD due to a lack of cases. However, these cases showed peculiar histologic features, that is florid vascular proliferation and scattered IL-6 and CD54 positive mononuclear cells in interfollicular areas. However, follicular dendritic cell hyperplasia or FDC dysplasia is not significantly relevent to negative cases. It is possible that an intimate functional relationship exists between the dysregulated immune system and the vascular proliferation mediated by IL-6 or other angiogenetic factors such as CD54 expressed in activated monocytes (15). KSHV may be incidentally infected in immunologically deteriorated MCD patients, rather than considered as an etiologic agent. KSHV positive MCD cases are expected to show unique features discriminative of KSHV negative cases. Another herpesvirus, EBV should play a role in some cases of MCD (16). In the present study, only one EBV positive case was found and the positive cells were activated immunoblast-like lymphoid cells, which did not resemble plasma cells or interfollicular monocytes, and no specific pathologic findings different from other KSHV positive MCD were present. One possibility is that EBV is not coinfected with KSHV in a single cell population but concomitantly infected in different cell populations due to immune dysfunction, so it may not play an important role in pathogenesis.

In conclusion, MCD did not show significant difference from SCD in respect of FDC and vascular proliferation. KSHV was detected in four out of 22 cases (18%), and EBV was detected in only one. The KSHV positive cases were all multicentric, plasma cell type, and histologically characterized by florid vascular proliferation, scattered IL-6 and CD54 positive mononuclear cells in inferfol-

licular area.

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