Histiocytic Necrotizing Lymphadenitis

- A clinico-pathologic study of 45 cases with in situ hybridization for
Epstein-Barr virus and hepatitis B virus -

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Forty-five cases of histiocytic necrotizing lymphadenitis (HNL) or Kikuchi-Fujimoto disease were reviewed clinico-pathologically and studied for Epstein-Barr virus (EBV) and hepatitis B virus (HBV) by in situ hybridization to assess their causative role. Histologically, the lymph nodes typically showed relatively well defined paracortical lesions composed of large atypical mononuclear cells, histiocytes, and karyorrhectic nuclear debris. Mild to moderate degree of coagulation type necrosis was present in 24 cases. Clinical features did not vary greatly from previously described female preponderance, young age onset, subacute cervical lymphadenopathy, and frequent leukopenia, except for a few cases with recurrent disease over 8-9 years. Serologic tests revealed EBV IgG antibody in one case, HBV surface antibody in 11 cases and HBV surface antigen in 2 cases. In situ hybridization was performed on 41 cases using internal repeat 1 fragment DNA and EBV-coded small RNA (EBER-1) for EBV, and pan-HBV DNA probe for HBV detection, and showed that all cases were negative for EBV or HBV genome. Our results suggest EBV or HBV may not have causative role in the pathogenesis of HNL.

Key Words : Histiocytic necrotizing lymphadenitis, Kikuchi-Fujimoto disease, Epstein-Barr virus, Hepatitis B virus, In situ hybridization

INTRODUCTION

Histiocytic necrotizing lymphadenitis (HNL), or Kikuchi-Fujimoto disease, was first described in Japan in 1972 (Fujimoto et al., 1972; Kikuchi, 1972), and subsequently recognized in many parts of the world (Pileri et al., 1982; Turner et al., 1983; Koh et al., 1985; Dorfman and Berry, 1988). It is characterized by predilection for cervical lymph nodes of young women and paracortical necrotizing process histologically consisting of karyor-

rhectic nuclear debris, phagocytizing macrophages and apparently atypical transformed lymphocytes. The etiology of HNL remains unknown. Microorganisms such as toxoplasma (Kikuchi et al., 1977), Yersinia enterocolitica (Feller et al., 1983), Epstein-Barr virus (EBV) (Takada et al., 1980; Hollingworth et al., 1994), and human herpes virus-6 (HHV-6) (Eizuru et al., 1989; Sumiyoshi et al., 1993; Hollingworth et al., 1994) have been suspected of, but not proven to be the cause of HNL. Many investigators consider this lymphadenopathy as a hyperimmune condition induced by certain antigenic stimulation, most likely viral infection (Dorfman and Berry, 1988; Asano et al., 1990; Cho et al., 1991). We present a clinicopathologic review of 45 cases with in situ hybridization study to detect EBV and hepatitis B virus (HBV) genome.

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MATERIALS AND METHODS

Necrotizing lymphadenitis in the Surgical Pathology files of the Korea Cancer Center Hospital during a period from 1988 to 1993 were reviewed. Forty-five cases of HNL were selected after exclusion of tuberculosis or suppurative lymphadenitis. The medical records of these cases were reviewed for information about age, sex, clinical manifestation, and laboratory findings. In situ hybridization studies were performed on formalin-fixed, paraffin-embedded tissue sections of 41 cases. For EBV, 2 kinds of biotinylated probes were used: internal repeat 1 fragment DNA (Enzo Diagnostics, NY, USA) and EBV-coded small RNA (EBER-1) (Research Genetics, AL, USA). For detection of HBV genome, biotin-labeled pHBV DNA probe (KREATECH Biotechnology, Netherlands) was used. The microprobe capillary system was applied to the procedures. Ultra-probe kit (Biomedica, CA, USA) was used for visualization. For DNA in situ hybridization, five μ m sections of 41 cases were deparaffinized in xylene, rehydrated in absolute alcohol, digested with ribonuclease (Sigma, MO, USA) and pepsin (Biomedica, CA, USA), and denatured in formamide (Sigma, MO, USA). Hybridization with probes was performed at decreasing temperatures from 110°C to 85°C for 15 minutes. After washing, sections were treated with avidin-alkaline phosphatase and Fast Red TR salt. In EBER in situ hybridization, sections were deparaffinized, rehydrated, predigested with pepsin, and hybridized for 35 minutes at decreasing temperatures from 85°C to 50°C. After washing and endogenous peroxidase blocking, hybridization was visualized by streptavidin-HRP conjugate and diaminobenzidine. Lymph node tissue with EBV-positive Hodgkin's disease and HBV-positive hepatic tissue were used as positive controls.

RESULTS

Clinical Data

Thirty-four patients were female and 11 were male (F:M=3:1). The ages of 45 patients ranged from 11 to 66 (mean 29), with two-thirds in their 3rd to 4th decades. All patients presented with multiple or single cervical lymphadenopathy. Inguinal or axillary lymph node enlargement was accompanied in 8 patients. Seventeen patients complained of flu-like systemic symptoms. Durations of the lymphadenopathies, available in 37 cases, were 1-2 weeks in 13, 1-2 months in 17, 3-5 months in 3, and 1 year in one. Three patients

manifested recurrent diseases over 8-9 years. White blood cell counts, checked in 32 cases, ranged from 2400 to 7800/mm³. Twenty-two (49%) patients had leukopenia under 4800/mm³. Relative lymphocytosis (>48%) and relative monocytosis (>9%) were noted in 5 and 6 patients, respectively. Serologic tests showed positive hepatitis B surface antibody (HBsAb) in 11, positive HBsAg in 2, positive ASO in 6, positive EBV IgG antibody in 1 and cytomegalovirus (CMV) E1A antibody in 1.

Pathologic Findings

All the lymph nodes showed mottled appearance and architectural effacement by relatively well defined patchy or nodular lesions in the paracortical area (Fig. 1). On high magnification, the paracortical lesions were composed of large atypical mononuclear cells, occasionally with plasmacytoid features, karyorrhectic nuclear debris, and phagocytizing or non-phagocytizing macrophages (Fig. 2). Deposition of eosinophilic debris between cellular components was seen in 18 cases. In 6 lymph nodes, there were confluent acellular areas of coagulation type necrosis (Fig. 3). Absence of neutrophilic infiltration was the rule. Severity of necrotizing process did not correlate with the durations of manifestation or other clinical and laboratory findings (Table 1).

Table 1. Summary of clinical, laboratory and pathologic findings

Pathology*	I	II	III
No. of cases	21(47%)	18(40%)	6(13%)
Age(yr) range	11-55	15-50	18-66
mean	28.3	28.6	32.3
Sex(female:male)	15:6	15:3	4:2
Site			
cervical only	19	13	5
cervical & ing.	1	2	1
cervical & ax.	1	2	0
cervical, ing. & ax.	0	1	0
Sx. duration			
1-2 wk	4	8	1
1-2 mo	9	4	4
3-5 mo	2	1	
1 yr	1		
8-9 yr	1	1	1
Systemic sx.	5	8	4
WBC count			
<4800	10	9	3
≥4800	5	4	1
Serology			
HBsAb	5	5	1
HBsAg	1	1	
ASO	3	2	1
EBV IgG Ab	1		
CMV E1A Ab	1		

*I: karyorrhexis only. II: intercellular necrotic debris.
III: confluent coagulative necrosis. ing.: inguinal,
ax.: axillary. sx.: symptom

Fig. 1. A lymph node with histiocytic necrotizing lymphadenitis characteristically showing a well demarcated pale-looking lesion in the paracortical area at low magnification.

Fig. 2. A typical lesion of histiocytic necrotizing lymphadenitis composed of atypical, often immunoblastic lymphocytes, histiocytes, and karyorrhectic nuclear debris.

In situ hybridization, performed on 41 cases, revealed neither EBV nor HBV genome in any cases (Fig. 4).

DISCUSSION

HNL of Kikuchi and Fujimoto is now a well known

Fig. 3. A case with confluent coagulative necrosis.

Fig. 4. Negative staining of HNL on EBER in situ hybridization (left). Right: EBER-positive Hodgkin's cells as positive control.

disease entity. Since the initial description in Japan (Fujimoto et al., 1972; Kikuchi, 1972), HNL has been reported from various parts of the world (Pileri et al., 1982; Turner et al., 1983; Koh et al., 1985; Dorfman and Berry, 1988). Recent literature still shows the prevalence of this disease in countries of the Far East (Asano et al., 1990; Cho et al., 1991; Tsang et al., 1994; Kuo, 1995). HNL is most often presented as a self-limiting subacute course of cervical lymphadenopathy with or without generalized symptoms in young female

patients. The involved lymph nodes are histologically characterized by relatively discrete paracortical areas consisting of large atypical lymphocytes, macrophages and karyorrhectic debris. Differential diagnosis from malignant lymphoma is pathologically important.

In spite of its characteristic clinico-pathologic features, the etiology of HNL remains unknown. A form of T cell-mediated immunity seems to be related with the pathogenesis of HNL. According to many immunohistochemical studies, cellular components in the necrotizing areas of HNL are mostly T cells and macrophages (Turner et al., 1983; Kikuchi et al., 1986; Rivano et al., 1987; Unger et al., 1987; Dorfman and Berry, 1988; Asano et al., 1991; Treilleux et al., 1991). Suppressor T cells were reported to predominate over helper T cells (Turner et al., 1983; Rivano et al., 1987; Dorfman and Berry, 1988; Treilleux et al., 1991). Transformed and proliferative mononuclear cells, which are not only characteristics of this disease, but also the source of confusion with malignant lymphoma, were shown to be suppressor T cells (Asano et al., 1987, 1991; Oshima et al., 1991). This phenomenon of proliferation of transformed suppressor T cells is reminiscent of infectious mononucleosis. Atypical lymphocytes in the peripheral blood have been observed in up to 25% of the patients with HNL (Kuo, 1995).

Involvement of viral or other infectious organisms in the pathogenesis of HNL has been suspected for a long time. Presence of serum antibodies to toxoplasma, *Yersinia enterocolitica*, cytomegalovirus, and EBV was documented in some patients with HNL (Kikuchi et al., 1977; Takada et al., 1980; Feller et al., 1983; Kikuchi et al., 1986; Rivano et al., 1987; Shirakusa et al., 1988). However, presence of antibodies to those ubiquitous microorganisms was not sufficient for an evidence of the organisms as the causative agents. Recently, serological evidences of human herpesvirus-6 (HHV-6) infection were demonstrated in patients with subacute febrile illness with cervical lymphadenopathy (Niederman et al., 1988; Steeper et al., 1990). HHV-6 is a novel lymphotropic virus and known to induce morphologic changes in cultured lymphocytes (Lusso et al., 1988). Japanese investigators quickly paid attention to this virus as the etiologic agent of HNL. However, HHV-6 antigens and genomes were found in the lymph nodes not only with HNL but also with non-specific reactive lymphadenitis, by immunofluorescence, polymerase chain reaction (PCR) and in situ hybridization methods (Eizuru et al., 1989; Sumiyoshi et al., 1993). In contrast, Hollingsworth et al. (1994) reported that 15 tested cases of HNL

lacked HHV-6 template on PCR. They also studied EBV in 20 cases by PCR and in situ hybridization, and detected EBV genome in only a fraction of cases with a low number of positive cells (Hollingsworth et al., 1994).

Our study failed to reveal EBV genome in both necrotizing areas and resting lymphocytes of forty-one HNL cases by in situ hybridization using two types of probe, internal fragment DNA and EBER-1. In situ hybridization method is usually less sensitive than, but of advantage to PCR, especially in EBV study. Since EBV is universal, it is necessary to avoid laboratory contamination and to confirm the presence of genome in diseased cells. And EBER-1-RNA technique is known to be highly sensitive in EBV genome detection (Weiss et al., 1991). Our results on EBV in situ hybridization study negates a possible role of EBV in the pathogenesis of HNL.

We found that eleven patients in our study were serologically positive for HBsAb, and two were positive for HBsAg. The fact is not very surprising in this country where clinical and subclinical HBV infection is not uncommon. At the same time, prevalence of HNL in the Far East countries where viral hepatitis are prevalent might implicate involvement of a hepatitis virus in HNL. Hepatitis viruses harbor mild lymphotropism, and can induce transient leukopenia followed by relative lymphocytosis and atypical peripheral lymphocytes indistinguishable from those in infectious mononucleosis (Isselbach et al., 1994). Cervical or abdominal lymphadenopathy manifest in 10-20% of the patients with viral hepatitis (Cassani et al., 1990). However, our in situ hybridization study failed to demonstrate HBV DNA in lymph nodes with HNL.

The similarity of HNL to SLE, including frequent ultrastructural finding of intracytoplasmic tubuloreticular structures (Imamura et al., 1982; Eimoto et al., 1983; Shirakusa et al., 1988; Asano et al., 1991), suggests that this lymphadenopathy might be a hyperimmune condition, either autoimmune or induced by certain antigenic stimulation, most likely viral infection. In spite of lack of evidence for a specific virus as the cause of HNL, clinico-pathologic features are yet suggestive of viral involvement.

The clinico-pathologic findings of the present 45 cases were similar to those of previously reported cases, except for a clinically recurrent pattern over 8-9 years seen in 3 cases. This uncommon presentation had also been observed in our previous study (Cho et al., 1991). Histopathologically, prototype of the necrosis in HNL is karyorrhexis of apoptosis type. In addition to

karyorrhexis, 18 and 6 cases in our study showed minimal or obvious coagulative necrosis, respectively. This incidence of coagulative necrosis is similarly high as those of other large series (Tsang et al., 1994; Kuo, 1995). Kuo (1995) subtyped HNL into proliferative type, necrotizing type with any degree of coagulative necrosis, and xanthomatous type. He postulated that these were sequential changes and that three types differed in certain aspects of clinical features. We also suspected if confluent coagulative necrosis represented a later stage of the disease which might have shown only karyorrhexis at early phases, but could not find correlation between duration of the disease and degree of coagulative necrosis. Presence or absence of coagulative necrosis may simply represent variation of immune reaction among hosts.

The chronic nature of this disease, infrequent though, and several reports on recurrence, subsequent development of systemic lupus erythematosus, or even fatality (Dorfman and Berry, 1988; Chan et al., 1989; Asano et al., 1991) indicate a need for long-term follow-up in some patients with HNL.

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