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A Study of the Viral Etiology of Histiocytic Necrotizing Lymphadenitis (Kikuchi-Fujimoto disease)

Histiocytic necrotizing lymphadenitis (HNL) or Kikuchi's disease is a distinctive, self-limited disorder characterized by necrotizing cervical lymphadenopathy in young individuals. HNL is more prevalent among Asians and is a relatively common disorder among Koreans. A preceding fever, lymphopenia, and occasional skin rashes suggest a viral etiology and there have been sporadic reports of viral association. However, so far, no infectious agent has been proven to be etiologically related. In the present study, the authors examined HNL tissue samples for the presence of the genome of herpesviridae. A polymerase chain reaction was performed on 12 freshly frozen lymph nodes with HNL with a single pair of consensus primers selected within a highly conserved region of the DNA polymerase gene of the Epstein-Barr virus (EBV), designed to detect herpes simplex type 1 (HSV1), herpes simplex virus type 2 (HSV2), and cytomegalovirus (CMV) in addition to EBV. The amplified products of known sizes were then analyzed by a single restriction enzyme treatment for confirmation. No viral DNA was amplified in any of the 12 cases of histiocytic necrotizing lymphadenitis. The authors conclude that there is no evidence that HSV1, HSV2, CMV, or EBV plays any role in the pathogenesis of histiocytic necrotizing lymphadenitis.

Key Words : *Histiocytic necrotizing lymphadenitis, Kikuchi's disease, PCR, HSV1, HSV2, CMV, EBV*

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INTRODUCTION

Histiocytic necrotizing lymphadenopathy (HNL) is a fairly common disorder among Asians including Koreans (1, 2). First described in 1972 by Kikuchi (3) and Fujimoto (4), HNL has been reported throughout the world (1, 4-7). Patients are usually young female adults in excellent general condition with complaints of unexplained fever and cervical lymphadenopathy which is associated with pain in almost half of the cases (7). Occasionally, HNL presents with a skin manifestation (8). Splenomegaly can be seen in a minority of patients (5, 9). Laboratory tests might reveal an elevated erythrocyte sedimentation rate, neutropenia and atypical lymphocytosis. In most cases, HNL resolves spontaneously within 2 months (6), although rare recurrence has been reported (2, 7, 10). Systemic involvement and even a fatal outcome have also been reported (11, 12). Although its association with lupus erythematosus is well recognized (13), the etiology of HNL still remains unknown (6). In the present study, the authors investigated the presence of the viral genome of herpesviridae in 12 serial cases of histiocytic necrotizing lymphadenitis by polymerase chain reaction (PCR) using a consensus primer selected within a highly conserved region of viral DNA polymerase gene.

MATERIALS AND METHODS

Tissues

Twelve cases of HNL diagnosed from 1991 through 1993 with available frozen tissue were included in the study. The histologic diagnosis of HNLs were confirmed by 2 pathologists (JH, GG) in all cases. In each case, characteristic geographic necrosis containing karyorrhectic debris were present with proliferation of plasmacytoid monocytes, lymphocytes, monocytes, and immunoblasts. In no case, neutrophils were conspicuous. In parallel, an EBV-infected human B-cell line ARH-77, a known case of EBV-positive T-cell non-Hodgkin's lymphoma, a case of EBV-positive Hodgkin's disease, CMV-infected human embryo lung cells, and HSV I and HSV II cultivated in

a Vero cell line were tested as positive controls. The specimens were obtained fresh, snap-frozen in isopentane cooled in liquid nitrogen, and stored at -70℃ until used.

DNA extraction

Using a cryostat, five 10 μm sections were obtained from the freshly frozen lymph node tissues. The sections were incubated with proteinase K (200 μg/ml) at 56℃ for 5 min. After addition of an equal amount of saturated phenol (pH 8.0) and centrifugation at 12,000 rpm for 8 min, the supernatant was mixed with an equal amount of phenol-chloroform (25:24). After centrifugation, the supernatant was mixed with 2.5 volume of absolute ethanol, and was cooled to -20℃ for 1 hour. After removal of the ethanol, the pellet was washed with 70% ethanol, which was followed by recentrifugation at 12,000 rpm for 5 min. DNA was resuspended in 10 μl of D.W.

Polymerase chain reaction

PCR was done using two 20 oligomers deduced from the sequence of the DNA polymerase gene of EBV corresponding to the base sequences from 154979 to 154998, and 154475 to 154494 of EBV strain B95-8 (14) as primers. These primers amplify the sequences of CMV into 589 bp product, HSV-1 and HSV-II into 518 bp product, and EBV into 524 bp product (14, 15, 16).

P1 : 5'-CGACTTTGCCAGCCTGTACC-3'

P2 : 5'-AGTCCGTGTCCCCGTAGATG-3'

The presence of amplifiable DNA was evaluated by performing PCR amplification on all samples using primers of sequences of the β-globin gene as previously described (17). The PCR protocol utilized the hot start technique to prevent nonspecific annealing. The extracted DNA (2 μl) was admixed with 10X diluted PCR buffer (Perkin-Elmer, U.S.A.) containing 50 mM KCl, 10 mM Tris-HCl (pH8.3), 15 mM $MgCl_2$, 0.01% gelatin, 5% dimethylsulfoxide, 200 μM each of deoxynucleoside triphosphates, and 10 pmole each of oligonucleotides (P1, P1), and was heated to 95℃ in an automated thermal cycler (Perkin-Elmer GeneAmp 9600) for 5 min. After this step, 1.25 U of *Taq* polymerase (Cetus corp, U.S.A.) was added. The solution was covered with 100 μl of preheated sterile mineral oil. The amplification cycle, which consisted of 10 seconds of denaturation at 95℃, 10 seconds of annealing at 55℃, and 10 seconds of extension at 72℃, was repeated 40 times with final extension at 72℃ for 3 min.

Analysis of PCR products

The amplified product (8 μl each) was loaded onto a 2.5% agarose gel containing ethidium bromide and was subjected to electrophoresis at 80 volts for 20 min. After the electrophoresis, the gel was examined under UV light for the presence of bands corresponding to each member of Herpesviridae; 589 bp-sized product of CMV, 518 bp-sized products of HSV I and II, and 524 bp-sized product of EBV. When the bands of the desired sizes were present, 5 μl of the amplified product was digested with 2 units of *Sma*I or *Bam*HI in 2 μl of enzyme buffer (10X, Toyobo buffer, Japan) and 15 μl of D.W. at 30℃ in case of *Sma*I and 37℃ in case of *Bam*HI. The 589 bp product of CMV remains undigested with *Sma*I or *Bam*HI. *Sma*I cleaves the 518 bp-sized product of HSV1 into 476 bp and 42 bp chains, while *Bam*HI cleaves the 518 bp product of HSVII into 225 bp and 293 bp chains. The 524 bp product of EBV is digested into 422 bp and 102 bp chains by *Sma*I digestion and 273 bp and 251 bp fragments by *Bam*HI (18, 19).

RESULTS

There were 4 males and 8 females. Patients' ages ranged from 12 to 34 years (median age: 21 years). All patients presented with cervical lymphadenopathy. Pathologically, the cases consisted of 7 necrotizing, 3 proliferative and 2 xanthomatous types, according to Kuo's criteria (1995). All the tissue samples contained an ade-

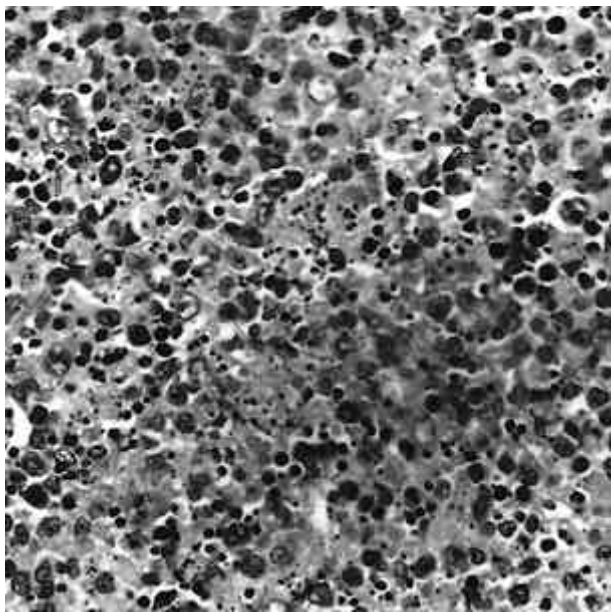

Fig. 1. H & E stain of case 1 (original magnification ×200). A case of Kikuchi's disease showing characteristic karyorrhectic debris and proliferation of plasmacytoid monocytes, histiocytes, immunoblasts and lymphocytes. Neutrophils are conspicuously absent.

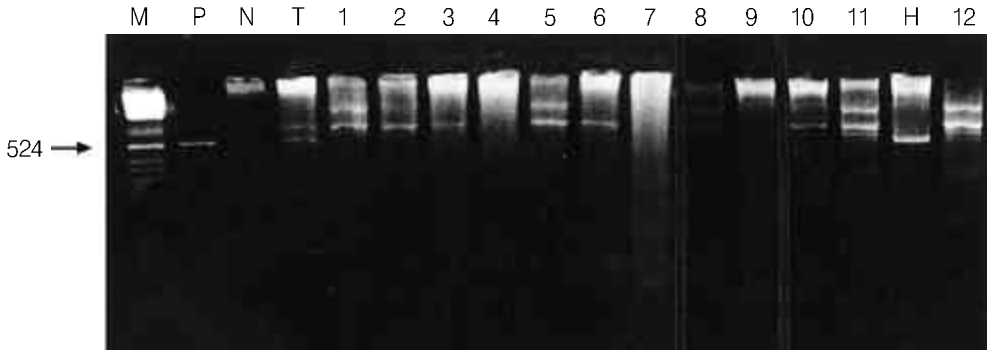

Fig. 2. Agarose gel electrophoresis of amplified products. Bands of 524 bp corresponding to Epstein-Barr virus were amplified in the EBV infected cell line and lymphomas with known EBV positivity, while in 12 cases of HNL, no viral genome was amplified.
(M: molecular marker. P: EBV infected human B cell line ARH-77. T: T-cell non Hodgkin's lymphoma with known EBV positivity. H: Hodgkin's disease with known EBV positivity. Lane 1-12: SNL)

quate amount of high molecular weight DNA for PCR. The results of the PCR study are illustrated in Fig. 2. No band corresponding to the viral DNA of CMV, EBV, HSV I or HSV II was amplified in any of the twelve cases of HNL. In the positive controls of EBV (lane P, T, and H), the PCR products of 524 bp corresponding to EBV were amplified, which were then cleaved by *Bam*HI and *Sma* into 273 bp and 251 bp, and 102 bp and 422 bp fragments, respectively (Fig. 3). The positive control of CMV, HSV I and HSV II showed bands of 589 bp, 518 bp and 518 bp products, respectively, and on digestion with *Sma*I or *Bam*HI, showed appropriate digestion pattern (data not shown).

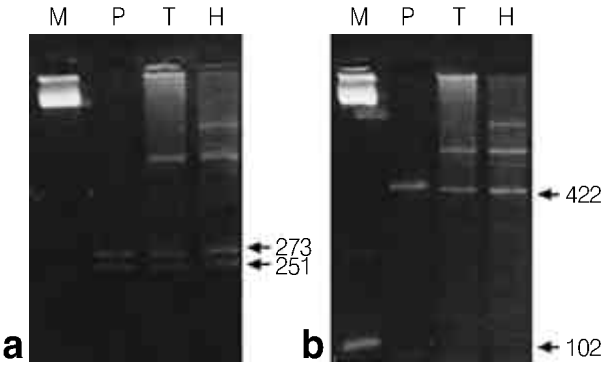

Fig. 3. Characterization of the PCR products of 524bp as EBV by restriction enzymes digestion.
a. *Bam* HI digestion into 273bp 251bp fragments
b. *Sma* I digestion into 422 bp and 102 bp fragments
(M: molecular marker. P: EBV infected human B cell line ARH-77.T-cell non Hodgkin's lymphoma with known EBV positivity. H: Hodgkin's disease with known EBV positivity. Lane 1-12: SNL)

DISCUSSION

HNL is a distinctive clinicopathologic entity histologically characterized by necrotizing lymphadenitis with karyorrhectic debris in the paracortical zone of lymph nodes (6). In the karyorrhectic focus, variable proliferations of immunoblasts, histiocytes, and plasmacytoid monocytes are observed (20). The karyorrhectic debris has been proven to be apoptotic bodies recently, and has been attributed to infiltrating cytotoxic T-lymphocytes (21). Sumiyoshi reported the presence of alpha interferon in histiocytes of HNL and interpreted this finding to be suggestive of a viral etiology (22). This and many of the clinical findings including fever, prodrome, self-limited nature, absence of response to antibiotics and lymphocytosis have lead numerous investigators to look into viral infection as a cause of Kikuchi's disease. Parvovirus B 19 infection has been observed in a case of Kikuchi's disease associated with systemic lupus erythematosus (23). Herpesviridae has been most extensively studied. Since the infection of most herpesviridae is lifelong with long latency and reactivation, they are not incompatible with the clinical presentation of Kikuchi's disease, in view of its onset in early adult life (as in the case of Epstein-Barr virus in developed countries), usual self-limited course with occasional fatal outcome, and rare but documented recurrences. Hirai identified the viral genome of Epstein-Barr virus in peripheral mononuclear cells in a Japanese female patient using PCR (24). Using PCR studies and in situ hybridization studies, Takano et al. did not detect EBV genome in the lymph nodes of Japanese patients with HNL (25). A study of American patients by Hollingswood et al. reported the detection of EBV but interpreted this finding to be insignificant

(26). That study also examined the presence of HHV 6, but did not detect any viral genome. The absence of HSV1, HSV2, EBV or CMV in HNL lymph nodes in the present study is in agreement with the previous studies. Since the sera of these patients have not been examined, we can not rule out the possibility of recent past infection with a very low viral burden below the level of detection by PCR or in situ hybridization. However, since the fevers of most patients are of recent onset, that is highly unlikely.

In conclusion, this study confirms the absence of HHV1, HHV2, CMV, or EBV genome in KFD. Future studies should be directed at other possible etiologic agents or immunologic abnormality.

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