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SHORT COMMUNICATION

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Analysis of herpesvirus genomes in Kikuchi's disease

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Abstract We examined the cervical lymph nodes of 30 patients with Kikuchi's disease and 15 patients with non-specific lymphadenitis, using Southern blot analysis and polymerase chain reaction (PCR) to identify human herpesviruses such as Epstein-Barr virus (EBV), cytomegalovirus, herpes simplex virus, and varicella-zoster virus. By Southern blot analysis, no virus DNA was recognized, but 16 of the 30 nodes from patients with Kikuchi's disease and 8 of the 15 nodes from patients with non-specific lymphadenitis showed amplified EBV DNA by PCR.

Key words Kikuchi's disease · Herpes virus
Lymph node

Introduction

Kikuchi's disease was first reported in 1972 as lymphadenitis with focal proliferation of reticular cells accompanied by extensive nuclear debris and numerous phagocytes (Kikuchi 1972). Since then, many cases have been reported in Japan (Fujimori et al. 1981; Fujimoto et al. 1972; Kikuchi et al. 1977, 1978, 1986, 1990). There have also been reports of patients from Europe, the United States and Asia (Ali and Horton 1985; Chamulack et al. 1990; Chan and Saw 1983; Feller et al. 1983; Turner et al. 1983).

Histologically, the lesion shows a focal proliferation of activated lymphoytes, and of histiocytes with extensive nuclear debris in the enlarged paracortex (Kikuchi et al. 1977, 1990). This disease, which mainly affects adolescents, causes swelling of cervical lymph nodes, leukopaenia, occasional skin rashes, and an elevation of lactate dehydrogenase and transaminase activities (Kikuchi et al. 1990). The histogenesis and pathogenesis of the lesion are still unknown but, because of its clinical features and some histological findings, viral infection is most probable. Epstein-Barr virus (EBV) (Takada et al. 1980), human herpesvirus-6 (HHV-6) (Eizuru et al. 1989; Kikuchi et al. 1992) and other viruses have been suspected but no causative virus has been conclusively identified.

Recently, we analysed the possible association between HHV-6 and Kikuchi's disease, but we could not demonstrate a specific relationship between them using Southern blot analysis, polymerase chain reaction (PCR), or in situ hybridization (Sumiyoshi et al. 1993). In the present study, to address our suspicions of a possible association between Kikuchi's disease and other herpesviruses such as varicella-zoster virus (VZV), herpes simplex virus (HSV), cytomegalovirus (CMV), and EBV, we examined biopsy specimens of cervical lymph nodes used Southern blot analysis and PCR to detect the virus genomes.

Materials and methods

A total of 30 samples of Kikuchi's disease were selected from the files in the Department of Pathology, Fukuoka University, Japan. Lymph node specimens were removed and fixed in formalin or B-5 solution, embedded in paraffin, and stained by haematoxylin-eosin, Giemsa, periodic acid-Schiff, Gomori's silver impregnation for reticulin fibers, or immunohistochemistry. Parts of the specimens were stored at −80° C in liquid nitrogen and/or in a deep freezer. In addition to patients with Kikuchi's disease, we selected samples from 15 patients with non-specific lymphadenitis and used them for comparison purposes.

For Southern blot analysis part of the frozen material was used. High-molecular-weight DNA was extracted with phenol/chloroform and precipitated with ethanol. We cleaved 10 μg of DNA with restriction enzymes (*Eco*RI and *Bam*HI) and fractionated it according to size by 0.8% agarose gel electrophoresis. The sample was denatured and filtered with ^{32}P-radiolabeled probes. More details of our examination methods have been described previously (Yoshida et al. 1989). We used probes for ^{32}P-radiolabelled virus DNA, which were made with amplified virus DNA of the infected culture cells (provided by Yohiti Minamishima, Toshio Minematu, Miyazaki Medical College, and Koichi Yamanishi, Osaka Univer-

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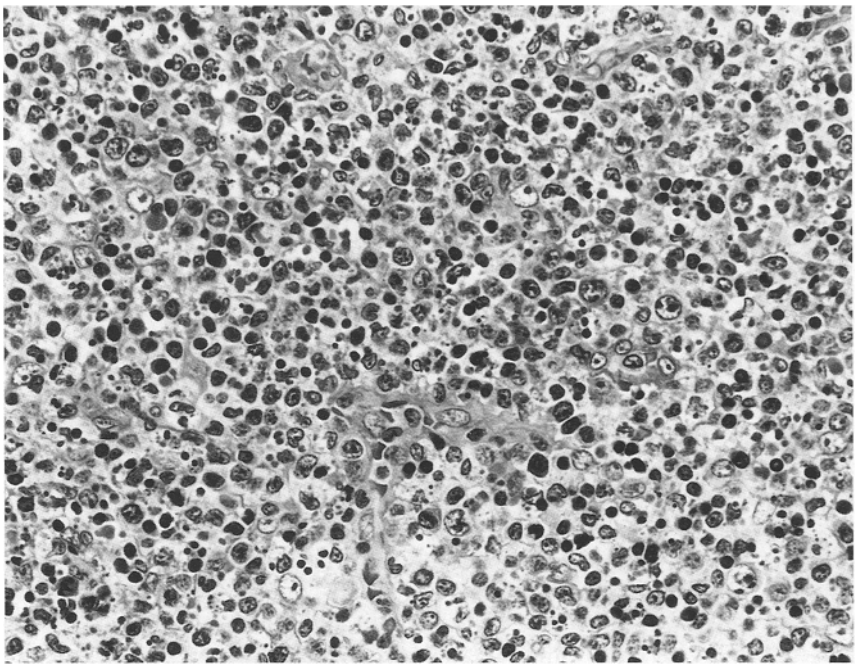

Fig. 1 Many transformed lymphocytes, including immunoblasts, plasmacytoid monocytes, numerous nuclear debris, and histiocytes with or without phagocytosis. HE, ×300

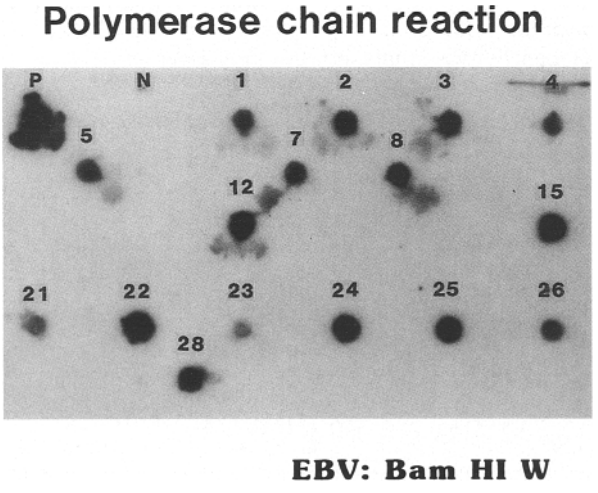

Fig. 2 Dot-blotting analysis with ^{32}P-labelled *Bam*HI W fragment of EBV in 30 of Kikuchi's disease case. Sixteen cases of Kikuchi's disease shows amplified EBV DNA. P: positive control for EBV DNA from EBV infected culture cells. *N*: negative control for placental DNA

sity) using PCR and radiolabelled with a-^{32}P-dCTP by the random hexamer primer technique.

Isolated DNA was used for PCR. Specific primers were synthesized, based on the published DNA sequences, and the primers used were as follows: for amplification of EBV, 5'-CCAGAGGTAAGTGGACTT-3' and 5'-GACCGGTGCCTTCTTAGG-3' (*Bam*HI W region) (Saito et al. 1989); for HSV, 5'-CATCACCGACCCGGAGAGGGAC-3' and 5'-GGGCCAGGCGCTTCTTGGTGTA-3' (DNA polymerase region) (Cao et al. 1989); for CMV, 5'GCAGAGCTCGTTTAGTGAACC-3' and 5'-GGCACGGGGAATCCGCGTTCC-3' (MIE region) (Yamaguchi et al. 1992); for VZV, 5'-CCGTATATGAGCCTTACTACCATTC-3' and 5'-GAGTTCATCAAACAGTGTGCTCGTG-3' (glycoprotein I gene region) (Kido et al. 1991). Amplifications were carried out with a GeneAmp DNA amplification regent kit and DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, Conn.). After amplification, one-hundredth of the reaction mixture was analysed by dot-blotting with the ^{32}P-labelled oligonucleotide probe (EBV: 5'-TTCTGCTAAGCCCAC-3'; HSV: 5'ACCGCCGAACTGAGCAGACAC-3'; CMV: 5'-CTCCTTAATACAAGCCATCCACATCTCCCG-3). The VZV probe alone was made with amplified virus DNA of the VZV-infected culture cells, using PCR and radiolabelled a-^{32}P-dCTP by the random hexamer primer technique.

Results

We examined lymph nodes from 45 patients: 30 with Kikuchi's disease and 15 with non-specific lymphadenitis. The patients with Kikuchi's disease are summarized below. Age distribution ranged from 12 to 53 years, with a mean age of 28.3 years. The ratio of men to women was 1.0: 1.7, showing a female predominance. All lymph nodes examined were obtained from the cervical area. Histologically, the affected nodes represented foci of transformed lymphocytes including immunoblasts, histiocytes with or without phagocytosis, plasmacytoid monocytes, necrobiotic cells, and extensive nuclear debris. Necrotic foci were present in almost all specimens. There were only a few plasma cells and granulocytes present in the affected foci many continued none of these cells (Fig. 1). On PCR examination (Fig. 2) 16 patients with EBV DNA were detected among the 30 patients with Kikuchi's disease. Likewise, we detected amplified EBV DNA in 8 of 15 patients with non-specific lymphadenitis. However, no patients were found to have CMV, HSV, or VZV DNA. On Southern blot analysis, we did not detect any herpesvirus DNA in any of the examined cases.

Discussion

Kikuchi's disease is a relatively common reactive lesion of lymph nodes in Japan and is readily mistaken for malignant lymphoma. It was first reported in 1972 as lymphadenitis with focal proliferation of reticular cells accompanying extensive nuclear debris and many histiocytes (Kikuchi 1972). Fujimoto et al. (1972) reported the same findings independently shortly thereafter. The characteristic clinical findings include localized lymphadenopathy on the neck; high fever; skin rash; leukopaenia; elevation of lactate dehydrogenase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase; and natural healing within several months (Kikuchi et al. 1990; Sumiyoshi et al. 1992). It primarily affects young adults. Because of these clinical manifestations, protozoa, microorganisms or viruses were thought to be causative agents. Elevated serum concentrations of 2'–5' oligoadenylate synthetase in the early stage of the disease (Kikuchi et al. 1990; Sumiyoshi et al. 1991a); the fragmentary appearance of tubuloreticular structures in the lymphocytes, histiocytes, and vascular endothelial cells (Eimoto et al. 1983; Imamura et al. 1982); and the recognition of numerous interferon-d possessing cells in the affected area (Sumiyoshi et al. 1991b) intensified the suspicion of viral infection. Some authors postulated the causative agents for Kikuchi's disease to be EBV (Takada et al. 1980) and HHV-6 (Eizuru et al. 1989) but no identification of a causative virus has been achieved. We recently analysed HHV-6 genomes in 27 patients with Kikuchi's disease, but our results indicated that the presence of HHV-6 genomes is not specifically related to Kikuchi's disease because the same genomes were also detected in other reactive lymph nodes (Sumiyoshi et al. 1993). We found some coexistence of Kikuchi's disease with high serum titers of EBV antibodies, but we could not substantiate the reports for detection of EBV from Kikuchi's disease using molecular biological methods. Because we suspected a possible association between other herpesviruses and Kikuchi's disease, we examined biopsy specimens of cervical lymph nodes to detect such other herpesvirus genomes as EBV, HSV, VZV, and CMV genomes, using Southern blot analysis and PCR. By Southern blot analysis we could detect no virus DNA from any of the examined cases. Using PCR, we detected amplified EBV DNA in some cases of Kikuchi's disease. These positive rates, however, were nearly the same as those of non-specific lymphadenitis. Based on recent experiments, it was possible to detect approximately one EBV-transformed cell per 100,000 uninfected-cell DNA equivalents. Therefore, we postulate that the presence of amplified EBV DNA identified by PCR comes simply from latent infected EBV in the lymphoid tissue.

We did not clarify the pathogenesis of Kikuchi's disease in this study. However, we obtained negative data demonstrating that the herpesviruses are not related to the pathogenesis of Kikuchi's disease. More studies for analysis of other viruses are needed to clarify the pathogenesis of Kikuchi's disease.

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