Analysis of human herpes virus-6 genomes in lymphoid malignancy in Japan

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Abstract
Ninety cases of malignant lymphoma and 56 cases of reactive lymphadenopathy were studied using Southern blot analysis and the polymerase chain reaction to identify human herpes virus-6 (HHV-6) DNA. This was detected in cases of lymphoid malignancy at a rate which ranged from 50-0% to 68-8%. There were no differences in rates for different types of lymphoid malignancies. Herpes virus-6 DNA was detected by PCR in lymphoid malignancies less frequently than in reactive lymphadenopathies. It was not detected in lymphoid malignancies using Southern blotting.

These results suggest that HHV-6 DNA was not related to lymphoid malignancy and was only a latent infection of non-neoplastic cells in tumour tissue.

Methods and Results
The lymph nodes obtained were fixed in B5 solution, embedded in paraffin wax, and stained with haematoxylin and eosin, Giemsa, periodic acid Schiff and Golgi's methenamine silver impregnation stain. We also immunostained B and T cells. Part of the specimens were stored at -80°C in liquid nitrogen or deep-frozen and these specimens were examined using monoclonal antibodies for B cells, T cells, and CD30. Samples of non-Hodgkin’s lymphomas were divided into B or T cell types using lymphocyte differentiation markers and classified according to the Lymphoma Study Group (Japan) classification. We used parts of the frozen material for DNA isolation. High molecular-weight DNA was extracted with phenol/chloroform and precipitated with ethanol. We cleaved 10 μg of DNA with restriction enzyme (EcoRI) and fractionated it according to size using 0-8% agarose gel electrophoresis. The samples were denatured and transferred to nylon membranes by Southern blotting and the filters were hybridised with 32P-radiolabelled HHV-6 DNA probes. The probe used for 32P-labelled HHV-6 DNA was made with amplified HHV-6 DNA of the HHV-6-infected cord blood (K Yamashini, Department of Virology, Research Institute for Microbial Diseases, Osaka, Japan) using PCR and radiolabelled with 32P using the random hexamer primer technique. Isolated DNA was used for PCR. Specific primers (P1: TAGGCTCAAGAACACATCTGCGAAGT, P2: TGTGCGCTTGTGCTGGACACT) were synthesised, based on the published DNA sequence, corresponding to the BamHI site of HHV-6 DNA (strain U1102). An amplification reagent kit and a DNA Thermal Cycler were used (Perkin-Elmer Cetus, Norwalk, Connecticut USA). Placental DNA was used as a negative control.

After 40 cycles of PCR amplification 10 μl aliquots of amplified product were detected by Southern blot analysis using 32P-labelled HHV-6 DNA probe. We examined lymph nodes from 90 patients with lymphoid malignancies and 56 patients with lymphadenitis.

The results are summarised in the table. All the samples were negative for HHV-6 DNA as determined by Southern blotting. Positive rates for detection of HHV-6 DNA by PCR ranged from 50-0% to 68-8%. No differentiation in detected rates were evident between Hodgkin’s and non-Hodgkin’s lymphomas, T cell and B cell lymphomas, and other non-neoplastic tissues.
Discussion

HHV-6 is a new member of the herpes virus group. The predominant affinity of CD4 positive T cells and a high incidence of latent infection with the virus suggest that HHV-6, like other such viruses, may cause specific lymphocyte related disease or may have some role in opportunistic infection. Recently, Josephs et al and Jarrett et al reported that HHV-6 DNA had been detected in three of 82 B cell lymphomas and two of 117 lymphomas using Southern blot analysis. Buchbinder et al and Torelli et al also reported detecting HHV-6 DNA in 23 of 25 different malignant lymphomas, three of 25 Hodgkin's lymphomas, and none of 41 non-Hodgkin's lymphomas using PCR methods. A definite association between malignant lymphoma and HHV-6 however, was still unclear.

In our study we found amplified HHV-6 DNA using PCR in 50-0-68-8 % of samples studied, but found no clear differentiation in rates of detection among different types of malignant lymphoma. Using Southern blot analysis, we were unable to detect any HHV-6 DNA. Using PCR, Kondo et al identified HHV-6 infected monocyte/macrophage cells in lymphadenopathies. We also clarified that HHV-6 infected CD68-positive monocyte/macrophage cells existed in a latent form, using double staining in situ hybridisation and immunohistochemistry methods. Therefore deduced that the presence of HHV-6 DNA shown by PCR in lymphoid malignancies was derived from latent infection. In this study we were unable to determine the HHV-6 positive cell subsets, but the HHV-6 positive cells might be infected in monocyte/macrophage cells as they are the same as in benign lymphadenitis.

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