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.

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Human herpes virus-6 (HHV-6) has been isolated from the peripheral blood cells of patients with various lymphoproliferative disorders.^{1,2} Yamanishi *et al* discovered that HHV-6 causes exanthema subitum,³ but the natural history of HHV-6 and the spectrum of related diseases are still unclear. The predominant affinity of CD4 positive T cells⁴ and a high incidence of latent infection with the virus suggests that HHV-6, like other herpes viruses, may cause specific lymphocyte-related disease or may have some role in opportunistic infection. Several reports suggest that this virus infection may be associated with atypical lymphoid proliferations.⁵⁻⁹

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 Gomori'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 Yamanishi, 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: TAGCCTCAGACAATCTGGCAAAGT, P2: TGTGGCTTTTGTCTGTTCCGACT) were synthesised, based on the published DNA sequence,⁷ corresponding to the *Bam*HI 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

Herpes virus-6 genomes in malignant lymphoma

Diagnosis	No of cases	PCR (%)	Southern blot
Hodgkin's disease	14	9 (64.3)	0
B cell lymphoma	29	18 (62.1)	0
diffuse large	7	4	0
diffuse medium	7	4	0
diffuse small	7	5	0
follicular	8	5	0
T cell lymphoma	41	24 (58.5)	0
diffuse large	8	5	0
diffuse medium	7	4	0
diffuse pleomorphic	10	5	0
lymphoblastic	8	5	0
AILD	8	5	0
anti HTLV-1 antibody positive	16	11 (68.8)	0
anti HTLV-1 antibody negative	10	6 (60.0)	0
Anaplastic large cell lymphoma	6	3 (50.0)	0
Benign lymphadenitis	56	55 (98.2)	0

anti-HTLV-1 antibody positive and negative cases.

Discussion

HHV-6 is a new member of the herpes virus group.¹ The predominant affinity of CD4 positive T cells⁴ 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 lymphomas⁸ and two of 117 lymphomas using Southern blot analysis.⁷ Buchbinder *et al* and Torelli *et al* also reported detecting HHV-6 DNA in 23 of 25 different malignant lymphomas,⁹ three of 25 Hodgkin's lymphomas, and none of 41 non-Hodgkin's lymphomas⁶ 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.¹⁰ 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.¹¹ We 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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- 1 Salahuddin S, Ablashi DV, Markham PD, *et al*. Isolation of new virus, HTLV, in patients with lymphoproliferative disorders. *Science* 1986;234:596–601.
- 2 Lusso P, Salahuddin SZ, Ablashi DV, *et al*. Diverse tropism of human B-lymphotropic virus (human herpesvirus-6). *Lancet* 1987;ii:243–4.
- 3 Yamanishi K, Toshiomi O, Kimiyasu S, *et al*. Identification of human herpesvirus-6 as a causal agent for exanthema subitum. *Lancet* 1988;i:1065–7.
- 4 Takahashi K, Sonoda S, Higashi K, *et al*. Predominant CD4 T-lymphocyte tropism of human herpesvirus-6 related virus. 1989;63:3161–3.
- 5 Jarrett RF, Gledhill S, Qureshi F, *et al*. Identification of human herpesvirus 6-specific DNA sequences in two patients with non-Hodgkin's lymphoma. *Leukemia* 1988;2:496–502.
- 6 Torelli G, Marasca R, Luppi M, *et al*. Human herpesvirus-6 in human lymphomas: identification of specific sequences in Hodgkin's lymphomas by polymerase chain reaction. *Blood* 1991;77:2251–8.
- 7 Borisch B, Ellinger K, Neipel F, *et al*. Lymphadenitis and lymphoproliferative lesions associated with human herpesvirus-6 (HHV-6). *Virchows Arch (Cell Pathol)* 1991;61:179–87.
- 8 Josephs SF, Buchbinder A, Streicher HZ, *et al*. Detection of human B-lymphotropic virus (human herpesvirus-6) sequences in B cell lymphoma tissue of three patients. *Leukemia* 1988;2:132–5.
- 9 Buchbinder A, Josephs SF, Ablashi DV, *et al*. Polymerase chain reaction amplification and in situ hybridization for the detection of Human B-lymphotropic virus. *J Virol Methods* 1988;21:191–7.
- 10 Kondo K, Kondo T, Okuno T, *et al*. Latent human herpesvirus-6 infection of human monocytes/macrophages. *J Gen Virol* 1991;72:1401–8.
- 11 Sumiyoshi Y, Kikuchi M, Ohshima K, *et al*. Human Herpesvirus-6 genomes in histiocytic necrotizing lymphadenitis (Kikuchi's disease) and other forms of lymphadenitis. *Am J Clin Pathol* 1993;99:609–14.

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Inaccurate haemoglobin estimation in Waldenström's macroglobulinaemia: unusual reaction with monomeric IgM paraprotein

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Abstract

Automated blood counts from a patient with Waldenström's macroglobulinaemia repeatedly failed critical limit standards set for mean cell haemoglobin concentration and mean cell haemoglobin. Haemoglobin estimation was higher than that suggested by clinical examination, symptoms, and the spun haematocrit. This was found to be due to an interaction between the Coulter lysing agent and monomeric IgM paraprotein in the

patient's plasma, creating a precipitate which was optically dense at 525 nm.

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Automated blood analyses have greatly increased the availability and accuracy of full blood counts. Haemoglobin measurements are made by converting haemoglobin to cyanmethaemoglobin and then measuring absorbance at 525 nm. The mean cell