

**BRIEF REPORT**

Expression of Cell-Homologous Genes of Human Herpesvirus-8 in Human Immunodeficiency Virus-Negative Lymphoproliferative Diseases

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**HUMAN HERPESVIRUS-8 (HHV-8) GENE EXAMINATION**

The **HHV-8** genome encodes for genes homologous to human cellular genes such as interleukin-6 (IL-6), Cyclin-D, BCL-2, and IL-8 receptor (G-protein-coupled receptor [GCR]). We used reverse transcriptase-polymerase chain reaction to study the expression of these viral genes in lymphoproliferative disorders associated with HHV-8 infection. None of these genes was expressed in 1 case of benign, localized Castleman’s disease (CD), and only viral IL-6 and viral Cyclin-D were transcribed in 2 cases of benign lymphadenopathies with giant germinal center hyperplasia and increased vascularity. In contrast, all 4 genes were transcribed in 1 case of multicentric CD of plasma cell type with aggressive clinical course and in 1 primary effusion lymphoma cell line. Our study provides the evidence that various HHV-8 genes, homologous to cellular genes involved in control of proliferation and apoptosis, may be differently expressed in different lymphoid disorders in vivo.

**RESULTS AND DISCUSSION**

The results of the expression studies are summarized in Table 1 and in Fig 1. Expression of all 4 HHV-8 transcripts was undetectable in the HHV-8 DNA-negative samples (not shown; Fig 1).

Our study reports, for the first time, that (1) HHV-8 genes, homologous to cell genes, may be transcribed in lymphoid tissues in vivo, out of the KS and acquired immunodeficiency syndrome (AIDS) settings; (2) the expression of such viral genes is apparently different in the different lymphoproliferative diseases of our series, associated with HHV-8 infection, namely in benign (reactive lymphadenopathy and localized CD

**PATIENTS AND METHODS**

Although the patient with localized CD of HV type had a benign clinical course and is still well, 5 years after diagnosis, without therapy, the patient with MCD of PC type suffered from a severe systemic disease, with massive lymphadenopathies and constitutional symptoms, resistant to treatment, and died 2 years after diagnosis from an opportunistic infection.

All of the tested samples harbored HHV-8 DNA sequences as detected by PCR, and the viral load was roughly similar to that found in 10 KS biopsies examined. Total RNA was also isolated from the HHV-8-infected PEL cell line, HBL-6 (kindly provided by Dr G. Gaidano, University of Eastern Piedmont, Novara, Italy), from the HHV-8-negative/Epstein-Barr virus (EBV)-positive Burkitt’s lymphoma cell line, Raji, from 10 HHV-8-negative lymph node biopsies. Expression of all 4 HHV-8 genes, namely vIL-6, v-cyclin-D, vBCL-2, and vGCR, was examined by standard RT-PCR technique performed on 1 µg RNA, after elimination of contaminating genomic DNA with RNase-free DNase, followed by phenol-chloroform extraction, as described elsewhere. Positive results were evaluated after hybridization of the first amplified product with a specific internal oligonucleotide probe (v-cyclin-D and vGCR; Fig 1B and D) or after reamplification of the first amplified product and visualization on ethidium bromide (v-IL-6 and vBCL-2; Fig 1A and C). Contamination by genomic DNA was excluded, because we failed to identify PCR products when using the DNase-treated RNA preparations in the absence of the RT reaction. As a standard, the actin gene was amplified from the same reverse transcribed samples (not shown).

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of HV type), atypical (multicentric CD [MCD] of PC type), and malignant (PEL) lymphoproliferative diseases. Our findings, although obtained on these few, rare cases, also suggest that the pattern of expression of HHV-8 genes homologous to cellular genes involved in cell proliferation (v-cyclin-D and vGCR) and apoptosis (vIL-6 and vBCL-2) may influence the nature of the lymphoproliferative process associated with this herpesviral infection.

Consistent with the transcription mapping of HHV-8 genome in the BC-1 lymphoma cell line using Northern blot analysis, our study may also suggest that, in the 2 cases of benign lymphadenopathies, the expression of v-cyclin-D and vIL-6 reflects a predominantly latent HHV-8 infection. Conversely, the expression of vGCR, documented in the case of MCD of PC type as well as in the HBL-6 cell line, suggests that HHV-8 is likely to replicate there, at least in a proportion of infected cells.

Expression studies on a larger series of different lymphoproliferative diseases are necessary to understand if the differential expression of some HHV-8 genes is one of the possible mechanisms of HHV-8–induced lymphoproliferation in vivo.5,7

REFERENCES


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