Human Herpesvirus-6 in Human Lymphomas: Identification of Specific Sequences in Hodgkin’s Lymphomas by Polymerase Chain Reaction

By Giuseppe Torelli, Roberto Marasca, Mario Luppi, Licia Selleri, Sergio Ferrari, Franco Narni, Maria Teresa Mariano, Massimo Federico, Luca Ceccherini-Nelli, Mauro Bendinelli, Giuliano Montagnani, Marcello Montorsi, and Tullio Artusi

In search of a possible involvement of the human herpesvirus type 6 (HHV-6) in human Hodgkin’s and non-Hodgkin’s lymphomas, we studied the levels of anti–HHV-6 antibodies in the sera of 94 cases by an immunofluorescence assay, as well as the presence of HHV-6 sequences in the affected tissues of 66 cases by polymerase chain reaction, using one set of primer oligonucleotides. Our results showed higher anti–HHV-6 antibody titers in human lymphomas than in normal blood donors, but the difference is statistically significant only when normal donors are compared with Hodgkin’s lymphoma cases. HHV-6 sequences were detected in 3 of 25 Hodgkin’s lymphomas and 0 of the 41 cases of non-Hodgkin’s lymphomas studied. The three cases positive for HHV-6 sequences belong to the nodular sclerosis-lymphocyte depletion histologic subtype and share remarkable similarities in their clinical features. Furthermore, Southern blot analysis of total genomic DNA obtained from the neoplastic tissues of two of the three patients showed the same restriction fragment length polymorphism. Our results suggest that: (1) the high level of anti–HHV-6 antibodies in Hodgkin’s disease is due to an activation of the immune system not related to the presence of HHV-6 sequences in affected lymph nodes; (2) the presence of HHV-6 sequences in human lymphoid tissues is not a frequent event, rather it is in fact a very rare event in non-Hodgkin’s lymphomas, while in Hodgkin’s cases it is more frequent than previously reported on the basis of Southern blot analysis; and (3) the presence of HHV-6 sequences in Hodgkin’s lymphomas may have a relation with the clinical presentation of the disease.

Human herpesvirus type 6 (HHV-6), the last human herpesvirus identified, was isolated in 1986 from six patients (five from United States and one from Jamaica) affected by lymphoproliferative disorders. Three of those patients were affected by lymphoma and two were seropositive for human immunodeficiency virus (HIV) antibodies. From a morphologic point of view, HHV-6 is an enveloped virion with an icosahedral nucleocapsid of 162 capsomers. The genome of HHV-6 is a linear, double-stranded DNA of about 170 kb, with a G + C content of approximately 40%. The genome of different isolates shows restriction fragment length polymorphism. To date, few data are available on the nature and function of HHV-6-specific proteins, although sera from infected individuals can recognize more than 30 virus-specific proteins in HHV-6-infected human T lymphocytes. Only recently the identification, cloning, and expression of the major capsid protein gene of HHV-6 were reported. HHV-6 efficiently infects not only CD4 T lymphocytes but also B lymphocytes, whereas cells of other lineages, such as megakaryocytes and glioblastoma cells, can be infected at much lower levels. Because the HHV-6 can infect human lymphocytes, it was tentatively classified as a member of the subfamily of the γ-herpesvirus, together with Epstein-Barr virus (EBV) and herpesvirus saimiri. However, there is now evidence that HHV-6 genome has important homologies with human cytomegalovirus (HCMV), the prototype of the β-herpesvirus subfamily. In host range studies of HHV-6 in several primate species, only human and chimpanzee T lymphocytes were found to be susceptible to HHV-6 infection. The prevalence of anti–HHV-6 IgG antibodies in the normal human population is very high, exceeding the 80% of the tested subjects in some surveys. This value, already present in newborn infants, decreases during the first 4 to 5 months of life and then increases again to reach the value found in adults by the age of 2 years. This behavior is consistent with a passage of maternal anti–HHV-6 antibodies to newborns and with a successive, widespread, and precocious primary infection. HHV-6 has been recognized as the etiologic agent of exanthem subitum, the so-called sixth disease of infants. The primary infection in seronegative adults has been related with different clinical pictures: chronic fatigue syndrome, fever with persistent lymphadenopathy, and hepatitis. HHV-6 was first isolated from lymphoproliferative diseases and HHV-6 sequences have been detected in few lymphoma cases. The role of HHV-6 infection in human lymphomas is still uncertain. Information on viral persistence, latency, reactivation, and sites of replication in vivo is needed to ascertain the role of HHV-6 in the etiopathogenesis of human lymphoid neoplasias. For this reason we decided to investigate the immune response against HHV-6 in Hodgkin’s lymphoma (HL) and non-Hodgkin’s lymphoma (NHL) cases, and the presence of HHV-6 sequences in a well-characterized series of human, uncultured lymphoma tissues obtained from Hodgkin’s and non-Hodgkin’s cases. The polymerase chain reaction (PCR) amplification technique was used because of its high sensitivity.

MATERIALS AND METHODS

Indirect Immunofluorescence Assay (IFA)

Replicating HSB-2 cells, treated with Polybrene (5 μg/mL; Sigma, St Louis, MO) for 2 hours, were infected with virus-rich culture supernatants from HHV-6/HSB-2 previously infected and dying cells. Five days later, large refractile cells, indicative of a...
successful infection, were prevailing in the culture. The cells were separated from cell debris on Ficoll (Sigma), applied to glass slides, and with fixed with 50% acetone. The slides were incubated with a control-positive, human serum and developed using a fluorescent-nated, goat antihuman IgG serum (Cappel-Organon Teknika, Veedijk, Belgium). IFA showed a characteristic, prevalently granular, nuclear staining of acetone-fixed cells. Antibody titration was performed double blind by two operators. The assay was performed on 200 sera from normal blood donors and on 94 sera collected from human lymphoma patients at the time of diagnosis. The sera used were stored at –20°C for no longer than 20 months.

Sources of Human Specimens and Clinical Histories

Genomic DNA was purified from 62 lymph node biopsies, from two fragments of surgically removed spleens, and from two cellular pellets derived from massive pleural effusions. All tissues were largely infiltrated by pathologic elements, while the cells from pleural effusions were more than 80% neoplastic. All the NHL cases were subjected to standard immunophenotyping procedures. All Hodgkin’s samples were from peripheral lymph nodes; in two cases the DNA extracted from peripheral blood mononuclear cells (PBMC), separated by Ficoll, was also examined. The clinical histories of the patients bearing the HHV-6 sequences were as follows.

Case 5. The patient, a woman born in 1960, was referred to the hospital in 1987 because of the presence of intermittent fever of unknown origin associated with left cervical lymphadenopathy. A standard chest radiography showed a bulky mediastinal involvement. The chest computed tomographic (CT) scanning confirmed the mediastinal mass, showing also a right hilar lymphadenopathy and the presence of small right pleural and pericardial effusions. The cervical lymph node biopsy showed a picture of nodular sclerosing Hodgkin’s disease (HD) of the subtype defined by Lukes as nodular sclerosis/lymphocyte depleted (NS-LD) and by Bennett et al as NS-LD with a prevailing “pleomorphic” basic pattern. The large pleomorphic Sternberg cells accounted for about 25% of the cellular elements. The patient, classified as stage II B with bulky mediastinal disease, was treated with associated chemotherapy (melphalan, vincristine, procarbazine, doxorubicin, bleomycin, vinblastine, and dacarbazine) and radiation therapy. The therapy was well tolerated and the patient reached a complete remission that still holds.

Case 12. The patient, a woman born in 1958, first observed bilateral cervical lymphadenopathy in August 1986 but underwent medical examination only 4 months later, complaining of intermittent fever, weight loss, night sweats, fatigue, and initial wheeziness. At the time of admission to the hospital, generalized lymphadenopathy and hepatosplenomegaly were present, together with bilateral pleural effusions and ascites. The biopsy of an axillary lymph node showed a histopathologic picture consistent with nodular sclerosing HD of the subtype NS-LD according to Lukes and NS-LD with a prevailing “fibrohistiocytic” pattern according to Bennett. The lacunar cells accounted for about the 25% of all cellular elements. The total body CT scanning showed mediastinal and retroperitoneal lymphadenopathy with hepatic and splenic involvement. The case was classified as a stage IV B advanced HD and treated with several chemotherapeutic agents (melphalan, vincristine, bleomycin, cyclophosphamide, epirubicin, and etoposide). Despite an initial response, the patient died 15 months after the diagnosis, with extranodal pulmonary involvement and massive pleural and pericardial effusions, despite the intensive chemotherapy applied.

DNA Extraction and Southern Blot Analysis

The DNA was extracted from the solid tissue specimens with the technique described by Enrietto et al and from cellular pellets as reported elsewhere. To confirm the lineage origin of the affected cells in NHL cases, DNA samples obtained from each specimen were digested with EcoRI, BamHI, and HindIII restriction endonucleases and were hybridized to probes corresponding to Ig heavy and light chain genes and to the T-cell receptor b-chain (TCR-b) gene, as described elsewhere. The same enzymes and the same standard Southern procedure were used in a restriction pattern analysis of the DNAs of the Hodgkin’s samples positive for HHV-6 sequences. The cleaved DNAs were hybridized with the plasmid pZVH14, containing a 9-kb segment of the HHV-6 sequence, and pSl76, containing two copies of the EBV Bam W internal repeated sequences, and with Pr-1 (Oncogene Science Inc, Manhasset, NY), a commercially available genomic restriction fragment representing a portion of the human ber gene.

Synthesis of Oligonucleotide Primers and Probes

Unmodified deoxyribonucleotides were synthesized on an automated solid-phase synthesizer (Applied Biosystems Inc, Foster City, CA, model 381A) by standard phosphoramidite chemistry. After several extraction cycles with ammonium hydroxide, the oligonucleotides were ethanol precipitated, lyophilized, and dissolved directly in twice-distilled water.

The oligonucleotides used in the HHV-6 PCR assays have the following sequence: (1) 5’ CCCCCATATGAGTTCTGCAC- CACATCTCTCCTC 3’ as sense primer; (2) 5’ TTCAGGGACCCT- TATGCTTATTGACGATGTCG 3’ as antisense primer; and (3) 5’ CGGTAAAAAATTTACACCTTCCATTCATTCT 3’ as internal probe.

For EBV PCR tests the sequences of primers (TC60 and TC51) and probe (TC62) were as specified by Saito et al.

PCR

One microgram of each DNA sample was included in a 100-μL reaction mixture containing 50 mmol/L KCl, 10 mmol/L Tris (pH 8.3), 2.5 mmol/L MgCl₂, 225 μmol/L dATP, 225 μmol/L dCTP, 225 μmol/L dGTP, 225 μmol/L dTTP, 200 μg/mL of bovine serum albumin, 50 pmol of each primer, and 2 U of thermostable Taq DNA polymerase (Promega, Madison, WI). The sample denaturation was performed at 94°C for 1 minute, the annealing at 53°C for 1 minute, and the primer extension step was performed at 68°C for 2 minutes. Thirty cycles of amplification were performed with an automatic thermal cycler (MJ Research, Cambridge, MA).

At the end of the amplification procedure, one fifth of the reaction mixture was subjected to electrophoresis on a 2% agarose gel. The DNA was then transferred to a nylon membrane (NEN, Boston, MA) by electroblotting, fixed to the membrane at 80°C for
2 hours, and hybridized overnight at 65°C in 5X SSPE, pH 6.4, 0.6% sodium dodecyl sulfate (SDS), 0.2 mg/mL sonicated salmon sperm DNA, with the specific oligonucleotide probe end-labeled, by a standard procedure, with a γ32P-ATP. At the end of the hybridization procedure the filters were washed in 2X SSPE, pH 6.4, 0.1% SDS for 15 minutes at room temperature and then in 5X SSPE, pH 6.4, 0.1% SDS for 1 hour at 60°C. The autoradiography of the membranes was performed for 2 and 7 days at −80°C. To avoid false-positive results, all procedures were performed in strict adherence to the recommendations of Kwok and Higuchi.7 The search for HHV-6 sequences was performed by a PCR procedure able to generate and amplify a segment of 186 nucleotides, part of the 9-kb HHV-6 sequence inserted in the pZVH14 plasmid.25 Dilution experiments, performed by diluting the pZVH14 in increasing amounts of normal human uninfected DNA, showed that, in our PCR conditions, it was possible to recognize one copy of the recombinant plasmid per 10,000 cell DNA equivalents. One positive (DNA from HHV-6-infected HSB-2 cells) and two negative (DNA from uninfected HSB-2) controls were used for each group of 10 samples examined. The test was discarded when one of the negative controls gave an even weak signal after a 7-day exposure.

The PCR procedure for EBV sequence detection was performed according to Saito et al,26 resulting in the amplification of a 122-nucleotide segment of the reiterated viral DNA sequence Bam W.

**RESULTS**

**HHV-6 Humoral Immune Response in Lymphoma Patients and Normal Individuals**

Indirect immunofluorescence assay on HHV-6-infected cells was performed to detect serum antibodies against viral proteins in 200 sera from normal blood donors (male-to-female ratio 1:1) with a median age of 37 years. Data obtained with sera at 1:20 dilution were disregarded because of the aspecific immunofluorescence detected in control uninfected cells. At a serum dilution of 1:40, 54% of the normal blood donors were clear-cut positive for the presence of anti–HHV-6 antibodies. This percentage decreases to 26% and 6% at dilutions of 1:80 and 1:160, respectively, as presented graphically in the histograms of Fig 1. Ninety-four sera collected from human lymphoma cases, of which 31 were derived from HL (mean age 55 years) and 63 from NHL (mean age 63 years) cases, were studied using the same procedure and the same pool of reagents. Males and females in both groups were almost equally represented. The percentages of positive cases at 1:40, 1:80, and 1:160 dilutions are 80%, 68%, and 16%, respectively, in Hodgkin's cases and 62%, 41%, and 19% in non-Hodgkin's patients (Fig 1). Our results indicated a threefold increase in the number of patients with high antibody titers (1:160) against HHV-6 in the population with HL and NHL as compared with the normal population. These results are particularly meaningful in light of a known decrease of antibody titers against HHV-6 after age 40 in the normal population.26 However, when a severe discriminating criterion is used, the statistical analysis shows that only the differences between the values of normal subjects and Hodgkin's patients are statistically significant ($X^2 = 16.0416$).

**Presence of HHV-6 DNA Sequences in Affected Tissues of Lymphoma Patients**

The presence of HHV-6 DNA sequences was investigated in the total DNA extracted, as described in Materials and Methods, from pathologic specimens derived from 66 cases of human lymphoma (Table 1). Forty-one were cases of NHL: 33 of the B lineage and eight of the T lineage. Twenty-five samples were obtained from HL cases. All 41 samples from NHL patients were negative for HHV-6 DNA sequences in two successive PCR tests. Three of 25 DNA samples extracted from lymph nodes of Hodgkin's cases were clearly positive for the presence of HHV-6 sequences by PCR (cases 5, 12, and 14; Fig 2). When the standard Southern blot analysis was used with the pZVH14 plasmid as probe, the lymph nodes of patients 5 and 12 were positive. In these two cases, by comparison with the signals obtained with a probe representing a single copy gene (human bcr), the copy number of HHV-6 sequences was evaluated as 1 per 5 to 6 cell DNA equivalents. DNAs from PBMC and from a lymph node of patient 14, obtained at the time of diagnosis, were subjected to PCR analysis. HHV-6 sequences were detected in both DNA, but the lymph node DNA appeared to contain a higher copy number of viral sequences. The DNA of PBMC from patient 5, already in complete remission, gave a strong positive signal in a PCR

---

**Table 1. Lymphoma Cases Positive for the Presence of HHV-6 Sequences by PCR**

<table>
<thead>
<tr>
<th></th>
<th>No. of Cases</th>
<th>HHV-6 +</th>
</tr>
</thead>
<tbody>
<tr>
<td>B lineage</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>T lineage</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>HLs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>MC</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>LP</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>NS/LD</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>NS/MC</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>NS/LP</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: LD, lymphocyte depletion; MC, mixed cellularity; LP, lymphocyte prevalence; NS, nodular sclerosis.
test as well as in a standard Southern assay. Interestingly, no anti–HHV-6 antibodies were detected in the serum of patient 5 at 1:40 serum dilution. The serum of patient 14, obtained at the time of diagnosis, yielded a positive IFA test at dilution of 1:80. Both patient’s sera were negative for anti-HIV antibodies (enzyme-linked immunosorbent assay [ELISA] test) and weakly positive for anti-EBV IgG antibodies (ELISA tests). The serum of patient 12 was not available for analysis. The research of EBV sequences in the lymph node DNA of the three HHV-6-positive patients was negative by Southern blot, but positive in all cases by PCR, although the autoradiographic signals obtained were very faint (data not shown). These results suggest that, at least in the lymph nodes of patients 5 and 12, who were positive for HHV-6 sequences in Southern blot, the number of EBV sequences is remarkably lower than that of HHV-6 sequences. The same tests were negative when performed on the PBMC DNA of cases 5 and 14. These data are summarized in Table 2.

**Restriction Analysis of HHV-6 Sequences Found in Lymphoma Cases**

The lymph node DNAs of a control normal individual and of cases 5 and 12, as well as the PBMC DNA of case 5, were digested with EcoRI, HindIII, and BamHI restriction enzymes and hybridized with the pZVH14 plasmid. The negative control DNA did not show segments hybridizing with the probe used, whereas the restriction patterns obtained in the three positive samples, when compared with that obtained in the DNA of HSB-2-infected cells, showed some differences (Figs 3 and 4). The segments resulting from EcoRI digestion of infected HSB-2 DNA are all present in our cases. The HindIII digestion shows two bands, one of higher and one of smaller molecular weight compared with the 9-kb segment present in HSB-2 DNA, corresponding to the insert of the pZVH14 plasmid. The double digestion with EcoRI and HindIII (Fig 3) suggests that the 5′ Hind site is lost in the cases examined, and a new Hind site is present in the pZVH14 sequence, possibly within the 5′ internal EcoRI fragment, as already reported. The digestion of infected HSB-2 DNA with BamHI (Figs 3 and 4) shows, as expected, four fragments: two short (0.9 and 1.1 kb) and two far longer. These latter segments are so similar in length that only a very short exposure time allows the distinction of two bands in the high molecular weight region of the gel. In the DNA of the patients, after BamHI digestion, the 0.9- and 1.1-kb fragments are absent, while the two longest, almost comigrating, fragments are only a little longer than those from infected HSB-2 cells. This behavior is consistent with the
Fig 3. Autoradiography of case 5 lymph node DNA digested with different restriction enzymes and hybridized with labeled pZVH14 plasmid. Sample DNA, 10 μg, and 1 μg of infected HSB-2 DNA were subjected to 0.8% agarose gel electrophoresis in each lane. The restriction map of the 9-kb HHV-6 sequence inserted in pZVH14 is shown at the bottom of the figure.

Loss of two of the three central BamHI sites. The double digestion with BamHI and HindIII (Fig 3) confirms that only the extreme 5' BamHI site is conserved, near the new 5' HindIII site. In conclusion, as shown by Southern blot analysis, the 9-kb viral sequence studied in two of the three positive Hodgkin's cases differs from that of the original HHV-6 isolate because of the absence of at least three restriction sites (one for HindIII and two for BamHI), with the formation of a new one (for HindIII), all in the 5' portion of the sequence examined (Fig 3).

DISCUSSION

The fact that about 50% of normal blood donors show the presence of IgG antibodies against HHV-6-related antigens at a 1:40 serum dilution is in agreement with the
widespread seroprevalence, ranging from 60% to 85%,
reported in the normal population by several investigators
examining the samples to serum dilutions as low as 1:10.13,15
A possible immunologic cross-reactivity between HHV-6
and other herpesviruses, mainly HCMV,19 has been ruled
out by the large majority of investigators using indirect
immunofluorescence techniques.1,3,13,15 Moreover, a nonspe-
cific reaction able to influence the data obtained at a 1:40
dilution is unlikely. Uncertainties in interpreting immuno-
fluorescence tests performed at low serum dilutions have
already been reported in the literature.16,41 This ambiguity
may be due to the fact that HHV-6 antibody reactivity, as
shown by the ELISA test, is normally distributed from the
weakest to the strongest responses without a discrete level
of reactivity, as pointed out by Saxinger et al.13
In our series of 94 sera from human lymphomas, the
percentage of tests positive for HHV-6 antibodies is higher
than in normal blood donors as it was previously re-
ported,2,4,5 but only the HL cases show a strong statistically
significant difference (X2 = 16.0416). Because the infection
is a widespread phenomenon and the comparison is per-
formed between values obtained at a 1:40 and higher serum
dilutions, we conclude that the titer of anti-HHV-6 antibod-
ies and not the frequency of infection is significantly higher
in Hodgkin’s cases than in healthy subjects. A possible
reason for these high antibody titers might be that the
impairment of the cellular immune response, induced by
the HD, allows the reactivation of a latent infection leading
to higher antibody titers. A several-fold increase in HHV-6
antibodies has also been reported during EBV and HCMV
infection.14,44 Thus, a possibly different mechanism for
the raising of HHV-6 antibody titers in HD may act through a
new infection by other viruses. We do not have a satisfac-
tory explanation of this behavior; however, the serology
data indicate that the immune system of Hodgkin’s patients
is in some way activated against HHV-6 antigens. This
activation is likely to be independent of the presence of
HHV-6 sequences in lymph nodes, which seems to be a
rather rare event, as judged by the results of the present
and previous surveys.5,25 As reported above, HHV-6 is able to
infect not only lymphocytes but also cells of other lineages.
Then we may consider the possibility that a tissue different
from the lymph node acts as a reservoir of the virus.
Previous studies, performed with Southern blot analysis,
showed the presence of HHV-6 in only 5 of more than 150
cases of NHL and in 0 of 37 cases of HD.24,25 In contrast,
others described a high percentage of lymphoma cases
bearing HHV-6 sequences.34 Despite the use of a sensitive
technique, our findings suggest that the presence of HHV-6
sequences, as a rule, is not a frequent event in human
lymphoid tissues, contrary to what is suggested elsewhere.45
This presence is in fact a very rare event in human NHLs, as
previously reported, whereas in HD it is more frequent than
previously suggested by the standard Southern blot tech-
nique. The high number of negative tests suggests that
contamination was substantially absent from our PCR
assays, although the sensitivity of the technique was high, as
demonstrated by the dilution experiments reported in
Materials and Methods. On the other hand, we must keep
in mind that a positive PCR result does not either prove per-
se the presence of a complete viral genome or indicate the
integrated or episomal form of the virus. It is also impos-
sible to exclude that variant HHV-6, not hybridizing well to
our primers, can be present in the examined tissues. Moreover,
the positivity of a PCR test may be due to the presence of a
very small amount of infected cells, which may not belong to
the pathologic tissues in the sample examined. This warning
holds particularly true in the case of HD in which only an in situ
hybridization test, showing the presence of the infection in the Reed-Sternberg cells,
would indicate a direct involvement of the neoplastic
elements. However, in two of our cases the number of
HHV-6 copies was high enough to allow an easy identifica-
tion by standard Southern technique.
Our observations are suggestive of a possible role for
HHV-6 in the pathogenesis of some cases of HD. The three
young women found positive for HHV-6 sequences in our
survey show some obvious similarities in their clinical
pictures. All cases belong to the same histologic subgroup,
NS-LD, generally accounting for a small proportion (about
5%) of the cases and associated with a poor prognosis. No
patients belonging to other, more frequent, histologic
subgroups were found infected. Two of the cases had a
predominant bulky mediastinal involvement, while the
third patient showed mediastinal involvement as part of a
generalized disease. Mesothelial effusions (pleuropericar-
dial in two cases and pleuropertitoneal in the third) were
observed in the three patients, although at different times
in the course of the disease. The number of cases is so small
that several reasons may explain the coincidence of HHV-6
infection with these common clinical features. The histo-
pathologic pattern, eg, frequently associated with poor
prognosis, may involve a severe immune impairment, which
in turn may favor exogenous or latent infections. However,
one of our cases showed a 1:80 titer of anti–HHV-6 antibod-
ies, indicating that HHV-6 infection is not necessarily
associated with the absence of the immune response.
Although a relationship between the presence of HHV-6
and a particular subset of patients is not solidly established,
the possibility is raised, in our opinion, that HHV-6
infection may play a role in determining some basic clinical
aspects of a limited number of cases. A better understand-
ing of the relations between HHV-6 infection and HD
requires the study of a larger number of cases and more
information regarding the genomic structure of HHV-6, the
possible integration of the virus in the host genome, the
precise mechanisms of latency and reactivation of the
infection, and the influence of a latent infection on the
behavior of the immune system. Also, the results of a
coinfection with other lymphotropic viruses must be care-
fully considered for their possible impact on clinical picture
and prognosis. In fact, a latent EBV infection has been
rather frequently reported in Hodgkin’s cases, although the
meaning of this coexistence is still only matter of specula-
tion.46-48 EBV sequences are present in the three cases
reported here, which are also HHV-6 infected. Moreover,
cases of coinfection of HHV-6 with HIV^{1,4,9,50} and HTLV-I^{1,2} are reported, and the transactivation of HIV LTR by HHV-6 has been observed.\textsuperscript{53}

The restriction analysis performed on the HHV-6–positive cases showed several differences between the viral sequences present in the Hodgkin’s cases and the pZVH14 sequence of the original isolate. This is consistent with the reported polymorphism of HHV-6\textsuperscript{4,6} but it has to be pointed out that the restriction pattern is identical in the two cases and partially similar to that already reported in a case of NHL infected with HHV-6.\textsuperscript{29} As the region analyzed is only 6% of the HHV-6 genome, the virus isolation and a more extensive restriction analysis are required to discuss the possible existence of a new strain of HHV-6.

ACKNOWLEDGMENT

The authors are grateful to Dr R.C. Gallo, Dr G. Franchini, and Dr D.V. Ablashi for the generous gift of pZVH14 plasmid and of HSBl infected cells. The authors thank also Prof P. Faglioni for the statistical analysis and Dr G. Zanni for contributing clinical cases.

REFERENCES


12. Lusso P, Markham PD, De Rococo SE, Gallo RC: In vitro susceptibility of T lymphocytes from chimpanzees (Pan troglodytes) to human herpes virus 6 (HHV-6): A potential animal model to study the interaction between HHV-6 and human immunodeficiency virus type 1 in vivo. J Virol 64:2751, 1990


myeloid leukemia with a chromosome 22 breakpoint outside the breakpoint cluster region. Blood 70:1659, 1987
Human herpesvirus-6 in human lymphomas: identification of specific sequences in Hodgkin's lymphomas by polymerase chain reaction

G Torelli, R Marasca, M Luppi, L Selleri, S Ferrari, F Nanni, MT Mariano, M Federico, L Ceccherini-Nelli and M Bendinelli

Updated information and services can be found at:
http://www.bloodjournal.org/content/77/10/2251.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml