Human Herpesvirus 8 Infection in Patients With Cutaneous Lymphoproliferative Diseases

Elisabetta Trento, PhD; Concetta Castilletti, PhD; Carmela Ferraro, MD; Ilaria Lesnoni La Parola, MD; Anna Mussi, MD; Luca Muscardin, MD; Valentina Bordignon, PhD; Giovanna D’Agostio, PhD; Ada Amantea, MD; Antonio Mastroianni, MD; Franco Ameglio, MD; Joachim Fluhr, MD; Paola Cordiali-Fei, PhD

Objective: To investigate the prevalence of human herpesvirus 8 (HHV-8; Kaposi sarcoma–associated herpesvirus) infection in patients with lymphoproliferative skin diseases such as large-plaque parapsoriasis (LPP) and mycosis fungoides compared with inflammatory cutaneous conditions or healthy control subjects.

Design: A survey study was undertaken in 123 subjects with various clinical conditions.

Setting: All patients had been seen in the Dermatology Department of the San Gallicano Dermatology Institute, Rome, Italy, in the last 2 years.

Patients: Forty-five patients with inflammatory or autoimmune cutaneous diseases, 50 healthy control subjects, 10 patients with LPP, 12 patients with mycosis fungoides, and 6 patients with classic Kaposi sarcoma were included in the study.

Main Outcome Measures: The prevalence of HHV-8 infection was investigated with serologic studies using the gold standard assay based on body cavity–based B-cell lymphoma-1 cells latently infected with HHV-8. The presence of HHV-8 conserved sequence, corresponding to open reading frame 26, was also assessed in the peripheral blood and lesion tissue samples from patients with lymphoproliferative cutaneous diseases with nested polymerase chain reaction. The presence and distribution of cell types infected with HHV-8 in the lesion tissues was determined with immunohistochemical staining with the monoclonal antibody directed against the latent nuclear antigen-1 of HHV-8 encoded by open reading frame 73.

Results: In healthy control subjects and patients with inflammatory skin diseases, 13.9% were found to have antibody against HHV-8, consistent with the seroprevalence population in Italy. A highly significant association of HHV-8 infection and LPP was found (100%) compared with mycosis fungoides (25%). The peripheral blood mononuclear cells in 8 of 10 patients with LPP were found to harbor viral sequences at nested polymerase chain reaction, whereas none of them had a detectable serum viral load. All LPP lesion tissue samples were positive for HHV-8–encoded open reading frame 26, and the presence of HHV-8–infected cells was confirmed by immunohistochemistry profiles performed on paraffin-embedded tissues from 4 of 10 patients. The positive cell types included endothelial cells and the infiltrating dermal lymphocytes, characteristic hallmarks of LPP. Analysis of T-cell receptor γ chain rearrangements in lesion tissue from our patients confirmed the lack of a significant association between T-cell clonality and LPP.

Conclusion: These data suggest that HHV-8 may play a role in the onset of LPP, a disease whose cause and evolution are still undefined and which has often been considered the early stage of mycosis fungoides.

Arch Dermatol. 2005;141:1235-1242

©2005 American Medical Association. All rights reserved.
nodular Kaposi sarcoma, B-cell lineage immunoblastic cells in multicentric Castleman disease, and CD30-positive lymphoma cells in PEL. Furthermore, blood B lymphocytes, monocytes, and CD34-positive cells in patients infected with HHV-8 have been demonstrated to harbor viral sequences. Viral sequences have been found in CD3-positive cells of a pleural T-cell lymphoma and in PEL cells with a T-cell phenotype, raising the question of the possible pathogenicity of HHV-8 in T cells. The role of HHV-8 in the pathogenesis of other tumors remains controversial. There have been occasional reports of the association of HHV-8 with cutaneous T-cell lymphomas and nonneoplastic lymphoproliferative cutaneous conditions such as large plaque parapsoriasis (LPP), based on molecular analysis, although serologic assays have not been performed in the same patients. Serologic analysis in patients with various malignancies, hematologic or neurologic diseases, and autoimmune conditions have not shown significant association with HHV-8 infection. In the present study we used an immunofluorescence assay (IFA) based on BCBL-1 cells to investigate the prevalence of HHV-8 infection in patients with lymphoproliferative skin diseases such as LPP and mycosis fungoides (MF) in comparison with inflammatory cutaneous conditions. We also investigated in the same subjects the presence of HHV-8 conserved sequence corresponding to the open reading frame (ORF) in lesion tissue samples, peripheral blood mononuclear cells (PBMCs), and plasma, with the nested polymerase chain reaction (PCR). A significant association of HHV-8 infection and LPP was found compared with healthy control subjects or patients with inflammatory skin diseases. The same association was not found in patients with MF, who showed lower percentages of anti–HHV-8 positivity. Endothelial cells, epidermal cells, and lymphocytes of the dermal infiltrate in lesion tissues were positive at immunostaining of the ORF 73 HHV-8-encoded region. These data suggest that MF and LPP are different clinical entities, even though LPP has often been considered the early stage of the cutaneous neoplastic condition.

**METHODS**

**PATIENTS AND CONTROL SUBJECTS**

Fifty healthy control subjects and 78 patients were included in this study. All subjects tested were negative for anti-HIV antibodies. Patients had LPP (n = 10); MF (n = 12); cutaneous inflammatory disorders that included plaque-type psoriasis (n = 11), eczema (n = 11), urticaria (n = 8), and atopic dermatitis (10 pediatric patients); or autoimmune diseases that included subacute lupus erythematosus (n = 5), scleroderma (n = 5), and bullous diseases (n = 5). Diagnosis of LPP and MF was based on clinical evidence and histologic examination of skin lesion biopsy specimens. Patients with irregular erythematous patches larger than 2 cm in diameter without noticeable infiltration and no history of preceding concomitant cutaneous lymphoma were enrolled as having LPP. The histopathologic findings consisted of a sparse subepidermal infiltrate of lymphocytes and histiocytes and pronounced epidermotropism of atypical lymphocytes either singly or in clusters. Patients with LPP were included in the study within 1 year from their clinical and histologic examination; nevertheless, some patients referred had had clinical symptoms for 3 (patients LPP3 and LPP9) or 6 years (patients LPP2 and LPP8).

Special attention was given to the diagnostic criteria for early patch stage MF. Specifically, the presence of intraepidermal lymphocytes surrounded by lacunae, alteration of collagen, and vacuolar interface dermatitis were considered together with additional histologic features such as haloes of lymphocytes, Pautrier microabscesses, disproportionate exocytosis of lymphocytes aligned within the basal layer, and highly convoluted lymphocytes. Further investigations to detect the presence of a prevalent T-cell clone in the lesion tissue and in blood were performed with molecular analysis (see the “TCR-γ Chain Rearrangement” subsection of the “PCR Analysis” subsection in the “Methods” section). In the patients with MF, disease was diagnosed 7 years (patient MF4), 2 years (patients MF1, MF11, and MF12), or 1 year (patients MF2, MF3, MF5 through MF10) before they were included in the study. In patients MF6 and MF8, a diagnosis of follicular mucinosis had been made 2 years before the diagnosis of MF; patients MF7 and MF9 had a clinical diagnosis of LPP previously made at another institution and not based on histopathologic findings.

**ANTI–HHV-8 SEROLOGIC ANALYSIS**

The HHV-8–infected BCBL-1 line, derived from PEL, was cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum and 50 µmol/L of β-mercaptoethanol. Exponentially growing cells (3 × 10^6 cells/mL) were treated for 72 hours with 20 ng/mL of phorbol-12-myristate-13-acetate (TPA; Sigma–Aldrich Corp, St Louis, Mo). Ten microliters of treated cell suspension or of untreated cells was dropped on the wells of multwell slides, air dried rapidly, and fixed in cold acetone for 20 minutes. Fixed cells, treated and untreated, were incubated in 2 steps of 30 minutes each at room temperature in a humid chamber, with serial dilutions of each serum sample starting at 1:20 and subsequently, after thorough washings with cold phosphate-buffered saline solution, with fluorescein isothiocyanate (FITC)–labeled goat F(ab)2 immunoglobulin antihuman IgG (Southern Biotechnology Associates Inc, Birmingham, Ala).

Slides were examined by 2 different operators (E.T. and P.C.-F.) with a UV light microscope (Axioskop; Zeiss, Germany). The microscopic examination of TPA-treated and untreated cells enabled establishment of specific antibody reactivity, and a positive result was considered when reactivity to nuclear and cytoplasmic antigens was detected. A pool of serum samples collected from a group of 6 babies and a pool of serum samples from 6 patients with Kaposi sarcoma (human immune deficiency virus [HIV]–negative) were used as negative and positive controls, respectively, in each assay.

**OTHER SEROLOGIC ASSAYS**

Serum samples from all study participants were tested for Epstein-Barr virus and cytomegalovirus antibodies with an immunoblot assay (RecomLine Epstein-Barr virus IgG and RecomLine cytomegalovirus IgG, respectively; Mikrogen GmbH, Neuried, Germany). The serum samples were also tested for herpes simplex virus 1 and herpes simplex virus 2 antibodies with an enzyme-linked immunosorbent assay; (Capita Herpes Simplex Virus 1 IgG and Capita Herpes Simplex Virus 2 IgG; Trinity Biotech USA, Jamestown, NY).

**IMMUNOPHENOTYPING**

Lymphocyte subpopulations positive for CD3, CD3CD4, CD3CD8, CD19, and CD16 were analyzed with flow cytom-
DNA EXTRACTION

Peripheral Blood Mononuclear Cells

The PBMCs were isolated by gradient centrifugation from 5 mL of EDTA–whole blood, washed, divided into aliquots of 2.5 × 10^6 cells, and pelleted. DNA was obtained with a chemical method using the PureGene DNA isolation kit (Genta Systems, Minneapolis, Minn). In brief, 300 mL of lysis buffer was added to each cell aliquot in the presence of 6 µg of ribonuclease. After 5 minutes of incubation at 37°C, proteins were removed by 15-minute centrifugation at 13,000 g. DNA isolation proceeded as described for PBMC samples.

Tissues

All tissues were obtained with informed patient consent. Molecular analysis was performed on DNA isolated from paraffin-embedded lesion tissue samples used for histologic and immunohistochemical studies. Tissue sections (6 × 5 µm) were deparaffinized by adding 1 mL of xylene. After 5 minutes the sample was spun, washed twice with 100% ethanol, and kept overnight in lysis buffer containing proteinase K (100 µg/mL) at 37°C. DNA isolation proceeded as described for PBMC samples.

Cell Lines

To assess the specificity and sensitivity of the molecular assays for detection of HHV-8 sequences or rearrangements of the Vγ1, and Vγ2 regions of T-cell receptors (TCR), DNA was isolated from the BCBL-1 cells or from the monoclonal human leukemic T-cell lines Jurkat (ECACC 88042803) and HSB-2 (ECACC 85112801).

Plasma

DNA was obtained from plasma samples with a commercial kit (EXTRAgen; Amplimedical, Turin, Italy) using a chemical method. In brief, 300 µL of plasma was added to 1 mL of lysis buffer. After 10 minutes of incubation at 80°C, proteins were removed by 15-minute centrifugation at 13,000 g. DNA was isolated from supernatants by adding 100% ethanol and rehydrated with 50 µL of Tris hydrochloride buffer (10 mmol/L, pH 7.5), with 1 mmol/L of EDTA. The concentration and purity of DNA was determined by measuring the absorbance at 260 and 280 nm. The integrity of DNA was checked at 2% agarose gel electrophoresis and ethidium bromide staining.

PCR ANALYSIS

Successful amplification of a β-globin fragment was performed on DNA samples before specific PCR analysis to establish that they were adequate and that no PCR inhibitors were present. All samples were analyzed in duplicate.

TCR-γ Gene Rearrangement

The TCR-γ gene rearrangement was studied by using a guanine-cytosine–clamp nested PCR and denaturing gradient gel electrophoresis (DGGE). For amplification of Vγ1 and Vγ2 regions of the TCR-γ gene we followed the protocols described by Wood et al. To evaluate the sensitivity of the detection of TCR-γ gene rearrangements in our laboratory, a total of 2.5 × 10^6 Jurkat (or HSB-2) leukemia T cells and healthy control polyclonal PBMCs were mixed at different ratios (from 100% to 0.1%) and DNA amounts corresponding to 120 × 10^3 cells were amplified. Samples containing up to 3% of Jurkat or HSB-2 monoclonal cells, corresponding to 3600 cells, showed a clear band at DGGE analysis.

Human Herpesvirus-8

The HHV-8 genome in PBMCs was detected with amplification of the specific sequence of ORF 26 following a nested PCR protocol with the outer set of primers 5'-AGC AGG GAT TCC ACC AT-3' (forward, nucleotides 47287-47306) and 5'-TCC GTG TTG TCT AGC AG-3' (reverse, nucleotides 47519-47500), and the inner set of primers 5'-TAT TCT TCT GCA GCA GCT GTT GG-3' (forward, nucleotides 47373-47392) and 5'-TCT AGC TCC AGA CAT GTG C-3' (reverse, nucleotides 47510-47489). The positions are based on the Kaposi sarcoma–associated herpesvirus genome (accession U75698 in the NCBI gene bank at the National Center of Biotechnology Information [available at: http://www.ncbi.nlm.nih.gov/]). The PCR reaction mixture consisted of 400 ng of DNA, 10 mM tri hydrochloride (pH 8.3), 0.5 mM of magnesium chloride, 0.01% gelatin, 0.2 µmol/L of each primer, and 0.25 U of Taq polymerase (AmpliTaq Gold; Perkin Elmer, Foster City, Calif) in a total volume of 25 µL. Amplification was performed on an oil-free thermal cycler (GeneAmp model 9700; Perkin Elmer). By using AmpliTaq Gold, provided in an inactive state, an efficient hot start procedure was achieved: before the PCR heat step, 10 minutes at 95°C, followed by 35 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 45 seconds.

The PCR product was a single band fragment of 233 base pairs (bp). The nested PCR was performed using 0.5 µL of the first-round product with the inner primer set for an additional 30 cycles, under the same conditions as in the first round. The nested PCR product was a fragment of 138 bp. The amplification products were run on 2% agarose gels, stained with ethidium bromide, and acquired under UV light with a Polaroid high-resolution gel imaging system (Gel Doc System; Bio-Rad Laboratories, Hercules, Calif). To assess the sensitivity of HHV-8 ORF 26 detection by nested PCR amplification, DNA was isolated from a total of 2.5 × 10^6 BCBL-1 cells and Jurkat cells (as negative controls), mixed at different ratios (from 100% to 0.05%), and DNA amounts corresponding to 120 × 10^3 cells amplified. The ORF 26 was still detectable in the sample containing 0.1% HHV-8 infected cells, corresponding to 120 cells. The HHV-8 DNA in plasma samples was detected with a commercial kit (HHV-8; Amplimedical, Milan, Italy) with a sensitivity of 80 copies per milliliter of plasma.
IMMUNOHISTOCHEMICAL ANALYSIS

Paraffin-embedded sections were deparaffinized with xylene and 100% ethanol; microwaved in 10 mmol of citrate buffer, pH 6.0, at 780 W for 6 minutes; and stained with rat monoclonal antibody against the latent nuclear antigen encoded by ORF 26. Samples of DNA extracted from the BCBL-1 and T-cell (HBS-2) lines were used as positive and negative controls. The results are summarized in Table 1. The seroprevalence in 50 healthy control subjects, aged 35 to 75 years, was 13.9%. No significant differences were found in the patients with other inflammatory skin disorders, either analyzed as a single disease or altogether, showing the same percentage of positive subjects (13.9%) as in the healthy control group, with titers ranging from 1:40 to 1:80. A group of 10 serum samples collected from children aged 0 to 6 years with atopic dermatitis were analyzed, and all were negative. As positive controls, 6 serum samples from patients with Kaposi sarcoma (HIV-negative) were tested and found positive, with titers between 1:160 and 1:640. All serum samples from patients with a diagnosis of LPP were found to be anti–HHV-8 positive, with titers ranging between 1:80 and 1:160, showing a highly significant difference from control serum samples at statistical analysis. Among patients with MF, 3 of 12 were positive for anti–HHV-8, with titers comparable to those found in patients with LPP, with no significant difference compared with control subjects.

Table 1. Prevalence of HHV-8 Infection in Healthy Control Subjects and Patients With Cutaneous Inflammatory Disorders or T-Cell Lymphoma Evaluated With Serologic Analysis

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age, y</th>
<th>HHV-8 Antibodies*</th>
<th>Titer</th>
<th>Prevalence, %</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control subjects</td>
<td>25-75</td>
<td>8/50</td>
<td>1:20-1:40</td>
<td>13.9</td>
<td>...</td>
</tr>
<tr>
<td>LPP</td>
<td>35-65</td>
<td>10/10</td>
<td>1:40-1:160</td>
<td>100.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>MF</td>
<td>45-69</td>
<td>3/12</td>
<td>1:40-1:160</td>
<td>25.0</td>
<td>.46</td>
</tr>
<tr>
<td>Eczema</td>
<td>35-60</td>
<td>1/11</td>
<td>1:20-1:40</td>
<td>9.0</td>
<td>.55</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>45-60</td>
<td>2/11</td>
<td>1:20-1:40</td>
<td>18.2</td>
<td>.85</td>
</tr>
<tr>
<td>Urticaria</td>
<td>35-45</td>
<td>1/8</td>
<td>1:20-1:40</td>
<td>12.5</td>
<td>.79</td>
</tr>
<tr>
<td>Autoimmune diseases</td>
<td>30-52</td>
<td>2/15</td>
<td>1:20-1:80</td>
<td>13.3</td>
<td>.80</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>0-6</td>
<td>0/10</td>
<td>1:20-1:80</td>
<td>13.9</td>
<td>.17</td>
</tr>
<tr>
<td>Kaposi sarcoma</td>
<td>65-75</td>
<td>6/6</td>
<td>1:160-1:640</td>
<td>100.0</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: HHV-8, human herpesvirus 8; LPP, large-plaque parapsoriasis; MF, mycosis fungoides.
*The numerator indicates the number of participants with a positive result; the denominator, the total number of participants.
†χ² Test.

OTHER HERPESVIRUS SEROPREVALENCE

The seroprevalence of cytomegalovirus or Epstein-Barr virus (85% and 75%, respectively) was not significantly different from that in the healthy control subjects (83.4%).

TCR-γ CHAIN REARRANGEMENT

The DGGE analysis of the amplification products of the TCR-γ gene segments Vγ1-8 and Vγ9 was performed in all the patients with a diagnosis of LPP or MF and in 3 patients with psoriasis. The presence of a dominant T-cell clone had a different association with LPP (2/10) or MF (8/12) lesion tissue samples (contingency table analysis, Fisher exact test, P = .04). The 2 patients with LPP displayed rearrangement of the Vγ9 segment, and 1 of them demonstrated rearrangement of the Vγ1-8 segment. T-cell clonality was also detected in PBMCs, but clones were different from those found in lesion tissues. Peripheral T-cell clonality was found in 4 of 8 patients with MF with a prevalent T-cell clone in the lesion tissue; in 2 of them (patients MF8 and MF11), the DGGE analysis suggested the presence of the same T-cell clone in the tissue and in the PBMCs as found in the systemic stage of the disease. Blood cells and lesion tissue from 3 patients with psoriasis and blood cells from 2 patients with Kaposi sarcoma were also analyzed, with no evidence of T-cell clonality.

HHV-8 GENOME SEQUENCE

Lesion tissue biopsy specimens and peripheral mononuclear cells in 10 patients with LPP, 12 patients with MF, 3 patients with psoriasis, and 2 patients with Kaposi sarcoma were analyzed for the presence of HHV-8 genome sequence ORF 26. Samples of DNA extracted from the BCBL-1 and T-cell (HBS-2) lines were used as positive and negative controls (Figure 1). All lesion tissue samples from patients with LPP were found to be positive in the nested PCR reaction; in samples from patients LPP3 and LPP4, the viral sequence was already detectable after the first-round PCR. The ORF 26 sequence was also detected in the PBMCs of 8 of the 10
patients with LPP; samples from patients LPP3 and LPP5 were negative, whereas the PBMC sample from patient LPP4 was positive after the first-round PCR. Considering the MF group, in seronegative subjects, both lesion tissue and PBMC samples were negative for the ORF 26 sequence; in 2 (patients MF7 and MF8) of 3 (MF7, MF8, and MF9) seropositive patients, the HHV-8 sequence was found at nested PCR in PBMCs. In 1 of these patients (MF8), the lesion tissue harbored the virus (2 different lesions were analyzed and were found to be nested PCR–positive). This same patient had T-cell clonality in tissue and in blood with the same DGGE pattern.

Tissues and PBMCs from 3 patients with plaque-type psoriasis were also analyzed for the presence of ORF 26, with negative results while tissue samples and PBMCs from 2 patients with Kaposi sarcoma were positive, as expected (Figure 1). Results of the HHV-8 antibody status and PCR positivity in the different groups of patients are summarized in Table 2. In none of the patients tested with LPP, MF, or psoriasis was HHV-8 plasma viremia detected, whereas the plasma samples from the 2 patients with Kaposi sarcoma were positive.

**IMMUNOHISTOCHEMICAL ANALYSIS**

All LPP lesion tissue samples (patients LPP3, LPP5, and LPP7) showed positive nuclear staining for HHV-8 in epidermal cells, dermal lymphocytes, and endothelial cells of dermal vessels (patient LPP3 in Figure 2). Weaker staining and fewer positive cells were detected in a patient with HHV-8 infection, detected at serologic and molecular analysis, and MF. This patient has been considered affected with LPP for 5 years (patient MF9 in Figure 2).

**COMMENT**

We describe the study of the prevalence of HHV-8 infection in cutaneous lymphoproliferative diseases in comparison with healthy control subjects and inflammatory or autoimmune cutaneous diseases. By detection of specific antibodies and of the viral sequence ORF 26 in plasma, lymphocytes, and tissues in the same subjects, we showed a highly significant association between HHV-8 infection and LPP, a lymphoproliferative cutaneous disease whose cause and evolution are still undefined. We also demonstrate the presence of HHV-8–infected cells in the lymphocyte dermal infiltrate characterizing LPP. Endothelial cells of dermal vessels and some epidermal cells were also shown to harbor the virus.

The HHV-8 seroprevalence value in Mediterranean areas ranges from 15% to 20% in the HIV-negative population, it is significantly higher in HIV-positive subjects, ranging from 30% to 56%, and is 100% in all patients with the clinical form of Kaposi sarcoma. Thus, the positivity rate of 13.5% in our control group, including healthy subjects and those with inflammatory cutaneous conditions, is comparable with that reported by other studies from Italy. Also, the finding of 100% positivity in patients with Kaposi sarcoma is consistent with the accepted role of HHV-8 in the pathogenesis of that tumor. The lack of antibodies in children younger than 6 years is consistent with the hypothesis of sexual transmission of the virus. The IFA based on HHV-8–infected PEL-derived cell lines is considered the most sensitive test for seroprevalence studies. In fact, enzyme immunoassays with different purified viral antigens have

### Table 2. HHV-8 Serologic Analysis and Molecular Detection of HHV-8 Sequence Open Reading Frame 26 in PBMCs and Lesion Tissue From Patients With LPP, MF, Kaposi Sarcoma, or Psoriasis*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Anti–HHV-8 Positive</th>
<th>ORF 26–Positive PBMCs</th>
<th>ORF 26–Positive Lesion Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPP</td>
<td>10/10</td>
<td>8/10</td>
<td>10/10</td>
</tr>
<tr>
<td>MF</td>
<td>3/12</td>
<td>2/12</td>
<td>2/12</td>
</tr>
<tr>
<td>Kaposi sarcoma</td>
<td>5/5</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

Abbreviations: HHV-8, human herpesvirus 8; LPP, large-plaque psoriasis; MF, mycosis fungoides; PBMCs, peripheral blood mononuclear cells.

*The numerator indicates the number of participants with a positive result; the denominator, the total number of participants.
shown that predominant antigenic proteins can be identified in serum samples from patients with AIDS and Kaposi sarcoma but not in serum samples from patients with other HHV-8–related diseases, and the IFA is still needed to reach high sensitivity rates. In our study, 11 of 13 PBMC samples from HHV-8 seropositive subjects, even with very low reactivity titers, and none of the antibody-negative samples were found to harbor viral genome sequences, confirming the high sensitivity and specificity of the IFA.

Considering patients with inflammatory cutaneous diseases, we did not detect significant differences compared with the control subjects in the seroprevalence of HHV-8 in patients with psoriasis, eczema, or autoimmune systemic or bullous diseases.

In patients with early MF, whether or not associated with detectable lesion or peripheral clonality, a prevalence of HHV-8 infection similar to that in healthy control subjects or patients with inflammatory cutaneous diseases was found, excluding a role of HHV-8 in the cause of this neoplastic condition. A highly significant association was found between HHV-8 infection and LPP, a clinical condition often considered pre-MF. A significant association with other herpesviruses, such as cytomegalovirus, Epstein-Barr virus, and herpes simplex virus type, was not confirmed in the same patients, owing to the high percentage of antibody-positive subjects in all groups of patients and control subjects. Using an antibody against a latent phase–associated nuclear antigen-1, considered a reliable and sensitive marker for the immunohistologic diagnosis of early Kaposi sarcoma, we showed that the infiltrating bandlike lymphocytes in LPP are latently HHV-8 infected. Different from what has been described in Kaposi sarcoma or the other HHV-8–

Figure 2. Nuclear positive staining for human herpesvirus 8 (HHV-8)–encoded open reading frame 73 in epidermal cells, infiltrating lymphocytes, and endothelial cells of lesion tissue samples from patient 3 with large-plaque parapsoriasis (B and C) and patient 9 with mycosis fungoides (E). 1 of the 3 patients with concomitant mycosis fungoides and HHV-8 infection. Rat IgG was used as control for specificity (A and D); body cavity–based lymphoma-1 cells and HHV-8 latently infected cells were included as positive control (F).
associated diseases multicentric Castleman disease and PEL,\textsuperscript{12} we found positive cells in endothelial cells of dermal vessels and in some epidermal cells.

Large-plaque parapsoriasis can be characterized by scaly patches or slightly elevated plaques that resemble psoriasis; hence, the nomenclature. However, this description fits several inflammatory cutaneous diseases, and consensus definition of parapsoriasis is still not accepted.\textsuperscript{9,14} The hypothesis that LPP is a self-limiting form of disease precluding MF is a point that is particularly debated. Few long-term longitudinal studies have been performed in patients with LPP to clarify the evolution of this disease. A 9-year follow-up study did not confirm that parapsoriasis leads to MF, even in the presence of occasional T-cell clonality.\textsuperscript{35} With regard to our patients with LPP, in 2 cases we detected blood and tissue T-cell–prevalent clones, with different TCR rearrangements of $V_{\gamma 9}$ or $V_{\gamma 18}$, highlighting the existence of an immune stimulation.

No clear cause for LPP is known at present, but we think that the strict association of anti–HHV-8 antibodies and HHV-8 viral sequences in cutaneous lesions of patients with LPP strongly suggests that a viral role in the cause of LPP lesions should be considered. Studies are in progress to further elucidate the localization of viral proteins or particles in the uninvolved skin and in lesion tissue, where HHV-8 could contribute to the proliferation of T cells by direct infection\textsuperscript{12,15,16} or secretion of viral protein homologues to human cytokines.\textsuperscript{4}

The onset of cutaneous lymphoproliferative lesions in the HHV-8–infected subject could depend on host factors, similar to what occurs in patients with Kaposi sarcoma.\textsuperscript{46} In fact, inflammatory cytokines or immunosuppression are considered to promote virus growth in the HIV-1–negative host.\textsuperscript{14,47} The immune system, evaluated as CD4 or CD8 T-cell numbers, was not affected in our patients, which showed control of HHV-8 replication, inasmuch as viral particles could not be detected in their serum samples. A role of HHV-8 in the development of LPP toward MF cannot be excluded. One of the 3 HHV-8–infected patients with MF had an HHV-8–infected cell with MF8, with low CD4 numbers, had a aggressive disease with the same T-cell clone in 2 different lesions and in blood. This feature has been reported as suggestive of a poor prognosis\textsuperscript{48,49} in early MF.

Accepted for Publication: December 21, 2004.
Author Affiliations: Laboratory of Clinical Pathology (Drs Trento, Bordignon, D’Agosto, and Cordiali-Fei), Laboratory of Cutaneous Histopathology (Drs Muscardin and Amantea), and Dermatology Department (Drs Ferraro, Lesnoni La Parola, Musssi, and Mastroianni), San Gallicano Dermatology Institute Laboratory of Clinical Virology, Italian National Institute for Infectious Diseases (Dr Castilletti); Laboratory of Clinical Pathology and General Hospital “S Giovanni Calibita” (Dr Ameglio), Rome, Italy; and Dermatology Clinic, University of Jena, Jena, Germany (Dr Fluhr).

Author Contributions: Study concept and design: Lesnoni La Parola, Muscardin, Amantea, Ameglio, and Cordiali-Fei. Acquisition of data: Trento, Castilletti, Ferraro, Musssi, Bordignon, D’Agosto, Mastroianni, and Cordiali-Fei. Analysis and interpretation of data: Trento, Fluhr, and Cordiali-Fei. Drafting of the manuscript: Trento, Lesnoni La Parola, Bordignon, D’Agosto, Amantea, Fluhr, and Cordiali-Fei. Critical revision of the manuscript for important intellectual content: Ferraro, Musssi, Muscardin, Mastroianni, and Ameglio. Statistical analysis: Bordignon, Amantea, and Fluhr. Obtained funding: Cordiali-Fei. Administrative, technical, and material support: Trento, Castilletti, Lesnoni La Parola, Bordignon, Amantea, and Mastroianni. Study supervision: Cordiali-Fei.

Correspondence: Paola Cordiali-Fei, PhD, Laboratory of Clinical Pathology, San Gallicano Dermatology Institute, Via Elio Chianesi 53, 00144 Roma, Italy (cordiali-fei@lio.it).

Financial Disclosure: None.

Funding/Support: This study was supported by grant 2OD/1.12 from Concerted Action 2002 on HHV-8 Infection, Istituto Superiore di Sanità/Istituto San Gallicano, Rome.

Acknowledgment: M. Capobianchi, MD, provided the BCBL-1 line derived from PEL at the Laboratory of Clinical Virology of the Italian National Institute for Infectious Diseases.

REFERENCES


©2005 American Medical Association. All rights reserved.
20. Nagore E, Ledesma E, Collado C, Oliver V, Pérez-Pérez A, Aliaga A. Detection of
17. Rabkin CS, Bedi G, Musaba E, et al. AIDS-related Kaposi’s sarcoma is a clonal
14. Nogore E, Ledesma E, Collado C, Oliver V, Pérez-Pérez A, Aliaga A. Detection of Epstein-Barr virus and human herpes virus 7 and 8 genomes in primary cutane-
11. Wood GS, Tung RM, Haefner AC, et al. Detection of clonal T-cell receptor gamma gene rearrangements in early mycosis fungoides/Sézary syndrome by polymer-
10. Shapiro PE, Pinto FJ. The histologic spectrum of mycosis fungoides/Sezary syn-
8. Sarmati L, Carito T, Rossella S, et al. Human herpesvirus-8 infection in preg-
2. Nevala H, Kärenko L, Väkevä L, Ranki A. Proapoptotic and antiapoptotic mark-