Human Herpesvirus 8 in Italian HIV-Seronegative Patients With Kaposi Sarcoma

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Objective: To evaluate the prevalence of human herpesvirus 8 (HHV-8) DNA detection in a large series of human immunodeficiency virus–seronegative patients with and without Kaposi sarcoma (KS) from the central and southern regions of Italy where classic KS is prevalent.

Design: Samples of lesional, peripheral unaffected, and distant normal skin and peripheral blood mononuclear cells (PBMCs) from 33 patients with KS and PBMCs from 42 control subjects were analyzed using single and nested polymerase chain reaction techniques for the presence of HHV-8 DNA.

Patients: A total of 33 patients with KS not related to acquired immunodeficiency syndrome (26 patients with classic KS and 7 patients with iatrogenic KS) were studied. Furthermore, 2 control groups were enrolled. The first group consisted of 13 healthy volunteers, the second of 29 patients affected by different dermatological diseases.

Results: Human herpesvirus 8 sequences were found in 100% of lesional and perilesional specimens, in 33% of the distant normal skin samples, and in 69.6% of the PBMCs from patients with KS. A possible correlation between HHV-8 DNA in PBMCs and the clinical stage of the disease was observed. Moreover, the prevalence of viral DNA in PBMCs from the total control group was 23.8%. No viral DNA was detected in tissue biopsy specimens taken from the control group.

Conclusions: Our data suggest that HHV-8 could be a widespread virus, at least in Mediterranean regions where KS is more prevalent, such as southern and central Italy. As with other herpesviruses, it may be present lifelong in latent form somewhere in the body and may contribute to the pathogenesis of KS when other predisposing conditions are present.

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irst described more than 100 years ago, Kaposi sarcoma (KS) is a multicentric neoplasm for which the etiopathogenesis is still under discussion. Epidemiological data pointed to the involvement of an infectious agent preferentially transmitted by sexual contact.

DNA sequences of a novel herpesvirus were identified in 1994 in biopsy specimens of KS by representational difference analysis. The designations of KS-associated herpesvirus and human herpesvirus type 8 (HHV-8) were given.

Later, HHV-8 DNA sequences were identified using a polymerase chain reaction (PCR) technique in tissue lesions from all clinical forms of KS. Human herpesvirus 8 DNA also has been detected in peripheral blood mononuclear cells (PBMCs) of human immunodeficiency virus (HIV)–seropositive individuals with and without KS, B-cell body cavity lymphomas, multicentric Castleman disease, established B-cell body cavity lymphoma cell lines, genitourinary tract specimens from immunocompetent individuals, and sensory ganglia of HIV-seropositive patients with KS. The presence of HHV-8 DNA in lesions other than KS suggests that this new herpesvirus may be ubiquitous and capable of infecting a number of healthy people, although the site of its latency and/or persistence in healthy individuals has not yet been identified. Similar to other herpesviruses, this virus may remain latent throughout the host’s lifetime unless factors, such as a compromised immune system, favor its propagation.

See also pages 700 and 736

The main focus of this study was to evaluate the frequency of HHV-8 DNA detection in 33 HIV-seronegative patients with KS from central and southern Italy where classic KS is prevalent. We also analyzed the prevalence of this new viral agent in a control group that included 42 HIV-seronegative subjects from the same geographical regions.

In addition, Epstein-Barr virus (EBV) DNA frequency was investigated in the same KS specimens. Epstein-Barr virus is a member of the Gammaherpesvirinae subfamily with which HHV-8 shares 39% of its homological features; its presence in KS lesions as well as its possible interaction with HHV-8 need to be assessed.
PATIENTS, MATERIALS, AND METHODS

PATIENTS

We enrolled 33 patients with KS: 26 with classic KS (22 men and 4 women; age range, 30-85 years; mean age, 66.7 years) and 7 with iatrogenic KS (5 men who were organ transplant recipients; age range, 50-63 years; mean age, 53.6 years; and 2 women receiving steroid therapy for rheumatic diseases, aged 88 and 72 years). They attended our department of dermatology from June 1996 until May 1997. All patients with KS were HIV-1 seronegative. Diagnosis was confirmed using routine histological examination in all cases. The patients were divided into groups according to the type and diffusion of the disease as reported by Mitsuyasu and Groopman. The staging was made using the results of a physical examination and routine laboratory tests (complete blood cell count, lymphocyte subset in the peripheral blood, and blood chemistry profile), when warranted by clinical suspicion, chest radiography, abdominal ultrasonography, gastrointestinal tract endoscopy, and computed tomography of selected organs were performed.

After receiving oral informed consent, we obtained a peripheral blood sample from each of the patients, 20 samples of lesional skin, 11 samples of peripheral unaffected skin, 6 samples of distant normal skin, and 1 lymph node. Additional blood samples were obtained for 7 patients during a 6-month follow-up.

CONTROLS

Two control groups matched by age, sex, and geographical origin were enrolled: the first consisted of 13 healthy volunteers (mean age, 40.8 years) without any dermatological disease; the second group, 29 patients affected by different skin disorders (mean age, 65.4 years). None of the control subjects were receiving immunosuppressive therapy. Blood samples were analyzed from both groups. Moreover, 8 skin biopsy samples were obtained from patients undergoing dermatological surgery (4 consisted of peripheral normal skin from benign pigmented lesions and 4 consisted of cutaneous lesions taken from patients with chronic inflammatory diseases).

SAMPLE PREPARATION AND DNA EXTRACTION

Peripheral blood mononuclear cells were separated from heparinized peripheral blood by lymphocyte separation medium (Ficoll-Hypaque, Eurobio, Les Ulis, France) density gradient centrifugation and washed twice in phosphate-buffered saline solution. Aliquots of 1 x 10^6 cells were centrifuged and cell pellets were stored at −80°C until DNA extraction.

The skin specimens were immediately frozen and stored at −80°C until DNA isolation. The lymph node specimen was obtained from a formalin-fixed, paraffin-embedded lymph node excised 2 years earlier.

Genomic DNA isolation from tissue biopsy specimens and PBMCs was carried out using standard proteinase-K treatment followed with several phenol and chloroform-isomyl alcohol extractions. Nucleic acids were then precipitated in alcohol, washed with 70% (vol/vol) alcohol, and resuspended in sterile water. The concentration and the quality of the extracted DNA were determined by spectrophotometry (optical density) at 260 and 280 nm.

PCR DETECTION OF HHV-8 DNA

Genomic DNA (0.5-1.0 µg) from skin biopsy samples was assayed for detection of HHV-8 DNA using the published KS330 primer set with the following amplification protocol: 98°C for 13 seconds, 5 cycles at 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and another 30 cycles at 92°C, 60°C, and 72°C for 15 seconds, as described by Corbellino et al. Each PCR reaction, containing 40 pmol of each primer, 200 µM of deoxynucleoside triphosphates (Boehringer

RESULTS

All biopsy specimens from KS lesions and from peripheral and distant uninvolved skin were analyzed using single and nested PCR techniques. We obtained positive signals clearly visualized on ethidium bromide staining for all 20 (100%) KS tissue biopsy specimens (classic KS and iatrogenic KS) tested. Human herpesvirus 8 DNA sequences were also detected in 100% of the tissue samples obtained from 11 peripheral unaffected skin biopsy specimens, while 2 (33%) of the 6 distant normal skin samples tested positive for HHV-8 DNA (Table). Similar results were obtained when the skin lesion specimens were submitted to DNA amplification with the primers P1 and/or P2 amplifying a 720-bp fragment that spans the sequences of KS330.

Restriction fragment length polymorphism analysis of the 720-bp PCR products showed the expected restriction patterns for each endonuclease according to the published sequences of HHV-8 DNA (Figure). This result shows that amplified sequences of HHV-8 are conserved among strains from different patients.

Human herpesvirus 8 DNA sequences were also found in the peripheral blood samples of 23 (69.6%) of 33 patients with KS. We detected the HHV-8 sequences more frequently in the PBMCs from iatrogenic KS (85%) than from classic KS (62%).

In our study, HHV-8 DNA was not detected in some samples from 2 of the 7 patients followed up for 6 months, although the presence of viral DNA was investigated using a nested PCR technique with a comparable amount of DNA from different PBMC samples. Particularly, in 1 of these patients negative results were obtained in the first 2 PBMC specimens, while the following 2 samples were positive. In the other case, the first specimen tested positive for HHV-8 DNA sequences, while viral DNA was not detected in the second. Both patients were receiving therapy with interferon alfa-2b (3 MU 3 times per week for 3 months). The first patient, with an aggressive KS localized on his arm, became positive after treatment, although he had an almost complete regression of the disease. The second, with a diffuse KS, had a partial response, but he died during follow-up of cirrhosis related to hepatitis C virus infection.
logical disorders were tested for HHV-8 DNA using a
volunteers and 29 patients affected by various dermato-
tients (100%) with cutaneous and visceral involvement.
9 patients with disseminated cutaneous KS, and in all 3 pa-
tients with a cutaneous localized form of KS, in 7 (77%) of
We detected HHV-8 DNA sequences in 9 (56%) of 16 pa-
in the PBMCs from patients with KS in different clinical stages.

<table>
<thead>
<tr>
<th>Blood (PBMC)</th>
<th>Skin Lesion</th>
<th>Peripheral Normal Skin</th>
<th>Distant Normal Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic KS</td>
<td>1/14 (100)</td>
<td>7/7 (100)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>Iatrogenic KS</td>
<td>6/6 (100)</td>
<td>4/4 (100)</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>Dermatological patients</td>
<td>0/4 (0)</td>
<td>0/4 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>3/13 (23)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*HHV-8 indicates human herpesvirus 8; PBMC, peripheral blood mononuclear cells; KS, Kaposi sarcoma; and NA, not applicable.

We also observed a different HHV-8 DNA detection rate in the PBMCs from patients with KS in different clinical stages. We detected HHV-8 DNA sequences in 9 (56%) of 16 patients with a cutaneous localized form of KS, in 7 (77%) of 9 patients with disseminated cutaneous KS, and in all 3 patients (100%) with cutaneous and visceral involvement.

Peripheral blood mononuclear cells from 13 healthy volunteers and 29 patients affected by various dermatological disorders were tested for HHV-8 DNA using a nested PCR technique. The prevalence of the viral DNA in the total control group was 23.8%: HHV-8 DNA sequences were detected in 10 (23.8%) of 42 PBMC samples analyzed. We found 3 (23%) of the 13 healthy volunteers and 7 (24%) of the 29 dermatological patients to have HHV-8 DNA (Table).

Among the control patients positive for HHV-8 DNA, 1 had scleroderma, 2 had melanoma, 2 had psoriasis, 2 had a dysplastic nevus, and 3 were healthy volunteers.
The 3 HHV-8-positive healthy volunteers and 1 dermatological patient were from Sardinia, a region where classic KS is prevalent.

No viral DNA was detected in tissue biopsy specimens taken from patients without KS who had undergone surgical excision of dermatological lesions.

We also analyzed all KS specimens (skin biopsy specimens and PBMCs) with primers amplifying specific EBV DNA sequences. Epstein-Barr virus DNA was detected in 4 (20%) of the 20 skin lesion biopsy specimens from both classic KS and iatrogenic KS and in 3 (27%) of the 11 peripheral unaffected skin samples examined. No EBV-related sequences were detected in normal distant skin samples of patients with KS. The prevalence of EBV DNA detection in PBMCs from patients with KS was similar to that found in the tissue specimens (30%).

Beginning in December 1994, numerous reports have described HHV-8 DNA sequences in tissue lesions and PBMCs from patients with all clinical forms of KS, supporting the hypothesis that this new virus could play a role in the pathogenesis of KS.13-18,25,26,30,31

On the other hand, it has already been reported that HHV-8 DNA is present in a variety of non-KS tissues, raising the questions whether HHV-8 might be relatively common and whether its presence in certain pathologic conditions is incidental and unrelated to disease pathogenesis.13-18,25,26,30,31 Recently, serologic data have yielded initial estimates of the seroprevalence of HHV-8 infection, showing that antibodies against HHV-8 were detected not only in risk groups for KS but also in the general population.32-33

To evaluate whether this virus is restricted to patients with KS or is widespread in the healthy population and contributes to the development of KS only under particular conditions, we searched for HHV-8 DNA in tissue and blood samples from Italian HIV-seronegative patients with and without KS, since classic KS, a clinical form not related to HIV infection, is prevalent among the elderly population in Italy.

Similar to other studies, we detected HHV-8 DNA sequences in all KS lesions and peripheral uninvolved skin specimens investigated. Distant normal skin samples from patients with KS tested positive for viral DNA in 33% of the cases, although 500 ng of DNA was tested for each specimen using a nested PCR technique. Human herpesvirus 8–negative distant normal skin samples belonged to patients with very localized disease; this could be one of the reasons for our positivity rate being lower than previously reported.6,12,13,25,36-38 Moreover, no viral DNA was found in skin biopsy specimens of control patients examined in this study.

These findings suggest that HHV-8 may be specifically associated with KS lesions, in which the presence of the virus precedes development of KS. Alternatively, KS tissue could be a haven where the circulating virus proliferates and subsequently disseminates to the surrounding skin. Human herpesvirus 8 diffusion may be supported by a hematogenous spread of the virus as its detection in PBMCs could suggest.39,40

In our study, HHV-8 DNA was found in 69% of the PBMC samples from patients with KS. In agreement with other reports, our results showed that HHV-8 DNA was not always detectable in PBMCs, as it was in all lesions of patients with KS.10,13,21,37,41

We also observed, as other authors have, a possible correlation between HHV-8 cell-associated viremia and clinical staging of KS.37,41 In our experience, HHV-8 DNA detection seems to be related to the clinical behavior of the disease and, in fact, we were not able to detect viral sequences in patients with just a few KS lesions. Moreover, we found that in 2 of 7 patients followed up for 6 months, HHV-8 DNA was not always detectable in samples collected at different times irrespective of treatment, which is in accordance with other reports.41

In our study the detection rate of EBV DNA in both lesions and PBMCs from patients with KS was similar to that observed by other authors in patients with classic KS and slightly higher than that observed in a healthy population.20 The relevance of EBV infection in the pathogenesis of KS and its possible interaction with HHV-8 in the cases of coinfection remain a matter of speculation20 in view of the higher prevalence reported among the Ugandan population.42

Detection of HHV-8 DNA in our control groups showed a positivity rate (23.8%) higher than previously described.25,38,43 The dermatological patients were comparable to patients with KS regarding age, sex, and geographical origin; this could be the first difference from other studies in which healthy subjects or blood donors, probably younger, were enrolled in the control group. Moreover, among control subjects positive for HHV-8 DNA, 4 (40%) came from Sardinia and 3 (30%) from Latium, 2 regions where classic KS is prevalent. As described for African patients,44 a possible explanation is that our results reflect the rate of HHV-8 infections in...
the Italian population without KS, suggesting that the distribution of the virus could be correlated with the varying degrees of prevalence of KS in different regions. On the other hand, evidence has been accumulating to indicate that these HHV-8 sequences could be detected in patients without KS, and seroepidemiological data recently published seem to confirm this wider distribution of the virus. Our findings are consistent with these reports, supporting the hypothesis that HHV-8 latently might infect large groups of the population in particular areas, probably remaining undetectable in many individuals until reactivation occurs.

Our results are in accordance with the hypothesis that this virus could be an infective cofactor in the pathogenesis of KS when other predisposing conditions, such as alterations of the immune system and genetic susceptibility, are present. The HHV-8 infection, closely associated with all clinical forms of KS, could play a role in the tumorigenic process of KS, although it is likely that several factors are involved.

In conclusion, our results suggest that HHV-8, like other herpesviruses, could be a widespread virus at least in some geographical areas. It may be present lifelong in latent form somewhere in the body and may be unmasked by factors of different origin including senescence. Finally, the higher prevalence of HHV-8 among the Italian population compared with North Americans may reflect the higher rates of different origin including senescence.12,17,19,30 The HHV-8 infection, closely associated with all clinical forms of KS, could play a role in the tumorigenic process of KS, although it is likely that several factors are involved.

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REFERENCES


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