Molecular evidence of organ-related transmission of Kaposi sarcoma–associated herpesvirus or human herpesvirus-8 in transplant patients

Mario Luppi, Patrizia Barozzi, Gaia Santagostino, Raffaella Trovato, Thomas F. Schulz, Roberto Marasca, Davide Bottalico, Lucia Bignardi, and Giuseppe Torelli

In transplant patients, Kaposi sarcoma (KS)-associated herpesvirus or human herpesvirus-8 (HHV-8) infection is associated with the development of KS, primary effusion lymphoma and Castleman disease. Whether HHV-8 is either reactivated in the recipient or transmitted by the donor has been investigated so far only by serologic studies. Thus, we addressed the issue of HHV-8 transmission in the transplantation setting by molecular methods. We exploited the high level variability of the orf-K1 gene and the polymorphism of the orf-73 gene of the HHV-8 genome to assess the genetic relatedness of the HHV-8 strains identified in the posttransplant KS lesions that developed, simultaneously, 20 months after transplantation, in 2 recipients of twin kidneys from the same cadaver donor.

The 100% identity of nucleotide sequence of the most variable viral region and the presence of the same, single orf-73 type in both patients provides strong molecular evidence of organ-related transmission of HHV-8 in the setting of transplantation. (Blood. 2000;96:3279-3281)

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Study design

In August 1994, 2 Italian women, 46 and 45 years old, respectively, living in different cities, received renal grafts from the same cadaver donor, a 53-year-old Italian man. Both patients received immunosuppression with cyclosporin A (CsA) and methylprednisolone (MP), but, during the posttransplantation course, both were also treated with MP pulses for an episode of acute rejection. Azathioprine was also added to the immunosuppressive regimen in one case. Twenty months after transplantation, cutaneous KS of the lower extremities developed simultaneously in both patients. A detailed description of the clinical histories of the 2 transplant patients has been reported elsewhere. Reduction of immunosuppressive therapy has led to KS regression in both patients, which still persists.

Frozen biopsy specimens from the cutaneous KS lesions of both
patients, as well as the Ficoll separated peripheral blood mononuclear cells (PBMCs) collected about 4 years after the initial diagnosis of KS, were available for molecular analysis. DNA was purified after proteinase digestion and phenol chloroform extraction. HHV-8 DNA was amplified on the same material by polymerase chain reaction (PCR) with primers specific for the orf-K1 gene, as we previously described. The PCR products were subjected to direct automated sequence analysis, and phylogenetic analysis of the K1 gene was performed as we previously reported. PCR amplification of the fragment of the orf-73 internal repeat domain was performed as described by Gao et al. To avoid false-positive results, all procedures were performed in strict adherence to the recommendations of Kwok and Higuchi. Negative controls consisting of all reagents, except sample DNA, were also present during the DNA extraction and equalled or exceeded the number of samples assayed.

Results and discussion

HHV-8 DNA was detected by PCR in the cutaneous posttransplant KS lesion from both renal recipients. Sequence analysis of the 2 highly variable regions of the orf-K1 gene from the 2 patients showed 100% identity (Figure 1A) and phylogenetic analysis showed that the infecting strain belonged to clade C, which is rather common in Italy. The orf-K1 gene sequence results were also the same in the PBMCs collected from the 2 patients about 4 years after the initial diagnosis of KS. PCR of the orf-73 internal repeat domain showed a single band of the same size in both patients (Figure 1B). In the same assay, a single band of different size was detected in one classic KS and in one PEL specimen, indicating the occurrence of different isolates in these latter cases (Figure 1B).

Sera collected before and after transplantation from 28 recipients who had posttransplant KS develop, have so far been examined for anti–HHV-8 antibodies in 8 independent studies. Twenty-three patients, most of whom originated from endemic areas, were infected with HHV-8 before the graft, suggesting that KS was mainly due to virus reactivation, the occurrence of HHV-8 transmission from the donor should not be underestimated. Analysis of the genetic relatedness in the highly variable orf-K1 gene and the polymorphic orf-73 gene of HHV-8 isolates from the transplant recipients and their paired donors represents a useful tool to obtain a molecular tracing of HHV-8 transmission in this clinical setting.

Table 1. Updated review of serologic studies for anti–HHV-8 antibodies in patients with posttransplant Kaposi sarcoma

<table>
<thead>
<tr>
<th>No. of patients seroconverting to HHV-8 after transplantation</th>
<th>No. of patients with anti–HHV-8 Ab before transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1 (3)</td>
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<tr>
<td>0</td>
<td>2 (4)</td>
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<tr>
<td>4</td>
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<td>1</td>
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</tr>
<tr>
<td>1</td>
<td>0 (10)</td>
</tr>
<tr>
<td>Total no. of patients</td>
<td>23</td>
</tr>
</tbody>
</table>

HHV-8 indicates human herpesvirus-8; Ab, antibodies.

References


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