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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M.L., P.B., and G.S. equally contributed to the study.

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performed in strict adherence to the recommendations of Kwok and common in Italy.\textsuperscript{15} The showed 100% identity (Figure 1A) and phylogenetic analysis of the highly variable regions of the KS lesion from both renal recipients. Sequence analysis of the HHV-8 DNA was detected by PCR in the cutaneous posttransplant recipients, 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.\textsuperscript{15} The PCR products were subjected to direct automated sequence analysis, and phylogenetic analysis of the K1 gene was performed as we previously reported.\textsuperscript{15} PCR amplification of the fragment of the orf-73 internal repeat domain was performed as described by Gao et al.\textsuperscript{16} To avoid false-positive results, all procedures were performed in strict adherence to the recommendations of Kwok and Higuchi.\textsuperscript{18} 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.\textsuperscript{15} 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.\textsuperscript{3,10} 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.\textsuperscript{3,5,10} In the remaining 5 patients who seroconverted after transplantation, transmission of HHV-8 from the organ donor to the recipient was suggested.\textsuperscript{3,4} However, for 4 of these patients, originating from nonendemic areas, the source of infection was not determined, as serum samples from their paired donors were not available.\textsuperscript{4} For one patient, originating from an endemic area (southern Italy), serum from the living-related donor was seropositive for HHV-8, providing the only clear available example of organ-related transmission of HHV-8, although the serum was tested more than 2 years after transplantation (Table 1).\textsuperscript{3}

In our study, the finding of a 100% identity of nucleotide sequence of the most variable region of HHV-8 genome and the presence of the same, single orf-73 type in the posttransplant KS lesions of the 2 patients receiving twin kidneys from the same cadaver donor, provide strong molecular evidence of organ-related transmission of HHV-8. Although among populations with high HHV-8 seroprevalence, the development of posttransplant KS has been found to be mainly associated with HHV-8 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.

References


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 with anti–HHV-8 Ab before transplantation</th>
<th>No. of patients seroconverting to HHV-8 after transplantation</th>
<th>Reference no.</th>
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</thead>
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<tr>
<td>10</td>
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<td>(3)</td>
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<td>(10)</td>
</tr>
<tr>
<td>Total no. of patients</td>
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<td>5</td>
</tr>
</tbody>
</table>

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


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