Bone Marrow Failure Associated with Human Herpesvirus 8 Infection After Transplantation

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Abstract

Background Human herpesvirus 8 (HHV-8) infection has been linked to the development of Kaposi’s sarcoma and to rare lymphoproliferative disorders.

Methods We used molecular methods, serologic methods, in situ hybridization, and immunohistochemical analyses to study HHV-8 infection in association with nonmalignant illnesses in three patients after transplantation.

Results Primary HHV-8 infections developed in two patients four months after each received a kidney from the same HHV-8–seropositive cadaveric donor. Seroconversion and viremia occurred coincidentally with disseminated Kaposi’s sarcoma in one patient and with an acute syndrome of fever, splenomegaly, cytopenia, and marrow failure with plasmacytosis in the other patient. HHV-8 latent nuclear antigen was present in immature progenitor cells from the aplastic marrow of the latter patient. Identification of the highly variable K1 gene sequence of the HHV-8 genome in both the donor’s periph­eral-blood cells and the recipients’ serum confirmed that transmission had occurred. HHV-8 viremia also occurred after autologous peripheral-blood stem-cell transplantation in an HHV-8–seropositive patient with non-Hodgkin’s lymphoma. Reactivation of the infection was associated with the development of fever and marrow aplasia with plasmacytosis; there was no evidence of other infections. HHV-8 transcripts and latent nuclear antigen were expressed in the aplastic marrow but not in two normal marrow samples obtained before transplantation.

Conclusions Primary HHV-8 infection and reactivation of infection may be associated with nonneoplastic complications in immunosuppressed patients.

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Infection with human herpesvirus 8 (HHV-8) has been implicated in the development of Kaposi’s sarcoma, primary effusion lymphoma, and multicentric Castleman’s disease of the plasma-cell type. These conditions have been described most frequently in patients infected with the human immunodeficiency virus (HIV), but they have also been reported in transplant recipients, and immunosuppression is thought to be an important cofactor in their pathogenesis. Whether primary HHV-8 infection, reactivation of infection, or both lead to a nonneoplastic illness has yet to be determined. We evaluated the course of illness in two patients in whom a primary HHV-8 infection developed after kidney transplantation and one patient who had reactivation of HHV-8 infection after peripheral-blood stem-cell transplantation.

Case Reports

Patient 1

Patient 1 was a 61-year-old Italian man with end-stage renal disease of unknown cause who had been receiving hemodialysis since April 1988. He received a renal allograft from a cadaveric donor in December 1998. The donor was a 67-year-old Italian man who had died of a cerebrovascular accident. Serologic tests for HIV, hepatitis B virus, hepatitis C virus, and cytomegalovirus were negative. The donor’s HLA haplotype was A2, A11, B35, B51, DR5, DR6.

Serologic tests showed that Patient 1 was negative for HIV and hepatitis B virus but positive for hepatitis C virus. His HLA haplotype was A1, A2, B35, B51, DR5, DR6. Before transplantation his immunosuppressive regimen consisted of cyclosporine and methylprednisolone, and in March 1999, after transplantation, he received pulsed doses of methylprednisolone because of one episode of rejection. In April 1999 a diagnosis of Kaposi’s sarcoma involving the cervical lymph nodes, the tracheobronchial tract, and the gastrointestinal tract was made.

Patient 2

Patient 2 was a 44-year-old Italian man with membranoproliferative glomerulonephritis who received a renal allograft in December 1998 from the same cadaveric donor as Patient 1. Serologic tests were negative for HIV, hepatitis B virus, and hepatitis C virus. His HLA haplotype was A2, A11, B18, B35, DR5, DR6. He received an immunosuppressive regimen that included cyclosporine and methylprednisolone. The graft began to function immediately after transplantation, and serum creatinine levels were normal within two weeks after transplantation. In March 1999 an episode of acute rejection was successfully treated with pulsed doses of methylprednisolone, with partial improvement of renal function (serum creatinine, 2.0 mg per deciliter [177 µmol per liter]). On April 20, 1999, the patient presented with a temperature of more than 38°C and splenomegaly. The hemoglobin value was 6.8 g per deciliter, with a reticulocyte count of less than 1 percent; the white-cell count was 3600 per cubic millimeter; and the platelet count was 110,000 per cubic millimeter.

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In May 1998. A dose of $11.8 \times 10^5$ unmanipulated CD34+ cells followed by autologous peripheral-blood stem-cell transplantation with cyclophosphamide, doxorubicin, vincristine, and prednisone. The results of bone marrow failure associated with human herpesvirus 8 infection after transplantation.

METHODS

Serologic Studies

Serum samples from Patients 2 and 3 were collected several times, before and after transplantation, as indicated in Figures 1 and 2. Serum samples from Patient 1 were collected at the same times as those from Patient 2 (data not shown). The serologic assays have been described elsewhere.15-17

Histopathological, Immunohistochemical, and in Situ Hybridization Studies

Formalin-fixed and paraffin-embedded bone marrow specimens were obtained from core biopsies in Patient 3 as well as those from Patient 2 (data not shown). The serologic assays have been described elsewhere.15-17

Donor

Serum obtained from the donor one day before the kidneys were removed was positive for HHV-8 in five serologic assays. Antibodies against latent and lytic HHV-8 antigens were detectable on immunofluorescence assays, and antibodies against three different recombinant proteins, derived from ORF 65, K8.1, and 73, were detectable on enzyme-linked immuno-

Bone marrow failure associated with human herpesvirus 8 infection after transplantation.
Figure 1. Changes in the Platelet Count, White-Cell Count, Hemoglobin Level, Serum Level of Human Herpesvirus 8 (HHV-8) DNA, and Results of Tests for Antibodies against HHV-8 Antigen in Patient 2 from the Time of Renal Transplantation to Death. Therapy consisted of epoetin, granulocyte colony-stimulating factor (circles), and transfusions of platelets (squares) and red cells (stars).
Figure 2. Changes in the Platelet Count, White-Cell Count, Hemoglobin Level, Serum Level of Human Herpesvirus 8 (HHV-8) DNA, and Results of Tests for Antibodies against HHV-8 Antigen in Patient 3 from the Time of the Autologous Peripheral-Blood Stem-Cell Transplantation on Day 0 to Death.

Therapy consisted of corticosteroids, granulocyte colony-stimulating factor (circles), and transfusions of platelets (squares) and red cells (stars). A lytic immunofluorescence assay was used to detect HHV-8 antibodies.
sorbent assay and Western blotting. HHV-8 sequences (ORF 26 and K1) were also detected by a nested PCR assay in the DNA extracted from unseparated peripheral-blood mononuclear cells.

**Renal-Transplant Recipients**

Serum samples obtained from Patients 1 and 2 at the time of transplantation were negative for HHV-8 in five serologic assays, as were serum samples obtained three months before transplantation in the case of Patient 1 and one month beforehand in the case of Patient 2. The analysis of the first available post-transplantation serum sample, obtained four months after transplantation from Patient 1, at the onset of Kaposis's sarcoma, and from Patient 2 at the onset of symptoms, showed seroconversion. Two subsequent serum samples, obtained one week apart from both patients, were also positive for HHV-8 on all serologic assays used.

HHV-8 DNA (ORF 26 and K1 sequences) was not detectable in serum samples from the two patients at the time of transplantation or before the procedure, but it was detected by PCR assay in the three available serum samples collected after transplantation, both from Patient 1 (50 to 100 copies of HHV-8 DNA per milliliter, as determined semiquantitatively by serial dilutions of DNA from the patient) (data not shown) and from Patient 2 (50,000 to 100,000 copies of HHV-8 DNA per milliliter) (Fig. 1). Sequence analysis of the two highly variable regions of the K1 gene from these two patients and the donor showed that both the nucleotide and the amino acid sequences were identical, and phylogenetic analysis showed that the infecting strain belonged to clade C, which is rather common in Italy.28 (Additional information is available on our Web site at http://www.unimo.it/gisl/luppi/nobacknejm.htm).

HHV-8 LANA was expressed in the nuclei of about 3 percent of the bone marrow cells from Patient 2 (Fig. 3A and 3B). Cells expressing LANA appeared morphologically to be immature bone marrow cells. LANA was present in distinct subnuclear domains in a pattern similar to that seen in cultured primary-effusion lymphoma cell lines (Fig. 3D).18 No staining with this antibody was seen in bone marrow specimens obtained from core biopsies in the five controls (data not shown).

**Recipient of Autologous Peripheral-Blood Stem Cells**

HHV-8 DNA (ORF 26 and K1) was transiently detected by a nested PCR assay (50 to 100 copies of HHV-8 DNA per milliliter) in the serum obtained from Patient 3 two days and eight days after transplantation, the period during which he had an intermittent fever, but the levels became undetectable immediately thereafter (Fig. 2). HHV-8 DNA was also undetectable in the serum collected 84 days before transplantation. A PCR assay with degenerate (nonspecific) primers for the DNA polymerase gene of herpesviruses confirmed the presence of HHV-8 sequences in the patient’s serum. HHV-8 DNA (ORF 26 and K1) again became detectable in the serum obtained 62 days after transplantation, and the viral load was high enough (50,000 to 100,000 copies per milliliter) to be detectable even by a one-step PCR assay (Fig. 2). On the basis of the sequencing of the K1 gene, the HHV-8 variant in this patient was classified as clade A.28

HHV-8 DNA (500 to 1000 copies per milliliter), detected by a nested PCR assay in serial serum samples, persisted concomitantly with the bone marrow aplasia and peripheral cytopenia until the patient died (Fig. 2). HHV-8 DNA sequences were also detected by a nested PCR assay in the DNA extracted from the Ficoll-separated peripheral-blood stem cells collected on day 62 after transplantation. In situ hybridization studies also revealed a few HHV-8–infected cells that expressed the latent T0.7 transcript in the aplastic bone marrow obtained on day 62 (Fig. 4). No hybridization signal was detectable in the two histologically normal bone marrow specimens obtained in December 1997 and February 1998 after the diagnosis of non-Hodgkin's lymphoma (Fig. 4). Immunohistochemical analysis also revealed HHV-8 LANA only in the aplastic bone marrow (Fig. 3C). On morphologic examination, HHV-8–positive cells appeared to be immature bone marrow cells. The presence of antibodies against HHV-8 lytic antigens was documented by immunofluorescence assay in the serum obtained 84 days before transplantation and after transplantation, but no antibodies against the latent nuclear antigen or recombinant capsid (ORF 65) or membrane (ORF K8.1) proteins were found.

**DISCUSSION**

The findings in our three patients show that primary infection with HHV-8 or reactivation of infection may be associated with nonmalignant disease after transplantation. In one renal-transplant recipient, the sudden onset of persistent fever, splenomegaly, and marked cytopenia suggested the presence of an acute infectious disease. Extensive microbiologic testing before the patient’s death failed to identify any other agents. Subsequent studies showed seroconversion and high levels of viremia, indicative of a recent primary infection with HHV-8. Our finding of HHV-8 LANA, which is expressed in persistently (latently) infected cells,18,31 within immature bone marrow cells strengthens the causal relation between primary HHV-8 infection and the bone marrow failure in this patient.

We also documented the transmission of HHV-8 from a kidney donor to two transplant recipients by showing that seroconversion occurred almost simultaneously in the two transplant recipients and that the K1 sequences of the HHV-8 DNA were the same in the donor and the two recipients. The K1 gene of HHV-8 is highly variable and has been used to iden-
Figure 3. Expression of Human Herpesvirus 8 (HHV-8) Latent Nuclear Antigen in Bone Marrow Cells from Patient 2 (Panels A and B, ×450) and Patient 3 (Panel C, ×400) and an HHV-8–Infected Primary-Effusion Lymphoma Cell Line (Panel D, ×500).

Immunohistochemical analysis was performed with a polyclonal rabbit antibody against recombinant latent nuclear antigen 1 (LANA) of HHV-8 encoded by open-reading-frame 73 (ORF 73). Specimens were counterstained with hematoxylin. LANA was detected in the nuclei of immature bone marrow cells (arrows) from Patient 2 four months after kidney transplantation (Panels A and B) and Patient 3 two months after the transplantation of peripheral-blood stem cells (Panel C). The pattern of expression of LANA was similar in the nuclei of the HHV-8–infected BCBL-1 cells (Panel D). There was no staining of bone marrow specimens obtained from core biopsies in five controls or of normocellular bone marrow specimens obtained from core biopsies in Patient 3 in December 1997 and February 1998, before transplantation (not shown).
The simultaneous occurrence of disseminated Kaposi’s sarcoma in one renal-transplant recipient and bone marrow failure in the other suggests that the same virus may have a different pathogenic potential in different, although HLA-related, human hosts.

In Patient 3, the detection of antibodies against HHV-8 lytic antigen before transplantation suggests that the viremia was likely to be due to reactivation of the infection rather than to a primary infection with HHV-8. The detection of HHV-8–infected cells by in situ hybridization and immunohistochemical analysis in the aplastic bone marrow, in which plasmacytosis was evident, but not in two previous normal bone marrow specimens from the same patient, suggests that infection of bone marrow cells occurred as a result of viral reactivation and that the virus is myelosuppressive.

Among herpesviruses, Epstein–Barr virus has been implicated in aplastic anemia, and cytomegalovirus and HHV-6 have been reported to exert a myelosuppressive effect in vitro and to be largely responsible for delayed platelet engraftment in transplant recipients. Our findings suggest that HHV-8 can also cause bone marrow failure, at least in immunosuppressed patients after transplantation. The severity of the illness in our patients was most likely related to the immunosuppression and is not typical of a primary infection or a reactivation of infection in immunocompetent subjects. The plasma-cell infiltration in the aplastic bone marrow of these transplant patients may be associated with the HHV-8 infection.

Kaposi’s sarcoma is very rare after the transplantation of allogeneic or autologous bone marrow or peripheral-blood stem cells. However, we found that other complications associated with HHV-8 infection may occur occasionally in such patients, at least in our geographic area (the lower Po valley of northern Italy), where the seroprevalence of HHV-8 infection among blood donors is about 13 percent. The identification of a serologic response to HHV-8 in Patient 3 by the lytic immunofluorescence assay but not by the...
three other assays illustrates the drawbacks of relying on a single antibody assay to identify HHV-8 infection in HIV-negative patients without Kaposi's sarcoma.16,19

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