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Nonmalignant disease associated with human herpesvirus 8 reactivation in patients who have undergone autologous peripheral blood stem cell transplantation

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Fever, cutaneous rash, and hepatitis—for which an infectious cause was suspected—developed in an Italian patient with non-Hodgkin lymphoma after autologous peripheral blood stem cell (PBSC) transplantation. Polymerase chain reaction (PCR) with degenerate primers for the highly conserved DNA polymerase gene of herpesviruses detected herpesvirus sequences 100% identical to human herpesvirus-8 (HHV-8) in serial cell-free serum samples, collected immediately before or concomitant with the occurrence of clinical symptoms; no other common infections were documented. The presence of the HHV-8 genome (clade C) was confirmed by PCR with HHV-8–specific primers for orf 26 and orf-K1. HHV-8 viremia was undetectable either before transplantation or when the patient was clinically asymptomatic. Semiquantitative PCR analysis showed variations of the viral load correlating with the clinical status. Anti–HHV-8 antibodies were detected before and after transplantation by an immunofluorescence assay for lytic antigens. Active HHV-8 infection may be associated with nonmalignant illness after PBSC/bone marrow transplantation. (Blood. 2000;96:2355-2357)

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Introduction

Human herpesvirus-8 (HHV-8) primary infection and reactivation are associated with the development of Kaposi sarcoma, primary effusion lymphoma, Castleman disease, and plasmacytic proliferation in patients who undergo solid organ transplantation.[1-9] In contrast, in patients who undergo bone marrow (BM) and peripheral blood stem cell (PBSC) transplantation, Kaposi sarcoma is the exception.[10] Only one study has so far been conducted to investigate HHV-8 seroprevalence in patients after allogeneic BM transplantation. It did not find any association between the presence of antibodies to HHV-8 latent nuclear antigen before or after transplantation, to chronic graft-versus-host disease, or to overall BM transplantation survival.[11]

In this report we used a combination of polymerase chain reaction (PCR)-based assays, with degenerate and specific primers, and several serologic assays to describe a temporal association between HHV-8 reactivation and the development of fever, cutaneous rash, and hepatitis in an Italian patient who received autologous PBSC infusions.

Study design

In September 1996, a 35-year-old man was found to have B-cell non-Hodgkin lymphoma of Burkitt-like type, involving the jejunum and the mesenteric, laterocervical, and supraclavicular nodes. He underwent excision of multiple parts of the jejunum and received a first treatment with chemotherapy according to the BFM-LNH81 protocol (cyclophosphamide, prednisone, methotrexate, aracytine, teniposide, doxorubicin), achieving complete clinical remission. His PBSC were mobilized with cyclophosphamide and granulocyte colony-stimulating factor (G-CSF). Then he underwent autologous PBSC transplantation in July 1997. The conditioning regimen consisted of BEAM chemotherapy (carmustine, etoposide, aracytine, and melphalan). A dose of 4.5×10^6/kg unmanipulated $CD34^+$ cells was reinfused, and the patient's neutrophil count became greater than 0.5×10^9/L in 10 days, and his platelet count became 20×10^9/L in 12 days. Prophylactic treatment with ciprofloxacin, itraconazole, acyclovir, and immunoglobulin was administered. Seventeen days after PBSC reinfusion, the patient had intermittent fever higher than 38°C, asthenia, and increased levels of the liver enzymes aspartate aminotransferases (AST; 145 IU/L) and alanine aminotransferases (ALT; 255 IU/L). Two days later (day 19) the patient contracted a cutaneous maculopapular rash, which regressed in 4 days, and diarrhea, which lasted for 3 days. Liver enzyme levels reached a peak (AST, 263 IU/L; ALT, 367 IU/L) and then progressively decreased but maintained elevated, with variations, for 6 months (Figure 1). Chest radiograms were negative, and ultrasonographic examination of the abdomen only revealed homogeneous enlargement of

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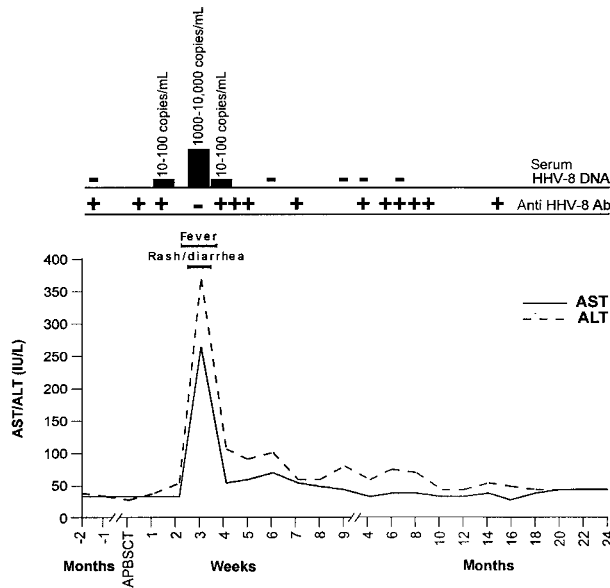

Figure 1. Clinical history and virologic study. Kinetics of AST and ALT, clinical signs, HHV-8 DNA (copies/mL), and anti-HHV-8 antibodies (Ab) in the patient, after autologous peripheral blood stem cell transplantation (APBSCT).

the liver. The patient has remained asymptomatic and in complete remission, and neither Kaposi sarcoma nor lymphoproliferative disease has been diagnosed in the posttransplant period, after a follow-up of approximately 2 years.

Results and discussion

Routine blood and urine cultures and serologies for common bacterial, fungal, and viral infections were negative. Nuclei acid extraction was performed in serial cell-free serum samples, as described.[12] Herpesvirus (HSV-1, HSV-2, VZV, EBV, HHV-6, HHV-7, CMV), adenovirus, polyomavirus (JC, BK) and hepatitis (A, B, C, G) virus DNA and RNA were not detected in the sera by PCR assays, using described protocols.[12,13] Using PCR with degenerate primers targeting the highly conserved DNA polymerase gene of herpesviruses,[14] amplification products of the predicted size of 236 base pairs were detected in the sera collected at days 12, 21, and 23 after transplantation, immediately before and concomitant with the clinical symptoms. The resultant PCR fragments were sequenced, and the alignment of the nucleotide and amino acid sequences revealed that the DNA sequences identified in the sera were 100% identical to the prototype HHV-8 DNA polymerase gene sequence (Figure 2).[14] The presence of the HHV-8 genome was further confirmed by PCR amplification of specific orf 26 and K1 sequences of HHV-8 in the same serum samples.[15-17] Nested PCR for the K1 gene detected as few as 50 HHV-8 copies, determined by serial dilutions of the HHV-8–positive BCBL-1 cell line. HHV-8 DNA was not detectable in the serum collected before transplantation. It was first detected by nested PCR (10-100 copies/mL, as determined semiquantitatively by 10-fold serial dilutions of patient DNA) in the serum collected at day 12 after transplantation (day +12), before the onset of fever and the increase of aminotransferase levels (Figure 1). HHV-8 viremia persisted throughout the duration of the clinical symptoms (Figure 1). Semiquantitative PCR analysis also showed that the viral load increased progressively to 1000 to 10 000 copies/mL at day +21, to be detectable even by 1-step PCR. Then it decreased to 10 to 100 copies/mL at day + 23 and was no longer detectable after the regression of clinical symptoms (Figure 1). HHV-8 sequences were also detected by nested PCR (orf 26 and K1) in the DNA extracted from the Ficoll-separated PBMC collected on days +21 and +23. Phylogenetic analysis and sequence analysis of the 2 highly variable regions of the K1 gene from this patient showed that the infecting strain belonged to clade C, known to be common in Italy.[17] This patient had antibodies to HHV-8 that were detectable by immunofluorescence assay for lytic antigens before transplantation (day −60) and in all but one serum sample collected after transplantation. Interestingly, no antibodies were detectable in the serum sample (at day +21) with the highest viral load. The patient had no detectable antibodies to the latent nuclear antigen or to recombinant capsid protein encoded by orf 65[18-20] or recombinant membrane protein encoded by K8.1.[21]

Thus far, HHV-8 infection has been involved in the development of neoplastic diseases in patients after transplantation.[1-9] Our study shows that active HHV-8 infection may be associated with nonmalignant pathologic events in PBSC recipients, at least in our geographic area (lower Po Valley, northern Italy), where HHV-8 seroprevalence in the blood donor population is approximately 13%.[22,23] The detection of anti-HHV-8 antibodies before transplantation suggests that HHV-8 viremia is likely to be caused by viral reactivation rather than by primary infection with HHV-8. The fact that HHV-8 viremia was detectable in serial cell-free serum samples collected immediately before or concomitant with the clinical symptoms, but undetectable in the serial sera collected after the regression of the symptoms and before transplantation, support a causal association between HHV-8 infection and the disease in this patient. HHV-8 reactivation occurred early (within the first 30 days) after transplantation, was transient, and was associated with an acute but self-limited clinical syndrome characterized by fever, hepatitis, and cutaneous rash—symptoms that have been noted in the context of other human herpesvirus infections. HHV-8 reactivation in this patient could not be prevented by prophylactic treatment with acyclovir, consistent with the reported in vitro resistance of HHV-8 to this and to the most common antivirals.[24,25] Finally, the identification of a serologic response to HHV-8 with only the lytic immunofluorescence assay suggests that using a single antibody assay may underestimate the true prevalence of HHV-8 infection in HIV-negative patients without Kaposi sarcoma and that at least some patients with "lytic immunofluorescence assay only" reactivity are genuinely infected with HHV-8. Thus, PCR appears useful for the early identification and monitoring of HHV-8 viremia and its associated complications in patients who undergo BM and PBSC transplantation.

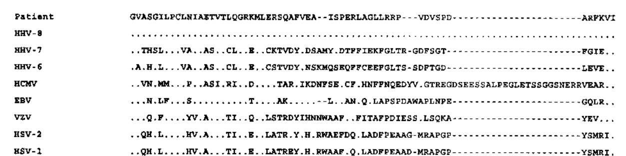

Figure 2. HHV-8 DNA polymerase sequence identified by degenerate PCR assay. Alignment of amino acid translations of sequences from the herpesvirus DNA polymerase gene obtained from the patient's sera, with analogous regions from the DNA polymerase sequences of other herpesviruses[14]: human herpesvirus-8 (HHV-8), human herpesvirus-7 (HHV-7), human herpesvirus-6 (HHV-6), human cytomegalovirus (HCMV), Epstein–Barr virus (EBV), varicella–zoster virus (VZV), herpes simplex virus type 2 (HSV-2), and herpes simplex virus type 1 (HSV-1). Dots indicate identity with the first sequence in the alignment.

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