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. Semi-quantitative 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.

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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. In contrast, in patients who undergo bone marrow (BM) and peripheral blood stem cell (PBSC) transplantation, Kaposi sarcoma is the exception. 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.

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 ciprofloxacine, 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
HHV-8 in the same serum samples. Nested PCR for the K1
sequences was 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. 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 (day +2) 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 or recombinant membrane protein
encoded by K8.1.21

Thus far, HHV-8 infection has been involved in the develop-
ment of neoplastic diseases in patients after transplantation.2−9
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 transplan-
tation 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 serum 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 reactiva-
tion 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
may underestimate the true prevalence of HHV-8 infection in
HHV-negative patients without Kaposi sarcoma and that at least
some patients with “lytic immunofluorescence assay only” reactiv-
ity 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.

Results and discussion

Routine blood and urine cultures and serologies for common
bacterial, fungal, and viral infections were negative. Nucleic 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 hepati-
tis (A, B, C, G) virus DNA and RNA were not detected in the
sera by PCR assays, using described protocols. 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, immedi-
ately 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. Nested PCR for the K1
genotype was 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 semiquantiatively by 10-fold serial dilutions
of patient DNA) in the serum collected at day 12 after transplanta-
tion (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

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).

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: human herpesvirus-8 (HHV-
8), human herpesvirus-7 (HHV-7), human herpesvirus-6 (HHV-6), human cytomega-
lovirus (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.
References

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