Transmission of Integrated Human Herpesvirus 6 through Stem Cell Transplantation: Implications for Laboratory Diagnosis

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We identified a stem cell donor with chromosomally integrated human herpesvirus (HHV)-6 and monitored the recipient for HHV-6 after transplantation. The appearance and subsequent increase in HHV-6 load paralleled engraftment and an increase in white blood cell count. Fluorescent in situ hybridization analysis showed integrated HHV-6 on chromosome band 17p13.3 in the donor and in the recipient after transplantation but not in the recipient before transplantation. The increase in viral load due to the genetic transmission of integrated HHV-6 could have been misinterpreted as substantial active infection and, thus, led to the administration of toxic antiviral therapy. We suggest that the confounding influence of integration be considered in laboratory investigations associating HHV-6 with disease.

Human herpesvirus (HHV)-6 persists in the host throughout life after primary infection, and polymerase chain reaction (PCR) analysis of peripheral-blood mononuclear cells (PBMCs) shows very low HHV-6 DNA loads in immunocompetent individuals, which is consistent with viral latency [1]. There are 2 variants of HHV-6, A and B, and they differ in genetic, epidemiological, and pathological properties. Virus reactivation can occur during immunosuppression, and there is growing evidence indicating that HHV-6 is pathogenic in transplant recipients.

An alternative form of HHV-6 persistence is characterized by abnormally high levels of HHV-6 DNA in PBMCs and integration of viral DNA sequences into chromosomes. Luppi et al. [2] first reported 3 individuals with high HHV-6 DNA loads in PBMCs and subsequently used fluorescent in situ hybridization (FISH) analysis to show the existence of integrated HHV-6B sequences on the telomeric extremity of the short arm of chromosome 17 (17p13) in all 3 cases [3]. Other investigators then reported 2 patients with lymphoproliferative disease with integrated HHV-6B sequences on chromosome band 1q44 (in one case) and 22q13 (in the other); integrated viral sequences were also found at similar chromosomal sites in family members, suggesting chromosomal inheritance [4, 5]. Tanaka-Taya et al. [6] identified 5 unrelated people with persistently high HHV-6 DNA loads (4 with variant B, and 1 with variant A) and an additional 5 members of their families with similar HHV-6 DNA loads in PBMCs. In 2 of the families studied, a mother and son plus a father and daughter were shown to have integrated viral sequences on chromosome band 22q13. This study suggested that the prevalence of integrated HHV-6 was 0.21%.

For human herpesviruses, the phenomenon of HHV-6 integration is unique, and its inheritance as a genetic element is completely novel. At present, it is not known whether integrated HHV-6 is associated with disease or, indeed, is capable of replication. We hypothesized that, if an individual with integrated HHV-6 became a stem cell donor, then genetic transmission of the viral sequences to the recipient could be misinterpreted as active HHV-6 infection after transplantation. We here report the first documented example of such a transmission event.

Patients, materials, and methods. A 47-year-old man with acute myeloid leukemia (subtype M1) in second complete remission was referred for an HLA-identical allogeneic stem cell transplant from his 37-year-old sister. PBMCs were harvested by apheresis from the donor after granulocyte colony-stimulating factor mobilization, with a yield of 4.05 × 10^6 cells/kg of recipient body weight. T cell depletion was performed by the addition of 10 mg of alemtuzumab to the donor cells before administration to the patient. Pretransplantation, both the donor and the recipient were seropositive for HHV-6 [7], and only the recipient was seropositive for cytomegalovirus (CMV).
(BioMerieux VIDAS; BioMerieux). CMV prophylaxis consisted of intravenous (iv) acyclovir (10 mg/kg 3 times a day) to day 13 after transplantation, followed by oral valaciclovir (2 g 4 times a day). The present study was approved by the local ethics committee, and the patient provided written, informed consent.

HHV-6 was monitored in whole blood by a real-time quantitative PCR assay that amplifies a region of the U67 gene. This PCR analysis was performed using an ABI 7700 sequence detector (Applied Biosystems) with the amplification primers U67F (5′-GGCTAGAACGTATTTGCTGCAGA-3′; forward) and U67R (5′-AATGTACGTCCCGAAATGG-3′; reverse) and a dual-labeled TaqMan probe, U67P (5′-[FAM]CGTTTCGGAGCTCAAGATCAACAATGT[TAMRA]-3′).

Primer sets that amplify sequences of each of the predicted open-reading frames (ORFs) of the unique region of the HHV-6 genome (from U2 to U100) were used in conventional PCR assays, in which DNA extracted from a donor lymphoblastoid cell line (LCL) (described below) was used as template. Variant typing of HHV-6 was conducted as described elsewhere [8].

An Epstein-Barr virus–transformed LCL was derived from donor PBMCs, and chromosome preparations of the donor LCL and the recipient’s bone marrow samples were made using established protocols [9]. A cocktail of 8 plasmids containing between 9-kb and 16-kb inserts of the HHV-6 genome (non-overlapping) were used as probes (7 of the plasmids were gifts from S. Schmid [Centers for Disease Control and Prevention, Atlanta, Georgia], and plasmid pZHV14 was a gift from R. Jarrett [University of Glasgow, Glasgow, United Kingdom]). The pool of HHV-6 plasmids was labeled with spectrum green dUTP (Vysis) by nick translation, and 100 ng of HHV-6 probe combined with spectrum orange–labeled telomere probe specific for the long arm of either chromosome 11 or 17 (Vysis) was added to the slide before incubation at 37°C overnight. After posthybridization steps were completed [9], chromosomes were counterstained with diamino-2-phenylindole. Fluorescence imaging was done using a SmartCapture FISH station (Digital Scientific).

**Results.** Before transplantation, the donor had a viral load of $8 \times 10^4$ HHV-6 genomes/mL of whole blood. The recipient was negative for HHV-6 DNA by PCR on days −7 and 0 but became positive on day 7, with a viral load of $2.4 \times 10^4$ HHV-6 genomes/mL of whole blood (figure 1). Neutrophil engraftment (>0.5 × 10⁸ cells/L of whole blood) occurred on day 10, and the viral load increased to $2.3 \times 10^4$ HHV-6 genomes/mL of whole blood. Platelet engraftment (>50 × 10⁸ cells/L of whole blood) was noted on day 12. Subsequent weekly testing showed a consistent viral load that ranged from $2.5 \times 10^4$ to $10 \times 10^4$ HHV-6 genomes/mL of whole blood (figure 1). The recipient was not given antiviral drugs that were active against HHV-6, because it was felt that he had probably acquired integrated HHV-6 DNA rather than an active viral infection.

On day 7, the patient was treated with meropenem for fever with neutropenia. Blood culture results were negative. Serial PCR surveillance of peripheral blood for CMV DNA detected a positive sample on day 38 (670 CMV genomes/mL of whole blood) and 2 consecutive positive samples on days 73 and 76 (517 and 475 CMV genomes/mL of whole blood, respectively), which met our criteria for preemptive therapy. The recipient was treated with iv ganciclovir (10 mg/kg/day), which was discontinued after 5 days when PCR for CMV DNA became negative. Grade 1 graft-versus-host disease of the skin developed 1 month after transplantation, and the recipient was treated with topical corticosteroids. He was no longer a patient at the transplant center 5 months after transplantation, and relapse occurred 3 months later.

PCR analysis of the donor LCL identified HHV-6A sequences and 94 of 94 predicted HHV-6 ORFs in the unique region of the viral genome. FISH analysis revealed integration of HHV-6 sequences on chromosome band 17p13.3 in the donor LCL (figure 2A and 2B). Similar analysis of a preparation of bone marrow obtained from the recipient 115 days before transplantation showed no evidence of integrated HHV-6 (figure 2C). In bone marrow obtained on day 88 after transplantation, HHV-6 sequences were identified on chromosome band 17p13.3 (figure 2D), and FISH analysis showed that the cells were of donor origin (100% XX karyotype).

**Discussion.** This report demonstrates that a stem cell recipient can become positive for HHV-6 DNA by PCR when engraftment occurs and the donor had integrated HHV-6. The pattern of results shown in figure 1 could easily be misinterpreted as active infection and prompt the administration of toxic antiviral drugs. An almost identical viral-load profile in this earlier article should consider returning to the patient,
if possible, and collecting samples, to determine whether the person has integrated virus.

FISH analysis of the donor LCL and posttransplantation bone marrow from the recipient confirmed that integrated sequences were present on chromosome arm 17p, a site where integration has previously been identified [3]. Essentially, the entire unique region (94 ORFs) of the viral genome (∼144 kbp), typed as variant A, was present.
The viral loads in blood associated with HHV-6 integration suggest that at least 1 viral copy exists per white blood cell (WBC) (~1 × 10^2 HHV-6 genomes/mL of whole blood). Tanaka-Taya et al. [6] also calculated that nearly 1 HHV-6 DNA genome equivalent exists per leukocyte. In our prospective studies in transplant recipients, transient viral loads associated with active HHV-6 infection have been in the order of 1 × 10^2 to 1 × 10^4 HHV-6 genomes/mL of whole blood [7, 11]. This significant difference in viral load between active infection (which most likely result from viral reactivations) and integration should facilitate their distinction when whole blood or PBMCs are tested by quantitative PCR. An exception may be primary infection in young children, in whom viral loads of >1 × 10^9 HHV-6 genomes/mL of whole blood have been reported during the acute stage of disease, as measured by a semiquantitative PCR assay [12].

Given the massive viral burdens observed in persons with integrated sequences, other clinical samples, such as cerebrospinal fluid (CSF) and serum (which are often used in laboratory investigations of HHV-6), may contain detectable levels of HHV-6 DNA. Serum from both the donor and recipient (obtained on day 17 after transplantation) had viral loads of 9.2 × 10^4 and 1.5 × 10^4 HHV-6 genomes/mL, respectively. We also identified viral loads of 3.9 × 10^4 and 3 × 10^3 HHV-6 genomes/mL in CSF from 2 other individuals with FISH-confirmed integrated HHV-6 (data not shown). The corresponding viral loads in whole blood were 6.8 × 10^5 and 1.8 × 10^5 HHV-6 genomes/mL, respectively.

It will be important to differentiate patients with active HHV-6 infection from those with integrated HHV-6, to prevent the latter from receiving unnecessary exposure to potentially toxic antiviral drugs. There are examples in the literature of persons with central nervous system (CNS) disease receiving antiviral therapy on the basis of the identification of HHV-6 DNA in CSF (either by qualitative or quantitative PCR) and, despite having a clinical response to the antiviral intervention, remaining positive for HHV-6 DNA by PCR [13, 14]. In one of these persons, the CSF viral load decreased from 1.1 × 10^5 to 2.6 × 10^4 HHV-6 genomes/mL after receipt of ganciclovir therapy, but this drop paralleled the reduction in CSF WBC count [13]. Corresponding serum viral loads were 3 × 10^5 and 1.2 × 10^4 HHV-6 genomes/mL, respectively. We would urge caution in interpreting such cases as representing true HHV-6 CNS disease, unless integration can be ruled out.

There have been a number of false leads and unproven associations between HHV-6 and disease, some of which may have been confounded by the inadvertent detection of integrated virus. Challoner et al. [15] used representational difference analysis (RDA) to identify novel DNA sequences in the brains of patients with multiple sclerosis (MS), compared with those in control tissue (PBMCs from healthy persons). An HHV-6 sequence was amplified from the brain of 1 patient with MS (MS-1). Limiting dilution analysis was conducted to estimate the HHV-6 load in MS and control brains. The brain from MS-1 produced a positive PCR result up to a dilution of 1:262,000, and a second MS brain was positive up to a dilution of 1:65,000. The other 47 MS and 51 control brains were positive up to a maximum dilution of only 1:16. CSF from MS-1 was also positive for HHV-6 DNA by PCR. In hindsight, it seems that MS-1 may have represented a case of viral integration, which would explain the ready identification of an HHV-6 sequence by RDA. We recommend that all studies associating HHV-6 with disease should exclude coincidental integration.

How common is HHV-6 integration? We identified patients with persistent (i.e., no negative blood samples) and strikingly high (~1 × 10^5 HHV-6 genomes/mL of whole blood) viral loads in 3 of 60 and 1 of 52 liver and renal transplant recipients, respectively [7, 11]. It is highly likely that these patients represent further cases of HHV-6 integration. We also identified 1 healthy person (of 25) with a consistent viral load of 1 × 10^6 HHV-6 genomes/µg of PBMC DNA [1]. FISH analysis of an LCL generated from this individual identified integrated HHV-6B sequences on chromosome band 11p15.5 (figure 2E). Tanaka-Taya et al. [6] estimated the prevalence of integrated HHV-6 to be 0.21% in their Japanese population. Further studies are required to determine the frequency of integration in different geographical populations.

As we have demonstrated, pretransplantation screening of donors can identify those with integrated virus and, thus, prevent the misdiagnosis of active infection in recipients after transplantation. It may be difficult to diagnose active infection (presumably with “normal,” nonintegrated virus) if it develops in persons with viral integration. Possibilities for such diagnosis include the use of virus isolation, the antigenemia test, and reverse-transcription PCR, although, for the latter, studies will include the use of virus isolation, the antigenemia test, and reverse-transcription PCR, although, for the latter, studies will be required to exclude the possibility of transcription from the integrated genome in vivo.

References


