Human Herpesvirus 6 Infections after Bone Marrow Transplantation: Clinical and Virologic Manifestations

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Human herpesvirus 6 (HHV-6) DNA levels in peripheral blood mononuclear cells were prospectively evaluated in 20 cytomegalovirus-seronegative allogeneic marrow transplant patients and in 10 healthy control subjects. Blood and saliva specimens obtained weekly for 3 months after transplant were evaluated by quantitative HHV-6 polymerase chain reaction. One of 20 patients experienced primary HHV-6 infection after marrow transplant (seroconversion, HHV-6 viremia, skin rash); 18 of 20 had increased peripheral blood mononuclear cell HHV-6 DNA levels consistent with asymptomatic reactivations, and 1 patient experienced a reactivation-associated skin rash. Genotyping revealed HHV-6 variant B DNA in all cases. Therapy with acyclovir or intravenous immunoglobulin was not correlated with lower HHV-6 DNA levels. Thus, asymptomatic HHV-6 reactivations appear to be common following allogeneic marrow transplantation. Among HHV-6-seronegative and viral DNA-negative patients, primary HHV-6 infection can ensue in association with self-limited clinical symptoms, including diffuse maculopapular rash.

Human herpesvirus 6 (HHV-6) causes life-long persistent infection in over 90% of persons before age 2 years [1, 2]. Most childhood infections are asymptomatic, but about 20% of children experience a self-limited fever that can be associated with rash (roseola), otitis media, and other clinical presentations [3–5]. On the basis of molecular and phenotypic characteristics, HHV-6 isolates are categorized into two groups, variant A (HHV-6A) and variant B (HHV-6B) [6–10]. Almost all symptomatic primary HHV-6 infections of childhood are associated with HHV-6B [11], whereas both HHV-6A and HHV-6B variants have been identified in the majority of healthy and diseased lung tissues [12] and lymph nodes from patients with AIDS-associated non-Hodgkin’s lymphoma [13]. HHV-6A is also occasionally isolated from the blood and other tissues of adults and children [14–17].

Most members of the human herpesvirus group (herpes simplex virus, cytomegalovirus [CMV], varicella-zoster virus, and Epstein-Barr virus) cause diseases in immunosuppressed hosts, such as marrow transplant patients. However, the pathogenic potential of HHV-6 infection after marrow transplantation remains uncertain. Increased isolation rates of HHV-6 from blood and marrow following marrow transplantation suggest more frequent reactivation during this period of acute immunosuppression [18, 19]. High levels of HHV-6 in lung tissues from marrow transplant patients with pneumonia suggest a possible pathogenic role in the respiratory system [20, 21]. Other studies have identified HHV-6 in marrow following transplantation, and some data support an association between HHV-6 infection and marrow dysfunction in vitro [22] and in vivo [17, 23]. Skin rash, a typical symptom of primary HHV-6 infection in children, has also been observed in some marrow transplant patients during periods of increased viral activity [24].

This prospective study was undertaken to define the replication activity of HHV-6 infection following marrow transplantation and to observe the clinical manifestations of active HHV-6 infection. The cohort was limited to CMV-seronegative marrow transplant patients so that the clinical manifestations of CMV infection and effects of consequent antiviral therapy, such as ganciclovir, would not confound interpretation of the HHV-6 data. The cohort was further restricted to allogeneic transplants, who tend to experience more HHV reactivations than patients receiving syngeneic transplants.

Methods

Subjects. Twenty patients undergoing allogeneic marrow transplantation at the Fred Hutchinson Cancer Research Center between 27 January and 9 October 1992 were enrolled. The enrollment criteria were CMV-seronegative recipient and donor; allogeneic, related donor; and age ≥18 years. A cohort of 10 healthy, age- and sex-matched volunteers were enrolled as controls.
Specimens and processing. Blood (20 mL, heparinized) and saliva (≤1 mL) were collected once before transplant and once each week after transplant for 12 weeks. The 10 controls donated blood and saliva specimens weekly for 12 weeks. The specimens from controls were processed concurrently with marrow transplant specimens using the same laboratory procedures. Peripheral blood mononuclear cells (PBMC) were isolated from the heparinized blood by density-gradient centrifugation (lymphocyte separation medium; Organon Teknika, Durham, NC), washed twice, and stored at -70°C as two dry pellets. Plasma from the density gradients was stored at -70°C. Saliva specimens, volumes of 0.1–1 mL, were brought to at least 0.4 mL total volume with PBS and stored in aliquots at -70°C.

Quantitative HHV-6 polymerase chain reaction (PCR) and subtype differentiation. All specimen handling and PCR set-up was done in a dedicated pre-PCR room to avoid contamination. DNA was purified from 0.4 mL of saliva or 1 PBMC aliquot by proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation [2]. The DNA was resuspended in 200 µL; 10 µL was used in each amplification reaction. HHV-6-specific, variant-common 5R primers 5R-A and 5R-B (150-bp product) and variant-common probe 5R-P were used for the initial PCR detection and quantitation of HHV-6 [2, 25]. Primers Hb-3 and Hb-4 and probe Hb-A were used for quantitative detection of the betaglobin gene [2]. For variant identification, HHV-6A- and HHV-6B-specific probes (LTP-A2 and LTP-B2, respectively) were hybridized to separately amplified PCR products derived from SIE-1/SIE-2 variant-common primers [12].

Amplification reactions (100 µL) contained 5 × 10¹⁴ molecules (0.83 μM) of each HHV-6 primer, 50 mM KCl, 10 mM Tris-HCl, pH 8.4, 200 µM each dATP, dTTP, dCTP, dGTP (Pharmacia, Piscataway NJ), 10% glycerol, 2 U of AmpliTaq polymerase (Perkin-Elmer, Norwalk, CT), purified specimen DNA, and 50 copies of a noncompetitive internal control template that amplified with the 5R primer set but hybridized with a control probe, Fly-C [26]. Negative controls consisted of ≥1 amplification containing DNA from uninfected CCRF-HSB-2 (ATCC CCL 120.1) cells for every 2 specimens evaluated, and ≥1 amplification with no added DNA per run. During the course of this study, 12 (1.8%) of 654 negative control amplifications were falsely positive for HHV-6 DNA. Runs containing false-positive controls were repeated.

Serology. An EIA was used to detect HHV-6 serum antibodies. Virion lysate from sucrose density gradient–purified virions, isolate U-1102 (Advanced Biotechnologies, Columbia, MD), was diluted to 5 μg/mL in carbonate buffer, pH 9.6, and added at 50 μL/well to 96-well plates (ELISA Easy Wash, Corning Glass, Corning, NY). After overnight incubation at 4°C, plates were washed three times with PBS/0.5% Tween-20 (PBS-Tween). Sera were serially diluted 2-fold in PBS with 4% goat serum (Sigma, St. Louis) from 1:100 to 1:51,200, and 100 μL was added to wells containing virus lysate or a mock lysate from HSB-2 cell culture. After 1 h at 37°C, plates were washed 3 times in PBS-Tween, then once with PBS. Goat anti-human IgG Fab fragment conjugated to peroxidase (Boehringer Mannheim, Gaithersburg, MD) was diluted 1:4000 in PBS with 4% goat serum and added at 50 μL/well. After 1 h at 37°C, plates were washed as before and bound complexes were detected with TMB substrate (Kirkegaard & Perry Laboratories, Indianapolis) according to insert instructions. Absorbance was read at 405 nm and plotted against dilution using a custom computer program. End-point titer was the reciprocal of the dilution at which the slopes of the antigen-positive wells and mock-infected wells deviated and the greatest dilution at which the absorbance value against viral antigen was at least 0.2 U greater than against mock antigen.

Statistics. Longitudinal analyses were based on 12-week follow-up. Each transplant patient had ≥1 pretransplant sample, so paired comparisons of pre- and posttransplant average HHV-6 DNA levels in PBMC and saliva and IgG serologic titers could be done using t tests on log-transformed data or Wilcoxon signed rank tests as appropriate. Unpaired comparisons between transplant patients and controls used t tests or Mann Whitney–Wilcoxon rank sum tests. When F tests indicated unequal variances, we used modified t tests that allowed for unequal variances. Ratio estimates were used when a single HHV-6 DNA value for a given person during a given time interval was needed. For example, the posttransplant HHV-6 DNA value for a person was the mean of the HHV-6 DNA equivalents during the 12-week posttransplant period divided by the mean of the cellular DNA equivalents. Use of means reduced the variance due to measurement error in both the numerator and denominator and gave lower weight to specimens with few cellular DNA equivalents.

For longitudinal analyses, we used a repeated measures model [27] that allowed for missing data, time-varying covariates, and correlation over time among the measurements from a given person. This correlation may take several different forms. Akaike’s information criterion [28] was used to choose a first-order autoregressive form that allows for higher correlation between measurements closer together in time than between more distant ones. Wald tests were used to assess significance of effects.
Results

Patient population. Graft-versus-host disease prophylaxis regimens and primary diagnoses for the 20 subjects (median age 34.5 years; range, 23–54; 6 females) are listed in table 1. The median duration of follow-up (93 days; range, 78–142) included a median of 9 days before transplant (range, 3–27) and 84 days after transplant (range, 70–118). No patients died during the study. Intravenous immunoglobulin was given to 12 of the 20 patients (median of 85 days each). No ganciclovir was used during the observation period (all patients were CMV-seronegative and received only CMV-negative blood products).

The 10 control subjects (median age 33 years; range 23–39; 4 female) each provided 12 weekly blood and saliva specimens. One control was not infected with HHV-6; viral DNA was not detected in PBMC or saliva in any of the 12 serial specimens, and no anti-HHV-6 antibodies were detected in all 4 serum specimens analyzed. This subject was eliminated from the comparisons between controls and marrow transplant patients.

Forty percent of the 264 marrow transplant PBMC specimens and 52% of the 109 nontransplanted control PBMC specimens contained detectable HHV-6 DNA. Every HHV-6-seropositive patient and control had >1 HHV-6 DNA-negative and 1 HHV-6 DNA-positive PBMC specimen during the observation period. Saliva HHV-6 DNA PCR positivity was found in 68% of 226 marrow transplant specimens and in 98% of 107 specimens from control subjects (excluding the uninfected control). HHV-6 IgG serologic titers were determined using 4 specimens from each subject: before transplant (time 0 for controls) and 1, 2, and 3 months after transplant (after enrollment for controls). Among the marrow transplant patients, 72 (90%) of 80 evaluated specimens were HHV-6-positive compared with 32 (89%) of 36 seropositive-specimens among the 9 HHV-6–infected control subjects. All 8 subjects who did not receive intravenous immunoglobulin had HHV-6 antibodies present.

Clinical findings: rash and fever during primary infection. Two of the 20 marrow transplant subjects (patients 6 and 16) experienced skin rashes and fevers related to HHV-6 infections. No other clinical complications were observed in association with laboratory evidence of active HHV-6 infection.

Patient 6, a 45-year-old man, had serologic and virologic evidence of primary HHV-6 infection following marrow transplantation. He was the only patient with completely negative HHV-6 DNA and serology results before transplant (figure 1). Since herpes simplex virus was cultured from his nasopharynx 10 days before transplant and intermittently after, he received acyclovir during posttransplant months 1 and 3. Salivary HHV-6 DNA was first detected on posttransplant day 8, providing the first evidence of HHV-6 infection. Eighteen days after transplant, he experienced a transient fever to 38.9°C, followed by a maculopapular rash on the scalp, face, chest, and back that persisted for 2 weeks. Salivary HHV-6 DNA was negative during the rash; however, PBMC HHV-6 DNA dramatically increased on posttransplant day 36, coincident with the first positive HHV-6 serology. The PBMC HHV-6 DNA level climbed thereafter to $2 \times 10^6$ per 10° PBMC on posttransplant day 63, the second-highest level in the entire study. His serologic titers continuously increased throughout the posttransplant period, ending with an antibody titer $>1:52,000$ on day 58, the highest antibody titer in the study. His clinical symptoms resolved shortly before the appearance of specific antibody. After appearance of HHV-6 antibodies, PBMC HHV-6 DNA levels decreased (figure 1).

Patient 16, the only other patient with unexplained rash, was a 23-year-old man. His PBMC and saliva were HHV-6 DNA-negative before transplant, and his pretransplant HHV-6 antibody titer was low-positive (1:200). Intermittent rashes appeared during the first 7 weeks after transplant: On day 2, erythematous body rash appeared that persisted for 1 day; on day 6, rash and 39.6°C fever occurred for 1 day; on day 15, diffuse maculopapular rash occurred, covering 60% of trunk and head, and lasting 2 weeks with fever to 40.2°C; and on day 40, he developed maculopapular rash on the abdomen, back, upper arms, and thighs that resolved in 1 week. The first evidence of HHV-6 DNA appeared in saliva, with increasing salivary HHV-6 DNA levels during weeks 4–9, peaking on posttransplant day 56 ($6.7 \times 10^6$ HHV-6/mL saliva). PBMC HHV-6 DNA first appeared on posttransplant day 55, although the

Table 1. Demographic and clinical data for 20 bone marrow transplantation patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Primary diagnosis</th>
<th>Antigen match</th>
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<tr>
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<td>48</td>
<td>M</td>
<td>CML-CP</td>
<td>M</td>
<td>CSP MTX</td>
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<td>CLL</td>
<td>M</td>
<td>None</td>
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<td>27</td>
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<td>CML-CP</td>
<td>M</td>
<td>CSP MTX</td>
</tr>
<tr>
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<tr>
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<td>M</td>
<td>DWDLI</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>AML</td>
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<tr>
<td>19</td>
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<tr>
<td>20</td>
<td>54</td>
<td>M</td>
<td>APL</td>
<td>M</td>
<td>Pred</td>
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</table>

* M, male; F, female.

b CML, chronic myelogenous leukemia; CP, chronic phase; AP, active phase; CLL, chronic lymphocytic leukemia; DWDLI, persistent diffuse well-differentiated lymphocytic lymphoma; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; APL, acute promyelocytic leukemia.

c Transplant antigen match between donor and recipient: M, match; MM, mismatch.

d Graft-versus-host (GvH) disease prophylaxis: CSP, cisplatin; MTX, methotrexate; Pred, prednisone.
subsequent peak of 2000 HHV-6/10⁶ PBMC was not particularly high for this cohort. At the end of the observation period, his HHV-6 antibody titer had increased by 4-fold over baseline levels. No viruses were cultured during the posttransplant period by routine diagnostic laboratory methods. While this patient demonstrated persistently high salivary and PBMC HHV-6 DNA levels after transplant, the initial HHV-6 seropositivity led us to classify this as reactivated infection.

**Evidence for HHV-6 reactivation after transplant.** The geometric mean posttransplant PBMC HHV-6 DNA level (378.3 HHV-6/10⁶ PBMC) was significantly higher than before transplant (2.1) and higher than in the nontransplanted controls (11.8) (P < .001 and P = .001, respectively). HHV-6 reactivations were not as clearly demonstrated by salivary HHV-6 DNA levels, although weekly saliva HHV-6 DNA levels correlated with PBMC HHV-6 DNA levels in the transplant patients (P = .02, longitudinal analysis). The geometric mean posttransplant saliva HHV-6 DNA level was higher than the mean pretransplant level (10,495 vs. 573 HHV-6/mL saliva, P = .02), but there was no significant difference between posttransplant and control mean salivary HHV-6 DNA levels (P = .33).

We found that PBMC HHV-6 DNA values were negatively biased when very few PBMC were available for analysis. For instance, figure 2A shows that leukopenia during weeks 1, 2, and 3 were associated with low PBMC HHV-6 DNA levels, whereas salivary HHV-6 DNA levels during the first weeks were similar to levels at other times (figure 2B). To compensate for the negative bias arising from leukopenia, the log of the cell equivalents was used as a covariate to adjust the mean HHV-6 DNA values (corrected mean). Corrected mean PBMC HHV-6 DNA values reduced the influence of negatively biased results, improving the validity of group comparisons between weeks after transplant.

Our adjusted model for transplant patients found no significant differences in HHV-6 DNA levels in the weeks following transplantation (P = .41). After compensating for effects of the low PBMC HHV-6 DNA levels, the corrected means of posttransplant weeks 1–3 were not significantly different from subsequent weeks. Average PBMC HHV-6 DNA levels for weeks 4–12 showed little variation (figure 2A). These data suggest that the timing of HHV-6 reactivation is not tied to the transplant event but instead occurs sporadically throughout the posttransplant period.

**Additional observations.** The geometric mean serologic titers in the transplant and control groups were not significantly different. Weekly means of the transplant group serologic titers were not related to weekly means of PBMC or saliva HHV-6 DNA levels (P > .1, longitudinal analyses), and serologic titers of each patient did not correlate with that person’s PBMC or saliva HHV-6 levels (P > .1, Pearson’s correlation). The group geometric mean pretransplant serologic titer (459) was only slightly lower than that after transplant (838) (P = .02).

HHV-6 DNA levels in PBMC were not significantly correlated with acyclovir therapy (P = .5, longitudinal analysis). Fifteen of the 20 transplant patients received intravenous acyclovir (200 mg/m², twice each day; average duration, 33 days) during the study period. Of 65 PBMC specimens obtained during acyclovir therapy, 49 (75%) were HHV-6 DNA–negative compared with 87 (52%) of 166 HHV-6 DNA–negative specimens obtained in the absence of acyclovir therapy. The geometric mean
HHV-6 DNA level in HHV-6-positive PBMC specimens obtained during acyclovir therapy was 5890 HHV-6/10^6 PBMC compared with a geometric mean of 1422 in the absence of acyclovir therapy (not significant).

The effect of HHV-6 DNA positivity on marrow suppression was examined by comparing blood cell counts obtained near the time of HHV-6 DNA positivity with counts obtained when HHV-6 DNA was negative. Longitudinal analyses found no significant relationships between PBMC HHV-6 DNA levels and levels of white blood cells, hematocrit, or PBMC in the same week or that immediately following after adjusting for leukopenia (P > .1, all analyses). A separate analysis considered...
only weeks 3–12 to avoid the transplant-induced leukopenia observed in the first 2 weeks after transplant, but there was still no significant difference in PBMC or hematocrit between HHV-6-positive and -negative time points (P > .1, both tests, Wilcoxon signed-rank test). Similar analyses using HHV-6 levels in saliva also showed no significant correlation between HHV-6 positivity in saliva and blood cell counts.

Discussion

In these longitudinal data, marrow transplantation was associated with a 40-fold increase in the average PBMC HHV-6 DNA level. In 18 of 20 patients, these increases occurred sporadically throughout the 3-month observation period without evidence of associated clinical symptoms. The data are consistent with a model of benign, periodic HHV-6 reactivation throughout the postmarrow transplant period.

Two patients experienced prolonged skin rashes and fever associated with increased HHV-6 activity, and 1 became symptomatic in the context of a primary HHV-6 infection. Previous studies [18, 24] also reported rash and fever in association with primary HHV-6 infections after transplant. Gathering evidence suggests that these relatively rare adult primary HHV-6 infections may cause self-limited skin rash and fever.

HHV-6 DNA was detectable by PCR in all 20 transplant patients and in 9 of 10 healthy controls. This is consistent with our previous data, which demonstrated HHV-6 DNA positivity in PBMC and saliva from 90% of healthy controls and from 100% of lung tissues from marrow transplant patients [2, 12]. Most previous studies of marrow transplant patients have relied on HHV-6 culture positivity to document HHV-6 reactivation, occasionally detecting HHV-6 in PBMC in 46% [19], 40% [18], and 37% [23] of patients after transplant. Although culture positivity is an important biologic marker, direct PCR, which produces quantitative results in a higher proportion of specimens, allowed us to perform longitudinal analyses and comparisons with controls. Kadakia et al. [19] attempted direct HHV-6 PCR using PBMC from marrow transplant patients but found no positivity among 26 longitudinally studied cases, despite culture positivity in some specimens. Since they used different PCR methods than used in our study, their lack of positivity may be related to methodologic differences.

The apparently random timing of HHV-6 reactivation after marrow transplant demonstrated in this study is consistent with previous findings. A longitudinal study of marrow transplant patients revealed HHV-6 DNA PCR positivity a median of 106 days after transplant (range, 8–130) [29]. A larger study of 26 marrow transplant patients demonstrated 16 HHV-6 culture-positive episodes with a median time to culture positivity of 49 days after transplant (range, 13–324) [19]. A study of 25 pediatric marrow transplant patients followed for 2 months showed a trend toward HHV-6 culture positivity in posttransplant week 3 [18]. This difference may be due to the ages of the study groups (children vs. adults).

The realization that post-transplant leukopenia raises the threshold for HHV-6 PCR detection explains why so many PCR-negative specimens were found during the first few weeks after marrow infusion (figure 2A). Specifically, we found that the apparent increase in PBMC HHV-6 DNA levels after post-transplant week 2 could not be interpreted as reactivation beginning at week 3. After adjusting the longitudinal analysis to compensate for the effects of leukopenia, HHV-6 reactivations were not more likely in posttransplant week 3 or at any other time. Previous reports of HHV-6 “reactivation” in posttransplant week 3 could be reexamined in light of this approach for handling leukopenic specimens.

Salivary HHV-6 DNA levels correlated with hematologic evidence for HHV-6 reactivation, but the high variability of salivary HHV-6 DNA measurements and the high levels of salivary HHV-6 DNA in healthy persons make saliva specimens less sensitive than PBMC for identifying individual transplant-related HHV-6 reactivations. Until improved standardization of salivary specimens is available, they appear to be unreliable markers of HHV-6 reactivation in individual cases.

All HHV-6 DNAs identified in our study population were variant B, consistent with most previous reports that showed predominately variant B after marrow transplant [16, 19, 23, 30, 31]. From a previous study, we know that most marrow transplant patients harbor HHV-6A in their lung tissues [12], suggesting that HHV-6A infection is frequent, even though it is not often detected in PBMC specimens. Also, previous case reports have identified HHV-6A in blood from marrow transplant patients, usually in association with severe illness [16, 29, 32]. Observations that most marrow transplant patients reactivate variant B in their blood and saliva suggests either that variant A is not often reactivated following marrow transplant or that variant A reactivations are generally limited to sites other than the blood and oral cavity. These data suggest that HHV-6A may be more organ-associated, whereas HHV-6B appears to be more blood-associated.

HHV-6 antibody titers did not reflect the general increase in PBMC HHV-6 activity following transplantation, a finding that is consistent with other studies [16, 18, 19, 24]. These results suggest that changes in HHV-6 antibody titers are not sufficient to document HHV-6 reactivation in the marrow transplant population.

Patients in this study did not experience significant clinical setbacks in association with their frequent increases in PBMC HHV-6 DNA levels. This contrasts with some previous reports proposing causal relationships between HHV-6 and various disease processes, including respiratory failure and death [29], marrow suppression [23], encephalitis [33], and pneumonia [34, 35]. We previously reported an association between pneumonia in marrow transplant recipients and high HHV-6 DNA levels in their lung tissues [21]. Further investigation revealed that the
HHV-6 DNA in lung tissues represented a mixed infection of both HHV-6 variants, and that neither variant predominated in cases of idiopathic pneumonia, decreasing the likelihood that HHV-6 actually causes pneumonia in this population [12]. In addition, Kadakia et al. [19] found no association between post-transplant HHV-6 reactivation and pneumonia, marrow suppression, or other major clinical problems. We propose that the current data are consistent with asymptomatic HHV-6 reactivations following marrow transplantation, and that associations between HHV-6 and disease should be stringently demonstrated in multiple, controlled studies before causality is considered.

We studied CMV-seronegative recipients and donors who received only CMV-seronegative blood products to reduce CMV-related complications that might be confused with clinical manifestations of HHV-6 infection. While this design clarifies observations related to HHV-6 reactivations, it prevents analyses of interactions between CMV and HHV-6. Therefore, this study does not rule out the possibility that concurrent CMV reactivation may modify the course of HHV-6 infection or vice versa.

In conclusion, asymptomatic hematogenous reactivations of HHV-6B were common throughout the postmarrow transplant period. No serious clinical complications could be attributed to HHV-6 infection, although self-limited skin rashes and fever were observed in 2 patients.

References

