Lymphotropic Herpesviruses in Allogeneic Bone Marrow Transplantation

By Fu-Zhang Wang, Helena Dahl, Annika Linde, Maria Brytting, Anneka Ehrnst, and Per Ljungman

Human herpesvirus-6 (HHV-6), human herpesvirus-7 (HHV-7), Epstein-Barr virus (EBV), and human cytomegalovirus (CMV) DNA were repeatedly assayed in peripheral blood leukocytes from 37 allogeneic bone marrow transplant (BMT) patients by polymerase chain reaction. Before BMT, HHV-6 DNA was detected in 8 (22%) patients. HHV-7, EBV, and CMV DNA were detected in 21 (57%), 10 (27%), and 1 (3%) patient, respectively. After BMT, HHV-6 DNA was detected in 26 (70%), HHV-7 in 21 (57%), EBV in 28 (76%), and CMV in 21 (57%) patients. Thirty-two (87%) patients were positive with more than one virus. HHV-6, HHV-7, and EBV DNA were found earlier than CMV DNA in most patients after BMT. The proportions of HHV-6-positive samples during the first 3 months after BMT were higher in the patients with either delayed granulocyte engraftment (P = .04, Fisher’s exact test) or delayed platelet engraftment (P = .001, Fisher’s exact test). The HHV-6 DNA in samples from the patients with delayed engraftment was confirmed to be variant B. The detection of any lymphotropic herpesvirus was not related to the development of acute graft-versus-host disease (aGVHD). High-dose acyclovir (ACV) prophylaxis significantly (P < .01) reduced the proportion of HHV-6-positive samples and tended to lower HHV-6 DNA levels (P = .06). Our data indicate that HHV-6 variant B can inhibit marrow engraftment and that high-dose ACV may be beneficial to engraftment after BMT by preventing HHV-6 reactivation. No relation between the proportions of HHV-7−, EBV−, and CMV-positive samples in the first 3 months and engraftment or aGVHD was found.

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Materials and Methods

Patients. Thirty-seven allogeneic BMT patients were evaluated. Patient characteristics are shown in Table 1. Acute GVHD was prophylaxed with cyclosporine and methotrexate. No T-lymphocyte depletion was performed. High-dose acyclovir (ACV) was given to 9 patients as prophylaxis against CMV infection. ACV was delivered in a dosage of 500 mg/m² intravenously three times daily, for 5 days before BMT until discharge, and then the prophylaxis was continued with oral ACV (800 mg, four times daily) or oral valaciclovir (2,000 mg, four times daily) for another 14 weeks.

Samples. EDTA blood samples were collected at weekly intervals from 1 to 2 weeks before and up to 4 weeks after BMT. Thereafter, blood samples were collected at 6 weeks and 2, 3, and 6 months and 1 year after BMT. Altogether, 270 samples were collected and assayed for HHV-6, HHV-7, EBV, and CMV DNA. The number of samples available at each time point is shown in Fig 1. Buffy coat from 20 healthy staff members (13 women, 7 men, mean age 36 years) was analyzed to establish the prevalence of HHV-6, HHV-7, and EBV DNA in healthy individuals.

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Table 1. Characteristics of the Patients

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>35 (range: 5-59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>24/13</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>9</td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
<td>13</td>
</tr>
<tr>
<td>Severe aplastic anemia</td>
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<tr>
<td>Acute lymphoid leukemia</td>
<td>4</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>2</td>
</tr>
<tr>
<td>Myelodysplastic syndrome/AML</td>
<td>4</td>
</tr>
<tr>
<td>Metachromatic leukodystrophy</td>
<td>1</td>
</tr>
<tr>
<td>Adrenoleukodystrophy</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myeloid leukemia.

Sample treatment. Buffy coat was collected as the source of PBL. RBCs were lysed with a lysis buffer containing 0.15 mol/L NH₄Cl, 10 mmol/L KHCO₃, and 10 mmol/L EDTA, pH 7.4. PBL were counted and frozen at −20°C as a dry pellet. Before running PCR, the pellet was suspended in a lysis buffer (10 mmol/L Tris-HCl, pH 8.3; 1 mmol/L EDTA, 0.5% Nonidet P 40, 0.5% Tween 20, 300 µg/mL proteinase K) at a concentration of 10⁷ cells/mL. The mixture was incubated at 37°C overnight and proteinase K was inactivated at 95°C for 15 minutes. For HHV-6, HHV-7, and EBV PCR, 5 µL of this preparation, corresponding to 5 x 10⁴ cells was used. DNA-free water was used in both sample treatment and PCR assays as the negative control. For semi-quantitation of HHV-6 DNA, the positive samples were diluted serially 10-fold in water until it became PCR negative.

General HHV-6 PCR. A nested PCR detecting a conserved region for both variants A and B of HHV-6 was developed. The outer primers have been reported previously. The total reaction volume was 50 µL containing 10 mmol/L Tris-HCl, pH 9.6, 2 mmol/L MgCl₂, 50 mmol/L NaCl, 0.5 mmol/L of each deoxynucleotide triphosphate (dNTP), and 1 U of Taq polymerase (Perkin-Elmer Cetus, Emeryville, CA). The 30 cycles used for both rounds consisted of 1 minute at 95°C, 1 minute at 55°C (first round) or 60°C (second round), and 1 minute at 72°C. After the last cycle of each round, the extension step was extended to 10 minutes at 72°C. The outer primers (H6-6 and H6-7) amplify a fragment of 230 bp. The inner primers (NH-6: 5'-TCC ATT ATT TTG GCC GCA TTC GT, and NH-7: 5'-TGT TAG GAT ATA CCG ATG TGC GT) amplify a fragment of 130 bp. The concentrations for outer and inner primers were 0.8 and 1 µmol/L, respectively. After the first amplification round, 2.5 µL of the reaction product was taken for the second round. Ten microliters of the second-round PCR product was electrophoresed on a 1.5% agarose gel containing 0.5 µg/mL ethidium bromide. The results were photographed under UV illumination and regarded as positive when a band corresponding to the 130-bp fragment was seen. Purified viral DNA from HHV-6 strain GS and strain Z29 was used for assessment of the sensitivity of the HHV-6 PCR. The detection limit of the general HHV-6 PCR is 20 to 30 genomes for both strains.

HHV-6 subtyping PCR. A nested PCR was used according to a previous report. The basis for this PCR is that HHV-6 variant B has an insert of 228-bp fragment in the putative immediate-early (IE) region compared with HHV-6 variant A. The PCR buffer was the same as that used for HHV-6 general PCR except that 0.4 mmol/L of each dNTP was used for both rounds. The outer primers (661: CAAAGCCTAACTGTGTATGT, and 662: TCTGCAATGTAATTCCAGTTTC) amplify a fragment of 325 or 553 bp for variants A and B, respectively. The inner primers (663: CTGGGCGGCCCTAATAACTT, and 664: ATCGCTTTCACTCTCATAAG) amplify a fragment of 195 bp for variant A or a fragment of 423 bp for variant B. All the primers were used at a concentration of 1 pmol/L. The PCR program was 30 cycles for both rounds consisting of 1 minute at 94°C, 1 minute at 50°C (first round) or at 60°C (second round)
and 1 minute at 72°C. After the last cycle of each round, the extension step was extended to 10 minutes at 72°C. HHV-6 subtypes were easily differentiated by the sizes of the PCR products.

**HHV-7 PCR.** A previously reported nested PCR system was used for the detection of HHV-7 DNA. The sequences of the primers are the same as reported. The final PCR product is a fragment of 264 bp. The PCR buffer has been modified into 50 μL containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 5 μM MgCl₂, 0.01% (w/v) gelatin, 0.5 mM dNTP (first round) or 0.3 mM dNTP (second round) of each dNTP and 1.25 U of Taq polymerase. The working concentration for all the primers was 0.4 mM/L. The thermal conditions for both rounds of reaction are the same: 94°C, 5 minutes, and then 30 cycles of 1 minute at 94°C, 1 minute at 60°C, and 1 minute at 72°C. The extension time of the last cycle was extended to 10 minutes at 72°C. Two and one-half microliters of the product of the first round was used as the template for the second round of PCR. The PCR product of the second round was determined by gel electrophoresis as described above.

**EBV PCR.** A nested PCR detecting a sequence in the BMHI K fragment of the EBV genome was used as described previously. The detection limit is four genomes. The results were regarded as positive when a band corresponding to the 209-bp DNA fragment was present in gel electrophoresis as described above.

**CMV PCR.** The CMV PCR was routinely performed for diagnostic purpose. Briefly, 5 mL of EDTA blood were used for leukocyte separation by sedimentation over Methocel (KEBO Lab, Spånga, Sweden). The leukocytes were counted; 2 × 10⁸ leukocytes were lysed in 50 μL of a buffer containing Nonidet P 40, Tween 20, and proteinase K by incubating at 56°C to 60°C for 1 hour, and the proteinase K was thereafter inactivated at 95°C for 10 minutes. Five microliters of the lysate was processed by a nested PCR in a volume of 50 μL. The primers used were from the conserved region of the CMV major IE gene. The outer primers IEA-2A and IEP-3B amplify a 721-bp long fragment and the inner primers IEP-3A and IEP-3B amplify a 167-bp long fragment. The results were analyzed on agarose gel as stated above. The detection limit of this PCR system is 10 gene copies.

**Serology.** Plasma samples drawn before BMT were analyzed for anti–HHV-6 and EBV IgG according to previously published methods. Anti-CMV IgG was analyzed by enzyme-linked immunosorbent assay (ELISA).

**Definitions.** Delayed granulocyte engraftment was defined as an absolute neutrophil count of 0.5 × 10⁹/L, first occurring on or after day 25 after BMT. Patients who needed platelet transfusions for more than 30 days were considered to have delayed platelet engraftment. Acute GVHD was defined according to Thomas et al.

**RESULTS**

**Viral DNA identification.** When 5 × 10⁶ PBL from 20 healthy individuals were examined, one (5%) of the healthy people was HHV-6 PCR-positive, which was confirmed to be variant B. Two (10%) were EBV PCR-positive and 9 (45%) were HHV-7 PCR-positive. Of all the samples drawn from the BMT patients during this study, HHV-6 DNA was detected in 68/270 (25%), HHV-7 DNA in 61/270 (23%), EBV DNA in 87/270 (32%), and CMV DNA in 54/270 (20%) samples. Before BMT, HHV-6 DNA was detected in 8/37 (22%), HHV-7 DNA in 21/37 (57%), EBV DNA in 10/37 (27%), and CMV DNA in 1/37 (3%) patients. During the study period after BMT, HHV-6, HHV-7, EBV, and CMV DNA were detected in 26/37 (70%), 21/37 (57%), 28/37 (76%), and 21/37 (57%) patients, respectively. Thirty-two (87%) patients were positive with the DNA of more than one virus. HHV-6 DNA was detected earlier than EBV in 12 patients, whereas the converse was found in 5 patients. HHV-6 DNA and EBV DNA were simultaneously detected in 4 patients. HHV-6 and EBV DNA were detected earlier than CMV DNA in 9 and 8 patients, respectively. CMV DNA was detected earlier than HHV-6 and EBV DNA in 2 and 1 patient, respectively. CMV DNA was simultaneously detected with HHV-6 and EBV in 1 patient each, respectively. HHV-7 DNA was detected earlier than HHV-6 and EBV in 4 and 6 patients, and later in 8 and 5 patients, respectively. HHV-7 DNA was detected earlier in 6 patients and later in 2 patients than CMV DNA. The kinetics of detection of the four viruses is shown in Fig 1.

**Relation between PCR results and serology.** Thirty-six of 37 patients (97%) were HHV-6-seropositive before BMT, and 26 (72%) of them were HHV-6 DNA-positive after BMT. The HHV-6-seronegative patient was HHV-6 DNA-negative after BMT. All 37 patients were EBV-seropositive before BMT, and 28 patients (76%) were EBV DNA-positive after BMT. All the 9 CMV-seronegative patients were CMV DNA-negative after BMT. Seven of them received bone marrow from seronegative donors and 2 from CMV-seropositive donors. Of the 28 CMV-seropositive patients, 16 of 20 (80%) with seropositive donors were CMV DNA-positive, and so were 5 of 8 patients (63%) with seronegative donors. HHV-7 serology was not performed.

**Semi-quantification of HHV-6 DNA after BMT.** When 5 × 10⁶ PBL were used for PCR, 26 of the 37 patients were positive. When dilutions corresponding to 5 × 10⁷, 5 × 10⁶, and 50 cells were used, HHV-6 DNA was detected in 6, 4, and 1 patient, respectively (Table 2). Patients with many HHV-6–infected leukocytes in one sample were more often positive in two or more consecutive samples (P = .007, Fisher’s exact test).

**HHV-6 variants.** Before BMT, HHV-6 variant B was found in 7 of the 8 HHV-6 PCR-positive patients. One positive patient could not be subtyped. After BMT, 18 of 26

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**Table 2: Relation Between the Levels of HHV-6 DNA, Consecutive Positivity, Delayed Engraftment, and aGVHD**

<table>
<thead>
<tr>
<th>Cells Used</th>
<th>Total No. of Positive Patients</th>
<th>No. of Consecutively Positive Patients</th>
<th>No. of Patients With Delayed Engraftment</th>
<th>No. of Patients With Grade II to III aGVHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>5,000</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>50,000</td>
<td>15</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Dilutions of lysis products corresponding to 50, 500, 5,000, and 50,000 PBL were used for PCR. Consecutive positivity was defined when two or more consecutive samples were positive. Patients with either delayed granulocyte or platelet engraftment or both were included. No aGVHD grade IV was observed in this group of patients. The negative patient was defined if HHV-8 DNA could not be detected during this study period when 50,000 PBL were examined.

Abbreviations: HHV-6, human herpesvirus-6; aGVHD, acute graft-versus-host disease; PCR, polymerase chain reaction; PBL, peripheral blood leukocytes.
HHV-6–positive patients were found to carry only variant B. HHV-6 variant A was detected in 4 patients early after BMT. Variant B but not variant A was detected in 2 of the 4 patients 1 and 3 weeks later, respectively. The third patient became PCR-negative 1 week later. The fourth patient was positive with both variants A and B in the third week after BMT, but changed to variant A 1 week later and became negative afterwards. None of the 4 patients received antiviral therapy, nor had they received ACV prophylaxis. No variant A was detected 4 weeks later after BMT. The samples from 4 patients could not be subtyped. No clinical complications could be linked to HHV-6 variant A during this study.

Detection of lymphotropic herpesviruses and delayed engraftment. Five patients had delayed granulocyte and 6 patients had delayed platelet engraftment. Of all the samples drawn within the first 3 months after BMT, the proportions of HHV-6–positive samples were significantly higher in the groups of patients with either delayed granulocyte engraftment (13/35 v 44/174, \(P = .04\)) or delayed platelet engraftment (16/36 v 41/173, \(P = .001\)) than that of those with normal granulocyte or platelet engraftment. The HHV-6 in positive samples from the patients with delayed engraftment was confirmed to be variant B. No correlation was found between engraftment and the proportions of HHV-7, EBV, and CMV DNA-positive samples in the first 3 months after BMT.

Detection of lymphotropic herpesviruses and the development of aGVHD. There was no significant difference in the detection of all the four viruses in the patients with aGVHD grades 0 to I and the 6 patients with aGVHD grades II to III. Of all the samples drawn within the first 3 months after BMT, HHV-6 DNA was detected in 45/173 (26%) samples from the patients with aGVHD 0 to I and in 12/36 (33%) samples from the patients with aGVHD II to III. The corresponding numbers for HHV-7 were 32/173 (18%) v 6/36 (17%), for EBV 55/173 (32%) v 6/36 (17%), and for CMV 42/173 (24%) v 10/36 (28%). Furthermore, there was no correlation between the severity of aGVHD and HHV-6 DNA levels in PBL (Table 2).

Central nervous system symptoms. A possible central nervous system infection by HHV-6 was noticed in 1 patient. The initial symptoms included moderate fever and confusion. Physical examination showed weakness in the left arm and increased reflex responses in the left leg. The patient was always tired and was bound to bed. Electroencephalogram and computed tomography scan showed moderate, diffuse abnormality. Analysis of the cerebrospinal fluid revealed a significantly increased protein level of 538 mg/mL (normal range: <320 mg/mL), but no cells. HHV-6 DNA was detected in PBL when DNA corresponding to 50 PBL was analyzed. HHV-6 DNA was also detected in cerebrospinal fluid, whereas DNA of CMV, EBV, and herpes simplex virus was not detected. There were no indications suggesting other pathogens. The patient’s condition improved after treatment with foscavir (45 mg/kg body wt, intravenously twice daily).

Effect of ACV prophylaxis on presence of lymphotropic herpesvirus DNA after BMT. In this study, 9 patients received high-dose ACV prophylaxis against CMV infection. HHV-6 DNA was detected in 3 (33%) of the patients receiving ACV prophylaxis compared with 23/28 (82%) of the group without ACV prophylaxis (\(P = .01\), Fisher’s exact test). HHV-7, EBV, and CMV DNA were detected in 4 (44%), 5 (56%), and 6 (67%), respectively, of the patients receiving ACV prophylaxis. The corresponding numbers in the patients without ACV prophylaxis were 17 (61%), 21 (75%), and 15 (54%), respectively. The proportion of HHV-6–positive samples within the first 3 months after BMT was significantly lower in the patients who received ACV (5/51 v 52/158, \(P < .01\)). None of the 9 patients in the ACV group had HHV-6 DNA detected when the samples were diluted at 1:10 compared with 11 of 28 patients in the group who did not receive high-dose ACV (\(P = .01\), Fisher’s exact test). There was no significant effect on the proportion of HHV-7, EBV, and CMV DNA-positive samples by high-dose ACV prophylaxis during the first 3 months after BMT.

Effect of antiviral treatment on the presence of HHV-6, HHV-7, EBV, and CMV DNA after BMT. Twenty patients were treated for CMV DNA-emia. Eleven patients were treated with ganciclovir (GCV) and 9 patients with foscarnet. The treatment response was analyzed on the patients only when there were blood samples collected within 2 weeks before and after the treatment. HHV-6–, HHV-7–, EBV–, and CMV-positive patients were defined positive only if they had two consecutively positive samples before treatment. In the GCV group, 1 of 4 HHV-6–positive, 1 of the 2 HHV-7–positive, and none of the 5 EBV-positive patients became PCR-negative. However, 6 of 11 patients became CMV DNA-negative after treatment. In the foscarnet-treated group, the 3 HHV-6–positive patients, the 2 HHV-7–positive patients, and 5 of 7 CMV DNA-positive patients all became negative after treatment. The 3 EBV-positive patients remained positive after foscarnet treatment.

DISCUSSION

Before BMT, HHV-6, HHV-7, EBV, and CMV DNA were detected in 22%, 57%, 27%, and 3% of the patients, respectively. The somewhat lower rates of HHV-6, HHV-7, and EBV DNA positivity found in this study compared with others18,26 may be explained by the different PCR protocols used. Our HHV-6 PCR results were in accordance with a previous report on BMT patients in which a similar amount of cells was used, and a nested PCR system also was applied.19 After BMT, HHV-6, HHV-7, EBV, and CMV DNA were detected in 70%, 57%, 76%, and 60% of the patients. There was no difference in the proportion of PCR-positive patients for HHV-6, EBV, and CMV when only the patients who were seropositive before BMT were included in analysis. Although there are no published data for detection of HHV-7 and EBV DNA in PBL after BMT, the CMV and HHV-6 results are compatible with previous reports.18,27 There were differences in the kinetics of detection of DNA for the four viruses after BMT. HHV-6, EBV, and HHV-7 DNA were detected several weeks earlier than CMV in most patients. Furthermore, although the frequency of detectable HHV-6, HHV-7, and CMV DNA gradually decreased during the second and third months after BMT, possibly because of antiviral therapy, the prevalence of EBV DNA was consis-
ently high during the study period. As expected, most patients were positive with more than one virus.

Most of the HHV-6 DNA positive patients were found carrying variant B in accordance with previous reports.\textsuperscript{9,16} HHV-6 variant A was found only transiently. It is possible that variant A may replicate or be latent in different cell types than variant B. This may explain why only a few healthy people were found carrying HHV-6 variant A when PBL were analyzed.\textsuperscript{28} Recently, Wilborn et al\textsuperscript{16} reported that 2 BMT recipients with HHV-6 variant A were continuously HHV-6 PCR-positive. However, it seems that, in general, HHV-6 variant B is more common than variant A after BMT, as well as in normal individuals.

Hematopoiesis is controlled by a complex network and can be suppressed by many viruses such as EBV, hepatitis B virus, parvovirus B19, certain flaviviruses, and CMV.\textsuperscript{29,30} A couple of reports have shown that HHV-6 variant B may be one cause of delayed engraftment after BMT, possibly by suppressing the response of progenitor cells to various growth and maturation regulating factors.\textsuperscript{31,32} Drobyski et al\textsuperscript{3} have reported that HHV-6 was isolated from patients suffering from delayed engraftment, whereas other viral or bacterial pathogens were absent. Although the number of patients in this study is small, HHV-6 variant B seems to be correlated to delayed engraftment. This is supported by the observation that some patients with delayed engraftment had more HHV-6-positive samples and higher HHV-6 DNA levels within the first 3 months after BMT. Furthermore, ACV prophylaxis significantly reduced the proportion of HHV-6-positive samples after BMT, and none of the treated patients suffered from delayed granulocyte engraftment. The appearance of EBV, CMV, and HHV-7 DNA was not related with delayed granulocyte or platelet engraftment. In addition, CMV DNA was mostly detected after engraftment in this study, and the contribution of CMV to delayed engraftment seems to be limited. Acute GVHD can be initiated through the recognition of major host histocompatibility complex or minor histocompatibility complex antigens. The contribution of viral infection to aGVHD is still not clear. Virus could possibly trigger or enhance aGVHD by shared antigen epitopes or by viral antigens expressed on cell membranes. HHV-6 infection of PBL can induce the production of tumor necrosis factor-\(\alpha\), interleukin-1\(\beta\), and interferon-\(\alpha\).\textsuperscript{33,34} These cytokines can cause increased major host histocompatibility expression and upregulate adhesion molecules. Conversely, viral infections also can be increased due to aGVHD or its therapy. In our study, no clear correlation between the appearance of either HHV-6 or EBV or HHV-7 or CMV DNA and aGVHD could be found after BMT. It was reported that high levels of HHV-6 DNA in lung tissue and continuous detection of HHV-6 DNA in PBL were significantly correlated with aGVHD.\textsuperscript{35,36} Unfortunately, whether HHV-6 DNA was detected before or after aGVHD was not given in either study, so the exact role of HHV-6 in aGVHD was not defined. The contribution of HHV-6 to aGVHD needs to be further studied because the virus appears earlier than the development of aGVHD in most patients.

HHV-6 infection of the central nervous system has been reported in previously healthy children and elderly people and in those with compromised immune function due to BMT or human immunodeficiency virus infection.\textsuperscript{35-37} The case of encephalitis mentioned in this study was most probably due to HHV-6 because the HHV-6 DNA level was extremely high in PBL and HHV-6 DNA was also detected in cerebrospinal fluid. In addition, there were no findings suggesting other pathogens.

Little is known about the effects of GCV and foscarnet treatment against HHV-6 and HHV-7. In this study, there is an indication that foscarnet is more effective against HHV-6 and HHV-7 than GCV. None of the agents, however, seem to have any effect on the presence of EBV DNA in PBL. GCV prophylaxis can strongly reduce the frequency of CMV infections after BMT but the survival is not improved.\textsuperscript{38-40} Prentice et al\textsuperscript{38} have reported that prophylaxis with high-dose ACV given intravenously followed by oral ACV not only significantly reduced the frequency and the severity of CMV infections, but also improved the survival.\textsuperscript{38} One possible explanation for the improved survival might be the significant reduction of HHV-6, as shown in the present study. Although it was reported that GCV is more effective than ACV against CMV and HHV-6 in vitro,\textsuperscript{41} the in vivo process may be more complicated. One important difference between ACV and GCV could be that GCV prophylaxis was instituted after engraftment, whereas ACV is given through the entire transplant procedure.\textsuperscript{38,40} However, the effect of GCV might also be compromised by its higher toxicity compared with ACV.\textsuperscript{38,40} We believe these results merit further study.

In summary, HHV-6, EBV, HHV-7, and CMV DNA can be frequently detected in the PBL from bone marrow recipients. HHV-6 subtype B may be one cause of delayed engraftment. One case of encephalitis possibly caused by HHV-6 was observed. HHV-7 does not influence engraftment. One case of encephalitis possibly caused by HHV-6 was observed. HHV-7 does not influence engraftment despite its similarity to HHV-6. In this study, it seems that HHV-6 is not related to aGVHD; however, the number of patients was small. One contributing factor to the improvement in survival associated with high-dose ACV prophylaxis might be the reduction of the load of HHV-6 in PBL after BMT.

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