Herpesvirus Type 6 in Patients Undergoing Bone Marrow Transplantation:
Serologic Features and Detection by Polymerase Chain Reaction

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To evaluate the potential role of human herpesvirus type 6 (HHV-6) infection in patients after bone marrow transplantation (BMT) we sequentially analyzed buffy coat leukocytes, oral lavage fluid, and urine from 57 patients for the presence of HHV-6 DNA by polymerase chain reaction (PCR) before and after 60 BMTs. Twenty-four patients undergoing autologous BMT and 36 with allogeneic BMT were studied. Thirty-six patients (60%) were PCR positive in one or more tests. The majority of PCR-positive patients had positive results only sporadically, in 1 (n = 23) or 2 weeks (n = 5). Six patients were positive in 3 to 5 weeks. In 2 patients, we found a high frequency of positive tests, in 7 of 7 and 10 of 10 weeks analyzed. Twenty-four patients (40%) remained PCR negative throughout the post-BMT period. There was a significant correlation between the results of HHV-6 PCR and the occurrence of acute graft-versus-host disease (aGVHD). In grade II-IV, 6 of 8 (75%) patients had 2 or more positive PCR tests, compared with 5 of 25 (20%) patients without or with grade I aGVHD (P = 0.1). There was no difference in the outcome of PCR tests with respect to the type of BMT or pre-BMT HHV-6 enzyme-linked immunosorbent assay titers. Restriction enzyme analysis of PCR amplificates from 18 patients showed HHV-6 variant B in 16 (88.9%) and variant A in 2 cases (11.1%). We conclude that HHV-6 DNA can be detected in 60% of the patients after BMT. HHV-6 DNA can be detected more frequently in patients with moderate and severe aGVHD than in patients without aGVHD or with mild aGVHD.

PATIENTS AND METHODS

Patients. Specimens were obtained from 57 patients after 60 BMT procedures (3 second transplantations). Thirty-three patients were male and 24 were female. The median age was 21 years (range, 2 to 53). Transplantations were performed for acute myeloid leukemia (AML) in refractory state (n = 2), complete remission 1 (CR1; n = 5), and CR2 (n = 5); acute lymphoblastic leukemia (ALL) in CR1 (n = 3), CR2 (n = 13), CR 3 (n = 8), first relapse (n = 1), and refractory state (n = 2); chronic myeloid leukemia (CML) in chronic phase (CP; n = 12) and accelerated phase (AP; n = 1); Hodgkin’s disease (HD; n = 1); germ cell tumor (n = 3); multiple myeloma (MM; n = 1); low-grade non-Hodgkin’s lymphoma (n = 1); and severe aplastic anemia (SAA; n = 2). Twenty-four transplantations were autologous, 36 were allogeneic from HLA-identical sibling donors (n = 31), relative donor (n = 1), and unrelated donor (n = 1). Three patients received grafts from family donors mismatched in 1 antigen. Conditioning was performed with cyclophosphamide (CY)/total body irradiation (TBI) (n = 8), CY/total nodal irradiation (TNI) (n = 1), busulphan (BU)/CY (n = 17), VP16/TBI (n = 23), and experimental protocols (n = 8). The second grafts were performed after BU/CY (n = 2) and CY (n = 1). Graft-versus-host disease (GVHD) prophylaxis was performed with cyclosporin and a short course of methotrexate. Marrow grafts were not T-cell depleted. Thirty-three patients were evaluable for acute GVHD (aGVHD). Two patients with allogeneic BMT were not evaluable for aGVHD because of early death. Grade 0 was observed in 18, grade I in 7, grade II in 7, and grade IV in 1 patient. Grade III aGVHD was not found. Treatment of aGVHD included prednisone (2 to 3 mg/kg body weight daily) and, if required, anti-T-lymphocyte globulin (Fresenius, Bad Homburg, Germany). Extensive chronic GVHD (cGVHD) was diagnosed in 2 patients; none of the patients developed limited disease. Treatment of cGVHD included either reinstitution or a dose increment of cyclosporin, prednisone, azathioprine, or methotrexate alone or in combination. aGVHD and cGVHD were graded according to the recommendations of Glucksberg et al’ and Shulman et al14 respectively. Specimens (buffy coat, urine, and oral lavage fluid) of 60 healthy blood donors (30 male, 30 female) served as a control. The median age was 40 years (range, 19 to 63).

Sample preparation. Blood, urine, and oral lavage fluids were

HUMAN HERPESVIRUS type 6 (HHV-6) was identified as a new member of the herpesvirus group in 1986.1 Primary infection occurs in early childhood, but is most often not noticed. In some patients it presents as exanthema subitum (roseola infantum).2 Furthermore, HHV-6 is thought to be associated with the chronic fatigue syndrome and multiple sclerosis, and there are anecdotal reports of a mononucleosis-like lymphoproliferative, febrile lymphadenopathy, hepatitis, meningoencephalitis, and a hemophagocytic syndrome.3-6 Primary infection is followed by conversion to positive antibody status. Antibodies are present in 60% to 90% of the general population. There are data suggesting that HHV-6 persists latently in salivary glands, lymph nodes, peripheral blood mononuclear cells, and monocytes/macrophages, and in the liver and kidney.7-13 It is likely that HHV-6 can be reactivated under conditions of immunosuppression, especially after organ transplantation. Little is known about the incidence and the onset of reactivation after allogeneic or autologous bone marrow transplantation (BMT) and the impact on the clinical course after transplantation. Because HHV-6 can possibly impair hematopoiesis, it is of special interest in the context of BMT.14,16

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collected at weekly intervals 1 to 2 weeks before BMT and up to 10 weeks after BMT until discharge or death. Buffy coat leukocytes were prepared from 10 mL heparinized peripheral blood by gravity sedimentation and lysis of red blood cells with 0.83% NH₄Cl and two subsequent washing steps. White blood cell counts were adjusted to 2 to 4 × 10⁶ cells per pellet. Cell pellets obtained by centrifugation of urine and oral lavage and buffy coat leukocytes were lysed by the addition of 50 μL of 50 mmol/L NaOH. After mineral oil overlay, lysates were heat denatured (10 minutes at 95°C), neutralized to pH 8.4 by the addition of 8 μL 1 mol/L Tris-HCl, and subjected to two cycles of freezing/thawing.

Antibody determination. For antigen preparation, HS-2 cells were infected with HHV-6, strain St W.⁷ When they had developed a prominent cytopathic effect, cells were pelleted and the remaining supernatant subjected to centrifugation (3 hours at 70,000g). The sediment consisted of virus material and contaminating cell detritus and was further purified by sucrose-density gradient centrifugation (6 hours at 100,000g). The gradient was fractionated and each fraction was analyzed concerning purity of virus material by negative contrast electron microscopy. Fractions containing virus material of sufficient purity were pooled, diluted in carbonate buffer (50 mmol/L, pH 10.1), and coated to Dynatech Immulon (Denkendorf, Germany) microtiter plates at a concentration of 12 μg/mL (15 hours at 4°C). After washing, the plates were blocked by 2% bovine serum albumin (BSA) in Tris-MCl (200 mmol/L, pH 8.5). All sera were tested in duplicate. Plates were read (SLT, Crailsheim, Austria) when the absorbance at 405 nm of the positive control had reached 1.000.

Detection of HHV-6 DNA by polymerase chain reaction (PCR). The following primer pairs were used: P1, 5'-GGGACCGGATC- GGAGGAAGC (21 mer); P2, 5'-CGACCCGATCGCTGACTCCC (20mer); P3, 5'-TCCGAAGCITCGGGTAACGGAGAACGA (25mer) and P4, 5'-ATTCAGGGCTCTAGAACCCTTCTGT (25mer). Primers P1 and P2 were used for the first round of 30 amplification cycles, which resulted in a fragment of 456 bp. The sequences of all primers are localized in a highly conserved region of the gene for the major antigenic structural protein (p100) of HHV-6.⁶ DNA present in 2 μL of cell lysate was used as a template for amplification in a 30-μL reaction mixture (3 mmol/L MgCl₂, 100 μmol/L of each dNTP, 0.3 μg of primers P1/P2, 1.25 U Taq polymerase). For reamplification, 1 μL of the first amplification reaction was transferred to a new reaction mixture (200 μmol/L of each dNTP, 0.3 μg of internal primers P3/P4) for a second round of 25 amplification cycles. This PCR resulted in a 187-bp product. The amplification products were separated on agarose gels and visualized with ethidium bromide. The specificity of the amplification products was demonstrated by Southern blot hybridization using "random primed" digoxigenin-dUTP labeled 187-bp PCR fragments from plasmid pMF340-11 carrying a 1.4-kb EcoRI insert of HHV-6 p100 gene as a probe in a luminescence immunoassay (Boehringer Mannheim Biochemica, Mannheim, Germany). A 170-bp fragment of the β-interferon gene sequences was amplified as a performance control.¹⁷ To determine the reproducibility of our assay system and to define the likelihood of sampling errors, we analyzed buffy coat, oral lavage, and urine samples of 4 patients. Two patients were PCR positive at initial testing and 2 patients were negative. Each sample was tested 5 times in separate experiments, amounting to a total of 60 PCR reactions. In all cases the results were consistently positive or negative as the initial test. In urine samples, we observed variations in the intensity of the PCR amplificates that were without influence on the final result. The detection limits of the PCR were determined in serial dilutions of the plasmid pMF340-11 in salmon sperm DNA and lysates from uninfected HS-2 cells. The limits were 5 × 10⁷ and 5 plasmid copies in the first and second rounds of amplification, respectively. A patient was called "positive" if one or more PCRs were positive independent of type of sample (buffy coat, oral lavage, or urine) in which the finding was obtained.

Identification of HHV-6 variants. To discriminate HHV-6 variants A and B, we sequenced nested 187-bp PCR products of pMF340-11 derived from strain U1102 as variant A material and DNA of strain R104 as a variant B material. In the amplified region strains, R104 and Z29 have an identical base sequence (Pellett et al.⁴ and Neipel, personal communication, 1993). On the basis of the DNA sequences obtained, we selected restriction enzymes that enabled us to distinguish between variants A and B according to the resulting digestion fragments. Dra I and Cfo I exclusively cleave the 187-bp amplification product of variant A, leading to fragments of 103 bp and 84 bp in the case of Dra I or of 115 bp and 72 bp in the case of Cfo I (Fig 1A). The amplificate of variant B is exclusively cleaved by Ksp6321 and Hph I and Ksp6321 leads to fragments of 137 bp and 50 bp, Hph I to fragments of 115 bp and 72 bp (Fig 1B). Because of its small size, the 50-bp fragment band was very faint and could not be shown (Fig 1B). For enzymatic digestion, amplified DNA was precipitated by the addition of 0.5 vol of 8 mol/L ammonium acetate and 2.5 vol of absolute ethanol. Digestions were performed over 8 hours at 37°C in a reaction volume of 20 μL containing

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**Fig 1.** Identification of HHV-6 variants was performed by restriction enzyme fragment analysis of 187-bp nested PCR amplificates of either variant A (upper panel) or variant B (lower panel). Enzyme digests were separated on 3.5% agarose gels (NuSieve) and visualized with ethidium bromide. Lanes 1 and 7 represent the size marker (100-bp ladder); also shown are digests of Dra I (lane 2), Cfo I (lane 3), Ksp6321 (lane 4), Hph I (lane 5), and EcoRI (lane 6). Variant A is cleaved by Dra I (lane 2) and Cfo I (lane 3); it is not cleaved by Ksp6321 (lane 4), Hph I (lane 5), and EcoRI (lane 6). Variant B is cleaved by Ksp6321 (lane 4) and Hph I (lane 5); it is not cleaved by Dra I (lane 2), Cfo I (lane 3), and EcoRI (lane 6).
10 U of either Dra I or Ksp632I (Boehringer Mannheim Biochemica).

Statistical evaluation. Differences between patient subgroups were analyzed using the χ² test and the Mann-Whitney U-test.

RESULTS

Sixty graft procedures were evaluated in 57 patients. Altogether, 1,207 samples from patients were analyzed, 122 of which gave positive PCR results. Before BMT, 5 of 71 (7.0%) buffy coat samples, 5 of 71 (7.0%) urine samples, and 6 of 75 (8.0%) oral lavage samples were PCR positive. After BMT, 43 of 344 (12.5%) buffy coat samples, 36 of 333 (10.8%) oral lavage samples, and 27 of 313 (8.6%) urine samples were PCR positive (Table 1). The median observation time after BMT was 5 weeks (range, 2 to 10 weeks). As controls, 180 samples from healthy blood donors were examined and 10 samples were PCR positive. Nine of the patients (15.7%) were once or twice positive before BMT. After BMT, 24 patients were PCR negative throughout the post-BMT observation period, whereas 36 patients were PCR positive on one or more tests. PCR-positive patients could be subdivided into 23 patients positive in only 1 week, 5 patients positive in 2 weeks, 3 patients positive in 3 weeks, and 2 patients positive in 4 weeks. One patient each was positive in 5 of 7 weeks, 7 of 7 weeks, and 10 of 10 weeks analyzed post-BMT. Eleven of 76 (14.5%) patient-weeks were PCR positive before and 72 of 355 (20.3%) were positive after BMT, compared with 6 of 60 (10%) in healthy controls (Table 1). The comparison of results pre- and post-BMT and with controls did not show significant differences. One of 22 allogeneic bone marrow donors tested was PCR positive at the time of bone marrow harvesting.

The analysis of the results according to type of BMT (allograft or autograft) is summarized in Table 2. One or more positive PCRs were found in 10 of 24 (41.7%) autograft recipients, compared with 26 of 36 (72.2%) allograft recipients. This difference, although statistically significant (P = .033), remains questionable because allograft recipients were observed for a mean of 6.7 weeks per patient compared with 4.7 weeks per autograft recipient. However, median observation times were not different. Accordingly, the ratio of the number of weeks with positive PCRs and the total number of weeks analyzed with 13 of 112 and 59 of 242 after autograft and allograft, respectively, were not significantly different. Furthermore, there were no significant differences in the proportion of patients with 2 or more positive PCRs in both groups.

Because it is conceivable that HHV-6 may be reactivated depending on the degree of immunosuppression, it is of interest to study patients with and without aGVHD. Altogether, 33 patients were analyzed for aGVHD (Table 3). The proportion of patients with 2 or more positive PCRs was lower in the group without or with only mild (grade I) aGVHD than in the group with grade II to IV aGVHD (5 of 25 [20%] patients and 6 of 8 [75%] patients, respectively; P = .01). The median observation times of both groups were not significantly different, but the mean observation periods were slightly longer in patients with grade I-IV aGVHD than in patients without or with grade I aGVHD (7.7 weeks and 6.7 weeks per patient, respectively). However, also the comparison of the number of weeks with positive PCRs per number of weeks analyzed showed a significantly higher proportion of positive tests in patients with aGVHD II-IV (P = .03).

For the analysis of HHV-6 variants, we retrospectively tested stored samples of buffy coat (n = 22), oral lavage (n = 15), and urine (n = 13) of 18 patients and the one marrow

### Table 1. Results of PCR for HHV-6

<table>
<thead>
<tr>
<th>Time of Analysis (wk)</th>
<th>Samples Analyzed</th>
<th>Spec</th>
<th>n</th>
<th>%</th>
<th>Weeks Positive/weeks Analyzed</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2 and –1</td>
<td>BC 71</td>
<td>75</td>
<td>5</td>
<td>7.0</td>
<td>78</td>
<td>11</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>OL 75</td>
<td>6</td>
<td>8.0</td>
<td>20.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UR 71</td>
<td>5</td>
<td>7.0</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+1 to +10</td>
<td>BC 344</td>
<td>43</td>
<td>12.5</td>
<td>355</td>
<td>72</td>
<td>20.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OL 333</td>
<td>36</td>
<td>10.8</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UR 313</td>
<td>27</td>
<td>8.6</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>BC 60</td>
<td>3</td>
<td>5.0</td>
<td>60</td>
<td>6</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OL 60</td>
<td>4</td>
<td>6.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UR 60</td>
<td>3</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: spec, kind of specimen; BC, buffy coat; OL, oral lavage fluid; UR, urine; NS, not significant (χ² test).

### Table 2. PCR Results in Relation to Type of Graft

<table>
<thead>
<tr>
<th>Type of Graft</th>
<th>No. of patients</th>
<th>Patients with ≥1 + PCR</th>
<th>Patients with ≥2 + PCR</th>
<th>Weeks positive/weeks analyzed</th>
<th>Median observation time (range) t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous</td>
<td>24 (100%)</td>
<td>10 (41.7%)</td>
<td>2 (8.3%)</td>
<td>13/112 (11.8%)</td>
<td>5 (2-8)</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>36 (100%)</td>
<td>26 (72.2%)</td>
<td>11 (30.5%)</td>
<td>59/242 (24.4%)</td>
<td>6 (3-10)</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

* χ² test.
† Weeks after transplantation.
‡ Mann-Whitney U test.

### Table 3. PCR Results in Relation to aGVHD

<table>
<thead>
<tr>
<th>Grade of aGVHD</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 = I</td>
<td>II-IV</td>
</tr>
<tr>
<td>No. of patients</td>
<td>25 (100%)</td>
</tr>
<tr>
<td>Patients with ≥1 + PCR</td>
<td>18 (72%)</td>
</tr>
<tr>
<td>Patients with ≥2 + PCR</td>
<td>5 (20%)</td>
</tr>
<tr>
<td>Weeks positive/weeks analyzed</td>
<td>35/168 (20.8%)</td>
</tr>
<tr>
<td>Median observation time (range) t</td>
<td>7 (4-10)</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

* χ² test.
† Weeks after transplantation.
‡ Mann-Whitney U test.
donor who was PCR positive at the time of BM harvest. We detected HHV-6 variant B in 16 patients (88.9%). Two patients had HHV-6 variant A. Interestingly, the latter 2 patients showed a high frequency of positive PCRs, namely in 5 of 7 and in 10 of 10 weeks (see below, patient H, unique patient number [UPN] 271), respectively. The marrow of one donor carried HHV-6 variant A. This marrow was filtered to patient B (UPN 223) (Fig 2C), who, after BMT, carried variant B from week -1 through week +6 and before his death converted to variant A.

Serum antibodies against HHV-6 were determined in 25 BM donors and in all patients before each graft procedure and consecutively in the posttransplantation period. Pre-BMT median ELISA values of patients were 0.670 (range, 0.073 to 2.218). Two patients were antibody negative (ELISA, <0.2). Median ELISA values of 25 BM donors were 0.712 (range, 0.354 to 1.827). There was no significant difference between the ELISA values of patients before allogeneic (median, 0.690; range, 0.148 to 1.763) or autologous BMT (median, 0.630; range, 0.073 to 2.218) or of healthy blood donors (median, 0.481; range, 0.056 to 1.043). Pre-BMT antibody levels did not correlate with the outcome of PCR results in the posttransplantation period.

A more detailed look at patients with positive PCRs shows the following. Besides the large group of 31 patients (52%) who were only sporadically positive 1 to 3 times after BMT, there were few patients exhibiting positive tests in 4 or more weeks. The course of 4 patients are described in the following.

Patient Z (UPN 170/230), a 31-year-old woman, was allografted for SAA with marrow from her HLA-identical, MLC-negative brother after conditioning with TNI (7.5 Gy) and 4 X 50 mg/kg CY. The posttransplant course was uneventful and without occurrence of aGVHD. She relapsed 10 months after BMT and received a second BMT from the same donor 12 months after the first BMT. Conditioning therapy then included antithymocyte globulin and 2 X 60 mg/kg CY. Subsequently, she developed grade II aGVHD present in weeks +3 and +4. She was PCR positive for HHV-6 in weeks +1 and +4 in the context with BMT1, but showed positive results in weeks +1 through +4 after BMT2 (Fig 2A). HHV-6 antibody levels remained stable during the early period after BMT1 and subsequently declined below threshold levels (<0.2) at relapse of SAA. In the context of BMT2, we observed an increase of antibody levels in parallel with the detection of HHV-6 by PCR over 5 consecutive weeks. HHV-6 variant was "A." She is alive and well 16 months after the second BMT.

Patient A (UPN 168/238), a 6-year-old boy, was autograft for ALL in CR3 after a conditioning regimen with fractionated TBI (12 Gy) and high-dose etoposide (60 mg/ kg). He relapsed 8 months after autologous BMT (ABMT). Subsequently, he received an allogeneic graft from his 2-antigen–mismatched, MLC-negative sister after a treatment with 4 X 4 mg/kg busulfan and 4 X 50 mg/kg CY. aGVHD grade II started in week +3 and lasted for 2 weeks. The further posttransplantation course was uneventful. He was PCR positive once (week +1) in the course after ABMT and in weeks +1 and +3 through +5 after second allogeneic BMT (Fig 2B). Before and after ABMT, HHV-6 antibodies were stable at a level around 0.5. However, after BMT2, antibody levels showed a sharp increase parallel with positive PCR results. HHV-6 variant was "B." The boy is alive and well 16 months after second BMT.

Patient B (UPN 223), a 29-year-old man, received a 1-antigen–mismatched, MLC-negative graft from his father for the treatment of CML in cP. The conditioning treatment consisted of fractionated TBI (12 Gy) and 4 X 50 mg/kg CY. aGVHD grade IV occurred on day +11 and persisted until death caused by interstitial pneumonitis (iPn) on day +50. The etiology of iPn could not be clarified by autopsy. HHV-6 PCR was positive in weeks -1 through +7 (Fig 2C). HHV-6 antibody levels were relatively high before transplantation. They showed a marked increase in the early posttransplantation period and subsequently decreased to threshold levels. Donor marrow was PCR positive at the time of harvest. It contained HHV-6 variant A. The recipient harbored HHV-6 variant B in weeks -1 through +6. In week +7, the week before his death from iPn, he converted to HHV-6 variant A.

Patient H (UPN 271), a 27-year-old man, who was treated for CML in cP, received a graft from his HLA-identical, MLC-negative identical. Conditioning treatment consisted of fractionated TBI (12 Gy) and 4 X 50 mg/kg CY. aGVHD grade I occurred on day +21 and lasted for 2 weeks. After discharge in week +7, he became febrile and was readmitted in week +9. The origin of the fever that then persisted over 5 weeks remained unknown. He was negative both in cyo-megalovirus (CMV)-PCR and CMV-antigen test. He finally defervesced under empiric antibacterial and antiviral therapy with ganciclovir and Ig. He was HHV-6 positive throughout the clinical course from week -2 to week +23. HHV-6 antibody levels fluctuated. After an initial increase in week +2 after BMT and a subsequent decrease, values formed a second peak in weeks +7 through +10. HHV-6 was of variant A. He now is alive and well 11 months after BMT (Fig 2D).

DISCUSSION

Herpesviruses persist throughout life after primary infection. Virus proliferation occurs either spontaneously or under conditions of immunosuppression. Reactivation can lead to illnesses that typically differ in their clinical presentation from disease associated with primary infection. After BMT, reactivated herpes simplex virus (HSV), varicella zoster virus (VZV), and CMV frequently cause serious, sometimes life-threatening disease. HHV-6 is a recently discovered member of the herpesvirus family. The chief manifestation of widespread primary infection, erythema subitum, occurs in early childhood. Anecdotal reports incriminate HHV-6 in the development of iPn, pyrexia, and skin rash. Relatively little is known about its behavior in immunosuppressed patients after BMT. Likewise, the contribution of HHV-6 to posttransplantation morbidity is still unclear. More recently, a close correlation between a high HHV-6 DNA content in lung biopsies and the development of iPn after BMT was described.

We performed PCRs to demonstrate HHV-6 DNA in oral
lavage, urine, and buffy coat leukocytes to study its role in patients after BMT. Overall, the finding of a positive PCR was a relatively rare event. Twenty-four patients (40%) were PCR negative throughout the post-BMT observation period. In this context it has to be considered that, with the exception of 2, all patients were HHV-6 antibody positive. Both antibody-negative patients remained PCR negative post-BMT. The majority of PCR-positive patients were positive only sporadically. Twenty-three patients harbored detectable amounts of HHV-6 in buffy coat leukocytes, or excreted it into urine or oral lavage fluid on only one occasion. Several positive tests in sequence, as seen in symptomatic CMV infection, were observed in only a few patients. Two patients were exceptional in so far as they were PCR positive before and during the whole observation period after transplantation of 7 (patient B, UPN 223) and 10 weeks (patient H, UPN 271), respectively (Fig 2A and B).

The most prominent finding was the correlation of positive HHV-6 PCR in buffy coat, with the severity of aGVHD. Six of 8 (75%) patients with aGVHD grade II-IV compared with only 5 of 25 (20%) patients without or with grade I aGVHD had two or more positive PCR tests (P = .01; Table 3). Similarly, the percentage of weeks with positive tests over the total number of weeks analyzed is higher in patients with aGVHD grade II-IV (35.4%) than in patients without or with grade I aGVHD (20.8%) (P = .03). These findings are compatible with those of Cone et al, who demonstrated a correlation of high levels of HHV-6 DNA in lung biopsy material with the presence of idiopathic iPn and increased severity of aGVHD. In our study group, 15 of 33 patients developed aGVHD grade I-IV. No specific clinical condition could be associated with HHV-6. Two patients died of iPn during the observation period after BMT. This was caused by CMV in one case, whereas the etiology remained unknown in the other. An autopsy was not performed. Antibody response to HHV-6 does not show characteristic differences if groups after allogeneic or autologous BMT or patients with or without aGVHD are compared. However, the look at the course of individual patients can give insight into the physiology of HHV-6 infection (Fig 2). Patients Z (UPN 170/230) and A (UPN 168/238) both experienced aGVHD during their second BMT, showed a higher frequency of positive PCR, and simultaneously experienced an increase in antibody levels. Patient B (UPN 223) developed aGVHD grade IV and lethal iPn accompanied by a decrease of antibodies to threshold levels. A similar observation has been described by Cone et al. Antibody titers at the time of open-lung biopsy were significantly lower in patients with idiopathic iPn. At the moment, we do not know if this is a consequence of the course of HHV-6 infection, of aGVHD, or of the intensive immunosuppressive treatment of aGVHD.

Little is known about the incidence of HHV-6 variants A and B in the general population and in BMT patients and about their clinical relevance. It is a matter of discussion whether infections with variant A and B differ in their clinical manifestations. We could detect variant B in 16 of 18 patients (88.9%) and variant A in only 2 patients (11.1%). One of these patients, who received marrow from his donor carrying variant A at BM harvest, first expressed variant B and, before his death, converted to variant A. It is of note that both patients carrying variant A were the ones who continuously were PCR positive throughout the post-BMT observation period. It will have to be shown if this phenomenon is characteristic for variant A. Recently, Drobyski et al could exclusively detect HHV-6 variant B in clinical isolates from 16 patients undergoing BMT.

Recently, new properties of HHV-6 were reported that can be of special relevance for patients after BMT. First, it was shown that HHV-6 may infect hematopoietic progenitor cells and thereby cause a reduction of burst-forming unit-erythroid, colony-forming unit--granulocyte-macrophage, and colony-forming unit--granulocyte, erythrocyte, monocyte, megakaryocyte growth. This suggests that HHV-6 may participate in the posttransplantation graft-failure syndrome that was, so far, attributed to CMV infection. Second, HHV-6 may increase posttransplantation immune deficiency because it can infect CD4+ and CD8+ T lymphocytes and natural killer (NK) cells.

In conclusion, HHV-6 can be demonstrated by PCR in buffy coat, oral lavage fluid, and urine in about half of the patients after BMT. It can be demonstrated more frequently in patients with aGVHD. It is highly likely that this is caused by reactivation of latent virus as observed with CMV, VZV, and HSV infection. It is conceivable that either aGVHD itself or the immunosuppressive effect of BMT-associated treatment creates a vicious circle of impairment of the immune system in which reactivated HHV-6 infects and destroys NK cells and T lymphocytes and thereby aggravates the pre-existing immunodeficiency. However, because of the low incidence of serious complications in our study group, we cannot conclude if HHV-6 as demonstrated by PCR has implications on the clinical outcome.

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Herpesvirus type 6 in patients undergoing bone marrow transplantation: serologic features and detection by polymerase chain reaction

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