Human herpesvirus 6 (HHV-6) infection was studied in 82 bone marrow transplant (BMT) recipients (72 allogeneic, 10 autologous). All recipients and 30 donors were seropositive for HHV-6 antibody at the time of bone marrow transplantation. Thirty-one recipients (37.8%) had HHV-6 viremia 2–4 weeks after transplantation. The incidence of HHV-6 viremia was significantly higher among allogeneic BMT recipients than in autologous BMT recipients ($P = .011$). Therefore, the following analyses of allogeneic BMT recipients were carried out ($n = 72$). Geometric mean antibody titers (log_{10}) were significantly higher in recipients without viremia than in those with viremia ($1.84 \pm 0.39$ vs. $1.61 \pm 0.42$; $P = .022$). Logistic regression analysis demonstrated that leukemia or lymphoma is an independent risk factor ($P = .031$) for HHV-6 viremia. Rash occurring within 1 month after transplantation was observed in 17 (54.8%) of 31 recipients with HHV-6 viremia but in only 8 (19.5%) of 41 recipients without HHV-6 viremia ($P = .001$).

In previous case studies of posttransplantation HHV-6 infection, polymerase chain reaction (PCR) was used to detect HHV-6 DNA in PBMC [6, 9, 17, 21]. However, this method may detect the viral genome in latently infected PBMC, giving a false-positive result. Also, inhibitors present in the samples may prevent the PCR from working, resulting in false-negative findings. Other major obstacles to accurate detection of posttransplantation HHV-6 infection include false positives caused by cross-reactivity between HHV-6 and -7 antibodies [22–24] and false negatives due to the lack of an appropriate antibody response against the virus in immunosuppressed recipients.

A more accurate way to detect active HHV-6 infection in immunosuppressed persons is to isolate virus from PBMC [25]. The isolation of HHV-6 from humans is difficult, because HHV-6 replicates efficiently in phytohemagglutinin-stimulated human umbilical cord blood mononuclear cells (PBMC) before bone marrow transplantation and subsequent viral infection [20], little is known about risk factors for posttransplantation HHV-6 infection.

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Transplant recipient guardians gave consent for patient study participation.

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Patients and Methods

Patient characteristics. Eighty-two patients received a BMT in the Division of Hematology-Oncology, Children’s Medical Center, Japanese Red Cross Nagoya First Hospital, and the Department of Pediatrics, Nagoya University School of Medicine. Patient characteristics relating to age, sex, type of graft, and underlying disease are summarized in Table 1.

Patient treatment. Details of the conditioning regimen and GVHD prophylaxis are described elsewhere [26, 27]. In brief, patients with hematologic malignancy were conditioned with melphalan (180 mg/m²) plus busulfan (16 mg/kg) or TBI (12 Gy) with the high-dose chemotherapy. Patients with severe aplastic anemia were conditioned with cyclophosphamide (50 mg/kg) and ATG (15 mg/kg). GVHD prophylaxis consisted of methotrexate with or without cyclosporine. Patients received intravenous gamma globulin preparations weekly during the first 3 months as prophylaxis for CMV infection. Oral acyclovir at a dose of 300–1000 mg was given daily. Preemptive therapy against CMV infection with ganciclovir was given after a positive antigenemia assay, as described later.

Experimental design. EDTA-treated peripheral blood was collected from the donor and the recipient at the time of transplantation and was collected biweekly from the recipients until 2 months after transplantation (time of transplantation and 2, 4, 6, and 8 weeks after transplantation). Because some samples from unrelated donors could not be obtained, in total we analyzed 30 donor samples. Clinical data were collected retrospectively and were assessed to determine associations with HHV-6 viremia.

Isolation and identification of HHV-6. The procedures for isolation and identification of HHV-6 have been described elsewhere [3]. In brief, PBMC were cocultured with cord blood mononuclear cells in culture medium. Viruses were identified primarily by morphologic changes in cultured cells (i.e., characteristics of pleomorphic, balloonlike large cells). Virus isolation was confirmed by specific immunofluorescence staining with a monoclonal antibody (MAb) to HHV-6 (OHV-3; provided by T. Okuno, Department of Microbiology, Hyogo College of Medicine, Hyogo, Japan). Since this MAb is variant B specific [28], all isolates that showed a positive reaction against the antibody were considered to be HHV-6 variant B.

Serologic assay to detect HHV-6 antibodies. Antibody titers to HHV-6 were measured by indirect IFA, as described elsewhere [29]. The representative strain of HHV-6 variant B (FG-1) isolated from PBMC obtained from an exanthem subitum patient was used as the standard antigen. The antibody titer was defined as the reciprocal of the serum dilution showing specific fluorescence. HHV-6 antibody titers of all recipients and of 30 donors were determined at the time of transplantation.

Criteria to determine association between HHV-6 viremia and clinical features. The clinical features examined for their relationship to HHV-6 viremia were rash resembling acute GVHD, pathologically confirmed GVHD, interstitial pneumonia, encephalitis or encephalopathy, CMV disease, and recovery of neutrophils and platelets.

We suggested earlier that HHV-6 plays an important role in the development of rash that occurs within 30 days after transplantation [11]. Thus, if a rash occurred within 30 days after transplantation, we defined this as positive for the rash resembling acute GVHD in this study. Meanwhile, we defined GVHD by clinical [30] and pathological [31] criteria. Engraftment was defined as recovery of hematopoiesis of neutrophils and platelets. The criteria for neutrophil engraftment was an absolute neutrophil count ≥500 cells/μL for 3 consecutive days within the study period, counting the first day as the day of engraftment. The criterion for platelet engraftment was a platelet count ≥50,000 cells/μL for 2 consecutive days independent of transfusion within the study period, counting the first day as the day of engraftment.

We screened for the presence of the early CMV antigen by using the HRP-C7 method to detect early CMV antigenemia. This method detects the pp65 antigen of CMV in leukocytes. CMV disease was diagnosed by the presence of CMV antigenemia in patients with symptoms of the disease.

Statistical analysis. Initial comparisons between the type of graft and HHV-6 viremia were conducted using the χ² test. Unpaired comparisons between the recipient’s anti–HHV-6 antibody titer at the time of transplantation and the presence or absence of viremia were made by Student’s t test. To identify risk factors of posttransplantation HHV-6 viremia, continuous variables were compared by unpaired t test. Binary variables were examined by Fisher’s exact and χ² tests. Finally, we used multivariate logistic models to determine independent risk factors. Variables entered into the logistic models were those with a univariate probability value of P < .05. The association between HHV-6 viremia and each clinical event was assessed by Fisher’s exact test or χ² test. The probability of achieving >500 neutrophils/μL and >50,000 platelets/μL after transplantation was evaluated by the Kaplan-Meier method, with analysis by log rank test.

Results

Virus isolation and HHV-6 serology. All recipients and 30 donors were seropositive for the anti–HHV-6 antibodies at the time of transplantation. Of 82 recipients, 31 (37.8%) had posttransplantation HHV-6 viremia. HHV-6 was isolated 2 weeks after transplantation in 27 recipients and at 4 weeks after trans-
plantation in 4 recipients. As described in Patients and Methods, all isolates were confirmed as HHV-6 variant B by IFA, using type-specific MAb. No recipient had HHV-6 repeatedly isolated. No virus was isolated from the samples obtained at any other time.

**Risk factors for HHV-6 infection.** The type of graft had a major influence on the likelihood of HHV-6 viremia (table 2). The incidence of HHV-6 viremia was significantly higher among allogeneic BMT recipients than in autologous BMT recipients ($P = .011$). All 31 isolates were recovered from recipients who received allogeneic stem cell transplants; no recipient with an autologous BMT had viremia.

Therefore, we carried out the following analyses in allogeneic BMT recipients ($n = 72$). First, we looked for an association between the recipient’s pretransplantation HHV-6 antibody titer and subsequent HHV-6 viremia. The geometric mean antibody titer (GMT; $\log_{10}$) was significantly higher in recipients who did not develop viremia (GMT $\pm$ SD, 1.84 $\pm$ 0.39) than in those with viremia (1.61 $\pm$ 0.42; $P = .022$). The factors in table 3 were evaluated for their association with HHV-6 viremia and absence of viremia during the observation period. The risk factors for HHV-6 viremia with $P < .20$ were underlying disease ($P = .059$) and ATG treatment ($P = .123$). Other factors, including sex, age, type of donor, and TBI, were not associated with subsequent HHV-6 viremia. A multivariate logistic regression analysis was performed to determine whether leukemia or lymphoma was by itself a significant risk factor. As shown in table 4, leukemia or lymphoma was an independent risk factor ($P = .031$). HHV-6 viremia occurred more frequently in patients who underwent transplantation because of leukemia or lymphoma than in patients who did so because of other diseases, including myelodysplastic syndrome, aplastic anemia, solid organ tumors, metabolic diseases, and congenital immunodeficiency.

**Associations with clinical features.** Table 5 summarizes the association of HHV-6 viremia with various clinical features. These analyses were carried out in allogeneic BMT recipients. Rash resembling acute GVHD was observed in 17 (54.8%) of 31 recipients with HHV-6 viremia but in only 8 (19.5%) of 41 recipients without HHV-6 viremia ($P = .001$). Thus, rash was significantly associated with posttransplantation HHV-6 viremia. As shown in table 5, pathologically confirmed acute GVHD, interstitial pneumonitis, central nervous system involvement, and CMV disease occurred in a small number of recipients, but these clinical features were not statistically associated with posttransplantation HHV-6 viremia.

The effect of HHV-6 viremia on engraftment within the first 2 months after transplantation was assessed by univariate analysis (figure 1). Since administration of granulocyte colony-stimulating factor (G-CSF) affects recovery of neutrophil levels, we first looked for an association between HHV-6 viremia and neutrophil recovery in recipients with G-CSF treatment ($n = 59$). Recovery of neutrophil levels to $> 500$ cells/$\mu$L occurred after a median of 17.5 days (range, 12–60) in recipients without HHV-6 viremia and after a median of 17.3 days (range, 10–38) in recipients with HHV-6 viremia ($P = .396$). Next, we evaluated the effect of HHV-6 viremia on neutrophil recovery in recipients without G-CSF treatment ($n = 13$) and found recovery to $> 500$ cells/$\mu$L after a median of 24.6 days (range, 15–60) in recipients without HHV-6 viremia and after a median of 22.3 days (range, 12–35) in recipients with HHV-6 viremia ($P = .662$).

Similar results were obtained for the rate of recovery of platelet counts. There was no difference between recipients with and without HHV-6 viremia ($P = .443$). The recovery of platelet levels to $> 50,000$ cells/$\mu$L occurred after a median of 26 days (range, 8–60) in the recipients without HHV-6 viremia ($n = 51$) versus a median of 31 days (range, 16–60) in recipients with viremia.

**Table 2.** Human herpesvirus 6 (HHV-6) viremia in relation to type of graft.

<table>
<thead>
<tr>
<th>Type of graft</th>
<th>HHV-6 viremia (n = 31)</th>
<th>No HHV-6 viremia (n = 51)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous</td>
<td>0</td>
<td>10</td>
<td>.011</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>31</td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>

*NOTE. Data are no. of patients, except where noted otherwise.

*Fisher’s exact test, 2-tailed.

**Table 3.** Univariate analysis of association between each risk factor and human herpesvirus 6 viremia after allogeneic bone marrow transplantation.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Yes (n = 31)</th>
<th>No (n = 41)</th>
<th>RR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>17</td>
<td>1.029 (0.3–3.3)</td>
<td>.962</td>
</tr>
<tr>
<td><strong>Age, mean years ± SD</strong></td>
<td>8.8 ± 4.9</td>
<td>7.5 ± 4.8</td>
<td>1.013 (0.9–1.1)</td>
<td>.830</td>
</tr>
<tr>
<td><strong>Underlying disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia or lymphoma</td>
<td>27</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>22</td>
<td>3.931 (0.9–16.3)</td>
<td>.059</td>
</tr>
<tr>
<td><strong>Type of donor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unrelated</td>
<td>17</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Related (sibling)</td>
<td>14</td>
<td>22</td>
<td>1.259 (0.4–3.9)</td>
<td>.689</td>
</tr>
<tr>
<td><strong>Conditioning regimen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBI</td>
<td>26</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No TBI</td>
<td>5</td>
<td>18</td>
<td>1.302 (0.3–5.3)</td>
<td>.713</td>
</tr>
<tr>
<td>ATG</td>
<td>3</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ATG</td>
<td>27</td>
<td>33</td>
<td>0.287 (0.1–1.4)</td>
<td>.123</td>
</tr>
</tbody>
</table>

NOTE. ATG, antithymocyte globulin; CI, confidence interval; RR, relative risk; TBI, total body irradiation.

*Data are no. of patients, except where noted otherwise.
pression. Since HHV-6 remains latent in monocytes and macrophages after primary infection [32], PCR analysis, the most popular method used in previous studies, may give false positives by detecting viral DNA in latently infected PBMC. Cross-reactivity between HHV-6 and HHV-7 antibodies may lead to false positives in serologic tests [22–24]. Serologic tests may also yield false negatives, because immunosuppression in this patient population may prevent an antibody response. Although isolation of the virus from the patient’s peripheral blood is time consuming and labor intensive, we consider it to be a more reliable method of detecting active viral infection in BMT recipients than PCR or serologic assays. Thus, we used the virus isolation method to monitor active viral infection in this study of the clinical features of and risk factors for viral infection after transplantation.

The onset and duration of active HHV-6 infection we observed differed from those seen in earlier studies [6, 9, 17–19, 21] that used PCR analysis for monitoring active viral infection. The onset of active HHV-6 infection was widely distributed from early to late time periods after transplantation in those studies. Although some studies that used semiquantitative PCR assay for evaluation of the viral infection demonstrated kinetics of infection similar to our findings [9, 18], the duration of active viral infection in most of the prior studies was also longer than that seen in the present analysis. Although we collected samples up to 2 months after transplantation, the time during which virus was isolated was concentrated at 2–4 weeks after transplantation, as reported elsewhere [5, 11]. Of importance, no patient had HHV-6 repeatedly isolated, which provides evidence for a limited duration of active viral replication. PCR analysis may actually be too sensitive. It can detect HHV-6 DNA during low-grade active virus replication, which is below the level detected by virus isolation even during latent viral infection.

As we described earlier, the kinetics of viremia are well correlated with clinical features of exanthem subitum (primary HHV-6 infection) [3, 33]. In contrast to the virus isolation method, sensitive PCR assays detect the viral DNA in PBMC after the subsidence of fever in patients with exanthem subitum [34]. Therefore, as recently postulated by Carrigan and Knox [25], we emphasize that virus isolation is the best method for analyzing the relationship between active infection and clinical events.

Further study is needed to determine whether high-magnitude active HHV-6 replication, which is detected by virus isolation, occurs >2 months after transplantation.

In contrast to previous studies conducted by our group and others [20], in the present study, the pretransplantation anti–HHV-6 antibody titers of recipients who subsequently developed viremia were significantly different from those who did not. The present study analyzed a relatively large number of patients, compared with previous studies, which may explain this discrepancy. Significantly lower antibody titers were found in the patients with subsequent viremia ($P = .02$). Kadakia et al. [15] also demonstrated a significant negative association between salivary shedding of the virus and mean antibody titers. These results indicate that the level of immunosuppression in each patient might be associated with subsequent active HHV-6 infection after transplantation, as with CMV infection [35]. To clarify this issue in future experiments, an evaluation of the activity of HHV-6–specific cytotoxic T cells from such patients is needed.

We used an IFA in the present study. Antibody titers determined by this method are well correlated with neutralizing antibody titers [36]. Therefore, it is possible that neutralizing antibodies may play an important role in preventing active HHV-6 infection after transplantation. Immunoglobulin that contains high levels of anti–HHV-6 antibodies might be valuable as prophylaxis against active viral infection.

We believe that this report is the first to document allogeneic bone marrow transplantation as a risk for active HHV-6 infection, since all isolates were recovered from recipients of allogeneic BMTs. Allogeneic stimulation can induce CMV reactivation in latently infected monocytoid-derived macrophages in vitro [37]. The allogeneic activation of cells in bone marrow grafts may provide a microenvironment conducive to cellular differentiation and reactivation of HHV-6. However, we assessed only 2 factors (TBI and ATG treatment) in the conditioning regimens as risk factors for viral infection. In a future prospective study, we will evaluate other factors in the conditioning regimens to clarify the point. Moreover, to confirm our hypothesis, it is necessary to carry out multivariate analysis that includes large numbers of autologous BMT cases. As demonstra-

### Table 4. Results of logistic regression analysis to determine risk factors for human herpesvirus 6 viremia.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>RR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia or lymphoma</td>
<td>4.5 (1.1–17.9)</td>
<td>.031</td>
</tr>
<tr>
<td>ATG pretreatment</td>
<td>0.314 (0.1–1.5)</td>
<td>.141</td>
</tr>
</tbody>
</table>

NOTE. ATG, antithymocyte globulin; CI, confidence interval; RR, relative risk.

### Table 5. Relationship between human herpesvirus 6 (HHV-6) viremia and clinical features.

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Yes ($n = 31$)</th>
<th>No ($n = 41$)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash</td>
<td>17</td>
<td>8</td>
<td>.001</td>
</tr>
<tr>
<td>GVHD</td>
<td>5</td>
<td>2</td>
<td>.132</td>
</tr>
<tr>
<td>Interstitial pneumonitis</td>
<td>1</td>
<td>7</td>
<td>.126</td>
</tr>
<tr>
<td>CNS manifestation</td>
<td>0</td>
<td>2</td>
<td>.502</td>
</tr>
<tr>
<td>CMV disease</td>
<td>4</td>
<td>5</td>
<td>1.000</td>
</tr>
</tbody>
</table>

NOTE. CMV, cytomegalovirus; CNS, central nervous system; GVHD, graft-versus-host disease.

*a Data are no. of patients, except where noted otherwise.

*b Determined by $\chi^2$ or Fisher’s exact test.
ed in CMV reactivation [37], it is also necessary to elucidate the mechanisms of HHV-6 reactivation by an in vitro model.

Active HHV-6 infection after transplantation has been found in conjunction with several clinical events, including rash [4, 5, 8, 9], GVHD [6, 7, 10], pneumonitis [12, 13], encephalitis or encephalopathy [14], CMV disease [15], and bone marrow suppression [16–19]. We looked for an association between HHV-6 viremia and these clinical events. HHV-6 isolation was significantly associated only with rash that occurred within 1 month after transplantation ($P = .001$). Recently, we compared the relationship between rash and HHV-6 infection in 2 patient groups, those with early-onset and those with late-onset rash ($\leq 30$ days vs. $> 30$ days after transplantation) [11]. In that study, HHV-6 infection occurred more frequently in the patients with early onset of rash. Taken together with data from the present study, that finding suggests that HHV-6 viremia may play an important role in the pathogenesis of early-onset rash resembling acute GVHD.

In the present study, if both clinical and pathological criteria were fulfilled, we defined the patient as having acute GVHD. However, we consider accurate diagnosis of early onset of acute GVHD to be difficult on the basis of pathological analysis. Therefore, an association between acute GVHD and acute GVHD-like illness due to HHV-6 infection remains unsettled. A more in-depth analysis is necessary to explore the precise mechanisms of rash induced by HHV-6 infection. Moreover, the ability to distinguish between the HHV-6–related rash and that seen with acute GVHD is most important for clinicians. This will allow us to decrease the number of recipients who receive unnecessary immunosuppressive treatments.

We found no simple association between HHV-6 viremia and other clinical features that have been suggested to be positively associated with HHV-6 infection. There are 2 reasons to explain the findings. One is the difference in methods for evaluation of active viral infection, and another is the difference in patient ages. Most prior studies analyzed adult patients, but our study...
included children. Adults usually have more posttransplantation complications than children do.

In summary, active HHV-6 infection, determined by virus isolation, occurred frequently 2–4 weeks after transplantation. An accurate determination of active viral infection by virus isolation allowed us to identify the risk factors and clinical features associated with HHV-6 infection. The frequency of subsequent HHV-6 viremia is clearly higher in allogeneic BMT recipients than in autologous BMT recipients. Among allogeneic BMT recipients, pretransplantation antibody titers in recipients who subsequently developed HHV-6 viremia were significantly lower than those in recipients without viremia. Posttransplantation HHV-6 viremia occurred more frequently in recipients with leukemia or lymphoma than in recipients with other diseases. Only rash occurring within 1 month after transplantation was significantly associated with HHV-6 viremia. Discovering the pathophysiology of the rash and delineating the differences between the rash associated with HHV-6 viremia and that associated with acute GVHD are important topics for future investigation.

Acknowledgments

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References

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