RAPID COMMUNICATION

Human Herpesvirus 6 (HHV-6) Isolation From Bone Marrow: HHV-6–Associated Bone Marrow Suppression in Bone Marrow Transplant Patients

By Donald R. Carrigan and Konstance Kehl Knox

These studies tested the hypothesis that human herpesvirus 6 (HHV-6) can cause posttransplant bone marrow (BM) suppression in BM transplant (BMT) patients. Fifteen adult patients who received T-lymphocyte-depleted, allogeneic BM transplants and who developed posttransplant BM suppression were studied. Detailed chart reviews were used to divide the patients into two groups: (1) those with diagnosed BM suppression (DBMS) and (2) those with idiopathic BM suppression (IBMS). BM aspirates obtained from the patients at the onset of BM suppression were subjected to an HHV-6 isolation procedure using mitogen-stimulated blood mononuclear cells. BM specimens obtained from another population of BMT patients solely to document engraftment irrespective of their BM function were also subjected to the HHV-6 isolation procedure as controls. HHV-6 was isolated from 6 of 15 BM samples from BMT patients with BM suppression. BM samples from patients with IBMS were more likely to be positive for HHV-6 than those from patients with DBMS ($P < .01$). Also, HHV-6–positive BM were significantly more likely ($P < .05$) to come from patients with suppression of more than one BM lineage than HHV-6-negative BM. Finally, samples of BM from an unselected series of BMT patients studied without regard to their BM function were less likely ($P < .01$) to be positive for HHV-6 than patients with IBMS.

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BONE MARROW transplantation (BMT) is being increasingly used for the treatment of a variety of diseases, including hematologic malignancies, solid tumors, immunodeficiency disorders, and marrow failure syndromes. Viral infections are a major cause of morbidity and mortality in BMT patients and one of the important ways in which viral infections can manifest is invasion of the grafted BM with a concomitant decrease or delay in hematopoietic reconstitution. Cytomegalovirus (CMV) is the pathogen most often associated with this complication.1

Human herpesvirus 6 (HHV-6) is a β-herpesvirus closely related to CMV.2,3 The primary target cells of HHV-6 appear to be CD4+ T lymphocytes and macrophages,4,5 although a number of other cell types have been reported to be infectable as well.6 Over the past several years, the potential for HHV-6 to serve as a pathogen in BMT patients has begun to be explored. Reactivation of HHV-6 can be frequently detected in the blood of BMT patients after transplantation.7–10 However, few clinical correlations have been made except for concurrence of HHV-6 viremia and graft-versus-host disease (GVHD)10 and rash.11 Other studies of HHV-6 infection of tissues from BMT patients have indicted the virus as causing interstitial pneumonitis11,12 and fatal encephalitis.13

Other work has suggested a role for HHV-6 in the suppression of BM function in the posttransplant period in BMT patients.14 In that study, four of five allogeneic BMT patients with severe, posttransplant idiopathic BM suppression (IBMS) were found to have active HHV-6 infection of their BMs. Further support of the hypothetical role of HHV-6 as a cause for BM suppression was obtained by observing the ability of the virus to suppress the proliferation and differentiation of BM cells in vitro.15 Also, other in vitro studies have shown that exposure of BM precursors to HHV-6 inhibited their abilities to respond to growth factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3).16

The studies described here were designed to test two major predictions of the hypothesis that HHV-6 can cause BM suppression in BMT patients. First, if HHV-6 is the cause of BM suppression, it should be isolatable from samples of BM from BMT patients with IBMS more frequently than from BM samples from BMT patients with diagnosed BM suppression (DBMS). Second, if BM infection by HHV-6 suppresses the function of the BM, then HHV-6 should be isolated more frequently from the BMs of patients with suppressed BM function than from BMs from patients unselected for their BM function. The results obtained confirmed both of these predictions.

PATIENTS AND METHODS

Patients: Two groups of patients were studied. Group I was a series of 15 adult allogeneic BMT patients selected only for the presence of clinically significant BM suppression. Selected characteristics of these patients are described in Table 1. Aspirates of their BMs obtained near the time of onset of BM suppression were subjected to a procedure aimed at the isolation of HHV-6. Criteria used for determining BM suppression are presented below. All of these patients were seropositive for HHV-6 as determined by an enzyme-linked immunosorbent assay.17 After the HHV-6 isolation data were complete, the charts of the patients were carefully reviewed to determine which of the patients had IBMS and which had DBMS. Specific causes for BM suppression that had to be excluded before a diagnosis of IBMS could be made included immunologic rejection of the graft, autoimmune antibody production, concurrent infection with CMV or other agent, drug toxicity, peripheral consumption of erythrocytes or platelets, GVHD, and recurrence of original disease. If any of
these factors were present at the time of BM suppression, a diagnosis of DBMS was given.

Group 2 was a series of 22 consecutive allogeneic BMT recipients from whom BM aspirates were obtained about 1 month after transplant for documentation of engraftment. Information concerning the level of BM function present at these patients at the time the aspirates were obtained was not taken into consideration in any way. All of these patients were seropositive for HHV-6. These BM aspirates were subjected to the same HHV-6 isolation procedure used with the first group of patients. These aspirates were obtained as part of a prospective study of HHV-6 infections in allogeneic and autologous BMT patients (Drobyski et al, manuscript in preparation). Virologic data for these patients are complete. However, detailed review of their records to assess BM function at the time of the HHV-6 isolation attempt is still underway.

All patients in both groups received T-lymphocyte-depleted BM grafts and pretransplant conditioning essentially as described previously.\textsuperscript{11,14} Chronic myelogenous leukemia (CML) was the predominant original disease in both groups (47% in group 1 and 50% in group 2), with acute myelogenous leukemia comprising the second most common disease (14% in group 1 and 20% in group 2). The balance of the patients in both groups had similar proportions of acute lymphocytic leukemia; HD, Hodgkin’s disease; RM, related, HLA-matched donor; RPM, related, HLA partially matched donor; NR, unrelated donor; GNC, ganciclovir.

**Table 1. Summary of BMT Patient Characteristics and HHV-6 Isolations**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)/Sex</th>
<th>Original Disease</th>
<th>Type of BMT</th>
<th>Time From BMT to BM Biopsy (d)</th>
<th>HHV-6 Isolation From BM</th>
<th>Diagnosis of BM Suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34/F</td>
<td>CML</td>
<td>RM</td>
<td>75</td>
<td>–</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>2</td>
<td>31/M</td>
<td>MDS</td>
<td>RPM</td>
<td>64</td>
<td>–</td>
<td>Rejection</td>
</tr>
<tr>
<td>3</td>
<td>44/M</td>
<td>CML</td>
<td>NR</td>
<td>75</td>
<td>–</td>
<td>Macrocyclic anemia</td>
</tr>
<tr>
<td>4</td>
<td>32/M</td>
<td>CML</td>
<td>NR</td>
<td>167</td>
<td>+</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>5</td>
<td>51/M</td>
<td>AML</td>
<td>RM</td>
<td>34</td>
<td>+</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>6</td>
<td>44/F</td>
<td>AML</td>
<td>RPM</td>
<td>279</td>
<td>+</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>7</td>
<td>23/M</td>
<td>NHL</td>
<td>RPM</td>
<td>360</td>
<td>–</td>
<td>Sepsis</td>
</tr>
<tr>
<td>8</td>
<td>39/M</td>
<td>CML</td>
<td>RM</td>
<td>117</td>
<td>+</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>9</td>
<td>21/M</td>
<td>ALL</td>
<td>NR</td>
<td>77</td>
<td>–</td>
<td>CMV</td>
</tr>
<tr>
<td>10</td>
<td>45/M</td>
<td>AML</td>
<td>NR</td>
<td>26</td>
<td>+</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>11</td>
<td>28/M</td>
<td>HD</td>
<td>NR</td>
<td>27</td>
<td>+</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>12</td>
<td>45/M</td>
<td>CML</td>
<td>NR</td>
<td>134</td>
<td>–</td>
<td>Autoimmune, CMV/GNC</td>
</tr>
<tr>
<td>13</td>
<td>21/M</td>
<td>CML</td>
<td>RPM</td>
<td>61</td>
<td>–</td>
<td>Autoimmune</td>
</tr>
<tr>
<td>14</td>
<td>46/M</td>
<td>NHL</td>
<td>NR</td>
<td>18</td>
<td>–</td>
<td>Rejection</td>
</tr>
<tr>
<td>15</td>
<td>31/M</td>
<td>CML</td>
<td>RPM</td>
<td>629</td>
<td>+</td>
<td>Idiopathic</td>
</tr>
</tbody>
</table>

Abbreviations: MDS, myelodysplastic syndrome; AML, acute myelogenous leukemia; NHL, non-Hodgkin’s lymphoma; ALL, acute lymphocytic leukemia; HD, Hodgkin’s disease; RM, related, HLA-matched donor; RPM, related, HLA partially matched donor; NR, unrelated donor; GNC, ganciclovir.

Confirmation of every virus isolate as HHV-6 was performed by indirect immunofluorescent staining with an HHV-6–specific monoclonal antibody (9A5D12)\textsuperscript{9,15} and by the polymerase chain reaction using HHV-6 variant\textsuperscript{21} specific DNA primers or oligonucleotide probes.\textsuperscript{12,20} Uninfected control cells were included in every virus isolation procedure, and HHV-6 was never detected.

**RESULTS**

**Isolation of HHV-6 from the BMs of BMT patients with BM suppression.** HHV-6 was isolated from 6 of the 15 (40%) BM aspirates from patient group 1 (Table 1). The virus isolates from patients no. 5, 6, 8, 10, and 11 were variant B HHV-6, whereas the isolate from patient no. 15 was a mixture of variants A and B. All 6 of the patients who had HHV-6 isolated from their BMs had DBMS. Thus, 6 of 8 of the patients with IBMS were HHV-6–positive, compared with 0 of 7 patients with DBMS (P < .01). Of further interest was the observation that, of the 6 patients whose BMs were HHV-6–positive, 5 showed suppression of more than one BM lineage. In contrast, of the 9 patients whose BMs were HHV-6–negative, only 2 showed suppression of more than one BM lineage (P < .045). Neutropenia was the most common (5 of 6) manifestation of HHV-6–associated BM suppression, followed by thrombocytopenia (4 of 6) and anemia (3 of 6). These results are consistent with the conclusions that HHV-6 infection is associated with a substantial proportion (in these studies, 75%) of cases of DBMS in BMT patients and that the presence of HHV-6 infection in BM correlates with a more severe degree of BM suppression than is present in suppressed but HHV-6–negative BMs.

When the data in Table 1 were further analyzed, no significant associations could be shown between isolation of HHV-6 from a patient’s BM and their original disease, age,
or time after transplant that the BM sample was obtained. However, it was observed that patients with HHV-6 infection of their BMs were significantly (P < .02) more frequently the recipient of BMs from HLA-matched relatives (4 of 6) than those patients whose BMs were negative for HHV-6 infection (0 of 7).

With respect to histopathologic findings in the BM biopsies obtained at the same time as the aspirates used for virus isolation, no distinctive pattern could be discerned in the pathologic changes seen. All 15 of the BM biopsies showed varying degrees of hypoplasia of one or more cell lineages with the severity ranging from mild (patient no. 7) to aplastic (patient no. 15).

**Isolation of HHV-6 from BMs of unselected BMT patients.** When BM aspirates obtained from 22 allogeneic BMT patients exclusively to document engraftment at about 1 month after transplant were assessed for HHV-6 infection, 4 were found to be positive by isolation of the virus in cell culture. This HHV-6 isolation rate was not significantly different (P < .3) from that seen with the total population of BMT patients with BM suppression (6 of 15). However, when those patients with DBMS were excluded, because they represent a group strongly biased against HHV-6 isolation, the 6 of 8 HHV-6 isolation rate of the IBMS patients differed significantly (P < .01) from that seen in the unselected BMT patients. As will be described elsewhere (Drobyski et al, manuscript in preparation), at least 1 of these unselected patients with HHV-6 infection of their BMs had IBMS at the time the BM aspirates were obtained.

**DISCUSSION**

The ultimate goal of these investigations was to test the hypothesis that HHV-6 can cause posttransplant BM suppression in BMT patients. The first prediction of the hypothesis that was tested, ie, that HHV-6 infection should be more common in patients with IBMS than in patients with DBMS, was shown to be true. Further evidence that patients with HHV-6 infections in their BMs comprise a specific subset of BMT patients was the finding that they were significantly more likely to have suppression of more than one BM lineage than were the HHV-6 isolation-negative patients. The ability of HHV-6 to suppress the proliferation and differentiation of multiple lineages of BM cells has been shown in vitro.15

The second prediction of the hypothesis tested was that an unselected population of BMT patients should have a lower HHV-6 isolation rate from their BMs than patients selected on the basis of the presence of IBMS. In other words, selecting for IBMS should simultaneously select for a group strongly biased against HHV-6 isolation from samples of BM. This prediction was found to be true. Patients with IBMS had an HHV-6 isolation rate of 75% (6 of 8), compared with a rate of 18% (4 of 22) for unselected BMT patients (P < .01).

Although these findings to not definitively prove that HHV-6 can cause BM suppression, they, in conjunction with earlier observations, strongly support that idea and appear to define a distinctive clinical entity in allogeneic BMT patients. For convenience, this entity can be termed HHV-6-associated BM suppression (HBMS). It appears that HBMS most often presents as an idiopathic suppression of the BM that can involve either one or more than one BM lineage. Most frequently multiple lineages were involved, a fact that may help to distinguish HBMS from other forms of BM suppression because, at least in these studies, they most often involved only a single lineage. Also, HBMS appears to more frequently involve recipients of BMs from HLA-matched relatives than other forms of BM suppression. This finding probably reflects the increased risk for graft rejection, GVHD, and other complications of patients receiving unrelated or partially HLA-matched BM transplants. Finally, HBMS can apparently occur at any time after BMT because in these studies the time after transplant of the onset of the BM suppression ranged from 27 to 629 days. However, half of the cases of HBMS occurred during the first 5 weeks after BMT, suggesting that BM infections by HHV-6 can occur early in the posttransplant period. Frequent reactivation of HHV-6 within the first few weeks after BMT has been described.8,9

Because the BMT patients with BM suppression studied here were selected for their BM suppression, conclusions cannot be drawn with respect to the frequency of HBMS in BMT patients. Also, in the patients studied, a bias toward the inclusion of patients with IBMS cannot be dismissed because BM samples from patients with obvious causes for their BM dysfunction may not have been submitted for virologic analysis. Thus, the main conclusion to be drawn from these studies is that HBMS appears to be a definable syndrome, and it deserves inclusion in the differential diagnosis of IBMS in BMT patients.

**REFERENCES**

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Human herpesvirus 6 (HHV-6) isolation from bone marrow: HHV-6-associated bone marrow suppression in bone marrow transplant patients [see comments]

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