Bone Marrow Suppression by Human Herpesvirus-6: Comparison of the A and B Variants of the Virus

To the Editor:

The ability of human herpesvirus-6 (HHV-6) to cause disease in patients with immature immune systems and acquired immunodeficiencies is becoming increasingly recognized. A recent report in Blood provided strong evidence for a role of HHV-6 as a cause of severe bone marrow suppression in bone marrow transplant (BMT) patients during the posttransplant period. Interestingly, BM suppression has been observed as a frequent complication of primary HHV-6 infections in infants.

The term HHV-6 actually encompasses two distinct variants of the virus, designated the A (HHV-6A) and B (HHV-6B) variants. These variants differ from one another with respect to their cell tropism, genomic DNA sequences, and protein expression. It is clear that the vast majority of primary HHV-6 infections in infants and reactivated infections in BMT patients are due to HHV-6B. The relative pathogenic potentials of the two variants remain largely unexplored.

In BMT patients, the BM suppression associated with BM infection by HHV-6B appears to most commonly manifest as a long-term, moderately severe suppression of BM function that usually involves multiple lineages of BM cells (D. Carrigan, unpublished observations). Chronic BM suppression associated with persistent BM infection by HHV-6B in an immunologically intact individual has been described. In contrast, the only known instance of HHV-6A infection of the BM of a BMT patient was associated with the abrupt and dramatic appearance of aplastic anemia associated with an essentially empty BM at biopsy in a patient almost 2 years after his BMT.

These results suggest that the A variant of HHV-6 may possess increased pathogenetic virulence compared with the B variant of the virus. We sought to confirm or refute this hypothesis by means of a variety of in vitro assays aimed at determining the effects of the two virus variants on BM function. Use of these assays has been described previously from our laboratory. A methylcellulose-based human BM stem cell proliferation kit (GIBCO BRL, Gaithersburg, MD) was used to quantify colony-formation units (CFU). Stem cells evaluated by this procedure were the precursor to the granulocyte and macrophage lineages (CFU-GM), the erythroid burst-forming unit (BFU-E), and the multipotent precursor of the granulocyte, erythrocyte, monocyte, and megakaryocyte lineages (CFU-GEMM). The stromal cell outgrowth assay was performed as described. Macrophage outgrowth from bone marrow cells exposed to granulocyte-macrophage colony-stimulating factor (GM-CSF) was measured as described previously.

HHV-6SF, also known as the DC strain of HHV-6, was isolated in this laboratory and is considered to be a prototype of HHV-6B. Normal human peripheral blood mononuclear cells were purified from the blood of healthy donors by Ficoll-Hypaque density gradient centrifugation, stimulated with phytohemagglutinin (PHA), and used to prepare stocks of HHV-6SF. HHV-6FS, a prototype of the A variant of HHV-6, was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program and was grown in the HSB-2 line of human T-cell leukemia cells. Medium freshly obtained from infected cultures showing greater than 80% involvement with viral cytopathic effects were clarified by centrifugation and used as viral stocks. Mock viral preparations, ie, supernatant medium from parallel uninfected HSB-2 cell or PHA blast cultures, were used in all experiments to control for cellular products and cytokines. Both virus-containing and mock-infected materials were used at 0.5 mL per 10⁶ BM cells. This viral input represented

<table>
<thead>
<tr>
<th>Virus Variant</th>
<th>BFU-E (%)</th>
<th>CFU-GM (%)</th>
<th>CFU-GEMM (%)</th>
<th>Stromal Outgrowth (%)</th>
<th>GM-CSF Response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHV-6A</td>
<td>97% (2%)</td>
<td>98% (4%)</td>
<td>99% (1%)</td>
<td>94% (2%)</td>
<td>97% (1%)</td>
</tr>
<tr>
<td>HHV-6B</td>
<td>73% (7%)</td>
<td>43% (11%)</td>
<td>71% (9%)</td>
<td>61% (6%)</td>
<td>66% (2%)</td>
</tr>
<tr>
<td></td>
<td>P &lt; .04*</td>
<td>P &lt; .01*</td>
<td>P &lt; .03*</td>
<td>P &lt; .01*</td>
<td>P &lt; .01*</td>
</tr>
</tbody>
</table>

Values are the mean percent suppression observed in four or five independent experiments. The number in parentheses is the standard error.

* Means for HHV-6A and HHV-6B compared by unpaired t test.
† Means for HHV-6A and HHV-6B compared by Mann-Whitney test.
approximate multiplicities of infection of 0.001 TCID_{50} per cell and 1.0 TCID_{50} for HHV-6_{A5} and HHV-6_{BF}, respectively.

Using these techniques, a direct comparison of the innate virulence of a prototype A variant of HHV-6 (HHV-6_{A5}) and a prototype B variant (HHV-6_{BF}) was performed. Results are summarized in Table 1.

HHV-6_{B} was able to suppress BM function from 43% to 86% depending on the assay used. In contrast, HHV-6_{A}–mediated suppression ranged from 94% to 98% in the different assays, showing a significantly higher degree of virulence. This difference is even more striking when the virus inputs are compared. As stated above, in these experiments the input of HHV-6_{A} was 0.001 tissue culture infectious doses 50% (TCID_{50}) per BM cell, whereas the input of HHV-6_{B} was 1.0 TCID_{50} per cell.

These results show that the A variant of HHV-6 has an intrinsically increased virulence with respect to BM suppression compared with the B variant of the virus. Mechanisms involved in these differences between the variants remain unexplored, but the profound biologic effects of HHV-6_{A} associated with very low inputs of infectious virus suggest that indirect mechanisms such as inappropriate cytokine induction may be involved.

Donald R. Carrigan
Konstance Kehl Knox
Medical College of Wisconsin
Milwaukee, WI

REFERENCES


Bone marrow suppression by human herpesvirus-6: comparison of the A and B variants of the virus [letter; comment]

DR Carrigan and KK Knox