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moderately severe suppression of BM function that usually involves multiple lineages of BM cells (D. Carrigan, unpublished observations).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.

HHV-6A, 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-6A. HHV-6B, 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 10^6 BM cells. This viral input represented

<table>
<thead>
<tr>
<th>Virus Variant</th>
<th>Colony-Forming Unit Assay</th>
<th>Stromal Outgrowth</th>
<th>GM-CSF Response</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>BFU-E</td>
<td>CFU-GM</td>
<td>CFU-GEMM</td>
</tr>
<tr>
<td>HHV-6A</td>
<td>97% (2%)</td>
<td>90% (4%)</td>
<td>99% (1%)</td>
</tr>
<tr>
<td>HHV-6B</td>
<td>73% (7%)</td>
<td>43% (11%)</td>
<td>71% (9%)</td>
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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$_{A}$ and HHV-6$_{B}$, respectively.

Using these techniques, a direct comparison of the innate virulence of a prototype A variant of HHV-6 (HHV-6$_{A}$) and a prototype B variant (HHV-6$_{B}$) 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.

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

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