Lack of serologic association of human herpesvirus-8 (KSHV) in patients with monoclonal gammopathy of undetermined significance with and without progression to multiple myeloma

Dharam V. Ablashi, Louise Chatlynne, David Thomas, Dimitra Bourboulia, Matthew B. Rettig, Robert A. Vescio, Dimitri Viza, Parkash Gill, Robert A. Kyle, James R. Berenson, and James E. Whitman Jr

Because human herpesvirus-8 (HHV-8) DNA has been found in multiple myeloma (MM) patients by polymerase chain reaction (PCR), it was suggested that HHV-8 may play a role in the transformation of monoclonal gammopathy of undetermined significance (MGUS) to MM. Therefore, 362 MGUS sera with and without progression to MM were tested for IgG antibody to HHV-8. Only 7.8% of the MGUS sera contained HHV-8 antibody to lytic proteins, and IgG antibody to HHV-8 latent antigen was even lower than IgM antibody (2.9%). No differences were observed in the distribution of antibody to HHV-8 in sera from MGUS patients who progressed to MM. The seroprevalences of HHV-8 in MGUS (7.8%), MM (5.4%), and healthy donors (5.9%) were similar, thus arguing for the lack of epidemiologic evidence of HHV-8 participation in the pathogenesis of MM. MGUS patients were immune competent in response to Epstein-Barr virus (EBV) infection because 97% contained antibody to EBV virus capsid antigen. (Blood. 2000;96:2304-2306) © 2000 by The American Society of Hematology

Study design

MGUS sera were collected and frozen at −20°C by Drs M. B. Rettig, J. R. Berenson, and R. A. Kyle. Samples of MM, Kaposi sarcoma/human immunodeficiency virus-1 (KS/HIV), and classic KS were obtained frozen from Drs M. Kaplan, A. Friedman-Kien, D. Viza, and P. Gill. Donor sera were from a well-characterized panel used at Advanced Biotechnologies Inc (Columbia, MD). All sera were screened for antibodies to lytic proteins by the whole-virus lysate ELISA kit (Advanced Biotechnologies Inc). Positive sera were confirmed by IFA for lytic HHV-8 antigens. D. Bourboulia did the testing for IgG antibody to LANA by the IFA method of Simpson et al in the laboratory of Dr C. Boshoff in London. The EBV-VCA ELISA was done using native gp125 as a source of antigen. All samples were coded before testing.

Results and discussion

Table 1 shows the seroprevalence of antibodies to HHV-8 lytic proteins for MGUS, MM, KS, and healthy controls. Twenty-six of 362 MGUS sera (7.1%), 6 of 110 MM sera (5.4%), 20 of 20 KS sera (100%), and 6 of 102 sera of healthy blood donors (5.9%) were positive by ELISA for IgG antibodies to HHV-8 antigen. For confirmation, the positive samples were restested by IFA, and all tested positive except for 2 of the MGUS sera and 2 of the donor sera (Table 2). These results show that the prevalence of antibody to lytic HHV-8 proteins in MGUS and MM patients is similar to that observed in the healthy donor.
population (Table 1). To ensure that we were not missing patients who had antibody only to latent proteins, we also used the LANA IFA to test randomly picked MGUS sera (95), MM sera (65), KS sera (20), and donor sera (50). For the samples tested, 3 MGUS (3.2%), 3 MM (4.6%), 18 KS (90%), and 2 healthy donors (4.0%) were LANA positive. The prevalence of antibody to the LANA in MGUS and MM is even lower than that of antibody to lytic proteins (Table 1). Analysis of HHV-8 antibody status in MGUS patients with and without progression to MM indicated no change in seroprevalence (Table 3). This argues against any role for HHV-8 in the transformation or pathogenicity of MGUS to MM.

These data are consistent with other antibody studies done largely on MM populations showing a lack of epidemiologic evidence for HHV-8 involvement in MM.11,12,16,17,19,20 Chauhan et al22 reported the analysis of 53 MM sera and found no antibody to latent or lytic proteins of HHV-8; they did, however, find HHV-8 DNA by PCR in the MM tissues. Whitty et al17 also failed to detect HHV-8 antibody in 4 MGUS patients who subsequently developed overt MM. Two other MGUS sera in their study were antibody positive but the patients had no evidence of MM after 36 and 40 months, respectively. The only study reporting the presence of HHV-8 antibodies in MM patients is by Gao et al.21 Using an immunoblot method instead of IFA,16,19 they reported the detection of antibodies to ORF-64 and LNA antigens.21 With the use of IFA, one can observe the difference in staining patterns between nonspecific and specific reactivity, but with the immunoblot method, it is possible to score only positive and negative results. If any nonspecific reactivity survived the blocking step, it would appear on the immunoblot as a positive result. This difference in technique may account for the discrepancy in results with other researchers. Therefore, on the strength of our data and that of the other researchers mentioned earlier, we conclude that it is extremely doubtful that HHV-8 has a role in the progression to MM.

To establish that MGUS patients are competent to make antibody to herpesviruses, we also tested the same 95 MGUS sera for IgG antibody to EBV-VCA using a native EBV (gp125 protein) ELISA. Ninety-two of 95 MGUS sera tested (96.8%) were positive for EBV-VCA antibody, demonstrating that MGUS patients were immunocompetent and responded to EBV infection and other human herpesviruses, as shown previously in MM patients.12,17,19 Both EBV and HHV-8 are classified as human γ-herpesviruses.

The work done by Rettig et al1 was based largely on PCR HHV-8 DNA data, as opposed to the present report, which is a study of serologic antibodies. It is interesting to note that in studies done to compare the frequency of PCR data with that of serology,24,25 the percentage of HIV-1/KS patients positive for HHV-8 DNA in peripheral blood mononuclear cells by PCR is much lower than the percentage found positive for HHV-8 IgG antibodies by IFA for lytic proteins. It is therefore curious that Rettig et al1 could find cells positive for HHV-8 DNA by PCR, but could not detect HHV-8 antibodies in these patients. Berenson and Vescio22 attributed the lack of serologic responses to HHV-8 in MM to interpatient differences as well as to consistent changes in ORF-65 sequencing derived from MM as compared to primary effusion B-cell lymphoma and KS patients. They argued that because ORF-65 is responsible for a major part of the serologic response to HHV-8, deletion of a base pair likely results in a change in the protein product, and this may explain the lack of or low level of serologic response to HHV-8 in MM patients.2 Although such deletions may cause a change in the function of the protein, it is unlikely that they would cause much change in antigenic response.

In light of this mounting molecular evidence based on HHV-8 data and the fact that these same patients lack HHV-8 antibody, MM patients may carry another herpesvirus yet to be identified. This theoretical herpesvirus could share common sequences with HHV-8 but be immunologically distinct from HHV-8.22,26 In much the same way, HHV-8 and EBV share homologous DNA sequences,26 but lack serologic cross-reactivity.22

### Acknowledgments

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**Table 1. Distribution of HHV-8 (KSHV) IgG antibody in sera from MGUS, MM, KS, and healthy donors detected by whole-virus lysate ELISA and by IFA to latent antigens**

<table>
<thead>
<tr>
<th>Serum ID</th>
<th>ELISA positive/no. tested</th>
<th>Antibody to latent antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive/no. tested</td>
<td>No. positive/no. tested</td>
</tr>
<tr>
<td>MGUS</td>
<td>26/362 (7.1%)</td>
<td>3/95 (3.2%)</td>
</tr>
<tr>
<td>MM</td>
<td>6/110 (5.4%)</td>
<td>3/65 (4.6%)</td>
</tr>
<tr>
<td>Classic KS (positive control)</td>
<td>20/20 (100.0%)</td>
<td>18/20 (90.0%)</td>
</tr>
<tr>
<td>Healthy donors (adult)</td>
<td>6/102 (5.9%)</td>
<td>2/50 (4.0%)</td>
</tr>
</tbody>
</table>

ELISA and IFA to lytic antigens were performed according to the assay of Chatlynne et al.,22 using the HHV-8 ELISA and IFA kits from Advanced Biotechnologies Inc. For ELISA, the sera were diluted at 1:100, and for IFA, the sera were diluted at 1:40. IFA to lytic antigens was used to confirm the ELISA-positive sera for antibody to HHV-8. Antibody to latent antigens was tested by IFA using a 1:100 dilution according to the procedure of Simpson et al.23

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**Table 2. MGUS, MM, KS, and healthy donors testing positive by HHV-8 whole-virus lysate ELISA and then tested by IFA to lytic antigens for confirmation**

<table>
<thead>
<tr>
<th>Serum ID</th>
<th>ELISA positive sera tested by lytic IFA no. positive/no. tested (%) correlation between the two tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGUS</td>
<td>24/26 (92.3)</td>
</tr>
<tr>
<td>MM</td>
<td>6/6 (100.0)</td>
</tr>
<tr>
<td>Classic KS (positive control)</td>
<td>20/20 (100.0)</td>
</tr>
<tr>
<td>Healthy donors (adult)</td>
<td>4/6 (67.0)</td>
</tr>
</tbody>
</table>

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**Table 3. Analysis of MGUS patients’ sera for IgG antibody to HHV-8 lytic antigens by ELISA to whole virus**

<table>
<thead>
<tr>
<th>MGUS patients’ sera</th>
<th>No. tested</th>
<th>No. positive</th>
<th>% positive†</th>
<th>No progression to serious disease‡</th>
<th>Progression to serious disease in less than 5 years</th>
<th>Progression to serious disease in more than 5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>268</td>
<td>21</td>
<td>7.8</td>
<td></td>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>2</td>
<td>5.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Sera were tested against the HHV-8 virus by ELISA at Advanced Biotechnologies Inc. This preparation of the ELISA antigen contained 1.8 × 10⁹ viral particles per liter and 1.08 mg/mL of protein.

†Antibody-positive samples were confirmed by IFA to HHV-8 lytic antigens, using the procedure of Chatlynne et al.22

‡Serious diseases such as MM, amyloidosis, or Waldenström macroglobulinemia.
References

7. Ma H, Vescio R, DerDanielian M, Schiller G, Berenson J. The HHV-8 IL-6R homologue and interferon regulatory factor are frequently expressed in myeloma bone marrow biopsies whereas vIL-6 is rarely found [abstract]. Blood. 1998;92(suppl 1):515a.
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