Detection of Serum Antibodies to a Kaposi’s Sarcoma–Associated Herpesvirus-Specific Peptide


Kaposi’s sarcoma (KS)–associated herpesvirus/human herpesvirus type 8 (KSHV/HHV-8) may play an etiologic role in the pathogenesis of KS. In an attempt to assess KSHV/HHV-8 infection, an ELISA was developed using an 18–amino acid peptide from a putative minor capsid protein of KSHV/HHV-8 conjugated to bovine serum albumin. Overall, sera from human immunodeficiency virus type 1 (HIV-1)–positive patients with KS had a higher reactivity in the assay than did sera from HIV-1–positive patients without KS ($P = .018$). Of 35 HIV-1–positive patients with KS, 60% were antibody positive, compared with 27% of 33 HIV-1–positive patients without KS. Of 30 healthy blood donors, 20% were antibody positive. The ELISA responses did not correlate with antibody titers to Epstein-Barr virus. Given the homology and antigenic relatedness between KSHV/HHV-8 and Epstein-Barr virus, serologic assays involving unique KSHV/HHV-8 peptides may prove to be valuable in defining the epidemiology and clinical expression of this virus.

The wide variation in the incidence of Kaposi’s sarcoma (KS) among particular human immunodeficiency virus type 1 (HIV-1)–infected risk groups and other epidemiologic evidence has suggested KS might be caused by an infectious agent [1, 2]. The recent discovery of KS-associated herpesvirus/human herpesvirus type 8 (KSHV/HHV-8) and the detection of its DNA in nearly all KS lesions from people with AIDS and people in other patient groups [3] have provided evidence that this virus plays an important role in the pathogenesis of KS. Further evidence of its etiologic relationship to KS was provided by the observation that detection of KSHV/HHV-8 DNA in peripheral blood mononuclear cells (PBMC) of HIV-1–positive patients by polymerase chain reaction (PCR) is predictive of the subsequent development of KS [4, 5]. However, KSHV/HHV-8 DNA has also been reported to be prevalent in semen of healthy men [6, 7], and KSHV/HHV-8 sequences have been associated with diseases other than KS [8–10], indicating that the KS may arise in only a subset of infected people.

A complete description of the epidemiology of KSHV/HHV-8 and its possible role in the pathogenesis of KS and other diseases cannot be derived from surveys of clinical materials for viral sequences by PCR. PCR-based assays require that the specific tissue being examined contain viral genomes and, therefore, can fail to detect virus when viremia is intermittent or there are low genome copy numbers [5]. Moreover, as pointed out by others, the ease of sample contamination provides another potential drawback of PCR [11–13].

A specific and sensitive serologic assay for antibodies to KSHV/HHV-8 would reveal the demographics of infection with this virus without regard to disease manifestations or sites of infection. Development of a serologic assay has been hindered by the significant sequence homology between KSHV/HHV-8 and Epstein-Barr virus (EBV). Expression of KSHV/HHV-8 antigens in HBL-6 cells along with the removal of EBV–cross-reactive antibodies allowed the development of an IFA [14]. More recently, an EBV-negative KSHV/HHV-8–infected BCP-1 cell line was used by Gao et al. [15] to detect KSHV/HHV-8–related antibodies, and the presence of antibodies was found to correlate well with the presence of KS. Also, an IFA using isolated nuclei from a KSHV/HHV-8–infected BCBL-1 cell line was developed and provided evidence of KSHV/HHV-8 as a sexually transmissible agent [13]. While not ideal, these assays have yielded initial estimates of the seroprevalence of KSHV/HHV-8 infection.

In an attempt to mitigate the potential problems of antigen abundance and cross-reactivity, we developed an ELISA to detect KSHV/HHV-8–specific antibodies. It is based on an 18–amino acid peptide sequence (referred to here as P1) of a KSHV/HHV-8 minor capsid protein (an antigen expected to be exposed during lytic infection) [3, 14] that has no substantive homology to human herpesviruses or other known proteins.

Materials and Methods

Patient samples. Serum samples from 118 persons were analyzed in this study (table 1). Thirty-four were obtained from adult
patients enrolled in research protocols at the National Cancer Institute (NCI) from April 1985 to December 1996. An additional 64 samples were obtained from hemophiliacs, blood donors, and homosexual men as part of research protocols at the Viral Epidemiology Branch, NCI. Twelve samples with known titers for EBV, including ones from patients with documented EBV-associated lymphoproliferative diseases, were collected from patients enrolled in research protocols at the Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases. Serum samples from 8 children (ages 6 months to 5 years) were provided by N. Luban (Laboratory of Medicine, Children’s National Medical Center, Washington, DC).

Of 68 HIV-1–positive patients for whom sera were analyzed, 33 were KS negative, and 35 either had KS at the time of sampling (29 patients) or acquired documented KS within 1 year after the sample date (6 patients). Samples were stored at −70°C prior to testing. Data on the detection of KSHV/HHV-8 DNA in PBMC glutaraldehyde in PBS for 2 h followed by dialysis against PBS.

Table 1. Characteristics of 118 persons whose sera were analyzed in a study to detect serum antibodies to a KS-associated herpesvirus-specific peptide.

<table>
<thead>
<tr>
<th>Group, n</th>
<th>KS</th>
<th>HIV-1</th>
<th>Risk group</th>
<th>Median age (range), years</th>
<th>Race or ethnic group (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 8</td>
<td>−</td>
<td>−</td>
<td>Children &lt;5 years</td>
<td>4/4</td>
<td>Unknown</td>
</tr>
<tr>
<td>B, 30</td>
<td>−</td>
<td>−</td>
<td>Blood donors</td>
<td>22/8</td>
<td>W (21), A (8), H (1)</td>
</tr>
<tr>
<td>C, 35</td>
<td>+*</td>
<td>+</td>
<td>Homosexual males</td>
<td>35/0</td>
<td>W (29), A (4), H (2)</td>
</tr>
<tr>
<td>D, 9</td>
<td>−</td>
<td>+</td>
<td>Homosexual males</td>
<td>9/0</td>
<td>W (8), A (1)</td>
</tr>
<tr>
<td>E, 24</td>
<td>−</td>
<td>+</td>
<td>Hemophiliacs</td>
<td>23/1</td>
<td>W (16), A (8)</td>
</tr>
<tr>
<td>F, 12</td>
<td>−</td>
<td>−</td>
<td>Known EBV titers</td>
<td>7/5</td>
<td>W (10), A (1), H (1)</td>
</tr>
</tbody>
</table>

NOTE. HIV-1 = human immunodeficiency virus type 1, EBV = Epstein-Barr virus, W = white, A = African-American, H = Hispanic.

* Includes 6 patients who developed KS within 12 months following sample collection date.

was treated with N-succinimidyl S-acetylthioacetate (Pierce) to introduce sulfhydryl groups via a reaction through the primary amines of lysine. The activated peptide was combined with the modified BSA and incubated at 4°C overnight, followed by desalting on a PD-10 column (Pharmacia, Piscataway, NJ). The peptide conjugate (1 mg/mL in PBS containing 1 mM EDTA) was stored at −20°C in multiple aliquots until further use. Sequence analysis software (MacVector; Kodak, New Haven, CT) was used to determine the antigenic index for regions of the predicted KSHV protein [17]. This method combines the information on hydrophilicity, surface probability, and backbone flexibility in addition to secondary structure predictions to obtain a composite prediction for antigenicity.

**Rabbit antibody production.** For production of a rabbit polyclonal antibody to the peptide, the peptide was conjugated to keyhole limpet hemocyanin (Sigma, St. Louis) by reaction with 0.1% glutaraldehyde in PBS for 2 h followed by dialysis against PBS. After 10 mL of blood was drawn from 2 rabbits, the animals were injected subcutaneously with 50 μL of 0.5 mg/mL keyhole limpet hemocyanin conjugate emulsified with Freund’s adjuvant. The rabbits were boosted with three additional injections on days 14, 28, and 70. To affinity-purify antibodies directed to the 18–amino acid peptide, rabbit serum was applied to an NHS high trap column (Pharmacia) that contained 2 mg of the bound, unconjugated peptide. Unbound material was washed off the column, and the bound antibodies were then eluted with 100 mM glycine, pH 2.3, containing 0.5 M NaCl. The antibody preparation was neutralized with 1 M TRIS to a final concentration of 100 mM and stored at −20°C until further use.

The affinity-purified antibody was tested for its ability to detect the P1-BSA conjugate by Western blot analysis. Western blot was also done on the supernatants from cultures of KSHV/HHV-8–infected BCBL-1 cells, an EBV-negative line [18]. For TPA induction, 100 BCBL-1 cells were incubated with 20 ng/mL TPA for 48 h. The cells were then washed with fresh media and incubated for 2 more days. The supernatants were then collected, and virus was pelleted by centrifugation at 26,000 g for 2 h, washed once with PBS, and pelleted again. To verify the presence of KSHV/HHV-8 in the cells and their pelleted supernatants, PCR analysis was done on DNA extracts as described previously [16]. For Western blot analysis of the proteins, the pelleted material was solubilized in SDS sample buffer, boiled at 95°C for 5 min, applied to
a 14% polyacrylamide gel, and subjected to electrophoresis (Novex, San Diego) at 100 V for 2 h. The proteins were then transferred to nitrocellulose (Novex) for 2 h at 35 V. The blots were probed with affinity-purified rabbit antibody. Protein bands were detected with a goat anti-rabbit antibody conjugated to alkaline phosphatase.

**ELISA for KSHV/HHV-8 peptide.** An indirect ELISA, similar to a technique previously used to detect antibodies to influenza virus [19], was developed in order to detect serum antibodies specific for the peptide-BSA conjugate. Half of the wells in 96-well polystyrene plates (Easy Wash; Corning, Corning, NY) were coated with 0.05 μM peptide-BSA conjugate in PBS (BioWhittaker, Walkersville, MD). Control wells were coated with 0.05 μM pyrogen-poor BSA (Pierce) in PBS. Following an overnight incubation at 4°C, the plates were washed three times with 0.05% Tween 20 (Sigma, St. Louis) in PBS (PBST) using a microplate washer (Nunc, Roskilde, Denmark). Plates were read continuously at 405 nm using a microplate reader (Vmax; Molecular Devices, Menlo Park, CA) and were stopped with 1% SDS (Oncor, Gaithersburg, MD). Plates were again washed three times with PBST, and they were incubated for 1.5 h at 37°C. Serum samples, preincubated at a dilution of 1:12.5 with PBST for 1 h at room temperature, were serially diluted 1:1 into both peptide-BSA conjugate and BSA control wells (final dilutions between 1:25 and 1:12,800). As an internal control for each plate, duplicate wells were incubated with serum diluted 1:100 from a KS-positive, HIV-1-positive patient known to have KSHV/HHV-8 DNA sequences not (figure 1). No favorable antigenic element of the other virus was present (aa 142–179 and 186–203) differed substantially from the to a technique previously used to detect antibodies to influenza virus [19]. To improve the reactivity of peptide P1, it was coupled to BSA through the peptide’s natural amino acid sequence. Subsequently, we hypothesized that the low antibody reactivity to P1 alone was due to the conformation of the antigenic determinant, while the corresponding region for EBV is 40% amino acid identity and 73% similarity [3]. However, two regions of the ORF26 protein (aa 142–179 and 186–203) differed substantially from the EBV BDLF1 homolog and, therefore, were considered possible candidates for the development of an ELISA to detect KSHV/HHV-8 antibodies. The sequence between aa 186–203 of the KSHV/HHV-8 protein was chosen, in part, because it has no substantial cross-homology to other human herpesvirus proteins or other known proteins. Specifically, only 3 of the 18 amino acids were positionally conserved, and there was only 17% identity and a 44% overall similarity to the EBV homolog for this region encoded in the BDLF1 gene (figure 1), and sequence homology to the cytomegalovirus protein was even lower (5% identity and 22% similarity, not shown). Furthermore, sequence analysis (MacVector software) of the KSHV/HHV-8 protein [17] predicted that the region between aa 194–198 is likely to be a surface-exposed antigen, while the corresponding region for EBV is not (figure 1). No favorable antigenic element of the other peptide (142–179) was noted.

Initial experiments with the 186–203 P1 peptide alone, using dot blot analysis and ELISA, revealed weak reactivities with sera from KS patients whose PBMC were known to be PCR positive for KSHV/HHV-8 DNA [16]. To improve the reactivity of peptide P1, it was coupled to BSA through the peptide’s only free amino group, located at the amino terminus, as described in Materials and Methods. This conjugate is referred here as the P1-BSA conjugate. The signal obtained with the P1-BSA conjugate in both dot blot analysis and ELISA with sera from KSHV/HHV-8–positive patients was increased up to 10-fold over that for the peptide alone (data not shown). We hypothesized that the low antibody reactivity to P1 alone occurred because the conformation of the antigenic region of the peptide was altered upon binding or that this region was not accessible to the antibody. We further hypothesized that if P1 were part of a larger protein, more of the peptide would remain accessible even after binding.

The P1-BSA conjugate (average M., ~80 kDa by SDS-PAGE) was also recognized on Western blots, using an affinity-purified antibody from the rabbits immunized with P1 conjugated to keyhole limpet hemocyanin (figure 2A). Detection of this conjugate was substantially reduced by preincubation of the antibody with purified unconjugated peptide (figure 2B). Of importance, the affinity-purified rabbit antibody detected a 34-kDa (M.) protein on Western blots of pelleted supernatant of the TPA-activated BCBL-1 cell line but not pelleted supernatants from nonactivated cells, and this band was eliminated by preincubation of the purified rabbit antibody with purified unconjugated peptide (figure 2B). These results provide evidence that affinity-purified antibodies to P1 can detect.

**Results**

At the outset of this study, we sought to identify a unique region among the then known KSHV/HHV-8 open-reading frame (ORF) sequences that had a high probability of being recognized by antibodies with little cross-reactivity to other proteins. ORF26 of KSHV/HHV-8 encodes a 305–amino acid putative minor capsid protein that has significant overall homology to the 301–amino acid virion protein encoded by the BDLF1 ORF of EBV (49% amino acid identity and 73% similarity) [3]. However, two regions of the ORF26 protein (aa 142–179 and 186–203) differed substantially from the EBV BDLF1 homolog and, therefore, were considered possible candidates for the development of an ELISA to detect KSHV/HHV-8 antibodies. The sequence between aa 186–203 of the KSHV/HHV-8 protein was chosen, in part, because it has no substantial cross-homology to other human herpesvirus proteins or other known proteins. Specifically, only 3 of the 18 amino acids were positionally conserved, and there was only 17% identity and a 44% overall similarity to the EBV homolog for this region encoded in the BDLF1 gene (figure 1), and sequence homology to the cytomegalovirus protein was even lower (5% identity and 22% similarity, not shown). Furthermore, sequence analysis (MacVector software) of the KSHV/HHV-8 protein [17] predicted that the region between aa 194–198 is likely to be a surface-exposed antigen, while the corresponding region for EBV is not (figure 1). No favorable antigenic element of the other peptide (142–179) was noted.

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the native 34-kDa viral protein (predicted molecular mass, 34 kDa) from KSHV/HHV-8.

An ELISA was developed to test patients’ sera for the presence of KSHV/HHV-8 antibodies reactive with the P1-BSA conjugate. The ELISA was done using sera from 118 persons with or without KS (table 1). A dose-response curve was obtained for all patients by serial dilution of sera from 1:25 to 1:12,800. Representative dose-response curves for patients with high, medium, and low responses are shown in figure 3. These curves are all roughly parallel, and we elected to use the OD at a 1:50 dilution for further analyses. The overall results using data from the 1:25 or the 1:100 dilutions were essentially the same.

As shown in figure 4, sera from 8 children (group A; ages between 6 months and 5 years) reacted minimally with the P1-BSA conjugate (median OD, 0.193), consistent with the hypothesis that these children may not have been infected with KSHV/HHV-8 and did not possess substantial persisting maternal antibodies to the virus. By contrast, many of the serum samples from the HIV-1–positive, KS-positive patients (group C) had substantial reactivity in the ELISA (median OD, 0.525), and overall, their values were significantly higher than those for the children (median, 0.193; P = .03). Sera from some HIV-1–positive patients without KS, as a whole, was significantly less than that of the KS patients (median OD, 0.207; P = .018). A similar result was obtained if the KS patients’ seroreactivity was compared with that of the HIV-1–positive hemophiliacs without KS (median OD, 0.155; P = .004). Differences in reactivity dropped just below the level of significance (P = .051), however, if we compared the HIV-1–negative blood donors (group B) with the HIV-1–positive homosexual patients with KS.

Assuming that the ELISA results from children’s sera are indicative of negative responses to P1-BSA (group A, figure 4), we established a positive antibody response as being a value greater than the mean + 2 SD (OD of 0.36 at the 1:50 serum dilution). Using this cutoff, we found that 21 (60%) of the 35 HIV-1–positive patients with KS were positive (group C, figure 4), compared with 9 (27%) of 33 HIV-1–positive patients without KS (groups D and E, figure 4).

For this analysis, a patient was considered KS positive if KS was acquired before or within 1 year after the sample date, as indicated in Materials and Methods. Of interest, of the 6 KS-positive patients who did not have KS at the time the serum was collected but who were diagnosed with KS 12 months after the sample date, 3 tested positive for antibodies to P1-BSA (figure 4, group C, ○). Furthermore, 1 patient in the HIV-1–positive, KS-negative group, who reacted positively for P1-BSA, developed KS 13 months after the sample date.
To estimate the sensitivity of this ELISA for KSHV/HHV-8 infection, we examined the serologic findings for a subset of HIV-1–positive patients with KS whose PBMC were previously positive for KSHV/HHV-8 DNA by PCR. Of 14 such PCR-positive patients, 9 (64%) were also antibody positive by ELISA. By contrast, none of the 5 HIV-1–positive patients without KS who tested negative by PCR were ELISA positive for the KSHV/HHV-8 P1-BSA.

We looked for a relationship between the response in the ELISA for the KSHV/HHV-8 P1-BSA and the CD4 cell count among HIV-1–positive patients (with and without KS) for whom we had CD4 cell counts. However, no significant correlation was found ($r_s = 0.004; P = 0.98, data not shown). Further studies were directed at assessing the potential cross-reactivity of P1-BSA to anti-EBV antibodies present in patients’ sera. EBV is nearly ubiquitous in adults in the United States [20], and because of its high sequence homology with portions of KSHV/HHV-8, including the 34-kDa minor capsid protein containing P1, we considered it important to verify that the reactivity toward the P1 conjugate was not simply a reflection of antibodies to EBV in the patients’ sera. To do this, we measured antibodies to the P1-BSA conjugate in 12 patients whose sera spanned a wide range of EBV titers. We also determined the EBV titers of 6 patients identified as having a range of antibody response to P1-BSA. There was no significant correlation ($r_s = -0.345; P = 0.158$) between the response to the P1-BSA conjugate and the titer for the IgG antibodies to the EBV viral capsid antigen (figure 5). In fact, the correlation coefficient had a negative trend ($-0.345$), suggesting a tendency for patients with higher EBV antibody titers to have lower ELISA responses to P1 (figure 5). Moreover, 2 of the patients with the highest EBV titers had a very weak response to the P1 conjugate, while 2 patients seronegative for EBV...
Figure 4. ELISA response for various cohorts obtained at 1:50 serum dilution. Sera were serially diluted from 1:25 to 1:12,800 and incubated overnight in ELISA wells as described in Materials and Methods. ODs ≥2.0 are plotted as >2.0. Solid horizontal lines represent median values obtained for each group. Dashed line represents cutoff obtained from children’s sera for determination of positive response in ELISA. ○ in group D represent patients who acquired KS within 1–12 months following date of sample used in ELISA; ○ in group E represents patient who acquired KS 13 months after sample. HIV = human immunodeficiency virus type 1.

(titers for viral capsid antigen <1:10) had a moderate or high response to P1 (figure 5). Similarly, we found no correlation between the response to P1 conjugate and the titers of antibodies to other EBV antigens, including early antigens and EBV nuclear antigens (data not shown). Taken together, these results strongly suggest that antibodies to EBV proteins do not cross-react to P1-BSA and are not responsible for the observed ELISA responses.

Discussion

Until very recently, PCR analysis of DNA from patients has been the primary method of assessing infection with KSHV/HHV-8 [3–5, 7, 16, 21–23]. However, the conclusions drawn from these studies vary widely, and the prevalence of KSHV/HHV-8 infection remains uncertain. PCR can, because of low levels of viral sequences in the circulation or assayed tissue [11, 12], underestimate the proportion of those infected with KSHV/HHV-8, and PCR studies may not identify patients who harbor the virus only transiently or in selected anatomic sites. Furthermore, sample contamination is a recognized problem with PCR and results in an overestimation of the number of infected people. To better characterize the rate of KSHV/HHV-8 infection, indirect IFAs and immunoblot assays were recently developed to detect viral antibodies, using antigens from KSHV/HHV-8–infected cell lines [13–15, 24]. The IFAs, which are believed to detect latent KSHV/HHV-8 nuclear antigens, have yielded 80%–83% seropositivity for patients with KS, but <2% of normal blood donors were seropositive [13, 25]. Although these assays have yielded initial estimates of the seroprevalence of KSHV/HHV-8 infection, the antigens in these tests are not well defined, and the interpretation of the results are subjective. More recently, serologic tests utilizing recombinant capsid-related proteins from KSHV/HHV-8 were reported [26, 27].

In this study, we developed an ELISA to detect antibodies to a predicted peptide sequence of a putative minor capsid antigen of KSHV/HHV-8. If KSHV/HHV-8 is sexually transmitted, which has been supported [13], we hypothesized that young children were likely to be seronegative for this virus because they generally are for most other herpesviruses. Therefore, we assayed a cutoff value for a positive ELISA result as one that is significantly higher (2 SDs) than the mean results for the children. This assumption could lead to an underestimate of the true seroprevalence of KSHV/HHV-8, however, if some young children are infected by the virus or retain some maternal antibodies to it.

We found that KS patients had a higher level of antibodies than did subjects in other cohorts. Using the cutoff described, we detected antibodies in 60% of the HIV-1–positive patients with KS and 27% of the HIV-1–positive patients without KS. The results within the HIV-1–positive groups compare closely with those of Miller et al. [24], who reported that antibodies to KSHV/HHV-8 lytic antigens, as assayed by an immunoblot assay, were detected by Western blot in 67% of KS patients and in 13% of HIV-1–positive patients without KS.

Previous studies using PCR technology suggested that KSHV/HHV-8 DNA could be detected in circulating PBMC from 35% to 52% of KS patients [5, 16]. However, KSHV/
HHV-8 DNA could be detected in ~95% of KS lesions from patients with both HIV-1– and non–HIV-1–associated KS [4, 8]. In KS patients previously found to harbor KSHV/HHV-8 DNA in their PBMC, the detection rate of antibodies to the P1-BSA conjugate in the present study was 64%. Overall, these results would seem to indicate that the present assay, like that of Miller et al. [24], does not detect antibodies in all HIV-1–positive patients who may also be infected with KSHV/HHV-8. Why might this be? In regard to our assay, one possibility is that it is based on a restricted antigenic epitope to which patients with particular HLA haplotypes may not mount strong antibody responses. Another possibility is that HIV-1–positive patients have a limited ability to produce antibodies to new antigens [28], and this may have decreased the proportion of seropositive patients because of some who might have been first infected with KSHV only after being infected with HIV-1.

A third factor that could limit the sensitivity of our ELISA is that the peptide utilized is part of a KSHV/HHV-8 protein, which like its EBV homolog, is expressed only during lytic infection. The 34-kDa protein was detected by the purified rabbit antibody to P1 in only the TPA-stimulated cellular supernatant, which is consistent with it being a lytic protein (figure 2). If KSHV/HHV-8 lytic replication ends with resolution of the primary infection, then antibodies to P1 may eventually decline to undetectable levels. Of interest, 5 of 6 HIV-1–positive homosexual patients whose KS was first reported within 1 year after the sample date (and 1 additional homosexual patient diagnosed with KS 13 months after the sample date) had antibodies to P1-BSA, suggesting that a burst of lytic infection might closely precede the development of KS. Thus, the ELISA response of sera from HIV-1–positive homosexuals to P1-BSA may be predictive of the subsequent development of KS. Prospective studies will be needed to better understand the factors affecting the production of antibodies to P1-BSA during the development of KS. Nevertheless, it is possible that the ELISA may be more sensitive for detecting acute or reactivated infections with KSHV/HHV-8 than for recognizing long-standing latent infections.

A more provocative finding in the present study is that 20% of normal adult blood donors and 27% of hemophiliacs with HIV-1 infection had detectable antibodies to P1-BSA. Multiple groups have attempted to define the prevalence of KSHV/HHV-8 infection in the general population. Using PCR-based analysis of DNA from PBMC or semen samples and serologic IFAs, some have suggested that KSHV/HHV-8 infection is generally confined to patients at risk for KS. By contrast, other groups have reported a more widespread prevalence of infection, including the detection of KSHV/HHV-8 sequences in the skin and other tissues in conditions other than KS. Also, KSHV/HHV-8 sequences have been detected by PCR in 23% of semen samples from healthy donors from the United States and in 91% of semen samples from healthy donors from Italy (where KS is a more frequent malignancy in HIV-1–negative people than it is in the US). Overall, these studies have yielded somewhat conflicting results, and many questions remain concerning the prevalence of KSHV/HHV-8 infection, particularly in the general population.

If our ELISA is, in fact, specific for antibodies to KSHV/HHV-8, then it would predict that 20%–30% of the general US adult population is infected with KSHV/HHV-8. This is higher than the seropositivity rates recently reported using IFAs utilizing latent antigens or by using a recently developed recombinant capsid protein assay. However, it is in close agreement with a recent report by Lennette et al. indicating that 25% of adults (and 2%–8% of children) had antibodies to lytic HHV-8 antigens as detected by IFA. These results are also in accord with rates of seropositivity in the United States to herpes simplex virus 2, the prototypical sexually transmitted herpesvirus, but are substantially lower than the seroprevalence of cytomegalovirus, another lymphotropic herpesvirus. It is possible that the present ELISA overestimates KSHV/HHV-8 seroprevalence by virtue of cross-reactions to an as yet undetermined antigen. Cross-reactivity would have to be presumed if recent reports of KSHV/HHV-8–related antibodies in ~1% of North American blood donors are eventually proven correct. A number of the IFAs are believed to detect antibodies to latent, rather than lytic, antigens of KSHV/HHV-8, and, therefore, the differences seen between the results of our studies and those of others may reflect the abundance.
of different antibodies in the populations tested. In this regard, Lennette et al. [33], using an IFA, found that while about 25% of adult blood donors had antibodies to KSHV lytic antigens, none had antibodies to latent antigens.

In the present study, we utilized a synthetic peptide representing an apparently unique sequence in the predicted minor capsid protein of KSHV/HHV-8. Similar peptides have proven to be valuable in a number of other serologic assays [35–37] and, pertinent to the present study, have been utilized to reliably detect EBV-specific antibodies [38–41]. Our use of a selective peptide with little apparent cross-homology to EBV may potentially have avoided cross-reactivity that may otherwise occur with other larger, viral expressed or recombinant proteins (given the degree of overall sequence homology to EBV [14]). Future studies will determine to what degree the antibody reactivity to P1-BSA found in patients at low risk for KS represents cross-reactivity with other antibodies in serum.

When this study was undertaken, limited sequence information on KSHV/HHV-8 was available for use in the selection of specific peptides. We identified only one peptide (P1) that reacted with sera from 60% of KS patients. Now that the entire KSHV/HHV-8 genome has been sequenced [42], other KSHV/HHV-8–specific peptides could be used separately or in conjunction with the P1-BSA conjugate to develop highly specific and even more sensitive assays to detect KSHV/HHV-8 antibodies. Use of KSHV/HHV-8 antigens that are expressed during latency would be of particular interest.

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References


