Human Herpesvirus 8 Infection Occurs following Adolescence in the United States

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Most recent evidence suggests that human herpesvirus 8 (HHV-8) infection is restricted to persons with Kaposi’s sarcoma (KS) or to persons who may subsequently develop KS. To accurately determine the prevalence of infection in the United States, children and adults with AIDS were examined for evidence of HHV-8 infection to see whether HHV-8 (like other herpesviruses) would be readily detected in immunosuppressed persons. By use of nested polymerase chain reaction, DNA specific for HHV-8, Epstein-Barr virus, and cytomegalovirus was detected in blood leukocytes from 0, 26 (51%), and 9 (18%), respectively, of 51 children. Similarly, HHV-8–specific antibodies were not detected in analyses of sera from the children. By contrast, HHV-8 DNA was detected in 9 (27%) of 33 adult AIDS patients without KS. These findings suggest that the pattern of transmission of HHV-8 in the United States differs from that of other herpesviruses in that primary infection occurs predominantly in adults.

Human herpesvirus 8 (HHV-8), also known as Kaposi’s sarcoma (KS)–associated herpesvirus, is a newly described γ-herpesvirus with sequence homology to Epstein-Barr virus (EBV) [1]. Through both DNA and serologic assays, HHV-8 is associated with the diseases KS [1–6], body cavity–based lymphoma (BCBL) [7], and Castleman’s disease [8]. However, the prevalence of HHV-8 infection in the general population and, specifically, the mode and age of primary infection remain controversial. DNA analyses of semen from human immunodeficiency virus (HIV)–negative heterosexual men from the United States and Italy suggest that HHV-8 infection is widely prevalent (23% and 91%, respectively) in general populations [9, 10]. Serologic assays in healthy adult blood donors, however, have yielded conflicting results, with HHV-8–specific antibodies detected in <5% [3–5] and up to 25% [6] of persons tested. In immunocompetent children, seroprevalence has been reported to be 0 [5] and <4% [6]. Because herpesvirus infections are often reactivated with immunosuppression [11, 12], we hypothesized that HHV-8 might be more readily detected in an immunosuppressed population of children. Thus, we examined US children with AIDS for HHV-8 infection. For comparisons, we also tested the same children for presence of EBV- and cytomegalovirus (CMV)-specific DNA in peripheral blood mononuclear cells (PBMC). EBV and CMV are herpesviruses known to be widely prevalent and transmitted early in life. In addition, US adults with AIDS and without the diagnosis of KS (i.e., similar to the children in the degree of immunosuppression) were examined for evidence of HHV-8 DNA in PBMC. By comparing the prevalence of HHV-8 infection in immunosuppressed US children and adults, the aim of these analyses was to determine an accurate prevalence of HHV-8 infection in the general US population.

Materials and Methods

Patients. Fifty-one HIV-infected children with AIDS by Centers for Disease Control and Prevention criteria [13], who were enrolled in clinical trials in the HIV and AIDS Malignancy Branch (formerly part of the Pediatrics Branch), National Cancer Institute, were studied. Thirty-three adults with AIDS [14] and without KS, who were enrolled in clinical trials at the Laboratory of Immunoregulation, National Institute for Allergy and Infectious Diseases, were also studied. Demographic and clinical information for all of the patients was collected through chart review.

Detection of virus-specific DNA by polymerase chain reaction (PCR). PBMC were isolated by density gradient centrifugation of heparinized blood. Total DNA was isolated after overnight digestion at 55°C in 4 mL of solution containing 10 mM TRIS-HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 1% SDS, and 400 µg/mL proteinase K, followed by phenol-chloroform extraction and ethanol precipitation. The concentration of DNA was determined by optical density readings at a wavelength of 260 nm by use of a spectrophotometer. For positive viral DNA controls, DNA was...
isolated from the HHV-8+ EBV+ BCBL cell line BC-1 (gift of Ethel Cesaran, New York Hospital-Cornell Medical Center, New York) [15], the HHV-8+ EBV+ BCBL cell line BCBL-1 (NIH AIDS Reference and Research Reagent Program, Rockville, MD), or EBV-specific and CMV-specific DNA (purchased from Advanced Biotechnologies, Columbia, MD).

Oligonucleotide primers (Genosys Biotechnologies, The Woodlands, TX) used in PCR assays were as follows (5’–3’): HHV-8 ORF26 (outer sense), AGCTACAGTCGATCTACCCCA; HHV-8 ORF26 (outer antisense), ATCGTCAAGCCTGAGG; HHV-8 ORF26 (inner sense), GAAAGGATTCCCATTGTTG; HHV-8 ORF26 (inner antisense), ATCCGTTGTTCTACGGTCCA; EBV EBNA-1 (outer sense), CCAACAGCAGCCCG; EBV EBNA-1 (outer antisense), CAAAGGGGAGAGCTCAAT; EBV EBNA-1 (inner sense), TGGAGGAGGGCCAAAGAAG; EBV EBNA-1 (inner antisense), GACATTGTGAATAGCAAG; CMV IE1 (outer sense), TTTCGAAGTCCTCACCCCCAT; CMV IE1 (outer antisense), GTACCTAGTCGACTCTTG; CMV IE1 (inner sense), GGGAGTTGTATTG; CMV IE1 (inner antisense), CGCGTTGGAAGGCAATT; CMV IE1 (outer antisense), GTACTTACGTCACTGTG; CMV IE1 (outer sense), TTTCCAAGTCTCAGAT; CMV IE1 (inner antisense), AAGCTGTAAGAATT; CMV capsid protein encoded by ORF65) were tested by an ELISA on BCBL, the type of lymphoma previously linked with HHV-8.

Detection of HHV-8–specific antibodies directed against latent and lytic antigens. For the 51 children, clotted blood samples were centrifuged, and sera were collected and stored at −20°C until assayed for HHV-8–specific antibodies. Antibodies directed against latently expressed HHV-8 antigens were assessed by an immunofluorescence assay, whereas antibodies directed against a lytically expressed antigen (i.e., a truncated recombinant minor capsid protein encoded by ORF65) were tested by an ELISA on two separate occasions, both as described in detail [5]. Briefly, for the immunofluorescence assay, sera at a 1:200 dilution (previously determined to represent the best dilution for avoiding nonspecific staining and for detecting specific staining) were incubated with BCP-1 cells, which are BCBL-derived cells latently infected with HHV-8, subsequently incubated with fluorescein isothiocyanate– conjugated rabbit anti-human IgG (Dako, Carpinteria, CA) after a series of washes, and examined by an immunofluorescence microscope. For the ELISA, sera were tested at 1:100 dilution, and the cutoff value to define reactive sera was selected as SSD above a mean value derived from previously tested nonreactive sera. Sera from KS patients and from healthy blood donors (previously determined to be HHV-8 antibody–positive and antibody–negative, respectively, by both assays) were used as controls when testing the children’s sera for this study. If results from these experiments were indeterminate, Western blots were performed for further clarification, also as described in detail [5]. The serologic assays as described here are capable of detecting HHV-8–specific antibodies in 80%–85% of KS patients [5].

Results

Demographic and clinical information for the children and adults is outlined in table 1. The median age for the children was 7 years (range, 0.5–18); 10 children were between the ages of 13 and 18 years, 12 between the ages of 8 and 12 years, 16 between the ages of 3 and 7 years, and 13 under the age of 3 years. For the adults, median age was 37 years (range, 26–55). The median CD4 cell count for the children and adults was 185/mm³ (range, 2–2140) and 119/mm³ (range, 4–200), respectively. Interestingly, 19 children had biopsy-proven lymphoma or lymphoproliferative disease, although none had BCBL, the type of lymphoma previously linked with HHV-8 [7]. Of note, none of the children studied reported sexual
ORF65 ELISA and indirect immunofluorescence assays and that seropositivity correlates with HHV-8 DNA positivity in PBMC by PCR [5].

Discussion

We found that US children with marked immunosuppression showed no evidence of HHV-8 infection as determined by use of all currently accepted techniques for detecting this virus. These data are consistent with HHV-8 seroprevalence studies previously reported for healthy children [5, 6] and are in contrast to transmission patterns reported for most other herpesviruses (e.g., infection with the prototypic γ-herpesvirus EBV and the prototypic β-herpesvirus CMV had already occurred in at least 51% and 18%, respectively, of our childhood patient population). Of note, we recognize that the prevalence of HHV-8 infection in children from east and central Africa may show a different pattern compared with that in US children, since KS occurs not infrequently in young central Africans, and HHV-8 seroprevalence in healthy Ugandan adults has been reported to be 50%–60% [4, 5].

To our knowledge, this is the first comparative detection study of HHV-8 DNA in the blood of children and adults with AIDS. Although we compared different age groups with similar levels of immunosuppression, we acknowledge that these groups also differed in their sexual activity. To prove that HHV-8 is a sexually transmitted virus, as suggested by our study and many others, it will be important to compare HHV-8 prevalence in sexually active adolescents and young adults with that in their sexually inactive counterparts.

Our results are derived from a population of immunosuppressed children. Considering that most herpesvirus infections are reactivated and more readily detectable during times of immune compromise [11, 12], we believe that our pediatric AIDS cohort gives an accurate measurement of the prevalence of HHV-8 in US children. We are aware that there may be unidentified demographic differences between children with AIDS and healthy children and that those factors might preclude direct comparisons of these populations. However, HIV infection and immunosuppression appear to be the only two differences when our children are compared with their healthy counterparts. Thus, our findings suggest that primary HHV-8 infection occurs following adolescence in the United States.

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References


