

Antibody Reactivity to Latent and Lytic Antigens to Human Herpesvirus–8 in Longitudinally Followed Homosexual Men

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Tests for human herpesvirus–8 (HHV-8) often disagree on which individuals are infected. To evaluate HHV-8 antibody changes over many years, 245 homosexual men enrolled in a prospective study between 1982 and 1999 were studied. Samples collected annually were evaluated by immunofluorescence to HHV-8 latent nuclear antigen and by an ELISA for antibody against the HHV-8 lytic antigen K8.1. When positive, samples from most subjects were persistently reactive in both assays. Titers to both lytic and latent antibodies increased for many years, usually in parallel, and were higher in human immunodeficiency virus–infected men. The increasing titers and expanding epitope recognition suggest ongoing, low-grade replication of HHV-8. Use of multiple assays is important in determining prevalence in asymptomatic populations. Because persons with more advanced infections have higher antibody titers, they are easier to detect, which may skew our understanding of the epidemiology of HHV-8.

Since the discovery of human herpesvirus–8 (HHV-8, also called the Kaposi sarcoma–associated herpesvirus) in Kaposi sarcoma tissue [1], this virus has been the subject of intense investigation. Despite remarkable advances in molecular studies, much remains unclear about its epidemiology. Soon after discovery, it was found that almost all patients with Kaposi sarcoma have HHV-8 antibodies [2–5], usually with relatively high titers [6–8]. Detection of asymptomatic HHV-8 infection has been much more problematic. In interlaboratory comparisons, using standard panels of samples

from general populations in America and Europe [7, 9], all laboratories generally detected antibodies in the patients with Kaposi sarcoma, but there was much less agreement about results in asymptomatic persons. Similarly, serial samples from some studies [10–12], but not all [13, 14], have yielded inconsistent serologic results in individuals thought to be positive.

In the absence of a reference standard, establishing the true infection status in asymptomatic persons is problematic. Culturing HHV-8 has proven to be difficult. By use of polymerase chain reaction (PCR), most investigators find <10% of seropositive, asymptomatic Europeans and Americans to be viremic [15–17], and, when detected, the virus is usually present in minute amounts. Furthermore, in serially followed subjects, PCR-detected viremia may be found only intermittently [18]. Even among persons with Kaposi sarcoma who have PCR-positive tumors, virus levels in blood and saliva are modest, and only one-half or fewer are found to be viremic [19–21].

We examined consistency within and between latent and lytic HHV-8 antibody assays and how antibody

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Informed consent was obtained from participants, and guidelines for sample use from the US Department of Health and Human Services and National Cancer Institute (NCI) were followed.

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levels changed over time. To this end, we studied samples collected annually for almost 2 decades from a cohort of homosexual men, a population in which the prevalence of HHV-8 antibodies is high [2–5, 8–11].

METHODS

In 1982, we began a longitudinal study of homosexual men in New York and Washington, DC, in response to the emerging AIDS epidemic. Details of enrollment, follow-up, and human immunodeficiency virus (HIV) testing have been presented elsewhere [22, 23]. Blood samples were sought at annual visits, except that Washington participants were not seen in 1983. Surviving HIV-infected subjects continued in follow-up study throughout the 1990s. Follow-up was discontinued on most HIV-negative subjects in the early 1990s, although some were retained as study control subjects. We have previously reported a study of this cohort in which we described HHV-8 prevalence and incidence, using an immunofluorescence antibody (IFA) assay for latent antigens [11]. In that study, we noted a peak in incidence in the early 1980s.

The main objective of the current study was to examine the longitudinal dynamics of the antibody response. Serial samples were screened for antibody to lytic and latent HHV-8 antigens. The methods used in our laboratories have been described in detail elsewhere [4, 11, 17]. An IFA assay was done using HHV-8 infected BCBL-1 [11] or BCP-1 [4, 17] cells with samples diluted 1:100. This assay detected reactivity to a latency-associated nuclear antigen encoded by *orf73*. The K8.1 assay was an ELISA using K8.1, a lytic phase surface glycoprotein, done on serum samples initially diluted 1:20. Because of sample depletion or equivocal reactions, results for only IFA or K8.1 were available for a few samples. The term “persistent” is used to describe sequential observations that were all or almost all of the same type, allowing for an occasional divergent result considered to be a sample or laboratory error.

Subsets of antibody-positive subjects were then titered. To avoid bias, we titered all HIV-negative subjects for whom we had at least 6 HHV-8-positive samples and a similar number of HIV-infected HHV-8-positive subjects. Because the objective of the study was to determine changes in asymptomatic men, none of those titered was reported to have developed Kaposi sarcoma. Titers were done at 2-fold dilutions in the IFA assay with BCP-1 cells and 4-fold dilutions in the K8.1 assay. Slopes were determined from \log_{10} transformed titers on positive samples, starting from the first sample positive by that assay; the significance of the titer change in individuals was calculated by linear regression (SAS software version 8.2; SAS Institute).

RESULTS

Tests were done on 1529 samples from 245 subjects. The mean time span was 8.1 years, with a mean of 6.9 samples per person. Of these subjects, 134 (55%) were prevalently HIV-infected or became HIV-infected during the study. Thirty-one (23%) of the HIV-infected men were reported to have developed Kaposi sarcoma during the study, but none of 111 HIV-negative men developed the disease.

We classified 130 men (53%) as being persistently HHV-8 seronegative. This group included 2 men who were never seropositive while participating but who were reported to have developed Kaposi sarcoma after they discontinued active participation. Among the 128 other seronegative subjects, there were 8 positive IFA results (1.0%) among 789 tests and 19 positive K8.1 results (2.3%) among 821 tests. These positive results were both preceded and followed by a series of negative results, and only 1 sample was positive by both tests. Presumably, they represent either false-positive reactions or mislabeled samples.

Sixty-seven (27%) men were prevalently HHV-8 seropositive, defined as positive by either assay on their first tested sample (figure 1). Sixteen (24%) were later reported to have Kaposi sarcoma. The first sample was both IFA- and K8.1-positive for 45 (67%) subjects, only K8.1-positive for 15 (22%) subjects, and only IFA-positive for 7 (10%) subjects, with the last group including 2 subjects not tested by K8.1 tests because no samples remained. Fifty-one (76%) of these men were persistently seropositive. In this expected-positive group, 9 samples had false-negative results by IFA and by K8.1 tests (2.4% each; generally not the same samples). Of 18 men with initially discordant results and multiple samples, 6 became positive by both tests in the second sample, 2 remained persistently positive only by K8.1 tests but did develop IFA reactivity 5 and 7 years later, 9 were persistently K8.1 positive but IFA negative for at least several years, and 1 was persistently IFA positive but remained K8.1 negative for at least 7 years.

Forty-eight (20%) subjects were classified as HHV-8 seroconverters, defined as being antibody-negative in 1982 but developing persistent antibodies in 1 or both assays thereafter (figure 2). Thirty-three (73%) subjects seroconverted by 1984. Thirteen seroconverters later developed Kaposi sarcoma. Of 41 subjects tested by both tests, 23 (56%) seroconverted to both assays at the same time, 11 (27%) seroconverted in the K8.1 assay first, and 7 (17%) seroconverted in the IFA assay first. Nineteen (40%) seroconverters had discordant antibody patterns in which one assay became persistently positive only years after the other, if ever, or else reverted to being persistently negative in 1 assay. Eleven of 19 were predominately K8.1 positive, and 8 subjects were predominately IFA reactive. At some point, reactivity by both assays usually occurred, but 5 subjects were never IFA positive while in the study.

Impact of HIV infection. Discordant serologic test results

reactivity at the time of sampling. HIV-infected men had higher reactivity in the last positive sample, both in optical density values and, among those titered, K8.1 and IFA antibodies than HIV uninfected men. In both HIV-infected and -uninfected men, reactivity increased between the first and last sample, whether measured by optical density or titer (data not shown). Profiles of HHV-8 antibody titers varied in both 16 HIV-negative and 13 HIV-positive subjects (representative examples in figure 3), but, in general, titers increased steadily over many years. Figure 4 shows the slopes of the titers over time for 27 men who had both IFA and K8.1 reactivity. Most (63%) had rising antibody titers in both IFA and K8.1 assays, and the increase was usually in parallel.

Subjects diagnosed with Kaposi sarcoma. The relationship between HHV-8 and Kaposi sarcoma was not the primary purpose of the present analysis. In brief, all subjects who developed Kaposi sarcoma were HIV-infected, and, of 134 HIV-infected men, 31 (23%) reported with Kaposi sarcoma. Accepting a positive result in either test as evidence of HHV-8 infection, Kaposi sarcoma was reported in 2 (2%) of 130 persistent negative subjects, 13 (27%) of 48 seroconverters, and 16 (24%) of 67 prevalent positive subjects. The 2 men who were negative for HHV-8 were both tested before developing Kaposi sarcoma but not after. Presumably, both seroconverted. Compared with asymptomatic subjects, men who developed Kaposi sarcoma

appeared to have generally similar profiles of qualitative results, with some being non- or late-reactors ($n = 7$) or seroreverters ($n = 2$), by either the IFA or K8.1 assay. Among 111 subjects who were never HIV-positive, none developed Kaposi sarcoma, although 17 were prevalently HHV-8 positive at study onset and 9 seroconverted to HHV-8. The risk of Kaposi sarcoma did not appear to be related to the relative timing of infection with HIV and HHV-8. Kaposi sarcoma was diagnosed in 6 (40%) of 15 subjects who were HHV-8 infected before HIV infection, 2 (15%) of 13 who seroconverted to both viruses at the same time, and 11 (50%) of 22 who seroconverted to HHV-8 after HIV infection (including 2 presumed to have become HHV-8 infected).

DISCUSSION

The use of serial samples over nearly 2 decades revealed the changing profiles of antibody reactivity in asymptomatic persons. Other studies have presented data on serial assay reactivity and performed antibody titers on selected cases in the years immediately preceding Kaposi sarcoma [12, 13, 24]. Although those investigators showed that titers increased immediately preceding or at the onset of Kaposi sarcoma, our study showed that antibody titers increased over time, even in asymptomatic subjects. In many studies, antibodies were also inconsistently

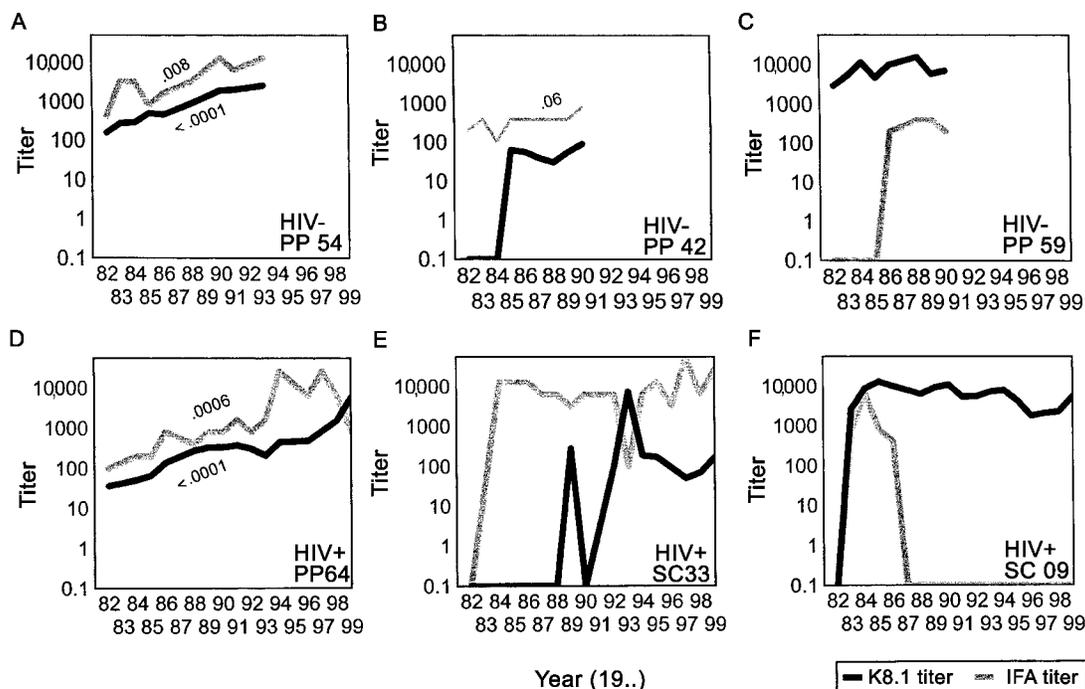


Figure 3. Titer changes in representative subjects over time. Identifiers link data to qualitative data presented in figure 1 (PP; prevalent positive) or figure 2 (SC; seroconverter). When titer trend was $P < .15$, the value is provided. Panels show in human immunodeficiency virus (HIV)-uninfected subjects: *A*, increasing immunofluorescence antibody (IFA) and K8.1 titers; *B*, delayed K8.1 response; *C*, delayed IFA response; and in HIV-infected subjects: *D*, increasing IFA and K8.1 titers; *E*, delayed K8.1 response; and *F*, loss of IFA reactivity.

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