BRIEF REPORT

Human Herpesvirus 8 Seroprevalence among Prostate Cancer Case Patients and Control Subjects

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(See the article by Engels et al., on pages 199–207, and the editorial commentary by Martin, on pages 173–5.)

To investigate a possible association between human herpesvirus 8 (HHV-8) and prostate cancer, we evaluated HHV-8 seroprevalence in 2 case-control studies. HHV-8 antibodies were detected by immunofluorescence with cells expressing lytic viral proteins and by enzyme immunoassays with recombinant viral structural protein (K8.1) and latent protein (latency-associated nuclear antigen–1; open reading frame 73), respectively. HHV-8 seroprevalence tended to be lower in patients with prostate cancer than in control subjects, but there was no significant difference in either study. These data imply that HHV-8 is not a major prevalent cause of prostate cancer.

Prostate cancer is the most common cancer among men in the United States and is third to lung and colorectal cancer as a cause of cancer death [1]. Known risk factors for prostate cancer include a family history of prostate cancer, age, and ethnicity [2]. Proposed cofactors for prostate cancer include prostatic inflammation, diet, and sexually transmitted agents [3]. In the United States, prostate cancer is more prevalent in African Americans than either whites or Asians, with some cancer registries reporting a ⩾30-fold difference between African Americans and Asians [4]. The increased risk seen in men of African descent is not limited to the United States. In a recent study from the Caribbean nation of Trinidad and Tobago, the prevalence of screening-detected prostate cancer was 3-fold higher among Tobago men of African descent than among US white men [5]. The reason for this increased risk is not known.

A recent study found an increased prevalence of antibodies against human herpesvirus 8 (HHV-8) among men with prostate cancer, compared with that among control subjects, in 2 populations [6]. In the Afro-Caribbean population on the island of Tobago, HHV-8 seroprevalence was significantly higher (>2-fold) among men with biopsy-proven prostate cancer than among age-matched control subjects who had normal levels of prostate-specific antigen (PSA) and normal digital rectal examinations (DREs). HHV-8 seroprevalence also was higher, albeit not significantly, among men in Pittsburgh who had advanced prostate cancer than among men with other types of cancer and unknown PSA and DRE status [6].

To further investigate a possible association between HHV-8 and prostate cancer, we evaluated HHV-8 seroprevalence in 2 case-control studies. In one of these, among African American men in Washington, DC, and Italian men in the Bologna region, patients with biopsy-proven prostate cancer cases were compared with control subjects with surgically treated benign prostatic hyperplasia (BPH) [7]. The second study was a population-based case-control study of prostate cancer among African American and white men in 3 regions of the United States [8]. HHV-8 antibodies were detected by immunofluorescence with cells expressing lytic viral proteins and by ELISAs with recombinant viral structural protein (K8.1) and latent protein (latency-associated nuclear antigen–1 [LANA-1]; open reading frame [ORF] 73), respectively.

Materials and methods. All patients scheduled for prostate surgery or biopsy between 1991 and 1994 at Malpighi Hospital, a community-based facility in Bologna, Italy, or at Howard University Hospital in Washington, DC, a community medical center that serves a predominately African American population, were eligible for the study unless judged by a trained member of the study team to be unable to provide consent or to respond to questions in an informed manner. This was a particular concern because of the advanced age of many patients. All but 2 eligible patients in Bologna and most in Washington (88%) were successfully enrolled in the study. Data were available only on enrolled individuals. Among the 156 African

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Table 1. Human herpesvirus 8 seroprevalence among patients with prostate cancer and control subjects with benign prostatic hyperplasia (BPH).

Location, group	Lytic IFA			K8.1 ELISA			ORF73 ELISA		
	Positive, %	*P*	OR (95% CI)	Positive, %	*P*	OR (95% CI)	Positive, %	*P*	OR (95% CI)
Bologna, Italy									
Case patients (n = 10)	40.0	1.00	1.08 (0.27–4.33)	20.0	1.00	0.813 (0.17–4.21)	20.0	.702	0.60 (0.12–3.03)
BPH control subjects (n = 34)	38.2			23.5			29.4		
Washington, DC, African Americans									
Case patients (n = 41)	17.1	1.00	0.88 (0.35–2.24)	7.3	.124	0.328 (0.10–1.11)	12.8	.116	0.43 (0.16–1.18)
BPH control subjects (n = 98)	19.0			19.4			24.5		

NOTE. CI, confidence interval; IFA, immunofluorescence assay; OR, odds ratio.

American subjects enrolled at Howard University, 41 of 50 with prostate cancer and 98 of 106 BPH control subjects were studied. The missing samples represent subjects from whom serum or plasma samples were unavailable. In Italy, 10 of 14 patients with prostate cancer were examined in the present investigation. A subset (34/104) of Italian control subjects was selected, by using 3:1 frequency-age matching to case patients, according to 5-year age groups. Not all case patients had 3 matched control subjects. Signed consent forms were obtained from each subject before the interview and venipuncture. Blood was separated by centrifugation, and plasma was stored at −70°C until testing.

The subjects for the present investigation were taken from a larger case-control study described elsewhere [8]. Case patients consisted of white and African American men covered by the population-based cancer registries of the Georgia Center for Cancer Statistics, the Metropolitan Detroit Cancer Surveillance System, and the New Jersey State Cancer Registry. Population control subjects were selected in the 3 geographic areas proportional to the expected age, sex, and race distribution of the combined cases. Control subjects <65 years old were selected by the Waksberg method of random-digit dialing [9]; older control subjects were selected by random sampling from the computerized records of the Health Care Financing Administration. For the present study we analyzed serum samples from 184 whites and 170 African Americans. There were 104 case patients with prostate cancer and 80 control subjects among whites and 95 case patients and 75 control subjects among the Americans. Signed consent was obtained from all subjects before blood sample collection. For both studies, informed, written consent was obtained using forms and procedures approved by the institutional review boards of the National Institutes of Health, the local participating sites, and the University of Pittsburgh.

Antibodies to lytic HHV-8 proteins were determined using a monoclonal antibody–enhanced immunofluorescence assay (IFA) as described elsewhere [10]. The cutoff value for positive seroprevalence was 1:100. HHV-8 antibody titers were determined using the same IFA on serially diluted serum samples (1:50–1:51,200). End-point titers were reported as the reciprocal of the last positive dilution. All samples were analyzed blinded, in duplicate, a minimum of 3 times. Agreement between the triplicate analyses was 98.7%.

HHV-8 antibody testing was performed using ELISAs to detect antibodies to the HHV-8 K8.1 structural glycoprotein (expressed during lytic viral replication) and ORF73 (also known as LANA-1, a viral regulatory protein expressed during latent infection). The LANA-1 protein is a full-length protein expressed in baculovirus, whereas the K8.1 protein is a full-length protein expressed in *Escherichia coli*. Serum samples were diluted 1:20 for the K8.1 ELISA and 1:100 for the ORF73 ELISA. ELISA methods have been detailed elsewhere [11].

Differences in HHV-8 seroprevalence were examined by χ^2 analysis or Fisher's exact test, as appropriate. Comparisons of mean HHV-8 titers by categorical risk factor variables were performed with the Mann-Whitney *U* test.

Results. We analyzed 2 separate cohorts using 3 different serological assays. The ELISAs detected antibodies directed against single proteins representing lytic (K8.1) or latent (ORF73) antigens, whereas the lytic IFA detected antibodies against all of the lytic antigens. The agreement between these assays was modest at best when the ELISAs were compared with each other or the lytic IFA was compared with the lytic K8.1 ELISA (κ = 0.34 and 0.25, respectively). There was low agreement between the lytic IFA and the ORF73 ELISA (κ = 0.13). Differences between assays have been noted previously and reflect differences in sensitivity and specificity [12]. In the present study, we used 3 separate assays to cover a wide range of viral antigens, as well as antibodies directed against both lytic and latent proteins.

In the first study (prostate cancer vs. BPH), all subjects from Bologna, Italy, were white, whereas all subjects from Washington, DC, were African American. Within each national-racial group, the seroprevalence of HHV-8, as determined by lytic IFA, was similar between patients with cancer and BPH control subjects (table 1). When the ELISAs were used, HHV-8 sero-

prevalence was lower, albeit not significantly so, among patients with prostate cancer than among BPH control subjects. Overall, the HHV-8 seroprevalence with all 3 assays was higher in Italy than in the United States, which is in agreement with previous studies demonstrating a higher infection rate in Italy [13–15].

HHV-8 seroprevalence in the US population-based study also tended to be lower, albeit not significantly so, among patients with prostate cancer than among control subjects (table 2). This trend was seen with each of the 3 assays and in both whites and African Americans. Among control subjects, HHV-8 seroprevalence was slightly, and nonsignificantly, higher among African Americans than among whites (table 2).

Discussion. The present study analyzed HHV-8 seroprevalence among 2 distinct cohorts. The first cohort represented men from the United States and Italy with biopsy-confirmed prostate cancer and men with surgically treated BPH. BPH and prostate cancer are considered to be 2 separate diseases of the prostate. BPH is confined predominately to the transition zone of the prostate, whereas prostate cancer is confined predominately to the peripheral zone. The onset and progression of BPH are not believed to be linked to prostate cancer. However, both diseases may share some similarities, including a role of inflammation in disease development and certain risk factors, such as age [16]. The second cohort consisted of African American and white US men with prostate cancer and control subjects with no history of prostate cancer.

The present analyses revealed no significant difference in HHV-8 seroprevalence between patients with prostate cancer and control subjects. This contrasts with the increased HHV-8 seroprevalence previously noted among patients with prostate cancer in the Caribbean island of Tobago [6]. There are several possible explanations for this discrepancy. First, the US/Italian cohort compared cancer with BPH, and we cannot rule out a role for HHV-8 in BPH development. It is noteworthy that the HHV-8 seroprevalence among patients with BPH was higher than expected (compared with that among healthy US men) with all 3 assays. In the second cohort (the US cohort), the control subjects were selected by telephone random-digit dialing or from the Health Care Financing Administration files, irrespective of PSA and DRE status. This is in contrast to the Tobago control subjects, who had normal PSA and DRE results. Thus, the control subjects may have had unrecognized prostate cancer. However, given the incidence rate of prostate cancer in the United States (237.7 cases/100,000 population among whites and 324.4 cases/100,000 population among African Americans), this is unlikely. Misclassification of case-control status would generally bias a true association toward the null.

A second possibility is that HHV-8 status was misclassified. Each of the 3 assays used in the analyses has imperfect sensitivity and specificity [12]. In the aggregate, however, they are complementary, testing for different HHV-8 antigens in different formats. Our consistent null results suggest that there is no association between prostate cancer and HHV-8 status as defined using these tests.

Finally, geographic heterogeneity may account for the null association between HHV-8 and prostate cancer in the present analyses. HHV-8 seroprevalence differs substantially in Italy, which is considered to be a region of endemicity, and in the United States [14, 15]. A nonsignificant 1.5-fold elevation in HHV-8 seroprevalence was previously found among patients with prostate cancer in Pittsburgh, whereas HHV-8 seroprevalence was nonsignificantly lower among patients with prostate cancer in Georgia, New Jersey, Detroit, and Washington, DC, in the present analyses. This diversity of sites suggests that HHV-8 is not associated with prostate cancer in the United States. Afro-Caribbean populations other than that in Tobago and those in Africa have not been evaluated.

In summary, we found no association between HHV-8 seroprevalence and prostate cancer in 2 case-control studies in the United States or in a very small case-control study in Italy, where HHV-8 is endemic. These negative results suggest that the elevated seroprevalence found in an earlier study may have

Table 2. Seroprevalence among case patients with prostate cancer and population-based control subjects in the United States.

	Lytic IFA			K8.1 ELISA			ORF73 ELISA		
Ethnicity, group	Positive, %	*P*	OR (95% CI)	Positive, %	*P*	OR (95% CI)	Positive, %	*P*	OR (95% CI)
Combined									
Case patients (*n* = 199)	18.1	.08	0.63 (0.39–1.04)	2.5	.544	0.64 (0.20–2.02)	3.0	.57	0.66 (0.23–1.91)
Control subjects (*n* = 155)	25.9			3.9			4.5		
White									
Case patients (*n* = 104)	18.7	.37	0.71(0.36–1.43)	2.9	1.00	0.76 (0.17–3.40)	1.9	1.00	0.77 (0.13–4.44)
Control subjects (*n* = 80)	24.4			3.8			2.5		
African American									
Case patients (*n* = 95)	17.5	.15	0.56 (0.28–1.14)	2.1	.656	0.52 (0.10–2.66)	4.2	.51	0.62 (0.17–2.20)
Control subjects (*n* = 75)	27.5			4.0			6.7		

NOTE. CI, confidence interval; IFA, immunofluorescence assay; OR, odds ratio.

been a chance association. Interestingly, the increase in seroprevalence among patients with prostate cancer in Tobago has remained significant after the analysis of additional samples (F.J.J. and C. H. Bunker, unpublished results). In all of our comparisons except for BPH versus prostate cancer in Bologna, Italy, there was >95% power to detect a 17% difference (the difference seen between case patients and control subjects in Tobago) in HHV-8 prevalence rates at $\alpha \leq .05$ (2-sided). There was 75.1% power in the Italian BPH versus cancer study. The strong serological association among men in Tobago and the lack of serological association in the present study among US men suggests a possible, yet unknown, environmental or genetic correlate of the virus with the risk of prostate cancer in Tobago.

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