Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2007 August ; 16(8): 1573–1580. doi: 10.1158/1055-9965.EPI-07-0134.

Plasma antibodies against *Chlamydia trachomatis,* human papillomavirus and human herpesvirus type 8 in relation to prostate cancer: a prospective study

Siobhan Sutcliffe[1], **Edward Giovannucci**[2], **Charlotte A. Gaydos**[3], **Raphael P. Viscidi**[4], **Frank J. Jenkins**[5], **Jonathan M. Zenilman**[3], **Lisa P. Jacobson**[1], **Angelo M. De Marzo**[6,7], **Walter C. Willett**[2], and **Elizabeth A. Platz**[1,6]

[1] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

[2] Departments of Nutrition and Epidemiology, Harvard School of Public Health and the Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

[3] Division of Infectious Diseases, Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, MD

[4] Stanley Division of Developmental Neurovirology, Department of Pediatrics, Johns Hopkins Medical Institutions, Baltimore, MD

[5] Department of Pathology, School of Medicine, and Departments of Infectious Diseases and Microbiology, School of Public Health, University of Pittsburgh, Pittsburgh, PA

[6] James Buchanan Brady Urological Institute and the Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Medical Institutions, Baltimore, MD

[7] Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD

Abstract

Traditionally, case-control studies of sexually transmitted infections (STIs) and prostate cancer have focused on gonorrhea and syphilis, with overall positive associations. More recently, researchers have begun to expand their focus to include additional STIs, such as *Chlamydia trachomatis*, human papillomavirus (HPV) and human herpesvirus type 8 (HHV-8) infections. Continuing this investigation, we examined each of these infections in relation to incident prostate cancer in a nested case-control study within the Health Professionals Follow-up Study. Prostate cancer cases were men diagnosed with prostate cancer between the date of blood draw (1993–5) and 2000 (n=691). Controls were men free of cancer and alive at the time of case diagnosis who had had at least one prostate specific antigen test between the date of blood draw and case diagnosis. One control was individually matched to each case by age; year, time of day and season of blood draw; and PSA screening history prior to blood draw (n=691). *C. trachomatis* and HPV -16, -18 and -33 antibody serostatus were assessed by enzyme-based immunoassays, and HHV-8 antibody serostatus was assessed by an immunofluorescence assay. No associations were observed between *C. trachomatis* (OR=1.13, 95% CI: 0.65–1.96), HPV-16 (OR=0.83, 95% CI: 0.57–1.23), HPV-18 (OR=1.04, 95% CI: 0.66–1.64) and HPV-33 (OR=1.14, 95% CI: 0.76–1.72) antibody

Corresponding author: Dr. Platz, Department of Epidemiology, Room E6132-A, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Baltimore, MD 21205. Tel: (410) 614-9674, Fax: (410) 614-2632, eplatz@jhsph.edu.

This article is not part of a series. The contents of this article were presented as an abstract at the American Association for Cancer Research: Frontiers in Cancer Prevention Research conference in Boston, MA in November, 2006. The authors do not have commercial or other associations that might pose a conflict of interest.

seropositivity and prostate cancer. A significant inverse association was observed between HHV-8 antibody seropositivity and prostate cancer (OR=0.70, 95% CI: 0.52–0.95). As this study is the first, to our knowledge, to observe such an inverse association, similar additional studies are warranted.

Keywords

Prostate cancer; *Chlamydia trachomatis*; human papillomavirus; human herpesvirus type 8

INTRODUCTION

In 1950, Ravich and Ravich (1) proposed that sexually transmitted infections (STIs) may contribute to prostate carcinogenesis. Since that time, several case-control studies have investigated this hypothesis with overall positive associations (2,3). Traditionally, these studies focused on gonorrhea on syphilis. However, more recent epidemiologic studies have begun to investigate additional STIs, reflecting the expanding number of recognized sexually transmitted pathogens over time. These include *Chlamydia trachomatis*, human papillomavirus (HPV) and human herpesvirus type 8 (HHV-8) infections. *C. trachomatis*is a putative, candidate infection because it is intracellular and is often asymptomatic in men, which may allow it to persist in the male genitourinary tract, and possibly ascend to the prostate, where it has been observed to infect prostate epithelial cells and elicit an intraprostatic inflammatory immune response(4–6). HPV and HHV-8 infections are putative candidates because they have both been associated with other cancers (cervical, vulval, anal and penile carcinomas and Kaposi's sarcoma)(7,8) and have both been detected (with some debate) in prostate tissue(9–13). Additionally, a recent study observed a correlation between HHV-8 protein expression and a macrophage/monocyte marker in prostate specimens, suggesting that HHV-8 infection may elicit intraprostatic inflammation(12). HHV-8 is also known to express viral interleukin-6 (vIL-6), a homolog of human IL-6 (8), which has been proposed to play a role in prostate cancer cell proliferation (14) and may thus contribute to prostate cancer progression. To date, results from prospective and retrospective epidemiologic studies of *C. trachomatis*, HPV and HHV-8 infections and prostate cancer are largely inconclusive, as many earlier positive findings have not been replicated in subsequent studies, and results are generally variable across studies, even those with similar study designs (15–26).

To further investigate associations between STIs and prostate cancer, we conducted a large, nested case-control study of *C. trachomatis,* HPV types 16, 18 and 33 and HHV-8 infections in relation to incident prostate cancer among participants in the Health Professionals Follow-up Study (HPFS). Histories of these infections were assessed by pre-diagnostic antibody serostatus to capture asymptomatic infections, which comprise a large proportion of *C. trachomatis,* HPV and HHV-8 infections, and symptomatic infections of possibly unrecognized origin. This last attribute is particularly important for *C. trachomatis* infection because it tends to be treated presumptively rather than specifically-diagnosed in men, and because chlamydia diagnostics have only been commercially-available since 1985, after many participants may have already been infected.

METHODS

Study population and design

In 1986, American male health professionals aged 40–75 were invited to participate in the HPFS, an ongoing, prospective study of cancer and heart disease in men. 51,529 health professionals agreed to participate by completing a mailed, baseline epidemiologic

questionnaire on demographics, lifestyle and medical history, and a semi-quantitative food frequency questionnaire. Since 1986, participants have completed questionnaires every two years to update exposure and disease information, and every four years to update dietary information. Information on death is obtained from the National Death Index, and the U.S Postal Service or next of kin in response to follow-up questionnaires. Between 1993 and 1995, HPFS participants were additionally asked to provide a blood sample for research purposes. 18,225 participants returned a chilled, EDTA-preserved blood specimen to the Harvard School of Public Health by overnight courier. Upon arrival at the school, specimens were centrifuged, separated into plasma, buffy coat and erythrocyte aliquots, and stored in liquid nitrogen.

All participants who provided a blood sample in 1993–5, who were free of reported cancer (except non-melanoma skin cancer) at the time of blood draw, and who provided valid baseline food frequency information were eligible for inclusion in the nested case-control study. Cases were defined as men diagnosed with prostate cancer between the date of blood draw and January 31, 2000 (n=691). Information on prostate cancer was obtained from biennial follow-up questionnaires, which requested that participants report medical diagnoses, including prostate cancer, in the prior two years. Over 90% of prostate cancer diagnoses were subsequently confirmed by medical record and pathology report review with permission from the participant or next of kin. Many of the remaining 10% provided supporting information (e.g., evidence of treatment) for their diagnosis. Information on disease stage (TNM) and Gleason sum was abstracted from medical records by trained study investigators using a standard form. Participants diagnosed with stage T1a prostate cancers (n=2) were not included as cases because, by definition, their tumors were detected at transurethral resection of the prostate for benign prostatic hyperplasia (BPH), and are especially prone to detection bias.

Controls were defined as men alive and free of a diagnosis of cancer (except non-melanoma skin cancer) at the time of case diagnosis. Controls were also required to have had at least one prostate specific antigen (PSA) test between the date of blood draw and the two-year interval of case diagnosis. No restrictions were placed on PSA concentration to avoid excluding men with non-cancerous prostate conditions associated with elevated PSA, such as BPH or prostatitis, as these conditions were not excluded from the case definition. Had restrictions been placed on control PSA concentration, a bias may potentially have been introduced if any of the infections considered were associated with either BPH or prostatitis. One control was individually matched to each case by age (±1 year), time (midnight -9 a.m., 9 a.m.-noon, noon-4 p.m., and 4 p.m.-midnight), season (January-March; April-June; July-September; and October-December) and year (exact) of blood draw, and PSA testing history prior to 1993–5 (yes/no).

This analysis was approved by the Human Subjects Committee at the Harvard School of Public Health and the Committee on Human Research at the Johns Hopkins Bloomberg School of Public Health.

Plasma antibody detection

C. trachomatis infection—*C. trachomatis* antibody serostatus was assessed by the Ani Labsystems *C. trachomatis* IgG enzyme immunoassay (EIA, Ani Labsystems, Helsinki, Finland) in the laboratory of Dr. Charlotte A. Gaydos. This assay detects IgG antibodies against synthetic peptides derived from variable domain IV of the major outer membrane protein of *C. trachomatis* serovars C, G, E and L2 (27,28). In a validation study, this assay had a sensitivity of 56.2% in culture-positive men and a specificity of 99.3% in children (calculated from Narvanen and colleagues' study (27)). Anti-*C. trachomatis* IgG antibodies are believed to be relatively persistent over time, as evidenced by stable antibody titers with

increasing age in a cross-sectional survey of elderly Finnish men (29), and persistent antibody seropositivity in women with chlamydial salpingitis, a complication of *C. trachomatis* infection (30).

All samples were tested once by EIA. Samples with signal to cut-off ratios between 1.0 and 1.4 were considered equivocal for anti-chlamydial antibodies, and those with signal to cutoff ratios ≥1.4 were considered positive for anti-chlamydial antibodies according to the manufacturer's instructions.

HPV-16, -18 and -33 infection—HPV-16, -18 and -33 IgG antibody serostatus were assessed by three in-house enzyme-linked immunosorbent assays (ELISAs) in the laboratory of Dr. Raphael P. Viscidi (31). These assays detect IgG antibodies against HPV-16, -18 and -33 virus-like particles, respectively. In previous studies, these assays had sensitivities of ≥50% in DNA-positive women, and specificities of ≥98% in virginal women and children. While some women in these studies lost antibody seropositivity over time, others have been observed to maintain antibody seropositivity for at least a decade, a phenomenon likely dependent, at least in part, on characteristics of their HPV infection(s) (e.g., duration) (32,33).

All samples were tested in duplicate with repeat duplicate testing for duplicates with optical density (OD) coefficients of variation (CVs) >25% and at least one value above the OD cut-point for seropositivity (defined below, n=44 for HPV-16, 42 for HPV-18, and 25 for HPV-33). Mean OD values were calculated for each participant based on duplicate test values or, in the case in which four replicates were tested, based on the average of the three values in closest agreement. OD cut-points of 0.85, 0.70 and 0.50 were used to assign seropositivity for HPV-16, -18 and -33, respectively, based on previously determined cut-points in self-reported female virgins (31).

HHV-8 infection—HHV-8 antibody serostatus was assessed by an in-house monoclonal antibody-enhanced immunofluorescent assay (IFA) against multiple lytic HHV-8 antigens in the laboratory of Dr. Frank J. Jenkins (34). In a comparative study of HHV-8 serologic tests, this assay had a sensitivity of 100% among patients with Kaposi's sarcoma, and an estimated sensitivity of 53.4% and specificity of 96.6% among blood donors (35). Once detected, anti-HHV-8 antibodies have been observed to persist for at least 17 years, and to increase in titer and range of epitope recognition over time (36), consistent with episodic viral reactivation of this life-long infection.

All samples were tested in duplicate and assessed microscopically by the same reader. In the case of discrepant duplicate results, a third replicate was tested and the results of the two replicates in agreement were used. Samples positive at a dilution of 1:100 were considered positive for anti-HHV-8 antibodies. Antibody titers were determined for positive samples using serially-diluted serum samples. End-point titers were calculated as the reciprocal of the last positive dilution.

For each assay, samples were tested in random case-control pair order, with case and matching control samples adjacent to one another, but in random within-pair order. Laboratory technicians were blinded to the case-control status of each sample. For HPV-16, -18 and HHV-8 testing, blinded samples of known serostatus were included in the testing sequence (two seropositive and two seronegative samples per assay) to assess the reliability of each serologic test. Known seropositive and seronegative samples were used as opposed to duplicate samples because of the expected low seroprevalence of each infection in this population. Reliability was high for each of these tests (kappa=1.00 for HPV-16, HPV-18 and HHV-8 infections). For *C. trachomatis* and HPV -33 testing, reliability was estimated

using duplicate, blinded quality control samples unselected for *C. trachomatis* and HPV -33 seropositivity (four samples per assay), due to difficulties in locating sufficient volumes of known seropositive serum (kappa=0.82 for *C. trachomatis* and 0.91 for HPV-33 infections).

Statistical analysis

To characterize participants and begin to investigate potential confounding, means and proportions of known or suspected STI and prostate cancer correlates or risk factors were calculated for prostate cancer cases and controls. Known or suspected STI correlates and risk factors included histories of gonorrhea, syphilis, trichomonosis and clinical prostatitis, ejaculation frequency from ages 20–29 and 40–49 (times/month), alcohol consumption from ages 18–22 (drinks/week), cigarette smoking before age 30 (pack-years), body mass index (BMI) at age 21 (kg/m^2), and vigorous physical activity in high school and college. Covariates previously observed to be associated with prostate cancer risk or progression in the HPFS cohort included race/ethnicity, cumulative family history of prostate cancer through 1996, height (inches), cigarette smoking between 1984 and 1994 (pack-years), total energy (kcal/day), alcohol (g/day), tomato sauce (servings/day), red meat (servings/day), fish (servings/day), calcium (mg/day), and energy-adjusted alpha-linolenic acid (g/day)in 1994, energy-adjusted fructose intake (g/day) in 1990, vitamin E (<15 mg/day, ≥15 mg/day) and zinc (<101 mg/day, ≥101 mg/day) supplementation, vigorous physical activity (metabolic equivalent-hours/week), and histories of vasectomy and diabetes mellitus type 2 as of 1994.

Associations between *C. trachomatis*, HPV and HHV-8 antibody seropositivity and prostate cancer were initially investigated by calculating antibody signal to cut-off ratio means, medians and proportions for *C. trachomatis* antibody serostatus, OD means, medians and proportions (based on OD quartiles among controls and OD cut-points for seropositivity) for HPV antibody serostatus, and proportions for HHV-8 antibody serostatus. Values were compared by paired t-tests, Wilcoxon signed-rank tests, McNemar's tests and likelihood ratio tests, as appropriate. Conditional logistic regression was used to calculate matched odds ratios and 95% confidence intervals for prostate cancer. Confounding was further explored by adding covariates individually and in combination to univariable conditional logistic regression models and comparing to univariable results. Covariates considered were those described above and other antibody serostatus (*C. trachomatis*, other HPV types and HHV-8, as appropriate). Detection bias was investigated by adding the number of PSA tests prior to case diagnosis to univariable models and comparing to univariable results. As none of the considered covariates altered any of the point estimates for exposures of interest, only known prostate cancer risk factors (race/ethnicity, family history of prostate cancer and age (matched)) were retained in the final multivariable model. Total prostate cancer was used as the outcome in the main analyses. Additional analyses using prostate cancer characterized by grade (low-grade (<7 Gleason sum) and high-grade (≥7 Gleason sum)) and stage (organ-confined (≤T2 and N0M0) and advanced (T3b or worse)) were also performed.

To investigate whether associations between STIs and prostate cancer varied by factors postulated to influence prostatic inflammation, stratified analyses were performed by aspirin use since age 20, lifetime cigarette smoking, and, in the case of *C. trachomatis* antibody seropositivity, age as a surrogate measure for availability of sulfonamide antibiotics at the time of *C. trachomatis* acquisition. Although *C. trachomatis* infection had not yet been identified as an STI when sulfonamide antibiotics were first introduced in 1937, it may still have been cured by these antibiotics (which possess anti-chlamydial activity) if acquired concurrently with gonorrhea, a known and prevalent STI at the time. Variation by underlying genetic susceptibility to prostate cancer was investigated by performing analyses stratified by age at prostate cancer diagnosis and family history of prostate cancer. All stratified analyses, with the exception of those for age, were performed by unconditional

logistic regression including terms for exposures of interest and matching variables (age, time of day, season and year of blood draw, and PSA screening history prior to blood draw). The statistical significance of any observed stratum-specific differences was assessed by including an additional cross-product term in the regression model and evaluating this term by the Wald test.

Prior to conducting the present analysis, we performed power calculations to determine the magnitude of minimum detectable associations for each infection using estimates of antibody seroprevalence from the literature. Given a sample size of 691 cases and controls, we estimated sufficient power (80%) to detect observed magnitudes of association between 1.38 and 2.57, assuming a range of antibody seroprevalences from 1.7–30% among controls(15,16). We also performed analyses to determine the effect of non-differential misclassification of exposure on our ability to detect associations. As stated earlier, the sensitivities of the assays used in the present study, which were similar to those used in most previous studies, were low for detecting current uncomplicated or transient infections, and may have been even lower for detecting past uncomplicated or transient infections, especially in men. However, we expected that the sensitivities of these assays might be higher for detecting infections of potentially greater relevance for prostate carcinogenesis, such as infections of longer duration that might be more likely to ascend to the prostate, repeated episodes of infection, and those with complications, such as prostatitis. This expectation was based on the observation that women with chlamydial salpingitis, a complication of *C. trachomatis* infection, women with HPV infections of longer duration, men with HHV-8 infections of longer duration, and individuals with Kaposi's sarcoma, a complication of HHV-8 infection, are more likely to have detectable or higher antibody titers than individuals with uncomplicated or transient infections (30,32,35,36). In the case that our expectation of higher sensitivities did not hold, we also performed analyses to determine the magnitude of minimum detectable associations using lower sensitivity assays. Assuming sensitivities of 50% and specificities of 97%, we estimated sufficient power to detect a true magnitude of association of 2.00 (or observed magnitude of association of 1.61), given an observed seroprevalence of 9.6% among controls.

RESULTS

Of the 691 prostate cancer cases included in this analysis, the majority were organ-confined (83.9% of 614 cases with prostate cancer stage information) with Gleason sums between 5 and 7 (14.4% Gleason sum 5, 38.8% Gleason sum 6, and 28.9% Gleason sum 7 of 623 cases with grade information). The mean age at diagnosis was 68.9 years (range: 47.7 to 84.3 years). The mean time from blood draw to diagnosis was 3.1 ± 1.7 years. When compared to controls, prostate cancer cases were more likely to report a family history of prostate cancer, less fish consumption, a slightly greater number of PSA tests before prostate cancer diagnosis, histories of trichomonosis and clinical prostatitis, and lower frequencies of ejaculation from ages 20–29 and 40–49 (Table 1).

Chlamydia trachomatis infection

No differences were observed in the distribution of signal to cut-off ratios for *C. trachomatis* antibody serostatus between prostate cancer cases and controls. Four percent of cases were seropositive for *C. trachomatis* infection as compared to 3.5% of controls (Table 2). Null results were also observed after multivariable-adjustment, and for prostate cancer characterized by grade and stage (Table 3 and data not shown). Too few seropositive participants were diagnosed with advanced stage cancer to investigate its association with *C. trachomatis* antibody seropositivity. In stratified analyses, no differences were observed in the association between *C. trachomatis* antibody seropositivity and total prostate cancer across strata of aspirin use, cigarette smoking, age as a surrogate measure for availability of

antibiotics, age at prostate cancer diagnosis, and family history of prostate cancer (all p-interaction >0.20).

Human papillomavirus infection

No differences were observed in the mean or median antibody concentration between prostate cancer cases and controls for HPV-16, -18 or -33. Among cases, 7.5% were seropositive for HPV-16, 6.1% for HPV-18, and 7.2% for HPV-33, while among controls, these values were 8.8%, 5.8%, and 6.4%, respectively (Table 2). When type-specific HPV information was combined, null results were also observed for any HPV seropositivity and number of HPV types (Table 2). Adjustment for potential confounding variables did not alter any of the results (Table 3 and data not shown). Null results were also observed for low-grade and organ-confined prostate cancer. A significant inverse association was observed between HPV-16 seropositivity and high-grade prostate cancer (Table 3). Too few participants were diagnosed with advanced stage prostate cancer to investigate potential associations between HPV seropositivity and advanced stage disease. In stratified analyses, no consistent patterns were observed across strata of cigarette smoking, age at prostate cancer diagnosis, and family history of prostate cancer for HPV-16, -18 and -33 (data not shown). A non-significant pattern of decreasing magnitudes of association with increasing aspirin use since age 20 was observed for each HPV type (p-interaction for all HPV types combined=0.16). No other differences were observed when information on HPV types was combined (all p-interaction >0.20).

Human herpesvirus type 8 infection

Prostate cancer cases were significantly less likely to be HHV-8 antibody seropositive than controls (13.5% versus 18.0%, respectively), although no differences were observed in the distribution of antibody titers between seropositive cases and controls (Table 2). Inverse results were also observed after multivariable-adjustment, and for low-grade and organ-confined prostate cancer (Table 3 and data not shown). Suggestive inverse associations were also observed for high-grade (Table 3) and advanced stage prostate cancer (OR=0.48, 95% CI: 0.16–1.42), although the number of seropositive men diagnosed with advanced stage disease was small (n=7). In stratified analyses, null to suggestive positive as opposed to inverse associations were observed for men who used aspirin infrequently since age 20 (OR=1.10, 95% CI: 0.58–2.08, p-interaction=0.17), men ≥74 years of age (fourth quartile of age at prostate cancer diagnosis, OR=1.31, 95% CI: 0.72–2.39, p-interaction=0.02), and men with a family history of prostate cancer (OR=1.28, 95% CI: 0.64–2.55, p-interaction=0.11); inverse associations were observed for all other strata of men (data not shown).

DISCUSSION

In this large, nested case-control study of male health professionals, no associations were observed between *C. trachomatis* and HPV -16, -18 and -33 antibody seropositivity and prostate cancer, with the possible exception of a significant inverse association between HPV-16 seropositivity and high-grade prostate cancer. For HHV-8, significant inverse associations were observed for total, organ-confined and low-grade prostate cancer. Suggestive inverse associations were also observed for HHV-8 antibody seropositivity and high-grade and advanced-stage disease. None of the observed associations were altered by adjustment for histories of other STIs, and correlates/risk factors of STIs or prostate cancer.

Chlamydia trachomatis infection

Our null finding for *C. trachomatis* antibody seropositivity is consistent with findings from one previous nested case-control study (15), but differs from those from two additional studies, one of which observed a significant inverse association between *C. trachomatis*

antibody seropositivity and prostate cancer across three study sites (22), while the other observed a suggestion of an inverse association between self-reported history of chlamydia and prostate cancer (23). Findings from the second study are, however, difficult to interpret because of the low reported cumulative incidence of chlamydia. This low incidence likely underestimates the true incidence because a) chlamydia is frequently asymptomatic in men; b) chlamydia diagnostics were only commercially-available after 1985; and c) self-reported incidence of chlamydia was much lower in this study population than reported incidences of gonorrhea, urethritis and epididymitis, the latter two of which are caused, to a fairly large extent, by *C. trachomatis*.

Our observed *C. trachomatis* seroprevalence of 3.5–4.0% is lower than previously published estimates, many of which were estimated in general male Scandinavian populations ranging in age from 18 to 97 years (15,22,29), and were based on use of the micro-immunofluorescence (MIF) assay, which is considered to be a more specific but less sensitive test than the Ani Labsystems EIA used in our study (28,37–39). To our knowledge, no estimates of *C. trachomatis* seroprevalence exist in the general male U.S. population, either based on the MIF assay or EIA, to allow for comparisons between men from the same geographic location. Therefore, it was difficult to compare our estimate to others in the literature and speculate as to reasons for their differences. We did, however, consider how our observed seroprevalence might have influenced our ability to detect statistically significant associations between *C. trachomatis* infection and prostate cancer. Given our findings, we are unlikely to have missed strong positive or inverse associations, but we cannot rule out weak to moderate positive (i.e., <1.96) or inverse (i.e., >0.65) associations.

Human papillomavirus infection

Our null findings for HPV-16, -18 and -33 infections are consistent with findings from several previous studies, but differ from those from several additional studies that observed either positive associations, potentially inverse associations and associations of debatable significance (15–21,40). In our interpretation of study findings, we considered several non-causal explanations for our results, including limited statistical power and assay validity. With respect to the former, we believe that limited statistical power is unlikely to explain our null findings because our study was as large or larger than previous studies and had similar seroprevalences of infection. With respect to test validity, although the assays we used, which were similar to assays used in all previous studies, had relatively low sensitivities for detecting current or past uncomplicated infections, we expected that they might have higher sensitivities for detecting HPV infections of potentially greater relevance for prostate carcinogenesis, such as infections of longer duration that might be more likely to involve the prostate or repeated episodes of infection. In this case, we expected that the potentially higher sensitivities of the assays would lead to a lesser degree of non-differential misclassification of exposure and a lesser degree of attenuation. Even in the case in which our assays did not have higher sensitivities for detecting potentially more etiologically-relevant infections, we estimated that non-differential misclassification of exposure could only have resulted in a minor degree of attenuation (e.g., from an odds ratio of 0.78 to 0.84 for HPV-16 infection, and an odds ratio of 1.21 to 1.14 for HPV-33 infection, assuming sensitivities of 50% and specificities of 98%).

Human herpesvirus type 8 infection

Our significant inverse findings for HHV-8 antibody seropositivity and prostate cancer differ from hypothesized findings, particularly for advanced stage disease, and from positive or null findings from three previous studies (24–26). We considered several possible non-causal explanations for these differences, including selection bias, differential assay validity and confounding. First, we believe that selection bias due to differential loss of HHV-8+/

HIV+ co-infected AIDS patients before case-control selection is unlikely to explain our findings because only 36 AIDS deaths (0.5% of all deaths) were observed in the HPFS cohort as of 2000. Second, differences in assay sensitivity and specificity are also unlikely to explain our inverse findings because any of these differences should be non-differential by case-control status, and thus should only result in an attenuation of study findings towards the null. Third, confounding by Mediterranean heritage, a correlate of classic Kaposi's sarcoma (41), is unlikely to explain our inverse findings because adjustment for Southern European ethnicity did not alter any of the results. Finally, although some studies have observed inverse associations between AIDS and prostate cancer possibly due to decreased frequency of prostate cancer screening in HIV+ men (42,43), confounding by HIV/AIDS is also unlikely to explain our findings because of the low expected prevalence of HIV infection in this cohort, and because adjustment for prostate cancer screening did not alter any of the results. One possible, albeit speculative, causal explanation for our inverse findings is long-term HHV-8 mediated skewing of the immune response from T_H1 towards T_H2, possibly by HHV-8 chemokines, vMIP-I, -II and –III (8), which has been hypothesized to protect against prostate cancer (44). Other possible non-causal explanations include confounding by another factor associated with both HHV-8 antibody seropositivity and prostate cancer, or chance.

Our predominantly Caucasian study population comprised of cases with typically earlier stage disease differs in racial and prostate cancer stage composition from some of the other previously-investigated study populations. For instance, one of the two main study populations investigated by Hoffman and colleagues (25) was predominantly African-Caribbean, and both were comprised of a high proportion of advanced stage cases (high PSA concentration at diagnosis). Therefore, if positive associations are limited to men of African descent, perhaps due to differences in immune response or other genes, or advanced stage disease, this may explain our inverse as opposed to positive findings. We attempted to reduce the likelihood of detecting falsely-positive associations by a) designing our study specifically to compare cases and controls from the same source population (i.e., the HPFS cohort) to avoid selection biases; b) matching closely on age (and using a matched analysis) to reduce the likelihood of confounding by age, as age may be associated with both increased cumulative incidence of infection and prostate cancer; c) adjusting for race/ethnicity to avoid confounding by Mediterranean heritage (as mentioned previously) and African heritage, both of which are typically associated with higher HHV-8 antibody seroprevalence and varying prostate cancer risk (45); and d) not placing any restrictions on control PSA concentration to avoid excluding controls with BPH or prostatitis, as these may potentially be associated with HHV-8 or other associated infections.

In conclusion, no associations were observed between *C. trachomatis* and HPV -16, -18 and -33 antibody seropositivity and prostate cancer, whereas a significant inverse association was observed between HHV-8 antibody seropositivity and prostate cancer in this large population of American male health professionals. As this study is the first, to our knowledge, to observe such an inverse association, similar additional studies are warranted before conclusions can be made regarding this association.

Acknowledgments

We are grateful to Jill Arnold, Elizabeth Frost-Hawes, Mira Kaufman, Laura Sampson, Yan Lydia Liu, and Mildred Wolff for their continued help in conducting the Health Professionals Follow-up Study. We thank Billie J. Wood, Barbara Silver and Stefanie Morosky for performing *C. trachomatis*, HPV and HHV-8 antibody testing, respectively. We also thank Ligia A. Pinto and Dr. Allan Hildesheim for generous provision of HPV-16 antibody positive control serum, Dr. Patti E. Gravitt for advice regarding HPV antibody testing, and Dr. Stephen R. Cole for discussion related to this manuscript.

Siobhan Sutcliffe was supported by a Doctoral Research Award from the Canadian Prostate Cancer Research Initiative/Canadian Institutes of Health Research and the Fund for Research and Progress in Urology, John Hopkins Medical Institutions. This work was supported by research grants CA55075, HL35464 (Harvard), and P50CA58236 (Hopkins) from the National Institutes of Health, and the Summer Epidemiology Program Fund, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health.

References

1. Ravich A, Ravich RA. Prophylaxis of cancer of the prostate, penis, and cervix by circumcision. N Y State J Med 1950;50:1519–20.
2. Dennis LK, Dawson DV. Meta-analysis of measures of sexual activity and prostate cancer. Epidemiology 2002;13:72–9. [PubMed: 11805589]
3. Taylor ML, Mainous AG 3rd, Wells BJ. Prostate cancer and sexually transmitted diseases: a meta-analysis. Fam Med 2005;37:506–12. [PubMed: 15988645]
4. Shurbaji MS, Gupta PK, Myers J. Immunohistochemical demonstration of chlamydial antigens in association with prostatitis. Mod Pathol 1988;1:348–51. [PubMed: 2467288]
5. Abdelatif OM, Chandler FW, McGuire BS Jr. *Chlamydia trachomatis* in chronic abacterial prostatitis: demonstration by colorimetric in situ hybridization. Hum Pathol 1991;22:41–4. [PubMed: 1985076]
6. Poletti F, Medici MC, Alinovi A, et al. Isolation of *Chlamydia trachomatis* from the prostatic cells in patients affected by nonacute abacterial prostatitis. J Urol 1985;134:691–3. [PubMed: 4032572]
7. Human papillomaviruses. IARC monographs on the evaluation of carcinogenic risks to humans. Lyon: IARC; 1995.
8. Moore, PS.; Chang, Y. Kaposi's sarcoma-associated herpesvirus. In: Knipe, DM.; Howley, PM.; Griffin, DE.; Lamb, RA.; Martin, MA.; Roizman, B.; Straus, SE., editors. Fields' virology 2. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 2803-33.
9. Strickler HD, Goedert JJ. Sexual behavior and evidence for an infectious cause of prostate cancer. Epidemiol Rev 2001;23:144–51. [PubMed: 11588840]
10. Blackbourn DJ, Levy JA. Human herpesvirus 8 in semen and prostate. AIDS 1997;11:249–50. [PubMed: 9030373]
11. Diamond C, Brodie SJ, Krieger JN, et al. Human herpesvirus 8 in the prostate glands of men with Kaposi's sarcoma. J Virol 1998;72:6223–7. [PubMed: 9621094]
12. Montgomery JD, Jacobson LP, Dhir R, Jenkins FJ. Detection of human herpesvirus 8 (HHV-8) in normal prostates. Prostate. 2006
13. Kuczyk M, Serth J, Machtens S, Jonas U. Detection of viral HPV 16 DNA in prostate cancer and benign prostatic hyperplasia by quantitative PCR-directed analysis. Prostate Cancer Prostatic Dis 2000;3:S23. [PubMed: 12497133]
14. Platz EA, De Marzo AM. Epidemiology of inflammation and prostate cancer. J Urol 2004;171:S36–40. [PubMed: 14713751]
15. Dillner J, Knekt P, Boman J, et al. Sero-epidemiological association between human-papillomavirus infection and risk of prostate cancer. Int J Cancer 1998;75:564–7. [PubMed: 9466657]
16. Hisada M, Rabkin CS, Strickler HD, Wright WE, Christianson RE, van den Berg BJ. Human papillomavirus antibody and risk of prostate cancer. JAMA 2000;283:340–1. [PubMed: 10647795]
17. Adami HO, Kuper H, Andersson SO, Bergstrom R, Dillner J. Prostate cancer risk and serologic evidence of human papilloma virus infection: a population-based case-control study. Cancer Epidemiol Biomarkers Prev 2003;12:872–5. [PubMed: 14504197]
18. Hayes RB, Pottern LM, Strickler H, et al. Sexual behaviour, STDs and risks for prostate cancer. Br J Cancer 2000;82:718–25. [PubMed: 10682688]
19. Strickler HD, Burk R, Shah K, et al. A multifaceted study of human papillomavirus and prostate carcinoma. Cancer 1998;82:1118–25. [PubMed: 9506358]
20. Korodi Z, Dillner J, Jellum E, et al. Human papillomavirus 16, 18, and 33 infections and risk of prostate cancer: a Nordic nested case-control study. Cancer Epidemiol Biomarkers Prev 2005;14:2952–5. [PubMed: 16365015]

21. Rosenblatt KA, Carter JJ, Iwasaki LM, Galloway DA, Stanford JL. Serologic evidence of human papillomavirus 16 and 18 infections and risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 2003;12:763–8. [PubMed: 12917208]
22. Anttila T, Tenkanen L, Lumme S, et al. Chlamydial antibodies and risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 2005;14:385–9. [PubMed: 15734962]
23. Rosenblatt KA, Wicklund KG, Stanford JL. Sexual factors and the risk of prostate cancer. Am J Epidemiol 2001;153:1152–8. [PubMed: 11415949]
24. Sitas F, Carrara H, Beral V, et al. Antibodies against human herpesvirus 8 in black South African patients with cancer. N Engl J Med 1999;340:1863–71. [PubMed: 10369849]
25. Hoffman LJ, Bunker CH, Pellett PE, et al. Elevated seroprevalence of human herpesvirus 8 among men with prostate cancer. J Infect Dis 2004;189:15–20. [PubMed: 14702148]
26. Korodi Z, Wang X, Tedeschi R, Knekt P, Dillner J. No serological evidence of association between prostate cancer and infection with herpes simplex virus type 2 or human herpesvirus type 8: a nested case-control study. J Infect Dis 2005;191:2008–11. [PubMed: 15897985]
27. Narvanen A, Puolakkainen M, Hao W, Kino K, Suni J. Detection of antibodies to *Chlamydia trachomatis* with peptide-based species-specific enzyme immunoassay. Infect Dis Obstet Gynecol 1997;5:349–54. [PubMed: 18476184]
28. Clad A, Freidank HM, Kunze M, et al. Detection of seroconversion and persistence of *Chlamydia trachomatis* antibodies in five different serological tests. Eur J Clin Microbiol Infect Dis 2000;19:932–7. [PubMed: 11205630]
29. Koivisto AL, Isoaho R, Von Hertzen L, et al. Chlamydial antibodies in an elderly Finnish population. Scand J Infect Dis 1999;31:135–9. [PubMed: 10447321]
30. Stamm, WE. *Chlamydia trachomatis* infections of the adult. In: Holmes, K.; Sparling, P.; Mardh, P.; Lemon, S.; Stamm, W.; Piot, P.; Wasserheit, J., editors. Sexually transmitted diseases. New York: The McGraw-Hill Companies, Inc; 1999. p. 407-22.
31. Viscidi RP, Ahdieh-Grant L, Clayman B, et al. Serum immunoglobulin G response to Human Papillomavirus type 16 virus-like particles in Human Immunodeficiency Virus (HIV)-positive and risk-matched HIV-negative women. JID 2003;187:194–205. [PubMed: 12552444]
32. Dillner J. The serological response to papillomaviruses. Semin Cancer Biol 1999;9:423–30. [PubMed: 10712889]
33. Shah KV, Viscidi RP, Alberg AJ, Helzlsouer KJ, Comstock GW. Antibodies to human papillomavirus 16 and subsequent in situ or invasive cancer of the cervix. Cancer Epidemiol Biomarkers Prev 1997;6:233–7. [PubMed: 9107427]
34. Jenkins FJ, Hoffman LJ, Liegey-Dougall A. Reactivation of and primary infection with human herpesvirus 8 among solid-organ transplant recipients. J Infect Dis 2002;185:1238–43. [PubMed: 12001040]
35. Pellett PE, Wright DJ, Engels EA, et al. Multicenter comparison of serologic assays and estimation of human herpesvirus 8 seroprevalence among US blood donors. Transfusion 2003;43:1260–8. [PubMed: 12919429]
36. Biggar RJ, Engels EA, Whitby D, Kedes DH, Goedert JJ. Antibody reactivity to latent and lytic antigens to human herpesvirus-8 in longitudinally followed homosexual men. J Infect Dis 2003;187:12–8. [PubMed: 12508141]
37. Anttila T, Saikku P, Koskela P, et al. Serotypes of *Chlamydia trachomatis* and risk for development of cervical squamous cell carcinoma. Jama 2001;285:47–51. [PubMed: 11150108]
38. Morre SA, Munk C, Persson K, et al. Comparison of three commercially available peptide-based immunoglobulin G (IgG) and IgA assays to microimmunofluorescence assay for detection of *Chlamydia trachomatis* antibodies. J Clin Microbiol 2002;40:584–7. [PubMed: 11825974]
39. Bas S, Muzzin P, Ninet B, Bornand JE, Scieux C, Vischer TL. Chlamydial serology: comparative diagnostic value of immunoblotting, microimmunofluorescence test, and immunoassays using different recombinant proteins as antigens. J Clin Microbiol 2001;39:1368–77. [PubMed: 11283058]
40. Strickler HD, Schiffman MH, Shah KV, et al. A survey of human papillomavirus 16 antibodies in patients with epithelial cancers. Eur J Cancer Prev 1998;7:305–13. [PubMed: 9806119]

41. Franceschi S, Geddes M. Epidemiology of classic Kaposi's sarcoma, with special reference to mediterranean population. Tumori 1995;81:308–14. [PubMed: 8804445]

42. Biggar RJ, Kirby KA, Atkinson J, McNeel TS, Engels E. Cancer risk in elderly persons with HIV/AIDS. J Acquir Immune Defic Syndr 2004;36:861–8. [PubMed: 15213571]

43. Gallagher B, Wang Z, Schymura MJ, Kahn A, Fordyce EJ. Cancer incidence in New York State acquired immunodeficiency syndrome patients. Am J Epidemiol 2001;154:544–56. [PubMed: 11549560]

44. Platz, EA.; Drake, CG.; Leitzmann, MF.; Camargo, CAJ.; Giovannucci, E. Asthma and risk of prostate cancer in a large prospective cohort study. Abstract 206. Breckenridge, CO: Keystone symposia: Inflammation and cancer; 2005.

45. Platz EA, Rimm EB, Willett WC, Kantoff PW, Giovannucci E. Racial variation in prostate cancer incidence and in hormonal system markers among male health professionals. J Natl Cancer Inst 2000;92:2009–17. [PubMed: 11121463]

Table 1

Characteristics[*] of 691 prostate cancer cases and 691 matched controls in the Health Professionals Follow-up Study, 1993–2000

	Cases	Controls	p-value[†]
Mean age at blood draw (years)	65.8	65.7	Matching variable
Race/ethnicity (%):			
Southern European	22.0	20.4	
Scandinavian	10.6	9.7	
Other Caucasian	62.1	64.1	0.70
African-American	0.9	0.4	
Asian	0.4	0.3	
Other	4.0	5.1	
Family history of prostate cancer (%)[‡]	21.8	16.8	0.02
Mean height in 1986 (inches)	70.1	70.1	0.76
Smoked cigarettes in the past 10 years (%)	16.5	17.7	0.61
Mean intakes of:			
Total energy (kcal/day)	2012	2035	0.47
Alcohol (g/day)	11.8	11.5	0.70
Tomato sauce (servings/day)[§]	0.19	0.19	0.89
Red meat (servings/day)[§]	1.03	1.01	0.70
Fish (servings/day)[§]	0.31	0.33	0.04
Calcium (mg/day)	950	951	0.95
Alpha-linolenic acid (g/day)[‖]	1.12	1.12	0.93
Fructose in 1990 (g/day)[‖]	49.0	48.9	0.88
Vitamin E supplementation (≥15 mg/day, %)	37.5	34.4	0.28
Zinc supplementation (≥101 mg/day, %)	0.1	0.4	0.62
Regular (2+ times/wk) use of non-steroidal anti-inflammatory drugs (%)	48.8	46.9	0.52
Any vigorous leisure-time physical activity (%)	58.0	57.4	0.87
Vasectomy (%)	25.9	26.5	0.85
Diabetes mellitus type 2 (%)	6.2	5.8	0.82
Mean number of PSA tests before the date of case diagnosis	2.6	2.5	0.15
History of (%):			
Gonorrhea[**]	2.6	2.6	1.00
Syphilis[**]	0.1	0.0	1.00
Trichomonosis[††]	12.6	9.4	0.07
Clinical prostatitis[**]	22.0	18.0	0.07
Mean monthly ejaculation frequency from[**]:			
Ages 20 to 29	13.6	14.0	0.17
Ages 40 to 49	10.7	11.1	0.18
Consumed alcohol, ages 18 to 22 (%)[‡‡]	69.6	68.2	0.61

	Cases	Controls	p-value†
Smoked cigarettes before age 30 (%)§§	47.9	49.5	0.59
Mean BMI at age 21 (kg/m2)§§	22.8	22.9	0.24
Any vigorous physical activity (%) in**:			
High school	84.8	85.0	1.00
College	76.6	76.8	0.95

*Unless otherwise indicated, values are from the 1994 follow-up questionnaire.

†Assessed by paired t-test for continuous variables, McNemar's test for binary variables and likelihood ratio test for categorical variables.

‡Assessed in 1990 through 1996.

§Cumulative mean intake between 1986 and 1994.

||Adjusted for total energy intake.

**Assessed in 1992.

††Assessed by antibody serostatus from blood samples collected in 1993–5.

‡‡Assessed in 1988.

§§Assessed in 1986.

Table 2

Chlamydia trachomatis, human papillomavirus (HPV), and human herpesvirus type 8 (HHV-8) antibody concentration in 691 prostate cancer cases and 691 matched controls in the Health Professionals Follow-up Study, 1993–2000

	Cases	Controls	p-value*
C. trachomatis infection			
Mean S/CO	0.228	0.228	0.99
Median S/CO	0.076	0.083	0.49
Score (%):			
S/CO <1.0	94.8	94.8	
1.0≤S/CO <1.4	1.2	1.7	0.66
1.4≤S/CO <2.5	2.3	2.3	
S/CO≥2.5	1.7	1.2	
S/CO≥1.4 (%)	4.0	3.5	0.90
HPV infection			
HPV-16			
Mean OD	0.051	0.052	0.77
Median OD	0.038	0.037	0.59
OD >0.085 (%)†	7.5	8.8	0.44
HPV-18			
Mean OD	0.037	0.040	0.44
Median OD	0.030	0.029	0.14
OD >0.070 (%)†	6.1	5.8	0.91
HPV-33			
Mean OD	0.030	0.026	0.13
Median OD	0.019	0.018	0.19
OD >0.050 (%)†	7.2	6.4	0.60
Any HPV infection‡ (%)	15.5	16.5	0.66
Number of HPV infections‡ (%):			
0	84.5	83.5	
1	11.6	13.0	0.77
2	2.5	2.5	
3	1.4	1.0	
HHV-8 infection			
Positive§ (%)	13.5	18.0	0.02
Titer (among positive participants, %):			
≤200	49.5	43.6	
400	22.6	29.0	0.49‖, 0.73**
800	18.3	11.3	
≥1,600	9.7	16.1	

S/CO = signal to cut-off ratio; OD = optical density.

[*] Assessed by paired t-test for mean values, Wilcoxon signed-rank test for median values, McNemar's test for binary variables, and the likelihood ratio test for categorical variables.

[†] Based on OD results from virginal women.

[‡] HPV-16, -18 or -33.

[§] Titer ≥100

[||] Based on a comparison of the entire distribution of titers.

[**] Based on a comparison of high titers (≥1,600).

Table 3

Odds ratios (ORs) and 95% confidence intervals (CIs) of prostate cancer by *Chlamydia trachomatis*, human papillomavirus (HPV) and human herpesvirus type 8 (HHV-8) serostatus in 691 matched pairs nested in the Health Professionals Follow-up Study, 1993–2000

	Seronegative		Seropositive		
	Cases/controls	OR (95% CI)*	Cases/controls	OR (95% CI)*	OR (95% CI)†
Total prostate cancer:					
C. trachomatis‡	655/655	1.00	28/24	1.15 (0.67–1.99)	1.13 (0.65–1.96)
HPV-16§	639/630	1.00	52/61	0.84 (0.57–1.24)	0.83 (0.57–1.23)
HPV-18‖	649/651	1.00	42/40	1.06 (0.67–1.66)	1.04 (0.66–1.64)
HPV-33**	641/647	1.00	50/44	1.14 (0.76–1.72)	1.14 (0.76–1.72)
HPV-16, -18 or -33	584/577	1.00	107/114	0.93 (0.70–1.24)	0.92 (0.69–1.22)
HHV-8††	598/567	1.00	93/124	0.70 (0.52–0.95)	0.70 (0.52–0.95)
Low-grade (Gleason sum <7) prostate cancer:					
C. trachomatis‡	363/361	1.00	15/14	1.04 (0.50–2.16)	1.01 (0.48–2.11)
HPV-16§	349/350	1.00	31/30	1.04 (0.62–1.74)	1.05 (0.62–1.77)
HPV-18‖	359/363	1.00	21/17	1.29 (0.64–2.58)	1.29 (0.64–2.61)
HPV-33**	353/356	1.00	27/24	1.13 (0.64–1.98)	1.13 (0.65–1.99)
HPV-16, -18 or -33	321/320	1.00	59/60	0.98 (0.66–1.45)	0.98 (0.66–1.46)
HHV-8††	328/308	1.00	52/72	0.65 (0.43–0.98)	0.64 (0.42–0.96)
High-grade (Gleason sum ≥7) prostate cancer:					
C. trachomatis‡	228/228	1.00	11/8	1.34 (0.54–3.34)	1.34 (0.52–3.41)
HPV-16§	230/218	1.00	13/25	0.50 (0.25–1.00)	0.46 (0.23–0.94)
HPV-18‖	224/225	1.00	19/18	1.06 (0.55–2.05)	1.01 (0.52–1.97)
HPV-33**	226/230	1.00	17/13	1.31 (0.64–2.69)	1.29 (0.62–2.68)
HPV-16, -18 or -33	207/202	1.00	36/41	0.86 (0.53–1.39)	0.81 (0.50–1.33)
HHV-8††	210/199	1.00	33/44	0.72 (0.45–1.17)	0.76 (0.47–1.23)
Organ-confined (≤T2 and N0M0) prostate cancer:					

	Seronegative		Seropositive		
	Cases/controls	**OR (95% CI)***	**Cases/controls**	**OR (95% CI)***	**OR (95% CI)†**
C. trachomatis‡	487/482	1.00	18/16	1.08 (0.55–2.14)	1.05 (0.53–2.08)
HPV-16§	468/461	1.00	39/46	0.84 (0.54–1.30)	0.82 (0.53–1.29)
HPV-18‖	476/474	1.00	31/33	0.93 (0.55–1.57)	0.90 (0.54–1.53)
HPV-33**	469/475	1.00	38/32	1.19 (0.74–1.92)	1.18 (0.73–1.91)
HPV-16, -18 or -33	425/421	1.00	82/86	0.94 (0.68–1.32)	0.92 (0.66–1.29)
HHV-8††	440/413	1.00	67/94	0.66 (0.47–0.94)	0.66 (0.46–0.93)

*Estimated by conditional logistic regression. Cases and controls were matched on age; time, season and year of blood draw; and PSA testing history prior to 1993–5.

†Estimated by conditional logistic regression including terms for race/ethnicity (Caucasian, non-Caucasian) and cumulative family history of prostate cancer.

‡Signal to cut-off ratio ≥ 1.4.

§Optical density >0.085.

‖Optical density >0.070.

**Optical density >0.050.

††Titer ≥ 100.