Seroreactivity to Human Papillomavirus (HPV) Types 16, 18, or 31 and Risk of Subsequent HPV Infection: Results from a Population-Based Study in Costa Rica

Raphael P. Viscidi,1 Mark Schiffman,2 Allan Hildesheim,2 Rolando Herrero,3 Philip E. Castle,2 Maria C. Bratti,3 Ana Cecilia Rodriguez,3 Mark E. Sherman,2 Sophia Wang,2 Barbara Clayman,1 and Robert D. Burk4

1Johns Hopkins University School of Medicine, Baltimore, Maryland; 2Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, Maryland; 3Proyecto Epidemiológico Guanacaste, Costa Rican Foundation for Health Sciences, San José, Costa Rica; and 4Albert Einstein College of Medicine, Bronx, New York

Abstract

Whether antibodies to human papillomavirus (HPV) capsids, elicited by natural infection, are protective is unknown. This question was addressed in a population-based cohort of 7046 women in Costa Rica by examining the association between baseline seroreactivity to HPV-16, HPV-18, or HPV-31 virus-like particles and the risk of subsequent HPV infection at a follow-up visit 5–7 years after enrollment. Seropositivity to HPV-16, HPV-18, or HPV-31 was not associated with a statistically significant decreased risk of infection with the homologous HPV type [relative risk (RR) and [95% confidence interval (CI)], 0.74 (0.45–1.2), 1.5 (0.83–2.7), and 0.94 (0.48–1.8), respectively]. Seropositivity to HPV-16 or HPV-31 was not associated with a decreased risk of infection with HPV-16 or its genetically related types [RR (95% CI), 0.82 (0.61–1.1) and 0.93 (0.68–1.2), respectively]. Seropositivity to HPV-18 was not associated with a decreased risk of infection with HPV-18 or its genetically related types (RR 1.3; 95% CI 1.0–1.8). Thus, we did not observe immunity, although a protective effect from natural infection cannot be excluded because of the limits of available assays and study designs.

Introduction

Mucosal-genital human papillomaviruses (HPVs), of which there are >40 types, are very common sexually transmitted infections (1). Most infections are benign and are cleared spontaneously within 1–2 years (2). After a HPV infection, ~50% of women develop a systemic humoral immune response directed at the major viral capsid protein (3). Whether this response will confer protection against reinfection with the same and related types is unknown. To address this question, we tested blood samples from women enrolled in a population-based natural history study for seroreactivity to HPV types 16, 18, and 31 capsids and examined the association between baseline seroreactivity and subsequent detection of HPV DNA at the cervix.

Materials and Methods

Study Subjects. A population-based cohort of 10,049 women (94% of eligible women) was established in Guanacaste, Costa Rica, in 1993–1994. The women comprised a representative sample of the adult female population of Guanacaste. Details of the cohort have been reported previously (4). Briefly, participating women were administered a detailed questionnaire that included questions on sociodemographic and sexual history. A pelvic examination was performed for cervical cancer screening and for obtaining cervical cells for HPV testing. Approximately 15-ml blood samples were drawn and processed by centrifugation at the collection site, and the plasma specimen was frozen at −70°C. All participants provided informed consent in accordance with guidelines of the United States Department of Health and Human Services. The National Cancer Institute and local institutional review boards approved the study. The present study included 7046 women from whom an enrollment plasma sample and HPV DNA test result were available, and a HPV DNA test result was available at a follow-up visit between 5 and 7 years after enrollment or ≤5 years for women censored and treated for indications of high-grade cervical neoplasia (HPV-16, n = 363; HPV-18, n = 290, HPV-31, n = 288). Exclusion of the latter women from analyses yielded the same conclusions.

HPV DNA Testing. Cervical cells were tested for HPV DNA using MY09-MY11 consensus primer PCR with AmpliTaq Gold DNA polymerase and dot blot to detect genital HPV type-specific DNA as described previously (5).

HPV Virus-Like Particle (VLP)-Based Enzyme Immunoassay. HPV VLPs were prepared in Trichoplusia ni (High Five) cells (Invitrogen, Carlsbad, CA) from recombinant baculoviruses expressing the L1 and L2 genes of HPV-16 or HPV-31 or the L1 gene alone of HPV-18 and purified by density gradient ultracentrifugation and column chromatography techniques as described previously (6). IgG to HPV-16, HPV-18, and HPV-31 was measured by enzyme immunoassay as previously described with minor modifications (6). IgA to HPV-16 was detected using peroxidase-labeled goat antihuman IgA (Southern Biotechnology, Inc., Birmingham, AL). The cut points for seropositivity were determined from the reactivity of concurrently tested plasma samples from more than 500 self-reported virgins in the study population. The mean and SD of absorbance values for the virginal subjects were calculated, and

Received 6/19/03; revised 10/24/03; accepted 12/9/03.

Grant support: National Cancer Institute Grants NO2-CP-81024-50 (to R. P. V.), CA78527 (to R. D. B.), NO1-CP-21081, NO1-CP-33061, NO1-CP-40542, and NO1-CP-506535.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Raphael P. Viscidi, The Johns Hopkins Hospital, Blalock Room 1105, 600 North Wolfe Street, Baltimore MD 21287. Phone: (410) 614-1494; Fax: (410) 955-3723; E-mail: rviscid1@jhmi.edu.
values greater than the mean plus 2 SDs were excluded. The analysis was repeated on the remaining samples until no additional absorbance values could be excluded by this criterion (n = 33, n = 37, n = 47 excluded outliers for HPV-16, HPV-18, and HPV-31, respectively). A positive cut point of 3 SDs above the mean of this distribution was chosen. A strong positive cut point was defined as 5 SDs above the mean of this distribution.

Statistical Analysis. Relative risks (RRs) and corresponding 95% confidence intervals (CIs) were calculated directly.

Results

Of the 10,049 participants in the Guanacaste cohort, we excluded 1351 based on findings at enrollment, including 628 women who had a hysterectomy, 140 women who had a prevalent high-grade cervical intraepithelial lesion or cancer, and 583 women who were virgins. Of these 8698 women, we excluded an additional 1652 for the following reasons: refusal to undergo a pelvic examination (291 women) or lack of enrollment serology (36 women), enrollment PCR (23 women), or follow-up PCR (1302 women).

The baseline seroprevalence for the 7046 women analyzed in this report was HPV-16, 22.5%, HPV-18, 22.7%, and HPV-31, 21.8%. The association between HPV DNA positivity and seropositivity varied by type. For HPV-16, 40.3% of PCR-positive women were seropositive, and 15.3% of PCR-negative women were seropositive. The corresponding percentages for HPV-18 were 26.9 and 15.8% and for HPV-31 were 50.6 and 16.5%, respectively.

There were no significant differences in new infections with the homologous HPV type among seropositive and seronegative women (Table 1). For women who were HPV-16 DNA negative at baseline, 1.7% of HPV-16 seronegative and 1.3% of HPV-16 seropositive women had a new HPV-16 infection at the follow-up visit. For HPV-18, the corresponding rates were 0.7 and 1.0%, respectively, and for HPV-31, 0.8 and 0.7%, respectively. Similar results were obtained using the high cut point for seropositivity (Table 1). A high level of antibody to HPV-16 VLPs was associated with a 40% reduction in risk, but this was not statistically significant. Additional stratification of the women by level of antibody was not feasible because of the small number of new HPV infections.

For a subset of ~2000 women, representing those at highest risk for a high-grade cervical intraepithelial lesion or cancer, blood samples obtained 1 year after enrollment were also tested for reactivity to HPV-16, HPV-18, and HPV-31 VLPs. Higher risk was defined as a positive screening result, including a low-grade or equivocal cytological abnormality, a mild cervicographic visual abnormality, or a lifetime history of more than four sexual partners. Among women who were persistently seropositive for HPV-16 (234 women), HPV-18 (203 women) or HPV-31 (267 women), the number of women with a new infection with the homologous HPV type was 4, 2, and 2, respectively. Within the limitations of small numbers, persistently seropositive women did not differ from seronegative or one-time seropositive women in the rate of new infection with the homologous HPV type (data not shown). Because a previously published study found that IgA reactivity to HPV-16 VLPs was a correlate of immune protection (7), baseline blood specimens were tested for IgA class-specific antibodies to HPV-16 VLPs on a stratified sample of 1222 women selected based on baseline and follow-up HPV 16 DNA status. Five (6.9%) of 72 HPV-16 IgA seropositive women and 82 (7.6%) of 1078 seronegative women had a new HPV-16 infection (RR 0.91; 95% CI 0.38–2.18).

We also looked at type specific and group-specific cross protection (Table 2). Seropositivity to one of the three types was not associated with a reduced risk of new detection of the other two. Two hundred twenty-nine (4.4%) of 5162 HPV-16 seronegative women and 50 (3.6%) of 1379 seropositive women had new infections with HPV-16-related types (HPV-16, HPV-31, HPV-33, HPV-35, HPV-52, HPV-58, or HPV-67), yielding a RR of 0.82 (95% CI 0.61–1.1). A null association was also observed for HPV-31 seropositivity and new infections with HPV-16-related types. One hundred thirty-seven (2.6%) of 5212 HPV-18 seronegative and 52 (3.5%) of 1491 seropositive women who had new infections with HPV-18-related types (HPV-18, HPV-39, HPV-45, HPV-59, HPV-68, or HPV-70), yielding a RR of 1.3 (95% CI 1.0–1.8). A small but statistically significant increased risk of infection with HPV-18-related types was observed among women seropositive for HPV-16 (RR 1.4; 95% CI 1.1–2.0) or HPV-31 (RR 1.6; 95% CI 1.1–2.1). Considering all mucosal genital HPV types as a whole, women seropositive and seronegative for HPV-16, HPV-18, or HPV-31 capsids did not differ in the risk of a new HPV infection (Table 2).

<table>
<thead>
<tr>
<th>HPV DNA acquisitiona</th>
<th>Serostatus</th>
<th>No. of women</th>
<th>No. (%) DNA positive</th>
<th>Relative risk (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-16</td>
<td>Negative</td>
<td>5360</td>
<td>92 (1.7)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>1492</td>
<td>19 (1.3)</td>
<td>0.74 (0.45–1.2)</td>
</tr>
<tr>
<td></td>
<td>Low positive</td>
<td>444</td>
<td>8 (1.8)</td>
<td>1.1 (0.51–2.2)</td>
</tr>
<tr>
<td></td>
<td>High positive</td>
<td>1048</td>
<td>11 (1.0)</td>
<td>0.61 (0.33–1.1)</td>
</tr>
<tr>
<td>HPV-18</td>
<td>Negative</td>
<td>5384</td>
<td>37 (0.7)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>1565</td>
<td>16 (1.0)</td>
<td>1.5 (0.83–2.7)</td>
</tr>
<tr>
<td></td>
<td>Low positive</td>
<td>470</td>
<td>7 (1.5)</td>
<td>2.2 (0.97–4.8)</td>
</tr>
<tr>
<td></td>
<td>High positive</td>
<td>1095</td>
<td>9 (0.8)</td>
<td>1.2 (0.58–2.5)</td>
</tr>
<tr>
<td>HPV-31</td>
<td>Negative</td>
<td>5459</td>
<td>43 (0.8)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>1490</td>
<td>11 (0.7)</td>
<td>0.94 (0.48–1.8)</td>
</tr>
<tr>
<td></td>
<td>Low positive</td>
<td>344</td>
<td>2 (0.6)</td>
<td>0.74 (0.18–3.0)</td>
</tr>
<tr>
<td></td>
<td>High positive</td>
<td>1146</td>
<td>9 (0.8)</td>
<td>1.0 (0.49–2.0)</td>
</tr>
</tbody>
</table>

* The type-specific analyses excluded seropositive women that were DNA-positive at enrollment (6.5% of HPV-16 seropositive, 1.9% of HPV-18 seropositive, and 3.4% of HPV-31 seropositive).
Discussion

For many viruses, antibodies directed at epitopes exposed on the surface of viral particles are protective against infection (8). For papillomaviruses, this principle has been established by the demonstration in animal papillomavirus models that protection can be passively transferred to naïve animals by immune sera or purified IgG induced by immunization with VLPs (9, 10). VLPs are empty capsids formed by self-assembly of the L1 major capsid protein (11). They are structurally and antigenically very similar to authentic virions, and immunization with L1 VLPs induces antibodies directed against conformational epitopes displayed on the surface of viral capsids (11). HPV L1 VLP vaccines administered to human volunteers have generated high levels of antibodies to HPV capsids (11), and a HPV-16 L1 VLP vaccine was recently shown to reduce the incidence of HPV-16 infection (12, 13). It is therefore reasonable to ask whether anti-VLP antibodies elicited by natural HPV infection are a correlate of immune protection against reinfection.

Our study failed to demonstrate that serum antibodies to HPV-16, HPV-18, or HPV-31 capsids, elicited by natural infection, are associated with immune protection against reinfection with the homologous HPV type or the other two heterologous HPV types. We also did not find evidence for group-specific immune protection or protection against reinfection with genital mucosal HPV types as a whole. A previous study by Ho et al. (7) reported that a sustained high level of antibody to HPV-16 VLPs was associated with reduced risk for subsequent infection with HPV-16 and its genetically related types. Our study differed from the former in the demographic characteristics of the population, definition of immune status, duration of follow-up, and analytical method. The Guanacaste project is a population-based cohort of women ages 18 –94 years (median age, 37 years). In contrast, the Ho et al. (7) study included young women with a mean age of 20 years. With the exception of a subset of the women, we defined serostatus by a one-time measurement of anti-VLP antibodies. In the Ho et al. (7) study, the significant correlate of immunity was seropositivity at two or more time points. We measured new HPV infection at a single time point 5–7 years after enrollment, whereas the Ho et al. (7) study monitored women every 6 months for 3 years. In our analysis, we excluded women with a HPV infection of interest at baseline and compared rates of new infections in the remaining seropositive and seronegative women. In the Ho et al. (7) study, rates of new infections with HPV-16 or HPV-16-related types were compared in HPV-16 seropositive and seronegative women who had an initial infection with a different HPV type. Whether these differences explain the conflicting results of the studies will need to be addressed in future studies and analyses.

Given the experimental evidence for a protective role of antibodies to HPV capsids and the ~100% efficacy of an experimental VLP-based vaccine administered to human subjects in a Phase II trial, why did seropositive women in our study have the same risk of a subsequent HPV infection as the seronegative women? The most likely explanation may be the low levels of anti-VLP antibodies elicited after natural HPV infection. In two studies that compared vaccinated women to HPV-exposed unvaccinated women, the levels of antibody induced by HPV-16 infection were 50 – 60-fold lower than post-vaccination levels (12, 13). In addition, because new infections were detected 5–7 years after enrollment and serological testing, levels may have been even lower at the time women were re-exposed to HPV.

Our study has a number of limitations. Until we complete variant-level DNA testing of all specimens collected throughout follow-up on all women in the cohort, we cannot estimate what percentage of the newly detected infections may have been incident as opposed to recurrent. We did not examine the possibility that antibody may confer short-term protection or protection against persistent infection. Regarding the latter point, in the recent trial of an HPV-16 vaccine, 6 vaccine recipients were transiently positive for HPV-16 DNA at a single visit despite high levels of serum antibody (13). We did not measure local antibody levels at the cervix. Another limitation, and a difficulty for any study of immune protection, is the potential difference between seropositive and seronegative women in the risk of exposure to HPV because of differences in sexual behavior. If fewer exposures occur among seronegative women, the relative risk would underestimate the extent of protection provided by antibody. Greater exposure to HPV may be the explanation for the increased risk of new infection with HPV-18-related types that we observed in HPV-16 and HPV-31.

---

Table 2  Risk of subsequent type-specific, group specific or any type HPV infection at a 5–7 year follow-up visit in relation to baseline HPV-16, HPV-18, or HPV-31 seropositivity

<table>
<thead>
<tr>
<th>DNA acquisitiona</th>
<th>Seropositivity to</th>
<th>HPV-16</th>
<th>HPV-18</th>
<th>HPV-31</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. Seronegative</td>
<td>No. DNA positive</td>
<td>RR (95% CI)</td>
<td>Total no. Seronegative</td>
</tr>
<tr>
<td>HPV-16</td>
<td>Seronegative 5360 192</td>
<td>1</td>
<td>1</td>
<td>5314 88</td>
</tr>
<tr>
<td>HPV-18</td>
<td>Seronegative 5397 37</td>
<td>1</td>
<td>1</td>
<td>5384 37</td>
</tr>
<tr>
<td>HPV-31</td>
<td>Seronegative 5575 16</td>
<td>1.5 (0.84–2.7)</td>
<td>1</td>
<td>1565 16</td>
</tr>
<tr>
<td>HPV-16-Cladeb</td>
<td>Seronegative 5162 229</td>
<td>1</td>
<td>1</td>
<td>5100 217</td>
</tr>
<tr>
<td>HPV-18-Cladec</td>
<td>Seronegative 1379 50</td>
<td>0.82 (0.61–1.1)</td>
<td>1</td>
<td>1436 60</td>
</tr>
<tr>
<td>Any HPV</td>
<td>Seronegative 1475 55</td>
<td>1.4 (1.1–2.0)</td>
<td>1</td>
<td>1491 52</td>
</tr>
</tbody>
</table>

- a Among women who were DNA negative at enrollment for that (those) type(s).
- b HPV types 16, 31, 33, 35, 52, 58, and 67.
- c HPV types 18, 39, 45, 59, 68, and 70.
seropositive women. Finally, for some analyses, the sample size of new cases was small.

Our findings do not signify that there is no naturally induced protective immunity to HPV because serological tests may not identify all immune individuals. Approximately 50% of HPV-infected women do not seroconvert (14). In general, residual imperfections in HPV serology and DNA testing might have affected the results. Specifically, our HPV-18 assay demonstrated lower seroprevalence than the corresponding HPV-16 and HPV-31 results for unknown reasons. However, seronegative women may be protected against reinfection as a result of being primed for an anamnestic antibody response or by non-antibody-mediated effector mechanisms. Future studies of immune protection should include other measures of immunity such as numbers of antigen-specific T-helper and cytotoxic T cells. Our study may have implications for HPV vaccine development because if vaccine-induced antibody levels decline over time to the levels seen after natural infection, then vaccinated women may no longer be protected.

References


Seroreactivity to Human Papillomavirus (HPV) Types 16, 18, or 31 and Risk of Subsequent HPV Infection: Results from a Population-Based Study in Costa Rica

Raphael P. Viscidi, Mark Schiffman, Allan Hildesheim, et al.