Detection of Cervical Antibodies to Human Papillomavirus Type 16 (HPV-16) Capsid Antigens in Relation to Detection of HPV-16 DNA and Cervical Lesions

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A more sensitive luminescence immunoassay (LIA) for human papillomavirus type 16 (HPV-16) was developed and used to measure HPV-16 antibodies in cervical samples from 292 college-aged women who were examined at 4-month intervals. Of the 609 collected samples, IgG, IgA, and secretory piece-associated antibodies to HPV-16 were detected in 12%, 6%, and 8%, respectively, of samples tested. Cervical IgG antibodies were most strongly associated with HPV-16 DNA detected within the previous 12 months (odds ratio, 3.3; 95% confidence interval, 1.4–7.8). Secretory IgA (cervical IgA– and secretory piece–positive) was most strongly associated with detection of a squamous intraepithelial lesions 4–8 months earlier (odds ratio, 6.4; 95% confidence interval, 1.9–21.8). As with serum HPV-16 antibodies, there appears to be a several-month delay between cervical HPV infection and detection of cervical antibodies.

Certain human papillomavirus (HPV) types cause genital warts, and other types have been implicated in the etiology of most anogenital cancers [1–3]. It has been difficult to study the humoral immune response to HPV, because these viruses are difficult to propagate in the laboratory. To circumvent this problem, investigators have produced empty HPV capsids by use of vaccinia virus, baculovirus, and yeast expression systems [4–8]. These in vitro–produced HPV capsids appear to be structurally identical to authentic viral capsids [9] and have been used as antigenic targets for numerous serologic surveys of the immune response to HPV [10–16]. Serum antibodies to HPV-6 or -11 correlated with the detection of HPV-6 or -11 DNA and genital warts. Similarly, detection of serum antibodies to HPV-16 or -31 correlates with the detection of HPV-16 or -31 DNA and precancerous lesions of the cervix and anus [10–12, 15]. HPV-16 antibodies are more likely to be detected among subjects who are repeatedly positive for HPV DNA detection [11, 16]. The antibody responses seen are mainly type-specific and directed against conformational epitopes [10]. A prolonged median lag period for serum antibody development of ~12 months from the first detection of HPV-16 DNA has been noted, and the titer of detectable serum antibodies has been low (maximal, 1/200) [11].

Animal and human studies have begun to delineate the extensive role of the mucosal immune system in the prevention of disease [17]. Antibody production (particularly IgA combined with secretory piece) can be detected in mucosal tissues, including those of the female reproductive tract [18–20]. Antigenic stimulation at one mucosal surface leads to the development of an antigen-specific immune response at distant mucosal surfaces, indicating the existence of a common mucosal immune system [18]. Because HPV infects mucosal sites, it is plausible that a local antibody response may develop more quickly, be more vigorous (a higher titer of antibody) than a systemic response, and potentially protect the host from future infection. Early studies of the mucosal response to HPV infection used either bovine papillomavirus or a denatured HPV protein as antigen [21–23], which made interpretation of these studies difficult. A study by Wang et al. [24] demonstrated HPV-16 antibodies in cervical swab specimens from a group of women who were referred for colposcopy with abnormal Pap smears. Our study describes the development of a more sensitive luminescence immunoassay (LIA) to detect cervical HPV-16 antibodies. This LIA was then used to detect HPV-16 cervical antibodies among a cohort of college-aged women enrolled in a longitudinal study of the natural history of HPV infection.

Materials and Methods

Study population. The study population includes a sample of 305 female university students between 18 and 24 years of age who...
were enrolled in a study of the natural history of HPV infection at the University of Washington (Seattle). In brief, recruitment letters were sent to a random sample of freshman women university students, of whom 628 called the study assistant, were 18–20 years of age, and were residents of the state of Washington with plans to stay in the area during the next 3 years. Of 628 eligible women, 603 (96%) were enrolled in the natural history study, of whom 305 consecutive subjects participated in the present study of cervical immunity to HPV. Clinic visits were scheduled at 4-month intervals for 3–4 years. At each clinic visit, participants underwent a standardized interview, physical examination, collection of exfoliated cervical cells by cervical brush and spatula for Pap smear, collection of cervical and vulvovaginal swab samples for detection of HPV DNA, and blood draw (20 mL). From June 1994 until March 1996, cervical secretions were collected by the wick technique from a consecutive series of 305 women who provided a total of 696 samples [25, 26]. After collection, the samples were frozen until processed. Samples were then thawed and vortexed, and the wicks were removed before samples were centrifuged at 14,000 g for 15 min. The supernatant volumes were recorded, 50 μL was removed for testing of occult blood (Hemoccult; SmithKline Diagnostics, San Jose, CA), and 22 μL was removed for protein assay (BCA; Pierce, Rockford, IL).

**Detection of HPV DNA.** The presence of HPV DNA in cervical and vulvovaginal swab specimens was determined by polymerase chain reaction (PCR) with use of L1 consensus primers. In brief, primers PC04 and GH2 were used to prime the synthesis of a 256-bp fragment of the eukaryotic β-globin gene and primers MY09 and MY11 (MY09: CGTCCMARRGGAW ACTGATC; MY11: GCMCAAGGGWCATAAYAATGG [M = A + C; R = A + G; W = A + T; Y = C + T]) were used to prime the synthesis of a 450-bp fragment of the HPV L1 gene [27]. After amplification, the PCR products were electrophoresed, stained with ethidium bromide, and observed under ultraviolet illumination. Amplified samples demonstrating the 250-bp β-globin fragment were considered to have sufficient numbers of cells for analysis for HPV typing by dot-blot hybridization. The PCR products were transferred to nylon membrane and probed with a biotin-labeled generic probe designed to detect most genital HPV types. Specimens positive by generic probe were then tested with biotin-labeled type-specific oligonucleotide probes that included probes for HPV-16 [27]. Controls consisted of specific L1-primed PCR products from cloned HPV-16.

**Pap smear evaluation.** Smears were considered satisfactory if >200 easily visualized squamous cells were present. Smears were classified according to the Bethesda recommendations: negative, atypical squamous cells of undetermined significance (ASCUS), and low- and high-grade squamous intraepithelial lesions (LGSIL, HGSIL) [28].

**Assay of total IgG and total IgA concentration.** To produce a standard curve, purified human IgG and IgA (10 mg/mL; Sigma, St. Louis) were serially diluted 2-fold, starting with a dilution of 1:100 to a final dilution of 1:200,000 in sodium carbonate buffer (0.1 M, pH 9.6) in microtiter plates (Immulon 2; Dynatech Laboratories, Chantilly, VA). Cervical secretions were also serially diluted 2-fold, starting at a 1:10 dilution and incubated at room temperature overnight. The plates were washed with PBS and blocked with 10 mM Tris, pH 8.0, 0.15 M NaCl, and 0.5% Tween with 10% goat serum for 1 h at room temperature. Bound antibodies were detected by incubation with goat anti-human IgG (Boehringer Mannheim, Indianapolis) or goat anti-human IgA (Pierce) at a dilution of 1:1000 for 1 h at 37°C. The plates were washed with PBS and developed with Sigma 104 phosphatase substrate (Sigma, St. Louis; 4.33 mg/mL) in reaction buffer (0.1 M NaHCO3, pH 9.5; 0.01 M MgCl2) for 30 min at room temperature. The reaction was stopped by the addition of 1.5 N NaOH. The plates were read on an automated plate reader (Titertek Multiscan MCC/320; ICN Biomedical, Irvine, CA). The immunoglobulin concentration was determined by comparison with the standard curve made with the purified human IgG and IgA.

**Exclusion of collected cervical secretions.** A total of 87 samples (12.5%) were excluded per published guidelines [25], thereby excluding 13 women from analysis. The excluded samples included 41 (5.8%) that were positive for blood (12 being visibly bloody), leaving only 4% of samples subclinically contaminated with blood. Also, 34 samples (4.9%) were lost during storage, 5 samples (0.7%) were not fully absorbed (low volume [<100 μL] with a low protein concentration [<25 mg/100 μL] or low IgG and IgA concentration [<2 and <8 mg/μL, respectively]), and 7 samples (1%) were diluted with vaginal secretions (high volume [>500 μL] with low total protein [<250 mg/μL]). The demographics of the 13 subjects whose samples were eliminated did not differ from those of the entire study population (data not shown). Subjects (n = 292) provided an average of 2.1 adequately collected cervical specimens (n = 609) that were collected an average of 125 days apart.

**Detection of antibodies to HPV-16 capsids.** Serum IgG antibodies were detected by ELISA [11]. For the LIA to detect cervical HPV-specific antibodies, microtiter plates (Microlite-2; Dynatech Laboratories) coated with a 1:2000 dilution of H16.V5 monospecific antibody diluted in 0.1 M sodium carbonate buffer, pH 9.5, were used. The plates were incubated overnight at room temperature, washed, and blocked as above [11]. Purified HPV-16 capsids were added at a dilution of 1:50 in PBS, incubated at room temperature for 1 h, and washed. Secretions were added at a dilution of 1:4 in blocking buffer, incubated at 37°C for 1 h, and washed. The plates were then incubated with a 1:30,000 dilution of goat anti-human IgA conjugated with horseradish peroxidase (Pierce) for cervical IgA testing and goat anti-human IgG conjugated with horseradish peroxidase (Boehringer Mannheim) for cervical IgG testing for 2 h at 37°C. The plates were developed by the addition of chemiluminescence ELISA reagent (Boehringer Mannheim) and developed for 5 min. Relative light units per second were determined by a 1-s reading in a Laminate EG & G Berthold Microlumat LB 96 luminometer (EG & G, Berthold, Germany). Log transformation of the raw ELISA data was done, and then the median of the 3 wells with capsids was subtracted from the median of 3 wells without capsids. Secretory piece specific for HPV-16 capsid antigens was determined in a similar manner except that the second antibody was a rabbit anti-human secretory piece diluted at 1:1000 and incubated at 37°C for 1 h. This was followed by incubation with 1:10,000 dilution of goat anti-rabbit IgG conjugated to horseradish peroxidase at 37°C for 1 h. The plates were developed as noted above. Only the top 40% of ELISA values from cervical HPV-16 IgA screening were assayed for secretory piece, and the top 25% of the secretory piece ELISA values were considered positive.
Cut points for the cervical antibody levels were based on the LIA values from 19 women who had no previous history of sexual activity and whose cervical and vulvovaginal swabs were HPV DNA–negative by PCR. The cut point was calculated as the mean of the LIA values ±2 SD. Because of a lack of sufficient volume of cervical secretions of these negative controls, a similarly derived cut point for the secretory piece could not be determined. Secretory piece measurements were analyzed in quartiles, with the top quartile being defined as positive. Secretory IgA positivity was defined as a positive cervical IgA test and a positive secretory piece test.

Control sera from the Medical Center of Louisiana, New Orleans, were selected by their reactivity in the single-dilution (1 : 100) HPV-16 capture ELISA. These sera were tested in both the LIA and ELISA with 2-fold-dilution titers, starting at a dilution of 1 : 10 and ending at 1 : 10,240 (single wells each dilution). In addition, the 10 strongest-reacting sera were pooled and titered. The natural log–transformed optical densities (OD) were plotted versus sera dilution and area under the curve (AUC), and 50% maximal concentration (EC50) was determined for both the ELISA and LIA by Prism 2.01 curve-fitting program (GraphPad, San Diego, CA). Comparisons made with Student’s t test demonstrated a lack of correlation between the EC50 and the ELISA as well as between the LIA and the OD from a single 1 : 100 dilution of sera in the ELISA (data not shown). On the other hand, similar comparisons made by use of the AUC showed strong associations between these variables (see Results), and determination of the titer with use of the AUC was done to compare the performance of the ELISA versus the LIA.

Statistical analysis. Student’s t test was used to compare the EC50 and AUC measurements of the titers by LIA or ELISA and the OD determined by ELISA on a single dilution of sera. For the analysis of the cervical secretions, Student’s t tests were used to establish mean values, and χ2 tests were used to compare properties. Longitudinal analysis of cervical antibody, HPV DNA, and Pap smear result was done by use of generalized linear models for repeated measurements (observations) for a single subject.

Results

Development of the LIA. Because of the low levels of HPV-16 IgG antibodies found in the sera [11], it was necessary to develop a more sensitive immunoassay to detect HPV-16 capsid antibodies in cervical secretions. An LIA was developed similar to the ELISA, substituting a horseradish peroxidase–conjugated goat anti-human antibody as the second antibody. The immunoassay plates were then incubated with a chemiluminescence substrate, and the light emissions were measured in a luminometer. The sensitivities of the assays were compared on a group of 84 sera by determining end-point dilution titers by both assays. The samples were selected by being IgG-seropositive for HPV-16 in a previous capture ELISA at a 1 : 100 serum dilution. The assays were compared by determining the AUC for each serum sample. For all samples, the LIA showed a 1.9-fold-greater AUC than the ELISA did, with 75% of the sera scoring higher in the LIA than in the ELISA. The average (±SD) AUC for the ELISA was 1.38 (±0.91), compared with 2.09 (±1.41) for the LIA. The AUCs determined by the LIA and ELISA were highly associated with each other (r = .70; P < .0001). The AUC determined with the LIA was also highly associated with the OD determined by ELISA at a single 1 : 100 serum dilution (r = .73; P < .0001). Thus, because the LIA appears to be more sensitive than the capture ELISA in detecting HPV-16 capsid antibodies, it was used to detect HPV-16 capsid antibodies in cervical secretions.

Characterization of the study population and study samples. The 292 women who provided adequate cervical samples at enrollment into this study were predominately Caucasian (78%) and reported an average lifetime number of 2 sex partners (table 1). Twenty-two percent of the subjects had abnormal Pap smears (LGSIL and HGSIL), 16% had HPV-16 DNA, and 20% had serum HPV-16 antibodies (table 1). Of the 292 women from whom adequate cervical secretions were collected, 106 provided only 1 cervical sample and 186 provided multiple cervical samplings. The collected cervical secretions had an average volume of 238 μL, protein concentration of 2.28 mg/mL, total IgG concentration of 206 μg/mL, and total IgA concentration of 234 μg/mL. These values were comparable to those found by Ashley et al. [25], with a lower volume collected but a higher protein concentration. The parameters were not significantly different between subjects who were concurrently HPV-16 DNA–positive and those who were HPV-16–negative (table 2).

Detection of cervical HPV-16 antibodies. Overall, cervical IgG antibodies to HPV-16 were detected in 12% of the samples (75/609) and at least once for 24% of the subjects (69/292). Cervical IgA antibodies to HPV-16 were found in 6% of the
Cervical IgA detected
Secretory IgA detected

were 3.3 times (95% confidence interval [CI], 1.4±7.8) more detected 12 months prior to the cervical antibody measurement than were those women who were HPV-antibodies were significantly more likely to have detectable cervical HPV-16 IgG than were those women who were HPV-antibodies did not differ significantly between women who were HPV-16 DNA–positive at the time cervical secretions were obtained and those who were HPV-16 DNA–negative at the time of collection (table 2).

**Correlation of cervical HPV-16 antibodies with HPV-16 DNA status.** The ability to detect cervical IgG antibodies to HPV-16 was compared with the detection of HPV-16 DNA in earlier and later time intervals by use of the GEE analysis technique (table 3). Time zero represents the day on which cervical antibody measurements were made; women with HPV-16 DNA detected 4–12 months prior to the measurement of cervical antibodies were significantly more likely to have detectable cervical anti–HPV-16 IgG than were those women who were HPV-16 DNA–negative. For example, with HPV-16 DNA detected 12 months prior to the cervical antibody measurement were 3.3 times (95% confidence interval [CI], 1.4±7.8) more likely to have HPV-16–specific cervical IgG than were those without detectable HPV-16 DNA. Detection of cervical HPV-16 DNA during the same study visit as the cervical antibody measurement was also associated with cervical IgG detection (odds ratio [OR], 2.1; 95% CI, 1.0±4.7). By use of similar techniques, detection of cervical IgA antibodies was not associated with the detection of HPV-16 DNA. However, analysis based on the smaller sample of cervical samples that could be tested for secretory piece suggested a stronger association between secretory IgA detection and prior HPV-16 DNA detection, but this did not reach statistical significance (OR, 4.2; 95% CI, 0.8±21.3 at –4 months) (table 3).

**Correlation of cervical HPV-16 antibodies with detection of SIL.** Table 4 demonstrates associations between cervical antibodies and detection of SIL by the GEE technique. SIL detected 4 months prior to the antibody measurement was associated with detection of cervical IgG HPV-16 antibodies (OR, 3.6; 95% CI, 1.6±8.1). Furthermore, SIL detected 4–8 months prior to cervical antibody measurements was significantly associated with HPV-16 secretory IgA detection (table 4).

### Discussion

In this study, we report the development of a sensitive LIA to detect capsid antibodies to HPV-16. End-point dilution titers were determined with the same sera by means of the LIA and the ELISA. On average, the LIA was ~2-fold more sensitive than the ELISA. During this analysis, it was discovered that representing the titers as an AUC was more informative than as an EC50. Measurements of the AUC are traditionally used for analysis of pharmacokinetic data, and usually the AUC correlates with the EC50; however, exceptions have been noted [29]. In this case, the difference between the AUC and EC50 is most likely due to the inability to determine an accurate EC50 for some sera, especially those with low levels of HPV-16 antibodies. The titer of the HPV-16 antibody response expressed as the AUC was found to correlate strongly with the OD at a 1 : 100 dilution. This suggests that the OD at a single dilution

### Table 2. Characteristics of cervical secretions (609 total samples) among human papillomavirus type 16 (HPV-16)–positive and –negative women.

| Parameter                        | HPV-16 DNA–positive | HPV-16 DNA–negative | p¹  
|----------------------------------|---------------------|---------------------|------
| Volume collected, µL             | 247 ± 127 (27)      | 238 ± 101 (576)     | .66  
| Protein concentration, mg/mL     | 2.6 ± 2.0 (25)      | 2.3 ± 1.8 (532)     | .42  
| Total IgG, µg/mL                 | 129 ± 264 (25)      | 206 ± 419 (533)     | .36  
| Total IgA, µg/mL                 | 183 ± 398 (25)      | 235 ± 724 (533)     | .72  
| Cervical HPV-16 IgG–positive     | 6/27 (22)           | 68/576 (12)         | .06  
| Cervical HPV-16 IgA–positive     | 2/27 (7)            | 34/576 (6)          | .75  
| Cervical HPV-16 secretory piece–positive² | 3/10 (30)        | 54/226 (24)         | .57  
| Cervical HPV-16 IgG– and secretory piece–positive | 1/7 (14) | 11/161 (7) | .52  

**NOTE.** Data are mean ± SD (n) or no./total (%).

¹ At time of sample collection.

² Only top 40% of ELISA values from cervical HPV-16 IgA screening were assayed for secretory piece, and top 25% of secretory piece ELISA values were considered positive.

### Table 3. Associations between detection of human papillomavirus type 16 (HPV-16) DNA and cervical antibody, on the basis of concurrent and time-delayed measurements of HPV-16 DNA and cervical antibodies.

<table>
<thead>
<tr>
<th>Average time between DNA and cervical antibody measurement (months)²</th>
<th>Cervical IgG detected</th>
<th>Cervical IgA detected</th>
<th>Secretory IgA detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. HPV DNA–positive/total OR (95% CI)</td>
<td>No. HPV DNA–positive/total OR (95% CI)</td>
<td>No. HPV DNA–positive/total OR (95% CI)</td>
</tr>
<tr>
<td>−12</td>
<td>28/377 3.3 (1.4–7.8)</td>
<td>28/377 1.9 (0.5–6.8)</td>
<td>27/363 3.3 (0.6–17.5)</td>
</tr>
<tr>
<td>−8</td>
<td>32/437 3.1 (1.3–7.5)</td>
<td>32/437 1.6 (0.5–5.4)</td>
<td>31/419 3.3 (0.6–16.5)</td>
</tr>
<tr>
<td>−4</td>
<td>29/493 3.0 (1.4–6.5)</td>
<td>29/493 1.8 (0.5–6.1)</td>
<td>28/472 4.2 (0.8–21.3)</td>
</tr>
<tr>
<td>0</td>
<td>27/603 2.1 (1.0–4.7)</td>
<td>27/603 1.3 (0.3–5.7)</td>
<td>26/579 2.0 (0.2–15.6)</td>
</tr>
<tr>
<td>+4</td>
<td>19/515 2.0 (0.8–5.2)</td>
<td>19/515 0.9 (0.1–6.5)</td>
<td>19/494 2.9 (0.4–23.0)</td>
</tr>
<tr>
<td>+8</td>
<td>17/411 0.4 (0.1–3.2)</td>
<td>17/411 0.9 (0.1–7.0)</td>
<td>17/394 2.9 (0.3–23.6)</td>
</tr>
<tr>
<td>+12</td>
<td>16/317 0.4 (0.1–2.6)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**NOTE.** OR, odds ratio; CI, confidence interval; NA, not available.

² Time 0 represents day on which cervical antibody measurements were made; months – or + represent HPV DNA detection before or after antibody measurements.
The presence of antibodies directed against the role in an established viral infection, local antibodies may have cervical antibodies was not done. Although unlikely to have a HPV type.

may protect against new infection or reinfection with the same (SIL) may indicate the appearance of an immune response that detection of cervical antibodies. The presence of cervical an-
tibodies after detectable HPV-16 DNA or HPV-related disease
detection. One would expect that cervical antibodies would de-
tected in relation to DNA detection and SIL could not be examined. The immunassay methodologies also differed sub-
stantially; Wang et al. [24] used a direct ELISA to measure cervical IgA antibodies for the entire study population and tested for cervical IgG only those patients who were positive for cervical IgA antibodies.

Although our study was longitudinal in nature, the infre-
dent of cervical antibodies (<10%) meant that the kinetics of the cervical humoral immune response against HPV-
16 could not be analyzed in detail. Also, we could not determine where the detected cervical antibodies were produced. In gen-
eral, mucosal IgG is derived from the serum, whereas secretory IgA is locally produced [17, 19]. Future studies that include cervical biopsies may be able to test for specific HPV-16 anti-
body–producing cells by immunofluorescence [33] and may better delineate the production site of the cervical antibodies. In addition, the kinetics of cervical antibody production are somewhat similar between HPV-16 DNA detection and SIL detection. One would expect that cervical antibodies would de-
volve sometime after infection but that the lag period would be less or nonexistent for the development of HPV-related dis-
bases. These data do not corroborate this theory. One expla-
ation is that initial HPV infection leads to relatively high virus loads with high amounts of L1 antigen present, leading to subsequent cervical antibody detection. These initial levels of antigen subside either because of the lack of long-term HPV infection and/or because of an effective immune response against HPV. This may lead to a decreased ability to detect serum antibody to HPV. SIL represents disease caused by HPV and implies relatively higher levels of virus and L1 antigen, again leading to antibody production.

Finally, established HPV infection is most likely controlled by cellular immune mechanisms. HPV DNA detection and HPV-related disease have been found to be higher in patients with cellular immune defects due, for example, to having undergone renal transplantation or to HIV infection [25, 34, 35]. Although little is known about local cellular immune mecha-

### Table 4. Associations between detection of squamous intraepithelial lesions (SIL) and human papillomavirus type 16 (HPV-16) antibodies in cervical secretions, on the basis of concurrent and time-delayed measurements of SIL and cervical antibodies.

<table>
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<tr>
<th>Average time between SIL and cervical antibody measurements (months)</th>
<th>Cervical IgG detected</th>
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<tr>
<td></td>
<td>No. with SIL detected/total OR (95% CI)</td>
<td>No. with SIL detected/total OR (95% CI)</td>
<td>No. with SIL detected/total OR (95% CI)</td>
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<td>&lt;12</td>
<td>26/381 0.6 (0.1–2.3)</td>
<td>26/381 1.3 (0.2–10.0)</td>
<td>26/367 3.5 (0.4–29.8)</td>
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<td>8</td>
<td>29/442 1.9 (0.7–4.6)</td>
<td>29/442 1.7 (0.5–5.6)</td>
<td>29/442 6.4 (1.9–21.8)</td>
</tr>
<tr>
<td>4</td>
<td>26/495 3.6 (1.6–8.1)</td>
<td>26/495 2.0 (0.6–7.0)</td>
<td>26/495 4.8 (1.1–20.3)</td>
</tr>
<tr>
<td>0</td>
<td>30/609 1.5 (0.6–3.7)</td>
<td>30/609 1.1 (0.2–8.5)</td>
<td>30/585 3.5 (0.4–28.6)</td>
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<tr>
<td>+4</td>
<td>25/519 1.0 (0.3–3.3)</td>
<td>25/519 0.6 (0.1–5.1)</td>
<td>25/498 2.2 (0.3–18.0)</td>
</tr>
<tr>
<td>+8</td>
<td>28/416 1.6 (0.7–3.9)</td>
<td>28/416 1.2 (0.3–5.5)</td>
<td>26/399 NA</td>
</tr>
</tbody>
</table>

**NOTE.** OR, odds ratio; CI, confidence interval; NA, not available.

*a* Time 0 represents day on which cervical antibody measurements were made; months + or + represent HPV DNA detection before or after antibody measurements.

*b* Not all samples were tried for secretory IgA.
nisms and HPV, undoubtedly they are critically involved in the control of infection. Future studies examining the cellular immune mechanisms against HPV both in peripheral blood and in cervical tissue or fluids are warranted.

Acknowledgments

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References