Occurrence of IgA and IgG Antibodies to Select Peptides Representing Human Papillomavirus Type 16 among Cervical Cancer Cases and Controls

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ABSTRACT

We used an enzyme-linked immunosorbent assay to test sera from 186 cases of invasive cervical cancer and 172 age-matched controls for IgG and IgA antibodies to an human papillomavirus 16 E7 peptide and to peptide 245, representing an epitope in E2. Cases had significantly higher mean absorbance values than controls for both immunoglobulin isotypes to E7 and elevated mean values for IgG to peptide 245. Since absorbances were not normally distributed we analyzed cervical cancer risk for seropositive and seronegative women. Of the traditional cervical cancer risk factors, cigarette smoking, educational level, number of pregnancies, time interval since last Papanicolaou smear, and age at first intercourse influenced the distribution of seropositivity to some of the viral antigens. Adjusting for these variables, the odds ratios of cervical cancer associated with IgG to E7 was 5.28 [95% confidence (95% CI) = 2.4-11.6] and that with IgA to E7 was 2.67 [95% CI = 1.3-5.3]. IgG to peptide 245 was less strongly associated, odds ratio 1.68 [95% CI = 1.2-3.3], and IgA to peptide 245 was not significantly associated with disease. These findings suggest that antibodies to E7 are markers for invasive cervical cancer. However, seropositivity correlated poorly with clinical state, survival, or the presence of human papillomavirus DNA in the cancer tissue.

INTRODUCTION

HPV infection of the female and male genital tracts has been associated with precancers and invasive squamous neoplasms. Within genital lesions, HPV types 16, 18, 31, 33, 35, and 56 have been found to be associated with severe dysplasia and carcinoma of the cervix, vulva, and penis; whereas, HPV types 6, 11, 41, 42, and 45 have been found to be associated with benign condylomata of the cervix, vulva, penis, and perineal and perianal skin (1). HPV 16 has been proposed to be a major factor in the pathogenesis of cervical carcinoma (2, 3). This hypothesis is supported by the finding of HPV sequences of some type in up to 80% of invasive cancers and in approximately 50% of the cases the sequences are those of HPV 16 (2). In addition, the E7 gene product of HPV 16 is capable of transforming cells in vitro (4) and the HPV DNA in cancer cells is frequently integrated in a manner that enhances the expression of E7 in cervical cancers (5). In a recent study, HPV 16 DNA was found to be exclusively integrated in 39% of primary cervical cancers, predominantly episomal in 36% and integrated and extrachromosomal to a similar extent in 25% of the cancers, confirming the presence of integrated viral DNA in the majority of cases (6).

Because papillomaviruses do not replicate in tissue culture systems, relatively little is known about the immune responses in human genital viral infections. Baird (7) used disrupted bovine papillomavirus type 2 virions in an ELISA and detected antibody in a high proportion of patients with anogenital warts, CIN, and invasive cancers but not in children or adults without genital disease. These antibodies were assumed to be due to the “genera-specific” antigens shared by all types of papillomaviruses. A similar antigen was used by Dillner et al. (8) who found IgA antibodies in cervical secretions obtained from 8 of 9 women with CIN as compared with 6 of 24 women with normal Pap smears.

The serological response to specific HPV 16 antigens found on proteins E4 and E7 was recently reported using fusion proteins and Western blot assays (9). This study revealed that antibodies against the E7 fusion protein were 14 times more frequent in sera of cervical cancer patients than in sera of aged and sex-matched controls. Antibodies to E4 were found three times more often among patients with HPV-associated CIN and condyloma acuminate than among controls. These observations suggested that antibodies to E4 correlated with virus replication, while antibodies to E7 may represent a marker for invasive cancer. In another study utilizing specific antigens, Dillner et al. (10) used a number of synthetic peptides, predicted to be antigenic from HPV 16 sequence information, to survey sera for IgA antibodies. One peptide (245), a 19-residue peptide deduced from a region close to the carboxyl terminus of the E2 open reading frame, identified a serological IgA response in 73% of women with cervical neoplasia and in only 22% of normal women. Immunoaffinity-purified human IgA antibodies against the peptide also detected a nuclear antigen in cells containing HPV 16 DNA.

In a preliminary analysis of a case series, we found antibodies to peptide 245 and an E7 peptide of HPV 16 to occur more frequently among patients with CIN and invasive cancers than among women without papillomavirus-associated lesions (11). The present study was undertaken to confirm the findings for invasive cancer by evaluating the distribution of IgA and IgG antibodies to E7 and the E2 peptide 245 among cases of invasive cervical cancer and carefully selected controls. Information available from the study subjects permitted us to determine the influence of cervical cancer risk factors on the distribution of antibodies. We found an impressive difference between cases and controls in the occurrence of antibodies of either isotype to E7 and these differences persisted after adjusting for cervical cancer risk factors that influenced antibody distribution. The differences in the occurrence of antibodies to peptide 245 between cases and controls were not as substantial, suggesting that antibodies to E7 may represent markers for invasive cancer of the cervix.

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4 The abbreviations used are: HPV, human papillomavirus; ELISA, enzyme-linked immunosorbent assay; CIN, cervical intraepithelial neoplasia; Pap, Papanicolaou; PBS, phosphate-buffered saline; BSA, bovine serum albumin.
MATERIALS AND METHODS

Study Subjects. Details of the selection of the study subjects have been published elsewhere (12). Briefly, serum samples were obtained from patients with newly diagnosed invasive cervical cancer who attended the Instituto Oncologico Nacional, Panama City, Panama, between January 1986 and June 1987. After histological confirmation of the diagnosis, women between 18 and 69 years of age who had received no previous treatment for cervical cancer and who had resided in Panama for at least 6 months were identified as eligible for the study. One hundred ninety-seven (98%) of the cases participated. We also selected two age-matched controls for each case. Women with a prior diagnosis of cancer or who had undergone surgical removal of their uteri were not eligible to serve as controls. One control was randomly selected from the community in which her matched case resided and the other control was selected from inpatients of the hospital which had referred the case and was randomly selected from women admitted for nongynecological disorders. Ineligible as hospital controls were women admitted for endocrine-, circulatory-, psychiatric-, or smoking-related diseases. Hospital controls and community controls from countries in which both types were available were similar with respect to cervical cancer risk factors. Three hundred fifty-two (88%) eligible controls were enrolled. For this study, one of the pair of matched controls of each case was randomly selected.

The cases had a standard clinical evaluation, which included staging of the cancer by the oncology institute staff. A standardized interview was administered to all participants by a trained interviewer. Sufficient serum for testing was obtained from 186 cases and 172 controls. Biopsy samples of the lesions yielding sufficient DNA for HPV testing were obtained from 188 cases.

Analysis of Specimen DNA. Biopsy samples were frozen in liquid nitrogen and transported to the Gorgas Memorial Laboratory where they were stored at −70°C until shipment to McMaster University. Sufficient DNA for analysis was obtained for 91% of the enrolled cases (12). DNA probes derived from recombinant plasmids containing HPV 16 (13) and HPV 33 (14) sequences were radiolabeled by the random primer method (15) using [32P]dCTP. The biopsy DNA was analyzed by the Southern blot method using 20 µg of DNA digested with BamHI, then electrophoresed on 1% agarose gels, and transferred to nitrocellulose paper by the Southern blot method as modified by Wahl et al. (16). The nitrocellulose papers were prehybridized in a solution containing 50 mM Tris-HCl, pH 7.5-5-fold Denhardt's solution [0.1% (w/v) Ficoll-0.1% (w/v) polyvinylpyrrolidone-0.1% (w/v) bovine serum albumin]-5-fold standard saline citrate (1 x SSC = 0.15 M NaCl, 0.015 M sodium citrate)-20% (v/v) formamide-200 µg/ml of sonicated and heat-denatured salmon sperm DNA. Hybridization was carried out at 42°C (40 to 45°C) in the same solution modified to contain 20 mM Tris-HCl, pH 7.5-5% (w/v) dextran sulfate-32P-labeled DNA probe (2 x 106 cpm/cm2 of nitrocellulose paper). After 16 h of incubation at 42°C, the nitrocellulose papers underwent four 1-h washes in 2-fold standard saline citrate containing 0.1% sodium dodecyl sulfate at 65°C (35°C to 20°C), and then they were exposed to X-ray film for 7 days. The autoradiographs were independently examined by 3 observers and considered HPV DNA positive when all observers agreed.

Peptide Antigens. Two HPV 16 peptide antigens were used: peptide 245 (10) was obtained from Integrated Protein Technologies, Inc., Toronto, Canada. Peptide 245, of the E2 Open Reading Frame, had an amino acid sequence of HN-His-Lys-Ser-Ala-Ile-Val-Thr-Leu-Thr-Tyr-Asp-Glu-Trp-Gln-Arg-Gsp-Gln-Cys-OH (10). The E7 peptide represented the entire 98 amino acids predicted from the HPV 16 E7 ORF (17) and was synthesized in one of the participating laboratories (Institute of Molecular Virology, St. Louis University).

ELISA. The ELISA was performed by a standard method (18). Briefly, each microtiter plate (Nunc; Kamstrup, Roskilde, Denmark) was coated with one of the two synthetic peptides, E7 (5 µg/ml) and peptide 245 (20 µg/ml), diluted in carbonate buffer, pH 9.6, and incubated overnight at 4°C. Following the removal of unadsorbed peptides, the plates were blocked with PBS-Tween-1% BSA and incubated for 1 h at 37°C. The patients' serum samples were diluted in PBS-Tween-1% BSA (serum to be tested for IgA was diluted 1/20 and serum to be tested for IgG was diluted 1/500) and then incubated for 1 h at 37°C. Mouse anti-human IgA (Serotec, Oxford, England) or IgG (Caltag Laboratories, San Francisco, CA) antiserum was then added to the washed wells and incubated 1 h at 37°C. After the wells were washed, goat anti-mouse antiserum alkaline phosphatase conjugate (Southern Biotechnology Associates, Inc, Birmingham, AL), diluted 1/200 in PBS-Tween-1% BSA, was added and incubated for 1 h at 37°C. The substrate [5 µl diethanolamine buffer + one Sigma 104 Phosphatase Substrate tablet (Sigma Chemical Co., St Louis, MO), dissolved 5 min] was then added to each washed well and incubated 30 min at 37°C. The reaction was stopped with 2 M NaOH and the plates were read at 410 nm.

Inspection of the values indicated that the distributions were not normal but highly skewed with values reaching 4.0-6.1 A. Therefore, the data were examined using two cutoff points for positivity: the use of outliers only as positive and the use of the mean of the control group (calculated minus outliers) plus 2 SD. The first method of positivity determination involved plotting the data in stem and leaf displays (19). The outside values, those which fall outside the fences, were considered positive. The second method to determine positivity involved calculating the mean of the control group values minus the outliers. Two SD were then added to that value to determine the cutoff point value.

Statistical Test. The influence of cervical cancer risk factors on the distribution of antibodies was evaluated by categorizing the study subjects according to selected risk factors and testing for statistically significant differences in antibody positivity. Categories included ≤40, 40-49, 50-59, and >60 years of age; none, 1-6, and >7 years of age; 1, 2, 3, and 4 or more lifetime sex partners; >20, 18-19, 16-17, and <16 years of age at first coitus; 0-3, 4-7, and ≥8 pregnancies; <48 months, >48 months, and never as duration since last Pap smear; ever or never smoked cigarettes; and ever or never used oral contraceptives. The differences were analyzed by the χ2 statistic, Fisher's exact test, or χ2 for linear trend. Odds ratios, as approximations to relative risk, were used to estimate the risk of cervical cancer associated with IgA and IgG antibody response to peptides E7 and 245. Multivariate logistic regression was used to adjust for factors found to influence antibody reactivity in the univariate analyses. Mantel-Cox statistics were used to compare the survival distributions between patients with and without antibodies (20).

RESULTS

Cases were found to have higher A values than the controls for both antigens and both immunoglobulin isotypes (Table 1). These differences were statistically significant for antibodies to the E7 peptide and IgG to peptide 245 but not for IgA to peptide 245. The findings suggest an association between cervical cancer and antibodies to E7 but not with antibodies to peptide 245. When the A values obtained for the community controls and the hospital controls were compared by Fisher's exact test for each antigen and each immunoglobulin isotype, no difference was detected between the two types of controls.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Immunoglobulin</th>
<th>Study group*</th>
<th>Mean</th>
<th>SEM</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E7</td>
<td>IgA</td>
<td>Controls</td>
<td>0.133</td>
<td>0.008</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E7</td>
<td>IgG</td>
<td>Controls</td>
<td>0.193</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>245</td>
<td>IgA</td>
<td>Controls</td>
<td>0.151</td>
<td>0.009</td>
<td>0.090</td>
</tr>
<tr>
<td>245</td>
<td>IgG</td>
<td>Controls</td>
<td>0.187</td>
<td>0.014</td>
<td></td>
</tr>
</tbody>
</table>

* Analysis carried out on sera from 186 cases and 172 controls.

a Probability of differences in mean values between cases and controls estimated by the Kolmogorov-Smirnov two-sample test.
Since the 
values were not normally distributed, seropositivity was examined using two end points (outliers and 2 SD above the control mean) as described in the "Materials and Methods." The occurrence of positivity among cases and controls according to these criteria are shown in Table 2. When the definition of outliers was used to define seropositive, 10–15% of cases and 2–6% of controls were positive. When the control group mean plus 2 SD was used to define seropositivity, 15–25% of cases and 6–11% of controls were positive. The presence of IgG antibodies against E7 was highly associated with cervical cancer at both end points (odds ratios, 5.9 and 5.0), as was IgA to E7 (odds ratios, 4.8 and 2.8). The strength of association with cervical cancer was considerably less for peptide 245 seropositivity. In subsequent analyses we used 2 SD above the control mean to define seropositivity.

The influence of cervical cancer risk factors (12) on the distribution of antibody reactivity was examined by univariate analysis using the control group of women. The results are summarized in Table 3. Among these control women, a significantly higher occurrence of IgA and IgG antibodies to peptide E7 was associated with history of cigarette smoking (odds ratios, 10.7 and 1.8, respectively). Older age groups had an increase in the occurrence of E7 IgA antibodies when compared to younger women, but this effect was lost after adjusting for smoking history. The duration since last Pap smear was associated with the occurrence of IgG antibodies to the E7 peptide; recent Pap smear reduced positivity. Interestingly, age of first coitus was inversely related to reactivity of IgG to peptide 245. For example, of the 44 women who first experienced intercourse by 16 years of age, 2% were positive, while this rate was 17% in 53 women who first experienced intercourse after age 19 years.

An analysis of the influence of cervical cancer risk factors on the distribution of antibodies to the peptides was also conducted using the entire study population. In addition to the factors associated among the control group, education level and number of pregnancies were associated with IgG to E7 in the entire study group (data not shown). The odds ratios for cervical cancer associated with reactivity to the peptides were reestimated after adjusting for these factors and the results are shown in Table 4. As before, significant associations were found for E7 IgG antibody, E7 IgA, and peptide 245 IgG. Peptide 245 IgA was not associated with significantly increased cancer risk. The adjusted odds ratios are similar to the unadjusted ratios.

There was no relationship between seropositivity to either peptide and clinical stage of disease (Table 5). Nor was there an apparent correlation between seropositivity and survival of patients through 1989 (Table 6). Similarly, there was no association between survival and the detection of HPV DNA in the tumor tissue.

Antibody status was available on 168 cases for whom information was available regarding the presence of HPV DNA 16 sequences in their cancers. The relationship between detection of antibodies and the presence of viral DNA in biopsies of cancer tissue is shown in Table 7. For IgA to E7, 16% of those negative by Southern blot were seropositive, while a significantly greater proportion (31%) of the DNA-positive cases had antibody. There were no significant associations between reactivity with the other isotypes of peptides and the presence of DNA.

Table 2 Occurrence of antibodies to HPV-16 peptides among cervical cancer cases and controls

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Immunoglobulin</th>
<th>Case</th>
<th>Control</th>
<th>% positive defined as outliers</th>
<th>% positive defined as 2 SD above the mean of controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>E7</td>
<td>A</td>
<td>10</td>
<td>2</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>E7</td>
<td>G</td>
<td>15</td>
<td>3</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>245</td>
<td>A</td>
<td>12</td>
<td>5</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>245</td>
<td>G</td>
<td>12</td>
<td>6</td>
<td>19</td>
<td>11</td>
</tr>
</tbody>
</table>

*Analysis of 186 cases and 172 controls.

Table 4 Effect of adjustment on the odds ratios of cervical cancer associated with the reactivity with peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Isotype</th>
<th>Reactive</th>
<th>Case</th>
<th>Controls</th>
<th>Odds ratios (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E7</td>
<td>IgA</td>
<td>–</td>
<td>147</td>
<td>157</td>
<td>1.00 (1.00–1.00)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td>39</td>
<td>15</td>
<td>2.78 (2.67–2.79)</td>
</tr>
<tr>
<td>E7</td>
<td>IgG</td>
<td>–</td>
<td>139</td>
<td>161</td>
<td>1.00 (1.00–1.00)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td>47</td>
<td>11</td>
<td>4.95 (5.28–5.61)</td>
</tr>
<tr>
<td>245</td>
<td>IgA</td>
<td>–</td>
<td>159</td>
<td>153</td>
<td>1.00 (1.00–1.00)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td>27</td>
<td>19</td>
<td>1.37 (0.98–1.92)</td>
</tr>
<tr>
<td>245</td>
<td>IgG</td>
<td>–</td>
<td>150</td>
<td>153</td>
<td>1.00 (1.00–1.00)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td>36</td>
<td>19</td>
<td>1.93 (1.68–2.28)</td>
</tr>
</tbody>
</table>

* Adjusted odds ratios were estimated by logistic regression in which factors found to influence antibody reactivity by univariate analysis were added to the model. These factors included age, number of pregnancies, smoking history, education level, age at first sexual intercourse, and history of Pap smear.

Table 5 Distribution of antibody according to clinical stage of cancer

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Isotypes</th>
<th>(N = 49)</th>
<th>(N = 73)</th>
<th>(N = 52)</th>
<th>(N = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E7</td>
<td>IgA</td>
<td>10 (20.4)</td>
<td>14 (19.2)</td>
<td>12 (23.1)</td>
<td>3 (25)</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>13 (26.5)</td>
<td>19 (26.0)</td>
<td>14 (26.9)</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>245</td>
<td>IgA</td>
<td>6 (12.2)</td>
<td>11 (15.1)</td>
<td>7 (13.5)</td>
<td>3 (25)</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>9 (18.4)</td>
<td>15 (20.6)</td>
<td>10 (19.2)</td>
<td>2 (16)</td>
</tr>
</tbody>
</table>

Table 6 Survival of cancer cases in relation to the presence of HPV 16 markers

<table>
<thead>
<tr>
<th>HPV marker</th>
<th>Marker negative group</th>
<th>Marker positive group</th>
</tr>
</thead>
<tbody>
<tr>
<td>E7 IgA</td>
<td>28/143 (19.6)</td>
<td>12/39 (30.8)</td>
</tr>
<tr>
<td>E7 IgG</td>
<td>30/136 (22.1)</td>
<td>10/46 (21.7)</td>
</tr>
<tr>
<td>245 IgA</td>
<td>35/147 (21.3)</td>
<td>7/27 (25.9)</td>
</tr>
<tr>
<td>245 IgG</td>
<td>35/147 (21.3)</td>
<td>5/35 (14.3)</td>
</tr>
<tr>
<td>HPV DNA*</td>
<td>24/103 (23.3)</td>
<td>13/61 (21.3)</td>
</tr>
</tbody>
</table>

* Survival information was available for 182 cases for whom antibody status was also known.

* HPV 16 DNA determined by Southern blot method of biopsy sample on 164 cases using the conditions described elsewhere (12).
Interestingly, most sera that contained antibody of one isotype to one peptide were negative for the other peptide. Thus, 60% of cases and 32% of controls were positive for antibodies of either isotype to at least one peptide (using the control mean plus 2 SD). Comparison of sera containing antibody to more than one isotype showed no specific patterns to the isotypes present. Cross-matching of heterologous isotypes where IgG and IgA reacted to E7 revealed that only 1% of the 15% positive sera reacted to both isotypes in the control group. Among cases this was 8% of 39% positive sera. IgA to E7 compared to IgG of peptide 245 revealed that only 1% of the 15% positive sera reacted to both isotypes in the control group. Further comparison of case and control sera suggests that the ratios of reactions to multiple isotypes or antigens varied but that the reactions of more than one isotype was a random event, not specific to peptide E7 or peptide 245. These reaction patterns suggest that an antibody response of one isotype does not predict reactions of this or other isotypes to other antigens.

DISCUSSION

This study was undertaken to determine whether the occurrence of antibodies to the E7 protein of HPV 16 was greater among women with cervical cancer than among age-matched control women. E7 appears to be a major transforming protein of HPV 16 and has been found to be one of the main viral proteins expressed in cervical cancer cells (5, 21). For comparison, we tested the sera against another synthetic peptide, peptide 245, which defined an epitope of the E2 open reading frame of HPV 16. This latter peptide was found to be part of larger proteins expressed in cancer cells and IgA antibodies to this protein have been found more frequently in sera from patients with papillomavirus disease and CIN than control women (10). We found a strong association of IgA and IgG antibodies to E7 peptide and invasive cervical cancer but only a weak relationship between risk and antibodies to peptide 245.

An analysis of the influence of cervical cancer risk factors on the distribution of antibodies among control women revealed a strong association between cigarette smoking and IgA antibodies to E7. A less impressive but still significant association existed between smoking and IgG antibodies to E7. These observations suggest that smoking may function as a cofactor in cervical cancer risk by altering the nature of the immune response in the cervical epithelium. As might be anticipated, a prior Pap smear reduced the probability of having IgG antibodies to E7. This is consistent with the expectation that successful treatment of preinvasive lesions would remove constant antigenic stimulation of E7 in dysplastic lesions. Unexplained is an association between age at first coitus and IgG antibodies to peptide 245. The magnitude of this association is not great and the association probably represents a chance observation. It is of note that adjusting for identified correlates of positivity did not appreciably change the magnitude of cervical cancer risk associated with the peptide antibodies, suggesting that the antibodies may represent primary risk factors.

Few reports describing the measurement of human antibodies to relatively defined HPV antigens have appeared in the literature (22–24). Thus, the biological significance of the antibody responses measured in our study is not well understood. Interestingly, our results obtained using a synthetic E7 peptide agree well with those of Jochmus-Kudielka et al. (9) who measured IgG and IgM antibodies to a bacteria-made E7 fusion protein of HPV 16 by Western blot. In a large study consisting of 88 patients with invasive cervical carcinoma and 147 controls, they detected antibodies in 20.5% of women with invasive cervical cancer and in 1.4–3.8% of control women; comparatively, we found 25% of cases and 6% of controls with IgG antibodies to E7 (using a cutoff point of the mean of the controls plus 2 SD). These authors compared the antibody reactivity to E7 and to an E4 fusion protein of HPV 16 and concluded that E7 represented a marker for invasive cervical cancer, while E4 reactivity appeared to correlate with vegetative viral lesions. Our comparison of antibodies to E7 with antibodies to a peptide representing an epitope of E2 of HPV 16 also supports the notion that antibodies to the E7 peptide are markers for invasive disease. Unfortunately, the biopsy specimens obtained from the patients included in the study by Jochmus-Kudielka et al. were not available for HPV DNA analysis.

A comparison of our results and those reported in the 1989 study by Dillner et al., in which the presence of peptide 245 IgA antibodies in patients with CIN or cervical carcinoma and donors known not to have CIN was examined, found poor agreement between the two studies (10). They detected antibodies in 73% of women with CIN or cervical carcinoma and in 22% of women known not to have CIN; whereas, in our study we identified the presence of IgA antibodies in 15% of patients with cervical carcinoma and in 11% of their controls. Reasons for this discrepancy may be found in the technological aspects of the two studies and in the definition of the survey populations. Different criteria for antibody positivity by the ELISA method were used in each of the studies. The survey populations differed in the definition of cases. Dillner et al. used women with CIN and a few cases of cervical cancer; whereas, our study consisted of incident cases of cervical cancer, i.e., only women with newly diagnosed invasive cervical carcinoma.

Unexpected was the poor correlation between antibodies and the presence of HPV 16 DNA in cancer biopsies of the cases. Equally surprising was the observation that most reactive sera were positive for only one isotype to one peptide. These observations suggest considerable variation in the host response to defined epitopes of HPV 16. One would anticipate that antibodies to the peptides among women whose tumors lacked HPV 16 DNA could represent false-negative assays for viral DNA or antibody responses to infections with related HPV types. However, the lack of antibodies in the sera of women with tumors containing HPV 16 DNA is harder to explain. It is possible that the patients may not have responded to the peptides because the proteins were not expressed in the tumors. It is also possible that the women lacked appropriate precursor
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The protocol used in this study was reviewed and approved by the Gargas Memorial Laboratory Human Subjects Committee and the Office of Protection of Research Subjects, NIH.

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