Serological evidence for protection by human papillomavirus (HPV) type 6 infection against HPV type 16 cervical carcinogenesis

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Introduction

The oncogenic types of human papillomavirus (HPV), in particular HPV types 16 and 18, are established as a major cause of cervical and other anogenital cancers (zur Hausen, 1996). Prophylactic vaccination against papillomaviruses using virus-like particles has been very successful in several animal model systems (Breitburd et al., 1995; Kirnbauer et al., 1996; Suzich et al., 1995), although it appears that the neutralizing antibodies conferring the protective immunity are only conferring type-specific protection.

For prediction of the likely effects of including one or several HPV types in a multivalent HPV vaccine, it is essential to know if the joint effects of infection with different HPV types are independent, antagonistic or synergistic. Synergistic effects in carcinogenesis have been demonstrated in the case of liver cancer risk after joint exposure to hepatitis B and C viruses (Donato et al., 1998) and there are also studies suggesting synergistic effects of joint exposure to several HPV types and herpes simplex virus in cervical carcinogenesis (Hildesheim et al., 1991).

Several prospective studies have shown that seropositivity for HPV-16 increases the risk for future development of cervical cancer (Lehtinen et al., 1996; Shah et al., 1997; Dillner et al., 1997; Vonka et al., 1999) and other anogenital cancers (Björge et al., 1997), and there are also studies that have found seropositivity to HPV-18 and -33 to be associated with invasive cervical cancer (Dillner et al., 1997; Wang et al., 1997). However, since the different oncogenic HPV types are similarly transmitted and since seropositivities for the different oncogenic HPV types thus are strongly associated with each other (Dillner et al., 1996), the possibility exists that the excess risks seen may be secondary to the excess risks associated with other HPV types. Indeed, in a study of non-cervical anogenital cancers, the risk associated with HPV-33 lost significance after adjusting for HPV-16 (Björge et al., 1997).

Population biology studies on co-existence of different serotypes of micro-organisms have suggested that vaccination against only some serotypes may, under certain circumstances,
be ineffective in protection against disease because other pathogenic serotypes may emerge (Lipsitch, 1997; May & Nowak, 1995). This scenario can only occur if interference exists between different virus serotypes.

Therefore, the study of possible interactions between HPV types is essential both for understanding of HPV-induced carcinogenesis and for prediction of the likely effects of different preventive measures.

Antagonism between HPV types was first suggested by Evans et al. (1992), who found decreased risk of development of cervical squamous intraepithelial lesions in women with anogenital wart history. Although early case-control studies reported that women with condyloma have an increased risk of cervical cancer (Chuang et al., 1984), a cohort study of women with condylomas found no evidence of excess cervical cancer risk, despite shared sexual risk factors (Sigurgeirsson et al., 1991). Also, it has been reported that there is little excess cervical cancer risk among HPV-16 seropositive women in populations with a high prevalence of sexually transmitted disease (STD), but there are highly elevated risks in populations with low STD prevalence (Dillner et al., 1997). Antibodies against certain broadly cross-reactive papillomavirus antigens also appear to be associated with a decreased cervical cancer risk (Dillner et al., 1994). Finally, an antagonistic interaction between seropositivity for HPV-16 and -6/-11 has been reported in a prospective study of invasive cervical cancer (Luostarinen et al., 1999).

The preferred methodology for the study of virus interactions is seroepidemiology. HPV infections are typically transient and focal (Evander et al., 1995). Therefore, studies using viral genome detection are difficult to interpret regarding past or present HPV exposure of the individual. In contrast, serum IgG antibodies against HPV are known to persist on long-term follow-up, even after clearance of viral DNA (af Geijersstam et al., 1996; Carter et al., 1996; Shah et al., 1997) and are preferred as markers of lifetime cumulative HPV exposure (Olsen et al., 1997).

In the present study, we investigated the joint effects of seropositivity for multiple HPV types in a large, previously characterized (Wang et al., 1997) seroepidemiological case-control study of incident, untreated cervical cancer.

Methods

**Study population.** Pre-treatment serum samples from 218 women with primary invasive cervical carcinoma admitted to the Department of Gynaecologic Oncology, Radiumhemmet, Karolinska Hospital, Sweden, and 219 age- and sex-matched healthy blood donor controls were obtained during the period 1989–1992 (Wang et al., 1997). In the present study, the HPV-16, -18 and -33 serological measurements are the same for the controls and for the 206 cases reported in the previous article by Wang et al. (1997). Data from 12 cases that were previously not confirmed to be invasive cervical cancers were, on review, found to indeed be invasive cervical cancers and were added to the case series in the present study. Six cases included in the previous study were, on review, found to be carcinoma in situ and four cases had no more serum available and were excluded. The controls are a 1:1 age-matched subset of the controls in the previous study, selected without knowledge of the serological results. The HPV-6 and -11 serological measurements as well as all statistical analyses are previously unpublished.

Most patients (n = 173) had squamous cell carcinomas, 109 patients had stage I disease according to FIGO (International Federation of Gynaecologists and Obstetricians) classification. Stage II cervical cancer was observed in 61 cases, stage III in 39 cases and stage IV in seven cases. Most cancers were poorly or moderately differentiated (86 and 97 cases, respectively). Twenty-one case patients had well-differentiated cancers and four patients had undifferentiated cancers.

**Laboratory methods.** Serum IgG antibodies to HPV-6, -11, -16, -18 and -33 capsids were measured using standard direct ELISA methods, developed and validated in previous studies (Kirnbauer et al., 1994; Heino et al., 1995).

The cut-off levels for determining seropositivity for the oncogenic HPV types from continuous OD values were preassigned and had originally been established using the data from previous studies (Dillner et al., 1995) by treating cervical cancer as a receiver-operated characteristic.

For HPV-16, -18 and -33, the preassigned cut-off levels (0.456, 0.253 and 0.294) were almost identical to the mean value of the case mean OD value and the control mean OD value, which has been used in some previous studies as a preassigned cut-off level (Heino et al., 1995).

For HPV-11, the cut-off level (0.142) was preassigned and, relative to internal standards, was the same as used in previous studies (Wikström et al., 1995). This level was originally arbitrarily assigned.

For HPV-6, three different cut-off level definitions were evaluated: (i) the mean value of case and control means (Heino et al., 1995) (0.302); (ii) above the third quartile of controls (0.368); and (iii) one standard deviation above the mean of controls (Strickler et al., 1997) (0.653). All three tested cut-off values gave similar results for cancer risk estimates as well as for interaction (see below). The cut-off value above the third quartile of controls was in-between the other evaluated cut-offs and was chosen for final analysis.

**Data analysis.** Statistika software was used for data analysis. Odds ratios (OR) and confidence intervals (CI) were calculated using logistic regression. OR were estimated for seropositivity to one virus, sero-positivity in case of negativity for all other HPV’s (non-interference risk) and for different combinations of joint seropositivity. For the multiplicative interaction model, expected and observed cervical cancer risks were compared and the statistical significance of interaction was tested by inclusion of an interaction term in the logistic regression model.

For the additive model of interaction (Rothman & Greenland, 1998), the relative excess risk due to interaction (RERI) was estimated. The RERI CI was estimated using Rothman’s modified regression model (Hosmer & Lemeshow, 1992). Since relative excess risk equals relative risk minus 1, there is evidence of interaction at the P < 0.05 level if the RERI 95% CI excludes zero.

Results

Seroprevalences were increased among cervical cancer patients for HPV-16 and also for HPV-18 and HPV-33 (Table 1). Among healthy controls, seroprevalences were about the same and less than or equal to 20% (Table 1). As previously reported, the highest risk for cervical carcinoma was observed for HPV-16 seropositivity (OR, 2.39). For all virus types, the point estimate of the risk was slightly higher for squamous cell carcinoma than for all cervical cancers (Table 1).
### Table 1. HPV seropositivity and cervical cancer risks

<table>
<thead>
<tr>
<th>Virus type</th>
<th>No. of cancer patients</th>
<th>No. of healthy controls</th>
<th>Risk of all cervical cancers [OR (95% CI)]</th>
<th>Risk of squamous cell cancer [OR (95% CI)]*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>HPV-6</td>
<td>167</td>
<td>51 (23.4%)</td>
<td>177</td>
<td>42 (19.2%)</td>
</tr>
<tr>
<td>HPV-11</td>
<td>178</td>
<td>40 (18.3%)</td>
<td>189</td>
<td>30 (13.7%)</td>
</tr>
<tr>
<td>HPV-16</td>
<td>142</td>
<td>76 (34.9%)</td>
<td>179</td>
<td>40 (18.2%)</td>
</tr>
<tr>
<td>HPV-18</td>
<td>157</td>
<td>61 (27.9%)</td>
<td>175</td>
<td>44 (20.1%)</td>
</tr>
<tr>
<td>HPV-33</td>
<td>162</td>
<td>56 (25.6%)</td>
<td>180</td>
<td>39 (17.8%)</td>
</tr>
</tbody>
</table>

* Analysis was restricted to the 173 patients with squamous cell carcinoma.

### Table 2. Non-interference cervical cancer risk for each papillomavirus type (positivity for one HPV type, when all other HPV types are negative)

<table>
<thead>
<tr>
<th>Virus type</th>
<th>No. of cancer patients</th>
<th>No. of healthy controls</th>
<th>Risk of all cervical cancers [OR (95% CI)]</th>
<th>Risk of squamous cell cancer [OR (95% CI)]†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>HPV-6</td>
<td>78</td>
<td>11</td>
<td>116</td>
<td>11</td>
</tr>
<tr>
<td>HPV-11</td>
<td>78</td>
<td>5</td>
<td>116</td>
<td>6</td>
</tr>
<tr>
<td>HPV-16</td>
<td>78</td>
<td>23</td>
<td>116</td>
<td>10</td>
</tr>
<tr>
<td>HPV-18</td>
<td>78</td>
<td>12</td>
<td>116</td>
<td>12</td>
</tr>
<tr>
<td>HPV-33</td>
<td>78</td>
<td>8</td>
<td>116</td>
<td>13</td>
</tr>
</tbody>
</table>

* Number of patients and controls negative for all tested HPV types.
† Analysis restricted to the 173 patients with squamous cell carcinoma.

### Table 3. Effect of excluding subjects seropositive for other HPV types on the cervical cancer-associated risk for specific HPV types

<table>
<thead>
<tr>
<th>Analysis with subjects excluded when positive for:</th>
<th>Cervical cancer-associated risk (OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPV-6</td>
</tr>
<tr>
<td>HPV-6</td>
<td>–</td>
</tr>
<tr>
<td>HPV-11</td>
<td>1.21</td>
</tr>
<tr>
<td>HPV-16</td>
<td>1.66</td>
</tr>
<tr>
<td>HPV-18</td>
<td>1.19</td>
</tr>
<tr>
<td>HPV-33</td>
<td>1.18</td>
</tr>
<tr>
<td>None</td>
<td>1.28</td>
</tr>
<tr>
<td>All other HPV types</td>
<td>1.48</td>
</tr>
</tbody>
</table>

The cervical cancer risk for one HPV type when all other viruses are negative (hereafter referred to as non-interference risk) was considerably higher for HPV-16 (OR, 3.42), but lower for HPV-33 (OR, 0.91) seropositive cases compared with the crude risks (Table 2). Seropositivity for one HPV type with concomitant negativity for all other HPV types was generally rare (Table 2). For example, although HPV-11 seropositivity was common, there were only a few subjects with that had HPV-11 seropositivity when the other HPV types were negative. Therefore, statistical power was markedly decreased for the non-interference risk calculations, especially for HPV-11.
Additive model: RERI = −1.67 (CI 95% −4.03 to 0.69),
Multiplicative model: expected risk, 5.59; observed risk, 1.64; expected/observed rate, 3/4 (P = 0.018).

(c) Interaction between HPV-16 and -33, all cervical cancers
Additive model: RERI = 1.47 (CI 95% −0.91 to 3.86). Multiplicative model: expected risk, 1.79; observed risk, 3.56; expected/observed rate, 0.53 (P = 0.23).

Table 4. Interactions between different HPV types

(a) Interaction between HPV-6 and -16, all cervical cancers
Additive model: RERI = −2.35 (CI 95% −4.65 to −0.04).
Multiplicative model: expected risk, 5.59; observed risk, 1.64; expected/observed rate, 3/4 (P = 0.018).

(b) Interaction between HPV-16 and -18, all cervical cancers
Additive model: RERI = −1.67 (CI 95% −4.03 to 0.69).
Multiplicative model: expected risk, 5.59; observed risk, 1.64; expected/observed rate, 3/4 (P = 0.018).

To investigate if differences between crude and non-interfered risks were attributable to specific combinations of viruses, we calculated the virus-specific OR values for cervical cancer excluding subjects seropositive to other viruses. An increased HPV-16-associated risk was mainly seen when HPV-6-positive subjects were excluded and, to a lesser extent, also when HPV-18-positive subjects were excluded (Table 3). The difference between crude and non-interfered risks for HPV-33 appeared to be mostly due to HPV-16 (Table 3).

Seropositivity for HPV-16 was not associated with any excess risk for cervical carcinoma among HPV-6-seropositive women (OR, 1.0). Interactions were investigated by analysis using the jointly negative cases as a reference group (Table 4). Antagonism was detected between HPV-6 and HPV-16, which was significant both in an additive model [RERI, −2.35 (95% CI −4.65 to −0.04)] and in a multiplicative model (P = 0.02). To investigate whether the choice of cut-off level for HPV-6 seropositivity might have affected the results, two alternative cut-off levels (one higher and one lower) were also investigated, with similar results. At the lower alternative cut-off level, the HPV-6-associated OR for cervical cancer was 1.24 (0.83 to 1.87) and the RERI between HPV-6 and HPV-16 was −2.34 (−3.74 to 0.81). At the higher alternative cut-off level, the HPV-6-associated OR for cervical cancer was 1.37 (0.68 to 2.01) and the RERI between HPV-6 and HPV-16 was −2.19 (−4.35 to −0.02).

A tendency for interaction was also found between HPV-16 and HPV-18: additive model, RERI = −1.67 (95% CI −4.03 to 0.69); multiplicative model, P = 0.08 (Table 4).

Discussion

Our analysis of joint effects found noteworthy departures from those expected, mostly in the case of HPV-16 combinations. Since HPV-16 is the most common papillomavirus type in cervical cancers, it is expected that by comparison a larger proportion of the virus exposures are causal and that studies on causal interference should have the best power to detect interference in the case of HPV-16. The strongest interaction detected was an antagonistic interference between HPV-6 and HPV-16, which is well in line with previous suggestions of antagonism between benign and malignant HPV infections (Evans et al., 1992; Sigurgeirsson et al., 1991; Luostarinen et al., 1999).

Because controversy exists among biostatisticians as to whether additive or multiplicative models should be used to study interactions (Greenland, 1993), we evaluated the data using both types of models. The results were similar and the antagonism was statistically significant in both models.

The co-factors that determine whether an HPV infection will be cleared or persist and progress to cancer are not firmly established, although smoking and parity are implicated. The possibility that non-causal (benign) HPV infections may act as a protective co-factor has been suggested by the so-called ‘plateau’ effect. This effect has been used to describe several phenomena: (i) HPV seroprevalences are directly dependent on sexual history only among women with low numbers of sexual partners (Dillner et al., 1996); (ii) HPV DNA prevalences
are similarly associated with sexual history only among low-risk women (Hildesheim et al., 1993); and (iii) the sexual behaviour-associated risk for cervical neoplasia is also most clearly seen among low-risk women (Kjaer et al., 1993).

Although several different explanations for the plateau effect are possible, the present study suggests that virus antagonism may be an explanation.

The serum antibody response to HPV capsids is established to be HPV type-specific, except for HPV-6 and -11 capsids that contain both type-specific epitopes and epitopes shared between HPV-6 and -11 (Christensen et al., 1996). Cross-reactivity between HPV-6 and -11 may be the reason why very few subjects were HPV-11-positive in the absence of seropositivity to other HPV types. On the other hand, the fact that the antagonism with HPV-16 was only seen with HPV-6 suggests a substantial type-specific component of the HPV-6 antibody response.

It is conceivable that antagonism between seropositivity to two papillomaviruses in cervical cancer might be at the level of the antibody response rather than on the infection itself, i.e. that among women with cancer (but not among controls), the presence of antibodies against HPV-6 would antagonize the ability to mount an immune response to HPV-16. However, there was no evidence of such impairment of antibody responsiveness. As can be seen in Table 4(a), among cases and among controls an identical proportion (43%) of HPV-6-positive subjects were also HPV-16-seropositive.

A tendency to antagonistic interactions was also observed in another virus combination: HPV-16 and -18. The possibility that some of the risk associated with HPV-16 or -18 is attributable to confounding by co-variation with the other type is not likely, since the cancer risks observed when positives for the other type were excluded were similar or greater than the crude risks, suggesting that the antagonism does reflect a biological interference.

This is in contrast to the results obtained regarding HPV-33. This virus is categorized as a moderately carcinogenic virus, since it is rather uncommonly found in invasive cancer tissue. Although HPV-33 seropositivity is strongly related to cervical cancer risk, HPV-33 positivity had no association with cervical cancer when subjects seropositive to other HPV types were excluded from analysis. This suggests that the risk associated with HPV-33 seropositivity is attributable to confounding by other HPV types. The observed tendency for a synergistic interaction between HPV-33 and HPV-16 was not statistically significant.

The present study has investigated only five common types of HPV. Taking into account the large number of HPV types, it is likely that more comprehensive studies of more HPV types may reveal various additional interferences between them. Considering the fact that virus interferences may be of relevance for understanding of HPV-induced carcinogenesis and for design and evaluation of vaccines, such studies seem warranted.

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