**Null Results in Brief**

Human Leukocyte Antigen Class I Alleles and Cervical Neoplasia: No Heterozygote Advantage


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**Introduction**

Genes encoding the HLAs are the most polymorphic known in humans (1, 2). HLA class I molecules (HLA-A, -B, -C) present foreign antigenic peptides to CTLs and play a crucial role in the host response to infectious diseases and other intracellular pathogens. Their diversity ensures that a wide range of antigenic peptides will elicit an immunogenic reaction, leading to subsequent clearance of a pathogen (i.e., heterozygote advantage). Heterozygote advantage at HLA class I loci has been demonstrated for some infectious diseases (e.g., hepatitis B and human immunodeficiency virus; 3, 4). To our knowledge, the hypothesis of an HLA class I heterozygote advantage has not yet been explored for HPV and cervical neoplasia. Therefore, to determine whether a similar advantage would hold true for HPV and cervical neoplasia, we tested the hypothesis in a subset of women from three populations in Costa Rica and the United States. Our hypothesis: that heterozygosity at HLA class I loci would protect from HPV infection and cervical neoplasia.

**Materials and Methods**

Participants were selected from three projects sponsored by the National Cancer Institute: (a) a 10,077-woman population-based cohort in Guanacaste, Costa Rica (5), (b) a 24,000-woman cohort in Portland, Oregon (6), and (c) a 529-woman multicenter study of histological subtypes of cervical neoplasia in the Eastern United States (7). For the present study, a total of 1,429 women with available DNA were typed for HLA class I, including 321 women from the Costa Rican cohort, 670 women from the Portland, Oregon cohort, and 438 women from the Eastern United States study. In Costa Rica, this subset of women typed included 60% of the women diagnosed with cancer. 77% of HSIL, 74% of LSIL/HPV+, and 48% of cytologically normal population controls. From the Portland cohort, HLA typing was completed for a subset of women including 88% of women diagnosed with HSIL, 74% of LSIL, and 95% of cytologically normal controls. In the Eastern United States study, HLA typing was conducted for 89% of women diagnosed with adenocarcinoma, 85% of squamous cell carcinoma, 83% of HSIL, 82% with adenocarcinoma in situ, and 69% of cytologically normal population controls. Our final analytic group consisted of cancer/high-grade lesions ($n = 473$), low grade lesions ($n = 275$), and cytologically normal population controls ($n = 681$). HLA class I loci were molecularly typed using PCR/single-stranded oligonucleotide primer (SSOP)-based protocols developed by the 13th International Histocompatibility Workshop with DNA from blood collected from each participant. Cervicovaginal samples were PCR-tested for HPV DNA as described previously (5–7). To test the hypothesis of a heterozygote selective advantage, we determined whether an increase in disease risk (e.g., LSIL, HSIL/cervical neoplasia) was observed for those who were homozygous at the HLA loci. In essence, we assessed whether those who did not exhibit a heterozygote selective advantage were at an increased risk for disease. We assessed zygosity at individual HLA loci (HLA-A, -B, or -C) and combined zygosity across the three class I HLA loci. The ORs and 95% CIs were calculated using SAS version 8 (SAS Institute, Cary, NC). Logistic regression analysis was performed to provide risk estimates adjusted by study to account for potential population differences. This study had 80% power (two-sided test of significance, $\alpha = 0.05$) to detect an OR of 1.4 for those homozygous at one HLA class I locus.

**Results**

Adjusted by study, we found a combined risk estimate for cancer/HSIL of 1.2 (95% CI, 0.8–1.7) for HLA-A homozygosity, 0.8 (95% CI, 0.5–1.4) for HLA-B homozygosity, and 0.8 (95% CI, 0.5–1.3) for HLA-C homozygosity, when compared with women heterozygous at the respective class I loci (Table 1). Compared with women heterozygous at all three loci, women homozygous at one HLA class I locus had a 0.8-fold risk (95% CI, 0.6–1.2) for cancer/high-grade lesions, adjusted by study. Women homozygous at two or three loci ($n = 69$), possessed a 1.1-fold risk (95% CI, 0.6–2.0) of cancer/high-

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2 The abbreviations used are: HLA, human leukocyte antigen(s); HPV, human papillomavirus; OR, odds ratio; CI, confidence interval; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

3 Internet address: http://www.ihwg.org/protocols/protocol.htm.
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grade lesions, compared with those heterozygous at all three loci. Null risk estimates were seen for individual HLA-A, -B, or -C homozygosity as well as for increasing numbers of homozygous loci when LSIL/HPV-16 cases were compared with population controls (data not shown). We also conducted analyses restricted to women positive for HPV-16; risk estimates remained null (data not shown).

Discussion

Because cytotoxic T cells are thought to be important in the control of HPV infection and destruction of HPV-infected cells, evading HLA class I-restricted cytotoxic T-cell response is one mechanism by which HPV can potentially remain undetected from immune surveillance. Theoretically, HLA class I heterozygotes will, in general, present a broader range of peptides than homozygotes; selective advantage for HLA class I heterozygotes has in fact been shown for hepatitis B and HIV (3, 4). That we did not find the same results for HPV and cervical neoplasia supports the notion that the immunological mechanisms by which HPV evades the immune system are at least somewhat distinct from that of other viruses. Unlike HIV, HPV is a relatively stable virus, inasmuch as evolution of HPV sequences is not readily observed and HPV variations within a person are considered rare. Furthermore, HPV maintains low viral gene expression and minimal systemic detectability, making HPV infection in general poorly immunogenic. However, we note that an effective immune response can in fact be mounted when HPV is detected, as evident from the protective associations observed for specific HLA loci and cervical cancer (6). One limitation of this study is the potential for confounding by population stratification, should specific alleles be associated with ethnicity. We do not believe this is likely to affect our analyses, which assesses overall HLA zygosities without regard to the specific allele. Nevertheless, our combined-risk estimates were adjusted by the study to account for any unmeasured population differences. In conclusion, evading immune surveillance through low immunogenicity may serve as an effective mechanism by which HPV appears to escape the host’s immune response. Data from our three populations suggest that the advantages afforded from a broad immune response for infectious diseases did not result in a decrease in risk for HPV infection and cervical neoplasia.

References

Table 1 Effect of homozygosity at each of the three HLA class I loci -A,-B, and -C and level of homozygosity on the risk for cancer/high-grade lesions, compared with individuals not homozygote for specified loci

<table>
<thead>
<tr>
<th>Study population</th>
<th>HLA-A homozygous for locus</th>
<th>HLA-B homozygous for locus</th>
<th>HLA-C homozygous for locus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n OR 95% CI</td>
<td>n OR 95% CI</td>
<td>n OR 95% CI</td>
</tr>
<tr>
<td>Guanacaste, Costa Rica (n = 242)</td>
<td>40 0.6 0.3–1.3</td>
<td>11 0.5 0.1–1.9</td>
<td>24 0.8 0.3–2.2</td>
</tr>
<tr>
<td>Portland, Oregon (n = 474)</td>
<td>82 1.5 0.8–2.7</td>
<td>39 0.5 0.2–1.5</td>
<td>60 0.8 0.4–1.7</td>
</tr>
<tr>
<td>Eastern United States (n = 438)</td>
<td>55 1.3 0.7–2.3</td>
<td>23 1.5 0.6–3.6</td>
<td>41 0.8 0.5–1.7</td>
</tr>
<tr>
<td>Combineda,b (n = 1154)</td>
<td>177 1.2 0.8–1.7</td>
<td>75 0.8 0.5–1.4</td>
<td>125 0.8 0.5–1.3</td>
</tr>
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</table>

a Excluding LSILs.
b Combined ORs adjusted by study.
c Number of individuals homozygous at specific locus.