Tumor Necrosis Factor a-11 and DR15-DQ6 (B*0602) Haplotype Increase the Risk for Cervical Intraepithelial Neoplasia in Human Papillomavirus 16 Seropositive Women in Northern Sweden

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Abstract
HLA genes have been shown to be associated with cervical intraepithelial neoplasia (CIN), a precursor of cervical cancer. The human papillomaviruses (HPV) types 16 and 18 are the major environmental cause of this disease. Because the immune system plays an important role in the control of HPV infection, the association of polymorphic HLA could lead to a different immune response to control the development of cervical cancer. The aim of this study was to analyze the association between CIN and a microsatellite polymorphism of tumor necrosis factor (TNFα) taking HPV exposure and CIN-associated HLA haplotypes into account. In a nested case-control study in northern Sweden, 64 patients and 147 controls matched for age and sex and derived from the same population-based cohort were typed for TNFa, HLA-DR, and DQ and assayed for antibodies to HPV types 16 and 18. TNFα polymorphism was not associated with CIN per se. However, there was a significant increase in the frequency of TNFα-11 among HPV16-positive and HLA DR15-DQ6 (B*0602) patients compared with HPV16- and HLA-DQ6-negative patients (odds ratios, 5.4 and 9.3, respectively). The relative risk for CIN conferred by the combination of TNFα-11, HLA-DQ6, and HPV 16 positivity was 15. Our study suggests that the TNFα-11 allele is associated with HPV16 infection and associated with CIN in combination with HLA-DQ6 but not by itself.

Introduction
Oncogenic HPV16 and -18 play a key role in the etiology of CIN, a precursor of cervical cancer (1–3). There is insufficient information on the immunological responses to oncogenic HPV infections and how they might influence the pathogenesis of virus-associated tumors. Several studies have suggested a possible role of HLA in the development of HPV-associated CIN (4, 5). HPV-transformed cells show an abnormal expression of HLA class I and II molecules (6, 7). Murine experiments have also shown that the MHC may contain tumor suppressor genes (8). Since HLA class I and class II molecules are highly polymorphic, they may present different sets of HPV-derived peptides to T cells. This points to a central role of HLA molecules in the control of HPV infection. To understand the role of HLA in the development of CIN, additional genetic markers within or in linkage to human MHC should be studied. Genes encoding for products involved in immune responses are located within the human MHC region, and despite the importance of HLA genes as a contributing factor in the pathogenesis of CIN, not many genes in the class III region have been studied in detail. Several genes occupy the MHC class III region. The MHC class III region encodes proteins with important known immune functions such as complement system (C2, C4), TNFα, and IFNγ (10). The TNF genes are located centromeric to HLA-B and telomeric to C2. TNFα (cachectin) and TNFB (lymphotoxin) proteins are inducible cytokines with a broad range of immunoregulatory and proinflammatory effects. TNFα in synergy with IFNγ mediates up-regulation of MHC class II antigens (9).

The TNF region contains several polymorphisms that are associated with different levels of TNF secretion and susceptibility to autoimmune and infectious diseases (10). The TNF locus is 12 kb in length and contains several polymorphic areas, including biallelic restriction sites and five microsatellites (TNF a-e) (11–13). The TNFα microsatellite contains CA/CT dinucleotide repeats and has the highest variety of 14 known alleles. The number of TNFα dinucleotide repeats correlates with TNFα secretion in mononuclear cells (14, 15).

There is a lot of evidence in the literature which shows that many individuals are HPV-positive, but few of them form CIN

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The abbreviations used are: HPV, human papillomaviruses; CIN, cervical intraepithelial neoplasia; TNF, tumor necrosis factor; OR, odds ratio; CI, confidence interval.

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Polymorphism in CIN Patients

In this paper, *TNFA* refers to the gene, *TNFA* to the alleles, and *TNF* to the protein.

### Materials and Methods

#### Study Design.

Since 1988, blood samples (plasma, erythrocytes, and buffy coat) have been collected from individuals participating in a population-based health-promoting project in the Västerbotten county in northern Sweden (for a detailed description of the Västerbotten project, see Refs. 16 and 17). A total of 64 patients with incident CIN and 147 controls from the same cohort matched for age and sex were included in this study. For a detailed description of case definition and selection of controls, see Ref. 4. Patients and matched controls were all derived from the same population-based cohort that participated in this nested case-control study. This form of selection of the cases and controls increases the strength of the study design. Genomic DNA was extracted from the buffy coat using the standard phenol-chloroform extraction protocol.

#### HPV Serology.

Serum samples were tested for antibodies against HPV16 and HPV18 capsids using the standard two-step ELISA method employing monoclonal antibodies to human IgG, as published previously (16).

#### Amplification of *TNFA* Microsatellites.

Analysis of the *TNFA* microsatellite polymorphism was done as described by Nedospasov et al. (12). The 5' end of the reverse primer (Pharmacia-Biotech, Uppsala, Sweden) was labeled with HEX fluorescent dye. The PCR fragment sizes were identified in a Hybaid ABI prism 373 DNA sequencer. An internal size marker (500 TAMRA; Perkin-Elmer) was added to each sample to allow accurate determination of allele sizes. We used the Perkin-Elmer software Genotyper 2.0 to study the Gene Scan output file.

#### HLA Class-II Genotyping.

The polymorphic second exon of the *HLA-DQA1* and *DQB1* genes were amplified in a Hybaid OmniGene thermal cycler (Woodbridge, NJ), under denaturing conditions. The membranes were hybridized with sequence-specific oligonucleotides, 3' end-labeled with 32-P dCTP, and washed in high stringency conditions before exposure to X-ray films, as described previously (18). The membranes were stripped of the labeled probes under alkaline conditions and reused for probing with other oligonucleotides.

#### Statistical Analysis.

Comparison of the allele frequencies between the patients and the control groups was done by the $\chi^2$ test with Yates correction or, when appropriate, with Fisher's exact test. Probability values corrected for the number of comparisons made ($P_c$) were also calculated and considered significant if <0.05 (19). Multivariable analyses were done using logistic regression (LogXout software) controlling for covariates.

### Results

The results from *TNFA* microsatellite polymorphism performed in 64 patients and 147 controls are shown in Table 1. None of the alleles of TNF were positively or negatively associated with the disease.

Of the 64 patients, 28 were seropositive for HPV16, and 36 were seronegative. The *TNFA-11* allele was significantly more frequent in HPV16 seropositive patients compared with seronegative patients (OR, 5.40; $P_c < 0.01$; 95% CI, 1.9–15.3), but this significant finding was not observed in HPV18-positive patients (data for HPV18 not shown). Similarly, the *TNFA-11* allele was also more frequent in DQ6-positive patients compared to DQ6-negative patients (OR, 9.31; $P = 0.01$; 95% CI, 2.9–28.9), but it was not significant after the $P$ was corrected for multiple comparisons (13 multiple comparisons for *TNFA*). *TNFA-12* was not observed in this population. Multivariate analysis was also performed to confirm our observations (Table 2).

We calculated the frequency of HPV16 seropositives, DQ6, and *TNFA-11* carriers of genetic polymorphism in patients (64) and controls (147), and found that these markers together were significantly increased in the patients compared to the controls (OR, 15.05; $P_c < 0.01$; 95% CI, 4.49–50.4; Table 2).

### Discussion

Many studies investigated the importance of HLA polymorphisms on CIN in different populations (3, 4, 20, 21). HLA
associations with CIN have been specific mainly for HPV type 16. This suggests that HPV 16 may alter the HLA-binding peptide sequences in order to escape immune surveillance (22), or that certain HLA isoforms present HPV16-derived peptides to T cells in an inefficient way. Loss of HLA class-I and -II up-regulation of HLA class-II expression is also observed in cervical carcinomas (6, 7). The association of HLA molecules with CIN may also be a result of linkage disequilibrium to other neighboring genes (TNFA, TNFB, HSP70, or MHC class-I chain-related genes) in the human MHC region.

In this study we analyzed the relation between HPV16-associated CIN and a microsatellite polymorphism in the TNF region. Sixty-four patients diagnosed with CIN grades I–III and associated CIN and a microsatellite polymorphism in the TNF chain-related genes) in the human MHC region.

Based on the above observations, the following hypothesis was tested:

In conclusion, the TNFa-11 allele is more frequent in HPV16-seropositive CIN patients and in HLA-DQ6-positive CIN patients. Together, these three markers confer high risk for the development of CIN in individuals in northern Sweden. These observations may have important implications in the understanding of the pathogenesis of the disease and in the design of vaccines for immunotherapeutic strategies.

Table 2: Analysis of TNFa-11, HLA-DQ6, and HPV16 seropositivity in CIN patients and controls

<table>
<thead>
<tr>
<th>Patients</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFa-11 positive</td>
<td>27/64 (42%)</td>
<td>58/147 (39%)</td>
<td>1.12</td>
</tr>
<tr>
<td>TNFa-11 positive adjusted*</td>
<td>27/64 (42%)</td>
<td>58/147 (39%)</td>
<td>0.9426</td>
</tr>
<tr>
<td>DQ6 positive</td>
<td>26/74 (35%)</td>
<td>32/164 (20%)</td>
<td>2.23</td>
</tr>
<tr>
<td>DQ6 positive adjusted*</td>
<td>26/74 (35%)</td>
<td>32/164 (20%)</td>
<td>2.26</td>
</tr>
<tr>
<td>HPV16 seropositive</td>
<td>33/74 (46%)</td>
<td>39/164 (24%)</td>
<td>2.58</td>
</tr>
<tr>
<td>HPV16 seropositive adjusted*</td>
<td>33/74 (46%)</td>
<td>39/164 (24%)</td>
<td>2.4426</td>
</tr>
<tr>
<td>TNFa-11 positive and HPV16 seropositive</td>
<td>18/63 (29%)</td>
<td>10/152 (7%)</td>
<td>5.68</td>
</tr>
<tr>
<td>DQ6 positive and HPV16 seropositive</td>
<td>15/74 (20%)</td>
<td>5/164 (3%)</td>
<td>8.08</td>
</tr>
<tr>
<td>TNFa-11 positive, HLA-DQ6 positive, and HPV16 seropositive</td>
<td>11/64 (17%)</td>
<td>2/147 (1%)</td>
<td>15.05</td>
</tr>
</tbody>
</table>

*NS, not significant.
* Adjusted for DQ6 and HPV16 in a multivariable logistic regression model.
* Adjusted for TNFa-11 and HPV16 in a multivariable logistic regression model.
* Adjusted for TNFa-11 and DQ6 in a multivariable logistic regression model.

References


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