Tumor Necrosis Factor α-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 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), TNF (α, β), and genes with less known immune involvement, such as heat shock proteins and MHC class-I chain-related genes. The TNF genes are located centromeric to HLA-B and telomeric to C2. TNFα (cachectin) and TNFβ (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/GT 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.
lesions. Thus the role of the immune system is critical to understanding how most patients repel HPV, or who may be at risk to form a CIN lesion. The aim of our study was to test the hypotheses that: (a) the microsatellite polymorphism of TNFα is associated with CIN; and (b) TNFα together with HLA-DQ6 and HPV16 or -18 seropositivity increases the risk for development of CIN in a population-based cohort in the Västerbotten county of northern Sweden.

In this paper, TNFA refers to the gene, TNFa to the alleles, and TNFα 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 TNFα 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 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 χ2 test with Yates correction or, when appropriate, with Fisher’s exact test. Probability values corrected for the number of comparisons made (Pc) were also calculated and considered significant if <0.05 (19). Multivariate 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; Pc < 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; Pc < 0.01; 95% CI, 2.99–28.9), 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; Pc < 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...
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>TNFa-11 positive adjusted(^a)</td>
<td>27/64 (42%)</td>
<td>58/147 (39%)</td>
</tr>
<tr>
<td>TNFa-11 positive adjusted(^b)</td>
<td>27/64 (42%)</td>
<td>58/147 (39%)</td>
<td>0.9426</td>
</tr>
<tr>
<td>DQ6 positive</td>
<td>DQ6 positive adjusted(^b)</td>
<td>26/64 (35%)</td>
<td>32/164 (20%)</td>
</tr>
<tr>
<td>HPV16 seropositive</td>
<td>HPV16 seropositive adjusted(^b)</td>
<td>33/74 (46%)</td>
<td>39/146 (24%)</td>
</tr>
<tr>
<td>DQ6 positive and TNFa-11 positive</td>
<td>16/62 (26%)</td>
<td>16/145 (11%)</td>
<td>2.8</td>
</tr>
<tr>
<td>TNFa-11 positive and HPV16 seropositive</td>
<td>TNFa-11 positive, HLA-DQ6 positive, and HPV16 seropositive</td>
<td>18/63 (29%)</td>
<td>10/152 (7%)</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>
</tbody>
</table>

\(^a\) NS, not significant. \(^b\) Adjusted for DQ6 and HPV16 in a multivariable logistic regression model.

associations with CIN have been specific mainly for HPV type 16. This suggests that HPV16 may alter the HLA-binding peptide sequences in order to escape immune surveillance (22), or that certain HLA isomers present HPV16-derived peptides to T cells in an inefficient way. Loss of HLA class-I and -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 147 matched controls all derived from the same population-based cohort participated in this nested case-control study. This form of selection of the cases and controls increases the strength of the study design. The sample size of the study was small. But using this strong study design, we observed a maximum risk in HPV16 seropositive patients with the haplotype TNFa-11 and HLA-DQ6. TNFa-11 gives a risk of 5.4 times more in HPV16-seropositive patients than in the seronegative ones. TNFa-11 was not found with higher frequency in HPV18 seropositive groups. The seropositivity for the HPV16 denotes either present or past infection with HPV type 16 virus. However, DNA typing for HPV16 in biopsies would only identify present viral infection.

TNFa-11 as a marker alone cannot be considered as a predisposition factor. When all three markers (HPV16 seropositivity, HLA-DQ6 positivity, and TNFa-11) were taken together, they give a risk 15 times greater than in the healthy matched controls. It can be speculated that the secretion of TNFa in HPV16-seropositive and DQ6 carriers is associated with the formation of CIN. But in the presence of HPV18 infection this does not happen, suggesting that there are differences in the pathogenesis of CIN mediated by HPV16 and HPV18 (21). HPV16 seropositivity is shown to be associated with an increased risk of squamous cell carcinoma. In contrast, risk associated with HPV18 seropositivity tended to be higher for cervical adenocarcinoma (23). Furthermore, the different expression of TNFa and IL-10 may be critical in the formation of the premalignant cervical epithelium and also in the limitation of local immune responses (24).

TNFa microsatellite alleles have been shown to be associated with different immune-mediated diseases such as rheumatoid arthritis (25), insulin-dependent diabetes mellitus (15), and myasthenia gravis (26) and in identifying acute graft rejection following renal transplantation prior to its development (27). Association of TNF microsatellites with disease may be a result of linkage disequilibrium with the extended MHC haplotypes or a direct biological impact of TNFa. The number of dinucleotide repeats in the TNFa are also shown to correlate with the secretion of TNFa, where the TNFa-2 allele is associated with high TNFa secretion and the TNFa-6 allele is associated with low secretion of TNFa (15). It is not clear whether the TNFa-11 allele is associated with high or low secretion of the TNFa.

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.

References


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