Association between Human Leukocyte Antigen Polymorphism and Human Papillomavirus 16-positive Vulval Intraepithelial Neoplasia in British Women

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ABSTRACT

Polymorphisms in human leukocyte antigen (HLA) genes have been implicated in the risk for developing human papillomavirus (HPV)-associated cervical neoplasia. By comparison with local cadaver controls typed for HLA class I (n = 946) and II (n = 144) antigens, HPV-16-positive high grade vulval intraepithelial neoplasia patients (n = 42) showed significantly different frequencies of HLA-A2 [odds ratio (OR), 2.1; confidence interval (CI), 1.4–3.9], HLA-B7 (OR, 2.6; CI, 1.4–4.7), HLA-DRB1*0401/minimize (OR, 0.1; CI, 0.03–0.5), HLA-DRB1*11 (OR, 3.3; CI, 1.4–7.1), HLA-DRB1*13 (OR, 0), HLA-DQB1*05 (OR, 0.2; CI, 0.05–0.6), and HLA-DQB1*0602 (OR, 4.6; CI, 1.5–14.0). With the exception of HLA-B7 and HLA-DRB1*11, these significant differences were also seen comparative to local HPV-16-positive cervical carcinoma patients (n = 114), suggesting a specific immunogenetic configuration that is independent of HPV-16 infection in high-grade vulval intraepithelial neoplasia. Such factors are important to the development of HPV vaccines for treatment of cervical and vulval neoplasia.

INTRODUCTION

Persistent infection with high risk HPV has been implicated in the etiology of malignant and premalignant disease of the female lower genital tract (1, 2). An active role for host immunity in the natural clearance of HPV infection is supported by the observation that allograft recipients taking immunosuppressive therapy and women with AIDS have an increased incidence of HPV-associated CIN and VIN (3). HLAs class I and II play an important role in regulating T-cell responses to viral proteins. Different HLA alleles present different peptides to the immune system, and it is likely that the range of alleles inherited by an individual is significant in determining the outcome of viral infections (reviewed in Ref. 4). Indeed, particular HLA alleles have been associated with susceptibility or resistance to HPV infection and cervical neoplasia (5–11), although many of these correlations have not been consistently strong or significant across different populations (4). However, in one recent study, a variant HPV-16 (E6 L83V) was seen to be associated with the DRB1*04-DQB1*03 haplotype in Swedish, Czech, and Italian women, irrespective of differences in the allele frequencies in these countries (12).

High-risk HPV-associated VIN is frequently a chronic, multifocal, high-grade disease that tends to recur after removal by local excision or laser therapy, and carries an appreciable risk of progression to invasive vulval cancer if left untreated (13). The importance of VIN relates to the symptoms it causes, the difficulty it poses to the clinician in treating it effectively and its increase in incidence over recent decades, particularly among young women (14). The study of HLA polymorphisms in HPV-associated LGIN has thus far been limited to women with CIN and cervical cancer. Here, we compare HLA class I and II polymorphisms in women with HPV-16-positive high-grade VIN with HPV-16-positive cervical carcinoma patients and controls. The objective of our study was to determine whether similar immunogenetic factors in HPV-16 VIN associate with susceptibility or protection as in cervical neoplasia. This is particularly important given the current drive to improve our understanding of the immunogenetic factors associated with female lower genital tract neoplasia in the climate of HPV vaccine development for both cervical and vulval disease (15).

MATERIALS AND METHODS

Patient and Control Subject Selection. Our study population comprised 50 consecutive patients with LGIN who were screened to take part in a Phase II HPV vaccine trial at St. Mary’s Hospital in Manchester (38 patients) and Addenbrooke’s Hospital in Cambridge, United Kingdom (12 patients). Patient blood samples and lesion biopsies were taken as part of the screening procedure for vaccine trial entry. All samples were obtained with written informed consent after local research ethical committee approval. Histological grading of all tissue specimens was performed by two independent consultant histopathologists with a special interest in gynecological oncology. Control HLA antigen frequencies were determined from cadaver organ donors from northwest United Kingdom [serology: 946 for HLA-A and HLA-B; DNA-based techniques: 144 for HLA-DRB1 and HLA-DQB1 as described previously (7)]. HLA antigen frequencies in the LGIN group were additionally compared with those seen in a cohort of cervical carcinoma patients from northwest United Kingdom (n = 188) recruited as documented in Ref. 7.

Preliminary analyses of the frequency of HLA specificities in the LGIN group as a whole (n = 50) showed some HLA antigens with significant differences in frequency compared with local controls. This finding was not altered when the Manchester and Cambridge datasets were analyzed separately, with the exception of HLA-B7. The data from these two groups of LGIN patients were pooled for subsequent analyses.

HPV Typing. DNA was extracted from the biopsies either by guanidium isothiocyanate extraction or proteinase K digestion. All specimens were positive for β-globin. HPV DNA was detected using GP5+/GP6+ consensus primers. The PCR products were either sequenced directly or genotyped using HPV type-specific primers for 6/11, 16, 18, 31, and 33 as described in Ref. 16. Amplification products were resolved on 2% agarose gels and visualized by ethidium bromide staining.

HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 DNA-based Typing by PCR Saline-Sodium Phosphate. DNA was extracted and purified from peripheral blood using the Dynabeads DNA Direct System 2 kit (Dynal A.S., Oslo, Norway) and processed according to the manufacturer’s instructions. HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 molecular typing was per-
formed using the Dynal Allset™ Saline-Sodium Phosphate low resolution kit (Dynal Biotech Ltd., Bromborough, United Kingdom) according to the manufacturer’s instructions.

Statistical Analysis. The χ² test was used to compare HLA antigen frequencies in the patient and control groups. Fisher's exact test was used when antigen frequencies were too small for the χ² test. A value of P < 0.05 was considered statistically significant.

RESULTS

Biopsy Pathology and HPV Typing. Our LGIN patient group comprised 42 women with biopsy proven HPV-16-positive VIN 3; 2 women with HPV-16-positive vaginal intraepithelial neoplasia 3; 2 women with HPV-negative VIN 3; 2 women with HPV-negative VIN 1 or normal vulval epithelium on current vulval biopsy but with a previous history of VIN 3 (confirmed histologically); 1 woman with HPV-18-positive VIN 3; and 1 woman with HPV-33-positive VIN 3. Of the 46 patients with biopsy-proven high-grade VIN, therefore, 44 were HPV-positive (96%) and 2 (91%) were HPV-16-positive. The high prevalence of HPV-16 infection in VIN was significantly different from the 61% observed for the local cervical cancers (n = 188, χ² = 15.64; P < 0.001).

HLA Class I and II Antigen Frequencies. Here, we present the immunogenetic analysis performed on the HPV-16-positive high-grade VIN patients (n = 42), although it is also true for the LGIN group as a whole. Tables 1 and 2 document the frequencies of the common HLA class I and class II antigens in HPV-16-positive VIN, HPV-16-positive cervical carcinoma patients, and local controls. The frequencies of HLA antigens in the HPV-16-positive high-grade VIN patients were initially compared with the control population (uncorrected χ² test unless otherwise stated). The following antigens were significantly increased in frequency in the HPV-16-positive VIN patients compared with the controls, respectively: HLA-A2 (67 versus 49%; P = 0.025); HLA-B7 (50 versus 28%; P = 0.002); HLA-DRB1*11 (26 versus 10%; P = 0.006); and HLA-DQB1*0302 (17 versus 4%; P = 0.011, Fisher’s exact test). The trend for an increased frequency of HLA-DQB1*0302 in the HPV-16-positive VIN patients (55 versus 38%; P = 0.056) did not reach statistical significance. Three HLA class II antigens were significantly reduced in frequency in the HPV-16-positive VIN patients compared with controls: HLA-DRB1*01010204 (5 versus 29%; P = 0.001); HLA-DRB1*13 (0 versus 17%; P = 0.005); and HLA-DQB1*05 (7 versus 31%; P = 0.002). These analyses are summarized in Table 3.
Secondly, the frequencies of these antigens in the HPV-16-positive high grade VIN patients were compared with a cohort of local HPV-16-positive cervical carcinoma patients. The following showed an increased prevalence in VIN: HLA-A2 (67 versus 41%; \( P = 0.05 \)); HLA-DQB1*03032 (55 versus 35%; \( P = 0.027 \)); and HLA-DQB1*03032 (17 versus 5%; \( P = 0.043 \), Fisher’s exact test). There was a trend in the VIN patients for an increased frequency that did not reach statistical significance of HLA-B7 (50 versus 39%; \( P = 0.20 \)) and HLA-DRB1*11 (26 versus 17%; \( P = 0.23 \)). The following showed a decreased prevalence in VIN: HLA-DRB1*01010204 (5 versus 20%; \( P = 0.02 \)); HLA-DRB1*13 (0 versus 12%; \( P = 0.018 \), Fisher’s exact test); and HLA-DQB1*05 (7 versus 25%; \( P = 0.013 \); Table 3).

Finally, we investigated the frequency of HLA antigens in the HPV-16-positive cervical cancer patients compared with local controls. Of the various class I and II antigens implicated in the VIN patients, only HLA-B7 (39 versus 28%; \( P = 0.02 \)) was significantly different in prevalence from the control group. Other comparisons as were as follows: a trend for an increase in HLA-DRB1*11 (17 versus 10%; \( P = 0.08 \)) and marginal decreases in HLA-DRB1*01010204 (20 versus 29%; \( P = 0.125 \)); HLA-DRB1*13 (12 versus 17%; \( P = 0.34 \)); and HLA-DQB1*05 (25 versus 31%; \( P = 0.33 \); Table 3).

In summary, HLA-B7 and HLA-DRB1*11 appear to show similar increased frequencies in both HPV-16-positive cervical cancer and VIN, whereas HLA-A2, HLA-DRB1*01010204, HLA-DRB1*13, HLA-DQB1*05, HLA-DQB1*03032, and HLA-DQB1*03032 show a stronger association with VIN than cervical cancer as evidenced by significant differences in antigen frequencies in VIN patients when compared with both HPV-16-positive cervical cancers and controls.

**DISCUSSION**

The LGIN patients in this study were recruited on the intention to treat high-grade disease with an HPV vaccine. They were consecutive patients in both Manchester and Cambridge, selected by pathology (high-grade LGIN) and subsequently typed for HPV. Although all local controls were from Manchester, we have no reason to believe that the frequency of HLA class I and II antigens in Manchester and Cambridge is significantly different. The cervical cancer patients were recruited consecutively at a single clinic at the Christie Hospital as previously described (7) between 1988 and 1996 and stratified for HPV type. The data suggest that there is an increased prevalence of HPV-16 infection associated with high-grade VIN compared with cervical cancer and are in keeping with recent studies of high-grade VIN (1). Single cases of VIN were detected with HPV-18 and HPV-33 (1 of 44, 2%) infections, which compares with a prevalence of 17% (31 of 188) and 3% (5 of 188), respectively, in the cervical carcinoma patients. It appears that VIN is much more homogeneous with respect to HPV type than cervical carcinoma.

For immunogenetic analysis, only the HPV-16-positive cases of VIN and cervical cancer have been compared. Strikingly, there appear to be different HLA associations in these two HPV-16-associated diseases. Historically, cervical neoplasia has been reported as showing an association with several HLA antigens, including HLA-A2 (17), HLA-B7 (7), HLA-B44 (18), HLA-DRB1*15 (11), and HLA-DQB1*03 (5). Evidence of HPV-16 specificity has been seen with HLA-DRB1*11 (7), HLA-DRB1*15 (DRB1*1501DQB1*0602; Ref. 11), HLA-DRB1*07 (9), HLA-DQB1*030205060708 (8), and HLA-DQB1*03032 (4). A decreased risk for cervical neoplasia has been associated with HLA-DRB1*13 (6, 8), but the mechanism appears to be independent of HPV type. These observations are supported by a meta-analysis by Konya and Dillner (4).

In this study, HPV-16-positive cervical cancer patients show increased frequencies of HLA-B7 and a trend for HLA-DRB1*07 (34 versus 24%; \( P = 0.11 \)) and HLA-DRB1*15 (41 versus 33%; \( P = 0.23 \)) as documented previously (7). By comparison, HPV-16-associated high-grade VIN shows a significant increase in HLA-A2, HLA-B7, HLA-DRB1*11, HLA-DQB1*030104, HLA-DQB1*03032, whereas there are significant decreases in the frequencies of HLA-DRB1*01010204, HLA-DRB1*13, and HLA-DQB1*05. Odds ratios have been used to provide a measure of these altered frequencies. Thus, compared with local controls, the following HLA antigens were associated with an increased probability of HPV-16-positive VIN, as documented in Table 4: HLA-A2 (2.6x); HLA-DRB1*11 (3.3x); and HLA-DQB1*03032 (4.6x). Other HLA antigens were associated with a decreased probability of HPV-16-positive VIN: HLA-DRB1*01010204 (10x); HLA-DQB1*05 (5x);
and HLA-DRB1*13 (where none of the VIN patients had the allele). VIN is an uncommon disease and this is a small study, which limits its statistical power, and is additionally complicated by the multiple comparisons required for analysis of HLA antigen frequencies. We have not used Bonferroni adjustments because this pilot study was designed to generate hypotheses relating to HLA class I and II polymorphisms in patients with HPV-16-positive high-grade VIN and any significant associations detailed here will clearly need confirmation from larger studies (19).

The surprising result in the context of VIN is the concurrence of so many HLA antigen associations previously implicated with marginal significance in cervical neoplasia. Studies that have looked for an influence of HLA polymorphisms in the development of cervical cancer have often pooled high-grade CIN with cervical carcinoma patients for the purposes of analysis. However, because up to 30% of high-grade CIN lesions regress spontaneously (20), it may be inappropriate to combine premalignant and malignant cervical disease in this manner. The resulting groups of patients are likely to be much more heterogeneous than the high-grade VIN patients studied here, who all had a diagnosis of HPV-16-positive VIN 3. Thus when comparing distinct HPV-16-positive cervical cancer and VIN patients, the significant differences seen for HLA antigens could reflect immunogenetic factors relevant to establishing a chronic, premalignant lesion. These factors appear to be independent of the HPV type of the infection.

In the natural history of the disease, HLA may influence susceptibility to HPV infection or the ability to clear the virus and avoid persistence, which is the key risk factor for progression (2). This effect is, in part, because of variation in the presentation of HPV-associated peptides through HLA class I or II molecules to CD8+ or CD4+ T-cell effectors, respectively. In HPV-16-positive cervical cancer and high-grade VIN, susceptibility and persistence is likely to be similar, but the genetic consequences for progression (other genetic changes and local factors) may be different. It seems probable that because of linkage disequilibrium in the HLA region, the associations described here may reflect other genetic factors. One possibility is some association with products of the class III region, for example, levels of tumor necrosis factor cytokine can be influenced by polymorphism (21), which may have different consequences in the cervix and vulva. This may prejudice early influences on the innate immune response initiated at primary infection. Thus there is likely to be a complex interplay of HLA factors, which may include polymorphic influence on specific T-cell activation and lesion cytokine production (22).

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