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HLA-Cw group 1 ligands for KIR increase susceptibility to invasive cervical cancer

Maureen P. Martin,
Cancer and Inflammation Program, Laboratory of Experimental Immunology, SAIC-Frederick, Inc. NCI-Frederick, Frederick, MD 21702, USA

Ingrid B. Borecki,
Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110, USA

Zhengyan Zhang,
Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO 63110, USA

Loan Nguyen,
Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO 63110, USA

Duanduan Ma,
Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110, USA

Xiaojiang Gao,
Cancer and Inflammation Program, Laboratory of Experimental Immunology, SAIC-Frederick, Inc. NCI-Frederick, Frederick, MD 21702, USA

Ying Qi,
Cancer and Inflammation Program, Laboratory of Experimental Immunology, SAIC-Frederick, Inc. NCI-Frederick, Frederick, MD 21702, USA

Mary Carrington, and
Cancer and Inflammation Program, Laboratory of Experimental Immunology, SAIC-Frederick, Inc. NCI-Frederick, Frederick, MD 21702, USA, Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology and Harvard University, Boston, MA 02114, USA

Janet S. Rader
Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110, USA, Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO 63110, USA

Janet S. Rader: jrader@mcw.edu

Abstract

Inherited genetic polymorphisms within immune response genes have been shown to associate with risk of invasive cervical cancer (ICC) and its immediate precursor, cervical intraepithelial neoplasia grade 3. Here, we used the transmission/disequilibrium test to detect disease-liability

Correspondence to: Janet S. Rader, jrader@mcw.edu.

Present Address: J. S. Rader (✉), Department of Obstetrics and Gynecology, Medical College of Wisconsin, Milwaukee, WI 53226, USA

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alleles and investigate haplotype transmission of *KIR* and *HLA* class I polymorphisms in a large family-based population of women with cervical cancer and their biological parents (359 trios). The effect of distinct human papillomavirus types was also explored. HLA-Cw group 1 (HLA-Cw alleles with asparagine at position 80), which serves as ligand for certain killer immunoglobulin-like receptors (KIR), was significantly overtransmitted in women with ICC (P=0.04), and particularly in the subgroup of women infected with high risk HPV16 or 18 subtypes (P=0.008). These data support the involvement of the *HLA-C* locus in modulating the risk of cervical neoplasia perhaps through its function as ligands for KIR, but functional studies are essential to confirm this hypothesis.

Keywords

Cervical neoplasia; HPV; HLA; KIR

Although the lifetime risk of contracting human papillomavirus (HPV) is 80% in the general population, most HPV infections are successfully resolved by the host immune system (Doorbar 2006). Even so, HPV infection is the most important risk factor for the development of intraepithelial lesions (CIN) and invasive carcinoma of the cervix (ICC) (IARC Working Group 1995). Moreover, persistent infection, which depends in part on the virus' ability to subvert the immune system, is one of the critical steps in the progression of the disease (Koshiol et al. 2008). HPV DNA can be identified in almost all specimens of invasive cervical cancer and in the vast majority of cervical intraepithelial neoplasia grade 3 (CIN3) (Bosch and de Sanjose 2007).

There is convincing evidence that points to the role of the immune response in viral clearance including the increased incidence of HPV-related diseases in individuals with immune deficiency (Chaturvedi et al. 2009), and the presence of an intense inflammatory infiltrate in the vicinity of regressing genital warts (Coleman et al. 1994). HLA class I and class II proteins are central to the host immune responses to viral infections and other pathogens. They are the most polymorphic genes in the human genome and variations in the peptide binding groove influence antigenic specificity. Numerous studies have evaluated the association of *HLA class I* genes (*HLA-A*, *HLA-B*, and *HLA-C*) and *class II* genes (*HLA-DQ* and *HLA-DR*) with susceptibility to cervical cancer (Neuman et al. 2000; Wang et al. 2001, 2002a, b), and they point to the importance of *HLA* in the pathogenesis of cervical neoplasia. Further, loss of HLA class I occurs in >70% of primary cervical cancers (Koopman et al. 2000), enabling HPV-infected cells to escape detection by the immune system. Other immune system genes such as *CD83* have also been implicated in susceptibility to cervical cancer (Zhang et al. 2007).

Natural killer (NK) cells are important elements of the innate immune system because they kill virus-infected cells and tumor cells. NK cells are regulated in part by activating and inhibitory killer immunoglobulin-like receptors (KIR), which recognize HLA class I molecules and convey either inhibitory or activating signals to NK cells. The interaction of inhibitory KIR (designated 2DL and 3DL) with specific HLA allotypes has been demonstrated (Long and Rajagopalan 2000; Reyburn et al. 1997).

Two case-control studies have examined the effects of variation at the *KIR* locus in women with CIN (Arnheim et al. 2005; Carrington et al. 2005). Carrington et al. found that specific inhibitory *KIR/HLA* ligand pairs were associated with decreased risk of developing cervical neoplasia, whereas the presence of the activating receptor *KIR3DS1* resulted in increased risk of disease (Carrington et al. 2005). The study by Arnheim et al. indicated that the inhibitory allele *KIR3DL1* associated with increased risk of CIN (although no mention is

made of the grade of CIN), while the activating allele *KIR3DS1* showed no association (Arnheim et al. 2005). These conflicting results led us to further examine the association of *KIR* and *HLA* genotypes with cervical cancer by analyzing a large population of affected women and family-based controls.

In order to assess whether *KIR3DL1/KIR3DS1* and *HLA class I* genes influence susceptibility to the development of CIN3 and ICC, we genotyped for the presence/absence of *KIR3DL1/3DS1* using PCR-SSP (Martin and Carrington 2008) and *HLA-Bw/HLA-Cw* group epitopes using sequencing analysis in cervical cancer trios (Table 1), which includes an affected woman and her biological parents. Due to the quality and/or quantity of available DNA, we were unable to perform full *HLA-B* and *HLA-C* allelic genotyping.

*HLA-C*w *group 1*, which serves as ligand for KIR2DL2/3, was significantly overtransmitted in the subset of women with ICC (P=0.04), while *HLA-Cw group 2*, which is the ligand for KIR2DL1, was under transmitted (Table 2). The significance of the association was further strengthened in the subgroup of women infected with HPV16- or HPV1618-associated HPV (P=0.008). There was no significant association of *KIR3DL1/S*1 or its *HLA-Bw4* ligand with cervical cancer (Supplementary Tables 1 and 2). We next examined the compound genotypes composed of *HLA-Bw*, *HLA-C* group, and *KIR*. No additional effect of *KIR* and/or *HLA-Bw* in combination with *HLA-C* group was seen (data not shown).

Due to the association between *HLA-C* variation and cervical cancer, we next evaluated a recently described polymorphism in the region, rs9264942, which is 35 kb upstream of *HLA-C* and was shown to associate with HIV-1 viral load set point levels (Fellay et al. 2007). This SNP also associates with the level of HLA-C mRNA in immortalized B cell lines derived from individuals of European ancestry (Stranger et al. 2005; Stranger et al. 2007) as well as cell surface expression of HLA-C (Thomas et al. 2009). There was no significant association between rs9264942 genotype and ICC in our study (N=612 trios; p=0.52), suggesting that level of HLA-C expression may not modulate risk of ICC (Supplementary Table 3).

The association between *HLA-Cw group 1* and increased risk of ICC in this study is in agreement with the previous study by Carrington et al. where *HLA-Cw group 2* was significantly associated with a decreased risk of developing CIN3/cervical cancer (Carrington et al. 2005). Interestingly, an association study of *HLA class I* alleles across several case-control and cohort studies identified *HLA-Cw*0202* (which is a group 2 allele) as protective in individuals with both high-grade Pap smears (OR 0.53, 95% CI 0.29–0.89) and low-grade Pap smears (OR 0.58, 95% CI 0.37–1.04) as compared with control subjects with normal cytologic cervical specimens (Wang et al. 2002a). Due to limited DNA resources, we were unable to perform full *HLA-C* allelic genotyping, and thus it was not possible to analyze individual *HLA-C* alleles in this study.

We replicated the *HLA-Cw* association in our family-based study using mostly women with invasive cervical cancer. The advantage of a family-based study is that it avoids false-positive results due to possible ethnic stratification that may affect the conventional case-control design (Cardon and Palmer 2003; Freedman et al. 2004). Furthermore, identification of adequate controls in a case-control study is problematic owing to the nearly ubiquitous, but heterogeneous, exposure to various HPV types and the ongoing risk of developing lesions with continued exposure. Family-based trios provide perfectly matched ethnic controls and obviate these other matching challenges. In addition, histologically confirmed tumor samples were available in our study, providing accurate diagnosis of stage as well as HPV type. While our cohort represents a relatively large data resource, it is not powered to find rather more subtle effects of variants on disease susceptibility. Power limitation may be

responsible for the failure to replicate the *HLA-B-KIR* association in this study that was previously reported for CIN3 previously (Carrington et al. 2005). In general, very large sample sizes are needed to achieve adequate power to detect interaction effects, depending on their strength. In addition, accurate determination of *KIR* haplotypes even with family data is inherently problematic since it is not always possible to determine gene copy number and therefore transmission. The predominance of invasive cancer in the current study might also contribute to the lack of *HLA-B* and *KIR* association. Indeed, a cervical cancer screening study in China uncovered unique risk factor profiles for CIN1, 2, and 3, such as number of pregnancies (Belinson et al. 2008). Thus, many host factors might promote the heterogeneity of cervical disease.

It is unclear how activation of NK cells might promote the development of cervical cancer. Previous studies have suggested that genotypes that theoretically increase activation of NK cells are beneficial in viral infections such as HIV and HCV, whereas they increase the risk for autoimmunity and perhaps cancers that have an inflammatory component like cervical cancer (reviewed in Kulkarni et al. 2008). This suggests that the host response to HIV and HCV differ from the response to HPV. This finding is not surprising because highly active antiretroviral therapy for HIV does not reduce the incidence of CIN or cervical cancer in women infected with both viruses (De Vuyst et al. 2008). Moreover, genetic and functional studies have shown that *KIR3DS1* protects against HIV infection even though it associates with increased susceptibility in cervical cancer (Carrington et al. 2008). Inflammation is an important response to infection, foreign elements such as tumor cells, and injury; however, on the flip side, chronic inflammation is also strongly associated with progression to certain cancers (de Visser et al. 2006). Indeed, inflammatory cells may be directly involved in malignant transformation and tumor progression by production of cytokines and other stimuli such as TNF-α, IL-6, and TGF-β that contribute to the proliferation and survival of tumor cells (Balkwill et al. 2005). The role of inflammation in the development of cervical cancer is not entirely understood. Thus, further work is needed to understand the role of inflammation and more specifically the role of NK cells and their receptors, in the development of cervical neoplasia.

The data presented here support the involvement of the *HLA-C* locus in modulating the risk of cervical neoplasia, as was implicated previously (Carrington et al. 2005). Overtransmission of *HLA-Cw group 1*, the ligands for KIR2DL2/3, in women with invasive cervical cancer, strengthens a model in which differential KIR-HLA-mediated interactions between effector cells and their targets alter the risk of cervical disease pathogenesis. However, functional studies are warranted in order to definitively define the role of KIR-HLA interactions in the pathogenesis of cervical cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of the study subjects

	Number of trios	Additional trios genotyped for rs9264942 (*N*=612)
Total	359	253
Race		
Caucasian	330	219
African American	28	32
Other	1	2
Age at diagnosis		
Mean (years) ± SD	34.19±7.64	34.16±9.75
< 40	265	174
≥40	93	64
Unknown	1	15
Histology		
CIN3	123	115
Squamous cell carcinoma	157	71
Adenocarcinoma	57	38
Adenosquamous	10	5
Other	12	9
Unknown	0	15

Three hundred fifty-nine trios were genotyped for *KIR*, *HLA-B*, and *HLA-C*. Each family consisted of one subject with CIN3 or ICC and both parents or one or more siblings. All individuals provided written informed consent, and the study was approved by the Human Research Protection Office at Washington University in St. Louis. Genomic DNA was extracted from either whole blood or buccal cells, as previously described (Zhang et al. 2007)

Table 2

Association of HLA-Cw group 1 or 2 with CIN3 and ICC

Stage	HPV[a]	Number of informative families	Minor allele frequency (Cw group 2)	Overtransmitted allele	*P* value
CIN3 + ICC	All	350	0.39	Cw group 1	0.55
CIN3 + ICC	16/18-related	235	0.39	Cw group 1	0.16
ICC	All	225	0.39	Cw group 1	0.04
ICC	16/18-related	165	0.39	Cw group 1	0.008

We used TRANSMIT v2.5.4 (Clayton 1999; Clayton and Jones 1999; Clayton and Lonjou 1997) software to analyze the family-based association data. The transmission/disequilibrium test (TDT) was used to test for linkage between the candidate marker(s) and the disease.

HLA-Cw group allotypes are defined based on the amino acid at position 80. Cw group 1 allotypes have asparagine, while Cw group 2 allotypes have lysine.

[a]Three hundred seventy-five cervical neoplasms were typed for HPV, as previously described (Li et al. 2003). *16/18-related* is defined as HPV 16, 31, 52, 18, or 45. Three hundred fifty-nine trios were genotyped for *KIR*, *HLA-B*, and *HLA-C*. Each family consisted of one subject with CIN3 or ICC and both parents or one or more siblings. All individuals provided written informed consent, and the study was approved by the Human Research Protection Office at Washington University in St. Louis. Genomic DNA was extracted from either whole blood or buccal cells, as previously described (Zhang et al. 2007).