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References

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Cutting Edge: Susceptibility to Psoriatic Arthritis: Influence of Activating Killer Ig-Like Receptor Genes in the Absence of Specific HLA-C Alleles

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NK cell activity is partially controlled through interactions between killer Ig-like receptors (KIR) on NK cells and their respective HLA class I ligands. Independent segregation of HLA and KIR genes, along with KIR specificity for particular HLA allootypes, raises the possibility that any given individual may express KIR molecules for which no ligand is present. Inhibitory receptor genes KIR2DL3 and KIR2DL1 were present in nearly all subjects sampled in this study, whereas their respective activating homologs, KIR2DS2 and KIR2DS1, are each present in about half of the subjects. In this work we report that subjects with activating KIR2DS1 and/or KIR2DS2 genes are susceptible to developing psoriatic arthritis, but only when HLA ligands for their homologous inhibitory receptors, KIR2DL1 and KIR2DL2/3, are missing. Absence of ligands for inhibitory KIRs could potentially lower the threshold for NK (and/or T) cell activation mediated through activating receptors, thereby contributing to pathogenesis of psoriatic arthritis. The Journal of Immunology, 2002, 169: 2818–2822.

Killer Ig-like receptor (KIR)5 recognition of HLA class I molecules contributes to the array of receptor-ligand interactions that determines NK cell response to its target (reviewed in Ref. 1). Binding of inhibitory KIR (designated 2DL and 3DL) to specific HLA molecules has been clearly demonstrated (reviewed in Ref. 2) and correlates well with their ability to inhibit NK cytolysis of target cells bearing those HLA allootypes. Specificity of inhibitory KIR for HLA-C allotypes is dictated to a large extent by the presence of asparagine or lysine at position 80 of the HLA-C molecule (3). KIR2DL1 recognizes group 2 HLA-C molecules, which have Lys80, whereas KIR2DL2 and KIR2DL3 prefer group 1 HLA-C molecules containing Asn80 (3). Studies performed in vitro indicate that this division of specificity is not necessarily strict and may depend to some extent on bound peptide (4, 5). However, the crystal structures of KIR2DL2-Cw*0301 and KIR2DL1-Cw*0401 suggest greater specificity in ligand binding than do the in vitro studies (6, 7).

Based on assays measuring target cell killing, activating KIR (designated 2DS and 3DS) can mediate NK cell activity through recognition of HLA ligands under some circumstances (8), but little if any direct binding of activating KIR molecules to their putative HLA ligands has been detected. Thus, the ligands for KIR2DS1 and KIR2DS2 (which exhibit high homology to the inhibitory KIR2DL1 and KIR2DL2/3, respectively) are not known, but candidate ligands include non-MHC molecules, such as foreign or microbial Ags expressed on infected cells, normal cell surface proteins that are aberrantly expressed, or complexes of pathogen-derived peptides bound to MHC class I molecules. Strong support for the possibility that non-HLA molecules behave as ligands for activating KIR derives from the recent identification of the mouse CMV m157 gene product as the ligand for the mouse activating NK cell receptor Ly49H, an interaction that leads to NK cell killing of the infected targets (9–14). The Ly49 family in mice is considered to be functionally equivalent to the KIRs (15). KIR3DL1 binds HLA-B allotypes that have the Bw4 epitope (determined by amino acid positions 79–83 of the molecule) (16), and HLA-B Bw4 molecules with isoleucine at position 80 (Bw4-80I) may be better ligands than those containing threonine at position 80 (17). The ligand for KIR3DS1 has not been determined, although the presence of this gene along with alleles encoding Bw4-80I has an epistatic protective effect on AIDS progression (18), suggesting that, like KIR3DL1, KIR3DS1 recognizes at least some of the Bw4 allotypes in HIV-positive individuals.

Diversity at the KIR locus has been generated by an evolutionary history of expansion and contraction, presumably due to combinations of duplication and unequal crossing over within the locus (19, 20). Up to 12 KIR genes appear to be expressed, and KIR haplotypes consist of various combinations of KIR genes (19, 21). The number of putatively expressed KIR genes present on a single
haplotype ranges from 7 to 11, depending primarily on the presence or absence of activating KIR loci (19, 21, 22). The most common Caucasian haplotype, the “A” haplotype (frequency of \(\sim 50\%\)), contains only a single activating KIR gene, KIR2DS4, and six inhibitory KIR loci (21). The remaining “B” haplotypes are very diverse and contain two to five activating KIR loci. Diversity in KIR gene content, KIR specificity for particular HLA allotypes, and the unlinked physical location of the KIR loci (19q13.4) relative to the HLA loci (6p21) give rise to the possibility that, for some KIR loci, any given individual may encode receptor only, ligand only, both receptor and ligand, or neither one.

NK cells have been implicated in the defense against infectious diseases through mechanisms involving cytotoxicity and cytokine production (reviewed in Ref. 23), presumably mediated in part by activating KIR molecules. As in other limbs of the innate and adaptive immune response, the benefits of such a defense system may be coupled to certain risks resulting in a situation whereby KIR genes conferring protection against one disease may predispose to another, perhaps less deadly, disease. NK cell activation mediated by KIR2DS1 and KIR2DS2.

### Materials and Methods

#### Study population

Three hundred sixty-six patients with PsA attending the University of Toronto Psoriatic Arthritis Clinic at the Toronto Western Hospital were studied after informed consent and approval by the Research Ethics Board of the University Health Network. Patients are registered in the clinic if they have an inflammatory arthritis associated with psoriasis. Two hundred ninety-nine controls from the Regional HLA Laboratory (University Health Network) were also studied. The majority were cadaveric donors and the remainder were normal healthy volunteers.

#### HLA class I genotyping

Genomic DNA was amplified using locus-specific primers flanking exons 2 and 3. The PCR products were blunted on nylon membranes and hybridized with a panel of sequence-specific oligonucleotide probes (see http://www.hwg.org/protocols/protocol.htm). Alleles were assigned by the reaction patterns of the sequence-specific oligonucleotide probes. Ambiguous typing results were resolved by sequence analysis.

#### KIR genotyping

Genomic DNA from patients and controls was genotyped for presence or absence of the following KIR genes: 2DL2, 2DL3, 2DL4, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, and 3DS1. Genotyping was performed using PCR amplification with primers specific for each locus (PCR-SSP). Internal control primers for a 796-bp fragment of the third intron of DRB1 were also included in each PCR. Two sets of primers were designed for each locus (see supplemental material\(^*\) for details of the KIR typing method and primer sequences).

#### Statistical analysis

Frequency differences between PsA and control groups were tested for significance by a two-sided Fisher’s exact test. Multifactorial analysis was performed by logistic regression (27) (PROC LOGISTIC, the SAS system; SAS Institute, Cary, NC). Due to the number of alternate factors being considered, an automatic stepwise selection was used to determine significant factors (\(p < 0.05\)).

### Results and Discussion

Increased frequencies of both HLA-Cw*0602 and -B*27 among individuals with PsA have been observed previously (26, 28). The frequencies of individuals with at least one copy of these alleles were also more common among the PsA samples studied here relative to individuals without PsA from the same demographic area (B*27, 19.4 vs 9.7%; Cw*0602, 31 vs 18.3%, respectively; Table I). Phenotypic KIR gene frequencies (i.e., the presence of at least one copy of each gene per individual) among the two groups

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\* The on-line version of this article contains supplemental material.
indicated consistently higher frequencies of activating KIR in the PsA group relative to controls, except for KIR2DS4, which is the only activating KIR present on the common A haplotype (21). The presence of multiple activating KIR genes is characteristic of the B haplotypes, and certain pairs of activating KIR genes are known to be in linkage disequilibrium with one another (20). Thus, the increased frequency of most activating KIR among individuals with PsA could be due to hitchhiking of these genes with a single activating KIR that confers disease susceptibility.

The inhibitory KIR2DL2 and KIR2DL3 segregate as alleles of a single locus, which was present in all individuals tested, and KIR2DL1 was observed in nearly all individuals. In contrast, their activating counterparts, KIR2DS2 and KIR2DS1, were observed in only a fraction of all samples tested (35–56.4%). KIR2DS1 was more frequent in the disease group (46.3%) than in the control group (35%), and represented the greatest gene frequency difference between the two groups.

Because KIR2DL1 interaction with group 2 HLA-C molecules on target cells would theoretically inhibit an activating signal mediated by KIR2DS1, we hypothesized that an effect of KIR2DS1 would be greatest among individuals who are missing group 2 HLA-C ligands for KIR2DL1. Therefore, we determined the various combinatorial frequencies of KIR2DS1 with Cw*0602 (a group 2 HLA-C allele that is strongly associated with PsA) or other group 2 HLA-C alleles (Fig. 1A). Individuals with no Cw*0602 and no KIR2DS1 appeared to be relatively protected (55% in controls vs 38% in PsA), corresponding with an increased frequency of individuals with KIR2DS1 plus Cw*0602 in the PsA group (14%) relative to controls (8%). The frequency of individuals with HLA-Cw*0602 but without KIR2DS1 was increased by ~6% in the PsA group relative to controls. KIR2DS1 was increased by 5% in the disease group when group 2 HLA-C alleles (including Cw*0602) were missing. However, in the presence of group 2 HLA-C alleles (excluding Cw*0602), KIR2DS1 frequencies were nearly identical in the disease and control groups. A significant difference (p = 0.001) between the PsA and control groups was observed in a trend test of protective to susceptible genotype frequencies (i.e., frequencies of Cw*0602-negative individuals with 1) no KIR2DS1, 2) KIR2DS1 with group 2 Cw alleles, and 3) KIR2DS1 without group 2 Cw alleles). These data suggested that KIR2DS1 might contribute to the pathogenesis of PsA by influencing NK or T cell activity when ligands for KIR2DL1 are absent, a situation whereby a dominant KIR2DL1-mediated inhibitory signal cannot occur. A virtually identical pattern of HLA-C/KIR2DS1 genotype frequencies was observed when individuals with HLA-B*27 were removed from the analysis (p = 0.002, Fig. 1B).

Due to apparent effects of multiple variables at the HLA and KIR loci on risk of developing PsA, a multivariate regression analysis was used to test more rigorously for statistical significance of genotype frequency differences between the PsA and control groups. Variables tested in the analysis included HLA alleles shown previously to associate with PsA (HLA-B*27 and -Cw*0602) and the three activating KIR genes (KIR2DS1, KIR2DS2, and KIR3DS1) that have inhibitory KIR homologs with known HLA ligands. Additionally, these three KIR genes in the absence of HLA ligands for their inhibitory counterparts (i.e., KIR2DS1 with no group 2 HLA-C alleles, KIR2DS2 with no group 1 HLA-C alleles, and KIR3DS1 with no HLA-B allele) were also used as covariates in the model. Significant effects were observed for both HLA-B*27 and -Cw*0602, confirming previous reports (26, 28) (Table II). Furthermore, in the absence of group 2 HLA-C alleles, KIR2DS1 was significantly associated with PsA (odds ratio (OR) = 2.2, p = 0.0025), as initially suggested by the frequencies shown in Fig. 1. A marginally significant effect of KIR2DS2 in the absence of group 1 alleles was also observed (OR = 2.3, p = 0.023), supporting the hypothesis that activating KIR may enhance development of PsA when ligands for the corresponding inhibitory
KIR are missing. KIR3DS1 in the absence of HLA-B Bw4-80I alleles showed no significant effect. As indicated by the multivariate regression analysis, the entire effect of KIR2DS1 and KIR2DS2 is attributable to having these alleles when ligands for the corresponding inhibitory KIRs are missing, and their effects are independent of HLA-B*27 and -Cw*0602.

The joint effect of the two activating genes KIR2DS1 and KIR2DS2 on PsA susceptibility is illustrated in Fig. 2. Individuals without either KIR2DS1 or KIR2DS2 are relatively protected against development of PsA (27% in PsA vs 40% in controls), whereas the frequency of individuals with KIR2DS1 and/or KIR2DS2 necessarily with the corresponding inhibitory KIR ligand is similar in patients and controls (45 vs 47%, respectively) (Fig. 2A). However, as indicated from the multivariate analysis, individuals who have KIR2DS1 and/or KIR2DS2 without the corresponding inhibitory KIR ligand are susceptible to PsA (27% in PsA vs 15% in controls; p = 1 x 10^-5). The effect is similar when subjects with HLA-B*27 and/or Cw*0602 are omitted from the analysis (Fig. 2B; p = 2 x 10^-5).

Absence of KIR2DS1 and KIR2DS2 was associated with protection against PsA, whereas these receptors conferred susceptibility to disease when HLA ligands for the corresponding inhibitory KIRs were missing. The presence of KIR2DS1 and/or KIR2DS2 along with HLA alleles encoding ligands for KIR2DL1 and KIR2DL2/3, genes that were present in nearly all individuals sampled, was neutral (Fig. 2). These data suggest that, in the presence of their HLA ligands, corresponding inhibitory KIR may neutralize the effect of the activating KIR. KIR2DS2 has been implicated in the pathogenesis of vascular damage among individuals with rheumatoid arthritis (24), and it would be of interest to determine whether this effect could be attributed to KIR2DS2 in the absence of alleles encoding HLA ligands for KIR2DL2/3.

Activating KIR molecules are known to bind poorly to HLA molecules compared with that observed for inhibitory KIR (29, 30), perhaps explaining the observed dominance of inhibition over activation of NK cells. The susceptibility effect of KIR2DS1 and KIR2DS2 in the absence of group 2 and group 1 HLA-C alleles, respectively, suggests that ligands for the KIR2DS2 molecules are not the same HLA molecules recognized by homologous KIR2DL receptors. Data from mice indicate that viral proteins structurally similar to class I molecules serve as ligands for activating receptors on NK cells (9–14), raising the possibility that activating receptors in humans may also recognize foreign molecules on target cells as opposed to recognition of self class I molecules.

The KIR locus appears to be particularly prone to molecular genetic mechanisms that result in dynamic fluctuation of gene content and allelic variation (19, 21). The potentially deleterious effects of activating KIR are certainly not lethal; therefore, these genes may remain and continue to induce or exacerbate pathogenesis of low mortality diseases such as PsA. Several HLA class I alleles are known to have either beneficial or deleterious consequences, depending on the type of disease. An example is the contribution of B*5301 to susceptibility in AIDS progression while protecting against severe malaria (31, 32). We recently reported an epistatic interaction between genes encoding the activating receptor KIR3DS1 and HLA-B molecules bearing a subset of Bw4 epitopes in protection against AIDS progression (18). Thus, KIR genes are likely to undergo opposing selection processes in a manner similar to that proposed for the HLA loci, ensuring persistence and perhaps expansion of genetic diversity at the KIR locus.

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Table II. ORs for individual and combinations of HLA and KIR types

<table>
<thead>
<tr>
<th>Allele/Gene Model</th>
<th>OR</th>
<th>p Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>B*27</td>
<td>2.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cw*0602</td>
<td>2.2</td>
<td>0.0003</td>
</tr>
<tr>
<td>KIR2DS1, no Grp2</td>
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<td>0.0025</td>
</tr>
<tr>
<td>HLA-C alleles</td>
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<td></td>
</tr>
<tr>
<td>KIR2DS2, no Grp1</td>
<td>2.3</td>
<td>0.0230</td>
</tr>
</tbody>
</table>

Other factors tested (KIR2DS1, KIR2DS2 and KIR3DS1 without consideration of the presence or absence of ligands for their inhibitory counterpart, and KIR3DS1 in the absence of HLA-B Bw4-80I) fell short of significance in the multivariate analysis (p > 0.1).
References


