Killer Cell Immunoglobulin-like Receptors in HLA-B27–Associated Acute Anterior Uveitis, with and without Axial Spondyloarthropathy

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PURPOSE. To determine associations between polymorphic genes that encode KIRs and their HLA class I ligands in patients with HLA-B27–associated acute anterior uveitis (AAU), with and without axial spondyloarthropathy (axial SpA).

METHODS. Molecular DNA typing methods were used to define the frequencies of variable KIR genes and their relevant HLA class I ligands in HLA-B27* (B27+) Caucasian subjects with AAU and 429 healthy Caucasian control subjects. The patients were evaluated for axial SpA based on their histories using published criteria.

RESULTS. Of 143 Caucasian subjects with AAU, 71 (49.6%) had features of axial SpA. The only difference between cases and controls in KIR gene frequencies was a trend toward fewer activating KIRs in subjects with AAU with axial SpA, which reached statistical significance for 2DS5 (P = 0.025, corrected P [P-cor] = 0.05; odds ratio [OR], 0.48; 95% CI, 0.25–0.90). The 3DL1+2Bw4 combination implicated in weak inhibition was more frequent in subjects with AAU than in control subjects (P = 2.73 × 10−28; Pc = 8.2 × 10−27; OR, 13.5; 95% CI, 7.73–23.68). The 2DL1+HLA-C2 combination was decreased in subjects with axial SpA compared with subjects with AAU without axial SpA (P = 0.022; Pc = NS; OR, 0.43; 95% CI, 0.21–0.88).

CONCLUSIONS. Evidence was found of a role for KIR-HLA combinations that trigger weaker inhibition in subjects with AAU. Furthermore, there was a trend toward fewer KIR3DS1, -2DS1, and -2DS5 in AAU patients with axial SpA, which have been implicated in NK cell activation. HLA-B27 without KIR2DS3 (and -2DS1 and -3DS1) may fail to trigger an early NK cell response to clear antigenic stimuli, which may in part contribute to disease pathogenesis. (Invest Ophthalmol Vis Sci. 2010; 51:1505–1510) DOI:10.1167/iovs.09-4232

Anterior uveitis is the most common form of intraocular inflammation in the community. In most cases, it is characterized by episodes of inflammation in the anterior segment of the eye that last less than 3 months (acute anterior uveitis [AAU]). One half to two thirds of the patients are HLA-B27* (B27+) and more than half have associated rheumatic diseases such as reactive arthritis or ankylosing spondylitis. Genes outside the major histocompatibility complex on chromosome 6 have also been found to be associated with AAU. The role of these genes in disease pathogenesis is unknown. It is likely that additional genes confer risk of disease, along with environmental triggers of an episode. Such environmental triggers most likely include microbial agents.

Killer cell immunoglobulin-like receptors (KIRs) are expressed on natural killer (NK) cells and some T cells, including activated CD8+ T cells that interact with class I HLA molecules (including HLA-B27). Fourteen KIR receptors have been identified. Ligation of the inhibitory (i) KIRs (3DL1-3, 2DL1-3, and 2DL5) inhibits activation of the cell expressing the KIR. The ligands for iKIR are specific class I HLA molecules. The ligands for the activating (a) KIRs (3DS1 and 2DS1-5), for the most part, have not been well described. Ligands for aKIR include class I HLA molecules in some cases (3DS1, 2DS1, and 2DS2), although they bind to class I HLA molecules with less avidity than the homologous iKIR. 2DL4 may trigger inhibition or activation. In murine models, virally encoded proteins may act as ligands for NK receptors that are functionally homologous to aKIRs. The balance of these inhibitory and activating signals determines whether the cell expressing KIRS is activated. When activated, a cell expressing KIRs may lyse the target cell or release inflammatory cytokines. The expression of HLA molecules is in part determined by the presence of infection or other cellular disease. Abnormal cells may express fewer class I HLA molecules, resulting in decreased iKIR-HLA ligand interactions and increased effector cell activation. KIR-HLA interactions may be further modified by specific microbial peptides presented by HLA molecules. Such KIR modulation of effector or regulatory cells is likely to play an important role, not only in the intrinsic immune response to microbes, but also in the transition to antigen-specific responses.

KIR genes have been shown to confer risk of several inflammatory and infectious diseases, including HLA class I-associated posterior uveitis, birdshot chorioretinopathy, and ankylosing spondylitis.
No statistically significant difference was observed in the frequencies of KIR genes in patients to that in control subjects has been fruitful, but is limited by the complexity of KIR genetics and KIR-HLA interactions. Unlike most genetic systems, including HLA genes, the number and type of KIR genes present varies substantially between individuals. As HLA and KIR genes are located on different chromosomes (6 and 19, respectively), there is independent assortment of HLA and KIR genes. It is therefore important to evaluate not only the frequency of KIR genes, but also the combination of KIR-HLA genes. There are also differences in the inhibitory strength of different iKIRs, and in the binding affinities and inhibitory strength of different iKIR-HLA combinations. Differences in the frequencies of weakly and strongly inhibitory iKIR-HLA combinations in patients and control subjects have been implicated in conferring risk of disease in birdshot chorioretinopathy.

HLA-B27 is part of the Bw4 family. Bw4 is an HLA supertype that is common to several HLA B alleles. Bw4 molecules act as ligands for KIRs, but the strength of this interaction depends on whether threonine (Bw4T80) or isoleucine (Bw4I80) is in position 80. The Bw4T80 subtype of HLA-B44 has been found to confer risk of birdshot chorioretinopathy mediated by KIR genes. As most patients with HLA-B27-associated disease have the HLA-B*2705 subtype, which also is Bw4T80, it is possible that HLA-B27 interactions with KIRs play a role in inflammatory diseases associated with HLA-B27. We examined the DNA from B27 patients with AAU, with or without a clinical history consistent with axial spondyloarthritis (axial SpA), to evaluate whether KIR genes and their HLA class I ligands in addition to HLA-B27 confer risk of disease.

**Materials and Methods**

**Study Subjects**

Informed consent was obtained from the subjects after explanation of the nature and possible consequences of the research. The AAU clinical phenotype was verified by careful review of ophthalmology chart.
TABLE 2. Frequency of KIR-Binding HLA Motifs in Subjects with AAU and Healthy Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls</th>
<th>Without Axial SpA</th>
<th>With Axial SpA</th>
<th>Control vs. AAU All</th>
<th>SpA vs. AAU All</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA C2</td>
<td>56.9 (257)</td>
<td>58.9 (124/130)</td>
<td>59.5 (95.5/160)</td>
<td>NS</td>
<td>0.012 (P = 0.29)</td>
</tr>
<tr>
<td>HLA C1</td>
<td>86.7 (352)</td>
<td>82.2 (122/130)</td>
<td>95.5 (95.5/160)</td>
<td>NS</td>
<td>0.040 (P = 0.29)</td>
</tr>
<tr>
<td>HLA Bw4</td>
<td>59.9 (257)</td>
<td>95.5 (95.5/160)</td>
<td>95.5 (95.5/160)</td>
<td>NS</td>
<td>1.00 (P = 1.00)</td>
</tr>
<tr>
<td>HLA A3/A11</td>
<td>36.8 (158)</td>
<td>90.8 (118/130)</td>
<td>90.8 (118/130)</td>
<td>NS</td>
<td>8.97 (P = 1.00)</td>
</tr>
</tbody>
</table>

The frequency (%F) of each HLA ligand is expressed as a percentage and defined as the number of individuals with a ligand (N) in the study group. The probabilities of HLA-C1, C2, and Bw4 were corrected by factor 2, and those of Bw4 I80 and Bw4T80 were corrected by factor 3.

The study was reviewed and approved by the Institutional Review Boards at the University of California, Los Angeles (UCLA), and Oregon Health & Science University, (Portland, OR), was HIPAA compliant, and adhered to the tenets of the Declaration of Helsinki.

DNA Extraction and KIR and HLA Genotyping

DNA was prepared from peripheral blood by the standard salting-out method or with a commercial kit (QIAamp blood kit; Qagen, Valencia, CA). The quality and quantity of DNA were determined by UV spectrophotometry, and the concentration was adjusted to 100 ng/μL.

The presence and absence of 16 distinct KIR genes was determined by using our recently developed duplex SSP-PCR (sequence-specific, primer-directed)-PCR typing system. The unique and unusual KIR genotypes were further confirmed by retyping with our alternative SSP-PCR typing method. Both of the typing methods were validated extensively in UCLA International KIR exchange reference DNA samples, which provided identical KIR genotyping results, indicating that the specificity and sensitivity of the two methods were comparable.

We examined the HLA genes of the HLA types that are known to interact with KIRs. They included HLA-A3/11, HLA-C (divided into two groups based on their interactions with KIRs, HLA-C1, and HLA-C2) and HLA-Bw4 (Bw4). HLA-A, B, and C typing was performed by either SSP-PCR amplification or sequence-specific oligonucleotide (SSO) hybridization methods with commercial kits (One Lambda, Canoga Park, CA). The KIR-binding HLA class I epitopes were predicted from the HLA typing results. If the HLA typing results were ambiguous, we typed the KIR-binding HLA motifs as described by SSP-PCR.

Data Analysis and Statistical Methods

Differences between control subjects and patients in the distribution of KIR genes, KIR genotypes, KIR-binding HLA motifs, and KIR-HLA gene combinations were tested by two-tailed Fisher exact probabilities (P), with P < 0.05 considered to be statistically significant. The probabilities were corrected (Pc) by using Bonferroni’s method of multiplying by the number of variables compared. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated for the comparisons showing significant differences between the patient and control groups.

RESULTS

The AAU cohort consisted of 160 unrelated patients (92 female and 68 male). The majority were Caucasian (n = 148), with the remaining subjects self-described to be of the following racial/ethnic groups: Asian (n = 5), Hispanic (n = 4), Native American (n = 2), and African American (n = 1). Only results from Caucasian subjects were used in these analyses. Although we used randomly selected Caucasian control subjects, the KIR frequencies in our control group did not differ from previously published KIR gene frequencies for B27+ Caucasian individuals (data not shown).

Among the 148 Caucasians, we were not able to successfully genotype 3 subjects, and we were not able to make a determination regarding axial SpA in 2; thus, our analyses are

notes (n = 153) or based on the expertise of the referring uveitis specialist (n = 7).

Only patients with AAU were included in the analysis. Most of them were seen in a uveitis referral service; hence, radiologic examinations for ankylosing spondylitis were not consistently available. The determination of SpA was made from examination of medical chart notes and applying the diagnostic criteria of Rudwaleit et al. in calculating a disease score. The Rudwaleit criteria estimate disease probability based on the likelihood ratios of individual spondyloarthritides parameters. The advantage of these criteria is that they allow assessment of early preradiographic axial SpA in the absence of available diagnostic imaging. A panel of 429 randomly selected, healthy Caucasians served as control subjects.

The study was reviewed and approved by the Institutional Review Boards at the University of California, Los Angeles (UCLA), and Oregon Health & Science University, (Portland, OR), was HIPAA compliant, and adhered to the tenets of the Declaration of Helsinki.
based on 143 subjects. Seventy-one (49.6%) subjects reported signs or features of axial SpA, with 72 lacking such features. It was determined that 60 met the criteria of Rudwaleit et al.36 for axial SpA with 90% diagnostic certainty, and 11 were scored as having axial SpA with 80% certainty. The axial SpA groups were combined for all analyses, as there were no differences found in KIR gene frequencies between the subjects who had 90% or 80% certainty.

There were no notable differences in the KIR genotypes between the subjects with AAU and the healthy Caucasian control subjects (Fig. 1). Table 1 shows the KIR gene frequencies in the patients and control subjects. There was a trend toward decreased frequencies of the aKIR genes KIR3DS1, -2DS7, and -2DS5 in the patients with both AAU and axial SpA compared with the control subjects that reached statistical significance only for KIR2DS5 (P = 0.025, Pe = 0.05; OR, 0.48; 95% CI, 0.25–0.90). This trend was also observed for KIR2DL5.

Table 2 shows the results for the relevant sets of HLA genes known to be ligands for KIRs. There was no difference in the frequencies of HLA-C1 or -C2 (C1, C2) and HLA-A3/11 between the patients with AAU and the control subjects. As all individuals who are HLA-B27+ are also HLA-Bw4+, the frequency of Bw4 was increased in the patients compared with that in the control subjects (P = 1.0 × 10−13, P = 2.0 × 10−12; OR, 13.83; 95% CI, 5.96–32.09), primarily because of the increase in Bw4+ (P = 8.97 × 10−50, P = 2.7 × 10−29; OR, 16.87; 95% CI, 9.02–31.52). We also compared the subjects with both AAU and axial SpA to subjects with AAU alone (Table 2). Those with AAU and axial SpA were more likely to have HLA-C1 (P = 0.04, Pc = NS; OR, 2.76; 95% CI, 1.05–7.26) and were less likely to have HLA-C2 (P = 0.012, P = NS; OR, 0.39; 95% CI, 0.19–0.79).

Table 3 shows the frequency of Bw4− subsets in the Bw4+ subjects with AAU and the Bw4− control subjects. The frequency of Bw4 (P = 1.39 × 10−13, P = 4.2 × 10−12; OR, 13.68; 95% CI, 5.81–32.2) was more common in Bw4− subjects (P = 2.5 × 10−13; OR, 9.36; 95% CI, 4.78–18.54), primarily due to the increased frequency of Bw4+Bw4 in the patients (P = 2.73 × 10−28, P = 8.2 × 10−27; OR, 13.5; 95% CI, 7.73–23.68). Conversely, Bw4+Bw4 was less common in subjects (P = 0.00,047, P = 0.0014; OR, 0.37; 95% CI, 0.21–0.66). Table 4 also shows the KIR-HLA combinations in patients with AAU with and without axial SpA. The subjects with AAU who had axial SpA were less likely to have the strongly inhibitory combination of 2DL1+2C than were subjects with AAU without SpA (P = 0.022, Pe = NS; OR, 0.43; 95% CI, 0.21–0.88).

**DISCUSSION**

We did not find the frequency of any KIR gene to differ between subjects with AAU without axial SpA and healthy control subjects. However, we found evidence of fewer aKIR genes in our subjects with AAU and axial SpA. It may be that HLA-B27+ without KIR2DS3 (and possibly 2DS1, 3DS1) fails to trigger early NK or CD8+ cell response to clear antigenic stimuli, which may in part contribute to the more chronic axial SpA. Virally encoded proteins have been implicated as acting as aKIR ligands,19,20 but it is likely that the microbial stimulus to the immune response in AAU are bacterial antigens at mucosal surfaces.3 An increased frequency of the aKIR gene 3DS1 was reported in ankylosing spondylitis in Asian20–27 and Caucasian28 patients when compared with B27+ control subjects. In these studies, only 3DS1 was examined. A more recent study failed to find an association between 3DS1 and ankylosing spondylitis in a British population.29 We examined 3DS1 along with three KIR genes that are in linkage disequilibrium with 3DS1: 2DS1, 2DS5, and 2DL5. All were decreased in our subjects with AAU and axial SpA, implying that our results have internal consistency. Although a possible explanation for this discrepancy is that we did not compare our results with those from B27+ control subjects, the KIR gene frequency of our control group did not differ from the Caucasian B27+ control group used in the study of ankylosing spondylitis.29 It may be that 3DS1 specifically confers a risk of axial disease that results in the radiologic findings that were used to diagnose ankylosing spondylitis in the previous studies.26–28

In two previous studies of KIR associations with ankylosing spondylitis, 3DL1 was decreased in patients, suggesting a role for decreased inhibition mediated by iKIRs in disease pathogenesis.27,28 There was no decrease in the frequency of any iKIR gene in our subjects or in another study of subjects with ankylosing spondylitis.29 However, we also examined class I HLA ligand-KIR combinations and found evidence of decreased inhibition in our subjects. The weakly inhibiting 3DL1+Bw4+ combination12–15 was more common in subjects with AAU, suggesting that less inhibition may play a role in the pathogenesis of AAU in B27+ individuals. In addition, less strong inhibition mediated by 2DL1+C2−11 increased the risk of axial SpA in subjects with AAU, consistent with the interpretation that less inhibition plays a role in clinical disease associated with the B27 allele. These HLA-KIR combinations were likely to be found in our cohort due to linkage disequilibrium between B27 and the relevant HLA-C ligands. Linkage disequilibrium is the tendency for genes close together on a chromosome to be inherited together rather than to be separated by crossover events during meiosis. It is nonetheless still possible that these combinations of alleles play a role in disease pathogenesis.

It is possible that the B27 molecule itself confers risk of disease in part through interactions with KIRs. Our data concerning Bw4+Bw4 and Bw4+Bw4 are consistent with a role for B27

<table>
<thead>
<tr>
<th>HLA Ligand</th>
<th>Bw4+ control (n = 257)</th>
<th>Bw4+ AAU (n = 137)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bw4Bw4</td>
<td>%F (N+)</td>
<td>%F (N+)</td>
</tr>
<tr>
<td>Bw4Bw4</td>
<td>46.3 (119)</td>
<td>11.7 (16)</td>
</tr>
<tr>
<td></td>
<td>7.28 × 10−12</td>
<td>2.2 × 10−11</td>
</tr>
<tr>
<td></td>
<td>0.15 (0.08–0.27)</td>
<td></td>
</tr>
</tbody>
</table>

The frequency (%F) of each Bw4-subset is expressed as a percentage and defined as the number of individuals having the Bw4 (N+) divided by the number of individuals (n) in the study group. Pe correction factor. 3.
### Table 4. Frequency of KIR-HLA Combinations in Subjects with AAU and Healthy Control Subjects

<table>
<thead>
<tr>
<th>AAU Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Controls</td>
</tr>
<tr>
<td>Without Axial SpA</td>
</tr>
<tr>
<td>All Subjects</td>
</tr>
<tr>
<td>KIR-HLA</td>
</tr>
<tr>
<td>Function</td>
</tr>
<tr>
<td>%F (N/n)</td>
</tr>
<tr>
<td>3DL2/A3</td>
</tr>
<tr>
<td>2DL1/C2</td>
</tr>
<tr>
<td>2DL2/Cl</td>
</tr>
<tr>
<td>3DL1/Bw4</td>
</tr>
<tr>
<td>3DL1/Bw4I80</td>
</tr>
<tr>
<td>3DS1/Bw4</td>
</tr>
</tbody>
</table>

| KIR-HLA |
| Function |
| %F (N/n) | %F (N/n) |
| 2DL3/C1 | 76.9 (330) | 73.1 (130) |
| 2DL2/Cl | 44.8 (192) | 38.8 (134) |
| 3DS1/Bw4 | 22.6 (97) | 27.5 (97) |

The frequency (%F) of each phenotype is expressed as a percentage and defined as the number of individuals having the genotype (N/n) divided by the number of individuals (n) in the study group.

P* correction factor, 8.

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and KIR interactions, although we could not demonstrate this effect directly in the present study. A threonine at position 80 (Bw4T80) results in a weaker interaction with the KIR3DL1 receptor than an isoleucine in position 80 (Bw4I80). The subjects in our current AAU cohort were B27+, and HLA-B*2705 is known to be the most common B27 subtype. HLA-B*2705 has threonine in position 80, making it one of the Bw4T80 molecules and is the likely explanation of the high frequency of Bw4I80 in our subjects. Bw4+ patients with AAU were more likely to have the less inhibitory 3DL1+Bw4T80 combination than the more inhibitory 3DL1+Bw4I80 combination compared with Bw4+ control subjects. There was also evidence that Bw4I80 was protective, although we had only 16 subjects who were Bw4I80. Bw4I80 was found to be protective in ankylosing spondylitis, as in our cohort, suggesting a role for decreased inhibition similar to what our data suggest. It appears likely that the presence of HLA-B*2705 overwhelmed the balance between Bw4T80 and Bw4I80 that could have been provided by other Bw4 genes. A study including B27+ subjects with AAU may shed additional light on the role of B27 and other Bw4 genes in disease pathogenesis.

In summary, we found evidence of decreased inhibition mediated by KIR-HLA interactions in subjects with B27-associated AAU, with or without axial SpA. Decreased KIR inhibition in subjects with AAU could allow the rapid onset of intraocular inflammation characteristic of AAU. Furthermore, we found a trend toward less aKIR receptors 3DS1, 2DS1, and 2DS5 in subjects with AAU and a history consistent with axial SpA. HLA-B27 without 2DS5 (and 2DS1 and 3DS1) may not trigger the early NK or CD8+ T-cell responses mediated by aKIRs needed to clear antigenic stimuli, which may result in an inflammatory response and clinical disease. Studying multiple genes could lead to positive results by chance. Although we adjusted for such multiple comparisons, further studies would be useful in confirming these findings.

### Acknowledgments

The authors thank Jinnell Lewis for assistance in enrolling subjects with AAU; the study participants for their cooperation; and the SpondyloArthritis Association of America, and the following collaborators for subject referrals: John Reveille, N. Kevin Wade, Eric Suhler, Friederike Mackensen, Atul Deodhar, Michael Weissman, Muhammad Khan, Gary Holland, John Kempen, Daryl Kurz, Guy Zimmerman/Albert Vitale, Mei-Ling Tay-Kearney, Alice Karpik, and William Samis.

### References


