A polymorphism within the interleukin 1 receptor antagonist (IL-1Ra) gene is associated with ankylosing spondylitis

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Abstract

Objective. Genetic factors that predispose individuals to ankylosing spondylitis (AS) are not fully understood, but are unlikely to be restricted to HLA-B27. Proinflammatory cytokines are implicated in the development of sacroiliitis. We have examined the allele frequencies of three polymorphic sites in the interleukin (IL)-1 genes in AS patients to investigate whether genetic regulation of IL-1 production could be implicated in AS pathogenesis.

Methods. DNA from 188 AS patients, 115 healthy controls and 81 HLA-B27-positive healthy controls, all from the West of Scotland, were examined with the polymerase chain reaction in a case-controlled study. Polymorphic sites in genes of the IL-1 family were examined, including the 86-base pair variable number tandem repeat within intron 2 of the IL-1Ra gene, and the restriction fragment length polymorphisms at positions 2889 in the IL-1α gene and 2511 in the IL-1β gene.

Results. No significant differences were seen at the polymorphic alleles in the IL-1α and IL-1β genes, but there was a significant increase in the carriage of allele 2 in the IL-1Ra in the AS patients compared with local controls (16% vs 8%, odds ratio 2.3, 95% confidence interval 1.2–4.4, P = 0.01).

Conclusion. This report of an association with a polymorphic site within the IL-1 locus and AS suggests that genes other than B27 may well be involved in the pathogenesis of AS.

KEY WORDS: Ankylosing spondylitis, Interleukin 1, Genetics, Restriction fragment length polymorphism, Variable number tandem repeat.

Ankylosing spondylitis (AS) is an inflammatory disorder primarily affecting axial spine and sacroiliac joints, but no specific cause has been demonstrated. There is an established association between AS development and the HLA-B27 gene within the MHC class I [1]. However, twin and family studies have suggested that genes outwith the MHC class I region are likely to be involved in AS development [2, 3].

Cytokines have been implicated in the pathogenesis of sacroiliitis in AS, mRNA for tumour necrosis factor α (TNF-α) being detected in biopsies from inflamed sacroiliac joints [4]. Other proinflammatory cytokines could also be implicated in sacroiliitis, including interleukin 1 (IL-1), but there is no direct record of their detection in joint biopsies. However, IL-1α and IL-1β up-regulate the acute-phase response, and are implicated in joint destruction in animal models [5]. The IL-1 receptor antagonist (IL-1Ra) prevents signalling through the IL-1 receptor by competitively inhibiting binding of IL-1 [6], and has been shown to prevent bone damage in animal models [5] and joint erosion in rheumatoid arthritis (RA) [7]. Recent studies have shown a linkage between the long arm of chromosome 2 and the development of AS [2]. As the genes in the IL-1 family are sited at 2q13, the alleles within them could represent markers for genes that may be implicated in AS pathogenesis [8].

There are several polymorphic well-characterized sites within the genes for IL-1α, IL-1β and IL-1Ra, some of which have been suggested to have an effect on cytokine production [9]. In particular, the IL-1Ra gene has a variable number tandem repeat (VNTR) in intron 2, with up to five variants depending on the number of repeats of the 86-base pair (bp) fragment. Allele 2 (with two repeat sequences) has been shown to be associated with increased production of IL-1Ra in vitro [8]. In clinical studies this allele has been linked with diabetic

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nephropathy [10] and systemic lupus erythematosus (SLE) [11]. In two other studies it has been linked with severe ulcerative colitis [12, 13], although in a third no association was detected with inflammatory bowel disease [14].

Within the IL-1α gene a biallelic polymorphism has been detected at position −889 [15]. In one European study the presence of the allele 2 has been linked with early-onset pauciarticular juvenile RA, but this has not been confirmed in a British study of oligoarticular juvenile chronic arthritis [16]. Similarly, a biallelic polymorphism has been detected at position −511 in the IL-1β gene promoter. However, no disease associations have been observed with either the C or T variant at this site.

As there are associations between the IL-1 gene family and various inflammatory diseases and a link has been identified in AS with the q region on chromosome 2, we decided the IL-1 gene family should be investigated in AS. We therefore analysed the frequency of these genetic polymorphisms within the three genes of the IL-1 family. Our studies show an association between AS patients and carriage of the IL-1Ra*2 allele.

Materials and methods

Patients and controls

One hundred and eighty-eight HLA-B27-positive AS patients were selected, all fulfilling the New York criteria [17]. Patients were screened for the presence of extraspinal features of AS, including uveitis and peripheral joint disease (not including hip and shoulder involvement) by direct patient interview and clinical examination. The mean age was 46 yr (range 17–76 yr) and the mean disease duration was 25 yr (3–58 yr). Male to female ratio was 3.4 : 1. 42% had a history of uveitis and 4% had inflammatory bowel symptoms. Thirty-two per cent had peripheral joint involvement separate from the axial skeleton [18].

Two populations of controls were examined. One hundred and fifteen healthy unrelated normal volunteers were taken from the Department of Tissue Typing panel at Glasgow Royal Infirmary. A second group of 81 HLA-B27-positive normal individuals were selected from those attending the Blood Transfusion Centre at Law Hospital, Carluke. Ethics committee approval had been obtained for these studies.

Blood samples were obtained from the patients and DNA was extracted using the Nucleon BACC2 genomic DNA extraction kit (Nucleon Biosciences, UK) as previously described [18].

Polymorphism screening

DNA was expanded over the IL-1α polymorphism at position −889, and the IL-1β polymorphism at position −511 amplified using the appropriate primers [12], and was cut with the restriction enzyme (NcoI) for the IL-1α and AvaI for the IL-1β restriction fragment length polymorphism (RFLP). Polymerase chain reaction (PCR) products were separated on an 8% polyacrylamide gel, which was stained with ethidium bromide and photographed on a UV transilluminator. The VNTR within intron 2 of the IL-1Ra gene was expanded by PCR using the appropriate primers [12] and the product was analysed on a 2% agarose gel. The alleles were identified by size by comparing the PCR products with a molecular weight ladder (Gibco BRL, Paisley, UK). Using this technique, allele 1 runs at 410 bp, allele 2 at 240 bp and allele 3 at 500 bp (Fig. 1), as previously reported [12].

Statistical analysis

To establish the primary differences in the frequencies of the IL-1Ra polymorphisms in which there may be more than two alleles, we used the Clump software [19]. In order to take into account the three analyses undertaken in this primary calculation, $P < 0.017$ was taken as significant. The use of approximately 380 patients and controls allowed an approximate power of 80% to detect a change of eight percentage points in allele frequency, assuming a control allele frequency of 0.08.

Further analysis of significant associations was undertaken by examining carriage rates (number of individuals with at least one copy of the test allele). For each polymorphism, $\chi^2$ analysis was undertaken using Minitab version 10 for PC. Odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. Subsequent secondary analyses were undertaken to compare the AS patients with control populations with and without HLA-B27. Because 12 analyses were necessary, a $P$ value of $< 0.004$ was taken as significant to allow for the number of comparisons under Bonferroni correction.

![Fig. 1. The PCR products from nine individuals amplified for the IL-1Ra polymorphism analysed on a 2% agarose gel. Lane 11 is the 123-bp molecular weight marker and lane 1 is a negative control. Lanes 2 and 5–9 represent individuals homozygous for allele 1 (410 bp). Lane 2 is an individual homozygous for allele 2 (240 bp) and lanes 4 and 10 represent individuals heterozygous for alleles 1 and 3 (410 and 500 bp).]
Table 1. Frequency of the IL-1Ra polymorphisms found in Scottish AS patients, the Scottish population sample and locally recruited HLA-B27-positive controls

<table>
<thead>
<tr>
<th>IL-1Ra</th>
<th>Scottish AS patients (n = 182) (%)</th>
<th>Combined Scottish controls (n = 191) (%)</th>
<th>Normal Scottish controls (n = 115) (%)</th>
<th>B27-positive Scottish controls (n = 76) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*1</td>
<td>147 (80)</td>
<td>173 (91)</td>
<td>104 (90)</td>
<td>69 (91)</td>
</tr>
<tr>
<td>1*2</td>
<td>14 (8)</td>
<td>1 (0.5)</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2*2</td>
<td>16 (9)</td>
<td>14 (7)</td>
<td>8 (7)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>1*3</td>
<td>5 (3)</td>
<td>3 (2)</td>
<td>2 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>1*</td>
<td>313 (86)</td>
<td>350 (92)</td>
<td>211 (92)</td>
<td>139 (91)</td>
</tr>
<tr>
<td>2*</td>
<td>46 (13)</td>
<td>29 (7)</td>
<td>17 (7)</td>
<td>12 (8)</td>
</tr>
<tr>
<td>3*</td>
<td>5 (1)</td>
<td>3 (1)</td>
<td>2 (1)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

Results

IL-1Ra polymorphisms

Only three of the possible five alleles at the IL-1Ra locus were seen in the Scottish AS patients and our two local control populations (Table 1). When we compared the frequencies of the IL-1Ra alleles by Monte Carlo simulation, the results from the AS patients were different from the combined normal West of Scotland population (P < 0.001). The AS patients were different in the overall frequency of alleles when each of the two separate populations were compared, whether with or without expression of HLA-B27 (P = 0.04 and 0.02 respectively). Further analysis showed that the carriage rate of allele 2 was higher in the AS patients than in the combined control population (16% vs 8%, OR 2.3, 95% CI 1.2–4.4, P = 0.01).

Although there is no direct reason to link the IL-1 gene cluster on chromosome 2 with HLA-B27 on chromosome 6, it is theoretically possible that some association exists. We therefore attempted to compare the frequency of allele 2 with those in the two normal populations separated by the presence of HLA-B27. The frequency of allele 2 was higher in the AS patients (16%) than in the 115 normal controls (8%, OR 2.3, 95% CI 0.91–5.8, P < 0.06). Despite the obvious numerical difference from each normal population, because these were secondary analyses neither reached statistical significance after correction for multiple comparisons.

IL-1x polymorphisms

The allele distribution for the IL-1x polymorphic site at position −889 in 184 AS patients was 66% for allele 1 and 34% for allele 2 (Table 2). A decrease in the 1*1 genotype was seen in the Scottish AS patients compared with the combined group of normal controls (45 vs 55%), but this did not reach statistical significance. Similarly, when we compared the AS patients with the two control groups separately, we noted no differences. There were no links between any allele at this polymorphic site and the alleles at IL-1Ra.

IL-1β polymorphisms

No significant differences were seen in the distribution of alleles and genotypes at the IL-1β −511 site between the AS patients and either of the control groups (Table 3). Allele 1 was seen in 62% of the AS patients and 63% of the combined group of normal controls. There were no differences in allele carriage between the AS patients and either control group after separate analysis. The high frequency of the IL-1Ra 1 allele in our study meant that we were not able to demonstrate any links between alleles at this IL-1β polymorphic site and the VNTR at IL-1Ra.

Clinical associations

To assess whether any of the common clinical features of AS were more commonly associated with the presence of the IL-1Ra allele 2, we compared the frequencies of alleles in the presence of peripheral joint disease and uveitis. In neither instance was IL-1Ra allele 2 more commonly found. Similarly, allele 2 was not more commonly detected in male or female patients, those with
a family history, or those with younger age of onset (<21 yr) of disease (data not shown).

Discussion
These data show that the IL-1Ra gene could have an important genetic contribution to AS. The allele 2 polymorphism was carried more frequently in our AS patients (16%) than the combined control populations (8%) in our study (P = 0.01). This observation has recently been corroborated by a study of Dutch AS patients, which showed an increase in carriage of the IL-1Ra 2 VNTR (53%) when compared with their normal population (39%) [20]. Although the difference was only an increase from 8 to 16% in the AS patients, the narrow confidence interval confirms the significance of our observations.

The frequency of the IL-1Ra 1 allele ranged between 69 and 73% in three recent studies of normal populations [12, 14, 21], but our analysis shows this frequency to be higher in our local population whether in the presence (91%) or absence (92%) of HLA-B27. Similarly, in 100 RA patients, also local to the West of Scotland, the IL-1Ra allele 1 was present in 91% [22], indicating that this is likely to be an accurate local representation of this allele frequency. The 1*1 genotype at the IL-1Ra VNTR in our local Scottish control group was also higher (90–91%) than in healthy controls in other parts of Europe (46–54%) [12, 14, 21]. This presumably reflects differences in gene inheritance dependent on the background of the local genetic pool, but also means that we are not able to confirm the previously published linkage between IL-1Ra allele 2 and IL-1β allele 2 [21]. Nevertheless, these comparisons highlight the importance of using locally recruited controls in comparative studies, as contamination of this population by recruits from outside the area could easily lead to a correlation being missed or created. The recent description of an intermediate frequency of 83% for allele 1 of this VNTR in the normal Czech population confirms that wider variations do occur within populations [23]. Interestingly, this study also supports our contention that the allele 2 may be more frequently found in AS patients, because an increase in allele 2 frequency was noted in juvenile arthritides, particularly in patients with oligoarticular disease and HLA-B27 [23].

Association studies and twin analyses have not previously examined the IL-1 locus in AS patients. However, the recent study of AS twins showed that one area on chromosome 2 marked by the microsatellite D2S160 is strongly associated with AS [2]. Although there are no published studies suggesting linkage between any alleles at D2S160 and any genes in the IL-1 family, their proximity (within 6 centimorgans) does raise the possibility that the IL-1Ra gene could represent a candidate gene in AS associated with the presence of this marker [24]. However, other important genes close to D2S160 could be more relevant, and under this circumstance the IL-1Ra association would be present simply because of linkage disequilibrium. Exact identification of the specific gene of interest can only come from detailed analysis of this region of chromosome 2.

Nevertheless, preliminary studies have suggested that the presence of the allele 2 VNTR IL-1Ra polymorphism is associated with altered (reduced) expression of mRNA for IL-1Ra in tissues from ulcerative colitis [25], but how this relates to protein synthesis remains uncertain in this situation. However, two other studies have shown that this allele is associated with increased IL-1Ra protein production. In one study, normal individuals possessing allele 2 produced higher IL-1Ra levels after stimulation of peripheral blood mononuclear cells with lipopolysaccharide, when compared with that produced by individuals only possessing allele 1 [9]. In a second study, IL-1Ra levels were higher in serum from patients carrying allele 2, particularly in the patients with the IL-1β −511*2 allele [21]. These studies suggest that the presence of the IL-1Ra allele 2 could be linked with high steady-state production of IL-1Ra and increased levels of IL-1Ra production in response to Gram-negative antigenic stimulation. Although this has not been specifically addressed in this study, it would imply that, in these AS patients, a poorer response to the standard levels of IL-1 would ensue.

Recent analyses, including our own in the West of Scotland [18, 26], have shown an increase in the frequency of the −308.1 allele in the promoter region of the TNF gene. However, these results are controversial because other case-control studies have failed to show any difference in carriage of the −308.1 allele in AS [27, 28]. Nevertheless, the −308.1 allele has been linked with less optimal TNF production after exogenous stimulation in studies using mRNA analysis [29] and protein production after stimulation [30]. Therefore, in the case of the expression of IL-1Ra allele 2, if this allele is also linked with increased IL-1Ra protein production, then because IL-1Ra prevents IL-1 signalling through the IL-1 receptor [9], the presence of this allele will limit IL-1-mediated signals to some extent. Whether this has significance in the context of AS remains to be established, but IL-1Ra has been shown to suppress the inhibition of IL-1-induced proteoglycan synthesis [31] in the annulus fibrosus. A predisposition leading to overproduction of IL-1Ra in some patients could therefore be implicated in preventing adequate repair at one site of tissue damage in AS.

Our AS patients in the West of Scotland appear to have two genetic factors associated with a reduced proinflammatory response: one linked with reduced TNF production and another associated with less effective action of IL-1. There is a precedent for such a combination of genetic effects in SLE, in which an association has been observed with more than one polymorphic site. Specific genotypes in the Bcl-2 and IL-10 genes each are associated with a moderate risk of the development of SLE. However, there appears to be
a synergistic effect when these two polymorphisms are present over and above the predicted summative effect of each [32]. Both Bcl-2 and IL-10 are implicated in apoptosis, a process that could lead to prolonged B-cell survival and persistent autoantibody production in SLE. It remains to be established whether a similar situation could be built up in AS patients covering more than one polymorphism linked with a less effective proinflammatory cytokine response.

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