Despite the discovery of the association between ankylosing spondylitis (AS) and HLA-B27 over thirty years ago, the precise role of the adaptive and innate immune system in the pathogenesis of this common rheumatic disorder has not been clarified.

Apart from genetic factors, bacteria are thought to be crucial in the pathogenesis of AS, the prototype of the spondylarthropathies. Over the past decades it has been shown that bacterial infections can trigger the onset of at least one type of spondylarthropathy, reactive arthritis (ReA). ReA occurs frequently after infectious diarrhoea caused by Campylobacter jejuni, or, can be caused by urogenital infections with Chlamydia trachomatis (for a review, see Zeidler and Schumacher*). However, so far no specific pathogen has been consistently linked to the development of AS.\(^1\)

The acquired and the innate immune systems play important roles in the host defence against pathogens. Innate immunity refers to the inborn resistance that is already present the first time a pathogen is encountered; it does not require prior exposure and is not modified significantly by repeated exposures to the pathogen over the life of an individual. Acquired immunity refers to resistance that is weak or absent on first exposure, but that increases dramatically with subsequent exposures to the same specific pathogen.

Cluster of differentiation 14 (CD14) and Toll-like receptor 4 (TLR4) are, together with the MD-2 protein, key receptors for the sensing of bacteria. The CD14 C-260T and TLR4 A896G single nucleotide polymorphisms are associated with aberrant signal transduction for bacterial agonists.

**Methods:** The distribution of the CD14 C-260T and TLR4 A896G polymorphisms was studied in genomic DNA from 113 unrelated white Dutch AS patients and 170 ethnically matched healthy controls. The diagnosis of AS was made according to the modified New York criteria. The CD14 C-260T and TLR4 A896G polymorphisms were genotyped by PCR-RFLP methods.

**Results:** No significant differences were found between patients and controls in the frequencies of the carriage of the less frequent CD14 C-260T allele (odds ratio 0.65; 95% confidence interval 0.37 to 1.15) or the TLR4 A896G allele (1.68; 0.67 to 4.19).

**Conclusions:** There is no evidence for involvement of the CD14 C-260T or TLR4 A896G polymorphisms in susceptibility to AS. An important role of bacteria and genetic predisposition of the innate immune system in cases of AS cannot be excluded by these findings. Therefore, studies of the surprisingly highly polymorphic candidate genes in this field should be continued.
Gly908Arg, or Leu1007fsinsC in the CARD15 gene. Both UC and CD can have disease manifestations that belong to the spondylarthropathies.

So far, there have been no studies addressing the possible association of the CD14-C260T SNP with AS, although carriage of the CD14-260T allele might increase the susceptibility of female patients to the development of chronic spondylarthropathy.16,17

The TLR4 gene is located on chromosome 9q32-3q3 at 115.8 Mb. Genome scanning in AS identified linkage to a region on chromosome 9q. Marker D9S1826 on chromosome 9q at 133.9 Mb achieved a lod score of 2.8 (p < 0.0005), and marker D9S1682 at 120.4 Mb achieved a lod score of 2.3 (p < 0.0005), both suggestive of linkage.18 Given the distance between the TLR4 gene and the D9S1826 and D9S1682 markers, it is unlikely that TLR4 can warrant the linkage to the region on chromosome 9. Nevertheless, similar to the situation in CD14, functional studies imply that TLR4 might be of relevance to AS.

The A896G SNP is located in the ectoplasmic receptor domain of TLR4, resulting in an aspartic acid to glycine substitution at position 299. Recently, the A896G SNP was shown to be associated with “an endotoxin hyporesponsive phenotype” in concert with other genetic changes or acquired factors that influence the complex physiological response to LPS.19 However, monocytes from individuals heterozygous for allele TLR4-299A do not exhibit a deficit in recognition of LPS from several bacterial strains.20 Moreover, the heterozygous TLR4 A896G polymorphism does not influence LPS induced human whole blood cytokine release6. Other agonists for TLR4 have not yet been tested functionally.

The present study was designed to study the frequency of the CD14 C-260T and TLR4 A896G SNPs in AS to find out whether these polymorphisms contribute to disease susceptibility and clinical manifestations.

PATIENTS AND METHODS

Subjects

After informed consent, 113 AS patients were recruited from the outpatient Department of Rheumatology of the Jan van Breemen Institute. All AS patients fulfilled the diagnosis of AS, according to the modified New York criteria.21 Controls were 170 randomly selected healthy blood donors from the Amsterdam region. All subjects were unrelated Dutch whites.

Methods

Genotyping the CD14 C-260T SNP (NCBI SNP cluster ID: rs2569190) was performed with forward primer 5'-TCACC TCCCCACCTCTCTT-3' and 5'-CCTGAGAACAT CCTTCCTGTTC-3'. Digestion overnight with HaeIII (New England Biolabs, UK) of the 107 bp amplicons was followed by electrophoresis on 4% agarose gels, staining with ethidium bromide, and visualization under ultraviolet light, and resulted in two fragments of 83 and 24 bp (C allele) or 107 bp (T allele).

Genotyping of the TLR4 A896G SNP (NCBI SNP cluster ID: rs4986790) was performed as described by Morré et al.22

Statistical analysis

Allele and genotype frequencies were tested for Hardy-Weinberg equilibrium by $\chi^2$ test. To compare frequencies, $\chi^2$ or Fisher’s exact test was used. The possible influence of interaction of the two studied polymorphisms in patients and controls was analysed by logistic regression. A two sided p value < 0.05 was considered significant. The magnitude of association was expressed as odds ratio with a 95% confidence interval (CI). Statistical analysis was performed by SPSS 10.0 for Windows.

RESULTS

Characteristics of the 113 AS patients are summarised in table 1. Genotype and allele frequencies in AS patients and controls for the CD14 C-260T and the TLR4 A896G SNP in table 2. The genotype frequencies in the control group did not deviate from HWE equilibrium. No significant differences were observed between AS patients and controls in the frequencies of carriage of the CD14-260T and TLR4 A896G alleles (table 2). Logistic regression analysis showed absence of modification of the odds ratios (OR). Thus, no interaction between the CD14 C-260T and TLR4 A896G polymorphisms was found (data not shown).

No significant associations were found between carriage of each of the alleles CD14-260T and TLR4 A896G with sex, existence in past or present of peripheral arthritis or acute anterior uveitis, age at first complaints, years between first complaints and actual diagnosis of AS, or the number of patients with at least one first degree family member with AS (table 3).

DISCUSSION

The studied SNPs reported to affect gene function in two genes that play a role in innate immunity, CD14 C-260T and TLR4 A896G, are not significantly associated with the susceptibility to or the clinical manifestations of AS.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Allele and genotype frequencies of the CD14 C-260T and the TLR4 A896G polymorphisms in ankylosing spondylitis patients and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14 C-260T</td>
<td>AS patients (n = 113)</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>111 (49.1%)</td>
</tr>
<tr>
<td>T</td>
<td>115 (50.9%)</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>23 (20.4%)</td>
</tr>
<tr>
<td>CT</td>
<td>65 (57.5%)</td>
</tr>
<tr>
<td>TT</td>
<td>25 (22.1%)</td>
</tr>
<tr>
<td>TLR4 A896G</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>219 (96.9%)</td>
</tr>
<tr>
<td>G</td>
<td>7 (3.1%)</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>106 (93.8%)</td>
</tr>
<tr>
<td>AG</td>
<td>7 (6.2%)</td>
</tr>
<tr>
<td>GG</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

No significant difference was found between patients and controls in the frequencies of the carriage of the CD14-260T (OR 0.65, 95% CI 0.37 to 1.15) or TLR4 A896G (OR 1.68, 95% CI 0.67 to 4.19) allele.

*Mean (SD, range), †median (interquartile range).*
Nevertheless, this study cannot exclude that specific phenotypic features of AS are related to TLR4 and/or CD14 genotypes. Therefore, it would be of interest to assess whether the CD14 C-260T and TLR4 A896G SNPs are associated with disease severity (C-reactive protein, ESR, Bath Ankylosing Spondylitis Disease Activity Index, Bath Ankylosing Spondylitis Functional Index), anatomical evolution (Bath Ankylosing Spondylitis Metrology Index, sacroiliitis, degree of ankylosis), or involvement of the gut. Moreover, although this study could not find a significant association between CD14 C-260T and TLR4 A896G and the clinical manifestations of AS, the number of patients in this analysis was too low to warrant a definitive conclusion.

Bacteria are thought to play a crucial role in the pathogenesis of AS. In animal models such as B27 transgenic rats, in ~50% of the cases, the presence of the bacterial flora is obligatory for development of inflammatory gut lesions and peripheral and axial inflammatory joint lesions, similar to AS. When raised in a germfree environment, inflammatory intestinal or joint disease does not develop until the normal gut flora is restored.23 Nevertheless, this study cannot exclude that specific pathogenic mechanisms that control inflammation in the gut.28 Recently, TLR4 and CD14 polymorphisms in ankylosing spondylitis237 have studied has revealed interactions of the TLR4 and CD14-896G allele with acquired immunity provides a good insight into the biology, and pathophysiology of inflammation.

**Table 3** Clinical characteristics of patients with ankylosing spondylitis (n = 113) in relation to carriage of the CD14-260T or the TLR4-896G allele

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TLR4 896G Carrier</th>
<th>Non-carrier</th>
<th>OR (95% CI)</th>
<th>p</th>
<th>CD14 260T Carrier</th>
<th>Non-carrier</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male</td>
<td>1/6</td>
<td>12/85</td>
<td>1.18</td>
<td>0.03</td>
<td>1.45</td>
<td>2/19</td>
<td>0.77</td>
<td>0.15</td>
</tr>
<tr>
<td>PA (+/-)</td>
<td>3/4</td>
<td>35/60</td>
<td>0.78</td>
<td>0.16</td>
<td>1.0</td>
<td>33/48</td>
<td>0.26</td>
<td>0.11</td>
</tr>
<tr>
<td>AAU (+/-)</td>
<td>5/2</td>
<td>34/57</td>
<td>1.0</td>
<td>0.2</td>
<td>0.11</td>
<td>31/48</td>
<td>0.75</td>
<td>0.11</td>
</tr>
<tr>
<td>Familial(+/-)</td>
<td>2/4</td>
<td>23/61</td>
<td>1.0</td>
<td>0.2</td>
<td>0.67</td>
<td>21/51</td>
<td>0.75</td>
<td>0.06</td>
</tr>
<tr>
<td>Age of first complaints, mean (SD)</td>
<td>25.83 (9.78)</td>
<td>25.64 (9.78)</td>
<td>0.57*</td>
<td>0.01</td>
<td>25.17 (9.39)</td>
<td>25.89 (10.37)</td>
<td>0.63*</td>
<td></td>
</tr>
<tr>
<td>Years to actual diagnosis, mean (SD)</td>
<td>9.08 (11.43)</td>
<td>9.27 (8.83)</td>
<td>0.77*</td>
<td>0.01</td>
<td>8.99 (8.79)</td>
<td>10.33 (9.74)</td>
<td>0.43*</td>
<td></td>
</tr>
</tbody>
</table>

AAU: acute anterior uveitis; PA: peripheral arthritis; familial, patients with at least one first degree family member with AS; +/−, present/not present; years to actual diagnosis, number of years between the first complaints and the actual diagnosis of AS. *Mann-Whitney test.
REFERENCES

6 Wright SD, Ramos RA, Tobias PS, Ullevij Rv, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1999;249:1413-3.