Class II MHC antigens in early rheumatoid arthritis in Bath (UK) and Madrid (Spain)


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Abstract

Objective. A number of studies have indicated that rheumatoid arthritis (RA) is a less severe disease in Mediterranean countries than in Northern Europe. We investigated whether differences in the frequency of class II MHC antigens might contribute to this variation in disease severity.

Methods. Typing at HLA-DR and -DQ loci was carried out at low and high resolutions by polymerase chain reaction amplification in patients with early RA of less than 6 months’ duration (68 patients in Madrid and 68 in Bath) and in control subjects (929 in Madrid and 226 in Bath). Only ethnic Spanish and British individuals were included as patients and controls.

Results. Shared epitope (SE) alleles represented 19.8 and 28.9% of the total number of class II MHC alleles in controls from Madrid and Bath respectively (P = 0.00001), this difference being largely due to increased numbers of DRB1*0401 individuals in the British subjects (P = 0.0000001). Analysis of the patients showed the expected increase in SE alleles when compared with their respective control groups (Madrid, 31.6 vs 19.8%; Bath, 42.6 vs 28.9%). In Bath the SE was mainly encoded by HLA-DR4 alleles (74.1%), while in Madrid it was encoded almost equally by DR4 (51.1%) and DR1 (44.7%) alleles. The risk of developing RA in carriers of SE alleles was similar in the two cities (Bath, odds ratio 1.83, 95% confidence interval 1.23–2.78; Madrid, odds ratio 1.87, 95% confidence interval 1.25–2.77), and was largely accounted for by HLA-DRB1*0401 alleles.

Conclusion. We conclude that rheumatoid patients in Bath differ from their Spanish counterparts in class II antigen expression and allele frequency. This may be explained partly by genetic differences between the control populations in the two centres, and may help to explain the greater incidence of more severe rheumatoid disease expression seen in RA patients in the UK.

Key Words: Rheumatoid arthritis, Early, Class II MHC, UK, Spain.

Introduction

Rheumatoid arthritis (RA) is a heterogeneous inflammatory disorder characterized by persistent inflammatory synovitis leading to joint destruction, and is sometimes associated with systemic complications. However, disease severity varies widely, and several studies and anecdotal reports indicate that the clinical presentation varies in different parts of the world, being less severe in Mediterranean countries such as Greece [1] and Spain [2] and more severe among northern Europeans [3]. Although interpreting the data is difficult, it is possible that these differences may be genetically determined.

RA is a complex polygenic disease, environmental and genetic factors contributing to both predisposition and the clinical course, but the relative importance of such factors in disease expression is unknown. The best-characterized genetic component that is functional in RA is the association of alleles of the major histocompatibility complex (MHC), in particular the genes encoding HLA-DR molecules. The use of DNA sequence analysis has shown that alleles associated with RA encode a conserved amino acid sequence in the third hypervariable region (HVR3) of the DR β chain, which has been referred to as the RA shared epitope (SE) [4]. However,
the association with certain HLA-DR alleles is subject to ethnic variation due to the different baseline frequencies of the alleles in the populations affected [5]. In Caucasian patients, RA is strongly associated with DR4, in particular with the HLA-DRB1*0401, *0404 and *0408 alleles [6, 7]. In Japanese, Chinese and Korean patients, the rheumatoid epitope is present in HLA-DRB1*0405 [8–10]. In Greece, Spain, Italy and Israel, the alleles HLA-DRB1 *0405, *0101, *0102 and *1001 have been found [11–17] and DRB1*1402 has been found in Yakima Indians [18].

Genetic differences may determine differences in disease expression, and it has been suggested that not all the alleles encoding the shared epitope are equivalent in terms of their association with susceptibility to or the severity of RA when present either as a single allele or when two alleles are present. Patients with more severe disease show increased frequency of alleles with the disease susceptibility cassette [13], are more likely to have the SE encoded by an HLA-DR4 allele than a non-DR4 allele [19–21] or have two of these alleles [6, 7, 22]. However, this does not seem to be true in all ethnic groups. Most African-American and Hispanic-American patients with RA do not express the shared epitope and disease severity is similar for patients with and without the epitope, independent of the allele dose [23, 24].

The present study was undertaken to investigate class II MHC polymorphisms among RA patients from two early synovitis clinics, in Bath (UK) and Madrid (Spain). We show that the frequencies of the SE and of HLA-DRB1*04 alleles are increased in British patients. These genetic factors may be responsible for the differences in the clinical course and disease outcome of early RA seen in the two centres.

### Patients and methods

#### Patients

The patients included in this study were consecutive patients with clinical evidence of inflammatory polyarthritis of <6 months duration, referred from primary care physicians to two early synovitis clinics (Royal National Hospital for Rheumatic Diseases, Bath, UK and La Paz University Hospital, Madrid, Spain), prior to the initiation of therapy with slow-acting drugs or steroids. Only patients who fulfilled the 1987 diagnostic criteria for RA of the American College of Rheumatology [25] and who were of Spanish (Madrid) or British (Bath) ancestry were considered for the study in each clinic.

#### HLA genotyping

Genomic DNA was extracted from whole peripheral blood by standard procedures. HLA class II (DRB1 and DQB1) alleles were studied by molecular procedures at both low and high resolution. Low-resolution typing was carried out by polymerase chain reaction with sequence-specific primers (PCR-SSP) [26, 27], and high-resolution typing by PCR and sequence-specific oligonucleotides (PCR-SSO) [28].

#### Statistical analysis

Allele frequencies were calculated by direct counting. HLA allele frequencies in RA patient groups and controls were compared using the χ² test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated as cross-product ratios of a 2 × 2 contingency table [OR = (a × d)/(c × b)] by the Woolf method as modified by Haldane [29].

### Results

Sixty-eight early RA patients were studied in Bath and 68 in Madrid. Control populations comprised 226 (Bath) and 929 (Madrid) persons of the same type of background and living in the same area as the patients.

The distribution of healthy controls with 0, 1 and 2 SE alleles is shown in Table 1. In Madrid, 38% of control subjects carried one or two alleles with the conserved HVR3 sequence, compared with 48% in Bath (P = 0.0001). These values represent 390 SE alleles (42% of the total number of alleles in Spanish controls) and 131 alleles (58% of British control alleles), respectively (P = 1 × 10⁻⁷). Clear differences were noted in SE distribution between the two control populations (P = 1 × 10⁻⁷) (Table 2), in particular the presence of HLA-DRB1*04 alleles (17.3% vs 7.7%). The increased expression of HLA-DRB1*04 alleles in Madrid was due to marked over-representation of HLA-DRB1*0401 and, to a lesser extent, *0404. Most controls in the two cities carried DR4-associated DQβ1*03 alleles, but the only difference found was that the DR4-DQβ1*0301 haplotype was much more prevalent in Bath than in Madrid (46% vs 19%, P = 0.00007) (Table 3).

Table 4 shows the number of RA patients with SE alleles. As in control subjects, there were differences in SE distribution between the two patient groups.

#### Table 1. Number of controls with SE alleles

<table>
<thead>
<tr>
<th>No. of alleles</th>
<th>Madrid</th>
<th>Bath</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>574 (62%)</td>
<td>118 (52%)</td>
</tr>
<tr>
<td>1</td>
<td>320 (34%)</td>
<td>85 (38%)</td>
</tr>
<tr>
<td>2</td>
<td>35 (4%)</td>
<td>23 (10%)</td>
</tr>
<tr>
<td>Total no. of controls</td>
<td>929</td>
<td>226</td>
</tr>
</tbody>
</table>

Global χ² test: χ = 18.17, P = 0.0001.

#### Table 2. Number and type of SE alleles in Spanish and British control subjects

<table>
<thead>
<tr>
<th>Total no. of alleles</th>
<th>Madrid</th>
<th>Bath</th>
</tr>
</thead>
<tbody>
<tr>
<td>*01</td>
<td>213 (11.4%)</td>
<td>50 (11%)</td>
</tr>
<tr>
<td>*0401</td>
<td>40 (2.1%)</td>
<td>53 (11.7%)</td>
</tr>
<tr>
<td>*0404</td>
<td>57 (3%)</td>
<td>22 (4.8%)</td>
</tr>
<tr>
<td>*0405</td>
<td>45 (2.4%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>*0408</td>
<td>4 (0.2%)</td>
<td>3 (0.6%)</td>
</tr>
<tr>
<td>*1001</td>
<td>31 (1.6%)</td>
<td>2 (0.4%)</td>
</tr>
<tr>
<td>*1402</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total no. of SE alleles</td>
<td>369 (19.8%)</td>
<td>131 (28.9%)</td>
</tr>
</tbody>
</table>

Global χ² test: χ = 76.12, P = 1 × 10⁻⁷.
The frequency of SE alleles is higher in the British population around Bath than in their Spanish counterparts around Madrid. In both centres, rheumatoid patients demonstrate a higher degree of SE expression than their respective control population. This over-representation of the SE in rheumatoid patients is largely due to the HLA-DRB1*0401 allele.

RA is a complex polygenic disease, environmental and genetic factors contributing both to its induction and to its clinical course. The best-characterized genetic component that is functional in RA is the association of alleles of the MHC, in particular with genes encoding HLA-DR molecules. All disease-associated haplotypes share a specific polymorphism encoded by the HVR3 of the HLA-DRB1 gene, suggesting that this common sequence is involved in the pathogenesis of RA [4]. HLA may be a factor in the initiation of the disease as its prevalence is high or low depending on the frequency of DRB1 alleles carrying the SE in the control population. The high prevalence of RA in Tlingit Indians may be explained by the high population frequency of the DRB1*1402 allele [30], while in Nigerians the low prevalence of RA may be related to the low frequency of alleles with the SE [31]. In Spain, the prevalence of RA is around 0.4% [32], while in Britain it has been reported to be 0.8% [33]. This difference is similar to the observed frequency of the SE in the Madrid and Bath control populations. There is evidence that HLA alleles are associated more with the severity of the disease than with its initiation. A low frequency of the SE in newly diagnosed patients with RA attending an early arthritis clinic has been described, and only a very weak association of disease with the SE has been found in community-based studies [34, 35]. In the present study, the frequency of the SE in our early synovitis clinics was lower than has been reported in chronic RA in Spain and the UK [17, 36].

The frequency of the SE seems to be higher in chronic RA patients in northern Europe and in whites in North America than in Mediterranean countries [13, 17, 36], and this is also true of early disease. In the present study, almost half of the Spanish and one third of the British patients with RA did not express the SE. Differences were also found in the alleles primarily associated with RA, reflecting the prevalence of these alleles in the control populations and suggesting that...
HLA-DRB1 genes play a more important role in the UK than in Spain.

The presence and dosage of HLA-DRB1 alleles with the SE is associated with more severe disease in most RA patients [37], including those in Spain [38]. Several authors have reported a hierarchy of disease susceptibility alleles, with a more pronounced association with HLA-DRB1*0401 and *0404 and a greater frequency of two of these alleles in patients with more severe disease [6, 7, 22], while those with milder disease were more likely to be DRB1*01 [20, 39]. From the above data it might be supposed that RA should be mild among populations in which the SE alleles encoding DR4 are less prevalent and primarily consist of non-*0401 or *0404. Our data are consistent with this hypothesis, as RA has been shown to be a less severe disease in Spanish than British patients [2]. We have shown that RA in Spain, from the very early stages, shows a lower prevalence of the SE, and when it is present it is encoded almost equally by DR1 and DR4 alleles. However, this is not true in all populations, as most African-American and Hispanic-American patients with RA do not express the SE and disease severity seems to be independent of its presence and dose [23, 24].

In the present study, we found that DR4-associated SE alleles were increased in both British and Spanish patient groups. DR1 alleles were similar to controls in Bath, but appeared to be more prevalent in Madrid, although this failed to reach significance with the present number of patients. These data reflect differences in the frequency of these alleles in the normal population and probably also the lower influence in this study of another SE allele which has been described as increased in Mediterranean countries [5]. MHC polymorphisms modify the peptide binding site and control the antigenic peptides presented to T cells. Given the different ethnic distribution of HLA-DR molecules, it is reasonable to speculate that different peptides may be related to RA [40], and disease-associated environmental factors may be different in the two countries.

It has been proposed that the inheritance of two SE alleles confers a much higher risk of RA [41]. In our study, this was true only in Spain, although the number of patients was low. In Spain, the relation between SE and disease was different, as shown by the lower frequency of the SE and its almost equal distribution between DR4 and DR1 alleles. Our data support the concept that the SE differs in its contribution to the rheumatoid process depending on the allele on which it is expressed. In the present study, DRB1*0401 clearly had a greater association with the disease than any other SE allele. Expression of this more ‘powerful’ allele as a single copy, together with its higher prevalence, may be sufficient to support or promote the disease in Bath, whereas in Madrid two alleles individually more weakly associated with RA might be needed to increase the genetic contribution to disease expression.

There is controversy as to whether HLA loci other than DR affect the susceptibility to and severity of RA. It has been proposed that the DRB1 locus is associated with protection against RA and that the RA-associated DRB1 alleles are merely permissive for the true disease susceptibility conferred by certain HLA-DQ alleles [42]. However, this has not been supported by others [43], as the strongest association in RA patients is with DRB1 genes rather than DQB1. Patients with more severe disease, including those with Felty’s syndrome, have a higher incidence of HLA-DR4-associated DQ7 (DQ7*0301) [20, 43, 44], but in Caucasians HLA-DRB1*0401 is in strong linkage disequilibrium with DQ7*0301 [43]. This extremely strong linkage disequilibrium between DQB1 and DRB1 alleles makes it difficult to determine their relative importance in predisposition to RA and the severity of the disease, but it is possible that both loci are important. Follow-up studies of the patient cohorts in Bath and Madrid may help to elucidate this problem.

It is clear that the shared epitope does not explain the entire genetic predisposition to RA and that susceptibility to and severity of RA are governed by a limited number of genes. It has been proposed that HLA accounts for only a third of the genetic susceptibility to RA [45] and that other genes must collaborate, but probably not in the same way in different populations. To identify other RA susceptibility loci, genome scans using a variable number of microsatellites have been carried out. In these studies, linkage to HLA was confirmed but was not restricted to the HLA-DR locus [46, 47]. The tumour necrosis factor (TNF) gene is in linkage disequilibrium with certain HLA-DRB1*04 alleles [48], and has been found to have an HLA-DRBI-independent role in predisposition to RA [49] and may influence radiological progression [50]. In both British and Spanish patients, TNF polymorphisms have been related to age at disease onset [51, 52]. Although the contribution of TNF polymorphisms to the initiation and outcome of RA is not clear [53], it is possible that they may act as a second MHC-linked influence which might be more important in some populations than in others.

In summary, a multitude of factors appears to influence the clinical course of RA, including different combinations of disease risk genes. We have shown in this study that class II MHC gene expression is different in early British and Spanish RA patients and may contribute to differences in disease progression and outcome.

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