CONCISE REPORT

Influence of HLA polymorphism on persistent remission in rheumatoid arthritis

E T H Molenaar, A E Voskuyl, I E van der Horst-Bruinsma, G M Th Schreuder, E Zanelli, B A C Dijkmans

Background: Several studies have reported an association between the presence of the shared epitope (SE) and susceptibility to rheumatoid arthritis (RA). Recent studies have shown that certain HLA-DRB1 alleles in combination with predisposing DQB1 and DQA1 alleles may protect against the development of RA. This model is known as the rheumatoid arthritis protection (RAP) hypothesis.

Objective: To determine the distribution of HLA-DRB1 and DQB1/DQA1 alleles in a cohort of patients with RA in remission and to determine the association between these HLA alleles and the persistence of remission.

Patients and methods: HLA-DRB1 and DQB1 typings were performed in 167 patients with RA in remission, defined according to the American College of Rheumatology criteria. The disease course, as defined by the persistence of remission during a follow up of two years, was compared between subgroups. According to the RAP hypothesis patients were divided into three subgroups: patients carrying predisposing DQ alleles, patients carrying predisposing alleles in combination with protective alleles (DQα+/DERAA phenotype), and patients lacking the predisposing alleles. According to the SE hypothesis, patients were divided into three subgroups based on whether they were carrying two, one, or no predisposing alleles [SE alleles].

Results: Predisposing DQ alleles along with a DERAA-bearing allele were present in 14 (8%) of the 167 patients. At least one SE allele was present in 116 (69%) patients; 34 of them (20%) were carrying two copies. The disease course was not significantly different between the subgroups according to the SE and RAP hypothesis, respectively.

Conclusion: The frequency of DQα+/DERAA combinations and of SE alleles in patients with RA clinically in remission was similar to that found in other RA populations. Persistent remission of RA was not associated with any particular HLA subtypes, indicating that HLA typing is not useful for predicting persistent clinical remission.

Rheumatoid arthritis (RA) is a chronic, inflammatory disease of unknown cause. Genetic factors play a part in the predisposition and in the severity of RA. Human leucocyte antigens (HLA) account for one third to one half of the total genetic contribution.

The shared epitope (SE) hypothesis presents an explanation for the susceptibility to RA. Several HLA-DRB1 alleles share a common amino acid sequence or epitope in the third hypervariable region of the molecule. Studies in different populations have reported an association between the presence of SE and the susceptibility to RA. In contrast, studies examining SE in relation to the severity of the disease have shown conflicting results. Although some of the conflicting results of previous studies may be due to differences in study design, study group, or in the outcome measures used, overall these results suggest that the SE hypothesis may not completely explain the course of disease severity.

Recently, a new hypothesis which accounts for the association of HLA class I with RA has been put forward. This hypothesis was originally based on investigations in mice and is presently known as the rheumatoid arthritis protection (RAP) hypothesis. In this hypothesis, susceptibility to RA is encoded by certain DQβ1/DQA1 combinations (referred to as DQ3 and DQ5, respectively), while protection from RA is mediated by certain DRB1 alleles, encoding a common DERAA amino acid motif in their third hypervariable region.

The combination of DQ3 or DQ5, or both, with the so-called “protective” DRB1 alleles, is hereafter referred to as DQα+/DERAA. The RAP hypothesis is proposed to explain predisposition and in particular protection from RA.

The RAP hypothesis was tested in patients with RA. Several studies have shown the significance of this model. Results from the studies performed so far suggest that the RAP hypothesis may explain susceptibility to RA, but it is not clear whether the RAP hypothesis may also explain differences in disease severity.

Based on observations in previous studies, it can be proposed that DQα+/DERAA-bearing alleles may be associated with a less severe disease. This study was performed to investigate the frequency of these alleles in patients with RA clinically in remission and to investigate whether patients carrying DQα+/DERAA-bearing alleles experienced a mild clinical course of the disease. In addition, we investigated whether the clinical course of the disease was more severe in the presence of the shared epitope.

PATIENTS AND METHODS

Patients

HLA typing was performed in 167 patients with RA clinically in remission, as defined by the modified American College of Rheumatology (ACR) criteria for clinical remission. For this study the criteria were modified by omitting the requirement of the term fatigue, so that a patient was classified as in remission when fulfilling four of the five remaining criteria. Disease activity was assessed every three months by the number of swollen joint counts (maximum 66) and the number of tender joints (maximum 68). From this a composite index of disease activity, the disease activity score (DAS), was calculated. No patients were being treated with corticosteroids and all were at least six months clinically in remission before entering the study.

Abbreviations: DAS, disease activity score; DMARD, disease modifying antirheumatic drug; RA, rheumatoid arthritis; RAP, rheumatoid arthritis protection; SE, shared epitope

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Table 1  HLA-DQ and HLA-DR genotypes in 167 patients with rheumatoid arthritis (RA) in remission at baseline

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE+ single dose</td>
<td>82 (49)</td>
</tr>
<tr>
<td>SE+ double dose</td>
<td>34 (20)</td>
</tr>
<tr>
<td>DQ\textsuperscript{RA+} single dose</td>
<td>86 (51)</td>
</tr>
<tr>
<td>DQ\textsuperscript{RA+} double dose</td>
<td>41 (25)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DQ</th>
<th>DRB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/3</td>
<td>0401/04/05/08/09 // 0401/04/05/08/09 // 0901</td>
</tr>
<tr>
<td>3/5</td>
<td>0401/03/04/05/08/09 // 0901 // 0101/02 // 1001</td>
</tr>
<tr>
<td>5/5</td>
<td>0101/02 // 0101/02 // 1001</td>
</tr>
<tr>
<td>3/x</td>
<td>0401/04/05/08/09 // 0403/06/07 // 0901 // 0101/02 // 1001</td>
</tr>
<tr>
<td>5/x</td>
<td>0101/02 // 1301/02/04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DQ\textsuperscript{RA+}/DERAA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3/5</td>
<td>0101 // 0402</td>
</tr>
<tr>
<td>3/x</td>
<td>0401/04/08/09 // 0402 // 0901 // 1102/03 // 1301/02/04</td>
</tr>
<tr>
<td>5/x</td>
<td>0101/02 // 1301/02/02</td>
</tr>
</tbody>
</table>

| DQ\textsuperscript{RA+/+} | 44 (26) |


RA predisposing alleles (DQ\textsuperscript{RA+}): DQ3 = DQB1*0301, *0302, *0303, *0304, *0401 or *0402 combined with DQA1*03.

DQ5 = DQB1*0501 combined with DQA1*0101 or *0104.


**HLA typing**

DNA was isolated from whole blood. Generic DRB1 typing was performed with the polymerase chain reaction and biotin labelled sequence-specific oligonucleotide method, as previously described.\textsuperscript{13} DRB1*04 subtyping was done by hybridisation with sequence-specific oligonucleotide on DRB1*04 specifically amplified material.\textsuperscript{14} DRB1*13 subtyping and DQB1 typing were performed by in-house designed sequence-specific primers.\textsuperscript{15}

**Statistical analysis**

Two analyses were performed to investigate whether the RAP and SE hypotheses could explain differences in the clinical course of RA, as defined by the persistence of remission during two years of follow up. One analysis was performed on a subdivision of the patients in three groups according to the RAP hypothesis, in which predisposing DQ3 and DQ5 molecules were referred to as DQ\textsuperscript{RA+}: (a) patients with phenotype DQ\textsuperscript{RA+}, or DQ\textsuperscript{RA+}/DERAA; (b) patients with phenotype DQ\textsuperscript{RA+}/DERAA; (c) patients with phenotype DQ\textsuperscript{RA+}. Another analysis was based on the shared epitope hypothesis. Accordingly patients were subdivided in three groups: (a) patients with “double dose” of SE; (b) patients with “single dose” of SE; (c) patients without SE.

Kruskall-Wallis or $\chi^2$ were used where appropriate. To evaluate a possible effect of covariate parameters on persistent remission, a logistic regression analysis was performed. Two sided p values <0.05 were considered to be significant.

**RESULTS**

The demographic and clinical characteristics indicate that a group of patients with established RA was studied: age (median 50 years), presence of rheumatoid factor (69%), presence of bone erosions on radiographs of hands or feet (77%). The most commonly used disease modifying antirheumatic drugs (DMARDs) at the time of inclusion were sulfasalazine, intramuscular gold, methotrexate, and antimalarial drugs. Parameters measuring disease activity were low (median DAS 1.0, range 0.0–2.9) as only patients satisfying the criteria for clinical remission were included in this study.

Table 1 shows that the majority of the patients carried SE and DQ\textsuperscript{RA+} alleles. Only 8% of the patients had one DQ\textsuperscript{RA+} allele in combination with a protective DERAA positive allele. Most patients with at least one predisposing DQ\textsuperscript{RA+} allele, also carried one or two copies of the SE positive DRB1 alleles. This distribution is due to linkage disequilibrium between HLA-DQ and DR alleles. DQ5 is linked to DRB1*0101, *0102, and *1001 alleles, while DQ3 is strongly associated with DRB1*0901 and DRB1*04 alleles. Thus every SE positive allele is linked to DQ3 or DQ5, representing predisposition for RA in the RAP hypothesis.

To investigate whether differences in HLA subtypes were reflected in clinical parameters, disease characteristics were compared between subgroups of patients according to the RAP and SE hypotheses, respectively. The percentage of patients remaining in remission during the two years of follow up was 52% in patients with phenotype DQ\textsuperscript{RA+}, or DQ\textsuperscript{RA+/+} (group I), 43% in patients with phenotype DQ\textsuperscript{RA+}/DERAA (group II), and 56% in patients with phenotype DQ\textsuperscript{RA+} (group III). No significant differences were found between these groups. In addition, no significant differences were found among the three subgroups according to baseline variables, except for the proportion of patients ever seropositive for rheumatoid factors, which was significantly higher in groups I and II than in the group without predisposition—that is, group III.

Disease characteristics were also compared between three subgroups based on the SE hypothesis: the proportion of patients remaining in remission was 53% in patients with double dose SE, 53% in patients with single dose SE, and 52% in patients without SE. The proportion of patients ever rheumatoid factor positive was significantly higher in patients with one or two SE alleles than in patients without an SE allele. The proportion of patients with erosions on radiographs...
of hands or feet was also significantly higher among patients with two SE alleles than among patients with one SE allele. With logistic regression analysis, no covariate association was found between persistent remission and rheumatoid factors, DMARD use, and HLA subtypes.

**DISCUSSION**

In this cohort of patients with RA in remission, with a clinical follow up of two years, DERAA-bearing alleles were found to be present in 8% of the patients. No association between persistent remission of RA and DQ*<sup>+</sup>/DERAA-bearing alleles as well as SE alleles was found.

The proportion of patients with RA in remission expressing the DQ*<sup>+</sup>/DERAA phenotype in this study falls within the range previously observed in other RA populations—that is, 4–7%, and healthy donors—that is, 9%. Although no control group was examined in this study, a cautious comparison with frequencies from other studies is advocated because all referred studies included only white patients with RA from the Netherlands.

The course of the disease, as measured by the proportion of patients with persistent remission during two years was not significantly different between the three subgroups as defined by the RAP hypothesis. The putative protective effect of DQ*<sup>+</sup>/DERAA combinations was tested in a few studies. An association between DQ*<sup>+</sup>/DERAA phenotypes and remission rate and radiological damage was found in patients with early RA. In contrast, the protective effect of DERAA-bearing alleles in combination with SE positive alleles on radiological damage could not be shown in patients with a predominantly longstanding disease, which is in line with the results from this study. The discrepancy between the results of these latter studies and those of Vos et al may be due to differences in disease duration in the study groups, suggesting that the protective effect of DQ*<sup>+</sup>/DERAA may be mostly exerted in patients with early RA.

The proportion of patients with RA in remission carrying one or two SE alleles is similar to that found in other RA populations, suggesting that patients with longstanding RA in remission are genetically not different from other patients with RA with longstanding disease. Contrasting results have been reported between the association of disease severity and SE. The association between the presence of SE alleles and disease severity is confirmed in our study, showing that the presence of rheumatoid factor ever and the presence of erosions is found more often in patients carrying an SE than in those without an SE. On the other hand, RA characterised by a “chronic”, “remittive”, “palindromic”, or “single flare” course was not associated with the presence of SE alleles. In line with the results of that study, our results show no association between SE alleles and persistent remission. This study has some limitations which may account for the results found. Firstly, a control group of patients with active RA was not included. Secondly, the possibility that the number of patients with RA in remission was too small to detect a substantial association between remission and certain HLA-DR and DQ subtypes cannot be excluded. Therefore in larger cohorts of patients with RA an association between remission and SE or DERAA motive might be found.

The main conclusion from this study is that persistent remission of RA is not associated with HLA subtypes according to the SE and RAP hypotheses, indicating that HLA typing is not clinically useful as a prognostic factor in predicting the persistence of clinical remission.

**REFERENCES**

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