Influence of non-inherited maternal HLA-DR antigens on susceptibility to rheumatoid arthritis

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Abstract

Objective—It has recently been observed that non-inherited maternal DR4 antigens (NIMAs) of DR4 negative rheumatoid arthritis (RA) patients were increased compared with non-inherited paternal DR4 antigens (NIPAs). The aim of this study was to determine the prevalence of non-inherited DR4 antigens and DRB1 alleles in parents of RA patients.

Methods—HLA-DR serology and DRB1 typing was performed in 97 RA patients and their parents. NIMA and NIPA frequencies were compared, stratified according to the presence of DR4 and/or the shared epitope (SE).

Results—In DR4 negative patients, NIMA DR4 was increased compared with NIPA DR4 (OR 3.10, 95% CI 0.76, 12.70). When combined with results from a previous study this increase was significant (OR 3.65, 95% CI 1.29, 10.31). The NIMA effect of SE positive DR4 subtypes in this study (OR 4.73, 95% CI 0.94, 23.8) was stronger than the NIMA effect of combined SE positive DRB1 alleles (OR 2.19 95% CI 0.36, 13.22).

Conclusions—The association between non-inherited maternal HLA-DR4 alleles and the susceptibility to RA was observed in two independent populations.

The susceptibility to rheumatoid arthritis (RA) is associated with the presence of certain HLA DRB1 alleles sharing a common sequence of aminoacids at position 70–74 of the DRbeta chain, known as the shared epitope (SE). Particularly the DRB1*0401 and *0404 alleles are strongly associated with RA. We have previously proposed that non-inherited maternal HLA-antigens, especially those of the mother (NIMA), can influence the susceptibility to RA. Evidence for this hypothesis was obtained by the finding of an increased frequency of a DR4 NIMA of DR4 negative RA patients (RR 3.5) compared with DR4 non-inherited paternal antigens (NIPAs). In the DR4 positive patients the NIMA DR6 was increased (RR 3.2) and the NIMA DR3 was decreased. No evidence for such a NIMA effect was found in another study that mainly analysed DR4 positive patients from multicase families.

The aim of this study was: (1) To test the NIMA hypothesis for the serologically defined DR4, DR3, and DR6 antigens in another population of RA patients. (2) To test the NIMA hypothesis for DRB1 alleles, taking into account the SE.

Methods

Ninety seven consecutive patients with RA fulfilling the 1987 ARA criteria were recruited in three outpatient clinics: 26 from Leiden (Leiden University Medical Centre), 48 from Amsterdam (Jan van Bremen Institute), and 23 from Nijmegen (University Hospital). The patients in Leiden were not included if they had participated in the former NIMA study. Both parents of all included patients had to be alive. All patients and their parents were interviewed to gain information about first degree relatives with RA. Blood samples were drawn from all patients and their parents to perform HLA class II typing.

HLA TYPING

Serological HLA-DR typing as well as generic DRB1 typing was performed with a polymerase chain reaction and biotin labelled sequence specific oligonucleotide (PCR-SSO) method as described previously. This method allows medium resolution DRB1 typing. DRB1*04 subtyping (DRB1*0401–0411) was performed by group specific amplification of DNA from all DRB1*04 positive subjects and hybridisation with relevant SSOs. In this way it was possible to distinguish homozygosity and heterozygosity for all DRB1*04 alleles. With the same technique the alleles known to express the shared epitope—that is, DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410, and *1001 were identified. DRB1*06 alleles were subtyped in *1001/*1302, *1303, and *1401. Differentiation between *1301 and *1302 was not possible.

The HLA DRB1 typings of 421 healthy organ donors served as controls for the HLA profile of the RA patients.

NIMA or NIPA were defined as serological DR antigens or DRB1 alleles present in respectively mother or father and not inherited by the patient.

Table 1 Comparison of frequencies of rheumatoid arthritis (RA) associated DRB1 alleles between RA patients and controls

<table>
<thead>
<tr>
<th>HLA</th>
<th>Patients</th>
<th>Controls</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*0401</td>
<td>56 (38)</td>
<td>94 (22)</td>
<td>4.73 (2.99, 7.55)</td>
</tr>
<tr>
<td>DRB1*0101</td>
<td>72 (74)</td>
<td>178 (42)</td>
<td>3.93 (2.40, 6.45)</td>
</tr>
<tr>
<td>DRB1*0405</td>
<td>58 (40)</td>
<td>95 (23)</td>
<td>5.10 (3.20, 8.13)</td>
</tr>
</tbody>
</table>

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To compare the NIMA and NIPA frequencies in non-RA families HLA-DR typing data from the healthy Dutch families of the previous study were used.

STATISTICS

The association between the presence and absence of HLA-DR3, DR4, and DR6 as a NIMA or a NIPA were calculated using odds ratios with 95% confidence intervals. This analysis was done in patients stratified according to the presence or absence of DR4. The frequencies of DRB1*0401 and/or *0404 or all SE positive alleles as NIMAs or NIPAs were calculated separately in the same way after stratification for the presence or absence of these DRB1 alleles or the SE. The data of the previous study were reanalysed calculating the odds ratio and confidence intervals instead of the relative risk. The odds ratio of the DR4 positive NIMA compared with DR4 positive NIPA in DR4 negative patients was calculated after combining the results of the previous study with this study.

Results

Most patients were women (85%) and, because of the selection for both parents alive, were young with a median age of RA onset at 29 years (range 16–53). The median age of disease onset did not differ among DR4 negative compared with DR4 positive patients (29 v 28 years, Kruskall-Wallis (KW) test p=0.51) or in compared with DR4 positive patients (29 v 28 years, KW p=0.29). The frequencies of the DR4, SE, and the SE positive DRB1*04 alleles *0401/*0404 alleles were increased in patients compared with healthy blood donors (table 1). The association of DR4 and DRB1*04 subtypes with RA did not differ much because most DRB1*04 subtypes were DRB1*0401 and *0404 alleles.

Table 2 Frequency of HLA-DR non-inherited maternal (NIMA) and paternal antigens (NIPA) in RA patients

<table>
<thead>
<tr>
<th>HLA</th>
<th>NIMA</th>
<th>NIPA</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR3</td>
<td>6</td>
<td>7</td>
<td>0.83 (0.25, 2.74)</td>
</tr>
<tr>
<td>DR4</td>
<td>8</td>
<td>3</td>
<td>3.10 (0.76, 12.70)</td>
</tr>
<tr>
<td>DR6</td>
<td>6</td>
<td>11</td>
<td>0.46 (0.15, 1.41)</td>
</tr>
</tbody>
</table>

DR4 positive patients (n=58)

<table>
<thead>
<tr>
<th>HLA</th>
<th>NIMA</th>
<th>NIPA</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR3</td>
<td>6</td>
<td>11</td>
<td>0.49 (0.17, 1.44)</td>
</tr>
<tr>
<td>DR4</td>
<td>10</td>
<td>9</td>
<td>1.13 (0.42, 3.04)</td>
</tr>
<tr>
<td>DR6</td>
<td>8</td>
<td>11</td>
<td>0.68 (0.25, 1.85)</td>
</tr>
</tbody>
</table>

The association between the presence and absence of HLA-DR3, DR4, and DR6 as a NIMA or a NIPA were also non-significant OR 4.73, 95% CI 0.94, 23.82, table 4).

NIMA compared with the SE positive NIPA patients lacking these alleles (OR 4.73, 95% CI 0.94, 23.82, table 4).

The effect of all SE positive alleles as a NIMA compared with the SE positive NIPA (OR 2.19, 95% CI 0.36, 13.22, table 5) was smaller than the effect of the SE positive DR4 subtypes (table 4).

To exclude a bias caused by other genetic factors that might play a part in multicase families, the family history of the patients was obtained. Thirteen patients belonged to multicase families: five (19%) from Leiden, four (8%) from Amsterdam, and four (17%) from Nijmegen respectively. A separate analysis of

Table 4 Frequencies of RA associated DR4 subtypes (*0401 and/or *0404) as NIMAs and NIPAs of *0401 and/or *0404 positive RA patients

<table>
<thead>
<tr>
<th>*0401/*0404−</th>
<th>*0401/*0404+</th>
</tr>
</thead>
<tbody>
<tr>
<td>patients (n=41)</td>
<td>patients (n=56)</td>
</tr>
<tr>
<td>NIMA</td>
<td>NIPA</td>
</tr>
<tr>
<td>*0401</td>
<td>4</td>
</tr>
<tr>
<td>*0404</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
</tr>
</tbody>
</table>

To exclude a bias caused by other genetic factors that might play a part in multicase families, the family history of the patients was obtained. Thirteen patients belonged to multicase families: five (19%) from Leiden, four (8%) from Amsterdam, and four (17%) from Nijmegen respectively. A separate analysis of
the NIMA frequencies in these patients was not possible because of the small number of DR4 negative patients (3 of 13).

Discussio

The increase of DR4 NIMAs compared with DR4 NIPAs of DR4 negative RA patients found in this study was in accordance with the previous study and does reach significance if the results of both studies are combined (OR 3.65, 95% CI 1.29, 10.31).

Focusing on the DR4 subtypes that were significantly associated with RA in the patients studied, the frequency of DRB1*0401 and/or *0404 NIMAs of *0401 and/or *0404 negative RA patients was increased compared with the NIPAs carrying these alleles. This increase (OR 4.73, 95% CI 0.94, 23.82) was stronger than that observed for DR4 (OR 3.10, 95% CI 0.76, 12.70). The effect of the SE positive NIMA of SE negative patients was less impressive (OR 2.19, 95% CI 0.36, 13.22).

Therefore we conclude that the risk of RA seems to be increased in patients who are genetically not predisposed (that is, DR4 and shared epitope negative) but have contact during their fetal period with DRB1*0401 and/or *0404 positive HLA antigens derived from the mother. Because we ascertained RA patients with a young age of onset (median age 29 years) as both parents had to be alive for patients to be included in the study, this conclusion may only be valid for RA with early onset.

To our knowledge there has been only one other study on the subject of the NIMA hypothesis and the onset of RA (see Table 5). This study showed no increase of either maternal DR4 or the HLA-DRB1 SE compared with the same non-inherited paternal antigens. These RA patients were all derived from multicase families, who might have a different genetic background than sporadic RA patients, the latter group constituting the majority of the RA patients. Of more importance is that there were too few DR4 negative patients in that study to allow an analysis of NIMAs of DR4 negative patients. As the DR4 NIMA effect was observed only in DR4 negative patients, the results of that study are not in contrast with our findings.

In conclusion, in DRB1*0401 and *0404 negative RA patients the frequency of DRB1*0401 or DRB1*0404 alleles is increased compared with their fathers. This suggests that maternal *0401 and/or *0404 positive cells, molecules or peptides may exert a predisposing effect on the development of RA in fetuses who are negative for these alleles.

We are very grateful to P D M de Buck and P W van Schendel, who participated in the preparation of this research project and collected many patients, W Verduyn who supervised the DRB1 subtyping and R v d Wijngaard who took part in collecting the patients. We would also like to thank J J van Rood for his support in all the stages of the study.