Evidence for a protective role of the human leukocyte antigen class II region in early rheumatoid arthritis

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Abstract

Objective. To analyse the distribution of predisposing DQ3 (DQB1*0304/DQA1*0303) and DQ5 (DQB1*0501/DQA1*0101), shared epitope encoding and protective DRB1 alleles in patients with early rheumatoid arthritis (RA) and undifferentiated arthritis (UA).

Methods. Consecutive patients enrolled in an early arthritis clinic were DNA-typed for HLA-DQ and DR. RA patients (n = 195), UA patients (n = 160) and controls from the same region (n = 306) were sorted according to their DQ-DR phenotypes: DQ3 vs DQ5 and the presence or absence of a protective DERAA-positive DRB1 molecule. The three groups were also sorted according to the shared epitope (SE) hypothesis.

Results. We observed the association of both DQ3 and DQ5 with RA. DQ3/3 homozygous individuals had the highest risk of developing disease [odds ratio (OR) = 20.00]. Conversely DQ5, but not DQ3, was associated with undifferentiated arthritis (OR = 2.15 vs 1.25). Consistent with these differences, DQ3-positive individuals had significantly more active disease at the first visit at the outpatient clinic than DQ5-positive patients. DRB1 alleles encoding a DERAA motif in their third hypervariable region provided a strong dominant protection against RA among DQ5-positive individuals and decreased arthritis activity among DQ3-positive patients. Individuals with SE-positive DR1, DR4 and DR10 alleles were also predisposed to RA, DR4/4 homozygous individuals having the highest risk of developing RA (OR = 11.00).

Conclusion. The DQ3-DR4/9 haplotypes are associated with RA. The DQ5-DR1/10 haplotypes are associated with less active disease, i.e. UA, and DERAA encoding DRB1 alleles modulate either predisposition to or the severity of RA. We propose that HLA polymorphism influences not only the initiation or perpetuation of RA but also protection against the disease.

Key words: HLA-DQ antigens, HLA-DR antigens, Peptides, Autoimmune disease, Immunogenetics.

A correlation exists between predisposition to RA and certain HLA-DQ3-DR4 and DQ5-DR1/DR10 haplotypes. The DRB1 alleles found in these haplotypes encode similar amino acid sequences in their third hypervariable region (3HVR), and this observation has been the basis of the shared epitope (SE) hypothesis [1].

In this model, the 3HVR motifs QKRAA, QRRAA and RRRAA of the DRB1*0101, *0102, *0401, *0404, *0405, *0408 and *1001 molecules constitute the molecular support for predisposition to RA. However, this model cannot explain, for instance, the differential associations of HLA-DR1 and -DR4 haplotypes in RA that have been described in many studies [2–4].

After a series of investigations in a mouse model of collagen-induced arthritis, which shows numerous similarities with RA, we have proposed a new hypothesis about the association between RA and the human leukocyte antigen (HLA) system [5, 6]. In this model, the predisposing alleles are DQ3 (DQB1*03/DQA1*03,
and DQB1*04: DQA1*03, but not DQB1*03: DQA1*05 and DQ5 (DQB1*0501: DQA1*0101 and DQB1*0501: DQA1*0104), which are in linkage disequilibrium with DRA/DQ9 and DRA/DQ10, respectively. Meanwhile, DRB1 alleles encoding the peptide motif DERAA in their 3HVR region modulate this DQ-mediated predisposition. Recently, we have demonstrated the value of this model in a cross-sectional study analysing two groups of RA patients and controls [7]. The major conclusions of this analysis were that DQ3 homozygosity conferred the highest risk of developing RA while DERAA encoding DRB1 alleles provided dominant protection in DQ5-positive individuals.

In this model, the DERAA motif of the protective DRB1 proteins is part of an antigenic determinant binding DQ3 and DQ5 molecules. Recently, Das et al. [8] have shown that administration of such a peptide, derived from the DRB1*0402 chain, could influence the course of collagen-induced arthritis in mice transgenic for a subtype of HLA-DQ3, DQB1*0302: DQA1*0301. A role for MHC peptides in immunoregulation is supported by the fact that a major fraction of natural ligands eluted from MHC class II molecules is constituted of MHC peptides themselves [9]. Moreover, MHC class II peptides can inhibit allorecognition in vitro [10, 11].

Most studies on the role of HLA in RA have been cross-sectional. However, prospective studies involving patients with early manifestations are necessary to better evaluate the contribution of genetic polymorphisms to RA. Only a few publications have reported such analysis. Specifically, three groups [12–16] have looked at the distributions of HLA-DRB1 alleles using high-resolution DNA typing in early arthritic patients, including patients not fulfilling the criteria for RA [17]. In these studies, the existence of possible protective alleles was not investigated. In the present study, we describe the first extensive analysis of the distribution of DQB1, DQA1 and protective DRB1 alleles in a large group of patients with early arthritis.

Methods

Patients

In 1993, a special Early Arthritis Clinic (EAC) was started at the Department of Rheumatology of the Leiden University Medical Centre, the only centre for rheumatology in a health-care region of more than 300 000 inhabitants. Its protocol was designed as a population-based study of arthritic patients. To this end, patients enrolled in this programme were required to have arthritis of at least one joint diagnosed by a rheumatologist in the department with a duration of symptoms of less than 2 yr. Patients with trauma-related arthritis were excluded. The general practitioners in the area could refer the patients to the outpatient clinic if at least two of the following features were present: joint pain, joint swelling and reduction of joint mobility. The patients could be seen at the EAC within 1 week. For every patient, routine diagnostic laboratory screening was performed [18]. On entering the study and yearly thereafter, a 54-joint count of swollen joints was obtained. The metacarpophalangeal, proximal interphalangeal and distal interphalangeal joints of each hand and the metatarsophalangeal joints of the foot were considered a single unit, so that a maximum of 22 swollen joints was measured. Diagnoses were made according to international classification criteria after 2 weeks, and were revised after 3 months and yearly thereafter if necessary [18]. Patients who could not be diagnosed with the available criteria were referred to as undifferentiated (UA). Five hundred and fifty-two HLA-DQ and -DR typed consecutive EAC patients completed 1 yr of follow-up. Three hundred and six Dutch cadaveric organ donors were used as HLA-DR and -DQ controls.

HLA typing

DNA isolation, DRB1 typing and subtyping and DQB1 typing were performed as described previously [19]. Patients and controls were sorted according to their DQ-DR phenotypes, i.e. one or two copies of DQ3, one or two copies of DQ5 and the presence or absence of a protective DERAA encoding DRB1 allele. Accordingly, they were divided into eight groups (Table 1). Patients and controls were also sorted according to the SE hypothesis: one or two copies of SE-positive DR4 and one or two copies of the DR1/10 alleles.

Statistics

To compare RA and undifferentiated arthritis patients with controls, odds ratios (OR) with 95% confidence intervals (CI) were calculated as described previously [7]. An arbitrary ratio of 1 was given to the DQx/x or DRx/x group when appropriate. Frequencies were compared between groups using the χ² test with Yates’ correction. For disease severity, the medians in the different groups of patients were compared using the Mann-Whitney U-test.

Results

Among the 552 HLA-typed patients that had been enrolled in the EAC and completed the 1-yr follow-up, 195 patients were diagnosed as having definite RA according to the 1987 ACR criteria [17] and 160 patients remained undifferentiated.

We first looked at the frequencies of the eight HLA phenotypes as defined in Table 1 in RA patients vs controls (Fig. 1a). No significant differences in the duration of symptoms before entering the EAC were observed among the eight different HLA groups (data not shown). To calculate the OR, an arbitrary value of 1 was given to the ratio of the number of patients with the phenotype DQx/x divided by the number of controls with the same phenotype. A large proportion of DQ3/x patients carried a DRB1*0401 allele (36 out of 58). The remaining 22 patients carried DRB1*0403(06:07), *0404, *0405, *0408 or *0401. Most of the DQ5/x patients were DRB1*0101-positive (26 out of 32). Both
Table 1. The eight HLA-DQ-DR phenotypes used in the present work and their corresponding \textit{DQ}B1-\textit{DQ}A1-\textit{DR}B1 genotypes

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<tr>
<td>DQ3/3$^a$</td>
<td>03 or 04/03 or 04</td>
<td>0301 (2)/0301 (2)</td>
<td>04 or 0901/04 or 0901 (except 0402)</td>
</tr>
<tr>
<td>DQ3/5</td>
<td>03 or 04/0501</td>
<td>0301 (2)/0101 (4)</td>
<td>04 or 0901/01 or 1001 (except 0103, 0402)</td>
</tr>
<tr>
<td>DQ5/5</td>
<td>0501/0501</td>
<td>0101 (4)/0101 (4)</td>
<td>01 or 1001/01 or 1001 (except 0103)</td>
</tr>
<tr>
<td>DQ3/x</td>
<td>03 or 04/x</td>
<td>0101 (2)/x</td>
<td>04 or 0901/x (except 0402)</td>
</tr>
<tr>
<td>DQ5/x</td>
<td>0501/x</td>
<td>0101 (4)/x</td>
<td>01 or 1001/x (except 0103)</td>
</tr>
<tr>
<td>DQ3/DERAA$^b$</td>
<td>03 or 04/x</td>
<td>04 or 0901/DERAA (or 0402/x)</td>
<td>DQ5/DERAA</td>
</tr>
<tr>
<td>DQ5/DERAA</td>
<td>0501/x</td>
<td>0101 (4)/x</td>
<td>01 or 1001/DERAA (or 0103/x)</td>
</tr>
<tr>
<td>DQx/x</td>
<td>x/x</td>
<td>x/x</td>
<td>Any allele</td>
</tr>
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$^a$RA-predisposing DQ3 phenotypes are the combinations of \textit{DQ}B1*03 or *04 and \textit{DQ}A1*03 alleles. RA-predisposing DQ5 phenotypes are the combinations of \textit{DQ}B1*0501 and \textit{DQ}A1*0101 or *0104.

$^b$DERAA encoding \textit{DR}B1* alleles are 0103, 0402, 1102, 1103, 1301 and 1302.

$x$ refers to any \textit{DQ}B1, \textit{DQ}A1 or \textit{DR}B1 allele other than those mentioned specifically.

DQ3/x and DQ5/x individuals were weakly, but significantly, predisposed to RA (OR = 3.04 and 2.56, respectively; Fig. 1a). Similar analyses were performed using the SE model but still taking into account the protective effect of DERAA encoding \textit{DR}B1 alleles. In this case, an arbitrary value of 1 was given to DRx/x individuals. As shown in Fig. 1b, DR4/x individuals tended to be more predisposed to RA than DQ3/x individuals (OR = 4.07 vs 3.04).

DQ5/5 and DQ3/5 individuals tended to be more predisposed to RA (OR = 4.48 and OR = 4.97, respectively) than DQ3/x and DQ5/x individuals. Only DQ3/3 homozygosity displayed a significant additive effect (OR = 20.00). Six of these DQ3/3 homozygotes were DRB1*0401/0401 homozygotes, two were DRB1*0404(8)/0404(8) homozygotes and eight were DRB1*0401(8)/0404(8) heterozygotes. Interestingly, nine of them carried a single SE encoding allele, five along with a \textit{DR}B1*0403 (06/07) allele and four along with a \textit{DR}B1*0901 allele. Therefore, DQ3/3 homozygosity predisposed to RA more than DR4/4 homozygosity (OR = 20.00 vs 11.00; Fig. 1a and b). As already reported using a smaller group of patients [19], DERAA provided dominant protection among DQ5-positive (or DR1 and/or DR10-positive) (Fig. 1a and b) but not DQ3-positive individuals (OR = 0.18 vs 2.84). In most cases, protection was conferred by either \textit{DR}B1*1301 or \textit{J}302, two common alleles in the Dutch population.

We then performed the same analyses using the 160 patients with UA (Fig. 1c and d). In contrast to the observations described above, the frequencies of genotypes DQ3/5 and DQ5/5 were not increased in this group. There was an excess of DQ3/3 homozygotes, but to a far lesser extent than in the group of RA patients (OR = 3.69 in UA vs 20.00 in RA patients). All six DQ3/3 UA patients carried two copies of SE-positive \textit{DR}B1 alleles. Interestingly, DQ5/x, but not DQ3/x, individuals were significantly predisposed to both relatively mild disease (OR = 2.15 vs 1.25; Table 2). Similarly, the numbers of DR1/x and DR10/x individuals were significantly increased in the UA group (OR = 1.90; Fig. 1b). In the EAC population, DQ5/x patients were significantly more predisposed to UA than DQ3/x patients (OR = 2.04, 95% CI 1.07–3.92). DQ3/DERAA individuals tended to be found more often in this group than DQ3/x individuals (OR = 2.18 vs 1.25).

Rheumatoid factor (RF) positivity and the existence of polyarthritis are two important criteria for the early diagnosis of RA, because at this stage only a few patients have nodules and/or erosive disease. For this reason, we looked at RF positivity and the number of swollen joints at the first hospital visit in the eight groups of HLA-typed patients (Table 2). Patients with two doses of predisposing alleles (DQ3/3, DQ3/5, DQ5/5) were more frequently RF-positive (65%) than patients with one dose (DQ3/x and DQ5/x: 41%) (OR = 2.71, 95% CI 1.40–5.30). These heterozygous patients were more often RF-positive than patients with no predisposing HLA phenotypes (DQx/x: 26%) (OR = 1.94, 95% CI 1.11–3.41) and patients with strong protection (DQ5/DERAA: 11%, only one patient out of nine).

On the basis of the results presented in Fig. 1, we specifically looked at the differences in the number of swollen joints at the first hospital visit between DQ3/x and DQ5/x individuals, along with the effect of the protective DERAA encoding \textit{DR}B1 alleles. When RA and UA patients were pooled, DQ3 was found to be significantly associated with a higher number of swollen joints than DQ5 (DQ3/x vs DQ5/x; P = 0.003). If DRB1*0401/x patients were discarded, the remaining DQ3/x patients still had a significantly higher number of swollen joints at the first hospital visit than the DQ5/x patients (P = 0.019). DERAA encoding \textit{DR}B1 alleles were significantly associated with a lower number of swollen joints among DQ3-positive individuals (P = 0.038). As expected, RA patients had a higher swollen joint count than UA patients (median 6.0 vs 2.0). In the group of RA patients, DQ3/DERAA individuals tended to have a lower count of swollen joints than DQ5/x individuals (median 4.5 vs 6.0), no DQ3/DERAA patients having more than seven swollen joints at the first hospital visit. In the group of UA patients, DQ5/x individuals had a significantly lower number of swollen
Fig. 1. Distributions of HLA-DQ3, -DQ5 and DERAA-bearing DRB1 molecules among RA (a) and undifferentiated arthritis (UA) patients (c) compared with controls according to OUR model, and distributions of HLA-DR4, DR1/10 and DERAA-bearing DRB1 chains among RA (b) and UA (d) patients compared with controls according to the SE model. Results are expressed as OR with the 95% CI and are shown on a logarithmic scale. DQx/x and DRx/x individuals were used as reference (OR = 1.00). Numbers of individuals in the different groups according to OUR model (a and c): DQ3/3: RA 25, UA 6, controls 4; DQ3/x: RA 58, UA 31, controls 61; DQ3/DERAA: RA 8, UA 8, controls 10; DQ5/5: RA 7, UA 2, controls 5; DQ5/x: RA 32, UA 35, controls 40; DQ5/DERAA: RA 1, UA 8, controls 17; DQ3/5: RA 14, UA 5, controls 9; DQx/x: RA 50, UA 65, controls 160. Numbers of individuals in the different groups according to the SE model (b and d): DR4/4: RA 16, UA 6, controls 4; DR4/x: RA 59, UA 23, controls 44; DR4/DERAA RA 6, UA 6, controls 8; DR(1/10)/(1/10): RA 7, UA 2, controls 5; DR(1/10)/x: RA 33, UA 33, controls 41; DR(1/10)/DERAA: RA 2, UA 9, controls 17; DR4/(1/10): RA 13, UA 5, controls 8; DRx/x: RA 59, UA 76, controls 179.
joints than DQ3/x patients ($P = 0.017$). In this group, DQ3/DERAA patients also tended to have a lower joint count than DQ3/x patients. Therefore, it may be concluded that the relative associations of DQ5/x and DQ3/DERAA with UA correlate with lower disease activity at the first hospital visit.

Discussion

Looking at the distributions of HLA-DQ and -DR alleles in patients with early signs of arthritis, we have demonstrated (i) the distinct contributions of the DQ3-DR4/DR9 and DQ5-DR1/DR10 haplotypes to early RA and UA, and (ii) the protective effect of DERAA encoding DRBI alleles on disease susceptibility in DQ5-positive individuals and early disease activity in DQ3-positive individuals. We have shown that, at early stage of disease, HLA class II phenotypes can already separate patients with distinct predisposition and disease activity, e.g. DQ3/x vs DQ5/x or DQ5/x vs DQ3/DERAA. This suggests that a significant fraction of UA patients (DQ5/x, DQ5/DERAA) represent a subgroup of individuals who will rarely fulfill the ACR criteria for RA or who will have a slowly progressive form of the disease (DQ3/DERAA). Indeed, DQ5/x and especially DQ5/DERAA individuals were not found to be predisposed to RA in a previous cross-sectional study [7]. Furthermore, we have shown that DQ3-positive patients already have more active disease by the time they reach an outpatient clinic and are predisposed to RA.

Until now, the association of DR10 with RA has been described mostly in populations, such as Southern Europeans, in whom this allele is not as rare as it is elsewhere [1]. In the present study, we observed that, in spite of the low frequency of DR10 in the Dutch population (1%; only two individuals in the control group), this allele was weakly associated with RA (2%; four cases in the DQ5/x group) but was significantly associated with UA (5%, eight cases in the DQ5/x group; OR = 9.70, 95% CI 1.84–68.04). Therefore, the association of DQ5 with UA is independent of its linkage with DR1 or DR10.

The SE hypothesis cannot explain the dichotomy between DQ3-DR4 and DQ5-DR1/DR10, and accounts at best for the distinct roles of the DRBI*04 alleles in RA [4, 20]. Others have already reported differences in RA association among SE encoding DRBI*04 alleles [2, 12, 14, 21]. In our study, DQ3/3 homozygous individuals are more predisposed to RA than SE-positive DR4/4 individuals and homozygous DRBI*0401/0401 or heterozygous DRBI*0401/0404 individuals. We believe that DRBI*0401 has a special role in disease severity. However, this effect is probably better seen among patients with longstanding disease.

In the present study, 36% (nine out of 25) of high-risk DQ3/3 RA patients carried a SE-negative DRBI*0403, *0406, *0407 or *0901 allele. These individuals, who are strongly predisposed to RA, are not included in the highest risk group according to the SE model (DR4/4). The association of DRBI*0901 with RA has been described in some populations [22, 23]. Recently, Wikitani et al. [24] found that DRBI*0901 homozygotes were predisposed to RA in Japan. Although this is difficult to confirm in Western countries due to the low frequency of the DRBI*0901 allele, we believe that the DQ3:3 genotype (DQBI*0303/DQ0303) of these DRBI*0901 homozygotes explains this association.

It is generally accepted that the role of HLA polymorphism in autoimmunity is to modulate the presentation of self-peptides to autoreactive T cells [25]. Thomas and Lipsky [26] have suggested that dendritic cells infiltrating the joints play an essential role in initiating RA. This model is supported by the fact that dendritic cells present self-peptides, including self-MHC peptides, very efficiently [27]. However, we propose that dendritic cells infiltrating the joints can also present self-peptides in order to protect against RA. This mechanism should operate at an early stage of RA. Consistent with this model, we have shown that HLA acts very early in
the disease process, at least before patients have reached an out-patient clinic. If the role of HLA were simply to present arthritogenic peptides to disease-eliciting T cells, there should not be a gene dose effect but rather a dominant mode of inheritance [28]. The gene dose effect of DQ3 on RA susceptibility suggests that DQ3-DRA-DRB4 haplotypes encode impaired immuno-regulation acting at an early stage of disease. Conversely, the association of DQ5 with this dysregulation is weaker and can be overcome by protective DRAA encoding DRB1 alleles. Many observations, starting with the lack of identified autoantigens in RA, support a model in which HLA acts not only on susceptibility but also on protection [28].

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