Association of HLA-DRB1*02 with osteoarthritis in a cohort of 106 patients

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Abstract

Objective. We have previously shown that the inflammatory cytokines tumour necrosis factor-α (TNF-α) and interleukin (IL) 6 or IL-1β are up-regulated in chondrocytes of patients with osteoarthritis (OA). However, the inflammatory responses associated with OA are of low grade and restricted. To investigate the involvement of the immune system in the pathogenesis of OA, we analysed patients for their HLA-DRB1 haplotypes.

Methods. Combining single-stranded oligo or sequence-specific primer typing procedures, 139 randomly selected controls and 106 OA patients were typed for their HLA-DRB1 alleles.

Results. The OA cohort showed statistically significant differences in the frequencies of the DR2 and DR5 alleles compared with the controls. While the frequency of the DR2 allele was elevated among the OA patients, the DR5 allele was negatively associated with the disease. The P values for differences from the controls were 0.0431 for DR2 and 0.0386 for DR5 and the odds ratios for the two alleles were 1.58 and 0.54 respectively.

Conclusion. The association of DR2 and DR5 with OA hints at linkage disequilibrium between HLA-DRB1 genes and genes involved in the pathogenesis of OA. Alternatively, DR2 has a direct role in restricting immunological responses to the low-grade inflammation characteristic of OA.

KEY WORDS: Osteoarthritis, Cytokines, HLA-DRB1 haplotypes.

Osteoarthritis (OA) is a degenerative joint disease and the most frequent cause of musculoskeletal disability in developed countries. Genetic as well as environmental factors contribute to the multifactorial aetiology of OA [1, 2]. Inflammatory episodes are generally accepted as a component in the disease course of most patients with symptomatic OA. However, inflammation is considered to be of low grade, with only low cell counts in the synovial fluid [3], patchy inflammatory changes in the synovia and a lack of large-scale systemic responses [4]. This low-grade inflammation is surprising considering the destructive changes in the joint, with major amounts of detritus accumulating.

The joint destruction of OA comprises loss of articular cartilage resulting from imbalance between cartilage breakdown and regeneration [5, 6]. We have shown previously that cytokines and growth factors involved in cartilage metabolism are up-regulated in the articular cartilage of OA patients [7]. This up-regulation resulted in two different phenotypes, which we called tumour necrosis factor (TNF)-αHi and TNF-αLo. The two phenotypes differed significantly in the expression of a number of cytokines and growth factors: high percentages of TNF-α+ and interleukin (IL)-6+ and low percentages of IL-1β+, TGF-β+ and IL-10+ chondrocytes are characteristic of the TNF-αHi phenotype while the TNF-αLo phenotype shows the opposite expression pattern, with low percentages of TNF-α+ and IL-6+ and elevated percentages of IL-1β+, TGF-β+, IL-4+ and IL-10+ chondrocytes [7]. The combination of low-grade inflammation and elevated levels of inflammatory cytokines associated with OA led us to investigate the involvement of the immune system in balancing inflammatory vs degenerative reactions. As disease-associated and protective HLA-DRB1 alleles have been described for the chronic inflammatory disease rheumatoid arthritis (RA) [8, 9], we set out to investigate the association between HLA-DRB1 alleles and OA. To this end, 106 OA patients and 139 controls were analysed for their DRB1 haplotypes.

Patients and methods

Study populations

Human articular OA cartilage samples were obtained from femoral heads, femoral condyles and tibial plateaux from a total of 106 OA patients with end-stage knee (n = 41) or hip (n = 65) OA who had undergone joint replacement surgery. The diagnosis of OA was based on clinical evaluations excluding metabolic
causes [7]. In order to be included as primary OA, the patient had to have several joints affected, including those of the hands. The criteria for secondary OA included a single affected joint with previous trauma or skeletal malformation being known. The patients were collected as two independent cohorts at two hospitals, the Hubertus Krankenhaus and the Immanuel Krankenhaus. The mean age of the 59 patients in the first cohort was 72.4 yr (range 57–90) and the female/male ratio was 2.1. The second cohort comprised 47 patients with a mean age of 68.2 yr (range 29–92) and a female/male ratio of 2.6. One hundred and thirty-nine randomly selected blood donors (mean age 38.2 yr, female/male ratio 1.4) were used as controls. Patients and controls were of Caucasian background but were matched neither for age nor sex.

**HLA typing procedures**

DNA was extracted from peripheral blood mononuclear cells (PBMC) from the controls and the second OA cohort and from chondrocytes from the first cohort using standard procedures. Briefly, cartilage was digested with 1 mg/ml collagenase type IV (Sigma) at 37°C overnight. Both isolated chondrocytes and PBMC were lysed in NP-40-containing buffer (0.2% NP-40, 0.9% NaCl in distilled water) and the nuclei were pelleted, resuspended in 5 ml SET buffer (150 mM NaCl, 5 mM EDTA (pH 8), 50 mM Tris, pH 7.5, 1% sodium dodecyl sulphate) and incubated with 0.1 mg/ml proteinase K for 12 h at 37°C. The genomic DNA was phenol-extracted and precipitated with ethanol. Alternatively, the Wizard DNA Purification Kit (Promega, Madison, WI, USA) was used.

Polymorphism at the *HLA-DRB1* locus was typed using genomic DNA, the polymerase chain reaction (PCR) and biotinylated single-stranded oligo (SSO) [10]. To distinguish between the alleles HLA-DRB1*05 and *06, additional PCRs with sequence-specific primer (SSP) were performed. For the identification of amino acids in positions 7–13 within the third hypervariable region, we used the 5’ primers TTTCTTGGAGTGACTCTACGTC and TTTCTTGGAGTACTCTACGGG in combination with the 3’ primer GH50 [10]. For the identification of amino acids in positions 52–60 and 65–71, the 5’ primer GAGTACTCTACGTCTGAG (amino acids 7–13) and 39 cycles, an annealing temperature of 69°C and 1.7 mM MgCl2 (amino acids 52–60 and 65–71).

**Statistical evaluation**

Odds ratios and *P* values for individual allele distributions were calculated, using the software SPSS 6.1 (SPSS, Chicago, IL, USA) [11]. *P*-values were obtained by the use of the χ² test (2×2 contingency tables). Continuity (Yates’) correction was applied if the expected frequency in a cell was below 5, resulting in the corrected *P* value (*P* corr). Global χ² tests were performed to compare *HLA-DR* haplotype distributions in our controls with those in patient cohorts and ethnically matched controls published elsewhere [12, 13].

**Results**

**DR2 is statistically significantly associated with OA**

In an effort to describe in more detail the involvement of the immune system in the pathogenesis of OA, we genotyped 139 controls and 106 OA patients for their *HLA-DRB1* haplotypes. As the controls were not matched for age, we compared them with ethnically matching control groups published elsewhere [12, 13] and found the *HLA-DRB1* haplotype frequencies to be very similar (*P* = 0.8). To account for possible homozygosities at the *DRB1* locus, we will refer to DR allele frequencies from here on. To lend more weight to results obtained from a relatively small number of patients, OA patients were collected as two independent cohorts from two hospitals. Each cohort was typed and analysed separately before a combined analysis of both cohorts was performed, which is shown in Table 1.

First, a global χ² test was used to compare allele frequencies between controls and the first patient cohort. This test revealed a significant difference in haplotype frequencies between cases and controls (*P* = 0.0176, data not shown), so that we did not think it appropriate to correct the *P* values obtained when trying to determine in which haplotypes the difference lay. Additional χ² tests were then performed to compare the allele frequencies for each of the 10 different *HLA-DRB1* haplotypes.

For DR2, the allele frequency was statistically significantly increased in the first cohort, and this increase was independent of the TNF-αHi or TNF-αLo phenotype described previously [2]. The frequency was 26.5% (31/117 alleles) compared with only 17% in the controls. In the second cohort, the allele frequency for DR2 was 21.7% (20/92 alleles) and was not different enough from that of the controls to reach statistical significance. However, the trend towards an elevated DR2 frequency in the first cohort was repeated in the second. The combined allele frequency of 24.4% for DR2 for both cohorts is again statistically significantly different from that for the controls, resulting in a *P* value of 0.0431. The odds ratio for the development of OA conferred by the DR2 allele was 1.58 (Table 1). In addition, 9.4% of the OA patients and only 2.9% of the controls were homozygous for DR2, raising the odds ratio conferred
by the double dose of OA-associated alleles to 3.5 (data not shown). In most instances, our typing method of combining SSO with SSP allows only the determination of groups of alleles rather than single alleles. Thus, 34 out of 51 DR2 alleles were *1501, *1502, *1504 or *1602. The increase in the allele frequency for DR6 observed in the first cohort was not detected in the second cohort.

**DR5 is negatively associated with OA**

Comparing the combined allele frequencies of both patient cohorts with the control frequencies revealed a statistically significant association with the DR5 allele, resulting in a P value of 0.0386 (Table 1). This association was negative and the odds ratio for protection from OA conferred by the DR5 allele was 0.54. Taken alone, neither of the two cohorts showed any significant difference from the controls.

**Discussion**

In previous studies we showed that the inflammatory cytokines TNF-α and IL-6 on the one hand and IL-1β on the other are up-regulated in articular chondrocytes from OA patients, resulting in two different phenotypes [7]. In detail, cartilage samples of the TNF-α+ phenotype showed between 4 and 24.8% (median 9.9%) IL-1α and between 9 and 39.8% (median 19.4%) IL-6+ chondrocytes. Samples of the TNF-α- phenotype contained between 4 and 24.8% (median 9.9%) IL-1α+ chondrocytes instead. In contrast, the non-OA control samples showed medians of 4.6% TNF-α+, 0% IL-6+ and 1.7% IL-1β+ chondrocytes [V. Moos, J. Sieper and B. Mueller, submitted for publication]. Despite the elevated levels of inflammatory cytokines characteristic of both phenotypes, there is strikingly little inflammation in OA joints and the inflammation is only patchy [4]. To investigate the involvement of HLA genes in this low-grade inflammation, we analysed 106 OA patients for their HLA-DRB1 genes. Interestingly, we found a statistically significant association between OA and the DR2 haplotype (Table 1). The odds ratio for the development of OA conferred by the DR2 allele is 1.58, and in this respect OA is reminiscent of multigenic diseases [14]. The complex trait is further supported when the two OA cohorts were analysed independently: the trend towards an increase in DR2 frequency was repeated in the second cohort even though statistical significance for the association between OA and DR2 was not quite reached. Contrary to the findings in RA, no common motif resulted from the alignment of OA-associated DR2 allele sequences (data not shown). We therefore do not expect a common pathogenetic role to be involved in the pathogenesis of OA. However, sequencing the OA-associated alleles in order to determine the exact haplotype and enlarging our cohort further may eventually reveal a shared epitope.

While the DR4 haplotype is associated with the chronic inflammatory joint disease RA, DR2 in RA has been described as neutral if not protective [8, 9]. The interesting observation here is that DR2 is positively associated with OA (Table 1). It is intriguing to speculate that DR2 as a haplotype allows the development of a degenerate joint disease with only low-grade inflammatory episodes while DR24 favours chronic inflammation.

The OA patients analysed here for their DRB1 haplotypes have previously been described phenotypically for their cytokine expression pattern [2]. However, the observed increase in the frequency of DR2 does not segregate with either the TNF-αHi or the TNF-αLo phenotype. We thus speculate that the association of OA with a certain HLA-DRB1 haplotype is greater than that of additional cytokine gene polymorphisms.

An association between DR2 and Heberden's nodes has been shown previously in a group of Ashkenazi patients [15] and a similar trend was described for Mexican mestizos [16]. However, it has been shown for

### Table 1. HLA-DRB1 allele frequencies in OA patients and controls

<table>
<thead>
<tr>
<th>HLA-DRB1 allele</th>
<th>Cohort 1 (n = 117)</th>
<th>Cohort 2 (n = 92)</th>
<th>Both cohorts* (n = 209)</th>
<th>Controls (n = 277)</th>
<th>P</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1</td>
<td>12.0 (14)</td>
<td>13.0 (12)</td>
<td>12.4 (26)</td>
<td>12.3 (34)</td>
<td>0.958</td>
<td>1.58</td>
</tr>
<tr>
<td>DR2</td>
<td>26.5 (31)</td>
<td>21.7 (20)</td>
<td>24.4 (51)</td>
<td>17.0 (47)</td>
<td>0.0431*</td>
<td>0.54</td>
</tr>
<tr>
<td>DR3</td>
<td>7.7 (9)</td>
<td>10.9 (10)</td>
<td>9.1 (19)</td>
<td>11.9 (33)</td>
<td>0.319</td>
<td></td>
</tr>
<tr>
<td>DR4</td>
<td>8.5 (10)</td>
<td>16.3 (15)</td>
<td>12.0 (25)</td>
<td>15.9 (44)</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>DR5</td>
<td>8.5 (10)</td>
<td>8.7 (8)</td>
<td>8.6 (18)</td>
<td>14.8 (41)</td>
<td>0.0386*</td>
<td>0.54</td>
</tr>
<tr>
<td>DR6</td>
<td>24.8 (29)</td>
<td>9.8 (9)</td>
<td>18.2 (38)</td>
<td>14.4 (40)</td>
<td>0.266</td>
<td></td>
</tr>
<tr>
<td>DR7</td>
<td>6.0 (7)</td>
<td>10.9 (10)</td>
<td>8.1 (17)</td>
<td>9.0 (25)</td>
<td>0.729</td>
<td></td>
</tr>
<tr>
<td>DR8</td>
<td>3.4 (4)</td>
<td>7.6 (7)</td>
<td>5.3 (11)</td>
<td>4.0 (11)</td>
<td>0.647b</td>
<td></td>
</tr>
<tr>
<td>DR9</td>
<td>1.7 (2)</td>
<td>1.1 (1)</td>
<td>1.4 (3)</td>
<td>0.7 (2)</td>
<td>0.751b</td>
<td></td>
</tr>
<tr>
<td>DR10</td>
<td>0.9 (1)</td>
<td>(0)</td>
<td>0.5 (1)</td>
<td>(0)</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>

*a*Analysis of the combined OA cohorts.

bCorrected for continuity.

n.d., not determined.

One allele in the controls and three alleles in the cases remained unidentified.

Statistically significant.
autoimmune diseases that HLA class II associations are different for the various ethnic groups and the same seems to apply to OA [17]. It cannot be ruled out that an association with DR2 or DR5 is only indicative of an association with a polymorphic gene linked to the DRB1 locus. Indeed, HLA and the gene encoding the α2 chain of type XI collagen (COL11A2) are tightly linked on the short arm of chromosome 6 (6p21.3) and evidence of linkage of female hip OA to HLA/COL11A2 has been shown [19]. It remains to be determined whether a specific COL11A2 gene polymorphism segregates with the DR2 and/or DR5 haplotypes.

Acknowledgements

This work was supported by a grant to VM from the Sonnenfeld Stiftung, Berlin, grants Mu 844/4 from the Deutsche Forschungsgemeinschaft to BM, and the Berlin Senate of Education and Research.

References

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