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The influence of HLA-DRB1 alleles encoding the DERAA amino acid motif on radiological outcome in rheumatoid arthritis

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Abstract

Objectives. To investigate the influence of HLA-DRB1 alleles encoding the QK/RRAA shared epitope (SE) on radiological outcome in rheumatoid arthritis (RA), and to determine whether it is modulated by alleles carrying the putative rheumatoid arthritis-protective (RAP) sequence DERAA.

Patients and methods. The association between erosive damage and HLA-DRB1 status was examined in 315 RA patients with a disease duration of 5–30 yr. Radiological outcome was measured by scoring X-rays of the hands and feet using the standard radiographs of Larsen (Larsen score). HLA-DRB1 typing was carried out using polymerase chain reaction methodology.

Results. Patients with two alleles encoding the QK/RRAA SE had significantly higher Larsen scores than SE-negative patients (96.9 *vs* 83.3; $P = 0.04$, after correction for multiple testing), with DRB1*0401/*0401 homozygotes demonstrating the greatest radiological damage (99.9). The lowest Larsen score (65.6) was observed in patients carrying the DERAA motif without an accompanying SE allele (RAP+/SE−). This was significantly lower than in patients with RAP+/SE+ (105.6; $P = 0.04$), RAP−/SE− (88.2; $P = 0.05$) and RAP−/SE+ (95.8; $P = 0.009$), after correction for multiple testing. There was no evidence that the RAP sequence was modulating the effect of the SE since radiological outcome in RAP+/SE+ patients was not significantly different to that in RAP−/SE+ individuals.

Conclusions. Our data support a possible role for DRB1 alleles encoding the DERAA motif in protection against severe erosive damage in patients lacking the QK/RRAA SE, but not in patients heterozygous for the SE. This suggests that DRB1 alleles encoding the SE have a dominant influence over 'protective alleles' and are not merely 'non-protective'.

KEY WORDS: Rheumatoid arthritis, HLA-DRB1, Shared epitope, Protective alleles, Radiological outcome.

Rheumatoid arthritis (RA) is a heterogeneous disease in which patients show considerable variation in disease progression and clinical outcome. The aetiology of RA is still unclear, and both environmental and genetic factors are believed to contribute to development of disease [1]. Genes that lie within the HLA class II region have been studied extensively, and several HLA-DRB1 alleles (DRB1*0401, *0404, *0405, *0408, *0101, *0102, *1001, *1402) have been shown to be associated with RA [2]. These DRB1 alleles all share a highly conserved amino acid sequence (QKRAA, QRRAA or RRRAA) in the third hypervariable region (HVR3) of the molecule, which forms part of the peptide binding pocket in the DR heterodimer [3]. This conserved sequence is commonly referred to as the RA 'shared epitope' (SE) [4], but although this association has been known for over 10 yr, the role of DRB1 molecules in RA aetiology remains unclear [5].

There has been considerable debate as to whether the HLA-DRB1 alleles influence susceptibility or disease severity [6]. Recently, it has been suggested that these alleles are associated with RA severity rather than susceptibility since the frequency of the trait in newly

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diagnosed patients in the community is similar to that in controls [7], while some prospective hospital-based studies have shown an association between HLA-DR4 and more severe disease [8]. In contrast, a recent community-based study suggested that DR4, and in particular DR1/DR4 heterozygosity, were related to susceptibility to RA rather than disease severity, and only in seropositive patients [9]. Such conflicting data may reflect the milder disease seen in community settings. In hospital-based studies of RA, data suggest that not all alleles carrying the SE provide equal risk of developing severe disease, with particular combinations of these alleles (e.g. DRB1*0401/0404) being associated with worse disease [10, 11].

Previous studies have suggested that certain HLA-DR phenotypes are protective, based on their reduced frequencies in RA cases compared with controls. The frequencies of DR2 (DRB1*15), DR3, DR5 (DRB1*11/*12) and DR7 haplotypes have all been shown to be decreased in epidemiological studies [2, 12, 13]. However, the effect of individual DRB1 alleles lacking the SE on disease severity is not clear. Recently, it has been suggested that the association of RA with some DRB1 alleles can be explained by their linkage with HLA-DQ alleles displaying affinity for similar 'arthritogenic' peptides [14]. According to this model, certain HLA-DQ alleles predispose to severe RA, but a self-peptide sequence (containing the DERAA motif) from the third hypervariable region of some DRB1 alleles (DRB1*0103, *0402, *1102, *1103, *1301 and *1302) can protect from disease if presented by DQ molecules. Studies in HLA-DQ8 transgenic mice showed a correlation between the DQ8-restricted T-cell response to DRB1 HVR3 peptides and non-association of the corresponding DRB1 alleles with RA in humans [15]. Further analysis of the immunogenicity of the DRB1*0402 HVR3 peptide in DQ8-transgenic mice demonstrated that the DERAA motif determined both DQ8 binding and T-cell receptor (TCR) recognition [16]. It was proposed that polymorphisms at positions 70 and 71 of the DRB1 molecules are likely to distinguish RA-predisposed from RA-protected individuals by inducing differences in their T-cell repertoire during thymic education.

The association of DRB1 HVR3 sequences with RA severity measures and their predictive value for clinical outcome may be of prognostic value. In order to test the hypothesis that certain HLA-DRB1 alleles protect against the development of severe RA, we have examined the relationship between disease severity and individual DRB1 alleles in combination with or without the QK/RRAA SE. We have specifically examined radiological damage as a measure of disease severity as this probably represents the most objective 'biological' and clinically important outcome measure. We have looked in particular at the effect of DRB1 alleles encoding the DERAA motif and investigated whether the influence of alleles encoding the QK/RRAA sequence is dominant over that of so-called 'protective' alleles.

Patients and methods

The association between HLA-DRB1 types and radiological severity was studied in 315 unrelated northern European Caucasians resident in north Staffordshire and suffering from RA (Table 1). They were recruited consecutively between 1986 and 1996 in a clinic established to examine the effects of slow-acting antirheumatic drugs. Chloroquine/hydroxychloroquine and dapsone were generally used first, followed by sulphasalazine, gold and D-penicillamine. Therapy was administered as clinically indicated. Details of some these patients have been described in previous studies [17, 18]. The ARA criteria of 1958 were recorded at the initial presentation and used as the basic definition of the group. The 1987 ARA criteria were documented retrospectively from data in the case notes. Since we were primarily interested in the association with long-term radiological outcome, we only included patients with a disease duration of 5 yr or more. These had all been reviewed annually for at least 5 yr and their disease had been extensively characterized. This enabled greater certainty of disease classification and assessment of disease progression and severity. Radiographic outcome, recorded at final review, was obtained by scoring X-rays of the hands and feet using the method of Larsen *et al.* [19]. Posterior–anterior radiographs of both hands and anterior–posterior radiographs of both feet were taken, and examined without knowledge of HLA-DRB1 genotype, by three experienced observers. All observers were trained in the use of the Larsen score. Inter- and intra-observer reliability was assessed as described previously [18], and showed no systematic bias between readings. Joint changes were classified by comparison with standard reference films and were graded 0–5, where 0 = normal, 1 = slight change (including soft-tissue swelling, osteoporosis or joint space narrowing), 2 = definite early abnormality, 3 = medium destructive changes, 4 = severe destructive changes and 5 = mutilating change. Operated joints were graded as scale 5. The final Larsen score was defined for each examination as the sum of the grades of the affected joints. The maximum possible score was 210.

HLA-DRB1 typing

HLA-DRB1 typing was performed by polymerase chain reaction-single-strand conformation polymorphism

TABLE 1. Characteristics of RA patients studied

Number	315
Gender	
Male	38.4%
Female	61.6%
Age (yr), mean (S.D.)	58.4 (10.3)
Age at onset (yr), mean (S.D.)	46.2 (11.0)
Duration (yr), mean (S.D.)	12.2 (5.2)
Rheumatoid factor	
Positive	61.9%
Negative	38.1%
Nodules	
Positive	18.4%
Negative	71.6%

TABLE 2. Association between Larsen scores and HLA-DRB1 type in RA patients

HLA-DRB1 phenotype	(*n*)	Larsen mean (S.D.)	Regression coefficient	S.E.	*P* Uncorrected	*P* Corrected
*01	(77)	92.7 (42.3)	−2.93	5.31	NS	NS
*15	(51)	92.4 (45.9)	−1.25	6.22	NS	NS
*03	(77)	85.0 (49.2)	−12.07	5.26	0.02	NS
*04	(211)	98.2 (41.5)	12.97	4.72	0.006	0.042
*11 or *12	(29)	96.0 (61.1)	−0.16	7.89	NS	NS
*13 or *14	(34)	86.2 (49.7)	−12.84	7.32	NS	NS
*07	(41)	87.7 (51.8)	−3.72	6.78	NS	NS
*08	(6)	88.3 (32.7)	ND	ND	ND	ND
*09	(13)	103.3 (39.8)	ND	ND	ND	ND
*10	(4)	60.0 (35.42)	ND	ND	ND	ND
*13 or *14 +/− SE						
*13 or *14/SE−	(16)	64.2 (46.1)	−12.84	7.32	0.04	NS
*13 or *14/SE+	(18)	105.8 (45.5)				

Regression coefficients with standard errors (S.E.)and *P* values (before and after correction for multiple testing) are shown for multiple regression analyses carried out to investigate the association between Larsen scores (dependent variable) and each phenotype (e.g. DRB1*01 *vs* non-DRB1*01). All analyses were corrected for age, sex and disease duration. Patients with DRB1*08, *09 and *10 were not considered because of the small sample size.

ND, not done.

NS, non-significant ($P > 0.05$).

(PCR-SSCP) using a panel of sequence-specific oligonucleotide probes selected from the set recommended by the 12th International Histocompatibility Workshop [20]. Probes were selected to define allele group specificity, and those carrying the RA 'SE' or RA 'protective' epitope. All probes were 5′ end-labelled with biotin and bound probe was detected by enhanced chemiluminescence (Amersham, UK). HLA-DRB1*04 subtyping was performed by PCR-SSCP [21] or using DRB1*04-specific LiPA strips (Innogenetics, Belgium).

Statistical analysis

The associations between individual HLA-DRB1 phenotypes/genotypes and Larsen score were assessed using multiple regression analysis which included the effect of the independent variables age, gender and disease duration. Where appropriate, correction for multiple testing was carried out using Holm's procedure [22, 23]. Interaction between SE and RA-protective (RAP) alleles was tested using multiple regression analysis with Larsen score as the dependent variable. The independent (explanatory) variables consisted of the interaction term RAP/SE as well as the individual main effects RAP+/− and SE+/−. Age, gender and disease duration were also included with the independent variables to correct for any influence of these confounders. All analyses were carried out using the Number Cruncher Statistical Package for Windows (NCSS Version 6.0.4). *P* values of <0.05 were considered significant.

Results

Association of HLA-DRB1 phenotype with radiological outcome

We initially stratified patients by HLA-DRB1 phenotype to examine whether it was possible to rank the amount of radiological damage in patients according to their DRB1 type. If any particular DRB1 allele was 'protective' in the presence of an SE allele, then this might be expected to be reflected by a lower Larsen score in patients with the 'protective' phenotype. Our data demonstrated that DRB1*04-positive patients ($n = 211$) had the highest Larsen score (Table 2), this being significantly different to that in patients ($n = 104$) lacking the *04 allele (98.2 *vs* 86.0; $P = 0.006$). This was still significant after correction for multiple testing ($P = 0.042$). Overall, the difference between phenotypes was not large. The lowest Larsen scores were found in patients carrying DRB1*03 (85.0) or DRB1*13/*14 alleles (86.2), but these were not significantly different from patients negative for these phenotypes, after correction for multiple testing. Patients with DRB1*08, *09 and *10 were not considered because of the low numbers involved.

Influence of the shared epitope on radiological outcome

We investigated whether the presence of an SE allele was having a significant effect on Larsen scores for each HLA-DRB1 phenotype. Apart from DRB1*01- and DRB1*04-positive patients, most DRB1 phenotypes divided into groups with or without an accompanying SE allele revealed a trend towards lower Larsen scores in the SE− groups. We have shown this only for DRB1*13/*14 (Table 2), the one phenotype to show a statistically significant difference ($P = 0.04$) between SE− and SE+ groups. However, this should be treated with caution since correction for multiple testing causes loss of significance.

Overall, patients positive for the SE had significantly higher Larsen scores than SE-negative patients (Table 3). This difference could be accounted for mainly by the lower Larsen scores of cases with DRB1*03,

TABLE 3. Influence of HLA-DRB1 SE status on Larsen score

SE+ phenotype	(*n*)	Larsen mean (S.D.)	Regression coefficient	S.E.	*P* Uncorrected	*P* Corrected
0101	(71)	94.6 (43.2)	10.84	7.28	NS	NS
0401	(171)	98.3 (43.1)	16.23	5.84	0.005	0.02
0404	(52)	94.3 (32.2)	9.54	8.71	NS	NS
0408	(12)	103.7 (47.3)	23.17	14.99	NS	NS
SE homozygotes						
0401/0401	(34)	99.9 (34.3)	20.21	9.10	0.03	NS
0404/0404	(4)	87.8 (25.1)	ND	ND	ND	ND
0401/0404	(24)	95.5 (36.1)	12.74	11.10	NS	NS
0401/0408	(6)	111.7 (48.9)	ND	ND	ND	ND
0101/0101	(5)	95.8 (19.5)	ND	ND	ND	ND
0401/0101	(25)	94.8 (45.0)	16.57	10.50	NS	NS
0404/0101	(6)	86.5 (31.8)	ND	ND	ND	ND
SE+/SE+	(109)	96.9 (39.0)	7.64	3.25	0.02	0.04
SE+/SE−	(141)	95.8 (43.6)	10.62	6.17	NS	NS
SE−/SE−	(65)	83.3 (53.3)				

Regression coefficients with standard errors (S.E.) and *P* values (before and after correction for multiple testing) are shown for multiple regression analyses carried out to investigate the association between Larsen scores (dependent variable) and each phenotype/genotype compared with SE−/−. All analyses were corrected for age, sex and disease duration. Patients with some SE homozygous genotypes were not considered because of the small sample size.

ND, not done.

NS, non-significant ($P > 0.05$).

DRB1*11/*12 or DRB1*13/*14 in the SE− group. In the SE+ group, there was no difference in radiological severity between individuals carrying one or two SE alleles. In patients homozygous for the SE, the highest Larsen score (99.9) was found in individuals with DRB1*0401/0401. This was the only SE homozygote combination which demonstrated a statistically different Larsen score to that in SE−/SE− individuals (Table 3), although this was lost after adjustment for multiple testing. Compound genotypes for the SE (DRB1*0401/*0101, *0401/*0404 or *0408) were not significantly different to DRB1*0401/*0401 or *101/*0101 homozygote combinations, although comparisons between some combinations were limited by small patient numbers.

Influence of the RA protective epitope on radiological outcome

The effect of DRB1*13 was of particular interest because the *1301 and *1302 alleles encode the so-called RAP sequence DERAA at positions 70–74 of their HVR3. This sequence is also encoded by the *0103, *0402, *1102 and *1103 alleles, although the majority (31/34) of DERAA-positive alleles in our particular RA population were accounted for by the DRB1*13 subtypes. When we compared all patients positive for DERAA against those lacking this sequence, the difference in Larsen scores (RAP+ *vs* RAP−, 89.1 *vs* 94.4) was not statistically significant ($P = 0.12$). It was not possible to examine the influence of RAP+ homozygosity since the vast majority of RAP+ patients (33/34) were RAP+/RAP− heterozygotes. In order to examine the influence of DRB1 alleles carrying the QK/RRAA SE, we grouped patients into those positive or negative for the DERAA sequence, and further subdivided them into groups positive or negative for the QK/RRAA SE (Table 4). The lowest Larsen score (65.6) was found in those cases carrying the DERAA sequence without an accompanying SE allele (RAP+/SE−), and this was significant (after correction for multiple testing) when compared with RAP−/SE− (88.2; $P = 0.05$), RAP+/SE+ patients (105.6; $P = 0.04$) and RAP−/SE+ (95.8; $P = 0.009$). There was no evidence to suggest that the RAP sequence was modulating the effect of the SE since radiological outcome in SE+ patients was not significantly different between RAP+ and RAP− patients. Thus, in the presence of the SE+, the RAP+ alleles have no effect, i.e. there is no interaction between them. A lack of interaction ($P = 0.12$) between the SE and RAP was confirmed by multiple regression analysis (Table 5) which included the interaction term between SE and RAP as well as the corresponding main effects (SE+/− and RAP+/− separately).

TABLE 4. Influence of RAP epitope (DERAA) and SE on Larsen score

	n	RAP− Larsen score mean (S.D.)	*n*	RAP+ Larsen score mean (S.D.)
All patients	281	94.4 (44.1)	34	89.1 (48.0)
SE−	51	88.2 (54.0)*	14	65.6 (45.9)
SE+	230	95.8 (41.5)‡	20	105.6 (43.2)†

P values (uncorrected) *P* values corrected for multiple testing.

*$P = 0.05$ $P = 0.05$ (*vs* RAP+/SE−)

†$P = 0.02$ $P = 0.04$ (*vs* RAP+/SE−)

‡$P = 0.003$ $P = 0.009$ (*vs* RAP+/SE−)

RAP = *0103, *0402, *1102, *1103, *1301, *1302.

TABLE 5. Multiple regression analysis to investigate whether the interaction between RAP and SE alleles is associated with Larsen score

Variable	Regression coefficient	S.E.	*P*
SE/RAP	23.62	15.41	0.12
SE	7.07	6.18	0.25
RAP	− 24.91	12.08	0.04

Multiple regression analysis with Larsen score as the dependent variable. The independent (explanatory) variables consisted of the interaction term RAP/SE as well as the individual main effects RAP+/− and SE+/−. The model was corrected for age, gender and disease duration by including these covariates with the independent variables. The *F* value for the model is 13.83 ($P < 0.0001$).

Discussion

In this study, we have examined the relationship between radiological outcome and HLA-DRB1 alleles in a large group of well-characterized patients with long-standing RA. We have confirmed previous findings that patients with DRB1 alleles carrying the QK/RRAA SE show more severe radiological change than patients lacking the SE [24]. Although the highest Larsen scores were found in DRB1*04-positive patients, they were not significantly higher than those in patients positive for DRB1*01, *15, *11, *12 and *09. This may be explained by many of these patients carrying a DRB1*04 allele on their other HLA haplotype. Comparison of patients carrying a DRB1*04 allele with all patients lacking these alleles revealed a significant difference in Larsen scores. Other studies looking at radiological progression as a measure of disease severity have produced conflicting results, with some reporting an association of DR4 with X-ray changes [25, 26] and some not [27, 28]. We should stress that our findings apply only to the more severe RA seen in hospital-based patients attending outpatient clinics, and cannot necessarily be extrapolated to milder, community-based RA.

In the case of DR1, our study differs from other studies where DR1 was associated with less severe radiographic changes or more benign disease [26, 29]. We found that the Larsen scores of patients with a DRB1*01 phenotype were not significantly different to those with DRB1*04. This was not due to the presence of an accompanying DRB1*04 allele in many DRB1*01-positive patients since comparison of Larsen scores in DRB1*01 groups with or without an accompanying DRB1*04 allele revealed no significant difference between them (data not shown).

In this study, patients who were homozygous for the SE demonstrated the highest Larsen scores. Although this was significantly higher than in the SE−/− group, it was not significantly different to patients carrying a single SE. The highest Larsen score was observed for DRB1*0401/*0401 homozygotes, which was significantly different to SE−/− cases, although significance was lost after correction for multiple testing. Compound SE genotype status (DRB1*0401/*0404 or *0408) was not associated with higher Larsen scores than homozygous SE combinations. Thus, the severity of radiological outcome in this particular group of patients does not appear to correlate with SE gene dosage or the combination of SE alleles. In contrast, studies where the severity of RA has been defined by extra-articular manifestations have shown a relationship between disease severity and SE dose, particularly compound heterozygosity [11, 30, 31], although the inheritance of two different SE-bearing alleles does not invariably associate with more severe disease [32]. It is worth noting that many studies in which severity has been linked with SE dose have used discrete data (e.g. the presence or absence of erosions, nodules, vasculitis, etc.), rather than the continuous data used in our study (Larsen score). The use of continuous data may account for the lack of an SE dose effect in our study since it takes into account the whole range of radiological severity in RA. Selection of patients may also lead to some disparity. For example, in a study by Weyand *et al.* [13], 46% of patients carried a double dose of the SE, with only a very small number (4%) being SE−/−. In our particular study, 34% of patients were SE+/+, while 20% were SE−/−.

It is noteworthy that in our particular population the mean difference in Larsen score between the different genetic groups was quite small. Even putatively high-risk (SE+/+) patients had a Larsen score only ∼16% higher than 'low-risk' (SE−/−) patients. Although statistically significant, this may not be very different clinically, and suggests that the clinical impact of the SE, in terms of radiographic damage, is not that great in patients with long-standing disease. The influence of other DRB1 alleles lacking the SE is even less clear. Previous studies have considered particular HLA-DRB1 phenotypes to be protective against the development of disease based on their reduced frequency in RA populations. However, this does not distinguish between protection and non-predisposition. From our study, it appears that HLA-DRB1 types lacking the SE are not equal with regard to their influence on radiological outcome. Our data suggest that certain HLA-DRB1 alleles (particularly DRB1*03 and DRB1*13) may provide protection against severe radiological damage, but this effect is largely lost in the presence of a single SE-carrying allele. Although DRB1*03 and DRB1*13 both appear to provide some protection, the effect is most marked in DRB1*13-positive individuals. Radiological severity in patients with other DRB1 types that have been considered to be protective (DRB1*15, *11/*12 and *07) was not statistically different to that in individuals with disease-associated DRB1*01 and *04 types, although there was an obvious trend towards lower Larsen scores in these groups when unaccompanied by a SE allele.

It has been suggested that DRB1 alleles carrying the DERAA sequence prevent the onset of RA [15, 16], although it is clear from our study that individuals carrying these alleles can develop the disease. Interestingly, DRB1*13 alleles which carry the DERAA sequence have been associated with reduced severity and/or protection in a number of other diseases, including hepatitis B [33, 34], multiple sclerosis [35],

sarcoidosis [36] and cervical carcinoma [37]. DRB1*13 has also been associated with non-progression of HIV infection [38]. The mechanism for this 'protection' has not been elucidated, although it has been suggested to be related to presentation of particular peptides, which is dependent upon amino acid residues (at positions 67 and/or 86) outside the DERAA motif (positions 70–74). In RA, it has been proposed that the binding of peptides derived from 'protective' alleles to particular HLA-DQ molecules could protect DRB1*04 heterozygous individuals. Our data support a role for DERAA-carrying DRB1 alleles in protection against the development of severe disease in patients lacking the SE epitope, but not in patients heterozygous for the SE. This does not, therefore, support the model of Zanelli *et al.* [14–16] in which presentation of a 'protective peptide' (i.e. containing the DERAA sequence) protects individuals unable to present a non-protective peptide derived from DRB1*01 or DRB1*04. Our results suggest that any 'protective' effect due to the presentation of a protective peptide can be overridden by the influence of the SE. This indicates that, in RA, DRB1 alleles carrying the SE are not merely non-protective, but have a dominant influence over so-called 'RAP' alleles. Our conclusions are based on a fairly large sample of RA patients, although the number of RAP+ patients was still relatively small. Thus, this study should be seen as exploratory, and although the significance of some results may be reduced or lost by the effects of multiple testing, it does indicate areas for future investigation in independent studies.

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