HLA-DRB1 Alleles Encoding an Aspartic Acid at Position 70 Protect Against Development of Rheumatoid Arthritis

DEREK L. MATTEY, PETER T. DAWES, MIGUEL ANGEL GONZALEZ-GAY, CARLOS GARCIA-PORRUA, WENDY THOMSON, ALI H. HAJEER, and WILLIAM E.R. OLLIER

ABSTRACT. ***Objective.*** To determine whether the association between rheumatoid arthritis (RA) and HLA-DRB1 is influenced by the amino acid residue encoded at position 70 (ß70) of the third hypervariable region (HVR3) of the HLA-DRß chain.

Methods. The frequencies of HLA-DRB1 alleles encoding different amino acid residues at ß70 were compared between patients with RA and controls in a population from the UK and in a confirmatory population from northwestern Spain. HLA-DRB1 typing was done by polymerase chain reaction methods on 476 clinic based patients with RA and 180 healthy controls from Staffordshire and Cheshire in the UK, and on 179 clinic patients and 145 controls from Lugo, Spain. Associations were investigated using chi-square analyses and regression analyses. The extended Mantel-Haenszel procedure was used for trend analysis.

Results. Carriage of 2 shared epitope (SE)+ alleles encoding a glutamine at ß70 (Q^{70SE+}/Q^{70SE+}) was associated with the greatest risk of RA in the UK and Spanish population (odds ratios 7.93 and 4.66, respectively), while possession of 2 SE– alleles encoding an aspartic acid at ß70 (D^{70SE-}/D^{70SE-}) was associated with the lowest risk (OR 0.23 and 0.34, respectively). In individuals carrying one SE+ allele and an accompanying D^{70SE-} allele there was no increased risk of developing RA [OR 0.93 (UK) and 1.30 (Spain)]. Possession of D^{70SE-} was more strongly protective than possession of Q^{70SE-}. Analysis of trend indicated that the strength of association of different DRB1 genotypes with RA could be ranked in order (from Q^{70SE+}/Q^{70SE+} to D^{70SE-}/D^{70SE-}) according to which amino acid residues were encoded at ß70, and whether or not they formed part of a SE sequence. The severity of radiographic damage could not be ranked in the same fashion.

Conclusion. The amino acid residue at position 70 of the HVR3 in HLA-DRß molecules influences susceptibility to RA. The strength of the association of DRB1 genotypes with RA is dependent not only on SE status, but also on which amino acid residues are encoded at ß70 of the DRB1 alleles. Presence of an aspartic acid residue at ß70 protects against development of RA. However, the severity of erosive damage does not appear to be associated with the amino acid substitution at ß70. (J Rheumatol 2001;28:232–9)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
HLA
SHARED EPITOPE
SUSCEPTIBILITY
ASPARTIC ACID

From the Staffordshire Rheumatology Centre, Stoke-on-Trent, England; Rheumatology Division, Hospital Xeral-Calde, Lugo, Spain; and the ARC Epidemiology Unit, School of Epidemiology and Health Sciences, Manchester University Medical School, Manchester, England.

Supported by the Arthritis Research Campaign, the Haywood Rheumatism Research and Development Foundation, and the European Commission (contract BMH4-CT-0396).

D.L. Mattey, PhD, Senior Scientist; P.T. Dawes, FRCP, Rheumatologist, Staffordshire Rheumatology Centre, Stoke-on-Trent, England; M.A. González-Gay, MD, PhD, Rheumatologist; C. García-Porrúa, MD, Rheumatologist, Rheumatology Division, Hospital Xeral-Calde, Lugo, Spain; W. Thomson, PhD, Lecturer; A.H. Hajeer, PhD, Lecturer; W.E.R. Ollier, PhD, Professor, ARC Epidemiology Unit, School of Epidemiology and Health Sciences, Manchester University Medical School, Manchester, England.

Address reprint requests to Dr D.L. Mattey, Staffordshire Rheumatology Centre, The Haywood, High Lane, Burslem, Stoke-on-Trent, Staffordshire, England ST6 7AG, UK. E-mail: d.l.mattey@keele.ac.uk

Submitted June 6, 2000 revision accepted August 31, 2000.

Several HLA-DRB1 alleles (DRB1*0401, *0404, *0405, *0408, *0101, *0102, *1001, *1402) are associated with susceptibility to rheumatoid arthritis (RA). These alleles all encode a conserved amino acid sequence (QKRAA, QRRAA, or RRRAA) at position 70–74 in the third hypervariable region (HVR3) of the molecule, which is commonly called the shared epitope (SE)[1]. This region forms part of the peptide binding pocket in the DR heterodimer[2]. Some DRB1 alleles lacking the SE have been suggested to be protective against development of RA. This is based on their reduced frequencies in RA cases compared to controls. Thus, the frequencies of DR2 (DRB1*15,*16), DR3 (DRB1*03), DR5 (DRB1*11,*12), DR7 (DRB1*07), and DR8 (DRB1*08) have been shown to be decreased in epidemiological studies of RA in different populations[3-9].

Many of these alleles encode an aspartic acid (D) at position ß70 of the HVR3, compared with a glutamine (Q) or arginine (R) in SE bearing alleles and some DR2, DR3, DR6, and DR9 subtypes[10].

Polymorphic differences between HLA-DRß chains may contribute to the selectivity of the immune response by influencing which particular peptides are bound by HLA-DR molecules. However, the exact role of HLA-DR molecules in the pathogenesis of RA remains unknown. It may involve presentation of "arthritogenic" peptides by these molecules or selection of autoreactive T cells capable of recognizing the pathogenic peptide. Alternatively, direct interaction between residues of the SE region and the complementarity-determining region (CDR) loops of the T cell receptor (TCR) ß chain may be capable of triggering T cell recognition[11]. Although DR subtypes may differ by only 1–4 residues in the HVR3, they may bind distinctly different groups of peptides[12]. Site directed mutagenesis has revealed striking differences between RA linked and unlinked DR types in selecting peptides that interact with the HVR3 region covered by positions 67–74[13]. Most differences were associated with a single amino acid exchange at ß71, and affected the charge of residues potentially contacting ß71. However, substitutions at positions 67, 70, and 74 have also been considered to be important[14]. The polymorphic residues at positions 70, 71, and 74 form part of the DR antigen binding p4 pocket, and substitutions at DR and peptide residues predicted to contribute to interactions within this region have the greatest effects on specificity of binding[14]. These data do not rule out contributions from other polymorphic positions outside the SE region in shaping the peptide repertoire. For example, the glycine/valine dimorphism at ß86 has an effect on the binding of natural peptide ligands[15]. This polymorphism has also been associated with susceptibility to RA, with alleles carrying a glycine at this position providing a higher risk than those with a valine[16-18].

Position 70 of the HLA-DRß chain can admit only glutamine (Q), arginine (R), or aspartic acid (D). All SE+ alleles possess a Q or an R at this position, although this is also the case for some SE– alleles (e.g., DRB1*1501, *03011, *0302, *1401, and *0901) that carry Q or R at ß70. A number of studies have indicated that not all SE+ alleles provide the same degree of risk of developing RA, and particular genotypic combinations of these alleles (e.g., DRB1*0401/0404) are associated with worse disease[19,20]. The reason for this is unclear, although it may indicate that carrying 2 variants of the SE [e.g., QKRAA (*0401) and QRRAA (*0404)] is important[21]. The influence of individual DRß molecules lacking the shared epitope on disease susceptibility is also unclear, although it has been suggested that alleles encoding a DERAA sequence at positions 70–74 provide protection against development of RA[22-24]. Zanelli, *et al* proposed that certain HLA-DQ alleles predispose to severe RA, but a self-peptide sequence (containing DERAA) from the HVR3 of some DRB1 alleles (DRB1*0103, *0402, *1102, *1103, *1301, and *1302) can protect from disease if presented by DQ molecules. We have shown that patients carrying a DRB1 allele with the DERAA sequence have less severe radiographic outcome, but this only occurs in the absence of a SE allele[25].

As well as those SE– alleles encoding the DERAA sequence, a number of other SE– alleles encode an aspartic acid at ß70 (D^{70}). These include DRB1*1601, *11011, *1201, * 0701, and *0801. However, not all SE– alleles carry D^{70}, so we investigated whether any variation of the strength of association of SE– alleles with RA was dependent on which amino acid residue was carried at ß70. We investigated this in 2 separate well characterized populations, one from the UK and one from NW Spain. We also investigated the association of RA with different genotypic combinations of alleles defined by the amino acid at ß70. Finally, we have examined the association between radiographic outcome and these genotypes in the patients with RA.

MATERIALS AND METHODS

UK population. HLA-DRB1 genotyping was carried out on 476 patients with RA and 180 healthy controls from the UK. All patients were northern European Caucasians resident in north Staffordshire, who had RA (Table 1). They were recruited consecutively between 1986 and 1996 in a clinic established to examine the effects of slow acting antirheumatic drugs. Therapy was administered as clinically indicated. Eighty-seven percent of patients were being treated with one or more disease modifying antirheumatic drugs (DMARD) that included hydroxychloroquine, sulfasalazine, gold, or methotrexate. About 5% of patients were being treated with corticosteroids. Details of some patients have been described[25–27]. All patients satisfied the American College of Rheumatology (ACR, formerly the American Rheumatism Association) 1987 ARA criteria for RA[28]. The control group comprised 180 ethnically matched healthy individuals with no history of inflammatory joint disease.

Spanish population. All patients and controls originated from the Galicia region of NW Spain, as described[29]. The patients (n = 179) were recruited from the Xeral-Calde Hospital (Lugo, Spain) and were periodically attending hospital outpatient clinics or were inpatients. Ninety-four percent of patients had been treated with one or more DMARD, including chloroquine, sulfasalazine, gold, cyclosporine, and methotrexate. The majority of patients (56%) were currently being treated with methotrexate, either alone or in combination with chloroquine or cyclosporine. All RA patients satisfied the 1987 ACR diagnostic criteria. Clinical details are shown in Table

Table 1. Demographic and clinical details of UK and Spanish RA populations.

	UK	Spain
Number	476	179
Mean disease duration, yrs	10.2	11
Male:female	175:301	51:128
Mean age at onset, yrs, ± SD	48 ± 12.1	49 ± 14
Erosions, %	85.9	74.7
Nodules, %	19.4	13.7
Seropositive disease, %	60.1	76.9

1. Controls (n = 145) were ethnically matched healthy volunteers from the same region.

Assessment of radiographic outcome. Radiographic outcome, recorded at final review, was obtained by scoring radiographs of the hands and feet using the Larsen method[30]. Scores were available on 373 patients from the UK only. Inter and intraobserver reliability was assessed as described[27] and showed no systematic bias between readings. Joint changes were classified by comparison with standard reference films[30]. 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. DNA was extracted from EDTA anticoagulated blood using a phenol-chloroform extraction procedure. HLA-DRB1 phenotypes were determined using a semiautomated commercial reverse dot blot method, INNO-LiPA (Abbott Laboratories UK), following the manufacturer's instructions. Reaction patterns were interpreted using Inno-LiPA software. HLA-DRB1*04 subtypes were identified using either single strand conformational polymorphism (SSCP) following amplification with DR4-specific primers or using DRB1*04-specific INNO-LiPA strips (Innogenetics, Belgium). The INNO-LiPA technology allowed intermediate to high resolution typing, although with some allelic combinations it was not always possible to provide an unequivocal assignment (e.g., DRB1*1401 or *1407 or *1410). However, because of sequence identities in the region of interest, this made no difference to the grouping of these alleles according to SE status and amino acid residue at ß70 (see below).

After identification, HLA-DRB1 alleles were stratified into 5 groups depending on the amino acid at ß70, and whether or not it formed part of the SE sequence. For example, alleles encoding the QKRAA or QRRAA sequence were designated Q^{70SE+}, while alleles encoding QARAA (DRB1*1501) or QKRGR (DRB1*03011, *0302) were designated Q^{70SE-}. The 5 groups and their corresponding DRB1 alleles are shown in Table 2.

Statistical analysis. The frequency distribution of SE genotypes and those encoding D, Q, or R residues at ß70 in the RA group were compared to that of controls by chi-square analysis of 2×2 contingency tables. Strength of association between RA and SE genotypes or genotypes encoding particular amino acid residues was estimated using odds ratios (OR) with 95% confidence intervals (CI). P values were corrected for multiple comparisons using Holm's procedure[31,32]. Analysis of trend was carried out using the extended Mantel-Haenszel procedure. The associations with Larsen score were assessed using multiple regression analysis that included the effect of the independent variables age and disease duration. All analyses were carried out using the Number Cruncher Statistical Package for Windows (NCSS v. 6.0.4) or the PEPI software package (v. 2.0) for epidemiologic analysis[33].

RESULTS

Influence of the shared epitope on susceptibility to RA. We initially examined the association of the SE with RA by stratifying patients and controls into 3 groups — SE+/SE+, SE+/SE–, and SE–/SE– (Table 3). In both UK and Spanish patients the frequency of RA patients carrying 2 SE alleles was significantly higher than in the control groups (OR 7.29, $p_c < 0.0001$ and OR 4.29, $p_c = 0.0006$, respectively). The frequency of RA patients carrying one SE allele was significantly higher in the Spanish patients compared with controls (OR 1.95, $p_c = 0.004$), but did not reach significance in the UK population (OR 1.39, $p_c = 0.08$). There was a strong negative association between the SE–/SE– genotype and RA in the UK (OR 0.19, $p_c < 0.0001$) and in the Spanish population (OR 0.30, $p_c < 0.0001$).

Influence of amino acid at position 70 on HLA-DRB1 genotype associations with RA. To determine whether susceptibilty to RA was influenced by the combination of alleles encoding different residues at ß70 we compared the frequencies of genotypes grouped according to SE status and amino acid residues at ß70. In genotypes carrying an SE+ allele we included Q^{70SE+} alleles only because the number of R^{70SE+} alleles was too small to consider in separate groups. As expected, carriage of 2 SE+ alleles (Q^{70SE+}/Q^{70SE+}) was associated with the greatest risk of RA in the UK and the Spanish population (Table 4). For individuals with SE+/SE– genotype we divided them into SE+ individuals with an SE– allele encoding a Q residue (Q^{70SE+}/Q^{70SE-}) and SE+ individuals with an SE– allele encoding a D residue (Q^{70SE+}/D^{70SE-}). Again we did not include R^{70SE+} or R^{70SE-} individuals in the analyses because genotypes with these designations contained such small numbers. Stratification of SE+/SE– individuals in this way revealed an increased risk of developing RA in Q^{70SE+}/Q^{70SE-} individuals in both the UK and Spanish populations (OR 1.96, $p_c = 0.012$ and OR 3.17, $p_c = 0.004$, respectively).

Table 2. Grouping of HLA-DRB1 alleles according to shared epitope (SE) status and amino acid residue encoded at position 70 (ß70). Only HLA-DRB1 alleles that were identified in the UK or Spanish populations are shown. In some genotypes with certain allelic combinations it was not possible to provide an unequivocal assignment (e.g., DRB1*1401 or 1407 or 1410, DRB1*1501 or 1503 or 1504). However, this made no difference to the ß70 amino acid designation in these individuals.

Designation by Residue at ß70, and SE Status	Amino Acid Sequence at Position 70–74	HLA-DRB1 Alleles
Q^{70SE+}	QRRAA	*0101, *0102, *0404, *0405, *0408
	QKRAA	*0401, *0409
Q^{70SE-}	QARAA	*1501
	QRRAE	*0403, *0406, *0407
	QKRGR	*03011, *0302
R^{70SE+}	RRRAA	*1001
R^{70SE-}	RRRAE	*1401, *0901
D^{70SE-}	DERAA	*0103, *0402, *1102, *1103, *1301, *1302
	DRRAA	*11011, *1201, *1305, *1601
	DRRAL	*0801
	DRRGQ	*0701

Table 3. Influence of the shared epitope (SE) on susceptibility to RA.

Genotype	RA, n (%)	Control, n (%)	OR (95% CI)	p_c
UK Population				
SE+/SE+	164 (34.5)	12 (6.7)	7.29 (3.82–14.21)	< 0.0001
SE+/SE–	221 (46.4)	69 (38.3)	1.39 (0.97–2.01)	0.08
SE–/SE–	91 (19.1)	99 (55.0)	0.19 (0.13–0.29)	< 0.0001
Spanish Population				
SE+/SE+	32 (17.9)	7 (4.8)	4.29 (1.74–11.06)	0.0006
SE+/SE–	88 (49.2)	48 (33.1)	1.95 (1.21–3.16)	0.004
SE–/SE–	59 (33.0)	90 (62.1)	0.30 (0.18–0.49)	< 0.0001

The OR with 95% CI were calculated from comparisons of genotype frequencies between RA patients and controls. P values were corrected for multiple testing using Holm's procedure.

However, SE+ individuals who carried a second SE– allele encoding a D^{70} residue had no increased risk of developing RA (OR 0.93, $p_c = 0.75$ and OR 1.30, $p_c = 0.69$).

In the UK and Spanish populations the carriage of 2 D^{70} alleles was associated with the lowest risk of RA (OR 0.23, $p_c < 0.0001$ and OR 0.34, $p_c < 0.0001$, respectively). A higher risk of developing RA in SE–/SE– individuals was found in those without a D^{70} allele (although the association was still negative). Analysis of trend indicated that the strength of association of different genotypes with RA could be arranged in order according to particular combinations of alleles encoding different amino acid residues at ß70, and whether or not they formed part of an SE sequence. The order of association giving the highest chi-square value and the highest cumulative OR in the trend analyses is shown for each population in Table 4. Exactly the same trend was seen in both populations from the highest association with Q^{70SE+}/Q^{70SE+} to the lowest with D^{70SE-}/D^{70SE-}.

Contribution of Q^{70SE+} (DR1) and Q^{70SE+} (DR4) to RA susceptibility. All Q^{70SE+} alleles are subtypes of either HLA-DRB1*01 or *04 (Table 2). The data on genotypes carrying Q^{70SE+} in Table 4 do not discriminate between the contributions of HLA-DR1 and HLA-DR4 to disease susceptibility, which may be different in different populations. To investigate this further we divided Q^{70SE+} alleles into DR1 and DR4 subtypes [Q^{70SE+} (DR1) and Q^{70SE+} (DR4)] as shown in Table 5. The data clearly indicate that Q^{70SE+} (DR4) has a significantly greater influence on RA susceptibility than Q^{70SE+} (DR1) in both the UK and Spanish populations. It is notable that the UK population has a significantly higher frequency of Q^{70SE+} (DR4) alleles than the Spanish population in Q^{70SE+}/Q^{70SE+} individuals (80.3 vs 66.7%), and this probably accounts for the stronger association of Q^{70SE+}/Q^{70SE+} with RA in the UK population. It is also important that in both populations carriage of a D^{70SE-} allele protects against the effect of an accompanying Q^{70SE+} (DR4) allele, and that it is more strongly protective than possession of a Q^{70SE-} allele.

Influence of amino acid residue at position 70 on erosive damage RA. Table 6 shows the Larsen scores of patients with different genotypes grouped according to SE status and amino acid residues at ß70. Overall there were no significant differences between any of the groups. Five of the 6 groups had very similar Larsen scores (range 83.2–94.0), with only the Q^{70SE-}/D^{70SE-} group showing a reduced score (65.9). Even so, this was not significantly different from any of the other groups after correction for age and disease duration. Since we had shown in SE–/– individuals that the DERAA sequence is associated with less severe radiographic outcome[24], we examined the Q^{70SE-}/D^{70SE-} and D^{70SE-}/D^{70SE-} groups for alleles containing this sequence. Such alleles were only found in the Q^{70SE-}/D^{70SE-} group. The mean Larsen score of this subset (n = 15) was 61.2, which was significantly lower than all other patients with RA (83.1; p = 0.02) after correction for age and disease duration.

DISCUSSION

As in many other studies we have confirmed in 2 separate ethnic populations that carriage of 2 SE+ alleles is strongly associated with RA requiring hospital treatment. In both populations the greatest risk is associated with the carriage of 2 HLA-DRB1*04 SE alleles. We have also shown that in individuals carrying one SE allele the strength of association with RA is influenced by whether the accompanying SE negative allele encodes a Q or D residue at ß70 of the HLA-DRß1 molecule. Thus individuals with SE+/SE– genotypes that include a SE– allele encoding a Q^{70} residue have an increased risk of developing RA, in both the UK and Spanish populations. This appears to be mainly associated with carriage of a Q^{70SE+} (DR4) allele. However, those SE+ (DR1 or DR4) individuals who carry an allele encoding a D^{70} residue have no increased risk of developing RA. Thus,

Table 4. Frequencies of genotypes designated by shared epitope (SE) status and amino acid residues encoded at ß70 of HLA-DRB1. Comparison between RA and controls.

UK Population Genotype Designation	RA, n (%)	Control, n (%)	OR (95% CI)	p_c
Q^{70SE+}/Q^{70SE+}	162 (34.0)	11 (6.1)	7.93 (4.06–15.9)	< 0.0001
Q^{70SE+}/Q^{70SE-}	117 (24.6)	26 (14.4)	1.96 (1.20–3.22)	0.012
Q^{70SE+}/D^{70SE-}	90 (18.9)	36 (20.0)	0.93 (0.59–1.47)	0.75
Q^{70SE-}/Q^{70SE-}	23 (4.8)	24 (13.3)	0.33 (0.17–0.63)	0.0005
Q^{70SE-}/D^{70SE-}	35 (7.4)	43 (23.9)	0.25 (0.15–0.42)	< 0.0001
D^{70SE-}/D^{70SE-}	17 (3.6)	25 (13.9)	0.23 (0.12–0.46)	< 0.0001
*Other genotypes	32 (6.7)	15 (8.3)		
Spanish Population				
Q^{70SE+}/Q^{70SE+}	30 (16.7)	6 (4.1)	4.66 (1.78–12.91)	0.0016
Q^{70SE+}/Q^{70SE-}	37 (20.7)	11 (7.6)	3.17 (1.49–6.91)	0.004
Q^{70SE+}/Q^{70SE-}	41 (22.9)	27 (18.6)	1.30 (0.73–2.32)	0.69
Q^{70SE-}/Q^{70SE-}	10 (5.6)	9 (6.2)	0.89 (0.32–2.47)	0.81
Q^{70SE-}/D^{70SE-}	24 (13.4)	32 (22.1)	0.55 (0.29–1.02)	0.12
D^{70SE-}/D^{70SE-}	20 (11.2)	39 (26.9)	0.34 (0.18–0.59)	0.0016
*Other genotypes	17 (9.5)	21 (14.5)		

*Other genotypes: any genotype containing R^{70SE+} or R^{70SE-} (e.g., Q^{70SE+}/R^{70SE+}, R^{70SE+}/R^{70SE+}, Q^{70SE+}/R^{70SE-}, Q^{70SE-}/R^{70SE-}, etc.).

The OR with 95% CI were calculated from comparisons of genotype frequencies between RA patients and controls. p values were corrected for multiple testing using Holm's procedure. Analysis of trend by the extended Mantel-Haenszel procedure was used to place the genotype categories in order according to their strength of association with RA. The order shown in the tables (Q^{70SE+}/Q^{70SE+} to D^{70SE-}/D^{70SE-}) was found to give the highest chi-square and cumulative OR in both the UK [chi-square 107.1, df = 1, $p = 4.1 \times 10^{-25}$, cumulative OR 5.9 (95% CI 4.4–8.1)] and Spanish population [chi-square 35.2, df = 1, $p = 3.0 \times 10^{-9}$, cumulative OR = 3.8 (95% CI 2.6–5.6)].

Table 5. Susceptibility to RA in different shared epitope (SE) positive genotypes carrying various combinations of HLA-DR4 and DR1.

UK Population Genotype Designation	RA, n (%)	Control, n (%)	OR (95% CI)	p_c
Q^{70SE+}(DR4)/Q^{70SE+}(DR4)	105 (22.1)	3 (1.7)	16.70 (5.0–66.8)	< 0.0001
Q^{70SE+}(DR4)/Q^{70SE+}(DR1)	50 (10.5)	7 (3.9)	2.90 (1.2–7.2)	0.042
Q^{70SE+}(DR1)/Q^{70SE+}(DR1)	7 (1.5)	1 (0.6)	2.67 (0.3–58.2)	1.00
Q^{70SE+}(DR4)/Q^{70SE-}	82 (17.2)	17 (9.4)	2.00 (1.1–3.6)	0.065
Q^{70SE+}(DR1)/Q^{70SE-}	35 (7.4)	9 (5.0)	1.51 (0.7–3.5)	1.00
Q^{70SE+}(DR4)/D^{70SE-}	70 (14.7)	30 (16.7)	0.86 (0.5–1.4)	1.00
Q^{70SE+}(DR1)/D^{70SE-}	20 (4.2)	6 (3.3)	1.27 (0.5–3.0)	1.00
Spanish Population				
Q^{70SE+}(DR4)/Q^{70SE+}(DR4)	13 (7.3)	0 (0.0)	∞ (2.2–∞)	0.007
Q^{70SE+}(DR4)/Q^{70SE+}(DR1)	14 (7.8)	4 (2.8)	2.99 (0.9–11.0)	0.24
Q^{70SE+}(DR1)/Q^{70SE+}(DR1)	3 (1.7)	2 (1.4)	1.22 (0.2–10.6)	1.00
Q^{70SE+}(DR4)/Q^{70SE-}	24 (13.6)	6 (4.1)	3.59 (1.3–10.1)	0.024
Q^{70SE+}(DR1)/Q^{70SE-}	13 (7.3)	5 (3.4)	2.19 (0.7–7.2)	0.56
Q^{70SE+}(DR4)/D^{70SE-}	26 (14.5)	15 (10.3)	1.47 (0.7–3.1)	0.78
Q^{70SE+}(DR1)/D^{70SE-}	15 (8.4)	12 (8.3)	1.01 (0.4–2.4)	1.00

The OR with 95% CI were calculated from comparisons of genotype frequencies between RA patients and controls. p values were corrected for multiple testing using Holm's procedure.

Table 6. Larsen scores in genotypes designated by shared epitope (SE) status and amino acid residues encoded at ß70 of HLA-DRB1. UK patients with RA.

Genotype Designation	n	Larsen Score (SD)
Q^{70SE+}/Q^{70SE+}	138	83.8 (46.1)
Q^{70SE+}/Q^{70SE-}	99	83.2 (47.9)
Q^{70SE+}/D^{70SE-}	73	84.5 (52.8)
Q^{70SE-}/Q^{70SE-}	16	94.0 (48.5)
Q^{70SE-}/D^{70SE-}	32	65.9 (51.4)
D^{70SE-}/D^{70SE-}	15	84.7 (55.5)

No significant differences in Larsen score were found between any of the groups (by multiple regression analysis with correction for age and disease duration).

the possession of D^{70SE-} has a dominant protective effect in SE+ individuals, and is more strongly protective than Q^{70SE-}. In SE–/SE– individuals there also appears to be a hierarchy of susceptibility to RA depending on the combination of alleles encoding different residues at ß70. In both the UK and Spanish populations the carriage of 2 alleles encoding D^{70} is associated with the lowest risk of developing RA, while the highest risk for SE–/SE– patients is in individuals without a D^{70} allele (although the association is still negative). Analysis of trend in both populations indicated that the strength of association of different genotypes with RA could be arranged in order (from Q^{70SE+}/Q^{70SE+} to D^{70SE-}/D^{70SE-}) according to which amino acid residues were encoded at ß70, and whether or not they formed part of a SE sequence.

Our data show that not all SE+ individuals have an increased risk of developing RA. This is consistent with findings by Zanelli, *et al*, who suggested that certain HLA-DRB1 alleles were protective against development of RA in SE+ individuals[22-24]. They proposed that such protection was achieved via presentation of a self-peptide sequence (containing D^{70}ERAA) from the HVR3 of some DRB1 alleles by certain HLA-DQ molecules. The role of HLA-DQ in predisposition to RA is controversial[24,34], and we did not address this issue. However, our data suggest that all D^{70} containing DRB1 alleles are protective, not just those carrying the DERAA sequence.

Other polymorphic positions in the HVR3 of HLA-DRß have been considered to be important in determining susceptibility to RA. In particular, the charge of the amino acid residue at ß71 is believed to be critical[13,14,35]. All RA associated alleles carry a positively charged residue at this position, either lysine (K) or arginine (R). The K substitution in particular is strongly associated with RA[36], although this is found only on one common RA associated allele (DRB1*0401). (Other rare alleles with the QKRAA sequence include DRB1*0409, *0413, *0416, and *0421.) All other RA associated alleles encode an R residue at this position. However, this is also commonly found on non-RA associated molecules so it seems unlikely that this polymorphic position is the most critical in determining susceptibility to RA. What appears to be more important is whether or not it forms part of a SE sequence. The same appears to be true for the uncharged Q or positively charged R residues at ß70. Either of these residues can be found in RA and non-RA associated alleles, but only those where the Q or R residues form part of the SE are associated with RA. In contrast, the negatively charged D residue is only found on alleles negatively associated with RA, and it never forms part of the SE.

The exact role of the SE region in RA remains to be elucidated. Our data support the view that the charge of the P4 pocket in the peptide binding site is a critical determinant of susceptibility to RA, since substitution with the negatively charged D residue at ß70 leads to the weakest association with disease. Nonetheless, some non-RA associated alleles maintain an uncharged Q or positively charged R residue at ß70. In these alleles the association with RA appears to be lost by substitution of one of the other residues within the SE region (usually ß71 or ß74). This may be due to an effect on the overall charge of the P4 pocket. However, it appears from this study that the SE– alleles that maintain a Q or R residue at ß70 still provide a stronger association with RA than SE– alleles that encode a D residue at this position.

A number of studies have shown the importance of amino acid residues at positions 67, 70, and 71 on peptide binding and/or T cell receptor (TCR) interactions[11,13,14,37-39]. Penzotti, *et al*[11] have suggested that the hydrogen bonding network around SE residues at ß70 and ß71 (in DRB1*0404) is intimately involved in direct TCR contact, and this interaction alone may be sufficient for TCR activation. In another study on HLA-DR1 molecules, ß67 and ß71 were shown to contribute to binding of a human immunodeficiency virus peptide with direct effects on TCR recognition, while ß70 appeared to be mostly engaged in TCR interactions[37]. It was further suggested that these polymorphic residues may select allele-specific peptides and influence the conformation of promiscuous peptides. Another study on HLA-DR1 molecules showed that substitutions at positions 67, 70, and 71 strongly affected specific T cell recognition of the promiscuous HA 306–318 peptide, and that residue 67 played an important role in determining conformation of the peptide presented to T cells[38]. Interestingly, all of the alleles with a D residue at ß70 have an isoleucine (I) or phenylalanine (F) residue at ß67, while all SE+ alleles have a leucine (L) residue in this position. The combination of residues at these 2 positions may thus have a crucial role in determining susceptibility to RA.

It has been suggested that the negative association between susceptibility to RA and alleles containing a D residue at ß70 could reflect a greater efficiency of these alleles to bind invariant chain (Ii), which results in a lower rate of presentation of self-peptides[9,40]. Invariant chain promotes correct folding of the HLA-DR heterodimer, and

prevents premature occupation of the antigen-binding groove by endogenous peptides. Reduced or defective binding of Ii by SE+ molecules could lead to premature occupation by and excess presentation of endogenous peptides. This alteration in function has been suggested as a possible mechanism by which these HLA-DR molecules are involved in the development of RA[40].

With regard to disease severity, it was surprising that no significant differences were found in Larsen scores between the different genotypes grouped according to SE status and amino acid residues at ß70 of HLA-DRß. From previous studies we would have predicted that individuals carrying 2 SE+ alleles would have had more radiographic damage than patients with a SE–/– genotype. In fact only one of the SE–/– groups (Q^{70SE-}/D^{70SE-}) showed evidence of a reduction in Larsen score, although this was not significantly different to any other group after correction for age and disease duration. However, the trend is consistent with our recent finding that SE– patients carrying a DRB1 allele with the DERAA sequence have less severe radiographic outcome[25]. In the present study all SE– patients with this sequence (n = 15) were found in the Q^{70SE-}/D^{70SE-} group. Further, the mean Larsen score (61.2) for the DERAA+ subset was significantly lower compared with all other RA patients (83.1). No patients carrying the DERAA sequence were present in the D^{70SE-}/D^{70SE-} group, which showed no overall reduction in Larsen score. The data suggest that in SE–/– patients it is mainly alleles carrying the DERAA sequence that are associated with less severe radiographic damage. Other studies have not divided SE–/– groups according to these categories, so it would be interesting to determine if this is a reproducible finding in other groups of patients.

Our data indicate that the amino acid residue at ß70 of the HVR3 in HLA-DRß molecules influences susceptibility to RA. The data confirm that carriage of an SE+ allele is an important susceptibility factor in RA, but the particular genotypic combination of SE+ alleles with other alleles is important in determining the strength of the association. Individuals carrying 2 SE+ alleles have the greatest risk of developing RA, while those carrying genotypes with a SE+ allele and a Q^{70SE-} allele have a greater risk than those with a SE+ allele and a D^{70SE-} allele. The presence of an allele encoding D^{70SE-} appears to protect against development of RA, with the best protection being provided by 2 D^{70SE-} alleles. The strength of the association of HLA-DRB1 genotypes with RA thus indicates a trend that is dependent on the amino acid residues at ß70, and whether or not they form part of a shared epitope sequence. Such an arrangement does not appear to predict erosive damage in RA.

REFERENCES

1. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis: An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987;30:1205-13.
2. Brown JH, Jardetzky TS, Gorga JC, et al. Three dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature 1993;364:33-9.
3. Ollier W, Thompson W. Population genetics of rheumatoid arthritis. Rheum Dis Clin North Am 1992;18:741-59.
4. Young A, Jaraquemada D, Awad J, et al. Association of HLA-DR4/Dw4 and DR2/Dw2 with radiologic change in a prospective study of patients with rheumatoid arthritis: preferential relationship with HLA-Dw rather than HLA-DR specificities. Arthritis Rheum 1984;27:20-5.
5. Larsen BA, Alderdice CA, Hawkins D, Martin JR, Mitchell DM, Sheridan DP. Protective HLA-DR phenotypes in rheumatoid arthritis. J Rheumatol 1989;16:455-8.
6. Hameed K, Bowman S, Kondeatis E, Vaughan R, Gibson T. The association of HLA-DRB genes and the shared epitope with rheumatoid arthritis in Pakistan. Br J Rheumatol 1997;36:1184-8.
7. Wakitani S, Murata N, Toda Y, et al. The relationship between HLA-DRB1 alleles and disease subsets of rheumatoid arthritis in Japanese. Br J Rheumatol 1997;36:630-6.
8. DeBaz H, Olivo A, Vazquez Garcia MN, et al. Relevant residues of DRB1 third hypervariable region contributing to the expression and severity of rheumatoid arthritis in Mexicans. Hum Immunol 1998;59:287-94.
9. Del Rincon I, Escalante A. HLA-DRB1 alleles associated with susceptibility or resistance to rheumatoid arthritis, articular deformities, and disability in Mexican Americans. Arthritis Rheum 1999;42:1329-38.
10. Marsh SG, Bodmer JG. HLA class II region nucleotide sequences, 1995. Tissue Antigens 1995;46:258-80.
11. Penzotti JE, Nepom GT, Lybrand TP. Use of T cell receptor/HLA-DRB1*04 molecular modeling to predict site-specific interactions for the DR shared epitope associated with rheumatoid arthritis. Arthritis Rheum 1997;40:1316-26.
12. Brown JH, Jardetzky T, Saper MA, Samraoui B, Bjorkman PJ, Wiley DC. A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. Nature 1988;332:845-50.
13. Hammer J, Gallazi F, Bono E, et al. Peptide binding specificity of HLA-DR4 molecules: correlation with rheumatoid arthritis association. J Exp Med 1995;181:1847-55.
14. Woulfe SL, Bono CP, Zacheis ML, et al. Negatively charged residues interacting with the p4 pocket confer binding specificity to DRB1*0401. Arthritis Rheum 1995;38:1744-53.
15. Davenport MP, Quinn CL, Chicz RM, et al. Naturally processed peptides from two disease-resistance-associated HLA-DR13 alleles show related sequence motifs and the effects of the dimorphism at position 86 of the HLA-DRß chain. Proc Natl Acad Sci USA 1995;92:6567-71.
16. Debaz H, Olivo A, Vasquez Garcia MN, et al. Relevant residues of DRß1 third hypervariable region contributing to the expression and to severity of rheumatoid arthritis in Mexicans. Hum Immunol 1998;59:287-94.
17. Gao XJ, Olsen NJ, Pincus T, Stastny P. HLA-DR alleles with naturally occurring amino acid substitutions and risk for development of rheumatoid arthritis. Arthritis Rheum 1990;33:939-46.
18. Taneja V, Giphart MJ, Verduijn W, Naipal A, Malaviya AN, Mehra NK. Polymorphism of HLA-DRB, -DQA1, and DQB1 in rheumatoid arthritis in Asian Indians: association with DRB1*0405 and DRB1*1001. Hum Immunol 1996;46:35-41.
19. Wordsworth P, Pile KD, Buckley JD, et al. HLA heterozygosity contributes to susceptibility to rheumatoid arthritis. Am J Hum Genet 1991;51:585-91.
20. MacGregor A, Ollier WER, Thomson W, Jawaheer D, Silman A. HLA-DRB1*0401/0404 genotype and rheumatoid arthritis:

Increased association in men, young age at onset and disease severity. J Rheumatol 1995;22:1032-6.
21. Ollier WER, Hajeer A. Does the HLA-DRB1 shared epitope really contribute that much to the development or severity of rheumatoid arthritis? In: Isenberg DA, Tucker LB, editors. Controversies in rheumatology. London: Marhn Dunitz; 1997:1-12.
22. Zanelli E, Gonzalez-Gay MA, David CS. Could HLA-DRB1 be the protective locus in rheumatoid arthritis? Immunol Today 1995;16:274-78.
23. Zanelli E, Krco CJ, David CS. Critical residues on HLA-DRB1*0402 HV3 peptide for HLA-DQ8-restricted immunogenicity. Implications for rheumatoid arthritis predisposition. J Immunol 1997;158:3545-51.
24. van der Horst-Bruinsma IE, Visser H, Hazes JM, et al. HLA-DQ-associated predisposition to and dominant HLA-DR associated protection against rheumatoid arthritis. Hum Immunol 1999;60:152-8.
25. Mattey DL, Hassell AB, Plant MJ, et al. The influence of HLA-DRB1 alleles encoding the DERAA amino acid motif on radiological outcome in rheumatoid arthritis. Rheumatology 1999;38;1221-7.
26. Hassell AB, Davis MJ, Fowler PD, et al. The relationship between serial measures of disease activity and outcome in rheumatoid arthritis. Q J Med 1993;86:601-7.
27. Plant MJ, Saklatvala J, Borg AA, Jones PW, Dawes PT. Measurement and prediction of radiological progression in early rheumatoid arthritis. J Rheumatol 1994;21:1808-13.
28. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
29. Hajeer AH, Dababneh A, Makki RF, et al. Different gene loci within the HLA-DR and TNF regions are independently associated with susceptibility and severity in Spanish rheumatoid arthritis patients. Tissue Antigens 2000;55:319-25.
30. Larsen A, Dale K, Eck M. Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. Acta Radiol (Diagn Stockh) 1977;18:481-91.
31. Holm S. A simple sequentially rejective multiple test procedure. Scand J Statistics 1979;6:65-70.
32. Aickin M, Gensler H. Adjusting for multiple testing when reporting research results: the Bonferroni vs Holm methods. Am J Pub Health 1996;86:726-8.
33. Gahlinger PM, Abramson JH. Computer programs for epidemiologic analysis: PEPI version 2. Stone Mountain, Georgia: USD Inc.; 1995.
34. de Vries N, van Elderen C, Tijssen H, van Riel PLCM, van de Putte LBA. No support for HLA-DQ encoded susceptibility in rheumatoid arthritis. Arthritis Rheum 1999;42:1621-7.
35. Dessen A, Lawrence CM, Cupo S, Zaller DM, Wiley DC. X-ray crystal structure of HLA-DR4 (DRA*0101, DRB1*0401) complexed with peptide from human collagen II. Immunity 1997;7:473-81.
36. Seidl C, Koch U, Brunnler G, et al. HLA-DR/DQ/DP interactions in rheumatoid arthritis. Eur J Immunogenet 1997;24:365-76.
37. Jurcevic S, Praud C, Coppin HL, et al. Role of polymorphic residues of human leucocyte antigen-DR molecules on the binding of human immunodeficiency virus peptides. Immunol 1996; 87:414-20.
38. L'Faqihi FE, Praud C, Yassine-Diab B, et al. Residue 67 in the Drß*0101 and Drß*0103 chains strongly influences antigen presentation and DR-peptide molecular complex conformation. Tissue Antigens 1998;51:10-9.
39. Doherty DG, Penzotti JE, Koelle DM, et al. Structural basis of specificity and degeneracy of T cell recognition: pluriallelic restriction of T cell responses to a peptide antigen involves both specific and promiscuous interactions between the T cell receptor, peptide, and HLA-DR. J Immunol 1998;161:3527-35.
40. Kirschmann DA, Duffin KL, Smith CE, et al. Naturally processed peptides from rheumatoid arthritis associated and non-associated HLA-DR alleles. J Immunol 1995;155:5655-62.