Patients Chosen for Treatment with Cyclosporine Because of Severe Rheumatoid Arthritis Are More Likely to Carry HLA-DRB1 Shared Epitope Alleles, and Have Earlier Disease Onset

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ABSTRACT. ***Objective.*** To determine whether patients with rheumatoid arthritis (RA) selected for treatment with cyclosporin A (CSA) because of severe disease are more likely to carry HLA-DRB1 alleles encoding the conserved "shared epitope" (SE) sequence.

Methods. The majority of patients (n = 178) were currently being treated with methotrexate (MTX), either alone or in combination with chloroquine and/or CSA. In about 30% of patients, treatment with CSA had been initiated because of limited response to MTX or MTX and chloroquine. Patients were treated as clinically indicated without knowledge of their HLA-DRB1 status. HLA-DRB1 typing was by a reverse dot blot method.

Results. Patients that had been treated with CSA were significantly more likely to carry an SE allele than patients not treated with CSA (81.5% vs 60.5%; OR 2.9, p = 0.006). Patients with 2 SE alleles were the most likely to have been treated with CSA. Results were still significant after correction for age, sex, and disease duration in a logistic regression model. There was no association between rheumatoid factor positivity and requirement for CSA therapy. Examination of individual SE alleles by multiple logistic regression analysis indicated that the strongest association was with presence of HLA-DRB1*0401 (p = 0.004). The DRB1*0401/*0404 genotype provided the greatest risk of requiring CSA treatment. Patients selected for CSA treatment had developed RA at a significantly earlier age than those not requiring CSA (44.4 vs 51.3 yrs; p = 0.004).

Conclusion. Patients requiring treatment with CSA because of severe RA were significantly more likely to carry an SE allele than patients not requiring such treatment. CSA treated patients were also more likely to have had earlier age of disease onset. These data provide further evidence that bearing the SE (particularly 2 alleles) is associated with development of severe RA. (J Rheumatol 2002;29:271–5)

Key Indexing Terms:
RHEUMATOID ARTHRITIS CYCLOSPORINE SHARED EPITOPE DISEASE SEVERITY

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by the destruction of multiple diarthrodial joints, often leading to severe disability. The progress of the disease varies considerably between individuals, but the reasons for this are still unclear. Until recently, conventional treatment in RA has often followed a particular hierarchy, initially involving nonsteroidal antiinflammatory drugs (NSAID) followed by various disease modifying antirheumatic drugs (DMARD) such as antimalarials, sulfasalazine, D-penicillamine or gold, methotrexate (MTX), and cyclosporin A (CSA). Low dose steroid treatment has sometimes been used in combination with DMARD, while cytotoxic drugs such as azathioprine and cyclophosphamide have been used in more severe patients no longer responding to other DMARD. More recently, severe disease has also been considered to be suitable for treatment with new biological agents active against tumor necrosis factor-α (TNF-α)[1,2].

Combination therapies in which 2 or more drugs are used simultaneously have been found to be effective in some patients with severe disease manifestations. One of these combinations using CSA with MTX has been found to be particularly useful in the treatment of refractory RA[3-5]. Cyclosporine is an immune suppressive agent that mainly affects the activation of T lymphocytes. The exact mechanism of action of CSA

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in RA is unclear, although a recent study has suggested that the therapeutic effect is achieved by correcting an imbalance in cytokines produced by T helper 1 (Th1) and Th2 cells[6].

In many studies development of severe disease has been associated with carriage of the HLA-DRB1 shared epitope (SE), a conserved sequence of amino acids found at positions 70–74 of the DRß1 molecule[7-13]. However, the association of the SE with severe disease is still controversial since not all studies show this[14-18]. Most investigations into the association between severe RA and the SE have examined the relationship between radiographic and/or functional outcome measures and SE status. However, other indices of severity have sometimes been used, including the presence of rheumatoid nodules and other extraarticular manifestations, or the number of DMARD used. We investigated whether patients chosen for treatment with CSA because of severe clinical manifestations were more likely to carry SE alleles.

MATERIALS AND METHODS

Patients. Patients with RA (n = 178) satisfying the 1987 American College of Rheumatology classification criteria[19] were recruited from the Xeral-Calde Hospital in Lugo, northwestern Spain, and were either attending hospital outpatient clinics or were inpatients. The cohort was made up of an unselected series of patients with RA seen over a period of about one year at the only rheumatology unit available for a population of almost 250,000 people. Over 90% of patients had been treated with one or more DMARD, including chloroquine, sulfasalazine, gold, MTX, and CSA. Patient demographic and clinical details are shown in Table 1. The majority of patients were currently being treated with MTX, either alone or in combination with chloroquine and/or CSA. Triple therapy was used only rarely with other drugs, and such regimes always included CSA. In roughly 30% of patients, treatment with CSA had been initiated because of the severity of their disease. In this regard, since July 1994, patients with a partial response to therapy with MTX alone or in combination with chloroquine have been switched to combined therapy with MTX with/without chloroquine plus CSA.

All patients were treated by the same group of rheumatologists (principally MAGG and CGP) following the same criteria for treatment with CSA plus MTX. As reported[20], combined therapy was prescribed in those patients showing a partial response to MTX in spite of receiving a maximal dosage (15 mg/week) for at least 3 mo. This was defined by persistent active synovitis as indicated by 6 or more swollen joints, 9 or more tender joints, and morning stiffness of more than 1 h. The therapeutic strategy was not influenced by the presence of erosive disease or rheumatoid factor (RF) status. Subsequent analysis of the Larsen score in CSA treated and untreated patients revealed that CSA treated patients had significantly more radiographic damage than untreated patients (Table 1). Further, the frequency of nodular disease in CSA treated patients was significantly higher than in untreated patients (Table 1).

Table 1. Demographic and clinical characteristics of patients with RA treated with or without cyclosporine (CSA). Comparisons corrected for age, sex, and disease duration using logistic or multiple regression analysis (nodules and Larsen score, respectively).

	Without CSA	With CSA
Number	124	54
Male:female	39:85	12:42
Mean age of onset, yrs (SD)	51.3 (14.8)	44.4 (11.9)
Mean disease duration, yrs (SD)	11.2 (10.1)	12.1 (6.4)
Nodular disease, %	8.9	24.1†
RF positive, %	75.8	77.8
Erosions*, %	70.2	85.2
Mean Larsen score (hands + feet)	1.74 (0.9)	2.51 (1.0)††

* Cortical bone erosions in hands and/or feet.
† p = 0.01, †† p < 0.0001.

The only selection procedure was to exclude those patients in whom CSA could not be prescribed because of complications of comedication. Thus patients with renal insufficiency or with hepatic function tests at least 2–3 times above the normal upper range were not included in the CSA or control group. Only 4 patients were excluded for these reasons. No patient had other contraindications for CSA therapy such as current or past malignancy or uncontrolled hypertension[21]. All patients were treated as clinically indicated without physician's knowledge of their HLA-DRB1 status. In the cyclosporine treated group, 52/54 patients were taking prednisone or an equivalent corticosteroid (a few received deflazacort). Prednisone or equivalent was taken by 106/124 patients from the control group. In the CSA group the prednisone dose used was the highest dose required to treat severe and active polyarthritis. The median dose in this group was 10 mg/day, although up to 20 mg/day was used in 4 cases. The dose was tapered whenever possible (i.e., when there was a clinical improvement). In the control group the majority of patients (n = 100) were taking 5–10 mg/day (median 7.5 mg/day).

Radiographic assessment. Twenty-six joints of hands and feet [wrists, 2, 3, 4 and 5 metacarpophalangeal (MCP) joints, 2, 3, 4 and 5 proximal interphalangeal (PIP) joints, and 2, 3, 4 and 5 metatarsophalangeal (MTP) joints] were evaluated in an unblinded fashion by 2 observers (MAGG, CGP), who were aware of the diagnosis of RA. Both investigators came to agreement on those plain radiographs on which their assessment differed. According to the Larsen method[22] joint damage was graded as follows: grade 0 = normal, grade 1 = slight abnormality, grade 2 = definite abnormality, grade 3 = marked abnormality, grade 4 = severe abnormality, and grade 5 = mutilating abnormality. The mean Larsen score (sum of the Larsen scores of all joints divided by the number of joints evaluated) was assessed for the hands and feet separately and for the hands and feet together.

HLA typing. DNA was extracted from EDTA anticoagulated blood using a phenol-chloroform extraction procedure. HLA-DRB1 typing was carried out using a semiautomated commercial reverse dot blot method, INNO-LiPA (Abbott Laboratories, Maidenhead, UK), following the manufacturer's instructions. Reaction patterns were interpreted using INNO-LiPA software. HLA-DR4 subtypes were identified using either single strand conformational polymorphism (SSCP) following amplification with DR4-specific primers or the INNO-LiPA technology[23].

Statistical analysis. The strength of association between severity of the disease (considered as requirement for cyclosporine) and SE alleles in patients with RA was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined using contingency tables by either chi-square or Fisher exact analysis. To examine whether the treatment with CSA therapy was associated with particular HLA-DRB1 SE alleles we also carried out multivariate logistic regression analyses with combinations of HLA-DRB1 SE alleles as independent (explanatory) variables. All analyses were corrected for age of disease onset, sex, and disease duration [age of disease onset was defined as the age when onset of symptoms was recalled by the patient (inflammatory pain, swelling, stiffness) before the diagnosis of RA was made]. 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[24].

RESULTS

Frequency of SE alleles in CSA treated and untreated patients. Patients that had been treated with CSA because of disease severity were significantly more likely to carry an SE allele than patients not treated with CSA (Table 2). Thus, 44 of 54 (81.5%) patients with RA who required treatment with CSA due to severity of the disease carried an SE allele compared

Table 2. RA patients selected for treatment with cyclosporine (CSA) because of severe disease were more likely to carry an SE allele.

SE Status	Without CSA, n (%)	With CSA, n (%)	OR (95% CI)	p
–	49 (39.5)	10 (18.5)	0.35 (0.2–0.8)	0.006
+	75 (60.5)	44 (81.5)	2.9 (1.3–6.7)	0.006
+/–	58 (46.8)	29 (53.7)	1.3 (0.7–2.6)	0.4
+/+	17 (13.7)	15 (27.8)	2.4 (1.03–5.7)	0.025
*0401/*0404	0 (0)	4 (7.4)	22.2 (1.6–∞)	0.016*

*Fisher's exact test (2 tailed) used to calculate odds ratio and p value.

with 75 of 124 (60.5%) who did not require such therapy (p = 0.006, OR 2.9, 95% CI 1.3–6.7). The results were still significant after correction for age, sex, and disease duration in a logistic regression model (p = 0.009). Patients with 2 SE alleles were found to be most likely to require CSA treatment. Of the 54 patients who received CSA due to disease severity, 15 (27.8%) carried 2 SE alleles compared with 17 of 124 (13.7%) who did not require this therapy (p = 0.025, OR 2.4, 95% CI 1.03–5.7).

Since compound heterozygosity for the SE has been associated with more severe disease[25-27], we also examined the association between CSA treatment and the frequency of various genotypic combinations. Patients carrying one of these combinations (DRB1*0401/*0404) were only found in the CSA treated group, and this was highly significant when compared to the untreated group (Table 2). No difference between CSA treated and untreated patients was found for any other genotype (data not shown).

There was no association between RF positivity (determined by nephelometry) and requirement for CSA therapy. Examination of individual SE alleles by multiple logistic regression analysis indicated that the strongest association was with carriage of HLA-DRB1*0401 (OR 3.1, p = 0.004) (Table 3). There were no significant associations with HLA-DRB1*01, *0404, *0405, or *0408 (p > 0.5).

Influence of age of onset on requirement for cyclosporine treatment. Multiple regression analysis indicated that those patients requiring treatment with CSA treatment had developed RA at a significantly earlier age than those not requiring CSA (44.4 vs 51.3 yrs; p = 0.004). This was not due to differences in disease duration of the CSA treated patients, since the regression analysis was corrected for current age and disease duration. Stratification of these patients into SE positive and negative individuals revealed no effect of the SE on the age of onset (Table 4). However, it is noteworthy that SE positive patients chosen for CSA treatment had a significantly earlier age of onset than SE positive patients considered not to need CSA therapy (p = 0.01).

Table 3. Multiple logistic regression model to show the association between DRB1 SE alleles and selection for cyclosporine treatment. Analysis corrected for age, sex, and disease duration. Selection for CSA treatment was the dependent variable.

	Coefficient	Standard Error	OR (95% CI)	p
Constant	–0.171	1.085		
DRB1*01	0.641	0.402	1.9 (0.9–4.2)	0.11
DRB1*0401	1.136	0.392	3.1 (1.4–6.7)	0.004
DRB1*0405	1.183	0.632	3.2 (0.9–11.2)	0.06
DRB1*0404 or *0408	0.287	0.544	1.3 (0.5–3.9)	0.6

DISCUSSION

There is still uncertainty whether the influence of HLA-DRB1 molecules on RA is due to effects on disease susceptibility or on disease severity and progression. The association with disease severity appears to vary between different ethnic populations. Thus, a number of studies have revealed an association between severe disease outcome and presence of the SE, particularly in populations from northern Europe and North America[8-10,12,13]. Recently, del Rincón and Escalante described a more rapid development of joint deformities in RA in Mexican Americans carrying the SE motif[11]. In contrast, in southern Europe, Valenzuela-Castaño, *et al* observed that the presence of SE in RA patients was not a good marker of longterm radiological severity[18]. Similarly, the RA SE was not a marker for RA severity in a Greek population[15]. However, in our unselected population of patients with RA from northwestern Spain who were uniformly treated, those patients requiring treatment with CSA because of severe disease were significantly more likely to carry an SE allele than patients not requiring such treatment.

In another study on this group of patients from Lugo we demonstrated that erosive disease was associated with presence of the SE, particularly HLA-DRB1*0101 and DRB1*04, while RF positivity was associated mainly with DRB1*0401[22]. In the present study the choice of CSA treatment was primarily associated with patients carrying DRB1*0401, although interestingly there was no association with seropositivity. The data in this study thus provide further evidence for an association between the SE and more severe disease in this particular population. Our group also described that RA in this region of Spain is immunogenetically different from that observed in southern Spain and Greece[23]. This immunogenetic difference may account for differences in the severity of the disease. In this regard, in a population from Eastern France, where immunogenetic studies also showed a

Table 4. Mean age of onset of RA in SE positive or negative patients treated with or without CSA.

SE Status	Without CSA, n	Age of Onset (SD)	With CSA, n	Age of Onset (SD)
–	47	50.3 (14.5)	10	42.0 (12.9)
+	77	51.9 (15.0)	44	45.0 (11.7)*

* p = 0.01 (compared with SE+ patients without CSA treatment).

different HLA-DRB1 susceptibility for RA from that observed in the south of France, the SE positive QKRAA motif (DRB1*0401) was also associated with more severe disease[12].

Several studies have suggested that more severe disease is associated with certain genotypic combinations of HLA-DRB1 SE alleles. In particular, compound heterozygosity (e.g., DRB1*0401/*0404) for the SE has been found in patients developing severe disease[25-27]. When we examined the frequency of various genotypes in patients treated with or without CSA we found a significant association of DRB1*0401/*0404 with the CSA treated group. Carriage of this genotype in these patients was also associated with a young age of onset (mean 36.2 yrs). Since none of the untreated group carried this genotype, it was difficult to provide an accurate assesment of risk, but Fisher's exact test suggested that patients carrying DRB1*0401/*0404 were over 20 times more likely to have been chosen for CSA treatment. This result needs to be treated with caution because of the low frequency of patients with DRB1*0401/*0404 in this population. Nonetheless, our data provide additional evidence that this particular genotype is associated with a substantially increased risk of developing severe disease.

Our data indicate that patients who were eventually considered to require CSA treatment had significantly earlier onset of disease. There was no apparent difference between SE positive and SE negative patients in this respect. However, it is interesting that SE positive patients chosen for CSA therapy had a much earlier disease onset than SE positive patients whose disease was not considered severe enough for CSA treatment. This suggests that a more severe disease outcome in SE positive patients is associated with an earlier age of onset.

It is possible that other genes, such as TNF-α, may influence disease severity, and there is evidence that interaction of TNF region polymorphisms with HLA-DRB1 alleles may promote more severe disease in some patients[28,29]. Therefore, in addition to this study we also investigated whether the association between selection for CSA treatment and the SE was influenced by interaction with TNF microsatellite markers. However, no evidence of such an interaction was found, nor was there any association with individual TNF microsatellite markers (unpublished observations).

We are aware that a retrospective study such as this will have certain limitations, and realize that other rheumatologists may use different criteria for treatment with CSA. Nonetheless, we believe that our data provide further evidence that presence of the SE (particularly 2 alleles) in the RA population of NW Spain is associated with the development of severe disease that is likely to require aggressive drug therapy.

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