HLA-DRB1*04 Alleles in Japanese Rheumatoid Arthritis Patients with AA Amyloidosis

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ABSTRACT. ***Objective.*** To compare the HLA-DRB1 shared epitope (SE) alleles in Japanese patients with rheumatoid arthritis (RA) and amyloid A (AA) amyloidosis versus those without AA amyloidosis.

Methods. The HLA-DRB1 alleles were genotyped for 91 RA patients without AA amyloidosis, 33 RA patients with AA amyloidosis, and 63 control subjects. HLA-DRB1 typing was performed by polymerase chain reaction, sequence-specific oligonucleotide probe hybridization method.

Results. Although a significant difference was not observed, the frequency of SE genotype was higher in RA patients with AA amyloidosis than in those without AA amyloidosis. All SE-positive RA patients with AA amyloidosis had *04 alleles (*0401, *0405, *0410), and a significant association of the presence of a double dose of *04 SE alleles with AA amyloidosis (OR 4.0, 95% CI 1.91–13.99) was observed.

Conclusion. Our data suggest that presence of double *04 SE is associated with a higher risk of developing AA amyloidosis in Japanese patients with RA. (J Rheumatol 2006;33:2120–3)

Key Indexing Terms:
AA AMYLOIDOSIS HLA-DRB1 SHARED EPITOPE RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown cause. One of the most serious outcomes is the development of extraarticular manifestations[1]. Genetic factors play a role in the predisposition and the severity of RA[2]. The most extensively studied genetic marker in RA is HLA-DRB1[3]. Several HLA-DRB1 alleles share a common amino acid sequence, which is commonly called the shared epitope (SE), in the third hypervariable region of the molecule[4]. Studies in different populations have demonstrated the association between the presence of SE and extraarticular manifestations in patients with RA[5]. Amyloid A (AA) amyloidosis is one of the most serious extraarticular manifestations of RA[6]. It is caused by amyloid depositions formed from serum amyloid A (SAA), an acute phase protein produced in response to inflammation[7]. Long-standing inflammation is necessary for the development of AA amyloidosis; however, not all patients with prolonged and markedly elevated SAA levels develop amyloidosis, which suggests a genetic predisposition for amyloidogenesis[8]. We investigated the association between DRB1 alleles and AA amyloidosis in Japanese patients with RA.

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MATERIALS AND METHODS

Patients. Table 1 presents the characteristics of RA patients in our study. One hundred twenty-four Japanese patients who met the American College of Rheumatology (ACR) 1987 classification criteria for RA[9] were recruited consecutively from the outpatient clinic of Kumamoto Rheumatic Center and Nagasaki Medical Center. Sixty-three healthy volunteers (33 women and 30 men) served as the control group. Written informed consent was obtained from each study participant. AA amyloidosis was diagnosed in 33 of the 124 patients with RA. The diagnosis of AA amyloidosis was confirmed by histology based on the presence of Congo red staining, greenish birefringence on polarizing microscopy, and anti-human amyloid A reactivity of tissue biopsy specimens. RA patients with AA amyloidosis exhibited clinical symptoms and signs associated with amyloidosis, including refractory diarrhea (20/33), renal manifestations, such as proteinuria and renal impairment (14/33), and cardiomegaly (6/33). All patients were diagnosed within the past 5 years.

The remaining 91 RA patients showed no symptoms supposed to be AA amyloidosis, such as proteinuria, gastrointestinal symptoms, and cardiac involvement. There was no significant difference in age, gender, or the presence of rheumatoid factor between the 2 RA patient groups. The study protocol was approved by the Ethics Committees of all 3 institutes.

HLA-DRB1 typing. Genomic DNA was extracted from whole blood. Typing for HLA-DRB1 was performed by the polymerase chain reaction and sequence-specific oligonucleotide probe hybridization method using Genoscience HLA-DRB1 kits (G&G Science Corp., Fukushima, Japan).

Statistical methods. Disease association with HLA-DRB1 allele was assessed by a single-allele cis-square test for a 2×2 contingency table (the conventional odds ratio method). The probability values obtained were corrected (Pc) for multiple testing (Bonferroni correction). Statistical analysis was performed with StatView software (SAS Institute, Cary, NC, USA).

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Table 1. Characteristics of patients with RA. Values are number (%) or mean ± SEM.

	No.	Age, yrs	Female/Male	Duration of RA, yrs	RF (%)	CRP, mg/dl
Patients with AA amyloidosis	33	63.0 ± 9.7	25/8	14.1 ± 10.2	29/33 (87.9)	3.6 ± 2.4
Patients without AA amyloidosis	91	61.9 ± 14.1	76/15	12.5 ± 10.9	73/91 (80.2)	2.0 ± 1.9

RF: rheumatoid factor; CRP: C-reactive protein.

RESULTS

The allele distributions of HLA-DRB1 in our 124 RA patients and 63 healthy subjects are summarized in Table 2. As demonstrated previously, the frequency of SE was significantly higher in RA patients, both with and without AA amyloidosis, than in healthy controls. The frequencies of HLA-DRB1 alleles were compared between RA patients with and without AA amyloidosis. There was a trend of higher frequencies of *0405 or *0410 and lower frequencies of *0101 in RA patients with AA amyloidosis, but results did not reach statistical significance (Table 2). Similarly, the frequency of SE was higher in RA patients with AA amyloidosis (SE+ 57.6%) than in those without amyloidosis (SE+ 44.5%); however, no significant differences were observed. Because our results suggest that the DRB1*04 SE allele might explain the association of SE and AA amyloidosis, we further evaluated the association between the presence of SE+ *04 alleles and AA amyloidosis in patients with RA. As shown in Table 3, all SE-positive RA patients with AA amyloidosis had *04 SE alleles (*0401, *0405, *0410). Further, when the populations were compared, a statistically significant association between the presence of 2 DRB1*04 SE alleles and AA amyloidosis was observed in patients (OR 4.0, 95% CI 1.94–13.99; Table 3).

Table 2. Distribution of HLA-DRB1 in 124 patients and 63 control subjects.

DRB1	Controls Alleles, No. (%) (n = 126 alleles)	RA Alleles, No. (%) (n = 182 alleles)	RA + Amyloidosis Alleles, No. (%) (n = 66 alleles)	p	p_{corr}
SE+					
*0101	7 (5.6)	14 (7.7)	0 (0)	0.024	0.576
*0401	2 (1.6)	7 (3.8)	6 (9.1)	NS	
*0404	0 (0)	1 (0.5)	0 (0)	NS	
*0405	18 (14.3)	53 (29.1)	28 (42.4)	0.048	NS
*0410	1 (0.8)	2 (1.1)	4 (6.1)	0.045	NS
*1001	1 (0.8)	1 (0.5)	0 (0)	NS	
*1406	4 (3.2)	3 (1.6)	1 (1.5)	NS	
SE–					
*0403	1 (0.8)	2 (1.1)	0 (0)	NS	
*0406	7 (5.6)	7 (3.8)	2 (3.0)	NS	
*0802	6 (4.8)	1 (0.5)	1 (1.5)	NS	
*0803	13 (10.3)	8 (4.4)	2 (3.0)	NS	
*0809	1 (0.8)	0 (0)	0 (0)	NS	
*0901	16 (12.7)	28 (15.4)	12 (18.2)	NS	
*1101	3 (2.4)	4 (2.2)	2 (3.0)	NS	
*1201	0 (0)	7 (3.8)	3 (4.5)	NS	
*1202	2 (1.6)	4 (2.2)	0 (0)	NS	
*1301	2 (1.6)	0 (0)	0 (0)	—	
*1302	9 (7.1)	7 (3.8)	0 (0)	NS	
*1401	6 (4.8)	2 (1.1)	0 (0)	NS	
*1403	3 (2.4)	1 (0.5)	0 (0)	NS	
*1405	4 (3.2)	0 (0)	0 (0)	—	
*1501	12 (9.5)	7 (3.8)	0 (0)	NS	
*1502	8 (6.3)	21 (11.5)	0 (0)	0.004	0.096
*1602	0 (0)	2 (1.1)	5 (7.6)	0.016	0.384
SE+ total	33 (26.2)	81 (44.5)	38 (57.6)	NS	
SE– total	93 (73.8)	101 (55.5)	28 (42.4)	NS	

SE: shared epitope bearing the amino acid sequences QK/QR/RRRAA at position 70–74 of the third variable region. NS: not significant.

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Table 3. Frequency of shared epitope alleles among RA patients with or without amyloidosis and healthy controls.

Genotype	Healthy Controls, n = 63	RA, n = 91	RA + Amyloidosis n = 33	p	OR (95% CI)
2 SE alleles					
*04/*04	1 (1.6)	9 (9.9)	12 (36.4)	0.0052	4.0 (1.94–13.99)
*04/SE	1 (1.6)	8 (8.8)	1 (3.0)	0.447	
SE/SE	1 (1.6)	0 (0)	0 (0)		
1 SE alleles					
*04/x	18 (28.6)	36 (39.6)	13 (39.4)	0.991	
SE/x	9 (14.3)	10 (11.0)	0 (0)	0.119	

*04: DRB1-04 shared epitope (*0401, *0404, *0405, *0410). SE: non-04 shared epitope (*0101, *1001, *1406). x: non-SE allele. Odds ratio (OR), 95% confidence interval (95% CI), and p values were calculated by RA group and RA plus amyloidosis group.

DISCUSSION

The development of RA could be due to the combination of genetic susceptibility to disease and exposure to an environmental trigger[10]. RA is now thought to be associated with a conserved sequence of amino acid in HLA-DRB1 alleles, known as the RA shared epitope[4]. However, the SE appears to be associated with RA chronicity and severity more than with susceptibility[11,12]. AA amyloidosis is a serious complication thought to be related to the chronicity and severity of rheumatoid inflammation[13].

Our results suggest the particular importance of DRB1*04 SE genotypes in RA patients with AA amyloidosis. Our data indicate that RA patients with double *04 SE alleles have an increased risk for AA amyloidosis (Table 3). The association between HLA-DR4 and RA is well known, and a higher prevalence of HLA-DR4 was described in RA patients with AA amyloidosis[14]. Our data, which indicate that HLA-DRB1*04 SE may contribute to the association of AA amyloidosis with RA, are consistent with those of the previous study.

The AA protein that forms AA amyloid fibril is derived from the circulating acute phase reactant, SAA, which is synthesized by hepatocytes under transcriptional regulation by proinflammatory cytokines[15]. Sustained high circulating levels of SAA are prerequisites for the development of AA amyloidosis. In Japan, AA amyloidosis is seen more often than in North America, and genetic predisposition is thought to be involved in the occurrence of amyloidosis[8]. Our study showed that Japanese RA patients with AA amyloidosis are more likely to have double *04 SE alleles and less likely to have non-*04 SE alleles. Our data suggest that a certain SE genotype could be a genetic risk factor for AA amyloidosis in RA patients.

The heterogeneity of DRBI*04 in RA is well known, and there are a growing number of studies on the distribution of this allele in different ethnic groups. DRBI*0401 (and *0404) account for the DRBI association with RA in Caucasians[16], whereas DRBI*0405 was found mainly in Asian patients with RA[17,18]. Our data demonstrating the high frequency of *0405 in Japanese RA patients are consistent with these previous findings.

DRB1*0401 (*0401-homozygous or *0401/*0404 compound homozygous) was found in patients with severe RA or RA with extraarticular features[19]. Weyand, *et al* also observed DRB1*04 alleles in patients with severe, erosive RA with extraarticular features, such as vasculitis and Felty's syndrome, while DRB1*01 were observed at a higher frequency in patients with less severe RA[20]. The presence of 2 SE-positive DRBI alleles in patients with RA has been shown to be associated with disease severity[21]. In another study, a double dose of DRBI*04 SE alleles was associated with progressive joint damage in northern European Caucasians[22]. Therefore, it is likely that an association between double dose of *04 SE and AA amyloidosis could be attributed to the RA severity in these patients.

Although the association of *04 SE with AA amyloidosis in RA patients was indicated in this study, the mechanisms by which *04 SE confers susceptibility to AA amyloidosis remain to be determined. Tumor necrosis factor-α (TNF-α) is considered to be one of the most important cytokines in the inflammatory process in RA[23]. The human TNF-α gene is located within the class III region of the MHC[24]. This chromosomal location raised the possibility that the association of RA and DRBI may be partly attributed to polymorphism within the TNF gene[25]. Recently it was reported that a particular TNF-α polymorphism is associated with RA severity through an interaction with HLA-DRB1[26]. Maury, *et al* reported that circulating TNF-α is significantly increased in RA patients with amyloidosis[27]. It is possible that the observed association between AA amyloidosis and *04 SE may be due to the linkage disequilibrium between TNF-α and HLA-DRB1 loci. Further large-scale studies are needed to define the relationship between DRBI and TNF-α gene in RA patients with AA amyloidosis.

HLA genes are likely involved in shaping the T cell repertoire. SE-positive HLA-DR alleles may shape the T cell reper-

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toire by presenting self-peptide to CD4-positive T cells[28]. The T cell repertoire in patients with RA is markedly contracted[29], and HLA-DRB1*04 alleles are thought to be associated with extraarticular manifestations in RA by affecting T cell repertoire formation[30]. Although the precise mechanism for *04 SE association with AA amyloidosis is still unknown, it is possible that *04 SE may influence the expression of AA amyloidosis by affecting T cell selection or activation.

In conclusion, ours is the first study evaluating the role of HLA-DRB1 genes in the association of AA amyloidosis with RA. HLA-DRB1*04 SE alleles tend to be associated with AA amyloidosis and could predict risk for AA amyloidosis in Japanese patients with RA.

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