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ORIGINAL ARTICLE

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Antibodies to type II collagen and their association with HLA DR1 alleles in Japanese patients with rheumatoid arthritis

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Abstract To investigate whether immunological responses to type II collagen (CII) play an important role in the pathogenesis of rheumatoid arthritis (RA), the presence of anti-CII antibodies was examined by enzyme immunoassay in 130 Japanese patients with RA, 10 systemic lupus erythematosus (SLE) patients, and 30 healthy subjects. In addition, the HLA-DRB1 genes of 40 RA patients were determined, and their association with positive findings of anti-CII antibodies was examined. A significantly high frequency of positive findings of anti-CII antibodies was detected in sera from RA patients (19%, $P < 0.05$) in comparison with that in sera from healthy subjects (3%). High frequencies of DRB1*0405 and 0101 alleles were observed in the 40 RA patients examined (40.0% and 30.0%, respectively). Patients with DRB1*0101 had a significantly higher rate of positive findings of anti-CII antibodies than those without DRB1*0101 (66.7% and 28.6%, respectively, $P < 0.05$). No such association was observed for DRB1*0405. From these findings, we suggest that immunological responses to CII may play an important role in the development of arthritis in some RA patients.

Key words Antibody · HLA · Rheumatoid arthritis (RA) · Type II collagen (CII)

Introduction

Rheumatoid arthritis (RA) is generally considered to be an autoimmune disease. However, the target antigens (autoantigens) responsible for the pathogenicity remain unclear. Type II collagen (CII), a molecule which is abundant in articular cartilage, is one of the major candidates, because peripheral T cells reactive to CII and antibodies to CII can be found in the blood or synovial fluid of RA patients,[1–4] and oral administration of CII to RA patients can induce tolerance and reduce the number of swollen joints.[5] However, because anti-CII antibodies have been found in only a small proportion of RA patients,[6,7] the importance of immunological responses to CII in the pathogenesis of RA has yet to be clearly established.

The presence of RA and of certain HLA class II alleles are associated, which supports a role of autoimmunity in the onset of RA. If immunological responses to CII play a significant role in the pathogenesis of RA, there may be some association between HLA alleles and the presence of anti-CII antibodies. Indeed, some studies have demonstrated such associations, although other studies did not show any association.[8–12]

To investigate whether CII is one of the important immunological targets in patients with RA, we examined the positivity rate of anti-CII antibodies, and the association between the presence of serum anti-CII antibodies and HLA alleles in Japanese RA patients.

Patients and methods

Patients

Sera from 130 Japanese patients with RA, who all fulfilled the 1987 classification criteria of the American College of Rheumatology,[13] were investigated. Sera from 30 healthy subjects were used as controls, and sera from 10 patients with systemic lupus erythematosus (SLE) were used as disease controls. Written informed consent was obtained from each subject before the collection of a blood sample, and the study design was approved by the Ethical Committee of the University of Tsukuba.

HLA typing

HLA–DRB1 types were assigned for 40 RA patients, randomly selected from 130 patients, using the Dynal RELI

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SSO HLA-DRB Test (Dynal UK, Wirral, UK). DR4 types, which could not classified by this kit, were subtyped for DRB1*0403, 0406, 0407, 0405, and 0410 by the polymerase chain reaction – sequence-specific primer (PCR–SSP) method.[14]

Measurement of anti-CII antibodies

Bovine CII was purchased from the Collagen Research Center (Tokyo, Japan). IgG antibodies to bovine CII were measured by ELISA.[2,4] Serum obtained from a collagen-induced arthritis rat was used as a standard. The amount of anti-CII antibody in this serum, diluted 1:12800, was defined as 1 antibody unit. A standard curve was generated for each assay, and the concentration of anti-CII in the samples was expressed in antibody units. A sample was considered positive for anti-CII when the antibody unit was greater than the mean +2 SD (0.091) of samples from healthy subjects.

To confirm the validity of this ELISA, an inhibition assay was performed.[2,4] Serum samples were diluted 1:50 with normal goat serum (GIBCO BRL) buffered with crystalline Tris (0.1 M) and NaCl (0.15 M), pH 8.0, containing CII (100 μg/ml), 1 h before the addition of serum to microtiter plates. The samples were then measured by ELISA, as described above, and the decrease in values of antibody units was calculated.

Statistical analyses

Fisher's exact test was used to analyze the relationship of the positivity of anti-CII antibodies between RA, SLE patients and healthy subjects, and in a given HLA–DRB1 type.

Results and discussion

Anti-CII antibodies were present in sera from 25 of the 130 RA patients (19%) included in this study, while only 1 out of 30 healthy subjects (3%) was positive for these antibodies (Fig. 1). All sera from the 10 SLE patients were negative (Fig. 1). The positivity of anti-CII in RA patients was significantly higher than that in healthy subjects ($P < 0.05$). Inhibition assays showed that the antibody units values of all positive sera from RA patients fell by 80% or more, confirming the validity of the ELISA (Fig. 2). Although we investigated whether there was any correlation between the positivity of anti-CII antibodies and the disease duration, the values of rheumatoid factor, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and family history of RA, there was no significant association between the presence of anti-CII antibodies and these clinical and laboratory features (data not shown).

DRB1*0405 (16/40, 40.0%)- and DRB1*0101-positive patients (12/40, 30.0%) were present at high frequencies in the RA patients studied (Table 1). A significant increase in the frequency of anti-CII antibody positivity was observed in patients with DRB1*0101 (66.7%, $P < 0.05$, Table 2). Other HLA–DRB1 alleles did not show any association with the positivity of anti-CII antibodies (Table 3).

Although many studies have reported the presence of anti-CII antibodies in sera from RA patients,[3,15–19] they have not been found in all RA patients.[3,20–22] In our study,

Table 1. Frequencies of HLA-DRB1 alleles and association with the presence of anti-CII antibodies for rheumatoid arthritis (RA) patients

DRB1 allele	No. (%) of RA patients[a] ($n = 40$)	No. (%) of anti-CII positive[b] ($n = 16$)	Positivity of anti-CII[c] (%)
0101	12 (30.0)	8 (50.0)	66.7[d]
0401	1 (2.5)	0	–
0403+0406+0407	6 (15.0)	2 (12.5)	33.3
0405	16 (40.0)	5 (31.3)	31.3
0410	3 (7.5)	3 (18.8)	100.0
0803	6 (15.0)	3 (18.8)	50.0
0901	8 (20.0)	2 (12.5)	25.0
1001	1 (2.5)	1 (6.3)	100.0
1101	1 (2.5)	1 (6.3)	100.0
1201	1 (2.5)	0	–
1202	1 (2.5)	1 (6.3)	100.0
1302	2 (5.0)	0	–
1401	2 (5.0)	1 (6.3)	–
1403	2 (5.0)	2 (12.5)	100.0
1501	2 (5.0)	0	–
1502	8 (20.0)	2 (12.5)	25.0
1602	1 (2.5)	1 (6.3)	100.0

[a] Percentage of positive DRB1 allele in all RA patients examined
[b] Percentage of anti-CII-positive patients with each DRB1 allele in all anti-CII positive RA patients examined
[c] Percentage of anti-CII-positive patients in all patients with each DRB1 allele
[d] Significance by Fisher's exact test ($P < 0.05$)

Table 2. Relationship between DRB1*0101 and the presence of anti-CII antibodies

	Anti-CII antibodies		Total
	Positive	Negative	
DRB1*0101			
Positive	8	4	12
Negative	8	20	28
Total	16	24	40

$P < 0.05$ by Fisher's exact test

Table 3. Relationship between DRB1*0405 and the presence of anti-CII antibodies

	Anti-CII antibodies		Total
	Positive	Negative	
DRB1*0405			
Positive	5	11	16
Negative	11	13	24
Total	16	24	40

No significance by Fisher's exact test

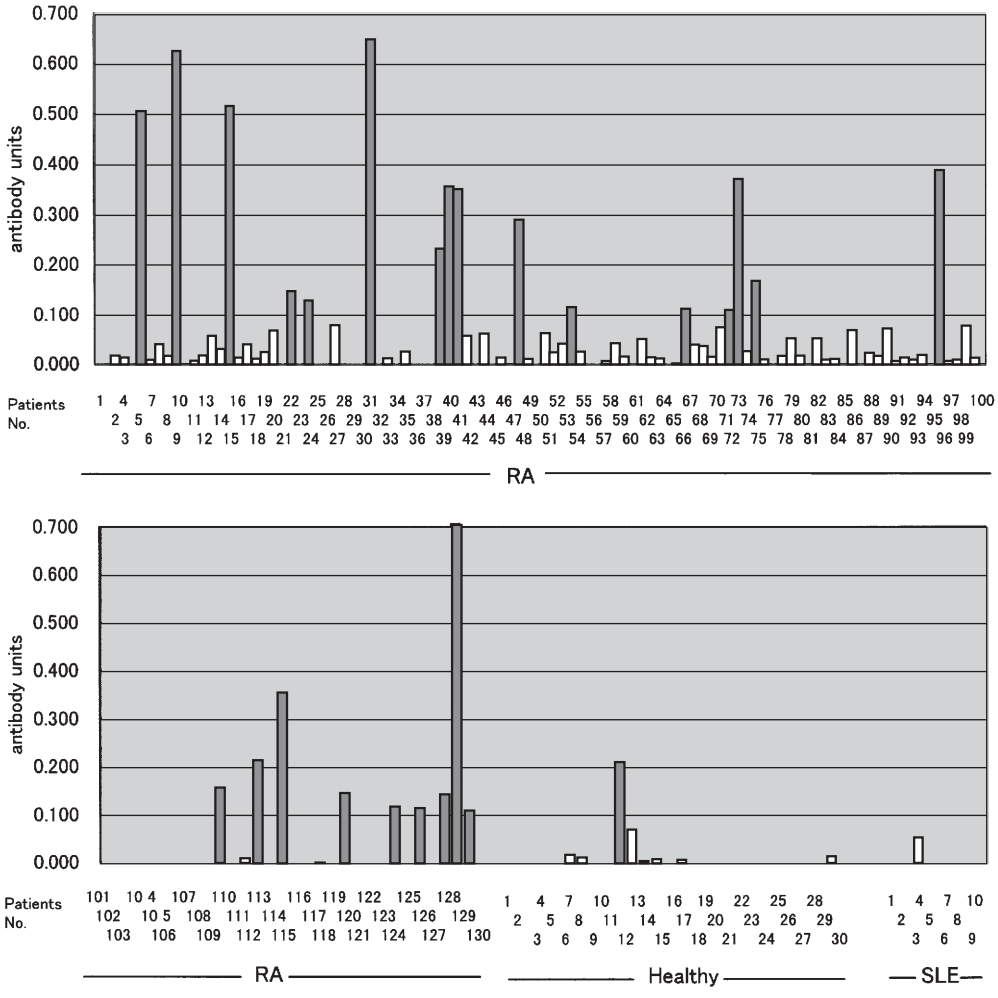

Fig. 1. Antibodies to CII in sera from rheumatoid arthritis (*RA*) patients, healthy subjects, and systemic lupus erythematosus (*SLE*) patients. Antibodies to CII in sera from 130 RA patients, 10 SLE patients, and 30 healthy subjects were measured by ELISA. The serum of a CIA rat was diluted 1:12800 and used as a standard for 1 antibody unit. *Shaded bars* indicate positive for anti-CII antibodies, which were greater than the mean +2SD (0.091) of the values for healthy subjects. *Open bars* indicate negative for anti-CII antibodies. Sera from 25 RA patients and from one healthy subject showed positive for anti-CII antibodies

the positivity of anti-CII antibody in RA patients was higher than that in healthy subjects. The inhibition assay confirmed that antibodies detected in the ELISA were indeed directed to CII. These results suggest that anti-CII antibodies were related to the pathogenesis of arthritis, at least in a subgroup of RA patients.

Many reports have shown an association between the presence of anti-CII antibodies and HLA allotypes.[8–12] Some investigators have suggested an association between HLA-DR1 and the presence of anti-CII antibodies.[12,23] A recent study by Cook et al.[23] showed that HLA-DR1 and DR7 were positively associated with the presence of anti-CII antibodies, while DR3 was negatively associated, although DR1 was positively associated only when DR7-positive patients were excluded. In our study, anti-CII antibodies were detected at high frequency in DRB1*0101 patients, and to our knowledge, this is the first report describing an association between the presence of anti-CII antibodies and HLA-DR haplotypes in Japanese RA patients.

Why were anti-CII antibodies detected at high positivity in DRB1*0101 RA patients? There are two possible explanations. Firstly, anti-CII antibody production may be mediated by other genes close to the DRB1*0101 locus. Secondly, DRB1*0101 may have the capacity to present the

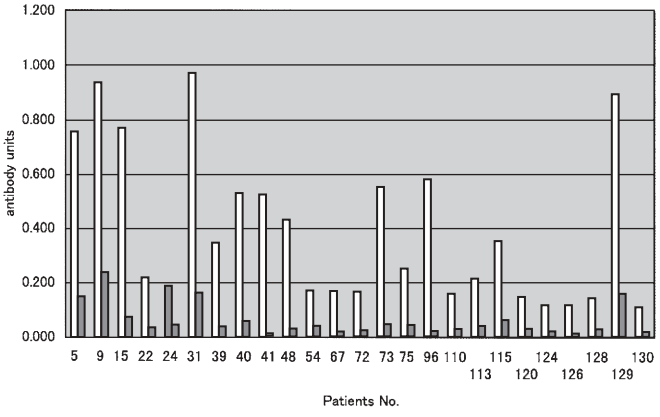

Fig. 2. Inhibition assay of anti-CII antibodies in sera from RA patients. Sera from 25 RA patients which indicated positive for anti-CII antibodies were treated with CII (100μg/ml) for 1h, and thereafter, titer of anti-CII antibodies were measured by ELISA. *Shaded bars* indicate antibody units after treatment with CII, and *open bars* indicate those without CII. The values of all sera decreased by 80% or more after treatment with CII

T cell epitope on CII and induce CII-specific T cell expansion, which in turn leads to the production of anti-CII antibodies. Although the answer is currently unknown, it is of interest that the dominant T cell epitope of CII in a DRB1*0101 transgenic mouse model was similar to that in DBA/1 (H-2q) mice, which is a collagen-induced arthritis (CIA)-susceptible strain.[24,25] Thus, CII-reactive T cells might help the production of anti-CII antibodies by B cells in DRB1*0101 RA patients as well as in CIA model mice. To elucidate this possibility, it is necessary to detect unique T cell epitopes on CII in the context of HLA-DRB1*0101, which would induce the production of anti-CII antibodies.

CIA resembles RA in its joint pathology, namely synovial proliferation, cell infiltration, cartilage erosion, and bone resorption.[6] Passive transfer of anti-CII antibodies can induce arthritis,[26] and complement depletion prevents the induction of arthritis in CIA mice.[27] These studies suggest the importance of anti-CII antibodies in the pathogenesis of arthritis in CIA mice. Our results, together with these previous studies, imply that anti-CII antibodies may also be important in inducing arthritis in DRB1*0101 RA patients.

In conclusion, our results have demonstrated that anti-CII antibodies are associated with RA patients with DRB1*0101, and that anti-CII antibodies may play an important role in the development of RA. Further examination of the mechanism of anti-CII antibody production and its consequences should shed light on the pathogenesis of RA.

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