HLA DR4 and rheumatoid arthritis in Japanese people

HIROO MAEDA,¹ TAKEO JUJI,¹ HIROSHI MITSUI,²
HIDEKICHI SONOZAKI² AND KATSUHIKO OKITSU²

From the ¹Blood Transfusion Service and the ²Department of Orthopedic Surgery, Tokyo University Hospital, Tokyo, Japan

SUMMARY Eighty-eight Japanese patients with rheumatoid arthritis and 104 normal Japanese persons were typed for HLA A, B, C, and DR antigens. The frequency of HLA DR4 was 70.5% in patients compared with 46.1% in normal controls ($P < 0.001$). However, a sex difference in the frequency of HLA DR4 in patients was noted. HLA DR4 was found in 80.6% of male patients, which was highly significant compared with controls ($P < 0.0005$), while only a borderline increase to 60.5% was found in female patients ($P < 0.05$). In addition, the frequency of HLA DR2 was remarkably low in male patients. These suggest the possible heterogeneity of rheumatoid arthritis in Japanese.

Since a strong association between ankylosing spondylitis and HLA B27 was reported,¹ HLA antigens in patients with other arthropathies have been extensively studied. Ankylosing spondylitis and its related diseases were confirmed to be associated with HLA-B27 in different ethnic groups.²⁻⁴ In rheumatoid arthritis most studies have failed to show an association with HLA A or B antigens.⁵⁻⁹ However, Stastny found an increased frequency of rheumatoid patients whose lymphocytes did not respond to one type of stimulator lymphocyte, now known as HLA Dw4, in mixed lymphocyte culture.¹⁰ Two other investigators confirmed this evidence in separate series of Caucasian patients.^{11, 12}

HLA DR antigens are the serologically detectable HLA D related antigens present on B lymphocytes and monocytes. By HLA DR typing an increase of HLA DR4 was also established in Caucasian patients with rheumatoid arthritis.¹³⁻¹⁶

In the study reported here Japanese patients with rheumatoid arthritis were typed for HLA DR antigens to see whether the same association was present as was the case in the Caucasian patients.

Materials and methods

Eighty-eight patients who were diagnosed as classical or definite rheumatoid arthritis according to the

criteria of the American Rheumatism Association¹⁷ were selected for this study. All were Japanese, aged from 25 to 73 years old (mean: 50.2), 36 male and 52 female. They have been followed up for 1 to 20 years at the Rheumatology Unit, Department of Orthopaedics, Tokyo University Hospital. All patients were tested for IgM rheumatoid factor at least every 3 months, and none had an abnormal titre of anti-DNA antibody and a positive LE test.

HLA TYPING

B lymphocytes were purified according to the thrombin-nylon wool method described by Danilovs *et al.*¹⁸ Mononuclear cells were separated from heparinised blood by Ficoll-Hypaque gradient centrifugation. After agglutination of platelets and monocytes with thrombin enriched lymphocytes were incubated in a plastic straw column containing nylon wool. Nonadherent cells were collected by dripping medium through the column, and adherent cells were recovered by squeezing the straw. Non-adherent cells were used for HLA A, B, and C typing and adherent cells for HLA DR typing.

The lymphocyte cytotoxicity test was performed according to a modified NIH standard method.¹⁹ The incubation was for 60 minutes at 37°C with antiserum and for 120 minutes at 22°C with complement. The rabbit complement was carefully selected not to have cytotoxicity against B lymphocytes.

ANTISERA

A panel of 100 antisera which covered 8 HLA A,

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Correspondence to Dr Hiroo Maeda, Blood Transfusion Service, Tokyo University Hospital, Hongo 7-3-1, Bunkyo-ku, Tokyo, Japan 113.

17 HLA B, and 6 HLA C specificities was used for HLA A, B, and C typing. All sera for HLA DR typing were absorbed with an equal volume of pooled platelets and confirmed not to react with T lymphocytes. For HLA DR1, DR2, DR3, DR4, DR5, DR7, and DRw8, at least 3 monospecific sera were used. HLA DRw6 and DRw9 were assigned by a positive reaction with anti-DRw3+6 and anti-DRw7+9 only in the absence of DR3 and DR7, respectively.

Anti-DR4 sera used were 7407.3 (Dr Mervart), CLB EB12 (Dr Engelfriet), 10552 (Dr Matsuyama), and T2297 (authors' laboratory). The former 2 sera were the key sera for DR4 in the 8th International Histocompatibility Workshop. Correlation coefficients among themselves and DR4 antigen are shown in Table 1. The antigen DR4 was assigned by a positive reaction with at least 3 out of the 4 sera.

Results

All the patients were typed for 31 HLA A, B, and C antigens (Table 2). A borderline increase of HLA Bw54 and HLA Cw1 was found in rheumatoid patients compared with controls. A difference in the frequencies of Bw54 and Cw1 was noted between the 2 sexes. No significant deviations were found in female patients, while Bw54 and Cw1 were found in 33.3% and 58.3% of male patients compared with 12.3% and 32.1% of controls, respectively. These increases in male patients were significant ($P < 0.01$).

Table 3 shows the frequencies of HLA DR antigens in rheumatoid patients and controls. The frequencies of DR4 were increased both in male and in female patients compared with controls. HLA DR4 was found in 80.6% of male patients and in 63.5% of female patients. These are significant increases compared with 46.1% for controls ($P < 0.0005$ for males, $P < 0.05$ for females). The relative risk of HLA DR4 for rheumatoid arthritis was 4.8 in males and 2.0 in females.

The frequency of HLA DR2 was decreased only in male patients. Only 4 out of 36 male patients were HLA DR2 positive compared with 37 out of 104 controls ($P < 0.02$).

Table 1 Correlation coefficients among anti-DR4 sera and with HLA DR4

Sera	7407.3	CLB EB12	10552	T2297
7407.3	—			
CLB EB12	0.90	—		
10552	0.90	0.94	—	
T2297	0.92	0.95	0.95	—
HLA DR4	0.92	0.95	0.95	1.00

$$r = \sqrt{\chi^2/n}.$$

Table 2 Percentage frequencies of HLA A, B, and C antigens in rheumatoid patients and controls

HLA antigens	Patients			Controls (n=106)
	Male (n=36)	Female (n=52)	Total (n=88)	
A1	5.6	1.9	3.4	1.9
A2	36.1	44.2	40.9	48.2
A3	2.8	0	1.1	0.9
Aw24	55.6	69.2	63.6	56.6
A26	19.4	26.9	23.9	21.7
A11	22.2	15.4	18.2	14.2
Aw31	16.7	9.6	12.5	17.0
Aw33	19.4	15.4	17.0	13.2
B7	2.8	17.3	11.3	11.3
B8	0	0	0	0
B13	5.6	1.9	3.4	3.8
B15	22.2	17.3	19.3	17.9
B17	0	3.8	2.2	0
Bw22	0	3.8	2.2	6.6
B27	0	1.9	1.1	0
Bw35	11.1	15.4	13.6	13.2
B37	0	1.9	1.1	1.9
Bw39	13.9	9.6	11.3	9.4
Bw44	19.4	7.7	12.5	12.3
Bw48	2.8	5.8	4.5	6.6
Bw51	8.3	11.5	10.2	16.0
Bw52	5.6	19.2	13.6	19.8
Bw54	33.3**	17.3	23.9*	12.3
Bw60	11.1	13.4	12.5	16.0
Bw61	13.9	15.4	14.8	20.8
Cw1	58.3**	36.5	45.5	32.1
Cw2	0	0	0	0
Cw3	47.2	48.1	47.7	44.3
Cw4	5.5	5.7	5.7	9.4
Cw5	0	0	0	0
Cw6	0	1.8	1.1	3.7

* $P < 0.05$, ** $P < 0.01$ (P not corrected).

Table 3 Percentage frequencies of HLA DR antigens in rheumatoid patients and controls

HLA DR antigens	Patients			Controls (n=104)
	Male (n=36)	Female (n=52)	Total (n=88)	
DR1	8.3	17.3	13.6	9.6
DR2	11.1**	36.5	26.1	35.5
DR3	0	0	0	0
DR4	80.6****	63.5*	70.5***	46.1
DR5	5.6	1.9	3.4	3.8
DRw6	2.8	5.8	4.5	12.5
DR7	2.8	0	1.1	1.9
DRw8	25.0	17.3	20.5	13.5
DRw9 (7J)	27.8	17.3	21.6	29.8

* $P < 0.05$, ** $P < 0.02$, *** $P < 0.001$, **** $P < 0.0005$.

Rheumatoid patients were subdivided into different groups according to the diagnosis, the year of onset, the presence of rheumatoid factor or rheumatoid nodules, the functional grade, and steroid treatment. The frequencies of HLA DR4 and DR2 are shown in Table 4. There were no significant differences between the frequencies of HLA DR4 in any subgroups compared with the combined data. A borderline negative association in patients was noted between HLA DR2 and steroid treatment ($P < 0.05$).

Table 4 Percentage frequencies of HLA DR4 and DR2 antigens in the subgroups of rheumatoid patients

	n	DR4	DR2
Sex			
Male	36	80.6	11.1
Female	52	63.5	36.5
Year of onset			
>35	70	72.8	25.7
<35	18	61.1	27.8
Diagnosis			
Classical	49	69.4	32.6
Definite	39	71.8	17.9
Rheumatoid factor			
Positive	70	72.8	25.7
Negative	18	61.1	27.8
Nodule			
With nodule	22	68.2	13.6
Without nodule	49	67.3	32.6
Functional grade			
I	6	83.3	33.3
II	35	68.6	22.8
III	36	72.2	30.5
IV	11	63.6	18.2
Therapy			
Steroid	18	72.2	5.5*
Nonsteroid	70	70.0	31.4
Total	88	70.5	26.5

*P<0.05.

Discussion

This report provides evidence that rheumatoid arthritis in Japanese people is strongly associated with HLA DR4.

So far the HLA Dw4 and DR4 association has been well established only in Caucasian patients with rheumatoid arthritis.¹⁰⁻¹⁶ Since most studies have failed to show a strong association between rheumatoid arthritis and HLA A, B, and C antigens,⁵⁻⁹ the HLA D or DR allele has been thought to be primarily concerned. This applies also to other autoimmune diseases such as coeliac disease, Graves's disease, and juvenile diabetes mellitus.³

The association between HLA antigens and these diseases has been generally thought to be due to the linkage disequilibrium between the HLA gene and the genes which predispose to the diseases. If so, it is important to establish whether the same association is present in other ethnic and racial groups having different distributions of HLA antigens.

In a joint report on rheumatoid arthritis during the 8th International Histocompatibility Workshop a strong association of rheumatoid arthritis with HLA DR4 was found in Japanese, Hungarians, Latin Americans, and Caucasians.¹⁶ However, there was some difficulty in assigning HLA DR4 in Orientals, because most of the anti-DR4 sera used in the workshop also contained activity for HLA DRw9, which cross-reacts with DR4 and DR7 and is common in Japanese.²⁰

In this experiment HLA DR4 was determined

accurately by 4 monospecific sera which correlated highly among themselves. A significant increase of HLA DR4 in rheumatoid patients was found. Interestingly, however, a sex difference in the frequency of HLA DR4 was present. The overall increased frequency of HLA DR4 was mainly attributable to male patients. The borderline significance of the association in female patients disappeared after the correction of P value by the number of antigens tested. Thus, the HLA DR4 antigen in male and female was given the relative risk of 4.8 and 2.0, respectively. A decreased frequency of HLA DR2 was also noted in male patients. These differences were not reported in Caucasian patients, indicating possible heterogeneity of rheumatoid arthritis among Japanese.

Increased frequencies of HLA Bw54 and HLA Cw1 are probably due to the effect of linkage disequilibrium with HLA DR4 in the Japanese population.

The same HLA DR antigen is thus associated with rheumatoid arthritis in both Caucasians and Japanese, and is also found in juvenile onset diabetes mellitus in Chinese.²¹ This means that the HLA DR gene and the gene which predisposes to the disease must be very close. The same combinations of antigens between HLA B and C loci, namely, HLA B15 Cw3, Bw35 Cw4, were reported in significant linkage disequilibrium in Caucasians, blacks, and Japanese.²² If the disease genes are as tightly linked to the HLA DR locus as the HLA B locus to HLA C, the same association should be found in 2 or 3 of the major racial groups.

Several investigators reported that HLA DR4 was more strongly associated with the severity of the disease^{11,14} or the presence of rheumatoid factor.^{15,16} However, there was no such correlation in our material.

In conclusion, the increase of HLA DR4 in male patients was significant after the correction of P value. This suggested possible heterogeneity between Japanese male and female patients with rheumatoid arthritis. Further study will be needed to show if the sex difference is true or occurred by chance alone.

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