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HLA Class I Antigens in Patients with Reproductive Autoimmune Failure Syndrome (RAFS)

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Summary. **The distribution of HLA Class I antigens was analyzed in 38 patients with reproductive autoimmune failure syndrome (RAFS), defined as patients who were positive for antiphospholipid antibodies and had experienced either recurrent fetal wastage and/or one or more experience of intrauterine fetal growth retardation with severe preeclampsia, and compared with the distribution in the general population in Niigata Prefecture, Japan. The distribution of HLA-A and -B antigens in patients with RAFS was not significantly different from that in the control group. The number of patients positive for HLA CW7 was significantly higher than that of the general population (132 normal healthy individuals) in this prefecture (Relative Risk; 3.47, 95% Confidence interval; 1.18-10.2). The compatibility of HLA antigens in spouses was also analyzed in 22 patient couples and 26 normal fertile couples, and there was no significant difference. Thus, it is suggested that HLA antigen systems might be involved in the genesis of reproductive autoimmune failure syndrome.**

Key words—reproductive autoimmune failure syndrome, HLA, Class I, antiphospholipid antibody.

INTRODUCTION

In view of the diversity of the immunological response in pregnant women, aberrations of immune response have been suggested as likely causes of pathologies associated with pregnancy. The possible involvement of the immune system in the pathophysiology of adverse outcomes of pregnancy such as spontaneous abortion, intrauterine fetal death, intrauterine fetal growth retardation and preeclampsia, has recently attracted increasing attention. Fetal wastage, fetal growth retardation and preeclampsia are major complications in pregnant women with systemic lupus erythematosus.[1] Recently, however, it has been reported that autoantibodies are linked to such reproductive abnormalities in women who are clinically completely asymptomatic. A possible link between antiphospholipid antibodies and such reproductive failure has been suggested[2-6] and a newly defined disease entity, reproductive autoimmune failure syndrome (RAFS), defined as recurrent fetal wastage including spontaneous abortion and intrauterine fetal wastage, intrauterine fetal growth retardation or preeclampsia with positive antiphospholipid antibodies, was reported.[7,8] Moreover, a therapeutic modality using immunosuppressive and/or anticoagulant agents has been reported to be efficacious for patients with RAFS.[9,10] In regard to the abnormal generation of autoantibodies such as those directed against phospholipid in these patients, they are believed to have aberrations in their immune regulation, and there is a possibility of linkage with HLA antigen systems. The immunological background of this disease entity, however, has not yet been analyzed. The HLA antigen system is known to be useful in examining the immunogenetic basis of some diseases.[11] In this study, the distribution of HLA Class I antigens in patients with RAFS was analyzed in Niigata Prefecture, Japan.

PATIENTS AND METHODS

Patients and controls

We defined "reproductive autoimmune failure syndrome" (RAFS) by the following criteria: patients who were positive for antiphospholipid antibodies and had experienced either recurrent fetal wastage and/or one or more instances of intrauterine fetal

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growth retardation with severe preeclampsia according to the modified concept proposed by Gleicher et al..[7] Thirty-eight patients with RAFS were examined for HLA-A, -B and -C antigens. Seven of these patients were affected with intrauterine fetal growth retardation, all of whom had complications of severe preeclampsia, mainly revealing severe hypertension. Intrauterine fetal growth retardation was defined as the gestational age-controlled birth weight below the tenth percentile, according to the standard curve by Brenner et al.,[12] corrected for sex and the number of deliveries. Thirty-one of these patients had recurrent fetal wastage and had experienced 2 or more consecutive spontaneous abortions and/or fetal deaths. All of patients were positive for antiphospholipid antibodies examined by a previously reported method of enzyme linked immunosorbent assay (ELISA).[9,13] The husbands of 22 of the patients were examined for HLA-A, -B and -C antigens, and the compatibility of the spouses in each couple was analyzed. As a control population, 132 healthy individuals were studied to determine the general distribution of HLA antigens in Niigata. Twenty-six normal fertile couples with at least two children and no history of recurrent fetal wastage or intrauterine fetal growth retardation were studied as a control group in the investigation of compatibility of HLA antigens between spouses.

Tissue typing

Peripheral blood lymphocytes were typed for HLA-A, -B and -C antigens by the standard microlymphocytotoxicity technique by Terasaki[14] using commercially available microtiter plates (One Lambda, Inc., Canoga Park, CA). The antisera used defined 14 A-locus antigens, 31 B-locus antigens, and 9 C-locus antigens. The antigen frequencies obtained from the patients were compared with those in the 132 healthy individuals.

Statistical analyses

Relative risk with 95% confidence interval was calculated to determine whether a significant difference existed between the HLA antigen frequencies in the patient group and those in the control group. The Chisquare method was used to analyze the significance of difference between the number of shared HLA antigens in patient couples and that in control couples. When the number of couples was less than 5, Fisher's exact test was used.

RESULTS

The types of HLA-A, -B, and -C antigens of 38 patients are listed in Table 1. There were no significant differences between the distributions of HLA-A and -B antigens in the patient group and that in the general population (Tables 2 and 3). Of the HLA-C antigens, however, CW7 antigen was significantly more frequent in the patient group compared with that in the general population (Relative risk; 3.47, 95% confidence interval; 1.18–10.2) (Table 4). The frequency of HLA CW7 antigens in patients with intrauterine growth retardation (Cases 1 through 7 in Table 1) was 28.6% (2 of 7 Cases), and that in patients with recurrent fetal wastage (Cases 8 through 38 in Table 1) was 19.4% (6 of 31 Cases). These frequencies were not significantly different. There were no significant differences in intra-couple compatibility between the couples with RAFS and the normal fertile couples (Table 5).

DISCUSSION

This study highlights an important point concerning the immunological involvement in the genesis of reproductive autoimmune failure syndrome (RAFS), in that frequencies of certain loci of HLA antigens in patients with RAFS were significantly different compared with those in the general population in the same district.

As pregnant women show a wide range of immunological responses, aberrations of immune function have been suggested to be possible causes of pathologies associated with pregnancy, such as spontaneous abortion, intrauterine fetal death, intrauterine fetal growth retardation and preeclampsia. For example, the immunologic demise of an allogeneic fetus by the mother is well known, and the efficacy of immunotherapy for patients with habitual abortion using the male patient's lymphocytes has been reported by several investigators, including the authors.[15,16] On the other hand, it has been recently reported that autoimmunity was linked with reproductive abnormalities, and a possible link between antiphospholipid antibodies in particular and reproductive failures has been suggested.[2–6]

A significantly high positive rate of antiphospholipid antibodies has been reported in patients who suffered recurrent spontaneous abortions,[3,4] and recent investigations have disclosed the same tendency in patients who exhibit intrauterine fetal growth retardation or preeclampsia.[6,8,17,18] One of the

Table 1. Phenotypes of HLA Class I antigens in patients with RAFS

Case No.	Obstetric history	HLA antigens		
		A	B	C
1	IUGR*	A24/A26	B 7 /B62	-/-
2	IUGR	A11/A34	B40/B54	CW 1 /CW 7
3	IUGR	A24/-	B40/B51	CW 3 /-
4	IUGR	A11/A24	B35/B62	CW 4 /-
5	IUGR	A 2 /A31	B39/B61	CW 7 /-
6	IUGR	A 2 /A24	B35/-	CW 3 /-
7	IUGR	A 2 /-	B 7 /B44	CW 1 /-
8	Recurrent fetal wastage	A26/-	B35/-	CW 3 /-
9	Recurrent fetal wastage	A24/-	B46/B52	CW 4 /-
10	Recurrent fetal wastage	A24/A26	B44/B51	CW 2 /CW 3
11	Recurrent fetal wastage	A24/A26	B51/B53	CW 7 /CW 8
12	Recurrent fetal wastage	A24/-	B52/B62	CW 3 /-
13	Recurrent fetal wastage	A24/A34	B44/B75	CW 7 /-
14	Recurrent fetal wastage	A 2 /-	B35/-	CW 9 /-
15	Recurrent fetal wastage	A 2 /A26	B39/B62	CW 7 /-
16	Recurrent fetal wastage	A 2 /A24	B52/B61	-/-
17	Recurrent fetal wastage	A 2 /A24	B51/B60	CW 3 /-
18	Recurrent fetal wastage	A26/A31	B35/B62	CW 3 /-
19	Recurrent fetal wastage	A 1 /A26	B35/B37	CW 3 /CW 6
20	Recurrent fetal wastage	A24/A33	B44/B61	CW 3 /-
21	Recurrent fetal wastage	A11/-	B51/B62	CW 4 /-
22	Recurrent fetal wastage	A31/A33	B 7 /B44	CW 7 /-
23	Recurrent fetal wastage	A 2 /A26	B35/B51	CW 3 /-
24	Recurrent fetal wastage	A11/A26	B61/B62	CW 3 /CW 4
25	Recurrent fetal wastage	A24/-	B48/B54	CW 1 /-
26	Recurrent fetal wastage	A31/A33	B44/B51	-/-
27	Recurrent fetal wastage	A 2 /A29	B35/B55	CW 1 /CW 3
28	Recurrent fetal wastage	A26/A31	B54/B62	CW 1 /CW 3
29	Recurrent fetal wastage	A31/-	B51/B60	CW 3 /-
30	Recurrent fetal wastage	A 2 /-	B62/-	-/-
31	Recurrent fetal wastage	A 2 /-	B46/B62	CW 1 /-
32	Recurrent fetal wastage	A 2 /A33	B 7 /B67	CW 3 /CW 7
33	Recurrent fetal wastage	A 2 /A26	B60/-	CW 3 /-
34	Recurrent fetal wastage	A26/-	B51/B61	CW 3 /-
35	Recurrent fetal wastage	A11/A33	B51/B62	CW 3 /CW 4
36	Recurrent fetal wastage	A11/A24	B52/B67	CW 7 /-
37	Recurrent fetal wastage	A24/A33	B44/B52	-/-
38	Recurrent fetal wastage	A24/-	B46/B62	CW 1 /CW 3

*Indicates intrauterine fetal growth retardation.

Table 2. Camparison of frequencies of HLA-A antigens in patients with RAFS and the general population

	RAFS (n=38)	General population (n=132)	
A 1	1 (2.6%)	3 (2.3%)	n.s.
A 2	13 (34.2%)	55 (41.7%)	n.s.
A 3	0 (0%)	1 (0.8%)	n.s.
A 11	6 (15.8%)	20 (15.2%)	n.s.
A 19	0 (0%)	0 (0%)	n.s.
A 24	16 (42.1%)	65 (49.2%)	n.s.
A 26	12 (31.6%)	33 (25.0%)	n.s.
A 28	0 (0%)	0 (0%)	n.s.
A 29	1 (2.6 %)	4 (3.0 %)	n.s.
A 30	0 (0%)	2 (1.5%)	n.s.
A 31	6 (15.8%)	13 (9.8%)	n.s.
A 32	0 (0%)	0 (0%)	n.s.
A 33	6 (15.8%)	20 (15.2%)	n.s.
A 34	2 (5.3 %)	1 (0.8%)	n.s.

n.s.: Not significant by the analysis of relative risk with 95% confidence interval.

Table 3. Comparison of frequencies of HLA-B antigens in patients with RAFS and the general population

	RAFS(n=38)	General population (n=132)	
B 7	3 (7.9%)	10 (7.6%)	n.s.
B 8	0 (0%)	1 (0.8%)	n.s.
B 13	0 (0%)	4 (3.0%)	n.s.
B 14	0 (0%)	0 (0%)	n.s.
B 27	0 (0%)	2 (1.5%)	n.s.
B 35	8 (21.1%)	22 (16.7%)	n.s.
B 37	1 (2.6 %)	0 (0%)	n.s.
B 38	0 (0%)	2 (1.5%)	n.s.
B 39	2 (5.3%)	17 (12.9%)	n.s.
B 40	2 (5.3%)	10 (7.6%)	n.s.
B 41	0 (0%)	0 (0%)	n.s.
B 42	0 (0%)	2 (1.5%)	n.s.
B 44	7 (18.4%)	22 (16.7%)	n.s.
B 45	0 (0%)	0 (0%)	n.s.
B 46	3 (7.9%)	6 (4.5%)	n.s.
B 47	0 (0%)	1 (0.8%)	n.s.
B 48	1 (2.6%)	9 (6.8%)	n.s.
B 51	10 (26.3%)	21 (15.9%)	n.s.
B 52	5 (13.2%)	21 (15.9%)	n.s.
B 53	1 (2.6%)	5 (3.8%)	n.s.
B 54	3 (7.9 %)	9 (6.8%)	n.s.
B 55	1 (2.6%)	4 (3.0%)	n.s.
B 56	0 (0%)	1 (0.8%)	n.s.
B 59	0 (0%)	1 (0.8%)	n.s.
B 60	3 (7.9%)	10 (7.6%)	n.s.
B 61	5 (13.2%)	17 (12.9%)	n.s.
B 62	12 (31.6%)	20 (15.2%)	n.s.
B 63	0 (0%)	1 (0.8%)	n.s.
B 67	2 (5.3%)	0 (0%)	n.s.
B 75	1 (2.6%)	0 (0%)	n.s.
B 76	0 (0%)	0 (0%)	n.s.

n.s.: Not significant by the analysis of relative risk with 95% confidence interval.

Table 4. Comparison of frequencies of HLA-C antigens in patients with RAFS and the general population

	RAFS (n=38)	General population (n=132)	
CW 1	7 (18.4%)	35 (35.7%)	n.s.
CW 2	1 (2.6%)	0 (0%)	n.s.
CW 3	19 (50.0%)	59 (44.7%)	n.s.
CW 4	5 (13.2%)	15 (11.4%)	n.s.
CW 5	0 (0%)	2 (1.6%)	n.s.
CW 6	1 (2.6%)	3 (2.3%)	n.s.
CW 7	8 (21.1%)	8 (6.1%)	*
CW 8	1 (2.6%)	2 (1.6%)	n.s.
CW 9	1 (2.6%)	1 (0.8%)	n.s.

n.s.: Not significant by the analysis of relative risk with 95% confidence interval.
*: Significant difference was observed by the analysis of relative risk with 95% confidence interval (RR; 3.47, 95% CI; 1.18-10.2)

Table 5. Number of shared antigens in HLA-A, -B and -C locus in patient couples with RAFS and in normal fertile couples

No. of shared antigens	Patient couples (n=22)	Normal fertile couples (n=26)
0	4 (18.2%)	4 (15.4%)
1	6 (27.3%)	11 (42.3%)
2	11 (50.0%)	7 (26.9%)
3	1 (4.5%)	4 (15.4%)
4	0	0

No significant difference was observed in the intracouple compatibility between the two groups.

possible explanations for the genesis of recurrent abortion, intrauterine fetal death and intrauterine fetal growth retardation by the antiphospholipid antibodies is the wide-spread formation of thrombi in the intervillous space, as evidenced by an experiment in which antiphospholipid antibodies or lupus anticoagulant suppressed the production of prostacyclin in the aortic endothelial cells of mice.[17]

The human leukocyte antigen (HLA) system was developed from a search for the typing of lymphocytes that could form the basis for transplantation immunology in humans. Although initial studies in humans showed only weak associations with some diseases,[18] subsequent studies have shown very strong associations with a number of diseases. For example, the case of ankylosing spondylitis provides the most striking example of an association between HLA and disease.[19] The frequency of the antigen B27 in patients was 90%, compared with 9.4% in controls. More recently, it has been shown that most autoim-

mune diseases have a significant relationship with the HLA antigen system. For example, the relationships between SLE and D/DR3, rheumatoid arthritis and D/DR4, and Hashimoto's disease and D/DR5 are well known.[11] The HLA antigen system contains a number of closely linked loci controlling a variety of immunological functions which include the determination of cell-surface molecules, immune response differences, components of the complement system, and possibly other related functions connected in general with cell-cell regulation as well as the roles of hormone receptors.

In this study, the distribution of HLA Class I antigens in RAFS was analyzed by using serological methods in comparison with the general population in Niigata Prefecture, Japan. A significantly high frequency of CW7 was observed in the patient group compared with the general population. This is, to our knowledge, the first report concerning the distribution of HLA Class I antigens in patients with RAFS. Although the possible involvement of such HLA antigen systems in the mechanism of the genesis of RAFS in not clear, it is possible that the diversity of immune functions which are mapped on to the HLA gene regions might function as promoting factors which generate autoantibodies in these patients. The compatibility of HLA antigens between spouses should be always taken into consideration concerning adverse pregnancies, such as spontaneous abortion, intrauterine fetal death and intrauterine fetal growth retardation. The results obtained in this study suggest that the compatibility of HLA antigens between patient couples was not implicated in the genesis of adverse pregnancies caused by antiphospholipid antibodies.

Recently, it was suggested that serologically defined antigens, especially Class II antigens, might be unreliable, and that gene typing of the HLA antigen system is mandatory to elucidate the relationship between the HLA Class II antigen system and diseases. We therefore are now analyzing the relationship between the genotype and phenotype of Class II antigens and patients with RAFS using the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method.[20]

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