MATERIALS AND METHODS

Patients and control

The HLA-A and -B antigens were determined by a microcytotoxicity method in 122 couples with unexplained recurrent abortion and in 60 normal fertile couples. All patients had experienced three or more consecutive first-trimester spontaneous abortions with the same partner. The patients were grouped into primary recurrent aborters and secondary recurrent aborters. Of the 122 patients, 106 had no other history of pregnancy (primary recurrent aborters), and the remaining 16 had experienced one delivery prior to consecutive abortion (secondary recurrent aborters).They were also divided according to positivity of MLR (mixed lymphocyte culture reaction)-blocking antibodies (MLR-BAbs). The HLA-A and -B antigens were also determined in 60 normal fertile women who had experienced two or more normal term deliveries and had no history of spontaneous abortion. The numbers of mismatched antigens in couples with unexplained recurrent abortion were determined, and compared with those in the control couples. Informed consent for this study was obtained from all individuals.

Analyses of HLA-A and -B antigens

Peripheral blood lymphocytes were typed for HLA-A and -B antigens by the standard microlymphocytotoxicity technique by Terasaki, using commercially available microtiter plates (One lambda, Inc., Canoga Park, CA, USA). The antisera used defined 13 A-locus antigens and 33 B-locus antigens.

Mixed lymphocyte culture reaction-blocking assay

The blocking effect of sera was investigated in one-way MLR between spouses. Lymphocytes were collected from heparinized blood via Ficoll-Hypaque gradient centrifugation. Mixed culturing of mitomycin C-treated stimulator cells of the husband and responder cells of the patient was performed for six days on a microtiter plate in RPMI 1640 containing either pooled human AB serum or tested serum. The cultured cells were harvested onto a glass fiber filter after a pulse time of 18 h with ^{3}H-thymidine. DNA synthesis was evaluated by liquid scintillation counting, and the blocking effect (BE) was calculated by the formula:

BE=(1-mean cpm of culture in tested serum/mean cpm of culture in AB serum) × 100 (%).

The significant level of the MLR-blocking effect was determined to be more than 22%, which was designated as positive for MLR-BAbs, as previously reported[17,18].

Scoring of the number of mismatched alleles

A two-antigen mismatch was scored when both of the paternal antigens at a locus differed from their partner's antigens for HLA-A and -B antigens, respectively. If one antigen was shared, one mismatch was scored. If antigens from both individuals were identical, a zero mismatch was recorded. A zero mismatch was also recorded in cases where the male was homozygous for an antigen shared by his partner. Thus, the number of mismatched HLA-A and -B antigens ranged from zero to four.

Statistical analysis

Student's T-test was used to analyze any significance in the difference between the number of incompatible HLA-A and -B antigens in the patient and control couples.

RESULTS

The mean numbers of incompatible HLA-A antigens in patient couples with recurrent aborters (n=122), and control couples (60) were 1.10±0.67, and 1.19± 0.68, respectively, showing no significant difference. The mean numbers of incompatible HLA-A antigens in patient couples with primary recurrent aborters (n=106) and secondary recurrent aborters (n=16) were 1.12±0.06 and 0.94±0.68, respectively. These numbers were not significantly different compared with those of the control couples (Table 1). The mean numbers of incompatible HLA-B antigens in patient couples with recurrent aborters (n=122) and control couples (60) were 1.57±0.0.60 and 1.64±0.48, respectively, and again were not significantly different. The mean numbers of incompatible HLA-B antigens in patient couples with primary recurrent aborters (n=106) and secondary recurrent aborters (n=16) were 1.56 ± 0.60 and 1.69 ± 0.60, respectively. These numbers were not significantly different compared with those of the control couples (Table 2).

The mean numbers of incompatible HLA-A and -B antigens in couples with recurrent aborters (n=122), and control couples (60) were 2.67 ± 0.97 and 2.83 ± 0.93, respectively; these were not significantly different. The mean numbers of incompatible HLA-A and -B antigens in couples with primary recurrent aborters (n=106) and secondary recurrent aborters (n=16) were 2.68 ± 0.98 and 2.63 ± 1.02, respectively. These numbers were not significantly different compared with those of control couples (Table 3).

Of the 122 patients, 105 patients were negative for MLR-BAbs, and the remaining 17 patients were positive

Table 1. Mean number of incompatible HLA-A antigens in patient couples with unexplained recurrent abortion and control couples

	Primary recurrent aborters	Secondary recurrent aborters	Primary and secondary recurrent aborters	Control couples
Number of couples	106	16	122	60
Number of incompatible antigens	1.12 ± 0.68	0.94 ± 0.68	1.10 ± 0.67	1.19 ± 0.68

The number of incompatible antigens was not significantly different between each couple and control couple.

Table 2. Mean number of incompatible HLA-B antigens in patient couples with unexplained recurrent abortion and control couples

	Primary recurrent aborters	Secondary recurrent aborters	Primary and secondary recurrent aborters	Control couples
Number of couples	106	16	122	60
Number of incompatible antigens	1.56 ± 0.60	1.69 ± 0.60	1.57 ± 0.60	1.64 ± 0.48

The number of incompatible antigens was not significantly different between each couple and control couple.

Table 3. Mean number of incompatible HLA-A and -B antigens in patient couples with unexplained recurrent abortion and control couples

	Primary recurrent aborters	Secondary recurrent aborters	Primary and secondary recurrent aborters	Control couples
Number of couples	106	16	122	60
Number of incompatible antigens	2.69 ± 0.98	2.63 ± 1.02	2.67 ± 0.97	2.83 ± 0.93

The number of incompatible antigens was not significantly different between each couple and control couple.

for MLR-BAbs.

The mean numbers of incompatible HLA-A antigens in patient couples with negative MLR-BAbs (n=105) and with positive MLR-BAbs (n=17) were 1.10±0.66, and 1.12 ±0.78, respectively; these were again not significantly different compared with those in normal control couples (Table 4). The mean numbers of incompatible HLA-B antigens in patient couples with negative MLR-BAbs (n=105) and positive MLR-BAbs (n=17) were 1.60± 0.60 and 1.41±0.62, respectively, totals which were not significantly different compared with those in normal control couples. (Table 5).

Table 4. Mean number of incompatible HLA-A antigens in patient couples with unexplained recurrent abortion who were positive for MLR-blocking antibodies and control couples

	Recurrent aborters negative for MLR-BE*	Recurrent aborters positive for MLR-BE*	Control couples
Number of couples	105	17	60
Number of incompatible antigens	1.10 ± 0.66	1.12 ± 0.78	1.19 ± 0.68

*MLR-BE, MLR-blocking effect; The number of incompatible antigens was not significantly different between each couple and control couple.

Table 5. Mean number of incompatible HLA-B antigens in patient couples with unexplained recurrent abortion who were positive for MLR-blocking antibodies and control couples

	Recurrent aborters negative for MLR-BE*	Recurrent aborters positive for MLR-BE*	Control couples
Number of couples	105	17	60
Number of incompatible antigens	1.60 ± 0.60	1.41 ± 0.62	1.64 ± 0.48

*MLR-BE, MLR-bloking effect; The number of incompatible antigens was not significantly different between each couple and control couple.

Table 6. Mean number of incompatible HLA-A and -B antigens in patient couples with unexplained recurrent abortion who were positive for MLR-blocking antibodies and control couples

	Recurrent aborters negative for MLR-BE*	Recurrent aborters positive for MLR-BE*	Control couples
Number of couples	105	17	60
Number of incompatible antigens	2.70 ± 0.66	2.53 ± 0.1.07	2.83 ± 0.93

*MLR-BE, MLR-blocking effect; The number of incompatible antigens was not significantly different between each couple and control couple.

The mean numbers of incompatible HLA-A and -B antigens in couples with negative MLR-BAbs (n=105) and positive MLR-BAbs (n=17) were 2.70±0.66 and 2.53± 1.07, respectively; these were not significantly different compared with those of normal control couples (Table 6).

DISCUSSION

As antigens expressed on the surface of fetal or placental tissues possibly induce the allo-immune response of the mother, recurrent spontaneous abortion, especially that of unknown etiology, has been assumed to be caused by

an immunological defect that elicits maternal allogeneic reactions against the fetus[19]. Previous investigations have demonstrated that maternal immunity during a normal pregnancy is characterized by a lack of strong maternal cell-mediated immunity[20,21,22], in conjunction with a dominant humoral immunity[17,23,24].

Recently, the so-called immunotrophic theory -- whereby some cytokines produced by maternal cells which recognize fetal antigens promote the proliferation of trophoblastic cells and sustain continuation of the pregnancy – has been thought to be significant for the immunological maintenance of pregnancy[1,2,3]. Moreover, some investigators have demonstrated the importance of a T helper 2 (Th2) bias for normal pregnancy, indicating the crucial role of the activation of maternal humoral immunity following the recognition of fetal antigens during pregnancy[4,5,6].

It has been recognized for many years that the human leukocyte antigen system plays an important role in the etiology of a number of diseases[25,26,27,28], and that it is also possible that HLA systems are involved in the genesis of human abortions. In this context, there have been reports concerning the frequency and sharing of HLA antigens in couples who experienced recurrent spontaneous abortions.

As for the frequency of HLA antigens in the recurrent spontaneous aborters, Christiansen et al. reported that maternal HLA DR1, DR3, and DR10 were genetic markers for pregnancy loss in Danish recurrent spontaneous abortion patients and their relatives[29]. Our group reported that the frequency of HLA-DPB1*0402 and -DPB1*04 in a patient group with unexplained recurrent abortion was significantly higher compared with normal fertile women and with the general population[30]. Concerning HLA class I antigens, Cauchi et al. reported that HLA-B35 was significantly lower in patients with unexplained recurrent abortion compared with a control population[9]. We also reported a significantly lower frequency of HLA-B35 in a patient group compared with the general population[31].

Earlier studies indicated significant HLA sharing between couples where unexplained recurrent abortions occurred[7,8,11,12]. On the other hand, some investigators demonstrated that the sharing of HLA antigens was not significantly higher among couples with unexplained recurrent abortion[9,14]. Recent DNA analyses have shown a lack of significant compatibility between patient couples compared with normal fertile couples[10,13,15,16], although Ober et al. pointed out the possibility of significant compatibility of the HLA-DQA1 and DQB1 alleles between patients and aborted fetuses using a PCR-SSO (sequence specific oligonucleotides) method[13].

All of these studies, however, were performed in Caucasian populations, and there are very few data concerning the Japanese population. In addition, the number of patients examined in these studies was not so high, i.e., the number of patient couples in these studies ranged from 4 to 85[7-16]. Thus, a study encompassing more subjects was thought necessary to draw a significant conclusion. We therefore analyzed 122 patient couples with unexplained recurrent abortion in the Japanese population.

It is said that recurrent abortion, even in unexplained cases, represents a heterogeneous syndrome, and the immunologic background of the patients with unexplained recurrent abortion should be taken into consideration when we analyze the immunologic conditions – such as the compatibility of HLA antigens between the couples. MLR-BAbs detected by mixed lymphocyte culture reaction-blocking assay between spouses are considered to be a very important factor implicated in the immunologically successful continuation of the pregnancy. For example, it has been reported that immunotherapy using paternal lymphocytes is beneficial for those patients with unexplained recurrent abortion, especially for those who were negative for MLR-BAbs following the significant appearance of MLR-BAbs[17,18,32-34]. For this reason, we divided the patient population with unexplained recurrent abortion into two groups, i.e., one group with negative MLR-BAbs and one with positive MLR-BAbs.The compatibility of HLA class I antigens were compared between the couples with negative MLR-BAbs and normal fertile couples as well as between those with positive MLR-BAbs and normal fertile couples. Neither comparisons, however, revealed any siginificant difference.

In conclusion, no significant compatibility related to HLA-A and -B antigens, was observed between patient couples with unexplained recurrent abortion and control couples even if the patient population was limited to that with negative MLR-BAbs. Thus, the compatibility of the HLA-A and -B antigen systems does not seem to be implicated in the genesis of recurrent abortion, as far as the Japanese population is concerned. However, it will be necessary to investigate the compatibility of HLA class II antigens, HLA-DR, -DQ and -DP antigens, to establish a definite conclusion as to the association between the recurrent abortions and compatibility of HLA systems. In this context, we are now analyzing these antigens in larger group of patient couples with unexplained recurrent abortion.

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