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MLR Reactivity against Spouse's Lymphocytes: Successful Cases with Unexplained Recurrent Abortion Treated by Immunotherapy with Spouse's Cells

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Summary. **Problem: What are the possible underlying mechanisms of immunotherapy for unexplained recurrent abortion using spouse's cells?**
Methods: The reactivity of lymphocytes against spouse's lymphocytes in a mixed lymphocyte culture reaction (MLR) was serially evaluated in married female patients with unexplained recurrent abortion who underwent immunotherapy.
Results: Reactivity against the spouse's lymphocytes significantly decreased after immunotherapy compared with that before therapy ($n=19$, $p<0.01$, Wilcoxon test). Reactivity during the first trimester of the new pregnancy was also significantly lower than that before immunotherapy in married female patients with a successful outcome ($n=9$, $p<0.05$, Wilcoxon test). Reactivity in MLR, however, was significantly higher in married female patients who subsequently had repeated spontaneous abortion ($n=4$, $p<0.05$, Wilcoxon test). Conclusion: It is suggested that the attenuation of the reactivity against the spouse's lymphocytes might be implicated in the successful pregnancy of patients with unexplained recurrent abortion who underwent the immunotherapy.

Key words—recurrent abortion, immunotherapy, MLR.

INTRODUCTION

Immunotherapy has been employed to treat recurrent aborters, using either paternal or third party lymphocytes.[1-6] Its efficacy has been reported by a number of authors, including ourselves. The underlying mechanisms of immunotherapy, however, have not yet been fully elucidated. We have reported that the blocking effect of a one-way mixed lymphocyte culture reaction (MLR-BE) significantly increased after immunotherapy for married patients with a negative MLR-BE,[5,7] and that the CD4/CD8 ratio significantly decreased.[8] We attempted to elucidate the mechanisms of this therapy by investigating the alteration of the reactivity of the patient's lymphocytes against the spouse's lymphocytes before and after immunotherapy and its relationship to the outcome of pregnancy.

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MATERIALS AND METHODS

Patients

Nineteen married patients were enrolled in this study after informed consent was obtained (Table 1). Twelve patients were primary recurrent aborters and the remaining seven were secondary aborters. None of the subjects showed any indication of genetic abnormality, Muellerian anomalies, hormonal deficiency, infectious diseases, metabolic disorders or autoimmune abnormalities (such as positive antinuclear antibodies or anti-phospholipid antibodies) in our systematic investigation for determining the cause of their recurrent abortions. All patients were healthy except for their history of recurrent abortions, and were negative for blocking antibodies, identified by a one-way mixed lymphocyte culture reaction (responder: patient; stimulator: spouse), in their sera. Each patient was injected with her spouse's lymphocytes with informed consent. In our initial protocol, patients were allowed to become pregnant after the appearance of blocking antibodies in their sera. Recently, however, patients were allowed to become

pregnant after two injections, on the basis of a finding that blocking antibodies could be induced in almost all cases by this procedure. All nineteen patients were given two injections approximately one month apart, according to the recent protocol.

Procedure for immunization with spouse's lymphocytes

Details of the procedure have been described elsewhere.[5,7] Briefly, lymphocytes from the spouse's heparinized peripheral blood were suspended in about 1 ml of normal physiological saline. This cell suspension was injected intradermally into the patient. Blood samples for the mixed lymphocyte culture reaction were collected sequentially from both patients and spouses.

Mixed lymphocyte culture reaction

Lymphocytes of the patients, spouses and two controls (investigators: Y.K. and K.T.) were collected from heparinized peripheral blood through centrifugation on a Ficoll-Hypaque gradient. The spouse's and controls' lymphocytes were treated with 50 μg/ml of Mitomycin C for 30 min. at 37℃ and washed three times with RPMI 1640. Mixed culturing of 1.0×10^5/well of the patient's lymphocytes as responder cells and the same number per well of either the spouse's or control's lymphocytes as stimulator cells was performed in six wells of a flat-bottomed microtiter plate (Corning 25860) in RPMI 1640 containing 10% inactivated pooled human AB serum at 37℃ in a humidified atmosphere containing 5% CO_2. The culture cells were harvested onto a glass fiber filter after a pulse time of exactly 18 h with H-thymidine (0.6 μCi/well, Amersham, Japan). DNA synthesis was determined by liquid scintillation counting.

To examine the change in the peak reactivity of the patient's lymphocytes against her spouse's lymphocytes after immunotherapy in three patients, the period of mixed lymphocyte culture was set at exactly 72, 96, 120, 144, 168 and 192 h. Because the time of peak reactivity did not change after immunotherapy as stated in RESULTS, the reactivity of the patient's lymphocytes against the spouse's lymphocytes in the 144-hour cultures was investigated. The lymphocytes were collected from patients before and approximately one month after immunotherapy, and during the first trimester of the new pregnancy.

Statistical analyses

A paired Wilcoxon test was used to determine the

Table 1. Patients' profiles and outcome of pregnancy after immunization

No. of patients	Age	Number of spontaneous abortions	Primary or secondary	Outcome of pregnancy after immunization
1	30	3	secondary	waiting for pregnancy
2	37	3	primary	success
3	29	3	primary	success
4	34	3	primary	success
5	27	4	primary	waiting for pregnancy
6	36	3	primary	waiting for pregnancy
7	25	3	primary	success
8	31	3	secondary	success
9	28	3	secondary	success
10	41	4	primary	abortion
11	37	4	secondary	waiting for pregnancy
12	29	3	primary	abortion
13	33	4	secondary	abortion
14	32	3	secondary	success
15	39	3	primary	success
16	27	3	primary	success
17	23	3	secondary	abortion
18	24	3	primary	success
19	37	3	primary	success

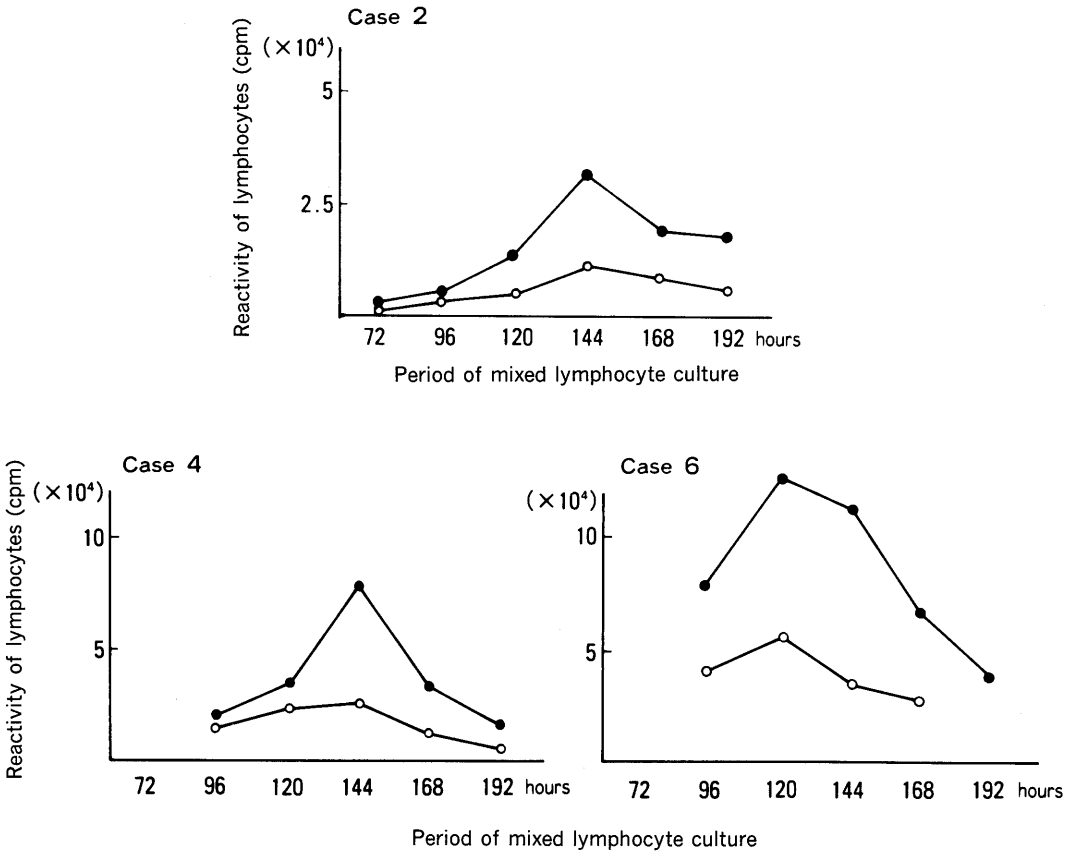

Fig. 1. Alteration of the time of peak reactivity against spouse's lymphocytes after immunotherapy. Closed and open circles indicate reactivity before and after immunization, respectively. Peak reactivity after immunization occurred after the same period of culture as before therapy in every case (after 144 h in two cases and 120 h in the third).

significance of differences between the pre and post-immunization reactivity of the patient's lymphocytes against the spouse's lymphocytes. The test was also used to determine the significance of the difference between the pre-immunization reactivity and that in the first trimester of the following pregnancy.

RESULTS

Outcome of pregnancy after immunization with spouse's lymphocytes

Of the nineteen patients, 15 have already become pregnant again, and the pregnancy continued successfully in 11 of these 15 (73.3%). The remaining four pregnancies resulted in repeated spontaneous abortion.

Alteration of the time of peak reactivity against spouse's lymphocytes after immunotherapy (Fig. 1)

As depicted in Fig. 1, peak reactivity after immunotherapy in three patients occurred after the same period of culture (after 144 h in two cases and 120 h in the third) as before therapy.

Alteration of reactivity against spouse's lymphocytes after immunization (Fig. 2)

The reactivity of the patient's lymphocytes against the spouse's lymphocytes significantly decreased after immunization with the spouse's lymphocytes ($p<0.01$, Wilcoxon test), while reactivity against the third parties' (Y.K. and K.T.) lymphocytes did not change significantly.

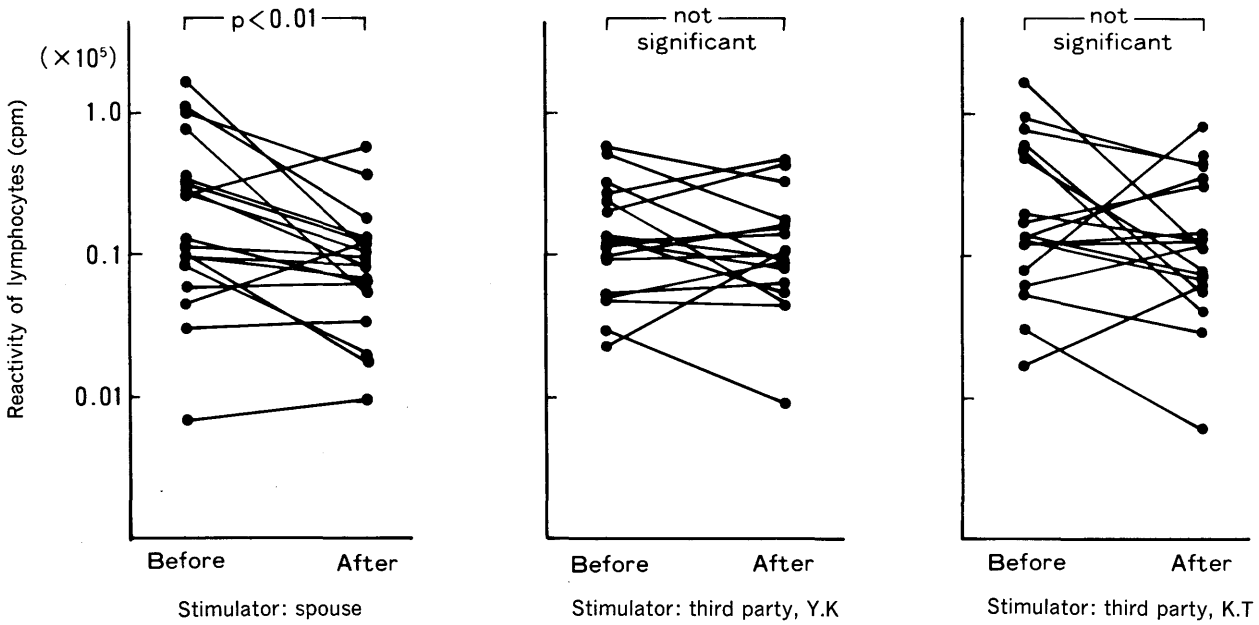

Fig. 2. Alteration of reactivity against the lymphocytes of the spouse and third parties after immunization. "Before" means before immunization and "After" means after immunization. The vertical axis indicates the reactivity of lymphocytes in MLR by logarithmic scale. The reactivity of the patient's lymphocytes against the spouse's lymphocytes was significantly lower after immunization, whereas the reactivity against the third parties' lymphocytes did not change significantly.

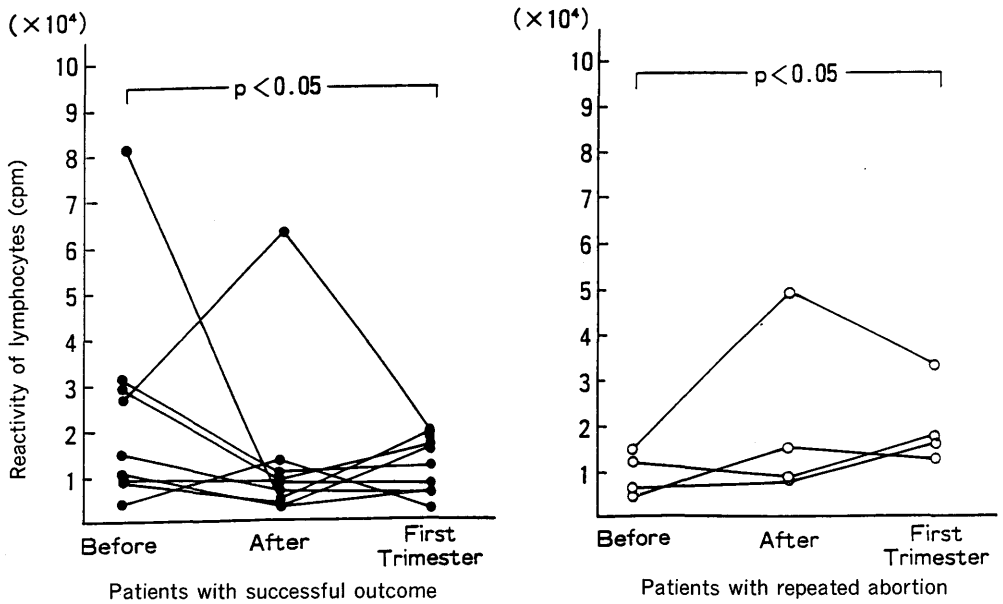

Fig. 3. Reactivity against the spouse's lymphocytes in the first trimester of the subsequent pregnancy. "Before" means before immunization and "After" means after immunization. Reactivity was significantly lower in the first trimester of the subsequent pregnancy compared with that before immunization in the nine patients with a successful outcome, but significantly higher in four patients with repeated abortion.

Reactivity against the spouse's lymphocytes in the first trimester of the subsequent pregnancy (Fig. 3)

The reactivity in the first trimester of the subsequent pregnancy was measured in nine patients with a successful outcome, and in four patients with repeated spontaneous abortion. The reactivity in patients with a successful outcome was significantly lower than that before immunization ($p<0.05$, Wilcoxon test), but significantly higher in patients with repeated abortion ($p<0.05$, Wilcoxon test).

DISCUSSION

This study reveals an important point involving the underlying mechanisms of immunotherapy for unexplained recurrent abortion. A successful outcome after immunotherapy for unexplained recurrent abortion has been reported by several investigators.[1-6] Mowbray et al. indicated the usefulness of paternal leukocyte immunization by a randomized double-blind trial.[2] Gatenby also reported the efficacy in a controlled trial.[6] Our study demonstrated that immunization with the spouse's lymphocytes improves the prognosis of pregnancy in patients with unexplained recurrent abortion, especially in patients without blocking antibodies in their sera.[5]

Although immunotherapy is considered useful, the underlying mechanisms have not yet been fully elucidated. We previously reported the production of blocking antibodies in a patient's serum which inhibited the response of autologous lymphocytes to transfused paternal lymphocytes.[7] In transplantation immunology, evidence has been presented over two decades that pre-transplant blood transfusion, especially when donor-specific, improves kidney graft survival and the immunoregulatory mechanisms have been extensively studied. MacLeod et al. reported the presence of non-cytotoxic antibodies in transfused patients which reacted with the Fc receptors on B lymphocytes.[9] Singal et al. described the presence of anti-idiotypic antibodies that were considered to react with autologous T cell receptors—which were generated against the donor's tissue antigens—and to induce the suppression of cell-mediated anti-allograft reactivity in transfused patients.[10]

The most interesting finding in the present study was the change in the patient's lymphocyte reactivity against her spouse's lymphocytes in the mixed culture reaction after the immunotherapy. Reactivity against a spouse's lymphocytes was specifically and significantly decreased approximately one month after the injections, and the reactivity during the first trimester of new pregnancy was significantly lower than that before immunization in patients in whom a subsequent pregnancy was successful. Our preliminary studies showed that cells with surface markers of suppressor increased on mixed lymphocytes cultured after immunotherapy compared with that before therapy (data not shown), and this might cause the attenuation of the lymphocyte reactivity. There are reports of decreases in some functional parameters of maternal cellular immunity or the appearance of blocking antibodies or anti-idiotypic antibodies during pregnancy that may be related to the survival of the fetus.[11-14] With respect to the relationship between the cellular immunity of immunized patients and the outcome of pregnancy, Gatenby et al. reported that the prolonged maintenance of $CD8^+$ suppressor T cells correlated with a successful outcome of pregnancy after immunization.[15] We also reported that the predominance of Ts/c (CD8) cells was maintained until the state of new pregnancy in patients who had a successful outcome after immunization with the spouse's lymphocytes.[8] Those results indicated that the predominance of Ts/c (CD8) cells, which was induced by sensitization with the spouse's lymphocytes, might be beneficial for successful continuation of the ensuing pregnancy in immunized patients. We clearly demonstrated the presence of blocking factors in the one-way MLR between spouses after immunization, and the continued presence of these blocking factors and the favorable natural course of the subsequent pregnancy have been reported elsewhere.[5,7,16]

The data in this study suggest that immunotherapy induces the specific immune regulation that suppresses the reactivity of the patient's cytotoxic activity against paternal antigens and sustains the successful continuation of the following pregnancy. Attenuation of lymphocyte reactivity during the first trimester of the successful pregnancy, however, could be understood as a result rather than a cause, and whether the absence of attenuation of the reactivity at preconception is predictive of subsequent spontaneous abortion should be investigated in a larger series.

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