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CASE REPORT

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# After 12 consecutive miscarriages, a patient received immunosuppressive treatment and delivered an intact baby

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## Abstract

**Aim:** An immune etiology for idiopathic recurrent miscarriage is an important issue because a fetus is allogenetically different from the mother. Type 1 T helper (Th1) and Type 2 (Th2) cells have important functions in immune responses and there is a general agreement that pregnancy is associated with Th2 cell dominance. The purpose of this case report is to establish the effectiveness of an immunosuppressive treatment for a patient who had 11 consecutive miscarriages despite several treatments, such as anticoagulation, that showed elevated Th1/Th2 cell ratios.

**Methods:** This patient visited our clinic following 11 consecutive miscarriages between 2009 and 2014 that occurred between 5 and 8 weeks' gestation. The Th1/Th2 cell ratio was evaluated after the 12th conception and she received an immunosuppressive treatment (tacrolimus; 1 mg/d).

**Results:** The Th1/Th2 cell ratio was elevated after the 12th conception, but the patient miscarried, with a normal karyotype of chorionic villi despite the immunosuppressive treatment. After the 13th conception, she began receiving treatment with 2 mg/d of tacrolimus at 4 weeks' gestation, which was continued until delivery.

**Conclusion:** For recurrent miscarriage cases that show an elevated Th1/Th2 cell ratio after achieving pregnancy, immunosuppressive treatment with tacrolimus could be effective.

KEYWORDS

immunological rejection, immunosuppressive agent, recurrent pregnancy loss, T helper type 1:2 cell ratio, tacrolimus

## 1 | INTRODUCTION

Type-1 T helper (Th1) and type-2 T helper (Th2) cells play important roles in immune responses, particularly in immune rejection and tolerance.[1,2] Hence, a method to achieve Th1/Th2 balance has been proposed to offset materno–fetal immune reactions during pregnancy. Pregnancy is generally associated with Th2 cell dominance except during instances of implantation and parturition. Over-reactive Th1 cell immune responses at the time of implantation have been associated with implantation failure, early pregnancy losses and repeated pregnancy losses[3-6] and can be compared to an allograft rejection.[7] Immunological rejection might be one of the causes of miscarriage[8] and several immunomodulation therapies such as prednisolone, γ-globulin therapy, and allogenic leukocyte immunization have been used for these types of patients. These therapies have their own demerits, which are disadvantages for both the patient and the fetus.

The patient described here was found to have an impaired Th2 dominance after the establishment of pregnancy. The administration of an immunosuppressive agent allowed the patient to continue her pregnancy, and she had a successful delivery.

## 2 | CASE REPORT

The histories of this patient's miscarriages are summarized in Table 1. She had received no treatment before her 1st and 2nd miscarriages. Because she had been diagnosed as a case of idiopathic recurrent miscarriage, she received empirical low-dose aspirin, low-molecular-weight heparin, prednisolone (5 mg/d), or intravenous massive immunoglobulin therapy between 2009 and 2014. For three out of these 11 miscarriages the fetal karyotype was subsequently found to be normal. There was no past medical, surgical, obstetric or gynecological history of note. Investigative screenings for recurrent miscarriage were performed on March 24, 2008, and all results were negative (Table 1).

This patient visited our clinic following 11 consecutive miscarriages between 2009 and 2014 that occurred between 5 and 8 weeks' gestation (Table 2). The peripheral blood Th1/Th2 cytokine producing cell ratio was measured at 8.9 (Th1=19.6, Th2=2.2). Th1 cells and Th2 cells were defined as $CD4^+$ lymphocytes with intracellular IFN-γ but without IL-4 ($CD4^+IFN\text{-}\gamma^+$) and $CD4^+$ lymphocytes with intracellular IL-4 but without IFN-γ ($CD4^+IL\text{-}4^+$), respectively, and the normal range of a Th1/Th2 cell ratio was set at less than 10.3 according to our previous report.[3] This Th1/Th2 cell ratio was re-checked just after confirmation of the 12th conception (14th day after a LH-positive day) and an elevation at 15.2 was detected (Th1=18.2, Th2=1.2; Table 3). The patient began to receive immunosuppressive treatment (tacrolimus; 1 mg/d) after the 12th conception, combined with low-dose aspirin and low-molecular-weight heparin. Unfortunately, she miscarried at 8 weeks gestation due to subchorionic hemorrhage, with a normotype of chorionic villi.

On her 13th conception, she received only immunosuppressive treatment (tacrolimus; 2 mg/d) that was started at 4 weeks' gestation after a home pregnancy test was positive, and she continued this dose until the day of delivery. A fetal heartbeat was confirmed at 6 weeks' gestation, but the fetal growth was small for gestational age. The pregnancy was monitored with serial ultrasonography and complicated by intrauterine growth restriction; therefore, the patient was moved from our clinic to the National Center for Child Health and Development. The patient's blood pressure was found to be elevated at around 24 weeks gestation, and she was treated with an antihypertensive drug. As a result of poor fetal growth velocity detected by serial ultrasonography and an inability to control her blood pressure, at 29 weeks a cesarean section was performed that resulted in the birth of a female infant weighting 748 g. Her physical condition improved rapidly after giving birth, and she was discharged 2 weeks after delivery. The baby girl was placed in the neonatal care unit (NICU), where her weight increased at a good rate, and she was discharged 3 months after birth without complications.

## 3 | DISCUSSION

This case report is interesting because it is a case of immunosuppressive treatment using tacrolimus for a patient who showed an elevated Th1/Th2 cell ratio after conception in recurrent miscarriages. In this case, the patient miscarried at 8 weeks' gestation despite receiving intravenous massive immunoglobulin (IVIG) following the 11th conception, which marked her longest period without miscarriage. Locally, we believed that IVIG could be effective for this case, and suspected that a cause of her miscarriages might be an immunological rejection. Therefore, we checked the levels of Th1 and Th2 cells in the non-pregnant period after the 11th miscarriage. However, she showed a normal Th1/th2 cell ratio, according to our criteria.[3]

On the 12th pregnancy, the patient received immunosuppressive treatment (tacrolimus 1 mg/d) with empirical low-dose aspirin and

**TABLE 1** Characteristics of the treatment for the patient's 12 consecutive miscarriages

| No. | Date | hCG[a] | GS[b] | Yolk sac | Embryo | FHM[c] | D&C | Crom[d] | LDA[e] | Heparin | PSL[f] | IVIG[g] | IM[h] | Miscarriage |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2007/11 | + | Yes | No | No | No | No | No | No | No | No | No | No | Yes |
| 2 | 2008/02 | + | Yes | Yes | Yes | Yes | Yes | No | No | No | No | No | No | Yes |
| 3 | 2008/09 | + | No | No | No | No | No | No | Yes | No | No | No | No | Yes |
| 4 | 2009/03 | + | Yes | No | No | No | Yes | No | Yes | No | No | No | No | Yes |
| 5 | 2009/11 | + | Yes | Yes | Yes | Yes | Yes | 46, XX | Yes | No | No | No | No | Yes |
| 6 | 2010/05 | + | Yes | No | No | No | No | No | Yes | Yes[i] | No | No | No | Yes |
| 7 | 2011/06 | + | Yes | No | No | No | No | No | Yes | Yes[j] | No | No | No | Yes |
| 8 | 2011/09 | + | Yes | No | No | No | Yes | 46, XY | Yes | Yes[k] | No | No | No | Yes |
| 9 | 2012/05 | + | No | No | No | No | No | No | Yes | No | No | No | No | Yes |
| 10 | 2012/09 | + | Yes | Yes | CRL:16[l] | Yes | Yes | 46, XY | Yes | Yes[k] | 5 mg | No | No | Yes |
| 11 | 2014/04 | + | Yes | Yes | CRL:15[l] | Yes | Yes | 47, XX+22 | Yes | Yes[k] | 5 mg | Yes | No | Yes |
| 12 | 2014/09 | + | Yes | Yes | Yes | Yes | Yes | 46, XX | Yes | Yes[k] | No | No | 1 mg | Yes |
| 13 | 2015/02 | + | Yes | Yes | Yes | Yes | No | No | No | No | No | No | 2 mg | No |

[a]hCG, the confirmation of serum human chorionic gonadotropin; [b]The presence of a gestational sac in the uterine cavity; [c]The confirmation of fetal heart movement; [d]Chromosomal analysis; [e]Low-dose aspirin (81 mg/d); [f]Prednisolone administration; [g]i.v. immunoglobulin administration (1 g/kg/3 d); [h]Administration of an immunosuppressive agent (tacrolimus, mg/d); [i]Heparin (2500 IU/d); [j]Heparin (5000 IU/day); [k]Heparin (10 000 IU/d); [l]CRL: crown rump length (mm).

**TABLE 2** Result of investigating screening for recurrent pregnancy losses (checked on March 24, 2008)

| Basal hormonal profile | Value | Antiphospholipid syndrome screening | Value |
|---|---|---|---|
| LH (IU/L) | 3.7 | IgM anticardiolipin antibody titers (U/mL) | <5000 |
| FSH (IU/L) | 10.2 | IgG anticardiolipin antibody titers (U/mL) | 1000 |
| Prolactin (ng/mL) | 6.3 | LAC (dilute Russell viper venom time) | 0.800 |
| Blood coagulation testing | | Anti-PE IgG (kininogen+) | 0.206 |
| PT (second) | 11.0 | Anti-PE IgM | 0.370 |
| APTT (second) | 25.6 | Anti-PS IgG | <0.500 |
| Protein S | 71.0 | Anti-PS IgM | 0.600 |
| Protein C (%) | 104.0 | β2GP1 antibody | <0.200 |
| Coagulation factor XII (%) | 138.0 | Autoimmune screening | |
| Full blood count | | Anti-DNA | <80 000 |
| White blood cells (/μL) | 4700.0 | Mitochondrial antibodies | <20 000 |
| Red blood cells (million/μL) | 372.0 | Thyroid antibodies (IU/mL) | 1200 |
| Hemoglobin (g/dl) | 11.7 | Viral screening | |
| Platelets (/μL) | 267,000.0 | Hepatitis B antigen | Negative |
| Random blood sugar count (g/dl) | 98.0 | Hepatitis C antibody | Negative |
| Thyroid gland profile | <20.0 | Human immunosuppressive virus | Negative |
| TSH (μIU/mL) | 1.7 | Karyotype analysis of both parents | |
| Free T3 (pg/mL) | 3.6 | Patient | 46, XX |
| Free T4 (ng/mL) | 1.5 | Partner | 46, XY |

APTT, activated partial thromboplastin time; FSH, follicle-stimulating hormone; GP, glycoprotein; Ig, immunoglobulin; LAC, lupus anticoagulant; LH, luteinizing hormone; PE, phosphatidylethanolamine; PS, phosphatidylserine; PT, prothrombin time; TSH, thyroid stimulating hormone.

low-molecular-weight heparin. As a result of this treatment, a subchorionic hemorrhage occurred and caused another miscarriage. Her Th1/Th2 ratio after the establishment of pregnancy was 15.2, which was due to a decrease in the Th2 cell level. She began a regimen of tacrolimus (1 mg/d) following a positive urinary pregnancy test (the 12th pregnancy). During the 12th pregnancy, her Th1 and Th2 levels were 18.2 and 1.2 with tacrolimus treatment (1 mg/d), respectively, and it was lower than that (Th2=2.2) before this pregnancy (non-pregnant status). As a consequence, her Th1/Th2 ratio was elevated (Th1/Th2=15.2). Although the patient received 1 mg/d of tacrolimus during the 12th pregnancy, this resulted in miscarriage regardless of her demonstration of a euploid chromosome (46, XX) from the chorionic villi sampling. Based on this data, we speculated that the cause of this miscarriage was a weakening of immunological tolerance. The immunosuppressive treatment using tacrolimus had not strengthened her immunological tolerance, and, instead, it had seemed to have weakened her immunological rejection (Th1 level). In this case, the decrease in immunological rejection that caused a miscarriage of the 12th pregnancy might have been a case of immunological rejection due to the weakening of her immunological tolerance despite the administration of an immunosuppressive agent. It was concluded that the dose of tacrolimus might not have been sufficient. Moreover, we were convinced that anticoagulant therapy was unnecessary for this patient.

There were no data concerning the Th1/Th2 ratio between the 12th and 13th pregnancies. However, the Th1/Th2 ratio was checked on October 1, 2012, on the day the patient was diagnosed with her 10th miscarriage (Table 3). Seven months after the 10th miscarriage, her Th1/Th2 was checked again, and it was confirmed that the Th1/Th2 ratio was less than 10.3, and lower than that at the previous check. When this patient was not pregnant, her Th1/Th2 ratio was less than 10.3. Therefore, the Th1/Th2 ratio was thought to have decreased to less than ten after her 12th miscarriage.

After the 13th conception, she began receiving treatment with tacrolimus of 2 mg/d at 4 weeks' gestation until delivery with no further anticoagulant therapy. During the patient's 13th pregnancy, the combination of tacrolimus and massive IVIG was an option for immunomodulation, but the patient did not agree to the use of IVIG due to its cost (1 million JPY/one treatment), therefore, we abandoned the combination of massive IVIG and tacrolimus, and decided to simply increase the dose of tacrolimus.

The embryo expresses paternal antigens that are foreign to the mother and therefore may be viewed as an allograft; in a normal pregnancy, the embryo is not rejected by the mother's immune system.[4] Antigens expressed on the surface of fetal or placental tissues possibly induce alloimmune responses by the mother, along with certain immunologic mechanisms that sustain the continuation of a normal pregnancy. Moreover, Th2 cell dominance is important for the maintenance of a normal pregnancy, and is a common phenomenon. With regard to immunological disorders as causative factors, it is assumed that recurrent pregnancy loss (RPL) could be caused by an increase in type 1 cytokines prior to implantation by essential immunological

**TABLE 3** Changes in the T helper 1 (Th1) and T helper 2 (Th2) cells before and after pregnancy

| Date | Status | Th1 (%) | Th2 (%) | Th1/Th2 | Tacrolimus |
|---|---|---|---|---|---|
| 2012-10-01 | 10th conception (6 weeks) | 18.8 | 1.7 | 11.1 | None |
| 2013-05-10 | Non-pregnancy | 19.6 | 2.2 | 8.9 | None |
| 2014-09-08 | 12th conception (8 weeks) | 18.2 | 1.2 | 15.2 | 1 mg/d |
| 2015-02-13 | 13th conception (4 weeks) | 14.6 | 0.9 | 16.2 | 2 mg/d |

status in the whole body, while recurrent implantation failure would induce the production of type 1 cytokines in the uterus, which would then be reflected in the whole body. A relative increase in the population of Th2 cells in a normal pregnancy is observed as a result of the suppression of type 1 cytokines production. The Th1/Th2 cell ratio in RPL patients who may have immunological disorders shows an excessive response to the fetus and tends to be elevated in general.[2,5] Initially, fetus antigens are recognized in early pregnancy and extensive numbers of antigens are transferred to maternal circulation in the second trimester. Theoretically, two peaks of immune responses to the fetus would be observed in patients who have either a failure of immunological tolerance or a suppression of immunological rejection.

In a previous study, changes in the percentage of Th1 cells, Th2 cells, and the Th1/Th2 ratio were reported in patients with unexplained recurrent abortions before and after immunotherapy with the husband's mononuclear cells.[8] In the present report, iimmunotherapy significantly increased the percentage of Th2 cells, while the Th1/Th2 ratio was significantly decreased in the total patient population, and the Th1/Th2 ratio was significantly decreased with immunotherapy in the total patient population. The present study demonstrated that Th2 cell dominance is important in order to avoid miscarriage. A worldwide meta-analysis study has concluded that immunization may be highly effective, although this is indicated only for a small number of patients.[8] Wegmann et al. proposed an immunotrophic theory,[9] whereby some cytokines produced by maternal cells, which recognize fetal antigens, promote the proliferation of trophoblastic cells and sustain pregnancy continuation.

There are several immunotherapy methods for treatment of unexplained recurrent pregnancy losses, such as IVIG, immunotherapy with the husband's mononuclear cells, and the administration of prednisolone. IVIG therapy is expensive, requires infusion, and might cause unknown viral infections. Immunotherapy using a husband's mononuclear cells presents the risk of graft-versus-host disease (GVHD), and the preparation of a mononuclear cell is a cumbersome procedure. Prednisolone therapy requires a high dose (20 mg/d) for treatment of unexplained recurrent pregnancy losses. These three immunotherapies all possess disadvantageous aspects for patients. On the other hand, tacrolimus therapy is safe and simple, and is much more convenient for patients.

Tacrolimus has been utilized throughout pregnancy for women who have received an allogeneic organ transplant, and many female recipients have given birth while taking tacrolimus.[10] Tacrolimus reduces peptidyl-prolyl isomerase activity by binding to the immunophilin FKBP12 (FK506 binding protein) and creating a new complex. This FKBP12-FK506 complex interacts and inhibits calcineurin, and further inhibits both T-lymphocyte signal transduction and IL-2 transcription.[11] The values for short-term immunosuppression and graft survival by patients are found to be similar between the two drugs. However, tacrolimus results in a more favorable lipid profile which may have important long-term implications given the prognostic influence of rejection on graft survival.[12]

In the present case, the cause of the patient's miscarriages was difficult to determine, because her elevated Th1/Th2 cell ratio was detectable only when she became pregnant. Tacrolimus is a major immune-suppressive agent for allogenic organ transplantation, but it has never been used for the treatment of recurrent miscarriages due to immunological rejection. This patient is an identical twin, and her sister had two children without a history of miscarriage. Based on the history of her sister, the cause of the patient's miscarriages was thought to be immunological rejection against the paternal antigen, which might have been acquired after birth.

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## DISCLOSURES

*Conflict of interest*: The authors declare no conflict of interest. *Human and Animal Rights*: All the procedures that were followed were in accordance with the ethical standards of the responsible committee of Sugiyama Clinic and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from this patient to be included in this case report. This article does not contain any study with animal participants that have been performed by any of the authors.

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