—Review—

Diagnosis and Treatment of Recurrent Miscarriage Associated with Immunologic Disorders : Is Paternal Lymphocyte Immunization a Relic of the Past?

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Abstract

Miscarriage is the most common complication of human pregnancy. Although the causes of recurrent miscarriage (RM) are various, immunologic aberrations between mother and fetus might be one of the causes of miscarriage. Immune responses fall into two categories: autoimmune and alloimmune. Currently, no appropriate diagnostic method has been established to identify alloimmune causes. We observed that NK cell activity in women with RM was higher than that in women without a history of miscarriage. Functional or quantitative analysis of NK cells could be used to identify alloimmune causes. Paternal lymphocyte immunization has been the most widely used treatment for alloimmune-mediated miscarriages. However, the latest Cochrane review by Scott reached the current conclusion that lymphocyte immunization therapy provided no significant beneficial effect over placebo in preventing further miscarriage. Approximately 70% of Japanese university hospitals are still performing paternal lymphocyte immunization. Is paternal lymphocyte immunization a relic of the past? Randomized controlled trials based on adequate patient selection in Japan should provide an answer to this question.

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Introduction

Of all clinically detected pregnancies, approximately 10～15% end in miscarriage during the first trimester. Although most are sporadic and non-recurrent, there is a subset comprising 2～5% of couples that suffer recurrent miscarriage (RM)[1]. The causes of RM are classified as genetic, endocrinologic, anatomic, immunologic, microbiologic, and environmental. At least 50～60% of sporadic miscarriages are the result of genetic disorders, but miscarrying pregnancies in women with RM are associated with a low incidence of genetic abnormality[2]. Much research has been conducted to identify the underlying mechanisms of RM, and accumulating evidence reveals that an immunologic mechanism is involved in some miscarriages. The majority of recent research has focused on immunologic causes, but controversy still exists over appropriate testing and treatment for RM due to immunologic causes. This review addresses the

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potential associations between alloimmune factors and RM, and the modern preventive treatment for RM, focusing on the diagnosis for alloimmune-mediated miscarriages and paternal lymphocyte immunization.

Miscarriage due to Alloimmune Causes

Successful pregnancy is an immunologic paradox. Despite the fetus being regarded as a semiallograft to the mother, the fetus can be successfully accepted by the mother for 280 days. Medawar suggested in 1953 that antigenic immaturity of embryos might protect them from allogeneic recognition. Indeed, classical class I molecules are not expressed on the cell surface of embryonic tissue. However, when class I antigen is absent, NK cells attack embryonic cells instead. Therefore, the simple lack of classical class I antigens on the trophoectoderm and later on invading cytotrophoblasts is inadequate to explain immunoprotection from T cells and NK cells. The presence of nonclassical, nonpolymorphic class I antigen, HLA-G or HLA-E, on the surface of extravillous trophoblasts explains the paradox[3]. Moreover, Wegmann *et al*. suggest that in pregnant women, cytokines produced by T helper 2 (Th2) cells predominate over those produced by Th 1 cells, resulting in successful pregnancy [4]. What is happening at the feto-maternal interface, human deciduas, is illustrated in **Fig. 1**. If such alloimmune-protective mechanism collapses, a pathologic situation develops, resulying in alloimmune-mediated miscarriage.

Diagnostic Tests for Miscarriages due to Alloimmune Causes

Despite the considerable attention paid to the role of immunology in reproductive failure, no appropriate diagnostic strategy has been established to identify alloimmune causes. In the early stage of investigation in this field, an immunologic miscarriage was thought to be a failure of the maternal allogenic recognition of the feto-trophoblast unit. Based on this theory, maternal antibody response, such as the facilitation reaction, was thought to be important for maintenance of pregnancy (Voisin GA). As early as the 1980s, the excess sharing of HLA antigens between spouses had been considered to be increased in recurrently aborting couples, and responsible for hyporesponsiveness to paternal antigens leading to miscarriage. In this regard, the absence of blocking antibodies, anti-paternal lymphocyte antibodies or anti-HLA antibodies were considered to be parameters of miscarriage for alloimmune cause. The facilitation theory collapsed with the discovery of major histocompatibility complex restriction, and it is now known that the pregnancy course of agammaglobulinemic women is absolutely normal. These facts suggest that antibody response is not necessary for maintenance of pregnancy. However, since there remains a patient population whose causes of miscarriages include antibody response, MLR blocking antibodies (MLR suppression test), anti-paternal lymphocyte antibodies (complement dependent microcytotoxicity test, flow cytometry cross match (FCXM))[5] are examined as laboratory tests for the detection of alloimmune disorders.

There is increasing evidence that the elevated proportion and activity of all peripheral blood NK cells are related to spontaneous abortions. Aoki *et al*. report that women with high preconceptional NK cell activity had a significantly higher abortion rate in the next pregnancy than women with normal levels of NK cell activity[6]. We also observed that NK cell activity in women with RM was higher than that in women without a history of miscarriage (data not shown). Kwak *et al*. observed the up-regulated expression of CD56+, CD56+/CD16+, and CD19+ cells in peripheral blood lymphocytes in pregnant women with recurrent pregnancy loss[7]. Moreover, Emmer *et al*. demonstrated that peripheral NK cell activity and CD56+CD16+cells increase during early pregnancy in women with a history of recurrent spontaneous abortion[8]. Thus, functional or quantitative analysis for NK cells could be used to monitor treatment and pregnancy outcome[9,10].

Hayakawa *et al*. report that the mean of the Th1 (IFN-γ-producing CD4 cells): Th2 (IL-4-producing CD4 cells) ratios in patients with RSA was significantly higher than in control subjects.

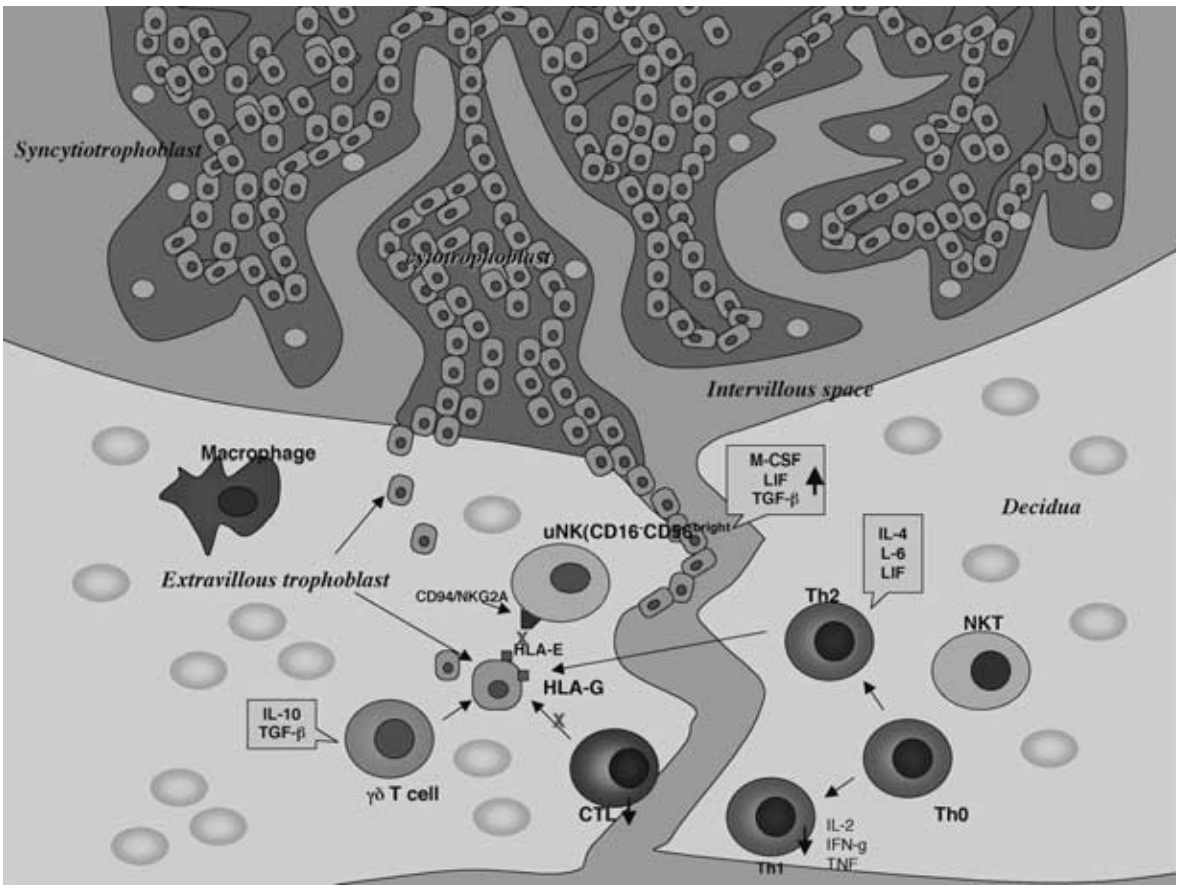

Fig. 1 Immunological events at the feto-maternal interface. In the decidua, MHC class I-positive extravillous trophoblast cells directly contact decidual immune cells. Extravillous trophoblasts have non-classical, non-polymorphic class I MHC antigens, HLA-G and HLA-E, which enable them to escape attack by maternal abT cells and NK cells.

The Th1/Th2 ratio is a candidate for identifying alloimmune causes in women with RM[11].

Treatment for Miscarriages due to Alloimmune Causes

Paternal lymphocyte immunization has been the most widely used treatment for alloimmune-mediated miscarriages. An alternative treatment is intravenous immunoglobulin therapy. Both therapies are still controversial in terms of effectiveness. This review focuses on the former treatment, allogeneic lymphocyte immunization.

a. Allogeneic (Paternal) Lymphocyte Immunization

Since no alloimmune mechanism has been shown to cause RM, we do not have appropriate arms to identify alloimmune causes, there is no adequate therapy for RM due to alloimmune causes. In the early 1980s, the predominant focus was allogeneic lymphocyte immunization, which was considered a promising and rational treatment because blood transfusion given before transplants was reported to decrease the rejection of organ allografts. Most early studies reported high success rates in the couples immunized. However, few studies had been performed by means of randomized controlled trials (RCT), and the first high quality RCT was performed by Mowbray *et al*. in 1985[12]. They observed a higher success rate in women given paternal lymphocyte immunization (17/22) than in women immunized with their own lymphocytes (10/27) (5.78 : 95%CI 1.63～20.5). Subsequently, several conflicting studies were published including negative trials in which treated patients did worse than the controls[13].

To address the uncertainties over the effectiveness and mechanism of paternal

immunization caused by these conflicting reports, a worldwide collaborative study was performed and published by the Recurrent Miscarriage Immunotherapy Trialists Group (RMITG) in 1994[14]. Nine randomized trials were evaluated independently by two data analysis teams to verify the conclusions. The percentage of live birth ratios were 1.16 (95%CI, 1.01～1.34) and 1.21 (95%CI, 1.04～1.37). These results were calculated based on a data set that included couples in whom the female partner had autoantibodies such as anticardiolipin and/or antinuclear antibodies, pre-existing circulating anti-paternal lymphocyte antibodies, and couples with one or more children. Therefore, supplementary analysis based on a data set excluding those with abnormal antibodies and those with prior live births has been subsequently performed. The outcome may be improved to give a live birth ratio of 1.45 (95%CI, 1.02～1.69) by selecting patients most likely to benefit from immunization therapy.

In 1999, the results of a large multicenter trial were published by Ober *et al*.[15]. They immunized 91 women with at least three miscarriages with paternal mononuclear cells and 92 women with sterile saline (control) and observed no statistically significant effects of treatment in primary or secondary recurrent aborters. Moreover, in the analysis of pregnant women, the success rates were even worse in the immunized group. They concluded that paternal lymphocyte immunization does not improve pregnancy outcome in women with RM, and that this therapy should not be offered as a treatment for RM. This report was quite sensational. The latest Cochrane review by Scott reached the current conclusion that lymphocyte immunization therapy provided no significant beneficial effect over placebo in preventing further miscarriage by analyzing 11 reliable studies including Ober's paper[16]. Several groups claim that there are a number of serious problems in this paper. Clark *et al*. points out the following problems. First, although the sample size was largest among previous RCTs with lymphocyte immunotherapy, the study size was still small. Second, patients with anti-nuclear antibodies (ANA) were not excluded. It was suggested in the RMITG study that immunotherapy of women with ANA and anticardiolipin antibodies could be harmful, and reduce the live birth rate. Women with positive ANA test might have other antiphospholipid antibodies such as antiphosphatidylethanolamine and/or antiphosphatidylserine antibodies, which would cause miscarriage per se. Third, there were 7 patients with abnormal karyotype abortuses in the immunized group. Since these pregnancies could not be modified by any medical intervention, if this population was excluded from the study, the results would be different. Clark *et al*. also mention the immunization method they used, and there are problems in cell number use for immunization, the storage of cells before immunization, and boosting. Since no clear conclusion has emerged, paternal lymphocyte immunization for the treatment of RM remains controversial[17].

Recently, the US Food and Drug Administration (FDA) agency issued a statement advising US clinicians that administration of such cells or cellular products in humans should only be performed as part of a clinical research project, and then only if an Investigational New Drug application is in effect. The adverse effects of alloimmunization are rare, but this therapy has the potential risks of transfusion reaction, graft-versus-host reaction, transmission of infection such as hepatitis B virus or HIV. Adverse neonatal outcome is also rare, but a case of neonatal alloimmune thrombocytopenia and intracranial hemorrhage in an infant whose mother received immunizations of paternal mononuclear cells has been reported[18]. Lymphocyte immunization therapy may have the potential risks of transfusion in the background that FDA announced the statement.

b. Paternal Lymphocyte Immunization Therapy in Japan

Clinicians in the U.S. currently have stopped this therapy since the FDA announced the statement. What is the situation in Japan? To address this question, we carried out a questionnaire survey of 80 domestic university hospitals in 2001. Surprisingly, approximately 70% of Japanese

Table 1 Protocols used for paternal lymphocyte immunization in Nippon Medical School

1. Indications
   a. Women with three or more consecutive spontaneous abortions with the same partner.
   b. Women with two recurrent spontaneous abortions with documented genetically normal fetus.
   c. Women with two recurrent spontaneous abortions with elevated peripheral blood NK cell activities.
2. Contraindications
   a. Women with more than one live birth with the same partner
   b. Women with malignant disease
   c. Women with positive APA, ANA
   d. Women with spouses who do not fit blood donor selection criteria
3. Protocol
   a. Lymphocytes are prepared by Ficoll-Paque centrifugation from husbands or partners and then 3,000 Gy irradiated. The cells are washed three times with sterile saline and resuspended in 1m*l* at a concentration of $4 \sim 7 \times 10^7$/m*l*. The cells are given intradermally 4 times at an interval of 2 ～ 3 weeks.
   b. The skin reaction at the injection sites is measured.
   c. If the skin reaction does not reduce after the 4th immunization, additional immunization is performed until it does.

university hospitals were still performing the paternal lymphocyte immunization therapy for the treatment of RM with alloimmune cause. The indication, patient selection, method of cell preparation and administration mode varied according to the hospital. In 2002～03, the committee for investigation of RM in Japan in the Japan society of Obstetrics and Gynecology performed a questionnaire survey of 229 randomly extracted fertility clinics regarding immunotherapy for RM. They found approximately 80% of clinics are performing immunotherapy. Furthermore, problems have emerged regarding the detailed analysis of the questionnaire. About 50% of clinics do not irradiate the cells used for immunization. A case of cutaneous graft-versus-host (GVH) reaction has been reported. In that case, it is not clear whether the cells for immunization were irradiated. Since non-irradiated cells may cause GVHD, lymphocytes prepared from the donor should be irradiated prior to administration.

The protocols used in Nippon Medical School are shown in **Table 1**. Overall protocols are similar to the protocols indicated by the clinical guidelines recommendation committee of the U.S. in 1997. As listed in **Table 1**, women with only 2 abortions are included if their peripheral blood NK cell activities are elevated. This indication is not listed in the U.S. protocols.

Secondly, we measured the diameter of erythemas (redness) at the injection sites as an indicator of the effectiveness of immunization. This is the reaction of delayed type hypersensitivity (DTH), which is a T cell reaction. When the diameter of erythemas is reduced after the 4th injection, the immunization is thought to be effective. In the US protocol, post-treatment testing is carried out to see if the anti-paternal lymphocyte antibodies are synthesized or not. Some Japanese university hospitals perform post-treatment testing using anti-paternal lymphocyte antibody production.

Conclusion

An adequate treatment for recurrent miscarriage may contribute to resolving the problem of the effect of the decreasing birthrate on Japanese welfare. Neither the inclusion criteria for immunization nor methods for judging the immunization effect, nor protocols of immunization, are unified in Japan. Is paternal lymphocyte immunization a relic of the past? Randomized controlled trials based on adequate patient selection in Japan should provide an answer to this question.

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