Review Article

Effective Treatment for Recurrent Pregnancy Loss

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Abstract:

Understanding the etiology of recurrent pregnancy loss (RPL) has been increasing in importance in reproductive medicine, especially in societies with low birth rates. Since the cause of human miscarriage is often complicated and multifactorial, effective treatment for the successful maintenance of pregnancy requires the comprehensive clinical management of recurrent wastages. Immunology for the maintenance of pregnancy is a very attractive field; however, the role of immunology during human gestation cannot be confirmed in couples with a history of recurrent pregnancy loss unless other clinical causes of miscarriage are especially ruled out.

Balanced chromosomal rearrangements have been found at an increased frequency in couples with recurrent pregnancy loss compared with the general population. The major anomalies are Robertsonian or reciprocal translocations, while most minor variants include pericentric inversion of chromosome 9. Both major and minor anomalies result in a spontaneous abortion rate of approximately 80-90%. Though the incidences of these chromosomal abnormalities vary in reported studies, a survey indicated an incidence of 9.8% (3.9% in male partners and 5.9% in female partners) among 1,639 couples with a history of recurrent pregnancy loss.

Congenital uterine anomaly resulting in a suboptimal uterine environment may influence ovum implantation and early fetal development and may be a candidate in the etiology of reproductive wastage.

A review on the relation between congenital uterine anomalies and the spontaneous abortion rate concluded that a precise diagnosis of uterine cavity deformity, especially arcuate uterus, is a key issue for determining the clinical prognosis during human gestation. After establishing diagnostic criteria for uterine cavity anomaly, 278 congenital uterine anomalies were detected in a series of 2,061 hysterosalpingographies (13.5%) performed in women with a history of recurrent pregnancy loss. Among these abnormalities, the incidences of spontaneous abortion were equally high among patients with arcuate uterus and those with partial septate uterus. A few studies on abortion mechanism in patients with congenital uterine anomalies have been reported. Histological findings using CD34-positive staining indicate the lack of a blood capillary system in both the uterine septum and the fundus muscle layer. The results of metroplastic surgery on congenital uterine anomaly are also controversial. Based on our fundamental findings of the maldistribution of uterine capillaries, we performed ideal metroplastic surgery in 173 women and significantly

reduced the spontaneous abortion rate from a pre-operative values of 93.2% to a post-operative value of 12.5%.

Anti-cardiolipin antibody was first described by Huges et al. as a pathogenic auto-antibody capable of causing recurrent pregnancy loss. Since then several other candidates for zwitterionic antiphospholipid antibodies to anionic phospholipids that are capable of inducing miscarriage such as phosphatidylethanolamine (aPE) and phosphatidylserine (aPS). In a multicenter study on antiphospholipid antibodies conducted at 15 institutes and hospitals between 2000 and 2002, significant levels of IgG and IgM for aPE were detected among women with a history of recurrent pregnancy loss. By establishing an enzyme-linked immunosorbent assay system, we determined that the IgG and IgM levels for aPE were significantly higher than those for other phospholipids antibodies during the first trimester recurrent spontaneous abortions. Coagulation factor XII, prekallikrein and the high molecular weight molecule kininogen have been reported as plasma contact proteins in the intrinsic pathway of the blood coagulation system. Deficiencies of these proteins are not associated with clinical obstetrical intrauterine bleeding, suggesting that these proteins possess anticoagulant and profibrinolytic activities. In patients with a precise diagnosis of these antibodies, antiphospholipid antibody syndrome has been effectively treated using oral low dose aspirin and/or heparin injection.

The beneficial effect of immunotherapy using the husband's or a third party's lymphocyte for the successful maintenance of pregnancy have been discussed for more than a decade yet still remain controversial. After ruling out other pathogenic etiologic factors for recurrent pregnancy loss, as discussed above, the beneficial effects of this form of immunotherapy have been revealed.

Spontaneous abortion is a common event and constitutes a major proportion of what has been referred to as pregnancy wastage[1]. Studies of various kinds of mammals have revealed that spontaneous abortion is an important selective mechanism for eliminating unfit products of gestation, since a high frequency of chromosomal errors has been consistently observed among abortuses. In general, habitual abortion is regarded as a sequence of three or more spontaneous abortions. Clinically the condition is defined as primary when all previous pregnancies have resulted in abortion, and as secondary when one or more pregnancies have proceeded to a period of fetal viability. In such reproductive wastage, the concept of a selective mechanism may not be applicable because many other etiologic factors other than chromosomal errors have been reported in these cases. An estimated 10-15% of known pregnancies terminate in spontaneous abortion; however, the incidence of three consecutive miscarriages in the same couple is extremely low, with a reported estimation of 0.34%[2]. Thus, couples with

a history of recurrent pregnancy loss (RPL) may have some specific etiologic factor or factors other than chromosomal errors in their fetuses. Although The American College of Obstetricians and Gynecologists has only approved two tests for the examination of RPL (parental chromosomal analysis and lupus anti-coagulant and anti-cardiolipin antibody assays[3]), other possible causes are comprehensively reviewed in this paper.

Chromosomal Abnormalities and RPL

Approximately 15-20% of recognized pregnancies end in a first-trimester spontaneous abortion. Many investigators have reported that chromosomal errors are common among such abortuses. In general, reproductive wastage is considered to be a random event for most women. However, in individuals with RPL, especially in the case of a sequence of three or more spontaneous abortions, the situation for the couple and their gynecologists becomes more serious, because

the possibility of success for subsequent pregnancies is very low.

Karyotypes are officially reported using the nomenclature developed by the International System for Human Cytogenetic Nomenclature (ISCN) in 1985. Using this standard nomenclature, chromosomal abnormalities in reproductive wastage have been clearly classified, as the causes of 50 % of embryonic death, 5 – 7 % of fetal losses, 6-11 % of stillbirth and neonatal deaths, and 0.9 % of live children[4,5]. The introduction of chromosome banding techniques has allowed the recognition of much smaller karyotype abnormalities and has demonstrated that a number of spontaneous abortions arise from the transmission of structurally aberrant chromosomes from the parents. Balanced chromosomal rearrangements have been found at an increased incidence in couples with RPL, compared with the general population[6,7]. The reported incidences of inborn chromosomal abnormalities in couples with a history of RPL are controversial and vary from 3 - 6 % [8,9]. By analyzing not only major chromosomal abnormalities resulting in translocations but also other chromosomal errors including mosaicism and chromosome inversion, Makino and his colleagues [6,7], reported a long-term study on 1,639 habitually aborting Japanese couples in a 22-year survey conducted between 1982 and 2004, and demonstrated incidences of chromosomal abnormalities of 3.9% among 1,635 male partners (63/1,635) and 5.9% incidence among 1,639 female partners (96/1,639) as shown in Table1. Chromosomal abnormalities are classified as major and minor variants. Table 2 shows the 72 (1.6-2.8%) of major chromosomal abnormalities (Robertsonian translocation and reciprocal translocation) that were detected and the 45 (1.0-1.7 %) of minor errors are most inversion (9), as well as 42 other abnormalities including mosaicism and triple X syndrome. Both major and minor abnormalities indicated approximately 80-90 % spontaneous abortion incidence of their past pregnancies in this study. On the other hand, prospective abortion rate in these abnormalities has reported with lower frequency of miscarriages[10]. Reciprocal or double segment translocations are a rearrangement of chromosomal materials in which breaks occur in two different chromosome and the fragments are exchanged before the breaks. Robertsonian translocation is another type of chromosomal translocation involving the long arms of two individual acrocentric chromosomes. The major chromosomal errors consist of these two translocations. No effective treatment for the prevention of subsequent miscarriage is available for these translocations. In many

Table 1 Incidence of chromosomal abnormalities in the couples with a history of recurrent pregnancy loss[7]

	total numbers examined	chromosomal abnormalities	%
male partners	1,635	63	3.9%
female partners	1,639	96	5.9%

Table 2 Different chromosomal abnormalities in both male and female partners with a history of recurrent pregnancy loss[7]

chromosomal abnormalities	wives(%) ¹⁾	husbands(%) ²⁾
Robertsonian Translocation	12(12.5)	2(3.2)
Reciprocal Translocation	34(35.4)	24(38.1)
Inversion 9	28(29.2)	17(27.0)
Others	22(22.9)	20(31.7)
Total	96(100.0)	63(100.0)

¹⁾n=1,639 , ²⁾n=1,635

cases with parental chromosomal abnormalities, genetic counseling is recommended for future pregnancies. Genetic amniocentesis and chorionic villi sampling have been proposed and prenatal genetic diagnosis (PGD) using cytogenetic analysis of fertilized ova during in vitro fertilization prior to embryonal transfer to the uterus could be another alternative, if ethical understanding of society could be obtained. Overall RPL arising from parental chromosomal error is clinically regarded as an inborn abnormality; however, it should be noted that intracytoplasmic sperm injection (ICSI) for the treatment of infertility may induce chromosomal abnormalities [11].

Congenital Uterine Anomaly and RPL

In women, uterine development starts during 4-5th week of gestation with the emergence of the metanephric ducts and their connection with the cloaca, followed by the union of the two Müllerian ducts during the 10th week of gestation. Fusion starts in the middle part of the conjugated area and extends caudally. By the 20th week of human gestation, the typical human uterine shape is completed as a result of cellular proliferation in the upper part of the original uterus and the dissolution of the cells in the lower part. Thus the fusion of the Müllerian ducts, which develop into a single reproductive tract in human, is induced at three different embryonal levels over three different periods of time. Therefore, a number of uterine malformations can easily be induced during these complicated developmental processes.

Clinically congenital uterine anomalies can be diagnosed using various procedures including

Fig.1 Classification for uterine cavity anomalies on hysterosalpingography [7,14-16]

Table 3 Incidence of congenital uterine anomaly in recurrent pregnancy loss

	n
Total Hysterosalpingography	2,061
Congenital Uterine Anomaly	278
Incidence	13.5%

hysterosalpingography, ultrasonography, hysteroscopy as well as magnetic resonance imaging. The reported incidence of congenital uterine malformation vary between 10-15 %, probably because of differences in the diagnostic procedures that were used[7,9,12,13-16]. Another possible explanation for this variation in reported incidences may be that many women with congenital uterine anomaly do not seek medical treatment, since most of them do not experience menstrual disorders after menarche. Conventionally, Jarcho's classification or a modified version is used to classify uterine cavity abnormalities[17]. However, the differentiation between the septate and the arcuate uterus is not clear in this classification or its modification[18]. As shown in Fig.1, Makino and his colleagues [7,14-16]. have been proposing, for last two decades, a new and practical classification for uterine cavity anomalies based on hysterosalpingography findings. To obtain precise diagnosis of the shape of uterine cavity, the longitudinal axis of the uterus and the surface of X-ray film should theoretically be

parallel during hysterosalpingography. The XM ratio proposed by Makino is an even more rational means of evaluating uterine cavity deformities, by revising the longitudinal length of the uterine cavity and fundus deformity on X-ray film. With their introduction of a ratio calculation, the position of a retroverted or anteverted uterus in the pelvic cavity can be easily revised. Makino et al. used the XM ratio to analyze the uteri of 2,061 women with a history of RPL and 278 congenital uterine anomalies (13.5%) in this group[7] (Table 3). A few studies have examined how congenital uterine anomalies may induce recurrent pregnancy loss, such as by limiting the space available for fetal growth in utero [19]. Vascularity [20], the suppression of enzymatic activity [21], and hormone responses of the endometrium[22] in subjects with congenital uterine anomaly have been also reported as important etiologic factors inducing recurrent reproductive wastage. However, another important result of Makino's study was that the frequency of spontaneous abortion was equally high in subjects with arcuate uterus and those with septate uterus [7,14-16](Table 4), thereby ruling out the space limitation hypothesis. Their results clearly demonstrated that the uterine vascular system was significantly poor in not only the uterine septum but also in the uterine fundus muscle layer of congenital uterine anomalies including cases with mild deformities, such as arcuate uterus[7,14-16]). The indications and efficacy of surgical treatment, such as metroplastic surgery, for uterine cavity deformity after recurrent pregnancy wastage are controversial, partly because of the lack of universal classification for congenital uterine anomalies and the lack of comprehensive clinical examinations for all possible etiologic factors capable of inducing reproductive wastage such as RPL prior to a discussion of the surgical indications. Furthermore, few cases of metroplastic surgery have been based on evidence of how this abnormality induces reproductive wastages. Upon completing their histological findings regarding congenital uterine anomalies, Makino introduced the idea of metroplastic surgery in which

Table 4 Pregnancy outcome in the couples with congenital uterine abnormalities

Shape of Ut.	n	total pregnancies	sp.abortions	full term deliveries
Arcuate Ut.	133	450	439(97.5%)	11(2.5%)
Septate Ut.*)	42	147	147(100 %)	0(0 %)
Septate Ut.	8	34	33(97.1%)	1(2.9%)
Unicornuate	5	14	12(85.7%)	2(14.3%)
Total	188	645	631(97.8%)	14(2.2%)

*partial septate uterus, Ut. : Uterus

Fig.2 Newly proposed metroplastic surgery based on basic histological findings

the endometrial tissue is reserved while the septum and uterine fundus muscle layer are completely resected and re-anastomosed (Fig.2). Using this new proposal for metroplastic surgery based on basic histological findings, the spontaneous abortion rate of 93.2 % (427 abortions out of 458 pregnancies) among 173 patients with congenital uterine anomalies was significantly reduced to 12.5 % of pregnancies after surgery (Table 5). This post-operative prognosis for pregnancy was also significantly better than that of a randomized un-operated group with uterine anomalies[16]. Transcervical resection (TCR) of the uterine septum by hysteroscopy is an attractive surgical treatment[23,24] 23, 24), however, the reported histological findings

indicate that the resection of the uterine fundus muscle layer with mal-distribution of the uterine vessels and re-anastomosis of the operated uterine fundus is incomplete with TCR.

Anti-phospholipid Antibody Syndrome and RPL

Before any discussion of RPL related to anti-phospholipid antibodies (aPL) or aPL syndrome is made, an overview of the physiological mechanism of blood coagulation and fibrinolysis is needed. Blood coagulation and fibrinolysis are two sides of the same coin in the normal blood coagulation pathway, and numerous factors play critical roles in several cascades. In normal pregnancy, this situation is shifted to overcome obstetrical bleeding. Therefore, the standard levels and values of these factors change during normal non-pregnant and pregnant periods. Based on these viewpoints, the concept of view that aPL and their related factors are only one part of the numerous factors influencing blood coagulation and fibrinolysis seems reasonable.

Harris *et al.* [25] first reported a reliable method for measuring anticardiolipin antibody and indicated a

Table 5 Pregnancy outcome pre- and post-metroplastic surgery in 173 cases with congenital uterine abnormalities

	Pre-surgery	Post-surgery
Total patients	173	173
Total pregnancies(%)	458(85.8)	112(50.9)
Average preg.number	3.09	1.27
Sp. Abortions (%)	1427(93.2)	14(12.5)
Full term deliveries %	1.3	63.4

Table 6 Various types of phospholipids

Negatively charged
Cardiolipin(CL)
Phosphatidylserine(PS)
Phosphatidylglycerol(PG)
Phosphatidylinositol(PI)
Phosphatidic acid(PA)
Neutral(twitterionic)
Phosphatidylethanolamine(PE)
Phosphatidylcholine(PC)
Sphingomyelin(SM)

Table 7 Phospholipids and corresponding binding proteins (cofactors)

Phospholipids	aPL	cofactors
PE	aPE	hK IK
PS	aPS	β_2 GP1
CL	aCL	β_2 GP1
Neg.charged PL	LA	prothrombin β_2 GP1

hK:high molecular weight kininogen,
IK:low molecular weight kininogen,
LA:lupus anticoagulant

Table 8 Anti-phosphatidylethanolamine antibodies in 139 patients with recurrent early (embryonic stage) pregnancy losses and 200 normal controls[26]

Ig	Independent aPEs		Dependent aPEs		Independent& dependent aPEs		total	
	RPL	controls	RPL	controls	RPL	controls	RPL	controls
IgG	7(5.0)	8(4.0)	16(11.5)	7(3.5)	5(3.6)	1(0.5)	28(20.1)	16(8.0)
IgM	8(5.8)	10(5.0)	8(5.8)	6(3.0)	1(0.7)	0(0.0)	17(12.3)	16.8(8.0)
IgA	2(1.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.4)	0(0.0)

$P < 0.001$
 $p = \text{NS}$
 $p = \text{NS}$

possible role of this antibody in general thrombosis and reproductive wastage. Since then, their findings have placed a promising spotlight on the diagnosis and treatment of RPL. However, an unfortunate side effect of the numerous investigations on anticardiolipin antibody is that for more than a decade, the majority of basic research and clinical attention has focused only on anti-cardiolipin antibody, and patients with other aPL have remained undiagnosed and untreated. Since the end of the last century, reports on the detection of other autoantibodies reactive to anionic phospholipids in patients with vascular thrombosis, thrombocytopenia and RPL have been gradually accumulating. Studies have also clarified that pathogenic aPL recognize phospholipids and/or epitopes on phospholipid-binding proteins (cofactors). To date, as shown in Tables 6 and 7, several phospholipids other than cardiolipin have been identified in the cell membrane, suggesting that autoantibodies against other phospholipids or contact proteins (cofactors) can be generated and may play similar important roles in blood coagulation and/or fibrinolysis. Of these, the zwitterionic phospholipid, phosphatidylethanolamine (PE) is a major component of both the outer and inner surfaces of the cell membrane and Sugi et al. [26] reported a significant relation between RPL and the anti-PE antibody (aPE) and its binding protein, kininogen. The authors indicated that

the IgG level of aPEs was significantly higher among 139 women with recurrent early pregnancy loss than among 200 normal control subjects (Table 8). Since kininogen-dependent IgG of aPE augments thrombin-induced platelet aggregation in vitro [27], kininogen likely binds to platelets and inhibits thrombin-induced platelet aggregation. The authors hypothesized that kininogen-dependent aPE may cause thrombosis in vivo as a result of the disruption of the physiological antithrombotic effects of kininogen. aPE was subsequently confirmed to recognize the kininogen domain 3 regions [28].

Early embryonic development requires a sufficient maternal blood supply through the placenta and disturbances in this flow can easily lead to embryonic mal-development or miscarriage. Therefore, thrombosis induced by aPL is a reasonable explanation for early RPL. However, histological placenta specimens obtained from patients with aPL syndrome exhibit various kinds of pathological changes, including inflammation and tissue degeneration as well as capillary thrombosis (Fig.3)[7]. Velayuthaprabhu *et al.* [29] immunized mice with a mixture of cardiolipin and β_2 GP1 and obtained a poor pregnancy outcome in immunized mice by facilitating thrombotic episode in the placenta. They concluded that one possible mechanism of stillbirth as a result of anti-cardiolipin antibodies (aCL) was the induction of thrombotic events in the placenta. In

Fig.3 Pathological changes of capillary thrombosis (A), and inflammation and tissue degeneration (B) in the placenta with aPL syndrome

addition to thrombosis, Halperin *et al* [30]. reported that the injection of human aPL positive sera into the uterus of pregnant rats rapidly and apoptotically destroyed intrauterine embryos, suggesting the existence of a novel factor in the aPL positive serum unrelated to aPL that exerts a detrimental effect to the conceptus without any relation to the aPL. Mulla *et al* [31] demonstrated that aPL triggers a placental inflammatory response via the Toll-like receptor 4/ myeloid differentiation factor 88 (TLR-4/MyD88) pathway, compromising trophoblast survival. D'Ippolito *et al* [32] also demonstrated that anti- β_2 -GP1 antibodies induced the activation of endothelial cells resulting in a proinflammatory state favoring the prothrombotic diathesis of this disease.

As indicated at the beginning of this chapter, numerous factors and cascades are involved in the mechanism of blood coagulation and /or fibrinolysis. In the fibrinolysis pathway, coagulation factor XII, prekallikrein and high molecular weight kininogen are known as plasma contact proteins in the intrinsic pathway of blood coagulation. However, deficiencies in these proteins are not associated, with clinical bleeding despite a marked prolongation of the *in vitro* surface-activated coagulation time, suggesting that these proteins possess anticoagulant and profibrinolytic activities. Therefore, a decrease in factor XII and kininogen-dependent aPE can cause thrombosis by suppressing the fibrinolytic pathway. The coagulation factor V Leiden mutation is physiologically suppressed during pregnancy by protein S and activated protein C; thus, this cascade is also involved in the blood coagulation pathway. Besides the pathological effects of aPL on blood coagulation and/or fibrinolysis, these antibodies are frequently detected in women with

recurrent *in vitro* fertilization-embryonal transfer (IVF-ET) failure[33]. The precise role of aPA in this mechanism by remains to be elucidated.

The pathophysiological immune state may generate not only aPL but also various kinds of other autoantibodies. For example, significantly higher levels of anti-saccharomycetes cerevisiae antibodies (ASCA) [34] and anti-Elastin antibodies [35] have been reported in women with a history of recurrent pregnancy loss.

The obstetrical management of women with aPL syndrome remains controversial. Despite recent advances in this clinical field, the ongoing controversies have been fuelled by our limited understanding of the multifactorial causes of aPL-associated pregnancy loss, as mentioned as above. A review of the reported results suggests that either anticoagulant and/or profibrinolytic agents should be at the core of obstetrical treatment. Rai [36] reported that heparin treatment should be a standard management for patients with RPL as a result of aPL syndrome and the author of the present review has reported that low dose aspirin treatment enabled a successful pregnancy maintenance rate of 64.7% success in the maintenance of pregnancy with aPL syndrome, while combination therapy with low dose aspirin and heparin injection yielded a successful pregnancy maintenance rate of 92.9% [7]. Administration of adrenal corticosteroids was once the treatment for RPL; however, steroid therapy can lead to several complications including premature labor, a low birth weight, and pregnancy induced hypertension as well as gestational diabetes. Furthermore, Laskin [37] indicated no significant difference between low dose aspirin administration and corticosteroid treatment with regard to the successful maintenance of pregnancy. A fourth choice for the treatment of RPL

with aPL is the administration of human IgG. Anti-phospholipid syndrome not only has pathological effects on pregnancy, but also is involved in the induction of severe ischemic effects on vital organs including the cerebral and cardio-vascular systems. Therefore, life-time management is essential for women with aPL.

Fetomaternal Immunology and RPL

Fetomaternal immunologic interactions are undoubtedly a key component into the successful maintenance of pregnancy. The survival and development of embryos and fetuses in the uterus in the presence of complicated immune networks is miraculous. Over the past half century, immunologists have discussed the survival of the semiallogenic conceptus in utero from the standpoint of immune suppression [38] and/or immune tropism [39]. Other discussions have focused on three different immunologic aspects: fetal, maternal and fetomaternal immunity. Several reviews have focused on each of these aspects with the goal of explaining immune mechanism of gestation. Through these discussions, it has become evident that human gestation occurs against a background of fragile and complicated immune networks.

Medawar [40] suggested that the fetus and placenta are immunologically inert and neutral, preventing the induction of a maternal immune response. Subsequent studies have indicated that major histocompatibility complex (MHC) antigens are unique markers as heteroallograft and human leukocyte antigen (HLA) are human analogue of the major histocompatibility complex. Weetman [41] reported that MHC class I and II antigens are not expressed on villous trophoblasts during any period of human gestation; consequently, the maternal immune networks do not recognize the conceptus as a semiallograft. The HLA are genes produced in MHC located on the short arm of chromosome 6. HLA class I consists of 17 different genes. Among them, HLA-A, -B and -C encode the major class Ia transplantation and HLA-E, -F and -G encode class Ib HLA antigens. HLA-G is expressed only in humans. This gene is expressed only in the extravillous cytotrophoblasts of decidual tissue but not in villous trophoblasts [42].

As indicated in the series of Wegmann's studies [39], immunological recognition, or immune tropism, is vital for the successful maintenance of pregnancy, with inadequate stimulation by a semiallograft possibly resulting in reproductive wastage. Many immunologic

studies have been based on this theory.

Natural killer (NK) cells, a type of lymphocyte produced in the bone marrow, are fundamentally involved in the immune response, specifically in cytotoxic actions. Two different subsets of human NK cells are classified according to the cell surface density of CD56. Most human NK cells are classified as CD56^{dim} and secrete low concentration of NK-derived cytokines. A minority of NK cells (approximately 10%) are classified as CD56^{bright}CD16^{dim/neg} NK cells and produce ample amounts of cytokines compared with CD56^{dim}. Using flow cytometry, NK populations can be precisely analyzed and classified [43]. The numbers of NK cells usually fluctuate in relation to endogenous hormone levels, physical exercise, psychological stress and many other factors. Therefore, the standard values for NK cell populations remain controversial. The dysregulation of NK cells in some obstetrical complications including RPL has been reported [44]. NK cells play an important role in both preventing the destruction of normal cells and killing target cells. These activities represent a fundamental action of immune tolerance exhibited by NK cells during pregnancy. Though the mechanism responsible for the immune tolerance of killer cells, especially uterine NK cells, to the conceptus in the uterine environment has been gradually clarified, the details remain controversial. Lachapelle [45] and Clifford [46] reported that the number of CD56^{dim}CD3⁻CD16⁺ NK cells is elevated in the uteri of women with RPL. The majority of NK cells related to alloresponses have been shown to express inhibitory receptors for self MHC-I [47], suggesting that NK cell licensing is relevant to observations in women with RPL.

Cyclic changes in NK cell populations have been observed in the endometrium suggesting hormonal interactions with immune cells [48]. Progesterone is a vital ovarian/placental steroid hormone during pregnancy, exerting luteotropic actions. Bulmer [48] demonstrated that alterations in the NK cell population paralleled local progesterone levels and Mori [49] summarized the progesterone and cytokine interactions in the endometrium during the pre-implantation period, shown in Fig.4. Estrogen, another important steroid hormone during pregnancy, also introduces an implantation window in collaboration with cytokines (Fig.5).

Cytokines may be viewed as an immunologic conversation tool between a mother and her fetus. Immune cell functions and many networks during human pregnancy depend on the secretions of various kinds of cytokines from three different immune cells,

Fig.4 Progesterone and cytokine interaction in the endometrium [49]

Fig.5 Estrogen and implantation window [49]

NK cells, macrophage and T cells in the endometrium. The role of cytokines in the successful maintenance of pregnancy and in RPL has been suggested by various studies [50-51]. T helper lymphocytes can be classified into 2 cell types, Th1 and Th2, according to their differences in cytokine production. In women, a Th2 dominant shift has been reported during normal pregnancy, decreasing Th1/Th2 ratio, while a Th1

dominant immune response has been detected in complicated pregnancies including RPL [52-53]. Summarizing the reported results for helper T cells and the secretion of cytokines, Th1 cells generate IFN- γ , TNF- α and IL-2 as pro-inflammatory cytokines, while Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-135 [54-55] as anti-inflammatory agents. Although information on cytokine gene polymorphisms related to

RPL has been accumulating, much of the reported data is inconsistent and remains controversial [56].

While basic research findings concerning immune network during human gestation have been accumulating and have revealed new areas of immunological study, an important target of immunology continues to be the development of clinical treatments for recurrent pregnancy losses. Without the clinical application of immunology and its beneficial effects, patients with recurrent pregnancy losses are likely to remain untreated. Immunotherapy using paternal lymphocytes and its beneficial effects on RPL have been discussed for decades. Through these debates, immunization with both paternal lymphocyte [57,58] and pooled allogeneic lymphocytes [59,60] have apparently improved the successful pregnancy rate; in one double-blind controlled study, a significant increase in successful pregnancy outcomes was reported among women who had been immunized with their husband's cells compared with women who had received autologous lymphocytes [58]. These early studies in the 1980s were subsequently confirmed by multicenter studies [61-62]. Despite these affirmative investigations of immunotherapy, however, the efficacy of this treatment remains controversial [63-65], mainly because of differing patient characteristics among the studies. A survey of the numerous studies that have examined immunotherapy clearly shows that the pre-treatment examinations, indications and immunotherapy procedures, and the evaluation of immunization outcome are too varied for a direct comparison. In short, patient selection is a key factor in elucidating the efficacy of immunotherapy. Makino [7] conducted immunotherapy in a relatively large group of patients (n=482) after screening for all other possible etiologic factors related to miscarriages and infectious diseases in the partners. A total of 4×10^7 irradiated leucocytes were injected intradermally and this procedure was repeated four times at intervals of 2-4 weeks. Throughout the course of immunization, the immune states of the immunized female partners were monitored using flow

cytometry [43]. By strictly maintaining these clinical procedures, a beneficial effect of immunotherapy was revealed as shown in Table 9.

Linking these complicated immune networks and their functional failure with human miscarriage, especially RPL, is a complex task. However, before diagnosing an immunologic etiology in couples with a history of RPL, excluding all other etiologic factors including chromosomal abnormalities, congenital uterine anomalies, and antiphospholipid antibody syndrome is essential, as discussed in the preceding chapters. Otherwise identifying a definite immunologic factor from among the many complicated immune networks in gestation as the true cause of RPL would be impractical.

Conclusion

A precise diagnosis of the etiologic factors of recurrent pregnancy loss is essential for the successful maintenance of pregnancy, and appropriate treatment should be selected after comprehensive examination of reproductive medicine has been completed.

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Table 9 Pregnancy outcome after immunotherapy[7]

Total immunized women	482
Pregnant	504
Live birth	322
On-going pregnancy	34
Miscarriages(%)	143 (30.8)
Ectopic pregnancy	5
Non-pregnant	119

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