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REVIEW

Manoj Kumar Pandey · Reena Rani · Suraksha Agrawal

An update in recurrent spontaneous abortion

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Abstract Recurrent spontaneous abortion (RSA) is defined as three or more consecutive pregnancy losses prior to the 20th week of gestation. The etiology of recurrent spontaneous abortion is often unclear and may be multifactorial, with much controversy regarding diagnosis and treatment. Reasonably accepted etiologic causes include, genetics, anatomical, endocrine, placental anomalies, hormonal problems, infection, smoking and alcohol consumption, exposure to environmental factors, psychological trauma and stressful life event, certain coagulation and immunoregulatory protein defects. Detection of an abnormality in any of these areas may result into specific therapeutic measures, with varying degrees of success. However, the majority of cases of RSA remains unexplained and is found to be associated with certain autoimmune (APA, ANA, ACA, ATA, AECA) and alloimmune (APCA, Ab2, MLR-Bf) antibodies that may play major role in the immunologic failure of pregnancy and may lead to abortion. Alteration in the expression of HLA-G molecules, T-helper-1 (Th-1) pattern of cytokines and natural killer (NK) cells activity may also induce abortion. Various forms of treatment like antithrombotic therapies such as aspirin and heparin, intravenous immunoglobulin (IVIg) therapy, immunotherapy with paternal lymphocytes and vitamin D3 therapy are effective mode of treatment for unexplained cause of fetal loss in women with RSA.

Keywords RSA (Recurrent spontaneous abortion) · Lymphocyte immunotherapy · Immunoglobulin (IVIg) therapy · Aspirin/Heparin therapy · 1α · 25 -dihydroxy-vitamin-D3 (VD3) therapy · APA (anti-phospho lipid antibody) · ANA (anti-nuclear antibody) · ACA (anti-cardiolipin antibody) · ATA (anti-thyroidantibody) · AECA (anti-endothelial cell antibody) · APCA (anti-paternal cytotoxic antibody) · Ab2 (anti-idiotypic antibody) · MLR-Bf (mixed lymphocyte reaction blocking antibodies) · Natural Killer (NK) cells · HLA-G · T-helper −1(TH-1) and T-helper −2(TH-2) cytokines

M. K. Pandey (✉)
Division of Molecular Immunology,
Cincinnati Children's Hospital Medical Center,
MLC 7021 TCH RF 5503 3333 Burnet Avenue,
Cincinnati, OH 45229-3039, USA
E-mail: Manoj.Pandey@cchc.org
Tel.: +1-513-6369408
Fax: +1-513-6365355

R. Rani (✉)
Department of Biology, University of Texas,
San Antonio, TX, USA
E-mail: Reena.Rani@utsa.edu

S. Agrawal (✉)
Department of Medical Genetics,
SGPGIMS, Lucknow, India
E-mail: suraksha@sgpgi.ac.in

Introduction

Recurrent spontaneous abortion (RSA) is usually defined as the loss of three or more consecutive pregnancies prior to 20–28 weeks of pregnancy [46, 68]. It affects up to 5% of fertile couples [44]. RSA can be classified into primary recurrent spontaneous aborters and secondary recurrent spontaneous aborters. Primary recurrent spontaneous aborters are those who have lost all previous pregnancies and have no live birth. Secondary recurrent spontaneous aborters are those who have at least one successful pregnancy irrespective of the number of pregnancies losses. Epidemiological studies suggest that the risk of subsequent pregnancy loss is approximately 24% after two clinical pregnancy losses, 30% after three and 40% after four consecutive spontaneous abortions [158]. In the vast majority of the cases, the etiology is unknown and several hypotheses have been proposed on the basis of available data. The causes could be chromosomal [165], genetic [191], anatomical [201], endocrinological [40], placental anomalies [99], infection [113], smoking and alcohol consumption

[68], exposure to environmental factors such as lead, mercury, ethylene oxide and ionizing radiations [143], and stress factors [187]. In addition to these certain autoimmune [204] and alloimmune factors [53] may also play major role in the immunologic failure of pregnancy in women with RSA. The immunological relationship between the mother and the fetus is a bi-directional communication determined on one hand by fetal antigen presentation and on other hand by recognition and reaction to these antigens by the maternal immune system. Immunological recognition of pregnancy is important for the maintenance of gestation. Inadequate recognition of fetal antigens or increased sharing of human leukocyte antigens with the father may inhibit the production of anti-HLA antibodies. Several investigators reported that decreased expression of antipaternal cytotoxic antibodies (APCA) [127], antiidiotypic antibodies (Ab2) [83] and mixed lymphocyte reaction blocking antibodies (MLR-Bf) [3] might lead to abortion in women with RSA. In addition, alteration in expression of HLA-G molecules [7], T-helper-1 (Th-1) pattern of cytokines [103] and natural killer (NK) cells cytotoxicity [10] may also induce abortion in women with RSA. Various forms of treatment like antithrombotic therapies such as aspirin and heparin [31], intravenous immunoglobulin (IVIg) therapy [65], lymphocyte immunization with paternal lymphocytes [129] and recently used 1alpha, 25-dihydroxy-vitamin-D3 (VD3] therapy [26] are effective treatments for alloimmune cause of RSA. Their effects are attributed to the appropriate induction of humoral factors [192], shift from Th-1 to Th-2 status [30] and result into significant inhibition in NK cell activity in women with RSA [98].

Uterine anomalies

Congenital uterine anomalies like septate uterus, bicornuate uterus and uterus didelphys resulting from incomplete fusion of Mullerian ducts are commonly associated with RSA [63]. A higher incidence [38%] of cervical incompetence was detected in women with a bicornate uterus [62]. The reported incidence of anatomic uterine defects range from 1.8 to 37.6%, and include cases of late abortion, immature delivery and minor mullerian (hypoplastic and arcuate uterus) defects [2]. The selection for surgical correction (metroplasty) must be individualized only in women with repeated late trimester losses or prematurity, because cervical cerclage is the primary choice of therapy for a bicornuate uterus. Among all major mullerian defects, a didelphic uterus yields the best prognosis [1]. The removal of intrauterine adhesions by hysteroscopic resection may benefit patients suffering with RSA [63]. The most commonly abnormalities noted on the hysterogram include a small T-shaped uterus with cavity constrictions [20]. Hysteroscopic lateral metroplasty may decrease pregnancy loss in women with RSA [122]. Cervical incompetence due to sudden rupture of membranes followed by painless miscarriage is one of the frequent causes of second trimester miscarriage. Cervical cerclage is the recognized treatment in these cases. However, its effectiveness is unclear [180].

Chromosomal abnormalities

Several studies investigated that most of the abortion in women with RSA may be due to de-novo numerical abnormalities, in particular autosomal trisomies for chromosomes 13, 14, 15, 16, 21 and 22, followed by monosomy X [70, 71, 178, 183]. Abortion in women with RSA may also be due to recurrent chromosomal anomalies on the fetus as a result of balanced aberration in one of the parent inherited by the offspring in an unbalanced form. The parental chromosomal aberration might be either a structural anomaly reciprocal or robertsonian translocations, or mosaicism for numeric aberrations. Recent studies have demonstrated extremely high ($>90\%$) skewed X-chromosome inactivation (the preferential inactivation of one of two X chromosomes in female cells) among women who experienced RSA [17, 196].

Carp et al [33] performed karyotyping in series of 125 embryos from women with RSA and found chromosomal aberrations in 36 cases only. Of these, two were structural anomalies that could have been inherited, and the other 34 (94%) were trisomies that might have occurred by chance. Approximately 29–60% of abortion in women with RSA was due to chromosomal aberrations of the embryo [23, 33, 125]. However, Carp et al [32] found a higher prevalence (10.8%) of parental chromosomal aberrations than found in literature (3–5%) [49]. Hence American colleges of Obstetrics and Gynecologists [8] and Royal colleges of Obstetrics and Gynecologists [164] officially recommended to karyotyping both maternal and paternal chromosomal aberrations in RSA couples. Fetal karyotyping is also recommended to determine if the incidence of fetal chromosomal aberrations is maternally or paternally derived. The introduction of fluorescence insitu hybridization (FISH) has enabled the chromosomal assessment of embryos. Various studies also suggested the initial applications of preimplantation genetic diagnosis (PGD) to prevent the unbalanced transmission of parental balanced translocations [41, 120, 172] that may useful to improve maternal age [86] and abortion in women with RSA [165].

Thrombophilic disorders

A successful implantation during pregnancy requires a balanced equilibrium between coagulation, fibrinolysis and vascular remodeling by the process of angiogenesis in order to avoid excess fibrin accumulation in placental vessels and intervillous spaces [28]. However, thrombosis in decidual vessels is reported to be one of

the major causes of RSA [13] and could be explained by excessive thrombosis of the placental vessels, placental infarction, and secondary uteroplacental insufficiency. Proposed mechanism include (i) inhibition of thrombolytic system, placental thrombosis and infraction due to deficiencies in the coagulation factors, protein C, protein S, antithrombin III [146, 171], fibrin stabilizing factor II (FXII) [24, 126] and fibrin stabilizing factor III (FXIII [9] (ii) mutations and polymorphism in several other procoagulatory factors such as plasminogen activator inhibitors-1 (PAI-1), plasminogen activator inhibitors-2 (PAI-2), coagulation factor V (FV) or prothrombin (FII) [93, 169, 207], angiotensin I–converting enzyme (ACE) [27].

The most common inherited thrombophilias are heterozygosity for the factor V Leiden (FVL) mutation (G1691 A) [25, 50, 57, 64, 109, 140, 161] and prothrombin gene mutation (*FII*, G20210) [144, 203] However, several studies did not find any relation between abortin and factor V Leiden mutation and Prothrombin gene polymorphism (G20210) in women with RSA [95, 132, 140, 151, 175]. A recent meat-analysis on the relationship between thrombophilia and fetal loss in 31 studies revealed that factor V Leiden mutation was associated with early and late recurrent fetal loss. However prothrombin G20210A mutation was only found to associated with early recurrent fetal loss, no association between methylenetetrahydrofolate (MTHFR) mutation and fetal loss has been reported [159]. Thus there is a weak association between thrombophilia and RSA, however, presumed relationship between thrombophilia and RSA has become sufficient to allow the presence of thrombophilias to be an indication for treatment with anticoagulant drugs [208].

Endocrinological abnormalities

Endocrinological abnormalities associated with RSA are polycystic ovary syndrome (PCOS), luteal phase defect, thyroid dysfunction and diabetes mellitus [156]. PCOS is essentially a problem of ovarian hyperandrogenism, probably resulting from a primary abnormality in androgen biosynthesis [80]. Hyperinsulinemia and obesity are common features. On ultrasound examination, the incidence of PCOS is very high in-patient with RSA, ranging from 44 to 82% [195]. Hyper-secretion of luteinizing hormone (LH) occurs in women with this syndrome. Increased LH secretions may have adverse effects on the developing oocyte or endometrium either directly or indirectly by causing an elevation in the levels of testosterone and estrogen [199]. Women with PCOS have a 25–40% increased risk of abortion [22]. The best management option is induction of ovulation by boosting follicle stimulating hormone (FSH) [80]. The luteal phase defect in women with RSA is characterized by inadequate endometrial maturation that results in a qualitative or quantitative disorder in corpus luteum function, characterized by inadequate progesterone production. However, its significance in RSA has been questioned because there are no accurate methods for its diagnosis, and no convincing evidence of correction with treatment [22]. The best and viable diagnostic test is late luteal phase endometrial biopsy, but it is invasive, uncomfortable and expensive [180].

Coulam and Stern [43] proposed progesterone therapy either as a vaginal suppository or an intramuscular injection. However, other treatment protocols include clomiphene citrate and gonadotrophins. Thyroid dysfunction and diabetes mellitus are other traditionally quoted causes of RSA, but their contribution to RSA is not clear [43, 87]. In one series, women with thyroid autoantibodies had an abortion rate of 17% compared to a control rate of 8.4% [176]. However, in insulin-dependent diabetic women the incidence of miscarriage in early pregnancy is 15% (with good glycemic control) and 45% (with poor control) [180]. Higher pregnancy loss in women with poor glycemic control may be due to the embryotoxic effects of hyperglycemia. However, glucose tolerance is warranted for women with RSA of second or third trimester pregnancy loss or with clinical signs of diabetes mellitus [43].

Microbial infections

Various infections may cause RSA, in second trimester of pregnancy but the role of infection in first trimester RSA is still controversial [115]. Since the main route of infection to the placenta and fetus is from the vagina and cervix, most studies have attempted to find an association between RSA and abnormal bacterial flora in the lower genital tract, vagina, and cervix. *Chlamydia trachomatis* could provoke abortion in women with RSA because of a strong immunological reaction to the specific bacterial protein [202]. Moreover, *C. trachomatis* infections of the cervix have been associated with second trimester abortions and premature membrane rupture [108]. Infections of mycoplasma [174], human cytomegalovirus [177], adeno-associated virus (AAV) [21] and human papillomaviruses (HPV) [76] in pregnant women are the main causes of RSA. However, recent findings of Matovina et al. [113] revealed that there is no role of *C. trachomatis*, *Ureaplasma urealyticum*, *Myeoplasma hominis*, HCMV, or AAV infections in women with RSA during first trimester of pregnancy.

Maternal diseases

Maternal diseases are associated with an increased risk of fetal loss. These include very rare connective tissue disorders such as marfan syndrome, ehler-danlos syndrome and pseudozanthoma elasticum. Hematological disorders like sickle cell anemia, dysfibrinogenemia, congenital hypofibrinogenemia, afibrinogenemia, Wilson's disease and hyperhomocysteinemia may also cause recurrent miscarriages in the mother [170]

Male factors

Hyperspermia (sperm count more than 250 million/ml) and oligospermia (sperm count less than 20 million/ml) are frequently associated with miscarriages. In a study by Buckett et al. [29] a hypo-osmotic swelling test score was found to be significantly lower in semen samples from men whose partners experienced unexplained first trimester recurrent abortions.

Toxic factors

The environmental toxins can cause abortions such as certain chemotherapeutic drugs and colchicine may cause recurrent miscarriages. Smoking, alcohol and coffee consumption have little effect on women with RSA. Only heavy users of these substances are at an increased risk for abortion. There is a clear statistically significant association with coeffe intake, cigarette smoking, alcohol consumption and abortion [14].

Psychological factors

Various studies have reported that immunological functions during pregnancy in women with RSA are affected by various psychological and stressful life events [81, 187]. Couples experiencing RSA are often frustrated and emotionally disturbed. Those caring for them must recognize the agony involved and deal with the situation. Recently, Arc et al. [12] correlated the high stress score and significant elevation in the numbers of $CD8^+$ T cells/activated mast cells in decidua of women with sporadic spontaneous abortion. They hypothesized that these activated immune cells induced the secretion of Th1 cytokines' which trigger the process that lead to vasculitis, affecting the maternal blood supply to the embryo. This is speculated to be a major abortion causing mechanism in depressed women with RSA. However, ''tender loving care'' has been reported to play an important role in the maintenance and success of pregnancy rate in women with RSA [181].

Autoimmune factors

Autoimmune factors represent the immunologic response of the mother to a pregnancy (self-immune problem) that can cause fetal rejection in 30% of women with RSA. Various autoantibodies that can cause abortion in women with RSA are anti phospholipid antibodies (APA) [204], antinuclear antibodies (ANA)[55], anti thyroid antibodies (ATA) [30], and anti endothelial cell antibodies [163] (Fig. 1). Recently Yamada et al. [204] have reported different types of anti phospholipid antibodies (Lupus anticoagulant, Anticardiolipin β2-glycoprotein IgG/IgM/IgA, anticardiolipin IgG/IgM/IgA, antiphosphatidylserine prothombin IgG/IgM, antiphosphatidylethanolamine IgG/IgM) in women with RSA. However, Benito et al. [19] and Malinowski et al. [106] reported a higher prevalence of anticardiolipin antibodies in women with first trimester pregnancy loss.

Phospholipid molecules are normal components of cell membranes and act like a glu that holds the dividing cells together, which is necessary for growth, and development of the placenta. The production of antibodies to phospholipid molecules may inhibit the development of placenta at the materno-fetal interface. These antibodies themselves do not cause miscarriage but can specifically damage the inner wall of the blood vessel, which allows blood cells to stick to the site of the injury and cause blood clot formation. The combination of blood clots and constricted blood vessels may impair blood supply to the fetus and placenta resulting in complete fetal demise or growth retardation in women with RSA [186, 197] (Fig. 1). Women with RSA of unknown etiology have a higher incidence of ANA [55] which indicated that there may be an underlying autoimmune process that affects the development of the placenta and can lead to early pregnancy loss. Histones are proteins, which combine with the DNA of the cell nucleus to govern the development of tissues. Antibodies to these histones mean the mother is developing immunity to histone components of DNA but the mechanism by which ANA can cause pregnancy loss is still not known.

Women with thyroid antibodies double their risk of miscarriage as compared with women without these antibodies [30]. Increased levels of thyroglobulin and thyroid microsomal (thyroid peroxidase) autoantibodies are associated with an increased miscarriage rate, and as many as 31% of women experiencing RSA are positive for one or both antibodies. Risk of fetal loss increases to 20% in the first trimester of pregnancy and there is also an increased risk of post-partum thyroid dysfunction. Therefore, antithyroid antibody should be routinely tested in women with a history of two or more losses of pregnancy who show thyroid abnormalities. During implantation, the trophoblast mononuclear cells infiltrate and surround the utero placental vessels while establishing the maternal blood supply to the fetus [141]. The cause of this infiltration is not known, but the migration of endovascular trophoblast may be inhibited by anti-endothelial cell antibody in women with RSA [163].

Alloimmune factors

The success of pregnancy in the face of potential maternal immune reactions has been largely attributed to the placenta, which appears to serve as an immunological barrier. The ability of the trophoblastic tissue to survive in the conditions of allograft rejection was initially attributed to its non-antigenic nature. But later it

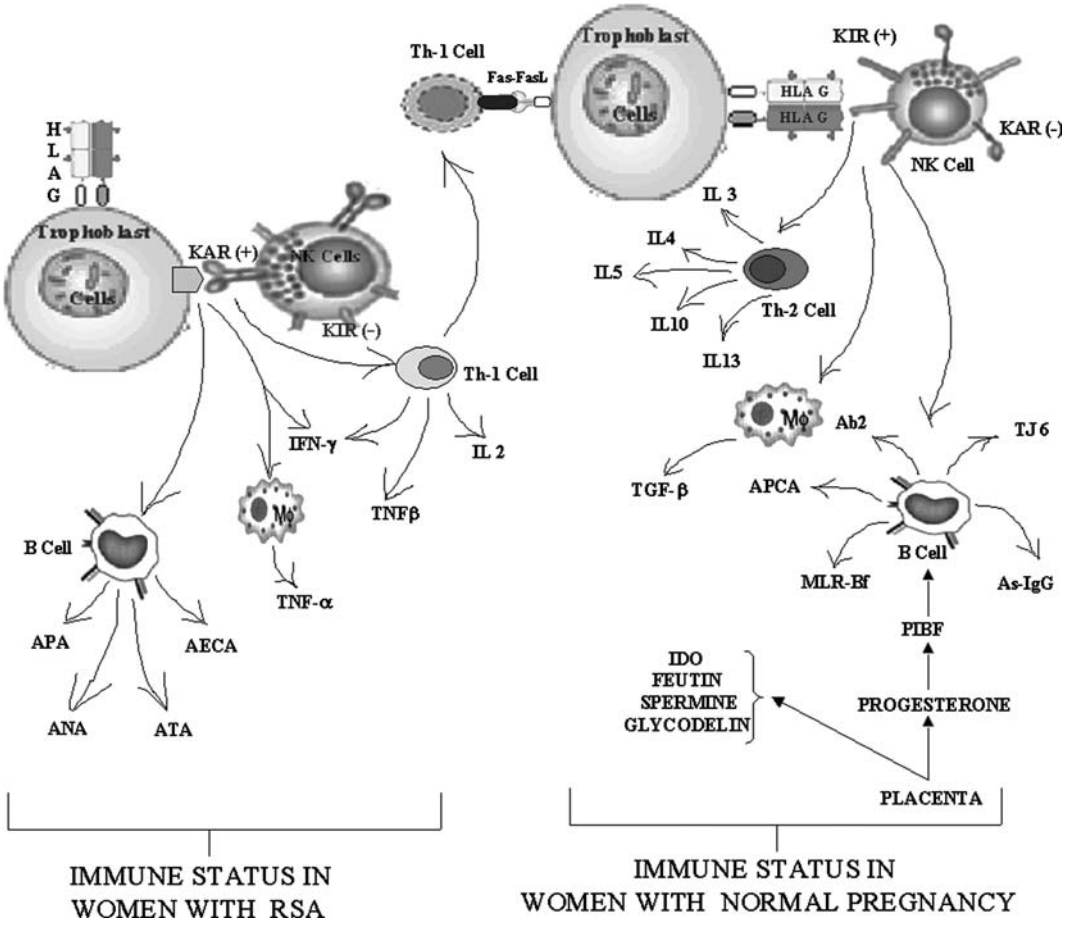

Fig. 1 Abortogenic and anti-abortogenic events in recurrent spontaneous aborters and women with normal pregnancy: During pregnancy HLA-G peptides are recognized by killer inhibitory receptor (KIR) of NK cells. This interaction down regulate the NK cell function by blocking its cytotoxicity, suppressed T-cells activity for production of Th-1 cytokines (TNF-α, TNF-β, IFN-γ, IL-2], inhibit the B-cell activity for expression of auto antibodies (APA, ANA, ATA, AECA) as well as, inhance B-cell function to produce alloantibodies (APCA, Ab2, MLR-Bf) and T-cell function to produce Th-2 cytokines (IL-3, IL-4, IL-5, IL-10, IL-13, TGF-β). However, HLA G peptides crosslinking with KAR of NK cells, induces the expression of Th-1 cytokines, autoantibodies and NK cell cytotoxicity as well as inhibit the expression of alloantibodies and Th-2 cytokines. Certain placental factors (IDO, spermine, feutin, glycodelin) and progesterone induced factors (PIBF, asymmetric IgG, TJ-6 glycoproteins) expressed during normal pregnancy act as a immunoregulatory molecules as they shift maternal immune response in Th-2 direction and make the pregnancy successful. Reduced expression of these molecules may lead to fetal loss in women with RSA. In addition, Fas and FasL interaction between maternal activated immune cells and implanted trophoblast triggers the programmed cell death for maternal activated immune cells and prevent the fetal loss

was investigated that trophoblast expresses major histocompatibility complex (MHC) antigens on its surface [200] which when recognized by maternal immune system triggers some alloimmune mechanism essential for the development of maternal immunotolerance [16, 45, 53, 61, 150]. However, RSA especially that of unknown etiology has been attributed to the following alloimmune characteristics that elicits weather the fetus will survive or reject during pregnancy.

Alloantibodies

When a woman becomes pregnant, maternal immune system usually recognizes the paternal HLA as different from her own and induce the expression of several alloantibodies (APCA, Ab2, MLR-Bf) that may coat the fetus and protect it from the cytotoxic maternal immune responses. However, absence or reduced expression of these alloantibodies during pregnancy may cause abortion in women with RSA [83, 116, 130] (Fig. 1). Regan et al. [157] have reported 10% APCA positivity in women with RSA as compared to 32% of women with normal pregnancy. Similarly, Hasegawa et al. [69] have reported less than 8.7% APCA positivity in women with poor pregnancy outcome. We have also demonstrated that APCA were present only in 8.5% of women with RSA as compared to 33–46% in women with normal pregnancy [5]. Anti-anti HLA antibodies (Ab2) and seemingly clonotypic antibodies recognize alloantigens receptor on T-lymphocytes and induce the suppression in alloimmune response during normal pregnancy [154, 155, 179, 184, 185]. We also evaluated the prevalence of Ab2 in women with RSA as well as in women with normal pregnancy and found a 30% Ab2 positivity in

women with normal pregnancy as compared to none in women with RSA which indicated that role of Ab2 is important for maintenance of pregnancies [83, 128].

A similar fetoprotective effect of MLR-Bf was observed [129, 152]. Tamura et al. [192] found MLR-Bf positivity in 82.4% women with normal pregnancy as compared to 10% of women with RSA. They also demonstrated that a blocking effect of MLR-Bf was inhances as the pregnancy progresses and once it is developed may also be helpful with subsequent pregnancies. Our study on time-kinetics of MLR-Bf during the course of a successful pregnancy showed maximum levels during the first trimester and a progressive decline through the subsequent trimesters and post-delivery [5]. We further investigated that whether the MLR-Bf was directed towards the paternal cells or not and found that MLR-Bf was specific to paternal cells only [5]. We have also investigated that MLR-Bf developed during pregnancy as well as in women with RSA after paternal lymphocyte immunotherapy is immunoglobulinG-3 (IgG-3) in nature [130] and protects the fetus from the maternal immune reaction. Several studies reported that MLR-Bf does not play protective role in the maintenance of pregnancy [47, 58, 84, 136]. However, there are many evidences which suggested that MLR-Bf expressed during pregnancy as well as in women with RSA after paternal lymphocyte immunotherapy prevents abortion and make the pregnancy successful [105, 106, 145, 152, 157, 210].

HLA-G molecules

HLA-G mRNA is identified in all extravillous trophoblast populations that are involved in the regulation of immune responses at the maternal–fetal interaction by allorecognition of NK cells [4, 89, 91, 134]. HLA-G is reported to presents the peptides that specifically activate either NK inhibitory receptors (KIR) or NK activating receptors (KAR) and induce the cytotoxicity that may influence pregnancy by altering cytokine profiles, suppression of T-cell subsets, and altered NK cell and monocyte function [104]. Several studies demonstrated that when HLA-G peptides are recognized by KIR of NK cells, it may first down regulate the NK cell function by blocking its cytotoxicity, secondly, suppressed the activity of certain T cells that may serve as an activator of $CD8^+$ cells and trigger the mechanism by which trophoblast grows or rejected [89, 117] (Fig. 1). Recently Haviid et al. [72] investigated the HLA-G polymorphism in couples with RSA and compared their results with normal fertile couples. Although no significant difference were found in the distribution of HLA-G alleles between controls and RSA couples, 15% of the women who aborted carried the HLA-G*0106 allele compared to 2% of those that did not. Aldrich et al. [7] also evaluated the role of HLA-G polymorphisms in 113 women with RSA and found a significant association with increased risk for RSA. Thus normal expression of HLA-G genotype during pregnancy may be important for healthy maternal fetal interactions.

Natural Killer cell activity

NK cells mediate nonmajor histocompatibility complex-restricted cytotoxicity of target cells and play a crucial role in immunologic defense and regulation. High preconceptional peripheral NK cell activity in women with RSA is found to associate with subsequent abortion [10]. Whereas, peripheral blood NK cell activities are significantly decreased during early pregnancy as compared to the non-pregnant status [97]. Absolute NK cell activity decreases in third trimester compared to the activity in normal non-pregnant controls and increased again during postpartum [77]. Studies on peripheral blood NK cells in women with RSA show that women sharing HLA DQ A1 alleles with their spouses have increased activities of $CD56^+$ cells in the peripheral blood as compared to women with normal pregnancies [96]. Increased numbers of $CD56^+$ cells were also documented in the endometrium of women with RSA when endometrial NK cells were compared in luteal phase endometrial samples from women with RSA and from normal subjects [39]. Endometrial biopsies of several women with RSA showed a significant increase of various cell populations ($CD4^+$, $CD8^+$, $CD14^+$, $CD16^+$/$CD56^+$) as compared to women with healthy live births [148]. However, deciduas of women with RSA also showed an increased number (40%) of $CD56^+/CD16^-$ cells that produce suppressor factors and cytokines [15]. Several studies demonstrated that activation of the maternal NK cells induces subsequent abortion in women with normal chromosomes [51, 205]. Thus other proposed mechanism for fetal loss in women with RSA related to the alteration in the NK cell activity by the induction of Th-1 pattern of cytokines [107, 134] (Fig. 1). Hence it is possible that suppression of NK cells activity may prevent maternal alloimmune reaction in the fetus.

T-helper-1 (Th-1) and T-helper-2 (Th-2) balance

A Th-1 type reaction in the maternofetal interface mainly triggers the inflammatory response with increase of interferon- gamma (IFNγ), tumor necrosis factor-beta (TNF-β), IL-2 and TNFα, which contribute to trophoblast toxicity and failure of pregnancy in women with RSA [11, 78, 103] (Fig.1). Significant levels of IL-12 was also found to associated with abortion in women with RSA as it induced the secretion of Th-1 cytokines and suppressed the secretion of Th-2 cytokines which is incompatible with a successful pregnancy [74, 209]. These Th-1 type cytokines may damage the placenta directly or indirectly via the activation of certain immune cells. TNF-α may cause fetal expulsion due to uterine contraction or may cause necrosis of implanted

embryos, alternatively TNF-α could act by occluding the blood supply to the conceptus. IFN γ has been shown to inhibit the secretion of granulocyte macrophage colony stimulating factor (GM-CSF) that promotes the growth and differentiation of the trophoblast during normal pregnancy as compared to pregnancy in women with RSA [137, 162]. The lack of GMCSF may be deleterious to the trophoblast whereas Perricone et al. [137] have observed its increased level after intravenous immunoglubulin (IVIg) given to pregnant women with RSA. Transforming Growth Factor β2 (TGFβ2) is also induced decidual anti-proliferative activity and is reduced in deciduas from women with RSA as compared to women with normal pregnancy [101]. Interestingly the activity of the decidua derived TGF-β2 is boosted by immunization that prevents abortion in mice [38]. In addition, Th-1 type cytokines induced NK cells and lymphokine activated killer (LAK) cells activity, however, Th-2 type reaction triggers the secretion of non-inflammatory cytokines (IL-3, IL-4, IL-5, IL-10 and IL-13] during normal pregnancy that promotes the success of pregnancy by alloantibody induction, counter inflammation and suppression of the NK cell activity [67, 79, 92, 138, 149, 166, 200] (Fig. 1).

Fas–FasL interaction

Several studies demonstrated that Fas or CD95 (type-I membrane protein of 45 kDa) is highly expressed on activated maternal immune cells (T cells and NK cells), where as Fas ligand (FasL) (type-II membrane protein of 37 kDa) is expressed on the surface of trophoblast [66, 82, 121]. The critical role of Fas/FasL interaction between activated mother's immune cells and trophoblast, during pregnancy initiates a cascade of events that culminates in the activation of caspases. It leads to apoptosis of activated immune cells that may block alloreactive responses and prevent the pregnancy failure [137, 167, 173]. This mechanism was generally regulated, by two specific proteins (Bcl-2 and bax) that can promote or inhibit apoptosis process [99, 167]. However, the decreased expression of bcl-2 and increased expression of bax in the deciduas are among of the characteristic features of pregnancy failure in women with RSA [90, 99, 100]. These findings suggested that FasL expressed on the surface of trophoblast cells could induce apoptosis in activated maternal (Th-1) cells. This may provide a mechanism for maternal immune tolerance to the fetus (Fig. 1). Hence reduction of Fas or FasL during pregnancy may be associated with fetal loss in women with RSA.

Hormonal activity

Some hormones like relaxin have been reported to favor the development of human Th-1cell response that induces abortion during pregnancy [138]. It is also reported that human placenta synthesizes a human placental growth hormone (HPGH) during pregnancy that could play an active role in feto-placental tolerance by acting on the immune system [59, 75]. Progesterone produced at high level during early pregnancy stimulates the production of TJ6 glycoprotein (55 kDa) in the uterus [56, 134]. The membrane form of TJ6 is expressed on B-lymphocytes during successful pregnancies (Fig. 1) and anti TJ6 binding antibodies destroy early pregnancy in mice. However TJ6 secreted protein bound to receptors of cytotoxic NK cells at the implantation site induces apoptosis and cause unfavorable outcome of pregnancy [42, 123, 160].

The immunomodulatory effect of progesterone may be thought to be the stimulation of progesterone induced blocking factor (PIBF) that may exert immuno-modulation by inhibiting NK cell activity and inducing the Th-2 response [189, 190]. However, its level decreases in women with RSA [35, 188]. PIBF induces the synthesis of asymmetric antibodies (IgG) during pregnancy, whereas its absence or decreased expression during pregnancy may cause abortion in women with RSA [92, 94]. Progesterone associated endometrial proteins or placental derived glycodelin found in epithelial glands of endometrium, endometriotic tissues, epithelial cells of umblical cord, human fallopian tube, decidua amniotic fluid, normal and ovarian tumours also act as immunosuppressive agent during pregnancy [213] (Fig. 1). On the other hand low levels of these proteins might lead to RSA and termination of pregnancy [142].

Indolamine 2,3-dioxygenase (IDO)

Munn et al. [119] showed that after several days of implantation, placenta starts to synthesize a tryptophan catabolizing enzyme, indolamine 2, 3-dioxygenase (IDO) that suppresses T-cell activity and protects the fetus from the adverse maternal immune response (Fig. 1) However inhibition of this enzyme during pregnancy leads to fetal loss in allogeneic pregnancies but not in T-cell deficient mothers. The mechanism of such fetal loss is still an immunological enigma that may be related to the consequences of inhibition of IDO on T-cell trafficking.

Spermine and feutin

Spermine and feutin are the plasma glycoprotein molecules that are present in amnions and counter regulate the immune response by inhibiting the expression of various proinflammatory cytokines that can damage the placenta [212] (Fig. 1). Fetal plasma glycoprotein feutin is required by the plasma glycoprotein, spermine to counter regulates TNF-α production during pregnancy [198].

Various treatment for women with RSA

Various forms of treatment have been introduced to treat women suffering with RSA. Before effective treat-

ment can be instituted, the cause of pregnancy loss must be determined. Once this has been determined the most suitable therapy can be recommended and applied. Heparin plus aspirin, aspirin alone, IVIg administration and immunization with allogenic lymphocytes are the most common treatments in women with RSA. Recently Jerjak et al [85] have used heparin/aspirin, aspirin alone, steroids, or combine therapy intravenous immunoglobulin and alloimmunization for the treatment of women with RSA, where he observed 80.5% success rate in immunized women.

Aspirin/heparin therapy

Among women with the combined problems of APA and elevated NK cells who become pregnant with preconception treatment, live birth rate is about 70%. The initial treatment of choice is usually low dose heparin and aspirin therapy. Heparin is a very large molecule and is unable to cross the placenta whereas, aspirin is able to cross the placenta. Normal placentation requires some degree of inflammation and the activation of the coagulation pathways permit the anchoring of chorionic villi. If inflammation and coagulation are involved in sustaining the fetus, it is contradictory that heparin, and aspirin could function as antiabortogenic drugs [31]. The other treatment modalities for antinuclear antibodies are prednisone, which suppresses the inflammatory process and stabilizes the cell.

Intravenous immunoglobulin (IVIg) therapy

Among women for whom pregnancy loss are due to immunological reason and has occurred even with the use of heparin and aspirin, IVIg therapy remains a safe alternative of treatment [36, 48, 182]. The basic effect of IVIg is to neutralize the cytotoxic effect of maternal immune response against the fetus. However, several studies demonstrated that IVIg suppress the activity of antiphospholipid antibodies, passively transferred blocking or antiidiotypic antibodies [34] that inhibit the binding of antiphospholipid antibodies to corresponding antigens, inactivate idiotype bearing B cells, down regulate the B-cell function [124], alter T-cell subsets, modulate, cell-mediated responses, perform blockade of immunoglobulin Fcg R on monocytes as well as reduced NK cell activity, cytokine production [52] and inhibit the complement activation [65, 118].

Lymphocyte immunotherapy

Various recent studies demonstrated that paternal lymphocyte immunization in women with RSA induce the level of humoral antibodies like APCA [114, 127], Ab2 [83] and MLR Bf [5, 6, 102, 129, 147, 152, 192] that were correlated with the success of pregnancy. In addition other benefits of this therapeutic approach was a non specific T-cell suppression [18, 112], decrease in the level of maternal IL-2 receptors [88], shift to Th-2 type immunity and suppression of NK cell activity [60, 73, 90, 131]. Thus there is popular belief that whenever there is failure of induction of this protective immune response, failure of pregnancy occurs [37]. We did a meta analysis of various randomized and nonrandomized clinical trials for lymphocyte immunization in women with RSA. Women with RSA who received paternal lymphocytes were considered as study group and those who received autologous lymphocytes, third party lymphocytes and normal saline were considered as control group. When we compared the success rate in pooled data of trials we found 67% success rate in paternal lymphocytes immunized women with RSA under study group as compared to 34% success rate in women with RSA of control group ($P < 0.05$) [131], This is in favor of the efficacy of paternal lymphocyte immunotherapy as a therapeutic approach for the treatment of women with RSA. However, the drawback of these studies is the small sample size and few of these trials have no control groups for comparisons. Women randomized to immunotherapy tended to be older and reported to be at higher risk of RSA than those randomized to other treatment (autologous cells, third party cells, saline) or no treatment. Although these differences were not significant, we cannot exclude a potentially worse prognosis in women allocated to immunotherapy since there were older women in this group compared with the control group.

1α, 25 –dihydroxy-vitamin-D3 (VD3] therapy

VD3 and its analogs were already known to be effective in the treatment of Th-1 immunity-mediated disease. Thus recently Bubanovic [26] proposed VD3 as new immunomodulatory agent for the treatment of women with RSA as it also belongs to the class of Th-1 immunity disease. He used a dose of 5–10 μg/kg of body weight of VD3 with or without immunosuppressive/anticoagulant therapy and found very encouraging results. The mechanism of VD3 activity, however, is not yet fully understood since this vitamin is pleiotropic but it is thought to down regulate the production of Th-1 cytokines, such as IL-2, IFN-γ, IL-1, IL-6, IL-8 [110, 212] as well as increased Th-2 associated cytokines in T cells from adults. VD3 also inhibits not only IL-12 generated IFN-γ production, but also suppresses IL-4 and IL-13 expression induced by IL-4 [111, 139]. As the effects of VD3 are very similar with immunomodulatory effects of IL-10, it was believed that VD3 could be used as local immunomodulatory drug for the treatment of RSA.

Risk and side effects of therapies used for treatment of women with RSA

When deciding whether immunization can be recommended as a treatment in women with RSA, potential

risk and side effects must be considered. There are various reported risk and side effects of different therapies used for treating the women with RSA that include transmission of infectious organisms (cytomegalovirus, hepatitis B and C viruses and the human immunodeficiency viruses). Antithrombotic therapies such as aspirin and heparin have not been uniformly successful and may be associated with fetal hemorrhage whereas, obstetrical complications such as preterm birth, premature rupture of the membrane and gestational diabetes are more common with prednisone [94]. Adverse effects of lymphocyte immunotherapy and IVIg immunization in women with RSA included headache, nausea, preterm labor, hypotension, paralysis, neonatal alloimmune thrombocytopenia, cerebral haemorrhagia, perinatal hypoglycemia and placental insufficiency, intrauterine growth retardation, graft versus host disease and mosaicism [135, 153, 194, 206]. Tanaka et al. [193] reported a very rare case of transient neonatal thrombocytopenia in infant was delivered by womenwith RSA immunized with paternal lymphocytes. Serological examination revealed that the thrombocytopenia was triggered by maternal anti HLA antibodies (anti-HLA IgG), which are easily absorbed by various fetal tissues. Isolated cell suspension used for immunization often contains erythrocytes, which may immunize women against paternal blood groups. However, this may also happen during normal pregnancy even without immunization. In early pregnancy approximately 50% of women experience vaginal bleeding despite the presence of a fetal heart beat and immunized women have a 7% incidence of intra uterine growth retardation (IUGR) compared to 14% in control patients. There is only 10% incidence of IUGR upon immunizations compared to 30% in control subjects [54]. We have been using lymphocyte immunotherapy for women with RSA for last 10 years without any serious side effect [6, 129]. Therefore, there is evidence that immunization may prevent rather than cause adverse effect.

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