



Fetal rejection: infertility and immunity

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Defective reaction toward fetal alloantigens could result in both recurrent spontaneous abortions (RSAs) and recurrent early pregnancy failures (REPFs), the latter existing in couples with unexplained infertility and multiple failures of implantation after *in vitro* fertilization embryo transfer. Immunological mechanisms leading to RSA and REPF seem to be different, although both syndromes probably have a genetic background that has not been identified so far. Despite the fact that antiphospholipid syndrome is a well-established cause of repeated pregnancy loss, the role of different autoantibodies existing in RSA and REPF patients needs to be elucidated. Immunotherapy is believed to correct the detrimental immune reactions; however, its real effectiveness and safety for the treatment of distinct forms of pregnancy loss need to be reconsidered.

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Fetal tissues, owing to the presence of paternally derived antigens on the surface of trophoblasts, are viewed by the maternal immune system as an allograft [1,2]. Maternal immune reaction, instead of being systemically depressed, was found to be a precisely regulated state of tolerance, acting especially at the materno–fetal interface. Mechanisms of fetal tolerance could be disrupted by certain unfortunate immunological events with both alloimmunological and autoimmunological backgrounds. They originate either from defective fetal allorecognition or from underlying immunological disturbances not directly connected with pregnancy, and could finally provoke reproductive failure in practically every trimester of pregnancy starting from the time of implantation. These are recurrent spontaneous abortions (RSAs) of 'unexplained' etiology, unexplained infertility and multiple failures of implantation after *in vitro* fertilization embryo transfer (IVF-ET) techniques (grouped together in this article as 'recurrent early pregnancy failures' [REPFs]), autoimmune causes of miscarriage and finally preeclampsia, although it will not be discussed in this review.

Recurrent spontaneous abortions

RSAs are defined as at least three consecutive pregnancy losses before the 20th week of

pregnancy and refer to 2–5% of couples in reproductive age [3]. The possible causes of RSA include uterine anomalies, cervical incompetence, endocrine disturbances, environmental factors, thrombophilias, antiphospholipid syndrome (APS) and parental chromosome abnormalities [4–6]. However, after all the above causes have been excluded, the clinician faces the situation when the rest of RSAs appear to have unknown etiology. Karyotype analysis of expelled fetal tissues indicating chromosomal anomalies explains approximately 35–55% of otherwise unexplained RSAs [6], but there is still a substantial group of patients aborting chromosomally normal fetuses, for whom explanation is not evident. The most probable cause of RSA in these patients is improper immunological recognition of fetal allograft by the maternal immune system, which, in most of them, is partner specific (allospecific) [7]. According to the sequence of consecutive miscarriages, RSA patients could be defined as primary aborters (having only miscarriages with the same partner), secondary aborters (having one liveborn followed by a series of miscarriages with the same partner) and tertiary aborters (having live birth in the middle of a series of miscarriages) [6,8]. Primary aborters are believed to present

with disturbed mechanisms of fetal allograft recognition and/or with defective induction of tolerance toward allograft, whereas in secondary aborters, defective production of anti-idiotypic blocking antibodies (i.e., asymmetric antibodies) is suspected [9]. Tertiary aborters are now considered to have distinct, probably genetic, pathology. They have a more favorable prognosis and respond differently to immunotherapy [6,8]. Identification of the type of RSA seems to not be sufficient to diagnose RSA of alloimmunological background. It is a broadly accepted opinion that one should first exclude all known, aforementioned causes of RSA, but there are still many controversies regarding how to identify and confirm alloimmunological pathology in such a selected group of women. The proposed tests included estimation of partner's histocompatibility, absence of mixed lymphocyte reaction-blocking antibodies, antipaternal lymphocytotoxic [10] and complement-dependent antibodies [8] or raised natural killer (NK) cells activity [11]. However, these tests have failed to prove their reliability beyond doubt [10]. The possible explanation is that one is searching for the indices of immunological pathology in patients who had an adverse past history of reproduction but during the diagnostic process are not pregnant and, moreover, looking for them in peripheral blood while the most detrimental processes take place inside the maternal–fetal interface. Another possibility is that 'alloimmune RSAs' are, in fact, a group of reproductive failures of heterogenic, so far not fully recognized, immunological causes.

The problem concerning RSA has been discussed for many years and plenty of interesting conclusions about the immunological causes of RSA have been extracted from studies performed on mice from classical abortion-prone mating of CBA/J female with DBA/2 male. The spontaneous abortion rate in CBA/J $\times$ DBA/2 mating is partner specific and range from 10 to 50% depending on the level of stress and bacterial contamination of breeding environment; however, it is much higher than the abortion rate in other matings, which usually do not reach 10% in the same standardized breeding conditions [6]. Although some of the detailed immunological mechanisms engaged in reproductive failure in mice are not identical with those found in humans, their understanding is very helpful, as profound scientific interference in the course of human pregnancy is impossible for ethical reasons. The CBA/J $\times$ DBA/2 model appears to adequately represent the situation that takes place in RSA patients aborting chromosomally normal embryos, as the frequency of chromosome abnormalities in mice is low [12]. Inside the maternal–fetal interface of aborting females, infiltrates of active immunocytes secreting increased amounts of proinflammatory Th1 cytokines IL-1, IL-12, TNF- α and IFN- γ have been found. These Th1 cytokines (particularly IFN- γ), being not balanced with proper Th2 and activity, could further stimulate trophoblastic and decidual overexpression of prothrombinase fgl2 activity [13,14], and could also induce endothelial cells of decidual vessels to secrete the neutrophil chemokine IL-8 [6].

IL-8-activated neutrophils, together with fgl2-triggered coagulation in placental sites, are capable of destroying vascular supply to the placenta [6], thus inducing abortion. The proposed mechanism for recurrent miscarriage is well documented; however, one should remember that Th1 overbalance is not necessarily the obligatory cause of RSA.

The major doubt concerning the crucial meaning of Th2 dysfunction for etiology of RSA appeared when it was shown that IL-4/IL-10 double-knockout mice were able to complete successfully their allogeneic pregnancies [15]. Consideration of this fact allows the conclusion that in allogeneic matings (other than abortion-prone CBA/J $\times$ DBA/2) some immunological mechanisms capable of compensating disturbed Th1/Th2 balance exist. Decreased numbers of both CD4⁺CD25⁺ T cells and IL-10⁺ Tregs, which could inhibit proliferation and IFN- γ secretion of effector cells, have been discovered in abortion-prone females [16], and depletion of Treg cells in normal pregnant mice provoked pregnancy failure [17]. Dysfunction of Treg cells triggers maternal cytotoxic reactions. Decreased levels of indoleamine 2,3-dioxygenase (IDO) were observed in mice with increased fetal losses, and administration of an IDO inhibitor (1-methyl-tryptophan) to pregnant mice led to abortion [18]. However, allogeneically mated IDO-knockout mice did not show a decrease in the size of litters [19], which means that, similarly to cytokines, IDO alone could not claim to the role of the key single anti-abortion regulator. Heme oxygenase (HO)-1 is another regulatory molecule engaged in murine gestation. Activity of HO-1 seems to be important for Treg function, as carbon monoxide (CO), being a product of HO-1, is able to exert antiproliferative effects on T cells and to downregulate pro-inflammatory cytokines secretion [20,21]. Blocking HO-1 abrogates both the regulatory activity and suppressive properties of CD4⁺CD25⁺Foxp3⁺ Treg cells [22]. Downregulation of HO-1 was observed in decidual and placental tissues from mice exposed to stress- or IL-12-triggered abortion [23] and HO-1-knockout mice showed impaired response to their embryos [24]. Systemic and local IL-4:IFN- γ and IL-10:TNF- α ratios in CBA/J $\times$ DBA/2J matings could be augmented by transfection with adenoviral HO-1 vector [25], and treatment of abortion-prone mice with HO-1 stimulator resulted in a diminished abortion rate [26]. These observations suggest that instead of viewing the cause of RSA only in the categories of disturbed cytokine action, one should pay more attention to alternative explanations, such as the dysfunction of Treg cells and HO- or IDO-dependent mechanisms.

Analogically, similar to abortion-prone mice, in RSA patients, both peripheral blood and decidual CD4⁺CD25^{high} Treg cells were decreased to nonpregnancy levels [27]. Furthermore, activated cytotoxic NK cells [28] could disturb secretion of IL-10 and TGF- β by $\gamma\delta$ T cells present inside decidua and, thus, enhance Th1 cytokine secretion, leading to abortion [29]. The existence of defective Th1/Th2 cytokine networks resulting from improper composition and function of immunocytes has been proven by many studies. In RSA women, elevated

concentrations of intracellular Th1 cytokine IFN- γ were reported in peripheral blood lymphocytes [30], while lowered levels of Th2 cytokine (IL-4 and IL-10) secretion were observed both in peripheral blood [30] and decidua [31]. Moreover, higher level of Th2 cytokines concurrent with downregulation of Th1 cytokines (IL-12, IL-18 and IFN- γ) were found to correlate with better prognosis for subsequent pregnancy success in the group of RSA patients [32]. Unopposed Th1 activity might in turn provoke activation of prothrombinase fgl2, similar to observations in mice [33]. Another possible factor capable of the enhancement of fgl2 activity might be decreased HO-1 expression observed in trophoblast and endothelial cells of decidual vessels of patients with RSA [34]. The disturbed IDO activity could also trigger procoagulatory changes as it was compromised on decidual and peripheral blood dendritic cells (DCs) in spontaneous abortion [35]. Both possibilities need elucidation

and confirmation in future investigations. The above-presented data strongly suggest that a cascade of adverse events provoked by defective allorecognition of paternal antigens could be responsible for the occurrence of RSA in both murine models and human patients (FIGURES 1 & 2).

Studies in murine models of RSA indicated that the tendency to miscarriage could be successfully reduced by immunization of CBA/J females with lymphocytes of BALB/c males or mating CBA/J females with BALB/c males before pregnancy with DBA/2 males [36,37]. Alternative management consisted of administration of Th2 IL-10 cytokine or anti-Th1 monoclonal antibodies into CBA/J females [38] or adoptive transfer of CD4⁺CD25⁺ T regulatory cells [16]. However, transfer of CD4⁺CD25⁺ Treg cells was effective only when Tregs were previously exposed to paternal antigens during pregnancy in normal, not abortion-prone, mating [16]. There are several

mechanisms by which aborting CBA/J females could benefit from immunotherapy. Passive immunotherapy by Th2 or anti-Th1 agents, as well as transfer of Tregs, restores the disturbed Th1/Th2 cytokine balance and adequate regulatory activity at the placental site. However, active immunization with lymphocytes from BALB/c males might exert multidirectional action and, currently, there is no consensus for which mechanism could have the dominant role. Lymphocyte immunization of aborting mice was shown to stimulate female decidual CD4 γ T cells for secretion of Th2 cytokines through the mediation of protolerance molecule CD200, where presence seemed to be obligatory for successful treatment [36]. It might also promote Treg activity by changing the cooperation between Tregs and DCs inside the maternal–fetal interface. Immature DCs by increasing IDO and IL-10 secretion favor Treg cells [39], which by direct cell–cell contact and ‘bystander suppression’, exerted by IL-10 and TGF- β inhibit maternal effector cells [24,40–42]. The investigations concerning this possibility are still lacking. Metabolic activity of HO-1 is another attractive but hardly known candidate for being a target for lymphocyte immunization.

Investigations performed on alloimmunization of abortion-prone mice allowed the suggestion that there might be a rationale to immunize RSA women using husband's or third-party donor leukocytes to prevent abortions [6,29,43]. According to some opinions [44], this form of treatment may be offered exclusively to primary

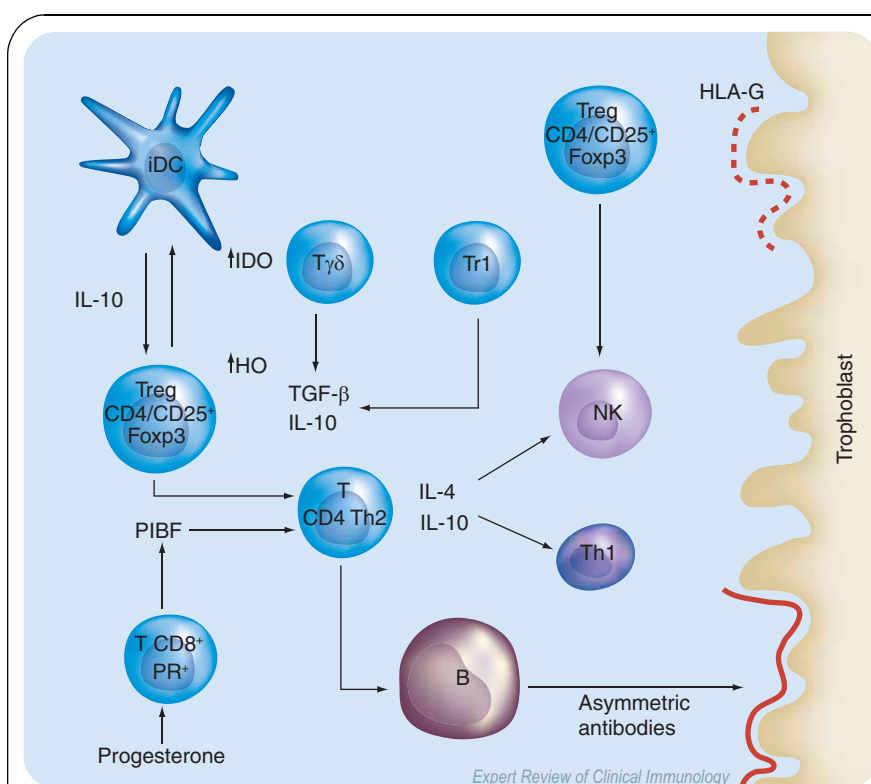


Figure 1. Immunological mechanisms responsible for local maternal tolerance toward fetal allotransplant. The presence of nonclassical molecule HLA-G on trophoblast prevents cytotoxic reactions directed against fetal alloantigens and is one of the triggering factors for protolerant immune reactions. The second stimulus is PIBF originating from stimulation by progesterone CD8⁺ T suppressor cells. One of the most important mechanisms is the interaction between iDCs and CD4⁺CD25⁺Foxp3⁺ Tregs inside decidua and uterus draining lymph nodes. iDCs through mediation of IL-10 are capable of inducing and sustaining Treg activity. Treg-induced IDO activity in DCs could in turn influence Treg cell function by upregulating HO-1, which exerts antiproliferative effects on T cells and downregulates proinflammatory cytokines secretion. Treg cells are also capable of inhibiting NK and Th1 cells by direct cell–cell contact. They may skew Th1/Th2 balance into Th2 responses by stimulating CD4⁺ T cells to IL-4, IL-10 and TGF- β secretion in the mechanism of ‘bystander suppression’. Decidual T γ δ producing TGF- β and Tr1 cells (CD4⁺ IL-10-secreting T lymphocytes) are other stimulators of a protolerant environment. Cytokines of Th2 activity not only inhibit cytotoxic maternal reactions, but also enhance production of asymmetric antibodies by B lymphocytes.

HO: Heme oxygenase; iDC: Immature dendritic cell; IDO: Indoleamine 2,3-dioxygenase; NK: Natural killer; PIBF: Progesterone-induced blocking factor.

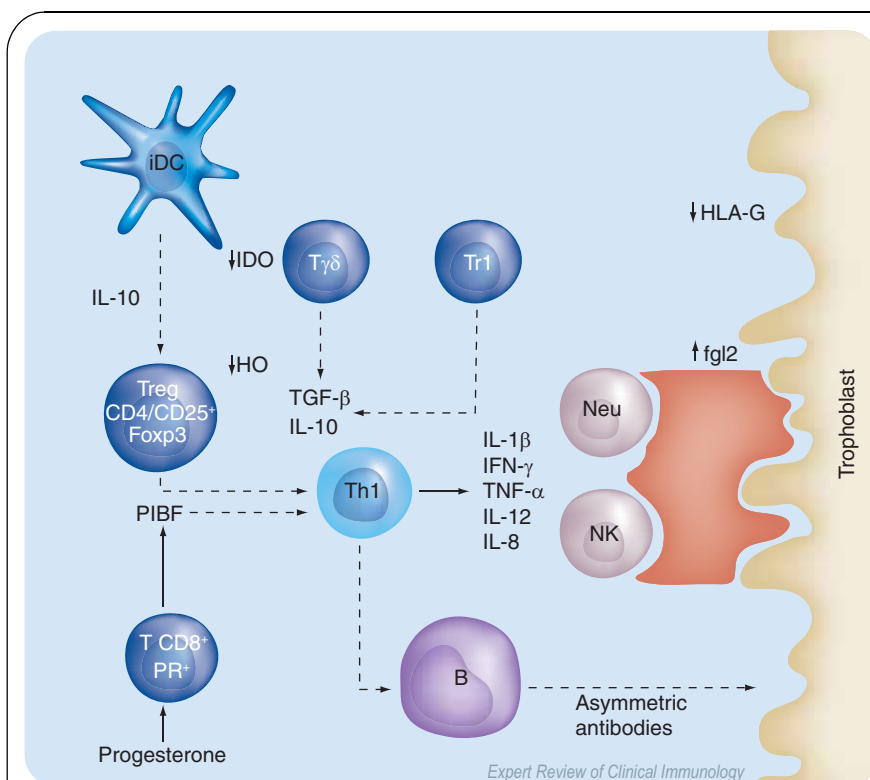


Figure 2. Disturbed immunomodulatory net inside the maternal–fetal interface leading to recurrent spontaneous abortions (RSA). Defective allorecognition of fetal antigens provoked by low level of HLA-G expression and/or genetic predisposition to aberrant pathway of some immunologic reactions could account for predisposition to RSA. The dysfunction of almost every element of local immunity described in FIGURE 1 could lead to an increase in maternal cytotoxic reactions, which consist of Th1-skewed cytokine balance together with NK cell attack and recruitment of activated neutrophils (Neu). All these factors may finally activate prothrombinase fgl2 and through blood coagulation inside the intervillous space deprive the fetus from maternal blood supply.

HO: Heme oxygenase; IDC: Immature dendritic cell; IDO: Indoleamine 2,3-dioxygenase; NK: Natural killer; PIBF: Progesterone-induced blocking factor.

aborters with low titers of coexisting autoantibodies (i.e., anti-nuclear antibodies [ANA] or anticardiolipin antibodies [ACA]). Another form of immunotherapy proposed for RSA women was passive immunization with intravenous immunoglobulins (IVIgs) containing asymmetric blocking antibodies from fertile donors [6]. This form of immunotherapy could probably benefit, especially secondary aborters and primary aborters, with high titers of coexisting autoantibodies [44]. The benefits of paternal lymphocyte immunization (alloimmunization) in RSA women was attributed to induction of nonspecific T-cell suppression [45], shifting cytokine activity toward Th2 type [46] and decreasing NK cell activity [47]. As RSA patients have been shown to have low levels of asymmetric blocking antibodies [48], passive immunotherapy using IVIg is believed to restore their normal levels, as well as to modulate excessive proinflammatory immunocyte activity and cytokine secretion [44,49]. Safety and efficacy of lymphocyte alloimmunization in RSA women are still a matter of discussion. There are concerns regarding possible distant complications, such as transfusion-related immunomodulation (TRIM) syndrome, which could adversely influence the future risk of cancer development [50].

Different protocols used for lymphocyte preparation and for performing the procedure of immunization raises the question of the credibility of comparisons between obtained results. Therefore, in 1994, Recurrent Miscarriage Immunotherapy Trialists Group (RMITG) performed a meta-analysis of randomized placebo-controlled or cohort-controlled trials devoted to alloimmunization. It showed that allogeneic lymphocyte immunization significantly increased (by ~16%) the chance of an alive-born baby among primary RSA patients without auto- and alloantibodies; however, it did not improve reproductive outcome in the group of secondary aborters [51]. In 1999, the placebo-controlled trial performed by Ober *et al.* [52] failed to show any advantage of lymphocyte alloimmunization but was later criticized because authors did not exclude RSA patients with significant autoantibody positivity, and used overnight stored lymphocytes, which could have adverse effects on their immunoactivity (discussed in [6]). The Cochrane meta-analysis performed in 2003 concluded that neither paternal lymphocyte immunization nor third-party donor lymphocyte immunization provided a significant effect over placebo in treatment of RSA patients [53] but, recently, Pandey *et al.* performed an analysis, which, in their opinion, advocates for alloimmunization [29]. Similar controversies con-

cerning IVIg therapy have been discussed, as many trials showed contradictory results of this treatment efficacy (reviewed in [44]). The Cochrane meta-analysis [53] once again failed to indicate any beneficial effect of IVIg therapy; however, statistical analysis was performed irrespective of the type of abortions, and there is well-documented suggestion that IVIg could be an effective management option for secondary aborters [44]. Existing controversies indicate that there is still a need to re-examine the pros and cons of lymphocyte alloimmunization [54,55].

Another possibility to enhance maternal tolerance toward fetus in RSA could be treatment with progesterone and/or progestogens (i.e., dydrogesterone), which by increasing progesterone-induced blocking factor secretion by CD8⁺ T cells could inhibit lymphocyte production of detrimental Th1 cytokines (IFN- γ and TNF- α), as well as cytotoxic effector responses, while augmenting the secretion of both Th2-type cytokines (IL-4 and IL-6) and asymmetric blocking antibodies [56–59]. Results of meta-analysis of three trials involving women suffering from at least three consecutive miscarriages commented in the Cochrane database [60] indicated that progestogen treatment showed a statistically significant decrease in

miscarriage rate compared with placebo or no treatment (odds ratio [OR]: 0.39). Recently, a novel treatment for RSA women has been proposed based on combined therapy of prednisone, acetylsalicylic acid, folate and progesterone [61]. In the opinion of the authors, this management option resulted in 77% live birth rate compared with 35% for women without any treatment; however, this needs confirmation in multicenter, randomized trials.

Both the presence of distinct forms of RSA and different reactions to immunotherapy could implicate a probable genetic predisposition to the pathway by which certain immunological reactions may exist and could influence pregnancy outcome. Existence of a genetic predisposition to RSA in humans was suggested by the observation that close relatives of pregnant women suffering from RSA also had an increased risk of pregnancy failure and that, in some cases, changing the partner reversed unfavorable pregnancy outcome [62]. We cannot exclude the option that RSA is caused by a single gene mutation; however, a more probable situation is polygenic inheritance [62]. Some of the candidate genes are those determining allotypes of receptor $\text{Fc}\gamma\text{RII}$ [63] or changing level of R80K alloantigen expression [64] on trophoblasts. Polymorphisms or mutations in these genes could adversely influence the protective efficacy of asymmetric antibodies or augment the possibility of NK cells attacking the trophoblast [64]. Polymorphism and inherited tendency to low expression of human leukocyte antigen (HLA)-G on trophoblasts [65,66] could also trigger the maternal cytotoxic response directed toward fetus. The same could result from the defective function of cytokines, as the relationship between high production of Th1 cytokines and certain genotypes has been observed [67–70]. The supposition that genetic predisposition to RSA is further supported by the fact that polymorphism of progesterone-receptor (PR) alleles resulting in types of PR having different affinities for progesterone may be associated with an increased likelihood of RSA [71]. Recently, genetically conditioned aberrant repertoire of genes encoding killing inhibitory receptors on NK cells [72], polymorphism of $\text{IFN-}\gamma$, IL-10-encoding genes [73,74], polymorphism of the IL-1 β promoter region [75] and the polymorphism of *HO-1* gene microsatellite size [76] have been found to correlate with RSA. Despite the growing evidence that RSA might have, at least in part, a genetic background, still many pieces of that puzzle are missing. The obtained data are insufficient to resolve the problem in general, and even more to show the potential genetic basis of observed clinical difference between primary and secondary RSA.

Recurrent early pregnancy failure

Although IVF-ET techniques have been shown to reverse unfavorable reproductive outcomes in many infertile couples, there are still couples in whom, despite obtaining good-quality embryos, successful implantation does not occur. Parental chromosomal abnormalities account for only approximately 2.5% of recognized causes of IVF-ET failure [77], and even when preimplantation genetic diagnosis to exclude intrinsic

embryo genetic aberrations prior to transfer was used, the implantation rate was less than 50% [78]. This observation raises the possibility that additional factors might contribute toward failed implantation and there is no doubt that the majority of them could be the result of the immunological disturbances occurring during the peri-implantation period. The suspected mechanism appears to differ from that observed during RSA because pregnancy failure in the cases of REPF occurs before the constitution of placental blood supply. Therefore, an aberrant receptivity of endometrium and/or defective function of immunological reactions inside the decidua at the implantation site could account for multiple failures of IVF-ET. One of the factors responsible for proper endometrial receptivity is IL-11 belonging to the IL-6-cytokine family. IL-11-receptor knockout mice are infertile owing to abnormal placentation caused by total lack of α -2-macroglobulin, which normally inhibits too profound trophoblast invasion [79]. Cytokine expression in the peri-implantation endometrium of fertile women indicated Th2-biased profile represented by decreased IL-12 and $\text{IFN-}\gamma$, while IL-4 and IL-6 increased [80]. These changes could be attributed to progesterone, of which serum and local levels were significantly raised during the luteal phase of the menstrual cycle [81]. Together with IL-4, it was found to stimulate decidual T-cell production of leukemia inhibitory factor (LIF) and macrophage colony-stimulating factor, which, in adequate concentrations, were essential for embryo implantation and early development [81]. An increase in IL-10 production a few days after implantation was also related to the success of pregnancy [82].

The positive role of Th2 cytokines, as well as proper regulatory mechanisms for the development of early pregnancy, has been confirmed in studies of infertile patients. Endometrium of infertile women indicated reduced expression of fork-head box protein-3 (Foxp3) mRNA, which suggested impaired differentiation of uterine T cells into $\text{CD4}^+\text{CD25}^+\text{Foxp3}^+$ Tregs phenotype [83]. Moreover, uterine luminal washing indicating higher concentrations of IL-18 and LIF, which suggested the existence of a proinflammatory biased environment, could predict poor endometrial receptivity and subsequent IVF-ET failure [84,85]. Some authors observed that peripheral blood lymphocytes of women with REPF presented with overexpression of activation markers CD69 and HLA-DR [86] and indicated raised intracellular Th1 cytokine expression manifested by increased $\text{TNF-}\alpha/\text{IL-4}$ and $\text{TNF-}\alpha/\text{IL-10}$ ratios [69,87]. Initial studies suggested that idiopathic infertile women with multiple REPFs after IVF-ET procedures demonstrated significantly higher percentages of peripheral blood cytotoxic CD56^+ NK cells, and the conception rate correlated positively with the CD56^+ NK cells percentage less than 12% [11,88]; however, recent investigations do not sustain that opinion [89]. Instead of number, rather the state of activation of both NK and natural killer T (NKT) cells determines unfavorable outcome in infertile women. An imbalance between killing activatory (CD161) and inhibitory (CD158a and CD158b) receptors on NK and NKT cells characterized women with REPF [90].

However, the conclusion that Th1 overbalance is a 'bad guy' that contributes to REPF seems to be an oversimplification in the light of other studies underlining the importance of Th1-skewed responses to pregnancy success. Investigations, in which decreased concentrations of both IFN- γ and IL-10 in uterine luminal washing has been found in women with recurrent implantation failure [91], have paid attention to a disturbed Th1/Th2 balance, which, instead of Th1 overbalance, may be responsible for REPF. IFN- γ secreted by uterine NK (uNK) cells is responsible for remodeling of decidual vessels and decidual integrity [92,93]. Although IL-18 was discussed as a proinflammatory Th1 cytokine, in the absence of IL-12 it could have Th2-inducing properties [94]. The implantation site of normal mice was infiltrated by IL-18⁺ uNK cells; however, IL-18 secretion inside decidua of abortion-prone females was significantly decreased [94]. Dichotomy of activity also characterized IL-15 which, besides procytotoxic activity directed toward uNK cells, might also have a growth factor role for immature uNK cells [94,95]. In IL-15-, IL-12p40- and IL-18-ablated mice, uNK cells failed to develop, which resulted in the improper modification of spiral arteries and decidua [94]. IL-12 could also have a stimulatory function in the synthesis of asymmetric antibodies [23]. Therefore, both defective uNK cell activation and uNK cell overactivation caused by dysfunction of IL-12/-15/-18 tripod could disturb vascular remodeling in the implantation site and provoke REPF (FIGURE 3) [95–97].

The reasonable conclusion concerning the pathological mechanism engaged in REPF, based on hitherto existing results, is that an inappropriate Th1/Th2 balance together with local cytotoxic effector activity may lead to defective implantation and/or vascular remodeling of the implantation site. The role of autoantibodies in this context could not be excluded [98], similar to disturbed endometrial receptivity, resulting from either genetic background [99] or from immunological and hormonal instability. Environmental factors, such as stress [100,101] or occult reproductive tract infection [102], could further influence predisposition to REPF by stimulating Th1 overactivity. There are still many controversies concerning the optimal and causative treatment of REPF. Several therapeutic options have been proposed including steroids and heparin, alone or combined with IVIg and even lymphocyte immunization; however, their usefulness has not been supported so far by results of controlled, randomized trials [98].

Autoimmune causes of miscarriage

There is no doubt that autoimmunity could account for pregnancy failure. The best known autoimmune cause of recurrent pregnancy loss is APS characterized by medium/high titers of ACA and/or lupus anticoagulant (LA) and/or antibodies against β_2 -glycoprotein I, as well as clinical signs of thrombotic disturbances and/or reproductive failure [103]. The

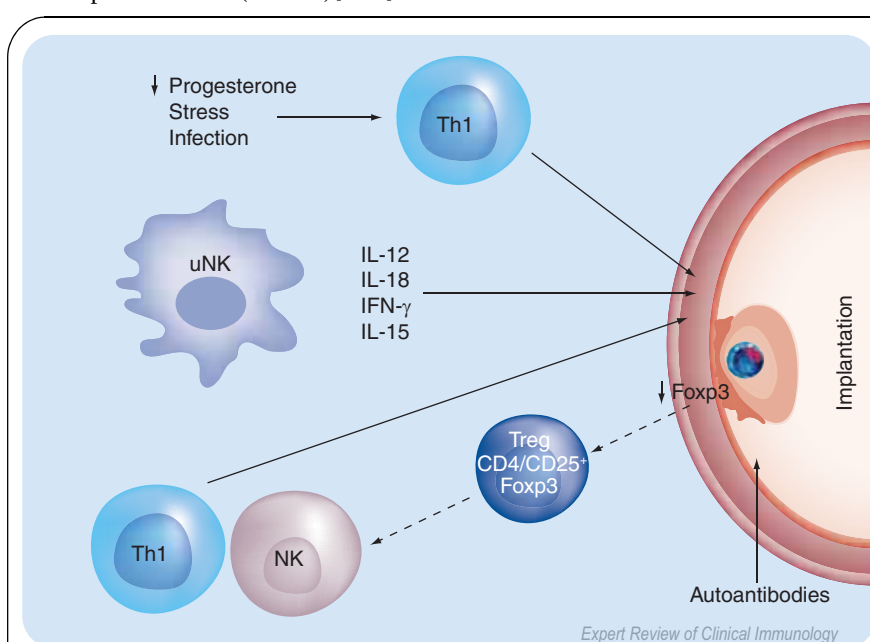


Figure 3. Immunological mechanisms considered as potential causative factors of implantation failure and recurrent early pregnancy loss. Low levels of progesterone decrease endometrium receptivity and negatively influence the Th1/Th2 balance leading to significant Th1 over-reactivity capable of killing the embryo. Stress and occult reproductive tract infection could trigger a similar cascade of events. Improper adjustment of uNK function represented by too low reactivity could result in disturbed angiogenesis in implantation site due to ineffective modulation by IL-12/IL-15/IL-18 tripod. In the case of exaggerated IL-12/IL-15/IL-18 secretion, these cytokines could directly kill the embryo. Low expression of Foxp3 on decidual cells may provoke insufficient CD4⁺CD25⁺ Treg protolerant activity followed by increased NK cells and Th1 cytokines over-reactivity. There is a possibility that autoantibodies might adversely influence the process of implantation by inducing trophoblast apoptosis and/or exerting the procoagulatory effects. NK: Natural killer; uNK: Uterine natural killer.

destructive mechanism of autoantibodies in APS is not alloantigen dependent, and consists of damage to phospholipid antigens on the surface of trophoblasts, followed by both disturbances of trophoblast migration and activation of procoagulatory factors at the maternal–fetal interface [104–107]. Some controversies concerning the quality and reproducibility of immune testing to recognize APS existed in the past [108] but we are now approaching some form of an agreement in this matter [109]. Consensus recommendations suggest that women with APS and a history of RSA or REPF or one or more late pregnancy losses who have no prior history of thrombosis should receive treatment with combination aspirin and low-molecular-weight heparin during pregnancy [110,111]. Therefore, one should consider that a connection between APS and pregnancy failure is well defined. However, greater criticism is involved in the interpretation of the presence of a plethora of autoantibodies in otherwise 'healthy' patients (those without clinical signs of autoimmune disease) presenting with recurrent pregnancy failures and defined as having reproductive autoimmune failure syndrome (RAFS) [112].

Evidence of ACA, LA and ANA was observed in many RSA patients [113]. Women suffering from REPF also demonstrated a higher prevalence of autoantibodies [98], including antiannexin V [114], antiphospholipid [107,115,116], ANA [116] and antithyroid (ATA) [117] autoantibodies. The presence of autoimmune thyroid disease was even suspected for provoking of Th1/Th2 balance disturbances with low production of IL-4 and IL-10 and high IFN- γ secretion by endometrial populations of lymphocytes [118], although pregnancy outcome in RSA women did not seem to be affected by ATA [119]. However, there is still a lack of certainty that observed autoantibodies cause the 'lion share' of unexplained pregnancy failures. Their presence could equally be an additional causative factor resulting from underlying systemic disorders [98] and an epiphenomenon. This further possibility points out that the presence of autoantibodies results from cross-reactivity between maternal tissue antigens and previously inaccessible fetal antigens exposed to the maternal immune system during the degradation of pregnancy remains in the course of abortion [120,121]. As microchimerism was found to correlate with the occurrence of autoimmune diseases [122], another possibility assumes that microchimerism during and after pregnancy might provoke autoimmune reactions directed against fetal autoantigens in the course of subsequent pregnancies in genetically predisposed subjects. The paucity of knowledge concerning the role of different autoantibodies, except of antiphospholipid ones, in the pathophysiology of RSA and REPF raises the question of the necessity and possible kinds of treatment in such patients. If we assume that both RSA and REPF are caused by defective allorecognition of pregnancy, and autoantibodies are just an epiphenomenon, there is probably no need to correct their level by immunosuppressive management. According to an opposite statement, the presence of autoantibodies may adversely influence the natural history of RSA and REPF and, therefore, should be corrected by immunosuppressive treatment with corticosteroids [123] or IVIg [124]. There is currently no broadly accepted and recommended solution in these cases, and the usefulness of different treatment options in RSA/REPF patients with the presence of autoantibodies should be tested during well-prepared controlled trials.

Expert commentary

There is no doubt that immunological disturbances constitute a great part of otherwise unexplained recurrent pregnancy losses. Despite the great progress in our understanding of immunopathology accounting for reproductive failure, there are still many topics that demand further studies. One of them, which has been extensively investigated recently, is the role of Tregs in establishing a tolerant state inside the maternal–fetal interface. Results of the studies performed on human Tregs indicate that proliferation of peripheral Treg cells depends on interactions with mature DCs expressing high levels of CD86 and CD28 molecules. Interactions between T cells and immature DCs via CD80 and cytotoxic T-lymphocyte antigen-4 (CTLA-4) molecules stimulate suppression [125–127]. Therefore, it seems reasonable to conclude that CD86 and CD80 costimulatory signals

have different abilities to interact via CD28 or CTLA-4 molecules, and that both balance in CD80 and CD86 expression on DCs and their type (mature vs immature) can regulate suppressive capacity [125]. Lipopolysaccharide-activated Treg cells can also inhibit reactive oxygen intermediates and cytokine secretion by neutrophils and promote their apoptosis. This kind of suppressive Treg cell capacity is modulated via Toll-like receptor-4 present on their surface [128]. The interactions between DCs and Tregs, as well as interactions between Tregs and neutrophils, are both promising areas of further investigation [129].

There is a reasonable conclusion, based on performed studies, that pregnancy failures occurring before (REPF) and after (RSA) the creation of maternal blood supply to the placental site have different pathophysiologies [54]. Taking into consideration that for the growing number of infertile couples the IVF-ET procedure becomes the only hope for successful reproduction, there is a burning need to fully recognize the mechanisms responsible for multiple failures of implantation. Studies on endometrial receptivity are a hopeful direction for improvement of pregnancy outcome in these cases, as nowadays it is obvious that the levels of endometrial preparation for implantation is one of the fertility-limiting factors of great importance. Recent studies devoted to the role of progesterone-dependent homeobox transcriptional factor HOXA-10, which stimulates progesterone-mediated proliferation of uterine stromal cells (and deficiency of which could predispose to implantation failure), represent this area of investigation [130]. There is no certainty that existence of autoantibodies without clinically apparent autoimmune disease could be the single causative factor in recurrent pregnancy failure; however, autoantibodies in high titers might interfere with other immune mechanisms and worsen the prognosis for pregnancy success.

To resolve this problem, prospective, randomized, controlled trials are urgently desired. They should answer three main questions: is there any difference in pregnancy outcome between RSA and REPF patients with and without the presence of autoantibodies; which autoantibodies and in what titers (except for APS) predict poor pregnancy outcome; and does steroid and/or IVIg treatment have any impact on improvement of pregnancy outcome in these cases? As autoantibodies may indicate presence of occult autoimmune disease or existence of genetic predisposition of such disease, the future trials should also answer the question of whether RSA and REPF women having raised autoantibodies present with increased risk of clinically evident autoimmune disease in the future. Immunotherapy methods and protocols are other points that need reconsideration, despite the fact that in some opinions immunotherapy could improve pregnancy outcome. Currently, there is no uniform rule for how to diagnose and treat the unexplained cases of RSA, and even more, of REPF patients. The same is true according to monitoring of the treatment efficacy. The treatment method for the main concern is lymphocyte alloimmunization, which, despite laboratory tests indicating its positive influence on some immunological mechanisms, is sometimes viewed as an inefficient and

potentially harmful option. The risk of the future TRIM syndrome development should, therefore, be checked in multicenter study. From another point of view, both alloimmunization and IVIg therapy are sometimes believed to be a remedy for any early pregnancy loss. Based on the opinion that RSA and REPF have different pathophysiologies, one should reconsider such a statement, as it rather issues from our medical helplessness than understanding of true causes. There are some new management options appearing on the horizon, such as etanercept (TNF- α blocker) [131], however, their usefulness for RSA cases needs further elucidation.

Five-year view

A growing body of evidence that some gene mutations and/or polymorphisms could manifest as predisposition to recurrent pregnancy failure points out the necessity for their identification and for investigating the connections between certain genotypes and corresponding immune phenotypes. Establishing the 'risk genes' separately for RSA, REPF and RAFS patients

would enable introducing the screening programs for couples from risk families. This would also enable adequate and precise diagnosis in couples suffering from previous unexplained pregnancy failure. The knowledge about underlying immunological pathology might help in creating the new, broadly accepted methods of qualification to therapy and its monitoring. Novel treatment options (e.g., manipulation with Tregs or gene therapy) could provide a safe and efficient method of improving reproductive situation in many infertile couples.

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Key issues

- Defective maternal allorecognition of fetal tissues could result in both recurrent spontaneous abortion (RSA) and recurrent early pregnancy failure (REPF) in couples suffering from an unexplained infertility and pregnancy failures after intravenous fertilization-embryo transfer techniques).
- Immunological mechanisms leading to RSA and REPF seem to be different. In RSA cases, they destroy maternal blood supply in the intervillous space, whereas in REPF, they do not allow proper angiogenesis in implantation site to occur and/or may directly kill embryonic tissues.
- There is a reasonable supposition that both RSA and REPF syndromes have a genetic predisposition that, however, has not been fully identified so far.
- The presence of autoantibodies could account for pregnancy failure (which is well established in antiphospholipid syndrome); however, the true meaning of plethora of autoantibodies existing in the RSA and REPF patients needs to be elucidated.
- Although some data seem to confirm that immunotherapy and immunomodulatory drugs are capable of correcting the detrimental for the pregnancy immune reactions, the belief that the same management might be equally effective in different immune pathologies is unjustified.

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