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Immunotherapy for recurrent pregnancy loss

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A B S T R A C T

When immunomodulation is used on an unselected population with recurrent miscarriage (RM), there is no improvement in the live birth rate. However, when the population is selected for a poor prognosis, or immune phenomena, immunotherapy has been shown to be effective. This review discusses four immunomodulatory agents, namely, paternal leukocyte immunization, intravenous immunoglobulin (IVIg), intralipid, and filgrastim. The presence of embryonic aneuploidy may confound the results of treatment, therefore creating an impression of futility when treatment may be highly effective in saving pregnancies that can be saved. Additionally, in an unselected population with RM, there is a relatively good prognosis of 60–80% for a subsequent live birth depending on whether the definition of $\geq$2 or $\geq$3 miscarriages is used. Hence, spontaneous prognosis must be taken into account, which has not been the case in previous trials.

This review discusses the possible immune-mediated mechanisms of pregnancy loss and the means whereby immunotherapy may modulate these mechanisms.

Introduction

Recurrent miscarriage (RM) is usually defined in North America, Russia, and Western Europe as two or more miscarriages before 20 weeks of gestation but in the UK as three or more consecutive miscarriages. All treatment modalities used for RM depend on immunomodulation for their effects. Aspirin

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is used for its anti-inflammatory effects, anticoagulants for their anti-inflammatory and anticoagulant effects, and steroids for their anti-inflammatory effects. This article describes active immunization with paternal leukocytes, passive immunization using intravenous immunoglobulin (IVIg), intralipid, and the use of growth factors such as G-CSF (filgrastim) to enhance placental and fetal growth and increase subsequent live births.

The problem is that all methods of immunotherapy have been assessed by an evidence-based approach in comparative trials, either randomized or blinded, or both, and compared to no treatment. Using this approach, it soon became clear that immunotherapy is not a panacea for treating all patients with RM. Unfortunately, there are no definite laboratory criteria by which immune-mediated mechanisms can be positively identified. Attempts have been made to use antipaternal complement-fixing antibodies and the number and killing activity of natural killer (NK) cells as selection criteria, but neither is sufficiently specific. Additionally, the effect of confounding factors such as embryonic structural malformations and embryonic genetic aberrations has not usually been taken into account.

This chapter discusses the efficacy, mode of action, and side effects associated with immunopotentiation in recurrent pregnancy loss.

Diagnostic criteria

As there are no definitive laboratory criteria for diagnosing immune-mediated miscarriages, immunopotentiation has usually been used on patients with no other established causes of RM. This approach has been used to prevent other presumptive causes of miscarriage from confounding the results. The author's criteria for excluding other presumptive causes of RM are as follows: (1) Normal karyotype of both parents; (2) Normal glucose tolerance test; (3) Normal uterine cavity as shown by hysterosalpingography, 3-D ultrasound, or hysteroscopy; (4) Normal thyroid function; (5) Normal serum prolactin; and (6) Negative antiphospholipid (aPL) antibodies. More recently, with the introduction of molecular technology to assess the genetic constitution of the embryo, immunotherapy is offered only to patients losing at least one euploid embryo. In sporadic miscarriages, up to 60% may have major chromosomal rearrangements [1,2]. However, in RM, the number varies widely from 29% in the author's series [3] to 90% when the definition of two or more miscarriages is used [4].

Most trials do not take into account factors affecting the prognosis of RM and fail to stratify accordingly. These prognostic factors are as follows: (1) The genetic status of the embryo. Women who miscarry chromosomally abnormal embryos have a 2.92 odds ratio for a subsequent live birth compared to women who miscarry chromosomally normal embryos [5,6]. (2) The number of previous miscarriages. Each miscarriage lowers the chance of a live birth by 24% [7]. Primary aborters who lose all their pregnancies have a worse prognosis than secondary aborters who have one or more live births and a string of miscarriages afterwards. The effects of immunotherapy are discussed herein according to the methodology used and the above-mentioned caveats.

Paternal leukocyte immunization

Efficacy of treatment

There are numerous meta-analyses of paternal leukocyte immunization (PLI). However, instead of clarifying the efficacy of treatment, they have obscured the results. The original meta-analysis was conducted by the “Recurrent Miscarriage Immunotherapy Trialists Group” (RMITG) [8]. This meta-analysis was performed on original patient data from an international register of 1753 patients who participated in double-blind trials carried out in 15 centers. The results were analyzed by two independent teams. The conclusion was that PLI was associated with a statistically significant 10% benefit. One team reported that ten patients needed treatment to obtain an extra live birth, whereas the second team reported that 13 patients required treatment to achieve an extra live birth. However, the RMITG drew other important conclusions as well as for the absolute treatment effect. Although the absolute benefit was 10%, it appeared that the benefit was 24% after correction of factors predictive of a

subsequent live birth. Two conclusions were drawn: First, immunization may be highly effective for a small proportion of patients who remain to be defined; therefore, diagnosis requires improvement. Second, the currently used regimens are suboptimal; therefore, the regimen requires improvement. In 1990, Carp et al. [9] showed that there was a subgroup in whom immunization was effective. This group consisted of primary aborters but not secondary aborters. The authors also showed that it is insufficient to only immunized patients, it is necessary to show that a response has occurred.

Since the RMITG meta-analysis, Jeng et al. [10] reanalyzed the RMITG data after the results were published. The RMITG meta-analysis compared the relative risk ratio of a live birth after immunization with that of nonimmunized patients using the DerSimonian and Laird (random effects model) equation [11]. By changing the method of analysis to an analysis based on the DerSimonian and Laird method, the previously reported benefit was lost. However, Jeng et al. [10] also published that after adjusting the data of maternal age and number of miscarriages, the increased chance of a live birth was statistically significant after immunization (RR = 1.17, 95% CI 1.01–1.36). Hence, the results of a meta-analysis depend on the statistical method used and subsequent analysis of subgroups of patient. A common odds ratio for all patients when taken as one group is not reliable and may lead to outliers (a minority of patients) missing treatment, which could alleviate their condition.

There have been two subsequent trends: to limit the indications for treatment to women with a poor prognosis and to widen the indications. Daya and Gunby [7] restricted their meta-analysis to the data retrieved from the RMITG register for women with primary miscarriages and no antipaternal complement-dependent antibodies (APCA) at initial testing. There was a 16% benefit in the subsequent live birth rate. Six women needed treatment to achieve one extra live birth. There was a statistically significantly increased chance of a live birth for any particular number of miscarriages. A meta-analysis [12] was performed on patients with primary and tertiary abortions with 5 or more previous miscarriages, who were negative for antipaternal complement-dependent antibodies and seroconverted as a result of immunization. The benefit was significantly increased. Three primary aborters and two tertiary aborters required treatment to achieve an extra live birth. No benefit was seen in secondary aborters. Unfortunately, there have been no trials comparing immunization according to immunological criteria such as the number or activity of NK cells or even trials limited to the loss of euploid embryos.

Ober et al. [13] in a subsequent trial widened the indications for treatment to include all patients with recurrent pregnancy loss including losses up to 29 weeks of gestation. Immunized patients had a worse prognosis compared to nonimmunized patients. However, on closer inspection of secondary aborters, the miscarriages were not always consecutive. There was no correction for karyotypic anomalies or seroconversion for APCA in primary aborters. Infertility for 1 year after immunization was considered as a treatment failure. A suboptimal dose of lymphocytes was used (200×10^6), and these lymphocytes were stored at 4 °C for up to three days, which further reduced their efficacy. Additionally, the trial by Ober et al. [13] included only few patients with 5 or more miscarriages. Hence, their results are not applicable to patients with a large number of miscarriages who are treated with more efficacious regimens.

Since the publication of Ober et al.'s [13] trial, Wong et al. [14], have published a meta-analysis of PLI in the Cochrane database. Ober et al.'s [13] trial was included in the meta-analysis. There was an odds ratio of 1.22 for a live birth after immunization, but this result was not statistically significant (95% CI 0.89–1.69).

If the data of the Cochrane database meta-analysis [14] are recalculated so that the primary question is "Does paternal leukocyte immunization raise the live birth rate if fresh cells are used," then there is a statistically significant benefit. Clark et al. [15] have published a meta-analysis updated the RMITG data and excluded the data of the Ober et al.'s [13] trial because of the very different regimen used in that trial. Clark et al.'s [15] meta-analysis showed a 9% statistically significant benefit when all patients are treated as a homogeneous group. Liu et al. [16] published an updated meta-analysis of 18 trials and reported a common odds ratio of 3.74 (95% CI 3.07–4.57).

Mechanism of action

The exact mechanisms of immunotherapy with paternal leukocytes have yet to be elucidated. Since Ober et al.'s [13] trial and the Cochrane database meta-analysis, PLI has fallen out of favor, and there is scant research on the mechanism of action. Suffice to say, immunization may have its effect by inducing a change in the balance between Th1 and Th2 cytokines [17], reducing the level of Th1 cytokines (IL-2, IFN-γ, TNF-α, and IL-6), while increasing the level of Th2 cytokines (IL-4, IL-10) [18]. Immunization by paternal cells has been shown to induce suppression of NK cell activity [18,19]. PLI may suppress T cell activity [20] and decrease maternal IL-2 receptors [21]. The Th-1/Th-2 balance is maintained by Th-17 cells, which enhance Th-1 responses, and Treg cells, which are associated with Th-2 responses. Immunotherapy may induce a decrease in the Th17/Treg ratio and the Treg bias, which may be beneficial for the maintenance of pregnancy. This is because there is a decrease in expression level of ROR gamma t, a transcription factor found in Th17 cells, and an increase in the expression of the Treg-specific transcription factor Foxp3 in the peripheral blood [22].

Pregnant women express higher levels of asymmetric antibodies (which possess a mannose-rich oligosaccharide residue bound to one of the Fab regions, making them unable to activate immunoeffector mechanisms) than nonpregnant women [23]. Women with pregnancy loss have significantly lower levels of asymmetric antibodies than normally fertile women [24]. PLI elevates the level of asymmetric antibodies [25].

Side effects

As PLI involves the administration of live allogeneic mononuclear cells, there has been concern over the possible side effects. The RMITG register contains the results of 1753 patients [8]. The conclusion drawn in the RMITG meta-analysis was that side effects seemed to be minimal. Kling et al. [26] reported a follow-up study of 2587 women treated in Germany from 1996 to 2003. They reported that acute side effects were comparable to those reported after intradermal vaccination for infectious diseases and that there were no cases of anaphylaxis, autoimmune, or graft versus host disease.

There is a risk of transmission of infection as with any transfer of blood products. Cytomegalovirus, hepatitis B and C viruses, HIV, and *Treponema pallidum* (syphilis) may be transferred. Hence, both partners should be screened before immunization to exclude these infections.

Maternal side effects include local erythema, irritation, swelling, and occasional blistering. These reactions are invariably transient but may last for up to two weeks. Autoimmune disease was seen in 8 of 1914 (0.4%) women in Kling et al.'s [26] series. However, 0.1% of the European and North American population develop some autoimmune disease per year. The RMITG meta-analysis [8] showed that the incidence of autoimmune disease following PLI (3/1149) did not exceed that of the control group (1/410). Hence, it is difficult to attribute autoimmune disease to immunization. There has been one case of autoimmune hepatitis in pregnancy reported to occur after PLI [27]. There is also a risk of sensitization to the minor blood groups. The white blood cell suspension used for immunization may be contaminated by erythrocytes. Hence, women may develop antibodies against paternal blood groups. In the case of Rhesus-negative women, the problem can be overcome by administering anti-D.

Perlman et al. [28] reported a case of neonatal alloimmune thrombocytopenia after PLI. The RMITG reported two cases of neonatal thrombocytopenia out of 1149 infants whose mothers were treated by allogenic leukocyte immunization.

Hence, side effects do not seem to be a major problem.

Intravenous immunoglobulin

Efficacy of treatment

With immunoglobulin (IVIg), there is a similar problem as that with PLI in terms of how to assess treatment. When all patients with three or more miscarriages are treated as one homogeneous group, there is no beneficial effect as shown in a systematic review in the Cochrane database [14]. However,

among all the causes of recurrent pregnancy loss, the ones that would be expected to respond to IVIg would be those that involve a mechanism that can be modulated by IVIg. IVIg would not be expected to increase the live birth rates in women who had aneuploid pregnancies or anatomic, hormonal, or thrombotic risk factors contributing to their losses. The etiological factors have been described by Coulam [29] as increased NK cell levels, an increased Th-1/Th2 cytokine ratio, and women losing euploid embryos. However, to date, no trial has been performed that restricts treatment to patients with the above-mentioned features. There have, however, been attempts to classify patients on a clinical basis. Hutton et al. [30] separated the series that were assessed in a previous version of the Cochrane database meta-analysis and analyzed the papers reporting secondary aborters as a separate subgroup; they found that IVIg had an almost statistically significant effect (OR = 1.71; 95% CI 0.99–2.95). Similarly, in Christiansen et al.'s [31] placebo-controlled trial, there was a 45% live birth rate regardless of whether IVIg was used or not. However, in secondary aborters, the live birth rate was 50% in IVIg-treated women compared to that of 23% in placebo-treated women. When the results of secondary aborters were pooled with those of Christiansen et al.'s previous [32] trial, IVIg was found to have a statistically significant beneficial effect in secondary aborters.

Additionally, the timing of IVIg administration is highly important. Coulam [29] analyzed 9 trials in which IVIg was administered; the author assessed the patients according to obstetric history alone or obstetric history and immunologic test results. Five trials involved IVIg administration before conception, and 4 of the 5 trials showed significant benefit in enhancing live birth rates. Five trials delayed treatment until pregnancy was established, and of these trials, none demonstrated benefit from treatment ($P = 0.04$, Fisher's exact test). Hutton et al. [30] published similar results. When IVIg was administered before pregnancy, there was a statistically significant benefit (OR = 2.02; 95% CI 1.04–3.92). Some of the trials involving IVIg administered the medication up to 8 weeks of pregnancy, with no ultrasound control as to fetal viability. Hence, IVIg may have been administered after fetal demise, too late to have any effect.

The author used IVIg in women with 5 or more miscarriages. In these cases, there was a statistically significant benefit of 20%. The author reserved IVIg for the most resistant cases.

Mechanism of action

IVIG has a number of potential mechanisms to prevent pregnancy loss. The anti-inflammatory effect of IVIg may be due to cytokine modulation. Andersson et al. [33] reported that when peripheral blood mononuclear cells are cultured in IVIg, there is significant inhibition of the production of the proinflammatory cytokines IL-2, IL-10, TNF-α, and IFN-γ. The altered cytokine levels after IVIg administration are due to interference with cytokine secretion or cytokine-specific blocking antibodies [34]. Graphou et al. [35] reported that IVIg enhanced the proportion of cells producing anti-inflammatory cytokines.

IVIg has been shown to depress the killing activity of peripheral blood NK cells [36,37].

Asymmetric IgG antibodies have been found in the serum of women with normal pregnancies; they bind to the placenta with specific activity to paternal antigens [25]. In RM, the levels of asymmetric IgG antibodies are lower, but IVIg returned asymmetric antibody levels to those seen in normally developing pregnancies [25].

The anti-inflammatory effect of IVIg may be due to interaction with the complement system [38]. aPL targeted to the placenta activates the complement system locally, generating split products that mediate placental injury, fetal loss, and growth retardation [39]. In laboratory animals, IVIg has been shown to inhibit the complement system [40]. However, this mechanism has not been shown in humans.

Side effects

IVIg is usually well tolerated. Most side effects are mild and usually related to the rate of infusion. Serious hypersensitivity reactions occur rarely but may include fever, vomiting, headache, shivering, skin rash, etc. Most of these symptoms regress when the speed of infusion is decreased. Patients with IgA deficiency may develop anaphylaxis if encountering IgA in the infusion. Increased serum viscosity may occur following IVIg therapy due to hyperproteinemia [41].

In women with hereditary thrombophilias, APS, or other prothrombotic states, IVIg, which is a procoagulant, may induce thrombosis. There are reports of thrombosis following IVIg infusion [41]. Therefore, in patients with prothrombotic states, an anticoagulant such as low-molecular-weight heparin should be administered concomitantly with IVIg. Acute renal failure has been reported usually when a sucrose-containing IVIg preparation was administered. Therefore, there is a contraindication to using sucrose-containing preparations of IVIg in diabetes. Currently, however, few IVIg preparations contain sucrose. There is concern about the transmission of viruses such as hepatitis B and C and HIV. However, the method of preparation and testing of the donors could prevent virus transmission. There is also concern with regard to prion transmission. Serious side effects are rare [42]. The main limitation of IVIg is its high price [42].

Intralipid

Efficacy of treatment

In 1988, Johnson et al. [43] carried out a randomized controlled trial involving trophoblast vesicles as active immunization for patients with RMs. As an inert intervention in the control group, intralipid (a 20% intravenous fat emulsion used routinely as parental nutrition) was used. There was no difference in the results between the immunized and control groups. Much of the criticism against Johnson et al.'s [43] study was that intralipid is not immunologically inert. There is evidence that intralipid administered intravenously may enhance implantation and maintenance of pregnancy. Coulam and Accacio [44] reported the results of 200 women with reproductive failure and elevated NK cell cytotoxicity treated with intralipid and compared them with the results of 242 age- and indication-matched women treated with IVIg. The overall live birth or ongoing pregnancy rate per cycle of treatment was 61% for women treated with intralipid and 56% for women treated with IVIg. The advantages of intralipid are that it is relatively inexpensive and is not a blood product. However, intralipid requires the same rigorous testing as other forms of treatment for recurrent pregnancy loss before it can be recommended as standard treatment. To date, there are few studies on this subject. Martini et al. [45] were not able to demonstrate a benefit in a retrospective comparative cohort study of women undergoing in vitro fertilization. However, there is no randomized trial of intralipid-treated subjects compared to controls in RM.

Mechanism of action

Intralipid decreases NK cytotoxicity both in vitro [46] and in vivo [47]. It contains soybean oil, egg yolk phospholipids, glycerin, and water. Fatty acids have been shown to affect NK cell activity through peroxisome proliferator-activated receptors (PPARs) [48], G-protein-coupled receptors [49], and CD1 receptors [50]. These mechanisms may be relevant in RPL, but further testing is necessary.

Filgrastim

Efficacy of treatment

Filgrastim is a cytokine growth factor (G-CSF). The main clinical use is in the treatment of neutropenia, such as chronic idiopathic or postchemotherapy neutropenia and to stimulate cell growth in hematopoietic stem cell donation or transplantation. The use of filgrastim in RPL is supported by a randomized controlled study [51]. The live birth rate was 82.8% in women treated with filgrastim compared to that of 48.5% in the control group ($p = 0.0061$). The number of patients who needed treatment for one additional live birth was 2.9. The strength of Scarpellini and Sbracia's [51] study lay in the inclusion criteria. They included only women with more than four previous miscarriages, failure of previous therapy for RPL, negative results for other known causes of RPL, and loss of a euploid embryo in the previous miscarriage. The authors reported that no infant showed any abnormalities.

As in other trials of immunotherapy, when the selection criteria were widened to include all patients with RPL irrespective of etiology and embryonic chromosomal analysis, the results were less clear. Zafardous et al. [52] broadened the selection criteria to include patients with two pregnancy losses (who have a good prognosis and are unlikely to show any benefit from treatment) and could not find a benefit from filgrastim therapy [52]. The above-mentioned two papers show how including a nonselected group of patients can confound the results of a trial that is designed to test treatment in appropriate patients.

Mechanism of action

Significantly increased β-hCG levels have been observed in filgrastim-treated pregnancies when compared with those in control pregnancies [51], which indicates a direct effect on the trophoblast. Alternatively, there may be an effect on lymphocytes. G-CSF has been reported to promote the mobilization and proliferation of lymphocytes, dendritic cells, and Treg cells [53,54].

G-CSF and its receptor are expressed in trophoblast cells throughout pregnancy [55,56]. The G-CSF receptor expressed in the trophoblast may activate different signal transduction pathways such as JAK/STAT, PI3K, and MAPKs, which, in turn, increase matrix metalloproteinase-2 and vascular endothelial growth factor secretion [57]. Additionally, Treg cell mobilization seems to be due to the regulation of the chemokine CXCL12 and its receptor CXCR4. G-CSF-mediated bone marrow stem cell mobilization has been reported to be dependent on the inhibition of the CXCL12/CXCR4 axis [58].

Summary

All treatment modalities of RM (aspirin, anticoagulants, hormone support) except pregestational testing for aneuploidy (PGT-A) have an immunomodulatory effect. Paternal immunization, IVIg, and filgrastim have been shown to have a beneficial effect in patients with a poor prognosis and when used appropriately. However, the results have been confounded by including a large number of inappropriate patients in trials. Thus, an impression of futility has been created. Because of this impression of futility, research into the mechanisms of immunologically mediated pregnancy loss and the mechanisms of action of immunotherapy has become less than that in previous years. Immune factors to select patients for treatment have relied on NK cell concentrations and its killing activity or cytokine concentrations and ratios. However, much work has to be performed to accurately determine the immune biomarkers that indicate which patients may benefit from treatment. It remains unclear why nature's transplant, pregnancy, is so successful. Once we learn this secret, we may know which immune manipulation is superior, as the methods of immunotherapy have not been compared with each other.

Conflicts of interest

The author has no conflicts of interest to report.

Practice points

- When used on an unselected population with RM, immunomodulation does not affect the live birth rate.
- When women with RM are selected for a poor prognosis, or immune phenomena, immunotherapy has been shown to be effective.
- Embryonic aneuploidy is a prevalent cause of RM, which confounds many trials of immunotherapy. Failure to account for embryonic aneuploidy has precluded showing a positive result of immunotherapy.
- There is no definitive biomarker to predict which patients respond to immunotherapy. NK cell concentrations and its killing activity are the most often used criteria but are probably not specific enough.

Research agenda

- Much research is needed to determine the immune and inflammatory mechanisms active in RM.
- Different types of NK cells require research to determine those that are relevant.
- Treg cells may be more important than other types of cells for promoting trophoblast growth.
- Different types of immunotherapy require further refinement to determine the dosage and the most efficacious regimens.

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