Intravenous Immunoglobulin: Effect on Infertility and Recurrent Pregnancy Loss

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Immunoglobulin for intravenous infusion is a preparation containing human gammaglobulin. The preparations are isolated from pooled normal serum by a chemical fractionation method using cold ethanol followed by acidification with non-organic acids at pH 4.0 with the addition of a minimal quantity of pepsin. The preparations therefore contain all the humoral immunoglobulin G antibodies normally occurring in the donor pool. The distribution of the IgG subclasses corresponds to that of normal serum. Immunoglobulin preparations are supplied as a lyophilized powder for reconstitution prior to infusion or they may already be constituted as a liquid.

IVIg was originally used for the treatment of agammaglobulinemia. However, the drug preparation is known to have immunomodulatory effects in a large number of conditions, such as immune thrombocytopenia, rheumatoid arthritis, Guillain-Barré syndrome, myasthenia gravis, multiple sclerosis, and infections such as human immunodeficiency virus and Parvovirus B19 infection.

The actions of IVIg have not been fully elucidated. However, the drug is known to have many actions. These have been summarized by Carp et al. [1]. Briefly, IVIg may modulate the effect of cytokines. When peripheral blood mononuclear cells are cultured in IVIg there is significant inhibition of production of the pro-inflammatory cytokines interleukins 2 and 10, tumor necrosis factor-alpha and interferon-gamma, and enhancement of the proportion of cells producing anti-inflammatory cytokines. IVIg reduces the number and the killing activity of peripheral blood natural killer cells. IVIg may inhibit the action of pathological antibodies by either the interaction of the Fc portion of immunoglobulin with Fc receptors or the Fab receptors, or by passively acting as anti-idiotypic. IVIg modulates the activation and effector functions of B and T lymphocytes, neutralizes pathogenic autoantibodies, and interferes with antigen presentation. The anti-inflammatory effect of IVIg may be due to interaction with the complement system. In laboratory animals, IVIg has been shown to inhibit complement.

A significant part of the *in vitro* suppressive activity is dependent upon the CD200 tolerance-signaling molecule, which is released from the surface of subsets of blood mononuclear leukocytes and may bind to IVIg [2]. CD200 is known to promote generation of regulatory T cells in mice [3]

IVIg has been used to improve the live birth rate in women with recurrent implantation failure at in vitro ferilization, unexplained recurrent pregnancy loss, and antiphospholipid syndrome. Treatment is based on the concept that implantation failure or pregnancy loss may be due to an aberrant immunological or inflammatory response involving cytokines and natural killer cells, or an autoimmune response as in antiphospholipid syndrome. Some of these mechanisms are explained below.

IVIg has numerous immunomodulatory properties, and may act at multiple sites involved in the immune response to pregnancy

Immune mechanisms in infertility

In implantation, a number of cytokines are known to play a specific role. Granylocyte macrophage colony-stimulating factor enhances trophoblast proliferation. Epidermal growth factor has been reported to be associated with the ability of the trophoblast to secrete human chorionic gonadotropin and human placental lactogen. IL-1 stimulates leukemia inhibitory factor, which is essential for implantation to take place. Leukemia inhibitory factor is associated with trophoblast proliferation. IL-6 releases matrix metalloproteinase-9, an endopeptidase that degrades the extracellular matrix during trophoblast migration into the endometrium. IL-15 increases trophoblast invasion, modulates MMP-1 and maintains uterine NK cells. IL-3 is associated with cytotrophoblast differentiation. IL-18 prevents implantation. In addition, NK cells are recruited into the decidua. TNFα can activate NK cells to lymphokine activated cells, which can attack

IgG = immunoglobulin G
IVIg = intravenous immunoglobulin G

IL = interleukin
MMP = matrix metalloproteinase
NK = natural killer
TNF = tumor necrosis factor

the trophoblast and cause its apoptosis. TGFβ can inhibit this activation. Injection of anti-tumor growth factor-beta2 antibodies induces resorptions of pregnancies in mice [4].

As stated above, immunoglobulin modulates the effect of cytokines and natural killer cells.

Immune mechanisms in unexplained recurrent pregnancy loss

In recurrent pregnancy loss, cytokines are known to have three main actions: they modulate NK cells, mediate the embryo's response to teratogens [5], and mediate coagulation. GM-CSF and epidermal growth factors have the same actions as those described above. IFNγ was found to be responsible for remodelling the spiral arteries to utero-placental arteries. IL-4 and IL-10 inhibit prothrombinase. IL-6 releases tissue factor, initiates clotting, and releases hCG. TNFα activates NK cells to lymphokine activated cells, mediates apoptosis and initiates clotting.

IVIg has not been shown to be beneficial in meta-analyses of large groups of heterogeneous patients, but is effective when patients are selected on the basis of immune testing, or a poor prognosis

Immune mechanisms in APS

Antiphospholipid antibodies are known to cause pregnancy loss directly, as injection of serum from mice with a high titer of aPL to naive mice induces resorbtion of pregnancies in the recipient [6]. The mechanism of action of aPL is mainly assumed to be thrombosis in decidual vessels, which might explain most of the internal medical ramifications of the condition. However, placental histology shows that most of the antibody is concentrated in the cytotrophoblast. The pathological features of aPL on the trophoblast include decreased vasculosyncitial membranes, increased synctial knots, and substantially more fibrosis, hypovascular villi and infarcts than in women without APS [7]. APS has also been shown to lead to pregnancy loss by other mechanisms, such as inhibiting placental hCG secretion [8], inhibition of trophoblast differentiation *in vitro* [9], complement activation [10], and cytokine imbalance – all of which may be responsible for some of aPL's actions. IL-3 is decreased in APS [11], and administration of IL-3 reduces fetal loss in experimental APS [12]. Alteration of the Th-1/Th-2 balance may be involved in the effect of anti-idiotypic antibodies on APS [13]. TNFα levels were significantly higher in patients with APS than in healthy controls [14]. Elevated levels of IL-6 and TNFα and a trend to lower IFNγ were found in patients with definite APS. [15].

Results of treatment

In cases of IVF implantation failure, IVIg has been used in an attempt to increase the pregnancy rate. Coulam et al. [16] reported a 50% pregnancy rate after IVIg. However, Balasch and co-authors [17] did not find this therapy to be beneficial. More recently, Elram and colleagues [18] reported a 38.9% implantation rate in patients sharing HLA antigens between spouses. However, the patient selection criteria were very different for these trials, making it difficult to compare the results. The author has used IVIg in a few patients with IVF failure, and the results have been impressive in certain patients. However, the small numbers cannot preclude these results occurring by chance.

Sher et al. [19] administered IVIg in a dose of 20 g together with heparin and aspirin to 89 women with at least 4 cycles of implantation failure. Fifty-two women were positive for aPL and they had a 42% live birth rate; 37 were negative for aPL and they had a 19% live birth rate. The authors concluded that IVF outcome is significantly improved in aPL-positive patients when treated with heparin/aspirin and IVIg, but this regimen did not improve the pregnancy rate in aPL-negative patients. In a subsequent paper [20], the same team reported that heparin/aspirin improved the IVF birth rate in cases of aPL antibodies, but that this regimen was insufficient if the aPL was either IgG or IgM directed against phosphatidyl ethanolamine or phosphatidyl serine. In these cases IVIg was also required.

A recent meta-analysis [21] of three published randomized controlled trials of IVIg in IVF failure patients shows a significant increase in the live birth rate per woman ($P = 0.012$). Relevant variables appeared to be selection of patients with abnormal immune test results as well as the properties and scheduling of the IVIg. Clark et al. [21] claimed that not all IVIg preparations are identical, and that some of the 'negative' trials may have used a biologically inferior preparation.

In unexplained recurrent pregnancy loss, the role of IVIg is controversial. The trend has been to take all patients with recurrent miscarriages (including patients with only two miscarriages) and to determine the live birth rates. Using this criterion, two meta-analyses did not show that IVIg had an effect [22,23]. However, the pooling of a large number of heterogeneous trials, including unsatisfactory trials due to different patient selection criteria or the use of biologically less potent IVIg preparations, into a meta-analyses can easily obscure significant benefits that occur in subgroups of patients. When patients are selected for a poor prognosis, either by immune testing [16], elevated blood NK T cells [24] or a greater number of miscarriages [25], the benefit reaches statistical significance. A recent meta-analysis [26] that analyzed all the IVIg trials found IVIg to be effective in secondary aborters, and in primary as well as secondary aborters when IVIg was administered prior to pregnancy.

In APS, IVIg inhibits the action [27] and production of aPL

GM-CSF = granulocyte macrophage colony-stimulating factor
IFN = interferon
hCG = human chorionic gonadotropin
APS = antiphospholipid syndrome
aPL = antiphospholipid antibodies

[28]. IVIg reduces the number of fetal resorptions in mice in which APS had been induced by immunization with aPL [29]. Caccavo et al. [27] reported the inhibition of binding of anti-cardiolipin antibody to cardiolipin by the F(ab')2 fragment from IVIg in a dose-dependent manner. Galli and collaborators [30] demonstrated the inhibition of lupus anticoagulant activity from the F (ab')2 fragment of IVIg. Additionally, IVIg lowers the levels of aCL after each infusion [31]. However, IVIg seems to have no advantage over heparins with regard to previous live births [32,33]. Furthermore, the incidence of late complications of pregnancy such as intrauterine growth restriction, preeclampsia, and prematurity seems to be reduced with IVIg [34].

IVIg can only act on pregnancies that are karyotypically normal. Aneuploid embryos at infertility treatment, or recurrent pregnancy loss can confound the results of IVIg, creating a false impression of futility

Chromosomal aberrations

Pregnancy failure is often due to chromosomally abnormal embryos. This is true in prolonged infertility and in pregnancy loss. I*n vitro* karyotyping (pregestational diagnosis) of the embryos of couples with implantation failure has shown that up to 66% may be chromosomally abnormal [35]. In unexplained recurrent pregnancy losses, the incidence of chromosomal aberrations varies from 25 to 60% [36-38]. The different incidence may be due to the different rate of successful karyotyping in different reports. In order to determine the true incidence of embryonic chromosomal aberrations, it is necessary to improve the technique of embryonic karyotyping. This may be achieved by chorionic villus sampling in failing pregnancies including blighted ova, or by embryoscopically directed placental biopsy [39]. Even in APS-related pregnancy loss, approximately 30% of abortuses are aneuploid [37,40]. IVIg can only be expected to be effective in pregnancies that are karyotypically normal. Hence, the presence of aneuploid embryos can confound the results of IVIg, creating an impression of futility, whereas the treatment may be effective in those pregnancies where it is able to help.

Conclusions

Much research is still needed on the effects and proper indications for IVIg in reproductive failure. However, its prohibitive cost will probably prevent it ever becoming a first-line drug. Its place is probably best reserved for severely affected patients who cannot be helped by simpler modes of treatment.

aCL = anticrdiolipin antibodies

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