Editorial Articles

A practical approach to the prevention of miscarriage: Part 2 - active immunotherapy

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Summary

Purpose: To present data suggesting that active immunization with lymphocyte immunotherapy is a treatment that has benefit in preventing miscarriage. *Methods:* Lymphocyte immunotherapy is given to women with a history of recurrent miscarriage or failure to achieve a successful pregnancy, despite several previous embryo transfers. Active immunization was combined with progesterone therapy. The lymphocytes were not refrigerated, but used fresh. *Results:* Compared to controls, i.e., progesterone therapy alone, the injection of paternal lymphocytes intradermally improved miscarriage rates and improved live delivered pregnancy rates per embryo transfer. *Conclusions:* The addition of progesterone treatment may act synergistically with lymphocyte immunotherapy, especially in primary aborters and tertiary aborters. However, it is important to use fresh – not refrigerated – stored lymphoctes.

Key words: Lymphocyte immunotherapy; Miscarriage; Refrigerated white cells; Progesterone induced blocking factor.

Introduction

Part 1 of "A practical approach to the prevention of miscarriage" emphasized the importance of progesterone supplementation and suggested that one of its main effects may be on the immune system.

There are data supporting the concept that one of the mechanisms involved in escape from immune surveillance, especially by natural killer (NK) cells in normal pregnancy, is through the hormone progesterone (P) [1]. A 34 kDa protein has been identified in pregnant women which can block NK cell mediated lysis of K562 tumor cells [2]. Because the expression of this protein by CD8+ T-lymphocytes (specifically gamma/delta T cells) needs P exposure for its expression, it was called the progesterone induced blocking factor (PIBF) [3].

There is evidence that PIBF may induce a shift from TH1 to TH2 cytokines [3, 4]. Progesterone receptors have not been demonstrated in normal T lymphocytes, yet these receptors have been found at a lower density than other tissues with P receptors (e.g., endometrium) in healthy pregnant women [5-7]. Liver transplants and blood transfusions have been shown to induce P receptors on these gamma/delta T cells, even in male patients [8]. Injection of paternal lymphocytes prior to ovulation has been shown to increase PIBF secretion in mid to late luteal phase in women exposed to the allogeneic stimulus of embryos following embryo transfer [9].

These data have led to the following hypothesis, as to at least one way that the fetus escapes immune rejection by NK cells: The fetal semi-allograft induces P receptors in gamma/delta T cells following trophoblast invasion. The interaction of these receptors with a high concentration of P causes the expression of PIBF by these gamma/delta T cells with induced P receptors. The PIBF is only made at the maternal fetal interface because that is where there is an adequate P concentration. Progesterone receptors in gamma/delta T cells are made throughout the body but the P level is insufficient to cause PIBF expression by gamma/delta T cells not at the maternal-fetal interface.

PIBF inhibits NK cell cytologic activity at least partially, by inhibiting the release of perforin from storage granules of NK cells [10]. PIBF also inhibits TH1 cytokines and favors TH2 cytokines, thus inhibiting cellular immune response and promoting hormonal response [2]. The suppression of the cellular immune system is limited to the maternal-fetal interface and this constitutes selective immune tolerance.

Szekeres-Bartho *et al*., by using an enzyme linked immunosorbent assay, measured PIBF in normal pregnancies, at the termination of pregnancy (at onset of labor, at time of miscarriage, and at the time of pre-term deliveries), and in the 10th week of women who subsequently spontaneously lost the pregnancy [11]. All women at pregnancy termination had sera PIBF levels lower than those of healthy pregnant women [11]. They also found that using a cut-off value of 197.5 ug/ml for PIBF, that 52 of 87 women who would eventually spontaneously abort, either immediately or up to 12 weeks later, had low PIBF levels [11].

Revised manuscript accepted for publication August 6, 2009

The same group using an immunocytochemistry method compared PIBF expression in women between the 9th and 40th week of gestation [12]. They found that the percentage of PIBF expressing lymphocytes in the peripheral blood of 96 healthy pregnant women was 67% vs 6.5% in 62 women with pathological pregnancies [12]. This group found over 90% of pathological pregnancies had PIBF levels below the established cut-off for normal pregnancies [12].

Theoretically, a low PIBF level may be caused by insufficient progesterone, or insufficient development of progesterone receptors on gamma/delta T cells. There are data that favor that the most common problem is the lack of progesterone, rather than lack of development of P receptors on gamma/delta T cells. One study evaluated PIBF levels in women supplemented with extra progesterone according to whether they miscarried or not [13]. The PIBF expression was similar in those having a miscarriage vs those who were successful in delivering a live baby [13].

Lymphocyte immunotherapy

Lymphocyte inoculation from the male partner's blood was given to women who had either failed to have a successful pregnancy following at least two in vitro fertilization (IVF) cycles with embryo transfers (ET), or had a history of recurrent (at least 3) miscarriages consecutively. The mean percentage of lymphocytes expressing PIBF was 2.9% before lymphocyte immunotherapy and 8.2% after the procedure. The percentage of women with PIBF expression in over 1% of the lymphocytes increased from 33.3% to 58%. There were 56% of the women without PIBF expression despite pregnancy and this decreased to 22% after lymphocyte injection [14].

There is controversy as to whether lymphocyte immunotherapy provides any benefit in preventing miscarriage with some studies finding a reduced rate [15-22], but others finding no benefit [23-25]. In fact, a meta-analysis concluded that there was no evidence that lymphocyte immunotherapy provides any benefit [26].

The possibility exists that the lymphocyte stimulus is insufficient to stimulate an increased level of PIBF from the gamma/delta T cells with the theoretical increase in P receptors, unless an increase in progesterone is also given. In one of our studies primary aborters with a history of recurrent miscarriages (3 or more) were randomly assigned to either progesterone supplementation from the early luteal phase throughout the first trimester vs progesterone therapy with the addition of lymphocyte immunotherapy [27]. Miscarriage occurred in eight of 14 (57.1%) given progesterone alone, vs six of 22 (26.0%) given combined therapy (Fisher's exact test, $p = .073$) [27].

When a couple has sex at the appropriate time or intrauterine insemination, failure to achieve a pregnancy may be related to failure to fertilize, failure of the zygote to cleave further, rejection of the embryo by the immune system, or other reasons for implantation failure. With in vitro fertilization and embryo transfer, failure to conceive is obviously not related to fertilization or embryo cleavage issues.

The efficacy of lymphocyte immunotherapy was evaluated in a group of women who averaged 4.3 previous embryo transfers without a live pregnancy and found in this matched pair study a live delivery rate of 16.2% (6/37) for those treated with progesterone only vs 51.3% (19/37) for those women who were given lymphocyte immunotherapy ($p < 0.01$, Fisher's exact test) [28]. This study evaluated only the first cycle of lymphocyte immunotherapy. In a larger retrospective study of all cycles receiving lymphocyte immunotherapy, the live delivery rate was 30.8% (39/94) per transfer vs 19.7% for controls [29]. For women with five previous failures the live delivery rate was 35.1% (13/37) vs 15.6% (10/64) [29].

Lymphocyte immunotherapy was evaluated in 20 embryo transfer cycles using donor oocytes, where the recipient was on estrogen/progesterone replacement having failed to have a live baby after three previous embryo transfers of embryos derived from donor oocytes. Six of the ten not receiving lymphocyte immunotherapy conceived, with one ectopic pregnancy and two miscarriages vs nine of ten demonstrating a clinical pregnancy with the addition of lymphocyte immunotherapy, and none aborted. The live delivery rate of 30% vs 90% was significantly different ($p < 0.05$, Fisher's exact test) [30]. This study is important because one concept is that the lack of progesterone receptors induced on gamma/delta T cells by the embryo/fetus may be related to a less immunogenic fetus due to sharing of histocompatibility antigens between the male and female couple. White blood cells are 100 to 1,000 times more immunogenic than in the fetus [29]. Using donor oocytes, theoretically if sharing of histocompatibility antigens played an important role, the foreign antigen from the donor egg source should overcome the problem. Thus these data favor more the concept that some women have a defective immune response to the relatively weak antigens stimulus of the fetus but may respond to a more potent white blood cell injection. Other data supports the conclusion that histocompatibility antigen sharing is not the reason for failure to suppress immune reaction of the fetus, especially DQ-alpha type II antigens [31].

One study has had a major impact on the use of lymphocyte immunotherapy in the United States. Unfortunately the study has also had a strong negative impact on its use [32]. Ober *et al*. found no reduction in the chance of a subsequent miscarriage, but actually found that there was a higher miscarriage rate in those females receiving paternal lymphocytes vs controls [32]. The miscarriage rate was 55.5% (37/68) for those receiving paternal lymphocytes vs 34.9% (22/63) for controls [32]. The patients from Ober *et al's* study, contributed to more than 20% of the patients used for a meta-analysis by Porter *et al*. published in 2006 [33]. The Cochrane review [33] found a miscarriage rate following lymphocyte immunotherapy of 35.5% (116/320) vs 41.0% (235/331) with an OR and 95% CI of 1.27 (0.92-1.74) which was not significantly different. However, if the data from Ober *et al*. was removed from the meta-analysis, the group

receiving lymphocyte immunotherapy would have had a miscarriage rate of 30.6% vs 42.1% in the controls [33]. These studies used for the meta-analysis were without any other therapy, e.g., progesterone, which facilitates lymphocyte therapy even more and thus our randomized controlled study was not included which would have further increased the benefit of active immunotherapy with lymphocyte injection.

One may question why remove the study by Ober *et al*. from the more recent meta-analysis? [32, 33]. Indeed, it is based on the study by Ober *et al*. showing a negative effect on a randomized double blinded study, that influenced the Food and Drug Administration in the United States to require an investigational new drug (IND) application to use immunization with paternal lympocytes [32]. Since the cost of the application was close to a million dollars, most reproductive centers no longer use this procedure in the United States. This is the basis of my statement that the Ober *et al*. study is one of the most influential of the studies since it abruptly stopped this treatment in the United States.

There have been many objections to the study by Ober *et al*. [34, 35]. One of the main objectives is that the other studies used for the meta-analysis used fresh paternal white cells, whereas Ober *et al*. stored the white cell prep at 4°C [32]. Clark *et al*. found that storing leukocytes at 4°C overnight can cause loss of effectiveness, related to shedding of surface CD200 molecules into the supernatant [36]. The CD200 molecules may be important in the induction of progesterone receptors in gamma/delta T cells. Though fresh white cell injections have been found to help decrease miscarriage rates in animals, the use of refrigerated white blood cells would either not lower the miscarriage rate or even make it worse [34-36].

Measurement of PIBF by commercial assay is not possible as yet, though an ELISA assay has been developed and has been submitted for approval to the European FDA. In my opinion, no other testing that is available at present, including natural killer cell levels or activity before pregnancy in the blood or endometrium, sharing of histocompatibility antigens, measurement of tumor necrosis factor alfa, interferon gamma, and TH1 promoting interleukins can be relied on to detect those women who would benefit from lymphocyte immunotherapy [37-41]. Similarly, I do not think there is sufficient evidence to make decisions on whether to use lymphocyte immunotherapy, or determine the efficacy of treatment by the detection or lack of detection of lymphocytotoxic antibodies.

These tests add a considerable amount of cost to the patient, especially since they are considered experimental and many third party payers do not reimburse for these tests. I think that the best indication is simply the history, i.e., recurrent pregnancy loss. Along these lines, the therapy to primary aborters with three consecutive losses does not have to be restricted. The treatment is safe (though a blood product is being injected without quarantine), blood is taken from a male partner with whom biological fluids are exchanged and the male partner is checked first for infectious diseases. The treatment should be inexpensive – how much can it cost to draw blood from the male partner, separate the lymphocytes and give intradermal injections? I actually think it would be appropriate to offer lymphocyte immunotherapy to a woman with her first pregnancy ending in a miscarriage, if the loss occurred despite progesterone therapy and testing of the fetus found a normal chromosomal constitution, especially if the fetus was a male with no risk of maternal contamination.

However, even if a woman had a chromosomal explanation for her first loss, if she indicated that psychologically she could not take another miscarriage unless she thought she had done everything to prevent one, she could be given active immunotherapy since it is not certain that the immune system caused the loss before organ abnormalities related to the chromosome abnormality of the fetus caused the death.

My general policy is if a woman presents with one or two miscarriages and has never been treated and no chromosome studies have been performed on the fetus, I would assume the stance that there was not a chromosome cause. I would offer progesterone therapy first, since it is inexpensive with minimal side-effects and would not compromise a new pregnancy, if it was not needed. If another miscarriage occurred and chromosome analysis was normal, or inconclusive, I would offer lymphocyte immunotherapy, if it was available. The group least likely to benefit from lymphocyte immunotherapy is secondary aborters, with the most efficacy for primary and tertiary aborters.

I think that once an ELISA assay for PIBF is available, management of women with a history of miscarriage will utilize this assay to determine the proper therapy. If progesterone therapy is not allowing the serum PIBF to attain a level found normal for non-aborting women at a certain stage of pregnancy, the dosage of progesterone would be increased. If the increase failed to improve the PIBF level, then lymphocyte immunotherapy would be given (if available) and PIBF remeasured.

Of course it would be beneficial if women who had a defect in the NK allorecognition system could be identified. Some studies are evaluating the possibility of a limited repertoire of Inh Kir receptors [42, 43]. Possibly future studies will hopefully identify women less likely to respond to the relatively weak allogeneic stimulus of the fetus.

The United States FDA funded Ober and his group over two million dollars for their study. Perhaps their decision to require such an expensive IND to use lymphocyte immunotherapy may be their belief that they should support the conclusions from the study [32]. They may not want to admit that they supported a flawed study, i.e., the use of refrigerated white cells. It is unlikely that in the present economy, the FDA will support a multicenter study performed using fresh lymphocytes, to give a true evaluation of the efficacy of this procedure. Hopefully, appropriate studies will be conducted outside of the United States. However, I hope such studies include a treatment arm of progesterone with the lymphocyte immunotherapy.

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