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Pathway to Parenthood

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THE IMMUNE SYSTEM AND ITS IMPACT ON SUCCESSFUL REPRODUCTION

One of the conversations that we frequently have with patients at Acacio Fertility Center concerns immune issues and the possible impact on their ability to conceive and have a baby. The conversation evolves like this:

Patient: *I asked my former doctor about the immune system and issues with conception and he/she said they do not believe in that.*

Dr.: Perhaps they should have instead said: "I do not know too much about the immune system and its impact on infertility."

Patient: *Why do you say that?*

Dr.: The immune system and reproduction is a very complex topic. There is a lot of information available and it can be very confusing. However make no mistake about it; the immune system is VERY IMPORTANT with respect to successful reproduction. For example, imagine taking a kidney from a person walking across the street and transplanting it into another person. Do you think it would be a successful transplant?

Patient: *No*

Dr.: That is the correct answer, but why do you say that?

Patient: *It would not take because they are different people.*

Dr.: That is correct – we call that rejection. This is the reason why doctors prescribe steroids to people who have undergone a heart transplant, for example, in order to minimize the rejection reaction. Now, imagine an embryo, how come it will take? It is an entirely different tissue made 50% from someone who is not related to the mother at all. How come it will take?

Patient: *Gee that's interesting – I never thought of it like that.*

Dr.: Well if you follow this logic, the question you should ask is "how come any pregnancy is successful?"

Patient: *Wow, that's true! How does this work?*

Dr.: The answer is that in the early stages of any pregnancy, there is a chemical dialogue going on between the embryos on the one hand, and the mother's immune system on the other. The embryo will secrete certain chemicals/proteins/cytokines and the mother's immune system will decide whether to "accept" this foreign tissue and allow it to implant. We call this "tolerance." So tolerance and rejection are opposite ends of the same spectrum. Well sometimes, the immune system does not work properly and this mechanism fails. We therefore have to resort to certain types of immune-modulation therapy to help the process.

Patient: *Are there any ways to test the immune system to see if I may have this problem?*

Dr.: Yes there are. We perform a series of blood tests that can help us decide if there are problems in the immune system that may need immune modulation therapy to help a pregnancy to implant.

That is what this piece is about – immune issues and pregnancy. We will take an extremely complicated and complex subject and help you to understand these mechanisms better.

Embryo quality and endometrial receptivity are the principal rate-limiting factors governing pregnancy success with assisted reproduction. It is estimated that the embryo is directly responsible for only one-third to one-half of IVF failures in young women. Inadequate uterine receptivity ("implantation failure") is most often the casual factor in these patients. We sometimes refer to this as the "**bun**" and the "**oven**." If you take a good bun (embryo) and put it into a malfunctioning oven (uterus), we cannot expect a satisfactory outcome. A rational approach to treatment requires the physician to differentiate between disorders of embryo production and uterine receptivity. Only with diagnosis-specific intervention directed at an identifiable cause of infertility can physicians justify providing patients with renewed hope of success in a subsequent attempt at assisted reproductive treatment.

Cellular elements of the innate and adaptive immune system are important regulators of implantation and early embryo growth. Pathologic events in cellular immunity have been linked to repeated implantation failure and recurrent pregnancy loss. Despite this clear understanding of the relevance of immune dysfunction to our clinical care of women with IVF failure, there has been little attention paid to improving outcome in these women. On the contrary, there has been a vicious debate, often without substance, regarding the veracity of immunotherapy.

In the course of this review we will examine the role of innate and acquired immunity in early reproduction. Epidemiologic tests of association will be applied to the thesis that innate immune dysfunction results in implantation failure and that immunomodulatory treatment may be appropriate in the care of these women. A close examination of this relationship permits a reappraisal of the debate concerning the utility of selective immunotherapy in the treatment of women with repeated IVF failure.

Integral Role of Lymphoid Tissues in Implantation

With respect to implantation of the early embryo, diverse cell systems participate in a complex network of events, including cells traditionally assigned to the endocrine, nervous, and reproductive systems, as well as immunocytes. The following is a summary of this extremely complex system of cells. Some of the specifics are specified, but the concepts are more important. Innate immunity, particularly NK cells and NKT cells, play a central role in this system. During implantation the system must validate the identity of and accept the fetal transplant ("tolerance"). Next is development of the placenta, and lastly, appropriate embryo growth and uterine invasion.

Immunocytes and their chemical messengers play a key role in this occurrence. This involves a concept referred to as the Th1/Th2 paradigm. Pregnant females and non-pregnant females have different cell types within their uteri. Non-pregnant endometrium displays a predominantly Th1 environment. It is hypothesized that Th2 cytokines modulate or prevent Th1 responses, improving fetal survival but impairing responses against some pathogens. Transformation to Th2 dominance is believed to contribute to maternal tolerance to the fetus.

Endometrial immunocyte populations vary dramatically during the menstrual cycle. In the first half of the cycle, T cell receptor (TcR) positive T cells are the predominate lymphocyte. NK cell cytolytic activity is normally maximal in the follicular phase. Proliferative phase (1st half of cycle) endometrium appears to be a Th1 dominate environment. The composition of lymphoid cells changes dramatically in the luteal phase peri-implantation interval and into early pregnancy.

Recent evidence offers the possibility that NK T cells serve as the "local data processing units" that determine the Th1/Th2 balance and help determine pregnancy success. The uterus has NK cells and NK T cells. NK T cells function as regulators of Th1/Th2 balance. In the uterus, unique sets of NK T cells direct Th1/Th2 balance in a manner distinct from other organs.

Network "communication and control" begin with the growth of follicles. Luteal production of progesterone stimulates local endometrial production of Th2 mediators. Studies have suggested that intrafollicular Th1/Th2 inputs may modulate embryo development prior to ovulation and through the early embryo development. It is possible that these T cells provide an initial cue in favor of creating favorable conditions for early embryo development.

"Network traffic" increases dramatically with fertilization. "Broadcast messages" announcing the presence of the embryo to the ovaries and other organs include early pregnancy factor that is detectable within 12 hours of fertilization and hCG that is detectable within several days. Paracrine (adjacent) signaling between the embryo and endometrial elements might be likened to "cross talk" over a local area network that help mediate implantation. Other important factors include Leukemia inhibitory factor (LIF) and Colony stimulating factor-1 (CSF-1 or macrophage CSF).

HLA-G is expressed on trophoblast and necessary for implantation. It is believed that it helps encourage the Th2 dominant environment. HLA-G expression may be stimulated by LIF. These bi-directional inputs, integrated by NK T cells and/or immunocytes, result in the sequential development of various cell types in a time-sequence specific manner. Hence the balance between these lymphocyte sets modulate the events of normal pregnancy and offer the possibility that pathologic deviations may lead to implantation failure and/or RPL.

Immunotrophism is a second mechanism through which endometrial immunocytes contribute to pregnancy success. Although fetal tolerance requires a Th2 environment, there is a Th1 influence in early pregnancy. A transient and focal inflammatory environment is required to facilitate trophoblast invasion and blood vessel growth prior to establishment of the Th2 anti-inflammatory environment.

As can be seen in this summary, the process is **EXTREMELY COMPLEX**. Fortunately, it is not essential to measure all of these different elements; in clinical practice, we can obtain useful information that allows us to make important therapeutic decisions, without having to know specific levels of each of these aforementioned markers.

Epidemiologic Tests of the Association of Immunopathology and Implantation Failure and the Efficacy of Immunomodulatory Therapies

David Clark and others have performed an elegant series of experiments characterizing reproductive loss in the mouse. Successful murine (mouse) pregnancy is associated with elevated levels of the Th2 cytokines, while pregnancy loss is associated with Th1 cytokines (TNF-alpha, gamma-interferon). Pregnancy failure may result from either pathologic establishment of a Th1 bias or failure of the Th2 "suppressor" elements. Two essential pathways have been described:

1. Peri-implantation losses (between implantation and formation of a vascularized placenta, analogous to occult losses in humans).
2. Early miscarriage.

Modulation of these systems to favor either pregnancy or abortion can be achieved by injection of cytokines, antibodies to cytokines or receptors, or biorespone modulators. These animal models have served to be extremely useful to enable clinical investigators to obtain an understanding of therapeutic interventions that may prove to be beneficial.

This model emphasizes a central role for innate immune cells (macrophages, dendritic cells, NK cell, NK T ells) in the regulation of immune balance at the maternal fetal interface. As one might have expected and as mentioned above, aberrant lymphoid cell function can cause significant problems with respect to successful reproduction.

Differences in cell populations have been found in women with IVF implantation failure, unexplained infertility, endometriosis patients and those with autoimmune thyroid disease (ATD) and reduced fertility. Abnormal LIF production has been documented by several investigators. Prolonged LIF into the late luteal phase is associated with IVF failure.

The recurrent pregnancy loss (RPL) literature offers analogous data regarding immunocyte profiles in preconceptual and pregnant tissues. These data suggest that the endometrial lymphocytes of recurrent spontaneous aborters harbor a distinct immunophenotypic profile that antedates implantation and that endometrial immunologic conditions are intrinsically altered in recurrent aborters. In contrast, others could not predict pregnancy outcome by immunohistochemical analysis of peri-implantation endometrium in women with recurrent abortion. Levels of the Th1 cytokines in peripheral blood are significantly greater in women with RPL compared with controls, along with other variations of immunologic cells.

The above review of some of the literature is intended to provide some insight into the complexity of this system. I trust you can see that to make the statement that we "do not believe in the importance of the immune system in the ability to get pregnant and maintain a pregnancy," is inappropriate. As complex as the system is, it would be equally inappropriate to pretend we have all the answers – as clearly we do not.

How can diagnostic tests aid in the diagnosis of these systems?

Cytokine (cell secretion) profiles in uterine flushing may provide a direct and palatable means to assess the uterine immune environment. Measurement of LIF in uterine flushing is reported to predict reproductive outcomes. A proportion of women with unexplained infertility have undetectable LIF in flushing fluid in the early and mid-luteal phase. Detectable levels of LIF in late luteal phase uterine flushing predict IVF failure. Further studies are required to validate these methods for clinical use.

Likewise, immune cell populations in luteal phase endometrial biopsies may offer more direct insight into endometrial immune homeostasis. Analysis of endometrial immunocyte profiles have been shown to be predictive of pregnancy outcome in both women with infertility and RPL. Given the association between the cytokine environment at the endometrial interface and integrin expression, it is possible that immunohistochemical determination of integrin expression may serve as an indirect marker for reproductive immune dysfunction.

Peripheral immunocyte profiles have bene shown to be predictive of reproductive outcome by some authors. Aoki, et al, demonstrated that pre-conceptional evaluation of NK activity in women with recurrent miscarriages in predictive of the risk of the pregnancy loss at the next conception. In contrast, Emmer was unable to find a difference in non-pregnant NK cell activation or NK cell subset between RPL and controls. Morikawa was unable to find a relationship between nonpregnant NK cell activity or

subsets and subsequent pregnancy outcome in a large group of women with RPL. However, no fertile controls were evaluated in this study.

The following are some studies that have examined this complex topic. Coulam prospectively measured peripheral CD56 cells in first trimester pregnancies of women suffering form infertility or RPL. The prevalence of women with persistent elevation of percent of CD56+ cells (>12%) was 18% among RPL and 39% in infertile women. The birth rate in 10 women with elevated CD56 cells who were treated with IVIG was 100%; as compared to a birth rate of 11% in women not receiving IVIG. Elevated CD56 counts were associated with normal chromosomes in women who aborted while women with normal CD56 counts had 68% abnormal karyotypes with miscarriage. Coulam subsequently reported that expression of TJ6 on CD19 and CD56 cells during the first trimester predicts viable pregnancy. Yamada found that high NK activity at 6 to 7 weeks of gestation predicted subsequent miscarriage of a chromosomally normal fetus.

Does immunomodulatory (medical manipulations to the immune system) treatment reverse immune-mediated implantation failure in humans?

Carp was the first to study the effect of IVIG (Intravenous immunoglobulin) on IVF outcome. There have been several studies of IVIG for the treatment of IVF implantation failure and a number of reports of less structured case series and trials combining the use of IVIG with other agents. Five authors found positive results, one reported equivocal outcomes, and two were unable to demonstrate a benefit of IVIG. In the early 1990's Kleinstein and De Placido performed placebo controlled trials of IVIG administration to unselected women with previous IVF failure. Kleinstein found that women treated with IVIG were significantly more likely to deliver than placebo (40% vs. 9%). In De Placido's study, implantation rates were significantly improved with IVIG (18% vs. 7% per embryo transfer), but the difference in pregnancy rates (33% vs. 24% in the placebo group) was not significant.

In 1994 Coulam described her experience with the use of IVIG for the treatment of women with repeated IVF failure. Twenty-nine women were categorized as either "efficient" or "poor" embryo producers. Each patient had suffered three or more failed IVF attempts. These women did not receive immunologic testing prior to study entry. IVIG treatment resulted in a live birth rate of 44% in efficient embryo producers. In contrast, IVIG did not benefit poor embryo producers (0% pregnancy rate). Scheer and Salazar reported the effect of IVIG on IVF outcomes in 30 patients suffering from recurrent IVF failure. Ninety percent of these women had one or more abnormal immune tests. Over the course of 40 treated IVF cycles, 79% of women became pregnant, 89% of pregnant patients delivered. Consistent with Coulam's experience, poor responders were unlikely to become pregnant (17% conception).

More recent studies have focused on outcomes in women with IVF failure and abnormal tests for NK cell function. Coulam reported the use of IVIG for the specific group of women with repeated IVF failure who had an elevated level of circulating CD56+ cells. The 32 women in this experience were efficient

embryo producers and each had at least three previous failed IVF procedures. Live birth rates following subsequent IVF with and without IVIG were 38% vs. 0%. Coulam and colleagues recently reported that treatment with IVIG was highly efficacious for the specific group of women that had elevated peripheral NK cytolytic activity and were suffering from repeated IVF failure. In this retrospective study, ongoing pregnancy rates for women treated with IVIG were 63% as compared to 4% in women with a positive NK assay that declined treatment.

Case reports and studies combining IVIG with other modalities suggest a beneficial effect of IVIG on recurrent IVF failure. Stricker reported pregnancies in 24 of 36 women treated with low dose IVIG in a mixed group of patients suffering from immunologic infertility or RPL, treated with either ovulation induction or IVF. Sher, et al. reported three positive studies using combined heparin, aspirin, and IVIG for treatment of women with APLA or antithyroid antibodies.

Negative studies were reported by Stephenson and Balasch. Stephenson randomly assigned 39 couples that suffered from recurrent IVF failure and were preparing for IVF to treatment with IVIG or saline. The patient population was not selected or screened with immunologic testing. No significant difference in live birth rates (19% vs. 17%) was found. Balasch treated 12 consecutive tubal infertility patients suffering from repeated IVF failure with high does IVIG. None of the patients became pregnant.

As demonstrated from the various findings of these studies, this treatment is still considered to be experimental.

Discussion

It is evident that lymphoid cells, particularly those comprising the innate immune system, play a central and multifaceted role in implantation. Immunocytes comprised a substantial proportion of cells during the time of implantation and early pregnancy. Cytokines, hormones that have traditionally been regarded as immune mediators, have profound influence on the success of early pregnancy.

Despite this understanding of the importance of immune cells in early reproduction, there has been relatively little research concerning the diagnosis and treatment of immune mediated implantation failure. The use of immunomodulatory therapies (IVIG or intralipid) in this indication has been extremely controversial.

The genesis of this controversy lies in the well-intended efforts of investigators to understand and treat repeated IVF failure. In the early 1990's considerable attention was paid to pathology of the adaptive immune system, particularly autoantibodies (i.e., antiphospholipid antibodies [APLA] and antithyroid antibodies). Although the role of antiphospholipid antibodies in the pathogenesis of recurrent

pregnancy loss is well accepted, the existence of a causal relationship with implantation failure is debated. Heparin treatment is reported to potentially be beneficial in these women.

Consequently, a furious debate erupted in the late 1900's over the relevance of APLA specifically, and immune pathology in general, in assisted reproduction. As a consequence of this debate authors on the "con" side have strongly asserted that immunotherapy is not indicated in recurrent IVF failure. This recommendation has its basis in and promulgates two fallacious observations:

> Fallacy #1: The literature suggests that autoantibodies are not causally related to implantation failure. Therefore, it is implied that implantation failure is not a result of immunopathology.
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> Fallacy #2: Immunopathology (i.e. autoantibodies) does not cause implantation failure. Therefore, immunomodulatory therapies are not indicated in the treatment of implantation failure.

It is clear that APLA are not the primary mediators of immune-based implantation failure. Autoantibodies may serve as a marker of generalized immune dysfunction which affects early reproductive performance.

It appears that innate immunity, not acquired immunity, plays a central role in modulation of endometrial Th1/Th2 balance that consequently determines the eventual success of pregnancy. The essential pathology is unknown (and indeed is likely multifactorial). Potential mechanisms may include:

1. Systemic immune disorders that inhibit a Th1 to Th2 shift by naïve T cells
2. Endometrial response that stimulates an early Th1 response or late Th1 response leading to placental thrombosis (recurrent miscarriage).
3. Aberrant HLA-G signaling resulting in a release of Th2 dominance.
4. Disruption of the time sequence of expression of cell-to-cell adhesion molecules that mediate implantation.
5. Failure of immunotrophic-mediated placentation.

Given the pivotal role of endometrial immunocytes as control elements of these processes, it is wholly unreasonable to suggest that there are no disease states involving these systems that lead to reproductive failure. There is not another organ system in which immune cells do not participate in disease. Why should we postulate that the reproductive tract is uniquely privileged? Accordingly, these reproductive lymphoid tissues remain targets for intervention with immunomodulatory therapies.

Likewise, it is wholly unreasonable to a *priori* suggest that IVIG or intralipid is not indicated in the treatment of selected patients with implantation disorders. Indeed, the preponderance of the available literature demonstrates a beneficial effect of this therapy for the treatment of women with recurrent IVF failure (in contrast to the literature concerning IVIG and RPL where the strength of the association is debated). Indeed, it appears that the association is strengthened when women who are poor embryo

producers are excluded and when IVIG is used in the specific indication of the treatment of women with abnormal measures of peripheral NK cell dynamics.

However, as complex as this system is, it would be wrong to suggest that there are not problems to be resolved. Indeed, there are problems with both our understanding of the underlying pathology and the clinical application of treatment for innate immune disorders and implantation failure. There is little human data to guide clinicians as to the specific disease states that need to be addressed. The literature describing the treatment of immunology based implantation failure does not meet the tests for evidence based practice. The test is straightforward: first to elucidate the pathology and second to apply diagnosis-specific treatments.

In this regard we are challenged. It is evident that immune-mediated implantation failure is an uncommon problem. While there is a conceptual framework in animal models, a corresponding understanding in human clinical practice is limited.

At present, treatment of "NK cell activation" is the only category of reproductive innate immune dysfunction that has been clinically explored and characterized. Coulam demonstrated that certain women with RPL and others suffering from recurrent IVF failure have activated peripheral NK cells. Similar findings have been obtained in RPL patients and infertile women. However, further studies are needed.

IVIG and intralipid have been successfully used to treat implantation disorders in this specific group of women testing positive for NK cell activation. These studies support the hypothesis that NK cell activation is causally related to recurrent IVF failure and that this treatment successfully ameliorates this condition.

The experience with IVIG or intralipid in the treatment of NK cell activation offers the hope that women with other implantation disorders may be successfully diagnosed and treated. Questions remain to be answered. What is the prevalence of immune-mediated implantation failure? Is immunologic implantation failure the result of local disruptions at the maternal-fetal interface, generalized autoimmune disease, or both? What are the appropriate treatments for this disorder; in particular is it possible to replace the use of IVF with a more suitable medication? To a large extent, we are hindered by the paucity of information in this area.

Without question there are women who suffer from repeated implantation failure. The data clearly indicates that immune dysfunction is the causal factor in some of these women. While clinicians have a firm desire to base their practice on indisputable knowledge (that is unavailable in this instance), some of these particular patients may not have children without intervention.

Women with findings consistent with NK cell activation should be offered the option of directed treatment with IVIG to address this pathology. It is likely that other patients suffer immune-mediated pathology that is not detected by NK assessment. In this instance, it is difficult to make a definitive

recommendation. It is important that the clinician and patient suffering from recurrent IVF failure should make an informed decision whether to proceed with empiric IVIG or intralipid therapy.

Many questions remain in this very complex area of reproductive medicine and unfortunately, in some instances, there are more questions than answers. The cost of IVIG is a significant obstacle (~$3500 per treatment x 2 treatments). To this end, new interventions, such as using intralipid therapy, are being evaluated. As with anything in medicine, benefits and risks must be evaluated. If all else fails with immune-modulation therapy and the couple generates nice embryos, consideration can be given to the use of gestational surrogacy – not an inexpensive treatment in and of itself but a very viable alternative.

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This handout is intended as an aid to provide patients with general information. As science is rapidly evolving, some new information may not be presented here. It is not intended to replace or define evaluation and treatment by a physician.