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Reproductive immunology: the relevance of laboratory research to clinical practice (and vice versa)

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In this issue of *Human Reproduction*, the paper by Gu *et al.* represents a landmark in reproductive immunology research. A new mechanism for evasion of maternal immune attack is described, one which explains an older theory but also itself represents part of a newer understanding of the maternal–fetal relationship. Although some of the methods may be relatively unfamiliar to many readers of this journal, the scientific endeavour is extraordinary and it is stimulating to consider how this is placed in the context of current theory and practice of reproductive immunology. In particular, this paper demonstrates, as an example, that there is a whole lot more to reproductive immunology than natural killer (NK) cells and immune therapy.

The field of reproductive immunology often arouses unusually emotive and diverse responses amongst clinicians, scientists and patients. At its core is the concept of the relationship between mother and conceptus which is critical for human reproduction. It is indisputable that the nature of the maternal–fetal interface determines the success or failure of pregnancy. However, our understanding of that interface, and our attempts to alter or improve it, are the cause of the controversy.

So why the controversy? Everyone has the same goal of trying to increase knowledge and improve outcome for patients. Clinical outcomes are the basis of all medical intervention, and the unfortunate truth is that some couples suffer apparently unexplained infertility or repeated miscarriage or IVF failure. Even with the most advanced genetic screening IVF produces, at best, 70% success rates when chromosomally normal embryos are transferred. It is perfectly reasonable to wonder if we are missing something. And from a patient perspective, is it not natural to want to do whatever *could* be possible to improve outcome next time? So there are considerable emotional, physical and financial costs driving the desire for immune testing and therapy.

Clinicians, by their nature, are simply trying to help too. Reproductive medicine has a long history of immune therapy, almost all empirical and unproven (in terms of randomized trials). But in the words of Peter Medawar, father of reproductive immunology, 'If a person a) is poorly, b) receives treatment intended to make him better, and c) gets better, no power of reasoning known to medical science can convince him that it may not have been the treatment that restored his health'. Problems arise when excessive claims are made, leading to patients potentially doing more expensive and even dangerous treatments where benefit is unlikely.

Research scientists are rigorous and objective, but often not involved in clinical care. There have, at times, been heated arguments between those interested in understanding, and those interested in treating. In reality, we must accept that there is currently a gap between the two. But we all do our patients no service whatsoever to pretend that we lie wholly in one camp or the other, and that one is somehow more relevant than the other. Clinicians and scientists (and their patients) need to appreciate the complexities and needs of each side, and this new scientific paper in this journal should be a catalyst for more open debate by all.

The 'placental sink'

The paper by Gu *et al.* describes a considerable amount of *in vitro* work using human placentas, trophoblast cell lines and trophoblast cell cultures, and gene knock-out mice. A wide array of laboratory methodologies from three different groups in both China and USA over a remarkable 8 years of research all provide supportive evidence for the main findings. The authors should be congratulated for their painstaking efforts to try to produce as definitive a result as possible. Even clinicians with little knowledge of the techniques used will appreciate the logic and depth of approach to the problem.

The background for the research was to increase understanding of how the placenta evades antibody attack in particular. Relatively little work on this has been done since the vague notion that the placenta acts as an 'antibody sink' to filter out potentially harmful anti-paternal antibodies (Gitlin and Morphis, 1969; Simister and Story, 1997). The 'placental sink' concept fitted the paradigm of the time, that the placenta is a passive protective structure that is largely 'unseen' by the maternal immune system.

This new body of work has established a mechanism for evasion of antibody attack, but also has wider implications for reproductive immunology. It has been shown that placental trophoblast (and endothelial) cells are capable of producing IgG, a significant portion of which is 'asymmetric' (glycosylated at one of its Fab arms). This asymmetric IgG can react with the Fc portion of other immunoglobulins, and also

to leucocytes. Asymmetric IgG does not trigger complement fixation phagocytosis or T-cell activation. Thus, the high local concentration of asymmetric placental IgG would be able to bind to maternal antibodies and leucocytes and thereby interfere with or block their potentially harmful reactions against the fetus. This new mechanism could explain the concept of the 'placental sink', and is also relevant to cellular attack (innate and adaptive). Indeed, it could be a major form of placental defence from maternal immune attack.

It is worth noting that numerous other mechanisms for fetal evasion from maternal immune attack have been described. These include the lack of expression on trophoblast of HLA-A and HLA-B, the immune modulating effect of HLA-G, the interaction between trophoblast HLA-C and maternal killer immunoglobulin-like receptors (KIR), the expression of indoleammine-2,3 dioxygenase which catabolyses tryptophan vital for T cell function, the presence of Treg cells, systemic suppression of NK cell numbers and function, and deviation of the maternal cytokine response towards type 2 (antibody-mediated) rather than the more dangerous type 1 (cell-mediated) (Trowsdale and Betz, 2006; Chaouat *et al.*, 2010). Given this list, it is immediately apparent that it is highly unlikely that one single mechanism is responsible for the success or failure of pregnancy. So what is the wider significance of this new study by Gu *et al.*?

Current paradigm for reproductive immunology

The interesting aspect of this study is that the protective mechanism is one of 'active exertion' rather than a 'passive escape' (Gu *et al.*, 2014). In other words, the placenta is an active immune entity that engages with the maternal immune system. This has been noted in other ways previously, such as the production, by trophoblast cells, of interleukin-4 (Sacks *et al.*, 2001), and is an example of a dynamic maternal–fetal relationship. This is, in essence, the problem with current immune empirical treatments which are derived from previous and apparently contradictory theories.

Until relatively recently, the overriding paradigm for understanding the maternal–fetal relationship was that described so eloquently by Peter Medawar (Medawar, 1953). Approaching the problem as an analogy with tissue transplantation immunology, in his 1953 paper, he proposed that (i) maternal and fetal circulations need to be separated, (ii) fetal (placental) cells should evade maternal immune recognition and (iii) there should be a general maternal immune suppression. The concept was that the maternal immune system is highly dangerous for pregnancy, and the success of pregnancy depends on the placenta 'evading' recognition and attack. This was the background to the theory of the 'placental sink'. It was also this paradigm which led to clinical application of immune suppression therapy in women with repeated miscarriage or in IVF failure. It seemed obvious to assume that if some maternal immune suppression is necessary, there may be some who will benefit from more.

But in the 1990s, as better research tools became available, it was increasingly apparent that not only is the placenta not passive, but also that the maternal immune system is not suppressed. Trophoblast cells encounter maternal immune cells directly in the uterus and also in the blood, and do indeed express HLA-C, HLA-E and HLA-G which can all be recognized by the maternal immune system. We now know of course that there is such an extensive release of fetal cellular material into the maternal circulation that it can be detected by a simple blood test for karyotype testing—a recent revolution for Down's syndrome screening (Lo, 2013). Moreover, the maternal immune system is patently not suppressed, and, on the contrary, activated in a number of ways (Sacks *et al.*, 2003, 2004; Chaouat, 2013).

Perhaps most controversy has revolved around NK cells. Research has clearly demonstrated that uterine NK cells are 'activated' rather than suppressed in normal pregnancy (Fu *et al.*, 2013; Moffett and Colucci, 2014), that they have receptors for trophoblast proteins (KIR and CD94) (Moffett and Loke, 2004), and that specific interactions between uterine KIRs and trophoblast HLA-C subtypes can result in deficient placentation and clinical disorders such as repeated miscarriage (Hiby *et al.*, 2008) and pre-eclampsia (Hiby *et al.*, 2004), and IVF failure (Alecsandru *et al.*, 2014). It is the inhibiting (rather than activating) form of KIR-HLA-C interactions which appears to be detrimental. In other words, uterine NK cells appear to play an active and necessary part in implantation. Medawar's paradigm has been completely overturned as we now consider the question of how the maternal immune system contributes to reproductive success, rather than prevents it (Moffett and Loke, 2004).

Maternal immune activation appears to be important for pregnancy success, presumably as it is a means for recognition of a pregnancy and appropriate alteration of maternal physiology. It has never been demonstrated that the maternal immune system 'rejects' a pregnancy. And given the wide array of protective features that do occur (of which the paper subject of this commentary is one), there is still no known mechanism for this to occur. Indeed, recent work has shown that even high levels of uterine NK cells are associated with a corticosteroid deficiency rather than immune attack, and that this may be the reason for any potential benefit from immune therapy (Kuroda *et al.*, 2013). So, this is why researchers are so frustrated with a persisting clinical empirical approach of immune therapy, which is universally suppressive (prednisolone, IVIG, intralipid, anti-TNFα) (Moffett *et al.*, 2004).

Immune therapy

There is no doubt that there is a strong clinical need for immune approaches (Kwak-Kim *et al.*, 2013). Patients can see that conventional reproductive medicine simply cannot guarantee success. They frequently use alternative practitioners in parallel with IVF or miscarriage care (Stankiewicz *et al.*, 2007). Anecdotally, their desire for immune investigation is often as much for a potential explanation as for treatment. In spite of research described above, it is of course still eminently possible that *some* women have an abnormal immune reaction to pregnancy that may be amenable to immune therapy. The real issue is—which ones? Many interested in immune investigation and therapy have probably done themselves a disservice by using poorly controlled tests, with poorly described reference ranges, resulting in treatment of almost certainly more women than necessary, and then overplaying the significance and overmarketing the positive outcomes. It is possible that NK testing, when done well, is able to target a particular group of women who may benefit from immune suppression (King *et al.*, 2010; Sacks *et al.*, 2012), and there are some preliminary data indicating potential benefit (Clark, 2008; Bansal *et al.*, 2012; Polanski *et al.*, 2014). It is possible that benefit occurred by chance or placebo, or by still unknown mechanisms that have nothing to do with immune suppression of NK cells (e.g. Kuroda *et al.*, 2013; Kwak-Kim *et al.*, 2014). But it is a necessary field for more and better research. Arguments that immune therapy does not make sense in the context of current knowledge of uterine NK

biology (Moffett *et al.*, 2004) are useful drivers for research. They do not exclude or disprove the possibility that it is beneficial to some women (Clark, 2008).

For practitioners of immune therapy though, there is a vital responsibility to be knowledgeable of current research. Reproductive immunology has changed so completely since immune therapy was first used, and awareness of this more dynamic relationship between mother and conceptus is likely to produce better results. For some, immune suppression may be wholly inappropriate and likely to reduce their chances. Thus, the immune tests used by clinics should be carefully assessed and appraised, and there should be a strong ambition to assess outcome as rigorously as possible. Whilst randomized trials are the goal, it is clear that they are very hard to complete in practice. So outcome studies of all kinds should be presented and debated (Clark, 2014). And the onus is on those practitioners to be honest and humble in discussing the relative lack of proof of their therapies. Immune therapy should not be controversial, although it is empirical.

Perhaps all of us—clinicians, scientists, patients—should remember another bit of advice from Peter Medawar: 'the intensity of the conviction that a hypothesis is true has no bearing on whether it is true or not'. The complexity of laboratory insights into the nature of the maternal–fetal interface should not be used to dismiss simplistic clinical empirical therapies, but rather to guide the development and interpretation of better clinical tests and more targeted therapies. It is our duty to our patients to embrace all these endeavours.

References

Alecsandru D, Garrido N, Vicario JL, Barrio A, Aparicio P, Requena A, García-Velasco JA. Maternal KIR haplotype influences live birth rate after double embryo transfer in IVF cycles in patients with recurrent miscarriages and implantation failure. *Hum Reprod* 2014;**29**:2637–2643.

Bansal AS, Bajardeen B, Thum MY. The basis and value of currently used immunomodulatory therapies in recurrent miscarriage. *J Reprod Immunol* 2012;**93**:41–51.

Chaouat G. Inflammation, NK cells and implantation: friend and foe (the good, the bad and the ugly?): replacing placental viviparity in an evolutionary perspective. *J Reprod Immunol* 2013;**97**:2–13.

Chaouat G, Petitbarat M, Dubanchet S, Rahmati M, Ledée N. Tolerance to the foetal allograft? *Am J Reprod Immunol* 2010;**63**:624–636.

Clark DA. Immunological factors in pregnancy wastage: fact or fiction. *Am J Reprod Immunol* 2008;**59**:277–300.

Clark DA. Popular myths in reproductive immunology. *J Reprod Immunol* 2014;**104–105**:54–62.

Fu B, Li X, Sun R, Tong X, Ling B, Tian Z, Wei H. Natural killer cells promote immune tolerance by regulating inflammatory TH17 cells at the human maternal–fetal interface. *Proc Natl Acad Sci USA* 2013;**110**:E231–E240.

Gitlin D, Morphis LG. Systems of materno-foetal transport of gamma G immunoglobulin in the mouse. *Nature* 1969;**223**:195–196.

Gu J, Lei Y, Huang Y, Zhao Y, Li J, Huang T, Zhang J, Wang J, Deng X, Chen Z *et al.* Fab fragment glycosylated IgG may play a central role in placental immune evasion. *Hum Reprod* 2015;**30**:380–391.

Hiby SE, Walker JJ, O'shaughnessy KM, Redman CW, Carrington M, Trowsdale J, Moffett A. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J Exp Med* 2004;**200**:957–965.

Hiby SE, Regan L, Lo W, Farrell L, Carrington M, Moffett A. Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage. *Hum Reprod* 2008;**23**:972–976.

King K, Smith S, Chapman M, Sacks G. Detailed analysis of peripheral blood natural killer (NK) cells in women with recurrent miscarriage. *Hum Reprod* 2010;**25**:52–58.

Kuroda K, Venkatakrishnan R, James S, Šucurovic S, Mulac-Jericevic B, Lucas ES, Takeda S, Shmygol A, Brosens JJ, Quenby S. Elevated periimplantation uterine natural killer cell density in human endometrium is associated with impaired corticosteroid signaling in decidualizing stromal cells. *J Clin Endocrinol Metab* 2013;**98**:4429–4437.

Kwak-Kim J, Han AR, Gilman-Sachs A, Fishel S, Leong M, Shoham Z. Current trends of reproductive immunology practices in in vitro fertilization (IVF)—a first world survey using IVF-Worldwide.com. *Am J Reprod Immunol* 2013;**69**:12–20.

Kwak-Kim J, Bao S, Lee SK, Kim JW, Gilman-Sachs A. Immunological modes of pregnancy loss: inflammation, immune effectors, and stress. *Am J Reprod Immunol* 2014;**72**:129–140.

Lo YM. Non-invasive prenatal testing using massively parallel sequencing of maternal plasma DNA: from molecular karyotyping to fetal whole-genome sequencing. *Reprod Biomed Online* 2013;**27**:593–598.

Medawar PB. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. *Society for Experimental Biology*. New York: Academic Press, 1953, 320–338.

Moffett A, Colucci F. Uterine NK cells: active regulators at the maternal–fetal interface. *J Clin Invest* 2014;**124**:1872–1879.

Moffett A, Loke YW. The immunological paradox of pregnancy: a reappraisal. *Placenta* 2004;**25**:1–8.

Moffett A, Regan L, Braude P. Natural killer cells, miscarriage, and infertility. *BMJ* 2004;**329**:1283–1285.

Polanski LT, Barbosa MA, Martins WP, Baumgarten MN, Campbell B, Brosens J, Quenby S, Raine-Fenning N. Interventions to improve reproductive outcomes in women with elevated natural killer cells undergoing assisted reproduction techniques: a systematic review of literature. *Hum Reprod* 2014;**29**:65–75.

Sacks G, Yang Y, Gowen E, Smith S, Fay L, Chapman M. Detailed analysis of peripheral blood natural killer cells in women with repeated IVF failure. *Am J Reprod Immunol* 2012;**67**:434–442.

Sacks GP, Clover LM, Bainbridge DR, Redman CW, Sargent IL. Flow cytometric measurement of intracellular Th1 and Th2 cytokine production by human villous and extravillous cytotrophoblast. *Placenta* 2001;**22**:550–559.

Sacks GP, Redman CW, Sargent IL. Monocytes are primed to produce the Th1 type cytokine IL-12 in normal human pregnancy: an intracellular flow cytometric analysis of peripheral blood mononuclear cells. *Clin Exp Immunol* 2003;**131**:490–497.

Sacks GP, Seyani L, Lavery S, Trew G. Maternal C-reactive protein levels are raised at 4 weeks gestation. *Hum Reprod* 2004;**19**:1025–1030.

Simister NE, Story CM. Human placental Fc receptors and the transmission of antibodies from mother to fetus. *J Reprod Immunol* 1997;**37**:1–23.

Stankiewicz M, Smith C, Alvino H, Norman R. The use of complementary medicine and therapies by patients attending a reproductive medicine unit in South Australia: a prospective survey. *Aust N Z J Obstet Gynaecol* 2007;**47**:145–149.

Trowsdale J, Betz AG. Mother's little helpers: mechanisms of maternal–fetal tolerance. *Nature Immunol* 2006;**7**:241–246.