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Neuroimmune-Endocrine Interactions during Early Pregnancy in an Autoimmune Context: Focus on Macrophage Activation

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Pregnancy · Autoimmune diseases, chronic · Neuroimmune regulation · Macrophages

Abstract
Neuroimmune-endocrine interactions seem to be central to the dialogue between the mother and the growing embryo during normal pregnancy. A proinflammatory Th1 microenvironment appears to be associated with embryo implantation but an excess of these cytokines may be deleterious. When normal gestation is subjected to stressful stimuli as those provided by a chronic inflammatory milieu, the activation profile of T cells and macrophages may be temporarily changed. Although much evidence supports the protective role of pregnancy in Th1 autoimmune diseases, the comprehension of the maternofetal interaction in an inflammatory context may serve to get more insight into pregnancy failures. Macrophages integrate multiple inputs and signals of neuroimmune-endocrine systems and they appear as major participants in either embryo implantation or loss. Changes at the macrophage level during gestation might help to understand their regulatory role in embryo implantation as well as to disclose their local and systemic pathogenic potential.

The success of implantation and pregnancy relies on an initial 'cordial dialogue' between maternal and embryonic/fetal tissues through a complex immune-neuroendocrine network whose comprehension entails an integrative approach.

Chronic diseases such as most autoimmune diseases of the mother can specially affect this interaction and, conversely, embryo implantation and pregnancy as a whole can modulate the outcome of ongoing diseases. Moreover, although in most cases pregnancy itself generates a protective milieu for the mother and the future newborn, as a stressing stimulus it may help to disclose subclinical pathological conditions or enhance the susceptibility to develop disease.

The concurrence of such a variety of factors, cell types and interactions makes pregnant women with autoimmune diseases a good example of the need for an interdisciplinary view and care by various specialized physicians and scientists.

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Immune-Neuroendocrine Interactions during Early Normal Pregnancy

Immune and neuroendocrine regulation of the maternal-fetal interface has a central role in both implantation and the development of the placenta by maintaining uterine quiescence and neovascularization and promoting maternal immunotolerance. The peri-implantation period is one of the critical periods along pregnancy where defects in the maternal-embryo interaction may have serious consequences with the risk of abortion. There are multiple causes of early abortions related to the hemostatic and endometrial defects, endocrine disorders, infections and autoimmune diseases of the mother with about 50% of abortions associated with an immunological origin [1]. According to some opinions, recurrent miscarriage is comparable to organ-specific autoimmune disease [2].

Wegmann [3] proposed a model for the immune regulation of implantation where the development of pregnancy required a Th2 environment and the Th1 response was deleterious. In this Th1/Th2 paradigm T lymphocytes from the mother tolerogenic to paternal antigens had a central role in the maintenance of gestation by promoting a Th2 response [3]. Moreover, allorecognition can increase the production of growth factors and hormones essential for embryonic and fetal development: HLA alloantigen recognition in uterus enhances leukemia inhibitory factor (LIF), stromal cell-derived growth factor-1 (SDF-1), ribonucleases and human chorionic gonadotropin [4]. In line with this, progesterone increases the receptivity of the endometrium for embryo implantation while it modulates the Th1/Th2 cytokine profile in favor of Th2 cytokines [5, 6]. However, recent concluding evidence supports a more complex model where the innate immune response and cytokines produced by both immune and nonimmune cells in the maternal-fetal interface regulate the overall process including tissue remodeling, neovascularization and host defense [7]. In this hypothesis, a microenvironment controlled by locally acting growth factors and cytokines, some of them under steroid control, appears to be necessary at the first stages of gestation for both implantation and uterine receptivity [7]. In fact, uterine NK cells (uNK), the largest leukocyte population in the human first trimester decidua, are recruited by progesterone which promotes uNK homing to the uterus. They express steroid receptors (ERβcx/β2 ERβ1), prolactin and glucocorticoid receptors [8]. Prolactin released by endometrial stromal cells induces differentiation and maturation of uNK. Thus, female sex steroids appear to regulate the number of uNK cells in both human and mouse models by recruitment of peripheral NK and also by proliferation of the existing uNK pool. Consequently, cytokine produced under these combined stimuli may influence the decidual and trophoblast microenviroment inducing local immunomodulation and controlling the trophoblast invasion.

In keeping with a model where the generation of an initial wave of proinflammatory cytokines and chemokines initiates and conditions the adaptive immune response and embryo survival, a β-chemokine such as RANTES (regulated upon activation normal T cell expressed and secreted) is required at immunoprivileged sites and peaked before the Th2 switch in fertile women but not in those with spontaneous resorption histories [9]. Similarly, alloimmunization of women with recurrent spontaneous abortion resulted in anti-CCR5 antibody production, increased production of RANTES, LIF, MIP-1α (CCL3), MIP-1β (CCL4) and SDF-1 [4, 10]. It has recently been proposed that CCL4 was important for the recruitment and/or retention of an immunosuppressive population of CD4+CD25+ T cells towards activated antigen-presenting cells [11]. Interestingly the number of regulatory T cells in decidua samples from spontaneous abortions is significantly lower compared to elective abortions [12].

Whilst a proinflammatory Th1 microenvironment appears to be inherent in implantation and early pregnancy, it is also clear that an excess of these cytokines may be deleterious [13, 14]. Conversely, although the balance might be restored by Th2 and Th3 cells, too much Th2/3 cytokines may not be healthy. Particularly TGF-β can inhibit trophoblast growth and invasion necessary for implantation through the inhibition of MMP-9 production and human chorionic gonadotropin secretion by human trophoblast [15].

Early Pregnancy and Autoimmune Diseases: Reciprocal Modulation

Some autoimmune rheumatic diseases provide the clinical counterpart of the interplay between pregnancy and the Th1/Th2 cytokine ratio in normal women. As an example to illustrate this, patients with rheumatoid arthritis, a typical Th1 chronic inflammatory disease, improve during pregnancy [16–18]. On the other hand, a high risk of a disease flare occurs during pregnancy in the preferentially Th2 systemic lupus erythematosus (SLE), leading to spontaneous abortion among other

complications [18, 19]. One potential mechanism underlying these clinical data is the capacity of cortisol, catecholamines, and estrogen to enhance IL-10 production, relative to TNF-α and IL-12; that is, during pregnancy the proinflammatory/anti-inflammatory cytokine balance favors humoral immune mechanisms that characterize SLE [18]. On the other hand, recurrent spontaneous abortions or a poor implantation record after multiple IVF cycles have been reported in women prone to develop Th1 responses [20] while, conversely, the cytokine shift to Th2 was reported accentuated rather than diminished in women with recurrent miscarriage [21] reflecting the complex nature of immune modulation of pregnancy at a systemic level. Cremaschi [pp. ■■■–■■■] presents in this issue a well-founded approach to the role of pregnancy in autoimmune thyroid disorders.

Not only does pregnancy modulate autoimmune disease activity but it can also induce or evidence autoimmune responses. Reports on fetal cells entering the mother's blood during pregnancy have raised the question of their potential role in autoimmunity. For instance, women that developed scleroderma after pregnancy had 30 times more fetal cells in their blood than healthy women did [22]. On the other hand, the percentage of microchimeric cells varies largely in Hashimoto's thyroiditis but appears to be absent from normal thyroid glands [23]. However, their potential role in autoimmunity is still unclear.

Spontaneous experimental models of autoimmune diseases may provide interesting evidence on the immune-neuroendocrine regulation of implantation in a systemic proinflammatory context. Although care should be taken when extrapolating murine data to the human situation [7], animal models offer the advantage of monitoring both pregnancy outcome and disease progress from day 0 in the absence of immunosuppressive or anti-inflammatory medication. They may also serve to analyze the differential effect that either single or multiple pregnancies have on several manifestations and targets of the autoimmune disease. Vice versa, the role of autoimmune mediators in the diverse processes involved in implantation and peripartum periods can be studied.

Spontaneous experimental models of autoimmune diseases generally present an altered HPA axis and hormone secretion profile that can influence pregnancy [24]. For instance, *lpr/lpr* mice are considered suitable models of SLE, as thoroughly discussed in the present issue by del Rey [pp. ■■■–■■■]. Among them, the MRL/*lpr* model has served to prove that skin disease disappeared after multiple pregnancies compared to virgin MRL/*lpr* animals although a more severe nephritis with shortened survival and higher blood pressure was observed [25].

Nonobese diabetic (NOD) mice provide another useful spontaneous model of two autoimmune diseases. Females in particular display a spontaneous Th1 autoimmune response against exocrine glands with a progressive loss of salivary secretion characteristic of Sjögren's syndrome [26]. Immune, nervous and endocrine factors seem to converge in the etiology of this autoimmune exocrinopathy that occurs before the onset of type I diabetes offering a suitable model to study such interactions at every disease stage [27]. Saravia's contribution [pp. ■■■-■■■] to this issue especially deals with neuroendocrine regulation in diabetic NOD mice. Virgin NOD females at the stage of exocrine dysfunction but still not diabetic presented an abnormal expression of nitric oxide synthase (NOS) and cyclooxygenase (COX) and a lower response to the neuroimmunopeptide VIP (vasointestinal peptide) in their uteri concomitantly with the onset of a Th1 systemic response [28]. Pregnancy in these mice has been associated with depressed inflammatory response and increased growth and function of Langerhans islets [29]. A higher resorption record in NOD mice has been preliminarily reported [30]. On the other hand, the effect of overt diabetes on pregnancy was reflected in a poor vascular bed development and altered endometrial lymphocyte recruitment associated with fetuses presenting a high rate of developmental defects [31]. Interestingly, pregnancy increased plasma glucose levels in overt diabetic mice [31] while the administration of pregnancy hormones to nonpregnant NOD females reduced an autoimmune attack on the pancreas [29] pointing to the differential effect of pregnant hormones or pregnancy as a whole on autoimmune response elements.

Macrophages at the Center of Immune-Neuroendocrine Circuits: Role in Early Pregnancy

Macrophages represent one of the main types of cells able to integrate inputs coming from the brain, the endocrine and the immune systems and to respond to these stimuli by producing a wide variety of mediators as rapidly as in seconds and/or for several days after challenge. This integrative capacity puts these cells in the center of the most diverse and complex regulatory mechanisms and processes: Macrophages are the link between innate and adaptive immune responses. They modulate immune responses by receiving and responding to media-

tors of the immune, endocrine and nervous systems. Hence, the proinflammatory cytokines IL-6, TNF-α and IL-1 secreted by macrophages are extremely pleiotropic serving different functions in disparate organ systems. Macrophages have, amongst others, serotonin, estrogen and acetylcholine receptors that can suppress the expression of proinflammatory genes through the NF-κB pathway [32]. During the course of pregnancy, macrophages with their ability to 'talk in many languages' have a crucial role as they provide the maternal-fetal dialogue with signals in both senses at the peri-implantation window and afterwards [33].

Given the role of macrophages to integrate immune, endocrine and nervous inputs and the relevance the immune response has for the implantation process with the participation of macrophages, the involvement of these cells in the maternal-embryo dialogue during a Th1 autoimmune response and the modulation of their activation profile by early pregnancy are the focus of the next sections.

During normal pregnancy macrophages constitute about 20–30% of decidual cells at the site of implantation and remain high throughout gestation suggesting that not only do they contribute to host defense but they also act in the maternal-fetal dialogue [34, 35]. In human cycling endometrium and at the maternal-fetal interface, glandular epithelium, stromal and trophoblast cells synthesize macrophage migration-inhibitory factor leading to the accumulation of macrophages in their vicinity.

Macrophages are described as necessary participants for setting up a suitable decidual microenvironment that promotes cell growth and inhibits deleterious inflammatory reactions [35]. Indeed, during early pregnancy, macrophages at the maternoplacentary unit are involved in the remodeling of uterine tissue [36].

The proinflammatory cytokines produced by macrophages such as IL-1, TNF-α, IL-6 and LIF participate in processes such as endometrial cyclic development, endometrial trophoblast interaction and endometrial tissue regeneration [37]. TNF-α is able to inhibit both the basal and insulin-stimulated release of prolactin from the decidua [38].The inflammatory response of TNF-α later induces the production of suppressor IL-10, but the high expression of TNF-α in women with recurrent spontaneous abortion failed to upregulate IL-10 expression [39].

Trophoblast-macrophage interactions contribute to the normal progress of gestation by modulating the Th1/Th2 switch and properly removing apoptotic bodies to prevent an inflammatory reaction that could lead to pregnancy failure [35]. It is also proposed that macrophages are 'educated' by trophoblast cells to create their own survival environment by the release of cytokines and chemokines [40]. The histological analysis of mice placenta showed a great number of macrophages localized close to apoptotic cells [35]. It is important to note that the environment where macrophages are committed to act is crucial: they clear out apoptotic bodies to prevent inflammation in normal conditions of gestation. Simultaneously, in an inflammatory milieu they can also flood the maternal-fetal interface with oxygen and nitrogen reactive species, TNF-α, prostaglandin E_2 among other inflammatory mediators at risk of interrupting gestation [41]. As sentinels of the innate response, macrophages can detect pathogens directly through Toll-like receptors, a subject also profoundly dealt with in this issue by Bornstein [pp. ■■■–■■■] and by Gomariz [pp. ■■■–■■■]. Sepsis leads to embryo resorption in humans [6] and macrophages found in resorption sites appeared preactivated to the synthesis of nitric oxide (NO) [42]. Consistent with this, in the CBA/JxDBA/2 mouse model of embryo resorption, the NOS-2 inhibitor aminoguanidine reduced resorption [42]. Both IFN-γ and LIF induce NOS-2 mRNA expression in trophoblast cells in vitro [43].

Recent evidence derived from a lipopolysaccharide (LPS)-induced embryonic resorption model in BALB/c mice indicates that NO and prostaglandins E_2, $F_2\alpha$ and thromboxane A_2 increase in uterus and deciduas with a dual role of NO: at low concentrations necessary for implantation [44], NO inhibited prostaglandin synthesis, while at high levels it increased COX-2 expression and caused abortions [45].

In this model LPS produces macrophage and granulocyte infiltration of the mesometrial decidua [46]. It has been also reported that macrophages are involved in the enhanced synthesis of NO and expression of iNOS and TNF-α mRNA associated with early pregnancy loss [41].

Effect of Pregnancy on Macrophage Activation Profile in Proinflammatory Conditions

Decidual macrophages (dMΦ) and their functional interaction are influenced by tissue-specific factors produced by trophoblast, glandular and stromal cells and by decidual lymphocytes comprising mostly uNK [47]. The lower expression of HLA-DR and costimulating CD86 molecules on early human dMΦ compared with peripheral blood CD14+ of pregnant and nonpregnant women suggests a less efficient antigen presentation within the

Table 1. Activation profile of macrophages upon LPS stimulation

	NOD at diestrus			NOD at diestrus + progesterone			Pregnant NOD at gestation day 9		
	IL-10, ng/ml	nitrites, μM	IL-12, ng/ml	IL-10, ng/ml	nitrites, μM	IL-12, ng/ml	IL-10, ng/ml	nitrites, μM	IL-12, ng/ml
Basal	1.6 ± 0.2	4.3 ± 0.3	1.2 ± 0.2	0.3 ± 0.1	1.0 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.1 ± 0.1
LPS	2.4 ± 0.6	20.0 ± 1.2	1.7 ± 0.5	0.7 ± 0.1	3.0 ± 0.3[a]	0.1 ± 0.1[a]	5.5 ± 0.6[b]	6.0 ± 0.3[a]	3.3 ± 0.6[a]
LPS+VIP	5.9 ± 0.3	3.8 ± 0.2	0.7 ± 0.1	0.8 ± 0.1[c]	4.0 ± 0.4	1.0 ± 0.3	5.4 ± 0.3	5.6 ± 0.1	1.2 ± 0.2

Isolated macrophages from NOD mice pregnant or at diestrus were obtained as previously described [48]. Macrophage monolayers were stimulated with 10 μg/ml LPS and 100 U/ml IFN-γ in the presence or absence of VIP (10^{-7} M) and progesterone (10^{-5} M) and incubated at 37°C for 24 h. Cell-free supernatants were harvested, cytokines determined by ELISA and nitrites by Griess [48]. Statistical significance of differences was determined by ANOVA. [a] $p < 0.01$ vs. corresponding values of LPS in NOD mice at diestrus; [b] $p < 0.05$ vs. IL-10 with LPS in NOD mice at diestrus and at diestrus + progesterone; [c] $p < 0.01$ vs. IL-10 with LPS+VIP at diestrus and in pregnant NOD mice.

decidua. A proposed model of dMΦ activation at the maternal-fetal interface maintains that trophoblast antigens activate proinflammatory responses reflected by an increase of TNF-α, IL-6, CXC ligands and NO. The competition with foreign trophoblast antigens for the binding site and internalization route of glycoproteins like TAG-72 appears to be an active mechanism for quenching the proinflammatory immune response of the dMΦ. Other proposed mechanisms could involve the interaction of HLA-G expressed on the extravillous cytotrophoblast and ILT-2/ILT-4 inhibitor receptors on the surface of dMΦ. The aberrant presence of activated macrophages in the decidua may be important in the preeclampsia etiology by limiting extravillous trophoblast invasion through TNF-α-mediated apoptosis and tryptophan depletion.

The activation of the systemic pool of macrophages of pregnant females in the course of an autoimmune disease context might also help to illustrate some interesting features about their local and systemic regulation. Compared with normal mice, macrophages from normally cycling prediabetic NOD females at a Sjögren's syndrome-like stage produce higher levels of nitrites and IL-12 at resting conditions [48]. In addition, NOD macrophages primed with bacterial LPS produced higher levels of NO and lower levels of IL-10. The neuroimmunopeptide VIP prevented NOS-2 induction by inhibiting NF-κB in normal mouse peritoneal macrophages primed with LPS [49]. In LPS-stimulated NOD peritoneal macrophages VIP increases IL-10 and it modulates NO production through IL-10 and PGE_2 [48].

However, pregnancy modifies the response of macrophages to LPS and the anti-inflammatory effect of VIP in these mice. Table 1 shows the activation profile of macrophages upon LPS stimulation on day 9 of pregnancy, at the peri-implantation window, compared with nonpregnant NOD macrophages. As mentioned above, progesterone alone can mimic some anti-inflammatory effects of pregnancy on the systemic response during an autoimmune disease. Thus, progesterone decreased both NO and IL-12 production in NOD macrophages at resting conditions and when stimulated in vitro with LPS. Table 1 also shows that in the NOD, pregnancy but not progesterone added in vitro modulated the anti-inflammatory response of VIP through an increase in IL-10.

The differential anti-inflammatory effect of progesterone and pregnancy on macrophages can also be observed in normal mice primed in vivo with an inflammatory stimulus like LPS: suppressive effects of progesterone on NOS-2 expression and NO production by murine peritoneal macrophages in response to LPS were reported [50]. Moreover, pregnant mice macrophages synthesized less NO than cells from nonpregnant animals when they were administrated with LPS [51] and the existence of a yet unknown type of progesterone receptor in murine macrophages has been suggested, the binding to which would be responsible for the anti-inflammatory effect of progesterone.

Concluding Remarks

Normal pregnancy is a physiological condition where hormones, immune factors and neurotransmitters orchestrate a dynamic interaction between the mother and the growing embryo. Early pregnancy challenges a variety of cells and molecules from the mother and the em-

bryo that need to concur and to interact efficiently for a successful implantation. Neuroimmune-endocrine interactions seem to be central to this dialogue. When the physiological condition of pregnant women is subject to stressful stimuli such as those provided by a chronic inflammatory milieu, the activation profile of T cells and macrophages may be temporarily changed. Although much evidence has shown the protective role of pregnancy on Th1 autoimmune diseases, the comprehension of the mechanisms for such an altered activation pattern at the macrophage level might help to understand their physiological role during gestation as well as to disclose their pathogenic potential. Macrophages integrate multiple inputs and signals of neuroimmune-endocrine systems and participate in early embryo implantation or loss. On this basis they might be explored as targets for pharmacological intervention in pregnant women with autoimmune diseases or susceptible to develop autoimmunity. This approach may also serve to get more insight into pregnancy failures in an inflammatory context.

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