Trophoblast Cells as Immune Regulators

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Abstract

Medawar, in the early 1950s, recognized for the first time, the unique immunology of the maternal-fetal interface and its potential relevance for transplantation. In his original work, he described the “fetal allograft analogy” whereby the fetus may be viewed as a semi-allogeneic conceptus that has evaded rejection by the maternal immune system. Although numerous hypotheses have been proposed to prove this observation, none have demonstrated that the maternal immune system is antagonist to the invading trophoblast. In the present manuscript we have reviewed recent studies demonstrating the expression and function of TLRs in trophoblast cells and based on this data we propose an alternative view for maternal-fetal immune interactions.

Introduction

Over fifty years ago the renowned transplant immunologist, Sir Peter Medawar, proposed the paradigm of why the fetus, as a semi-allograft, is not rejected by the maternal immune system. Subsequent studies demonstrated the presence of an active maternal immune system at the implantation site and this provided evidence to support Medawar’s original notion. As a result, investigators began to pursue the mechanisms by which the fetus might escape such maternal immune surveillance. Furthermore, alterations in these pathways in pregnancy complications, such as recurrent abortion and preeclampsia, where the immune system is thought to play a central role, have been used as further evidence for the Medawar hypothesis. As a consequence, since Dr. Medawar’s original observation, numerous studies have been performed in order to explain this paradigm, many of which have been centered on how the fetus and placenta fight against an active and aggressive maternal immune system. While varied hypotheses have been proposed in order to explain how the maternal immune system might be neutralized during normal pregnancy (see ref. 2 for review of the different hypotheses); none, however, have been able to convincingly demonstrate that in normal pregnancy there exists an immunological attack on the fetus.

The Immunology of Pregnancy

The finding of macrophages and neutrophils at the implantation site as early as the first week of implantation, as well as the high numbers of immune cells present at the maternal-fetal interface throughout pregnancy, have been taken as conclusive proof that the maternal immune system responds to the allograft fetus. Normal pregnancy is characterized by immune cells present at the maternal-fetal interface and it is the innate immune system that dominates the early pregnant decidua. 70% of decidual leukocytes are natural killer (NK) cells, 20-25%
are macrophages and approximately 1.7% are dendritic cells. From the adaptive immune system, B cells are absent, but T lymphocytes constitute about 1-3% of the decidual immune cells during the first trimester of pregnancy. Decidual NK cells are phenotypically distinct (CD56<sup>bright</sup>, CD16<sup>+</sup>), and unlike their circulating equivalents, uterine NK cells have a morphology similar to large granular lymphocytes and display low cytotoxicity. It is thought that these cells do, however, migrate in from the periphery. During the first trimester, NK cells infiltrate the decidua and accumulate around the invading trophoblast cells, however, as gestation proceeds these innate immune cells progressively vanish and are absent at term. Decidual macrophages are found located in the decidua basalis and paritalis and are densely distributed beneath the uterine epithelium that surrounds the fetus. These innate immune cells are also in close contact with extravillous trophoblasts. The observed migration of immune cells into the maternal-fetal interface during normal pregnancy, coupled with their close proximity to the invading trophoblast cells, prompted Medawar's hypothesis to be altered; yet strengthened. Thus, the maternal immune system is not responding to the fetus, but instead to the trophoblast.

**Pregnancy Represents an Allograft**

Medawar, in the early 1950s, recognized for the first time the unique immunology of the maternal-fetal interface and its potential relevance for transplantation. In his original work, he described the "fetal allograft analogy" whereby the fetus may be viewed as a semiallogeneic conceptus that has evaded rejection by the maternal immune system. Subsequently, a number of mechanisms were proposed to account for this lack of fetal rejection. The different hypotheses can be summarized into five main concepts: (i) The mechanical barrier effect of the trophoblast; (ii) Systemic suppression of the maternal immune system during pregnancy; (iii) The absence of classical MHC class I molecules in the trophoblast; (iv) A cytokine shift; and more recently (v) Localized immune suppression. Unfortunately, none of them can adequately explain or prove the existence of the fetal allograft analogy. Indeed, we know today that the trophoblast does not provide a mechanical barrier, since there is evidence for the trafficking of cells in both directions across the maternal-fetal interface. This includes the migration of maternal cells into the fetus, and the presence of fetal cells in the maternal circulation. Indeed, this is the case in almost all the immune privileged tissues, including the brain's blood brain barrier. Conclusive evidence has shown that immune cells circulate through all the parts of the brain indicating that mechanical barriers do not deter the migration of leukocytes into supposedly immune privileged sites. The hypothesis of systemic immune suppression has been challenged by recent studies clearly demonstrating that maternal anti-viral immunity is not affected by pregnancy. The clearest observation is that HIV<sup>+</sup> pregnant women do not suffer from AIDS-like disease argues against the existence of such nonspecific immune suppression during pregnancy. The expression of HLA-G, a monomorphic, trophoblast-specific HLA class I molecule was used to explain why trophoblast survives, despite the presence of abundant decidual NK cells. According to this hypothesis, the HLA-G molecule acted as a surrogate "self" class I molecule thus preventing NK cell killing. However, more recent studies by several groups have not supported this hypothesis. The definition of pregnancy as a "TH2 environment" was originally enthusiastically embraced and numerous studies tried to prove and support this hypothesis. It is now becoming increasingly clear that the TH1/TH2 nomenclature is a hindrance beyond CD4<sup>+</sup> T cell functions; and while there are strong evidences that anti-inflammatory cytokines, such as IL-10, are relevant for the success of pregnancy, it is also true that pro-inflammatory cytokines, such as IL-6 and IL-8, are produced during and necessary for normal pregnancy. Lastly, the idea that the trophoblast creates an immune privileged microenvironment by eliminating immune cells that pose a potential threat, specifically through the Fas/Fas ligand system, has been challenged by the lack of an immune response or fetal rejection in mice lacking Fas (lpr) or Fas ligand (gld). Our recent studies indicate that Fas ligand (FasL) is not expressed at the cell surface membrane of trophoblast cells, but is instead...
secreted via microvesicles to act on Fas expressing cells at locations away from the implantation site. The role of this functional secreted FasL is not fully understood and is under investigation. More recently, the role of IDO and T regulatory cells (Trg) have been proposed as potential mechanisms for the immunological escape of the fetus. Numerous groups are pursuing this hypothesis, and their results will determine whether this theory is valid.

In reviewing these different hypotheses we have observed that the field of Reproductive Immunology has always followed mainstream immunology; translating the findings from transplantation to explain the immunology of the maternal-fetal relationship. So far, all of these ideas have failed to conclusively prove the principle of semi-allograft acceptance by the mother and has produced confusion on the role of the immune system during pregnancy. Therefore, it is necessary that we reevaluate the basic question of reproductive immunology: Does the fetal/placental unit truly act as an allograft that is in continual conflict with the maternal immune system?

**Challenging the Medawar Hypothesis**

**Transplantation vs. Implantation**

Medawar's observation was based on the assumption that the placenta is a "piece of skin" with paternal proteins, which under normal immunological conditions, should be rejected. However, the placenta is more than just a transplanted organ. Our knowledge of placental biology has significantly increased over the last 50 years. We now know that the placenta is a complex organ, which has evolved from the original "egg cover". Pregnancy and implantation, contrary to "graft implants", has been taking place for more than 100,000 years. Therefore, from an evolutionary point of view it is difficult to conceive that the placenta and the maternal immune system still maintain an antagonistic status. Furthermore, as our understanding of role of the immune cells at the implantation site increases, we learn that many of these leukocytes are present for the protection and maintenance of the pregnancy, rather than rejection. Therefore, we propose that the trophoblast and the maternal immune system have evolved and established a cooperative status, helping each other against common enemies, such as infectious microorganisms. In the present review we will discuss some of the evidences suggesting that the immune system is critical for pregnancy success and that the trophoblast itself may function as a normal component of the innate immune system, so that together they can defend the maternal-fetal interface against invading pathogens and, as we will discuss, possibly take advantage of commensal microbes.

**Infection and Pregnancy**

Clinical studies have shown a strong association between certain pregnancy complications and intrauterine infections, suggesting that the innate immune response can affect the outcome of a pregnancy. Preeclampsia and intrauterine growth restriction (IUGR) are both thought to be associated with infection and a link between preterm labor and intrauterine infections is now well established. Indeed, infections have been reported as responsible for up to 40% of preterm labor cases. Furthermore, 80% of preterm deliveries occurring at less than 30 weeks of gestation have evidence of infection, suggesting that an intrauterine infection may occur early in pregnancy, preceding such pregnancy complications. Infection as a mediator of inflammation, therefore, represents an important and frequent mechanism of disease. However, inflammation is also necessary for normal implantation and parturition. Implantation is characterized by the production of chemokines, pro-inflammatory cytokines and other inflammatory mediators. Blockage of this inflammatory process in rodents results in implantation defects. In contrast, animal models of pregnancy complications demonstrate that inflammation is often an underlying cause (reviewed in Ref. 39). Understanding how the trophoblast and the maternal immune system regulate inflammation represents the core for understanding maternal-fetal immune interactions.
The Trophoblast and Implantation

The trophoblast is not a classical differentiated epithelial cell. Instead, it is an embryonic stem cell with the outstanding capacity of adaptation to changing environments, tissue remodeling and organ development. Embryonic implantation consists of three consecutive phases; apposition, adhesion and invasion, and in each of these steps the trophoblast will confront different cell types and microenvironments. The success of the pregnancy depends on how well the trophoblast responds and adapts to each of these stages. Starting from the process of cell attachment to the lumen of the uterus, followed by invasion into the decidua and finally to transformation of the spiral arteries, the invading trophoblast requires a high capacity to communicate with its cellular environment.

As already discussed, the human decidua contains a large number of immune competent cells such as macrophages, NK cells and T cells. These leukocytes, as well as the decidual stromal cells themselves, are capable of producing soluble cytokines and hormones, all of which are necessary for both the regulation of immune responses and the growth and development of the placenta. The appropriate communication between all these cellular components at the fetal-maternal interface is crucial for successful reproduction. In addition, the upper female reproductive tract including the uterine lumen, is often exposed to commensal bacteria and bacterial products from the lower tract. Such microflora may interact with the external layer of the blastocyst while it is at the luminal surface. Moreover, as the blastocyst invades the endometrium, bacterium may gain access into this maternal compartment. Thus, for successful implantation, the invading trophoblast must recognize its new environment consisting of maternal cellular/soluble components and foreign microbes, and consequently respond by sending out the appropriate signals that will facilitate its adaptation and growth. Several questions arise from these observations and will be discussed thereafter:

- How does the blastocyst respond to the presence of bacteria in the uterine lumen?
- How do the trophoblast and the maternal immune system prevent the invasion of bacteria from the uterine lumen into the decidual stroma during implantation?
- How does the trophoblast communicate with the maternal immune system to prevent pathogenic bacteria invading the uterus?

How the Trophoblast Recognizes and Responds to Microbes

The signaling loop that mediates innate immune responses to microorganisms is based on the sensing of conserved structural motifs, known as pathogen-associated molecular patterns (PAMPs) that are specifically expressed only by microorganisms. PAMPs include bacterial components such as lipopolysaccharide (LPS) and peptidoglycan (PGN), and viral components such as dsRNA. These motifs, expressed by both commensal and pathogenic microorganisms, are recognized by pattern recognition receptors (PRRs). The best known PRRs are the toll-like receptors (TLRs), which are expressed by cells of the myeloid and lymphoid lineages, as well as by epithelial and endothelial cells.

Toll-Like Receptors (TLR)

All living organisms are constantly exposed to microorganisms present in the environment and invasion by such foreign bodies must be controlled. Therefore, the proper recognition and response towards potentially pathogenic microorganisms must be in place. Recent studies have shown that the innate immune system has a greater degree of specificity that was previously thought and that it is highly developed in its ability to discriminate between self and infectious nonself. This discrimination relies, to a great extent, on a family of evolutionary conserved receptors, known as TLRs, which have a critical role in early host defenses against invading pathogens.

Toll-like receptors are transmembrane proteins, which have an extracellular domain containing leucine-rich repeat motifs. Each receptor differs in their ligand specificity. So while individually, TLRs respond to limited ligands, collectively the family of TLRs can respond to a
wide range of proteins associated with bacteria, viruses, fungi and parasites. TLR-4 was the first human Toll-like receptor to be identified and was subsequently found to be the specific receptor for the recognition of LPS. To date ten members of the TLR family has been identified in humans. TLRs 1, 2, 4, 5 and 6 and 9 appear to specialize in the recognition of mainly bacterial products, while TLRs 3, 7 and 8, in contrast, specialize in viral detection. While extracellularly, each TLR is distinct in their specificity, all receptors signal through a common pathway. Toll-like receptors have an intracellular domain which is highly homologous to the type-1 Interleukin-1 receptor (IL-1R) and is known as the Toll/IL-1R homology region (TIR). Both TLR and the IL-1R recruit and interact with the adapter signaling protein, myeloid differentiation factor 88 (MyD88). Following ligation of a TLR by its ligand, MyD88 becomes associated with the intracellular domain of the receptor through a TIR-TIR interaction. In turn, MyD88 through its DD recruits and activates the DD-containing serine/threonine kinase, IL-1R associated kinase (IRAK). Subsequent downstream activation of the NF-κB and MAP kinase signaling pathways occurs through activation of a kinase cascade and results in an inflammatory response characterized by the production of cytokines and chemokines. In addition, NF-κB and JNK activation induced by TLR-3 and TLR-4, can also occur via MyD88-independent pathways which can stimulate the production of type I interferons (IFNα and IFNβ) and trigger the expression of IFN-inducible genes.

**Toll-Like Receptors, Commensal Microbes and Pregnancy**

The upper reproductive tract was originally thought to be a sterile environment that was infrequently exposed to bacteria present in the ecto-cervix and vagina. However, recent studies indicate that epithelial cells of the female reproductive tract are exposed to bacteria through peristaltic contractions at a frequency not previously appreciated. Following mating, viable bacteria can also be transported with the semen towards the uterine lumen. In the nonpregnant uterus the endometrial epithelium functions as a protective barrier against such microorganisms present throughout the reproductive tract. However, it would be incorrect to suggest that the reproductive tract is in a constant state of low inflammation. On the contrary, the uterus and fallopian tubes have a relatively low incidence of chronic infections, suggesting the existence of physiological mechanisms that control inflammatory responses towards commensal bacteria. Several studies have identified TLR expression throughout the female reproductive tract (FRT). Pioli et al (2004) have shown expression of TLR1-TLR-6, MyD88, MD-2, and CD14 in both lower and upper FRT tissue. A recent study of in vivo expression of Toll-like receptors throughout the FRT epithelium reported expression of TLR-1, TLR-2, TLR-3, TLR-5 and TLR-6 in the lower tract, with TLR-4 found only on the epithelium of endocervical and uterine tissue and endometrial glands. Additionally, this study reported a secreted form of TLR-4 from endocervical glands. All these data indicate that the female reproductive tract, upon activation of TLRs, may produce cytokines and chemokines that can regulate the differentiation, maturation and recruitment of leukocytes in the underlying stroma.

The window of implantation or opportunity for the embryo to adhere to the endometrium is defined by specific changes in the expression of epithelial integrins and mucins, allowing close apposition between the blastocyst and the luminal surface. The expression of these adhesion molecules is cytokine dependent and thought to be induced by seminal plasma. Bacterial products transported through the reproductive tract in association with seminal plasma may constitute such pro-inflammatory stimuli needed for apposition of the blastocyst. It is, therefore, tempting to speculate that this bacterial stimulus may be essential for implantation. There are numerous observations both in humans and animals to support the priming role of the semen, although the focus of those studies have been on cytokines or factors present in the seminal fluid. An alternative priming factor may be from the commensal bacteria present in the vagina that may be carried into the upper tract by the seminal fluid. Once in the uterus, such bacterium may be recognized by the uterine epithelium through TLRs and induce this epithelial layer to produce cytokines and chemokines. Therefore, the presence of commensal...
bacteria within the uterine lumen may be essential for successful implantation in both its priming capacity and also in its ability to limit the growth of more virulent microbes.

The priming effect of commensal microbes may not be limited only to the uterine epithelia, but also to the developing blastocyst as it passes through the oviduct and into the uterus prior to, and during, implantation (Fig. 1). The trophoectoderm, the external layer of the embryo, may also recognize bacterial products through TLRs and respond to them through the production of cytokines.

Indeed, trophoblast cells throughout the whole of pregnancy express TLRs. Initial studies have reported mRNA expression of TLR-1 - TLR-10, as well as protein expression of TLR-2 and TLR-4, in term placenta. Since then we have observed that in first trimester placental tissues, functional TLR-2 and TLR-4 are highly expressed in the villous cytotrophoblast and extravillous trophoblast populations. Interestingly, while third trimester trophoblast cells express TLR-2 and TLR-4, these receptors are not expressed by first trimester syncytiotrophoblast cells. Together these findings have demonstrated that trophoblast cells expressing TLRs may respond to bacterial products. How then can the trophoblast tolerate LPS from commensal gram-negative bacteria but also react to the same LPS from a pathogen? One potential explanation is the compartmentalization of TLR-4. This compartmentalization can be either intracellular localization or cell type specific. In the first case, we found that, contrary to the classical membrane expression of TLR-4 on many of innate immune cells, such as macrophages, trophoblast cells express TLR mainly in the cytoplasm; suggesting that these cells may recognize LPS associated with bacteria that have been internalized. We have also found that the

Figure 1. Recognition and response: The trophoblast recognizes, through TLRs, microbes and the cellular components at the implantation site and responds to them through the production of cytokines and chemokines. A) During normal implantation, trophoblast cells secrete chemokines, such as MCP-1 and IL-8, promoting the recruitment of macrophages and NK cells which then protect the trophoblast against infection and facilitate trophoblast invasion. Figure and legend continued on next page.
Figure 1. Continued. B) Anti-viral properties of the trophoblast. Trophoblast cells, through TLRs, recognize viral products and actively initiate an anti-viral response by producing interferons and anti-viral peptides. C) In disease, the cross talk between the trophoblast and the maternal immune system is broken. This may arise from unrestrained infection or an excessive inflammatory response due to macrophage, NK cell and neutrophil activation. Both may result in elevated trophoblast apoptosis.
expression of TLR is cell type specific, according to its tissue localization. In first trimester placentas, the syncytiotrophoblast (the outer layer of the villi) is negative for TLR-2 and TLR-4, while the internal layer, the cytotrophoblast, is positive for both receptors. The lack of TLR expression by the outer trophoblast layer during the first trimester is analogous to studies of mucosal epithelial cells of the intestinal tract, which have been shown to express TLR-5 only on their basolateral side. These cells will only respond to a bacterium that has invaded the basolateral compartment from the apical side. Since a pathogen is characterized as a microorganism that breaches certain physical barriers, these observations have helped to explain how an immune response can be mounted against pathogenic, but not commensal bacteria. Similarly, a microorganism will only be a threat to the fetus if the TLR-negative syncytiotrophoblast cell layer is breached and the pathogen has entered either the decidual or the placental villous compartments. Therefore, the placenta may distinguish between pathogenic and commensal microorganisms during pregnancy. Once an infection has gained access to the TLR positive trophoblast cells, a response may be mounted. As described below, the type of pathogen and, therefore, the specific receptor activated may have a significant impact on the type of response generated by the cells of the placenta.

Cross Talk between the Trophoblast and the Innate Immune System

**TLRs and the Regulation of an Immune Response**

A fundamental feature of the immune system is to protect the host from foreign bodies or abnormal cells. This function resides on the innate immune system's capacity to coordinate cell migration for surveillance, to recognize and respond to invading pathogens, and to facilitate the efficient clearance of dying cells. The recognition of pathogens or inflammatory signals at the site of an infection by the innate immune system triggers the process of cell migration. Innate recognition of PAMPs through TLRs initiates an inflammatory response, characterized by the recruitment of immune cells to the site of infection in order to augment microbial killing and halt spread. Cell migration from the peripheral blood into inflamed tissues involves a tightly controlled sequence of events, which are mediated by the two type of signals; chemokines and cell surface adhesion molecules. Activation of TLRs by microbial products induces the expression of chemokine and their receptors which in turn regulate immune cell migration to the sites of inflammation. Key inflammatory chemokines produced during acute microbial infections include interleukin 8 (IL-8), growth related oncogene α (GRO-α), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), and RANTES (Regulated Upon Activation Normal T cell Expressed and Secreted). The trophoblast is able to produce all these chemokines; some of them constitutively, such as IL-8, MIP-1α, MCP-1 and GRO-α, and others only following activation of certain TLRs, such as RANTES. Chemokine production is critical for implantation in order to facilitate immune cell recruitment into the decidua for host defense, but also for what we, and others, believe is essential immunological support during pregnancy.

**Trophoblast Cells Regulates Macrophage Migration**

Using a two-chamber migration system, we, and others, have observed that trophoblast cells are able to chemoattract monocytes and NK cells (Fig. 2). These observations have shed new light on the cross-talk between trophoblast cells and the immune system. Instead of the maternal immune system responding to the invading trophoblast as foreign, it appears that the trophoblast, under normal conditions, is playing a central role in regulating leukocyte migration into the decidua, suggesting that the immune cells themselves are important for pregnancy. In vivo models suggest that the presence of NK cells within the endometrium is necessary for successful implantation and it has been postulated that NK cells play a role in the decidualization of the endometrium. Similarly, macrophages at the implantation site and throughout gestation are thought to benefit pregnancy. Decidual macrophages may efficiently
clear apoptotic cells, thus maintaining tissue homeostasis. Macrophages may also provide the trophoblast with soluble factors that will stimulate their growth and differentiation, as well as promoting the invasion and transformation of the spiral arteries.

As the trophoblast breaches the epithelial surface of the lumen during implantation, it may allow access of bacteria into the uterine stroma. While, acute inflammation is necessary for implantation, it is also critical to avoid chronic inflammatory responses that may be triggered by excessive bacterial invasion. To this purpose, we hypothesize that the trophoblast itself, promotes a local innate immune responses towards microbes within the stroma, thereby increasing protection (Fig. 1). Again using the in vitro migration system, upon ligation of TLR-4 by bacterial LPS, chemokine production by trophoblast cells is significantly increased and this further enhances monocyte migration (Fig. 2). Furthermore, the chemokine response following activation is differentially regulated depending upon the stimuli. When trophoblast cells are exposed to the TLR-3 agonist Poly(I:C), a synthetic analog of viral dsRNA, a potent chemokine response is induced, yet this profile is distinct from that triggered through TLR-4. These findings demonstrate that the trophoblast, just like an innate immune cell, can recognize and respond to microbes. As a result, the trophoblast can coordinate an immune response through the recruitment of innate immune cells to the site of infection.

Active Protection of the Trophoblast Against Viral Infection

The trophoblast can not only recognize microorganisms and initiate an immune response; it may also produce anti-microbial peptides and, therefore, actively protect itself against pathogenic organisms. Studies have demonstrated the expression of the antimicrobial human beta defensins 1 and 3 by trophoblast cells. Secretory leukocyte protease inhibitor (SLPI), which is a potent inhibitor of HIV infection and inducer of bacterial lysis, has also been found in trophoblast cells. The expression of TLR-3 by trophoblast cells may explain how the placenta regulates the expression of these antimicrobial factors. Indeed, stimulation of first trimester
trophoblast cells, through TLR-3 with Poly(I:C), promotes the production and secretion of SLPI and beta Interferon (IFN-β), two important anti-viral factors. These factors are the first line of defense against viral infections and have the potential to activate multiple intracellular pathways. Therefore, IFN-β and SLPI production by trophoblast cells in response to a viral infection at the maternal-fetal interface may represent a potential mechanism by which the placenta prevents HIV transmission to the fetus during pregnancy. Together, these observations support the concept that the trophoblast is able to protect itself and the fetus from infectious pathogens.

**TLRs and Pregnancy Complications**

While the trophoblast has protective properties towards infections and can coordinate the immune system for enhanced responses against microorganisms, infection, nevertheless, is a common mechanism of disease. Intrauterine infections have been associated with cases of preterm labor and other pregnancy complications, such as intrauterine growth restriction (IUGR) and preeclampsia. Thus, an infection at the maternal-fetal interface represents a significant threat to both fetal well-being, as well as the success of a pregnancy. While innate immune cells may be important during normal pregnancy for resolving infections at the maternal-fetal interface, these same leukocytes may contribute to the pathology of certain pregnancy complications (Fig. 1). In abnormal pregnancies, such as prematurity or preeclampsia, decidual tissues contain elevated levels of macrophages, neutrophils and NK cells and such leukocyte distributions are altered. Similarly, in animal models of preterm labor and pregnancy failure, where the delivery of microbial products are used to initiate disease, the decidua becomes infiltrated with these same innate immune cells. Such altered immune responses at the maternal-fetal interface may significantly impact the pregnancy.

Toll-like receptors may also function as a link between a dangerous immune response and pregnancy complications, many of which are associated with elevated placental apoptosis. Indeed, we have recently demonstrated that TLR-2 ligation by bacterial peptidoglycan directly induces first trimester trophoblast cells to undergo apoptosis, rather than to produce cytokines. In contrast, high levels of LPS acting through TLR-4, triggers first trimester trophoblast cells to produce pro-inflammatory cytokines, including TNFα and IFNγ, which in turn may induce trophoblast cell apoptosis. Therefore, while LPS does not directly induce trophoblast cell death, the intense inflammatory response generated by either the trophoblast or decidual immune cells following its activation may provide an alternative mechanism for the induction of trophoblast apoptosis. Thus, we predict that certain intrauterine infections during pregnancy may have either a direct or indirect effect upon trophoblast cell survival, depending upon which TLR is activated. We have also observed that TLR-2 expression in fetal membranes is significantly elevated in women with chorioamnionitis, while TLR-4 expression by interstitial trophoblast cells is increased in patients with preeclampsia. Since TLR-4 levels appear to be upregulated by pro-inflammatory cytokines, such as TNFα, altered TLR expression may exacerbate certain pathological mechanisms. Together, all these data suggest that while on one hand TLRs function as important sensors for the trophoblast, allowing it to coordinate the local immune response and promote cell invasion and placental formation; TLRs may also provide the bridge for placental recognition of danger signals and a subsequent shift in the type of response generated may have harmful consequences for the pregnancy.

**Summary**

Our studies provide an alternative perspective on the role of the maternal innate immune system and its interactions with the trophoblast during pregnancy. We believe that the field of Reproductive Immunology needs to reevaluate its focus, and modify the immunological paradigm of pregnancy from a graft-host interaction towards a symbiotic interaction. As we learn more about the regulation of the expression and function of TLRs during pregnancy we will better understand the cellular cross talk existing at the maternal fetal interface.
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