How does Toxoplasmosis affect the maternal-fetal immune interface and pregnancy?

Running title: Toxoplasma immune response and pregnancy

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Summary

*Toxoplasma gondii* is a zoonotic parasite which, depending on the geographical location, can infect between 10 to 90% of humans. Infection during pregnancy may result in congenital toxoplasmosis. The effects on the fetus vary depending on the stage of gestation in which primary maternal infection arises. A large body of research has focused on understanding immune response to toxoplasmosis, although few studies have addressed how it is affected by pregnancy or the pathological consequences of infection at the maternal-fetal interface. There is a lack of knowledge about how maternal immune cells, specifically macrophages are modulated during infection and the resulting consequences for parasite control and pathology. Herein, we discuss the potential of *T. gondii* infection to affect the maternal-fetal interface and the potential of pregnancy to disrupt maternal immunity to *T. gondii* infection.

1. Introduction

*Toxoplasma gondii* is a protozoan parasite with a global distribution. It is an important pathogen of humans and their livestock. In humans, it can cause abortion or a spectrum of clinical diseases dependent on the parasite isolate, the host immune status and whether infection is acquired post-natally or in utero \(^1,2\). *T. gondii* infection causes substantial losses due to abortion in livestock (including sheep, goats and pigs) and the ability of livestock to act as reservoir of disease is a public health risk \(^3\). Limited genetic variability has been recognised in *T. gondii* strains isolated from Europe and North America for some time. The vast majority of *T. gondii* isolates characterised to date can be classified as Type I, II and III \(^4\). Each of these canonical *T. gondii* lineages have different patterns of virulence as empirically determined in mice and supported by restriction length polymorphisms, isoenzyme analysis, large-scale genome sequencing and transcriptomics. However, more recent analyses of *T. gondii* isolates from diverse geographical regions, most notably South America has revealed a greater genetic diversity with at least 15 haplogroups that fall into 6 major clades \(^2,4-7\). The rapidly-replicating tachyzoite life cycle stage predominates for around 10-14 days post infection before differentiating into bradyzoites, which replicate more slowly and form cysts in tissues throughout the body \(^8\). Tissue cysts are long-lived and are generally not considered responsible for significant disease, although associations with seropositivity and some neuropsychiatric diseases have now been recognised \(^9\). Infection of immune-competent adults with these canonical strains of *T. gondii* has been generally considered self-resolving. This is largely due to a robust systemic immune response to *T. gondii* that controls, but does not eliminate infection \(^10\). However, tissue cysts are assumed to rupture from time to time and in people with immunodeficiencies disease reactivation occurs, causing neurological
and sometimes ocular or systemic disease. 

*T. gondii* can also cause congenital disease and abortion, almost exclusively in women infected for the first time during pregnancy. Congenitally infected individuals are also at risk of repeated disease reactivation, mostly evident through ocular and neurological disease, though the reason for this remains to be determined. It is now recognised that certain lineages and recombinant strains of *T. gondii* that are common in South America are responsible for severe disease in humans even when acquired as immune-competent.

**2. Immune response during *T. gondii* infection**

The major findings concerning systemic *T. gondii* immune response have come from experimental murine models, and much of this has been corroborated through clinical studies and *in vitro* studies. Challenges exist in extrapolating the data between studies as differences including the mouse strain, parasite strain and life-cycle stage used to infect, dose of inoculum and route of infection often varies. It is not usually ethically possible to test if the murine studies can be translated into humans as this would involve deliberately infecting humans. Nonetheless a consensus of the sequence of immunological events after infection emerges.

Following oral infection, enterocytes are the first line of defense against *T. gondii*, since this parasite is acquired mainly via oral ingestion in mouse, human and other intermediate hosts. Parasites disseminate within the lymphatic and circulatory systems and spread throughout the body. There is evidence that by invading dendritic cells (DCs) and macrophages, previously recruited by the chemokines secreted by infected enterocytes, *T. gondii* is able to cross endothelial barriers, thus allowing entry into sites such as the brain. Neutrophils, DCs, macrophages and natural killer (NK) cells, have been shown to play an important role in the innate immune response to *T. gondii* by controlling parasite multiplication. During acute *T. gondii* infection chemokines, belonging to the CC and CXC chemokine superfamilies and their receptors have an important role in the recruitment of NK, DCs, monocytes, neutrophils and in the induction of T helper (Th)1 and CD8+ cytotoxic T cells, as reviewed elsewhere.

The process of neutrophil recruitment and control of parasite numbers during the initial stages of *T. gondii* infection has been shown to be dependent on interleukin (IL)-17 signaling.
*T. gondii* has a unique cyclophilin 18 with the ability to bind C-C chemokine receptor type 5 (CCR5) and a number of ligands for toll-like receptors (TLRs) including, glycosylphosphatidylinositol-anchored proteins (GIPLs), heat shock protein 70 (HSP70) and profilin, which interact with TLR-2, 4 and 11. This results in induction of many cytokines including IL-12 produced by parasite-infected DCs, macrophages and neutrophils. Interferon (IFN)-γ acts in a synergic way with tumor necrosis factor (TNF)-α, inducing nitric oxide (NO) production by macrophages. NO can inhibit essential parasite mitochondrial and nuclear enzymes, thereby killing tachyzoites or inducing their conversion to the bradyzoite stage. IFN-γ also controls: (1) generation of reactive oxygen intermediates with toxoplasmacidal activity in human infected macrophages; (2) iron deprivation within *T. gondii* infected enterocytes, leading to inhibition of parasite replication; (3) tryptophan starvation through indoleamine-2,3-dioxygenase pathway, inhibiting *T. gondii* growth, and (4) activation of the p47 GTPases that bind to subcellular membranes, such as endoplasmic reticulum and Golgi bodies, thereby mediating disruption of the parasitophorous vacuole by exposing the parasite to the cytosol. However, an excessive Th1 response leads to tissue damage, which may lead to the development of pathology. Notably, both IL-4 deficient and IL-10 deficient mice have reduced survival following infection with *T. gondii* relative to wild-type mice as a likely consequence of reduced Treg and Th2 cell. Thus, the ability of the host to balance this response by maximizing pathogen clearance and minimizing immunopathology (Figure 1).

### 3. Congenital Toxoplasmosis

Congenital toxoplasmosis constitutes a major problem for human health, leading to severe implications for those affected from fetus to adulthood. However, it is known that the relative risk to the fetus depends on various factors, such as the mother’s immunological status and genotype, parasite genotype and virulence as well as the gestational period when infection is acquired. Other disease determinants include the infectious dose, whether infection is initiated with oocysts or tissue cysts or the occurrence of co-infections.

Primary *T. gondii* infection in the first trimester is associated with a high risk of several adverse pregnancy outcomes including abortion, stillbirth and premature birth, but with a low risk of congenital transmission. If pregnancy results in a viable child, *T. gondii* infection may cause a wide range of clinical manifestations, such as neonatal malformations that are severe enough to ultimately result in blindness, chorioretinitis, mental retardation, heart and...
brain defects, permanent neurological damage and death. If transmission occurs at later stages of pregnancy, abortion is unlikely, but the risk of congenital infection is increased. Under these circumstances, infected newborns will often be asymptomatic at birth, but essentially will all develop chorioretinitis later in life. If infection occurs prior to pregnancy, the risk to the unborn child is extremely low, even if the mother is re-exposed to another infection. However, there is a growing body of literature which demonstrates, in a small number of cases, that maternal disease reactivation can occur during pregnancy and sometimes result in adverse effects on the developing fetus. In addition, other cases have been reported in which pregnant women with immune suppression due, for example, to HIV infection, have resulted in congenital toxoplasmosis. Furthermore, several studies monitoring females with previously acquired T. gondii infection through pregnancy found maternal disease reactivation at the ocular site. The cysts stages are presumed to be the source of disease reactivation in all of the above scenarios. Altogether, these data suggest that in the vast majority of cases, a previous maternal infection protects against congenital transmission, even when exposed to a heterologous challenge. However, hormonal-induced alteration of systemic immunity occurs in some pregnancies and can result in disease activation and, occasionally, in vertical transmission.

4. Effect of T. gondii infection on implantation

Healthy pregnancy is highly dependent on appropriate immune responses from coitus to postpartum. Seminal plasma released during coitus contains a variety of immunologically active components including transforming growth factor (TGF)-β, CXCL8, IFN-γ and prostaglandin E. These mediators are responsible for changes in local gene expression, recruitment of monocytes, DCs, NK cells, T cells and expansion of Treg cells and, thus, sets the immunological context for the first encounter of the maternal immune system with paternal antigens. As the blastocyst undergoes implantation, macrophages are required to clear apoptotic maternal uterine cells. There is now evidence of progressive maternal immune regulation at the maternal-fetal interface comprising the fetus-derived placenta and the decidua. The development of a decidua is dependent on progesterone and is crucial for implantation. The blastocyst breaches the uterine epithelium, an event that is accompanied by inflammation and infiltration of decidual leukocytes, including NK cells, macrophages, dendritic cells and T cells. The success of implantation is dependent on the expression of a number of cytokines and chemokines including leukemia inhibitory factor.
(LIF), IL-6, IL-15, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-33, CXCL8, CXCL1, MIP-1α and RANTES and activation of signalling pathways like JAK-STAT, MAPK, Notch, Smad and PI3K. Macrophages, decidual NK cells (dNK), decidual dendritic cells (dDC) and other leukocytes are present in the endometrium at this stage and have been demonstrated to play important roles during implantation and, subsequently, promote tolerance to the fetus. Macrophages, decidual NK cells (dNK), decidual dendritic cells (dDC) and other leukocytes are present in the endometrium at this stage and have been demonstrated to play important roles during implantation and, subsequently, promote tolerance to the fetus. Treg cells expand in peripheral blood and lymphoid organs in the preimplantation period, migrating and, subsequently, accumulating in the decidual tissue. Besides their importance in ovarian homeostasis and ovulation, these cells have a central role in implantation, preventing maternal anti-fetal responses, and are thus determinant in the maintenance of pregnancy.

The potential for *T. gondii* to disrupt these early events in pregnancy has scarcely been studied. Nevertheless, in murine models, chronic *T. gondii* infection has been shown to increase reproductive failure. These alterations have been attributed to tissue cysts of *T. gondii* in various organs, especially in the brain, potentially causing damage in the hypothalamic–pituitary–gonadal axis and leading to alteration in the female estrus cycle. However, in other infection models, such as for *Trypanosoma cruzi*, inhibition of implantation and cell division has also been described. Nevertheless, *T. gondii* acute infection induces transient changes in systemic expression of many immune mediators, which in theory have the potential to antagonise normal immune changes during pregnancy (Figure 1).

5. **Effect of *T. gondii* infection on the developing placenta, decidua and fetus**

As pregnancy progresses there are a number of alterations in the maternal immune system, which facilitate fetoplacental development and prevent fetal rejection. Successful healthy pregnancy is highly dependent on specific regulation and balance of maternal immune responses within the decidua and placenta. This immune regulation enables growth and provides protection of the semi-allogeneic fetus, expressing paternal major histocompatibility antigens from maternal immune rejection. Dysfunction of this immune regulation by extrinsic factors, as in the case of infection, compromises pregnancy maintenance leading to adverse gestational outcomes.
*T. gondii* infection can have profound effects on the systemic maternal cellular immune responses and affects normal immune mechanisms at the maternal-fetal interface and can thus influence pregnancy outcome. *T. gondii* strains with different virulence are known to infect decidual immune cell populations and the placenta, a prime anatomical location to alter immune responses at the maternal-fetal interface. On the other hand, the immunomodulation during pregnancy may contribute to the development of an environment (dominated by Th2 and Treg cells) that facilitates the escape of *T. gondii* from the immune response, leading to increased maternal pathology or an increased likelihood of congenital transmission.

6. The placenta and *T. gondii* infection

Trophoblasts are epithelial-type cells that constitute the fetal placenta. Cytotrophoblasts act as stem cells for other trophoblast cell types: the syncytiotrophoblast and extravillous trophoblasts. The syncytiotrophoblast forms a multinucleated cell layer that is directly in contact with maternal blood, allowing an intimate interaction between mother and fetus. Extravillous trophoblasts have the ability to invade maternal tissues. These cells essentially function to protect the fetus from harmful substances, while facilitating the passage of nutrients and factors important for fetal development. The immune response within the placenta provides protection from infection and contributes to maternal tissue remodeling, which is fundamental for fetus development.

Human and experimental animal models have shown that trophoblasts are a component of the innate immune system, since they recognize pathogens, produce chemokines and cytokines, which influence the differentiation and migration of macrophages, NK, Treg and DCs at the decidua. Trophoblasts are responsible for the production of a variety of anti-inflammatory mediators, including TGF-β, IL-10 and Fas ligand. However, in some situations, trophoblasts may also initiate signals that promote fetal rejection. For example, studies have demonstrated that activation of TLR3 in the trophoblast, by Poly[I:C], a synthetic analog of viral dsRNA induces preterm delivery in mice. *In vitro* studies demonstrate that Poly[I:C] stimulation of both mouse primary trophoblast or human trophoblast induces a range of cytokines and chemokines (including MCP-1, RANTES, IL-6 and IL-12), supporting the idea that these cells function as an immune regulators and influence the differentiation and migration of immune cells. Although *T. gondii* does not have TLR3 ligands, it does have ligands that can activate TLR2,4 and 11 and these could...
have similar effects\(^8\). Indeed, infection may alter crosstalk, inducing TLR-mediated trophoblast inflammatory or apoptotic responses, influencing the recruited and resident maternal immune cells. In extreme cases, as during *T. gondii* infection, these cells shift from a protective to an aggressive phenotype promoting fetal rejection\(^{55,60}\). Notably in this respect, administration of the TLR4 ligand, lipopolysaccharide (LPS) to pregnant mice induces abortion through the induction of TNF-\(\alpha\) and nitric oxide synthase\(^{64}\).

Trophoblasts are also inextricably linked to parasite transmission to the fetus, since they are strategically located between maternal and fetal blood circulation systems and are efficiently infected with *T. gondii*\(^{54}\). *In vitro* studies using Bewo cells, a human trophoblast cell line, have shown that trophoblast susceptibility to *T. gondii* increases in response to high concentrations of macrophage inhibitory factor (MIF)\(^{65}\). In contrast, treatment of Bewo cells with IL-10 or TGF-\(\beta\) promoted *T. gondii* proliferation. In contrast to parallel experiments performed in HELA cells, the addition of IFN-\(\gamma\) to Bewo cells did not curtail *T. gondii* proliferation unless IL-10 or TGF-\(\beta\) were neutralised with specific antibodies\(^{66}\). Thus, IFN-\(\gamma\), was not able to control *T. gondii* proliferation in Bewo cells, contrary to expected, suggesting that this cytokine has different activities at the maternal-fetal interface, depending on other host factors\(^{66}\).

Recently, *ex vivo* experiments, using primary human trophoblasts isolated from placentas of both the second and third trimesters, have shown that syncytiotrophoblast cells are able to restrict *T. gondii* attachment and intracellular parasite replication in an IFN-\(\gamma\)-independent manner\(^{67}\). The T regulatory chemoattractant CCL22 expression has been associated with miscarriage\(^{68}\), and levels of this ligand were increased in primary human placental cells isolated from full-term placentas in response to *T. gondii* infection\(^{67}\). It has also been shown, that earlier in human pregnancy, using mid-gestation chorionic villous explants, trophoblasts was also able to restrict *T. gondii* attachment and increase CCL22 production\(^{67}\). Increased trophoblast apoptosis and necrosis occurring after *T. gondii* infection is dependent on inflammatory cytokines, such as IFN\(\gamma\), which can be a factor in determining pregnancy outcomes in women infected by this parasite\(^{69}\). In contrast, IL-10 is able to reduce *T. gondii*-infected trophoblast apoptosis levels\(^{69}\). If trophoblast invasion is excessive, which occurs during a *T. gondii* infection, IFN-\(\gamma\) has a dual function, thereby limiting parasite replication and promoting its removal\(^{40,66}\). Adhesion of infected monocytes to trophoblasts could also
promote placental and fetal infection. However, trophoblasts are able to modulate monocyte activity controlling *T. gondii* infection, thus promoting pregnancy maintenance\(^{70}\). Overall, the interaction between trophoblast cells and maternal-fetal immune cells, specifically macrophages are likely to determine *T. gondii* survival and putative vertical transmission \(^{65,66,70,71}\).

7. The decidua and *T. gondii* infection
The decidua results from proliferation and differentiation of stromal endometrial cells into decidual stromal cells (DSC) by a process called decidualization. These cells are important for embryo implantation, placentation and modulation of local immune cell functions that are essential for maternal/fetal tolerance and protection against infections. DSC are activated by TLR signalling, produce growth factors, such as G-CSF, cytokines including IL-6 and TNFα, as well as chemokines, like IL-8, CXCL1, and possess the chemokine receptors CXCR4. These chemokines induce the recruitment of T cells, monocytes and peripheral NK, modulating the activation profile at the decidua \(^{72,73}\). Depending on the decidua environment, DSC can contribute to a successful pregnancy or miscarriage \(^{74,75}\). *T. gondii* tropism is not clearly understood, although the decidua/trophoblasts interface is highly vulnerable to infection after parasite dissemination through maternal leukocytes \(^{54}\). As yet, there is no description regarding the interplay between DSC and *T. gondii* infection.

8. Decidual immune cells and *T. gondii* infection
The decidua is a highly dynamic immunological tissue composed of different maternal immune cell types, and is also modulated by placental-derived factors \(^{51,76}\). Recently, murine studies have suggested an important role for CCR5 and RANTES in abortion caused by *T. gondii* infection \(^{77}\). In fact, it is suggested that embryo loss results from alteration of decidual and trophoblastic function, induced by recruitment of macrophages, DCs and T cells in response to increased expression levels of CCR5 and RANTES, in implantation units from *T. gondii* infected mice \(^{77}\). Quantitative proteomic analysis of *T. gondii*-infected human decidual immune cells has revealed differential expression of protein levels involved in immune tolerance, fetal intrauterine growth and trophoblast invasion, essential biological processes during pregnancy. For instance, decreased levels of IL-1β and increased levels of Granzyme A and CCAAT/enhancer-binding protein β (C/EBPβ) expression were found in *T. gondii*-infected decidual immune cells compared with uninfected ones \(^{78}\). Decreased IL-1β levels
during *T. gondii* infection would affect the immune-balance at the maternal-fetal interface and restrict decidualization, placenta development and development of the fetus. Increased granzyme A levels is indicative of increased NK cells and cytotoxic T cells and again could affect decidualization and fetus development. C/EBPβ is involved in ERK1/2 signalling during decidualization in human and mice, and in macrophage activation. These observations suggest that, by interfering with the related-pathways to decidual immune responses, *T. gondii* infection can negatively affect pregnancy outcome.

8.1. Decidual NK cells and *T. gondii* infection

The NK cells present at the uterine mucosa and decidua, referred to as dNK cells, constitute the main leukocyte cell population during implantation and early pregnancy. These cells account for 70% of all decidual leukocytes and are functionally and phenotypically different from blood-circulating NK cells. Specifically, the majority of dNK cells are highly granulated and characterized as CD56bright/CD16− non-cytotoxic NK cells. These cells interact with the invading trophoblast cells, helping them to migrate and invade, thus contributing to the remodeling of decidual spiral arteries into high-conductance blood vessels. This allows sufficient blood flow at the maternal-fetal interface, which is crucial for a healthy gestation. In addition, these cells express angiogenic factors, like vascular endothelial growth factor and angiopoietin-2, contributing to the development of placental vascular bed. dNK cells also secrete a variety of cytokines, such as TNF-α, TNF-β, IL-10, IL-13 and GM-CSF. These immune cells are the main source of IFN-γ, which are essential for implantation and early placental progression. In recent years, several studies have demonstrated the ability of *T. gondii* to modify the immune profile of decidual cells and to invade and multiply within uterine dNK cells. *T. gondii* infection not only increases IFN-γ secretion by dNK cells, but is also associated with higher levels of trophoblast apoptosis, via the caspase-3 and caspase-8 mediated pathways. Additionally, *T. gondii* infection results in the increase of the expression of the human dNK cell-activating receptor (NKG2D), which may trigger a higher cytotoxic activity of dNK towards trophoblast cells. It is believed that these modifications contribute to abnormal pregnancy outcomes.
8.2. Decidual macrophages during *T. gondii* infection

Decidual Macrophages (dMϕ) constitute 20% of the decidual leukocyte population and represent the second most abundant immune cell-type population in decidua. dMϕ are recruited to the maternal-fetal interface by stromal and trophoblast cells, where they have specialized functions, such as decidual homeostasis, placental development and tolerance to the semi-allogeneic trophoblast. They also form a major line of defense against invading pathogens in the decidua and thus protect the fetus from infection. In vitro studies have shown that dMϕ differentiation and polarization are regulated by factors produced by trophoblasts such as IL-10, supressing IFNγ-induced Stat1 activation. During early gestation, dMϕ are also major producers of IL-10 and are able to supress and regulate the decidua immune response. dMϕ can decrease the cytolytic activity of dNK cells against invading trophoblasts. These leukocytes also play a role in the induction and expansion of Treg cells, contributing to the maintenance and support of uterine tissue homeostasis and remodelling.

dMϕ are able to recognize, phagocytose and eliminate pathogens. In addition, they also remove apoptotic cells and cellular debris and, therefore, promote normal fetal development and acceptance, preventing tissue damage and fetal rejection. *T. gondii* infection of human trophoblasts modulates trophoblast-macrophage crosstalk, in order to favor its establishment in the host cell. The polarization of dMϕ is modulated by *T. gondii* infection, but it is still unclear at present how this occurs during human pregnancy. Human and animal studies support the occurrence of both classical and alternative activation in macrophages responding to *T. gondii*. Macrophages have a variety of activation states, being able to adapt their functions to cytokine environmental changes, described as innate TLR ligation, classical/M1 or alternative/M2 activation states. Innate macrophages are activated after a microbial stimulus, through LPS stimulation or other TLR ligands, producing TNF-α, IL-6, IL-12 and NO. M1 macrophages are antigen-presenting cells, activated usually through a combination of LPS and IFN-γ, producing IL-12, IL-23 and reactive oxygen species. M2 macrophages can be induced by IL-4, IL-10, IL-13, IL-33, TGF-β and granulocyte-colony stimulating factor (G-CSF) and are important regulators of the wound-healing response, tissue homeostasis and adiposity.
M1 and M2 macrophages are often associated with polarised Th1 and Th2 responses, respectively. However, M1 and M2 functions can occur either in the presence or absence of T cells \(^{94}\). Additionally, during an inflammatory response, M1 and M2 activation profiles are present, suggesting a switch between M1 and M2, thereby highlighting the dual role of macrophages in initiating and, subsequently, resolving inflammation. Whether this switch occurs by local conversion of M1 macrophages to anti-inflammatory M2 cells, or by sequential recruitment from distinct precursor cell populations from the blood is currently under debate \(^{95}\). Several studies have shown that M2 activation profile includes several stages \(^{89,96,97}\). Thus, while classical macrophage activation can control *T. gondii* replication through induction of iNOS, alternative activation can control parasite replication through induction of arginase and depletion of arginine, since this parasite is auxotrophic for arginine \(^{89,98}\). M1 macrophages induced by a Th1 response in murine models exhibit cytotoxic and antimicrobial activities against *T. gondii* infection. This same response is also associated with immunopathology and adverse pregnancy outcomes \(^{89,99}\). In humans, during implantation, decidual macrophages consist of a M1/M2 heterogeneous activation profile population. This mixed decidual macrophage profile continues to predominate during the first trimester and at the beginning of the second trimester of pregnancy, coincident with the vasculature remodelling at the uterus. After placental growth, in the final stages of the second trimester, decidual macrophages exhibit predominantly an M2 polarized profile, promoting fetus maintenance and growth \(^{85,87,92}\).

Several studies have focused on macrophage activation profiles in normal and complicated pregnancy. Some of these have associated an imbalance of the macrophage polarization with *T. gondii* intrauterine infection, but failed to determine the context of macrophage polarity and the mechanisms promoting their dysregulation at the maternal-fetal interface \(^{87}\). Recently, it has been reported that human dMφ upon *T. gondii* infection showed upregulated M1 markers and downregulated M2 macrophage markers concomitantly with a downregulation of human leukocyte immunoglobulin-like receptor subfamily B member 4, an important factor in immune tolerance and immune regulation during normal pregnancy \(^{100}\). These alterations provide a possible explanation for the disruption of pregnancy \(^{39,100}\). Other infection models, such as malaria during pregnancy indicates that macrophage accumulation in the placenta is a key determinant in the immune pathology associated with this parasite \(^{101,102}\).
8.3. Decidual DCs during *T. gondii* infection

DCs are antigen-presenting cells priming T cell responses, but dDCs fail to initiate immunogenic T cell responses to placental antigens, thus contributing to maternal-fetal tolerance \(^{38,103}\). Despite the fewer number of human DCs at the decidua, they play determinant roles in normal and pathological pregnancies \(^{104,105}\). In addition, DCs are important for materno-fetal tolerance and are also involved in angiogenic responses at the maternal-fetal interface \(^{105}\). In normal human decidua, both immature myeloid – identifiable by their expression of DC-SIGN (CD209) – and mature myeloid DCs coexist \(^{105}\). In early human pregnancy the presence of DC-SIGN+ cells and dNK clusters indicate that the interconnection between these cell subsets is essential in pregnancy maintenance \(^{106}\). In fact, dNK cells induce apoptosis in DC-SIGN+ cells, constituting a mechanism of maternal-fetal tolerance \(^{106}\). Other models of infection, namely with *Coxiella burnetii* and *Brucella abortus*, revealed that myeloid dDCs are unable to mature in response to bacterial ligands, such as peptidoglycan or LPS, or to produce inflammatory cytokines. This would prevent development of effective immunity and favour pathogen multiplication \(^{107}\). However, contrary to these observations during bacterial infections, *in vitro* studies have shown that *T. gondii*-infected human dDCs produced IL-12 which increased the cytotoxicity of dNK cells as determined by increased expression of NKG2D and production of IFN-γ. The presence of increased IL-12 and IFN-γ levels would favor Th1 cells and suppress Th2 cells and thus contribute to disruption of pregnancy \(^{48}\).

8.4. Decidual T cells and *T. gondii* infection

The relative balance of lymphoid cells, such as Th1, Th2 and Treg cells, present at the decidua coordinate macrophage polarization and are vital for successful pregnancy \(^{108}\). In the past it was accepted that maternal-fetal immunological balance could be disturbed by *T. gondii* infection by disrupting a pregnancy-induced bias from Th2-type environment (associated with IL-4 and IL-10) toward Th1-type environment (associated with IFN-γ, TNF-α and IL-12 production), which has been demonstrated to be abortogenic. The discovery of new cytokines at the fetal/maternal interface which did not fit the Th1/Th2 paradigm led researchers to move away from this dichotomy and explore the Th1/Th2/Th17 and Treg paradigm in reproductive immunology \(^{109}\). Th17 cells secrete the cytokines IL-17, IL-21 and IL-22, promoting the inflammatory response during pregnancy in *T. gondii*-infected women \(^{17}\). Treg cells are key players present in the pregnant uterus, contributing to immune
regulation, decidualization, maternal immune tolerance and acceptance of the fetus. Increased Treg cells are positively correlated with the enzyme placental Indoleamine-2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) during pregnancy and are linked to maternal tolerance. IDO expression is induced by IFN\(\gamma\) and converts L-tryptophan, an essential amino acid during pregnancy, into kynurenine, which favours expansion of Treg cells. The importance of these events is evident as reduced levels of kynurenine and other downstream metabolites of tryptophan degradation as well as reduced transcripts for IDO and TDO are associated with fetal growth restriction. Furthermore, IDO expression is increased in the placenta during acute T. gondii infection at late gestation.

Recently, a decrease in the ratio Treg/Th17 in T. gondii-infected pregnant mice was reported, suggesting a role for this imbalance in T. gondii-induced embryo loss. During T. gondii infection, a decrease in maternal Treg cells has been reported to disrupt fetal tolerance and to be associated with pregnancy complications.

9. Progesterone and T. gondii Infection
Pregnancy-specific factors, such as maternal hormones, are able to modulate the maternal immune response and affect the activation of macrophages and lymphocytes. Progesterone (P4), one of the maternal hormones synthetized in the breast, endometrium, brain, ovaries and fetoplacental unit, plays a role during pregnancy by regulating immune cells, essential for the maintenance of pregnancy. Low levels of P4 in pregnant women infected with T. gondii provides another potential adverse effect of T. gondii infection on pregnancy. P4 is able to regulate selectively the expression of different genes associated with alternative macrophage activation. In this study, it has been shown that P4 treatment of murine macrophages selectively reduces transcription of mrc1 (coding for mannose receptor), but increases transcription of ym1, demonstrating plasticity in alternative macrophage activation with potential significant consequences for pregnancy. In addition, P4 contributes to a local Th2-associated cytokines production by murine fetoplacental tissues and recently it has been demonstrated to negatively regulate the differentiation of Th cells into Th1 and Th17. Therefore, it is possible that during T. gondii infection, low levels of P4 may be implicated in altered macrophage polarization and T cell responses, negatively affecting pregnancy. Moreover, during pregnancy, progesterone is able to induce the secretion of progesterone-induced blocking factor (PIBF) by progesterone-receptor-positive.
PIBF is able to modulate Th1/Th2 balance and contribute to the enhanced Th2 profile noted during pregnancy, characterized by increased production of IL-4 and IL-10\(^{121}\). Notably, PIBF can also downregulate NK cytotoxicity favoring gestation progression (Figure 1) \(^{121}\).

**10. Conclusion**

Successful pregnancy requires a delicate, fine-tuned equilibrium of maternal immune cells at the maternal-fetal interface to promote fetal tolerance. The activation of maternal immune effector cells by infection dysregulate this equilibrium and can lead to fetal loss or fetal infection. There is evidence that *T. gondii* affects the activation states of numerous cells that play pivotal roles at the FMI including dendritic cells, macrophages, NK cells and trophoblasts. Importantly, *T. gondii* disrupts the normal balance of T cell subsets during pregnancy favouring the development of Th1 and Th17 cells associated with parasite control rather than Th2 and Treg cells that are conducive to normal pregnancy. In recent years, scientific advances have contributed to the understanding of the interactions between fetal trophoblasts and maternal immune cells. The effect of *T. gondii* at the maternal-fetal interface is evident in human and murine models, although the mechanisms are not totally clarified. There is still a need for further research into the pregnancy-related mechanisms regulating dDCs, dNK cells, T cells and dMφ, upon *T. gondii* infection which might explain adverse pregnancy outcomes and pathological features of congenital toxoplasmosis \(^{122}\). Current prenatal and postnatal treatment can reduce the chance of congenital transmission and the severity of disease sequela, but not prevent either \(^4,123\). At present, no vaccine exists to prevent human disease induced by *T. gondii*. The only commercially available vaccine is ToxoVax\(^{®}\) (Intervet B.V.), which is based on live attenuated tachyzoites of *T. gondii* strain S48, but is only for veterinary use \(^{124}\). Greater understanding of the immune response at the local maternal-fetal interface could suggest new targets for therapeutic intervention of congenital toxoplasmosis.
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Figure Legends

Figure 1. The immune response developing to acute *Toxoplasma gondii* infection (green) and the immune response changes during pregnancy are mutually antagonistic (red). Physiological changes are in grey. (A) *T. gondii* have a number of pathogen-associated molecular patterns (PAMPs; including HSP70, GIPLS and profilin) which can ligate TLRs on host cells and a chemokine mimic, cyclophilin18, that binds CCR5 to induce activation of neutrophils, dendritic cells and macrophages. (B) IL-12 produced by these cells act on NK cells to stimulate them to produce IFN-γ. (C) Together, IFN-γ and IL-12 preferentially induce differentiation of Th1 cells. (D) Th1 cells secrete IFN-γ and, preferentially favour M1 macrophage activation. (E) Th2 cells and Treg cells play a role in reducing inflammation during *T. gondii* infection. (F) Pregnancy is associated with an increase in progesterone production that stimulates NK cells and T cells to produce PIBF, which downregulates NK cell activity and promotes Th2 expansion. (G) Interaction of NK cells with atypical HLAs (HLA-C, E and G) in the maternal-fetal interface inhibits NK cell activation. (H) indolamine deoxygenase (IDO) induced in monocytes results in degradation of tryptophan and the production of kynurenine which in turn expands Treg cells. (I) Th2 cells produce IL-4 and IL-13 which favours M2 macrophage activation. (J) Progesterone levels are known to be downregulated during *T. gondii* infection although it is not known if this is via direct effects or indirectly through inflammation. The exact interaction of pregnancy and the developing immune response to *T. gondii* infection will be dependent on when during pregnancy infection occurs.

Figure 2: Impact of *T. gondii* infection in pregnancy. Clinical consequences for the fetus and newborn and concomitant alteration to maternal immune mediators at the FMI and hormonal alterations (Blue: relative levels during normal pregnancy; Red arrows: effect of *T. gondii* infection on these levels during pregnancy and infection). nd: not described.
### T. gondii infection

- **A**: Cyclophilin18 & PAMPs
- **B**: Macrophages, Dendritic cells & Neutrophils
- **C**: NK cells
- **D**: IFN-γ, IL-12
- **E**: IL-13, IL-10, IL-4
- **F**: PI3K, PI4K
- **G**: CD4 T helper cells
- **H**: HLA-G, HLA-E, HLA-C
- **I**: CD4 T cells
- **J**: Progesterone

### Pregnancy

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#### Table: Consequences for fetus and disease manifestations in newborn

<table>
<thead>
<tr>
<th>Trimester</th>
<th>Clinical Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>High risk of abortion, stillbirth or premature birth</td>
</tr>
<tr>
<td>II</td>
<td>Intermediate risk of abortion, stillbirth, premature birth, blindness, chorioretinitis, mental retardation, neurological alterations or death</td>
</tr>
<tr>
<td>III</td>
<td>High risk of congenital infection. Low risk of overt clinical manifestations in newborn</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL2</td>
<td>+↑</td>
<td>+↑</td>
<td>+↑</td>
</tr>
<tr>
<td>IL-12, IFN-γ</td>
<td>+↑</td>
<td>+↑</td>
<td>+↑</td>
</tr>
<tr>
<td>IL-1β</td>
<td>++↑</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Granzyme A, C/EBPβ</td>
<td>+↑</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>NKG2D</td>
<td>+↑</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>TH2 &amp; Treg CD4 T cells</td>
<td>+↓</td>
<td>++↓</td>
<td>+++↓</td>
</tr>
<tr>
<td>Macrophage Activation</td>
<td>M1+↑, M2+↓</td>
<td>M1+↑, M2+↓</td>
<td>M1+↑, M2+↓</td>
</tr>
<tr>
<td>Tryptophan degradation</td>
<td>+↑</td>
<td>+↑</td>
<td>+↑</td>
</tr>
<tr>
<td>Progesterone</td>
<td>+↓</td>
<td>+↓</td>
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</tr>
<tr>
<td>Estrogen</td>
<td>+↓</td>
<td>+↓</td>
<td>+++↓</td>
</tr>
</tbody>
</table>

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