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The placenta in toxicology. Part II.

Systemic and local immune adaptations in pregnancy

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Running title: Immune regulation in pregnancy

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Pregnancy, Immune regulation, Tolerance, Leukocytes, Decidua, Placenta

Abbreviations

CTLA-4	cytotoxic T lymphocyte antigen 4
DC	dendritic cell
dNK cell	decidual NK cell
EVT	extravillous trophoblast
Foxp3	forkhead box p3
GM-CSF	granulocyte-macrophage colony-stimulating factor
IDO	indoleamine 2,3-dioxygenase
KIR	killer immunoglobulin-like receptor
M-CSF	macrophage colony-stimulating factor
NK	natural killer
PlGF	placental growth factor
Th	T helper
Treg cell	regulatory T cell
VEGF	vascular endothelial growth factor

Abstract

During pregnancy, the maternal immune system is challenged by the semi-allogeneic fetus, which must be tolerated without compromising fetal or maternal health. This review updates the systemic and local immune changes taking place during human pregnancy, including some examples in rodents. Systemic changes are induced by contact of maternal blood with placental factors and include enhanced innate immunity with increased activation of granulocytes and non-classical monocytes. Although a bias towards Th2 and regulatory T cell (Treg) immunity has been associated with healthy pregnancy, the relationship between different circulating T helper cell subsets is not straightforward. Instead, these adaptations appear most evidently at the fetal-maternal interface, where for instance Tregs are enriched and promote fetal tolerance. Also innate immune cells, *i.e.* NK cells and macrophages, are enriched, constituting the majority of decidual leukocytes. These cells not only contribute to immune regulation, but also aid in establishing the placenta by promoting trophoblast recruitment and angiogenesis. Thus, proper interaction between leukocytes and placental trophoblasts is necessary for normal placentation and immune adaptation. Consequently, spontaneous maladaptation or interference of the immune system with toxic substances may be important contributing factors for the development of pregnancy complications such as preeclampsia, preterm labor and recurrent miscarriages.

Introduction

The maternal immune system during pregnancy is changed in order to tolerate the semi-allogeneic fetus. This is especially important in species with a hemochorial placenta (for instance humans, monkeys and rodents), since this is the most invasive type of placenta, in which there is an intimate contact between the maternal immune system and fetal tissue (Meeusen *et al.* 2001). The immune adaptations include changes in local immune responses, *i.e.* in the uterine mucosa (decidua) (Trundley and Moffett 2004), and systemic changes (Chaouat 2003). Immediately after implantation the endometrium is infiltrated by fetal trophoblast cells and will develop into the decidua and ensure anchorage of the placenta and therefore proper fetal nutrition. However, this trophoblast invasion needs proper regulation in order to protect the integrity of the uterus. Therefore local immune cells, such as uterine natural killer (NK) cells, macrophages, gamma/delta and regulatory T cells, are present in the endometrium already before implantation and increase in numbers and adapt immediately following implantation (Trundley and Moffett 2004) (Fig 1). These immune cells are important regulators of the balance between tolerance of fetal trophoblast cells and limitation of their invasion (Lash *et al.* 2010; Nagamatsu and Schust 2010). After establishment of the placental circulation, *i.e.* between weeks 8-12 of gestation, the peripheral blood also comes into close contact with fetal cells in the intervillous space, *i.e.* the villous trophoblasts, which may affect the systemic immune response (Fig 1). The most evident changes seen in the systemic circulation are a shift from a Th1 towards a Th2 immune response and increased activation of innate immune cells (described in more detail in later sections). The present review will focus on cellular and molecular adaptations that take place in the circulation (systemically) and in the decidua and the chorioallantoic placenta (locally) during pregnancy. The recent contributions in the field will be highlighted, providing a background for understanding the effects of maladaptation as well as interference of the immune response in

pregnancy. In general, the review refers to studies in humans although information referring to studies in rodents is included as well.

Changes in the systemic immune response during pregnancy

After the establishment of the placental circulation, maternal blood is in close contact with the fetal villous syncytiotrophoblasts (Burton *et al.* 2010). Such contact with fetal cells may influence the maternal immune response. It has indeed been shown that the passage of maternal blood through the placenta activates inflammatory cells such as granulocytes and monocytes (Mellembakken *et al.* 2002). This may be due to contact of these immune cells with fetal trophoblast cells during placental passage. However, villous trophoblasts have been shown to produce and shed various factors, like cytokines (Naruse *et al.* 2010; Sacks *et al.* 2001) syncytiotrophoblast microfragments (Redman *et al.* 2012), fetal cells (Bianchi *et al.* 1996) and pregnancy hormones, *e.g.* progesterone, estrogen or human chorionic gonadotropin, into the maternal circulation (Fig 1). All these factors may affect the immune system (Dong *et al.* 2008; Han 1975; Piccinni *et al.* 1995; Southcombe *et al.* 2011). Recently, studies have indeed shown the presence of factors in the plasma of pregnant women that activate maternal immune cells such as monocytes *in vitro* (Faas *et al.* 2008; Faas *et al.* 2010b).

Pregnancy-induced changes in the systemic innate immune response

It has long been established that innate immune responses change during pregnancy. This has most often been shown by higher numbers of circulating monocytes and granulocytes, resulting in increased numbers of total leukocytes during pregnancy (Kuhnert *et al.* 1998; Siegel and Gleicher 1981; Veenstra van Nieuwenhoven *et al.* 2002). However, there is

substantial evidence that not only the numbers of these innate immune cells are increased, but they also show phenotypic and functional activation. Various studies have for instance shown upregulation of activation markers, such as CD11b or CD64, on monocytes and granulocytes (Davis *et al.* 1998; Luppi *et al.* 2002b; Naccasha *et al.* 2001; Sacks *et al.* 1998). In accordance, it has recently been shown that changes take place in monocyte subsets during pregnancy. Increased numbers of non-classical monocytes (CD14⁺CD16⁺ or CD14⁺⁺CD16⁺) and decreased numbers of classical monocytes (CD14⁺⁺CD16⁻) have been found in the third trimester of human pregnancy (Melgert *et al.* 2012). The nonclassical monocytes can be considered to be more pro-inflammatory than the classical subset, since they have been shown to produce increased amounts of pro-inflammatory cytokines, such as TNF and IL-1 β (Ancuta *et al.* 2009; Cros *et al.* 2010; Ziegler-Heitbrock *et al.* 2010). Their pro-inflammatory association was also illustrated by the fact that their numbers increased during inflammatory conditions, such as during sepsis (Fingerle *et al.* 1993). Interestingly, these phenotypic changes observed in monocytes during pregnancy appear to be similar to changes seen in septic patients (Fingerle *et al.* 1993; Sacks *et al.* 1998). Various other studies have shown that monocytes also show functional changes during pregnancy, for instance cytokine as well as oxygen radical production change following activation *in vitro* (Luppi *et al.* 2002a; Sacks *et al.* 1998; Veenstra van Nieuwenhoven *et al.* 2003a). Also, granulocyte function is changed during pregnancy: granulocytes show increased production of oxygen radicals (Sacks *et al.* 1998) and increased phagocytic activity (Barriga *et al.* 1994). The number of dendritic cells (DC), which are antigen presenting cells, on the other hand, were found to be decreased during pregnancy (Cordeau *et al.* 2012; Darmochwal-Kolarz *et al.* 2003; Shin *et al.* 2009; Ueda *et al.* 2003). However, it has been shown that these cells show increased tolerogenic properties (Darmochwal-Kolarz *et al.* 2012): upon stimulation IFN α production and costimulatory molecules such as CD54 and CD86 are decreased (Cordeau *et al.* 2012), while

the expression of tolerance-associated molecules, *e.g.* CD200 and CD200R, are increased during pregnancy (Darmochwal-Kolarz *et al.* 2012). Finally, the number of circulating NK cells is decreased during pregnancy (Kuhnert *et al.* 1998; Watanabe *et al.* 1997; Veenstra van Nieuwenhoven *et al.* 2002; Veenstra van Nieuwenhoven *et al.* 2003b). Moreover, their production of IFN γ is decreased in pregnant as compared with nonpregnant women (Veenstra van Nieuwenhoven *et al.* 2002) and it has been shown that, similar to the shift towards Th2 cells (described in the next section), there was also a shift towards NK2 cells (NK cells producing type 2 cytokines) during pregnancy (Borzychowski *et al.* 2005). It is now generally accepted that the innate immune response is activated during pregnancy.

These changes in the innate immune response appear to be important for normal pregnancy, since pregnancy complications, like preeclampsia or intrauterine growth retardation, are associated with deviations from pregnancy-induced adaptations in the innate immune response. During preeclampsia, both neutrophils and monocytes are even further activated, phenotypically and functionally, as compared with normal pregnancy (Gervasi *et al.* 2001; Mellembakken *et al.* 2002; Redman *et al.* 1999; Sacks *et al.* 2003; Sacks *et al.* 1998; Sakai *et al.* 2002; Veenstra van Nieuwenhoven *et al.* 2008). These data show that aberrant activation of the innate immune response during pregnancy results in pregnancy complications, suggesting that the adaptation of the innate immune response is tightly regulated.

Pregnancy-induced changes in the systemic adaptive immune response

Not only the innate immune response is changed during pregnancy, also the adaptive immune response shows changes. The concept proposed by Wegmann *et al.* in 1993 (Wegmann *et al.* 1993) that a healthy pregnancy is accompanied by a decreased T helper 1 (Th1)/Th2 ratio has been confirmed by many others (Naccasha *et al.* 2001; Sacks *et al.* 1998; Saito *et al.* 1999;

Veenstra van Nieuwenhoven *et al.* 2002). However, although it is generally recognized that a shift away from the Th1 response is important in certain stages of pregnancy, it has now also been shown that type 1 cytokines, such as IFN γ or TNF are not only detrimental to pregnancy, but also play an important role in placental development. It has for instance been shown that they are needed in triggering pregnancy-induced spiral artery remodeling (Ashkar *et al.* 2000; Zhang *et al.* 2003). Moreover, the immunological paradox of pregnancy appears to be much more complicated than previously thought. Th17 cells and regulatory T (Treg) cells have now also been implicated in the complex immune regulation during pregnancy (Ernerudh *et al.* 2011; Saito *et al.* 2010). However, the exact role of circulating Treg cells during pregnancy remains unknown, since inconclusive results have been found. Several studies have demonstrated that Treg cells are essential for promoting immune tolerance and initially, circulating Treg cell numbers were reported to increase during pregnancy (Aluvihare *et al.* 2004; Sasaki *et al.* 2004; Somerset *et al.* 2004). However, more recent studies have shown that peripheral blood Treg cells are not altered or even decreased during pregnancy (Mjosberg *et al.* 2009; Tilburgs *et al.* 2008), indicating that they may predominately exert their tolerogenic functions locally.

Only a few studies have been performed on Th17 cells in pregnancy and also these results are inconclusive: some studies have shown that circulating Th17 cell numbers are not different in pregnant as compared with non-pregnant women (Nakashima *et al.* 2010; Toldi *et al.* 2011) while others have reported them to be increased during pregnancy (Liu *et al.* 2011). The contradicting results may be due to differences in methods or patient selection, which may have a great impact given the limited number of studies performed to date. Therefore, future studies should not only focus on the role of Th1 and Th2 cells, but also on the role of Treg and Th17 cells during pregnancy. This is more important since findings of the numbers of Treg cells during preeclampsia are conflicting. Some authors show that in preeclampsia the

numbers of circulating Treg cells are decreased compared with healthy pregnant women (Darmochwal-Kolarz *et al.* 2012; Prins *et al.* 2009) while others did not find different numbers of Treg cells between preeclamptic patients and normal pregnant women (Hu *et al.* 2008; Paeschke *et al.* 2005). Some of the inconsistencies regarding Treg cells may refer to how they were defined (Mjosberg *et al.* 2009). Although there is disagreement on the role of Treg cells in preeclampsia, there is agreement that preeclampsia is associated with a shift of the Th1/Th2 balance towards Th1 immune responses in severe preeclampsia (Boij *et al.* 2012; Borzychowski *et al.* 2005; Darmochwal-Kolarz *et al.* 1999; Darmochwal-Kolarz *et al.* 2002; Makhseed *et al.* 1999). In mild preeclampsia, however, it was shown that the maternal immune response is shifted towards a type 2 immune response (Veenstra van Nieuwenhoven *et al.* 2008). Therefore, as for innate immune responses, it also appears important for normal pregnancy to tightly regulate the Th1/Th2 balance.

Maternal consequences of the adapted systemic immune response

Although the maternal immune response adapts to pregnancy, most pregnant women experience a healthy pregnancy and do not show an increased susceptibility to most infections. This suggests that the immunological changes do not dramatically affect the integrity of the mother. However, it has been shown that pregnant women are more sensitive to certain infections that depend on Th1-mediated responses, such as the risk of developing clinical disease after infection with poliovirus or hepatitis A virus or an increased infectivity of cytomegalovirus (Weinberg 1984), herpes (Fink *et al.* 1993) or malaria infection (Lagerberg 2008). Also, influenza virus infections, including the recent pandemic H1/N1 influenza, lead to a higher morbidity and mortality in pregnant women (Louie *et al.* 2010). Notably, pregnant women seem to respond properly to influenza vaccination (Fisher *et al.*

2012; Steinhoff *et al.* 2010) and vaccination against influenza, as well as to other infectious agents, is widely and strongly recommended in order to protect both the mother, the fetus and the neonate (Munoz and Ferrieri 2012). In addition to the effects of pregnancy on infection susceptibility, it has long been known that changes in the expression of autoimmune diseases are obvious during pregnancy: rheumatoid arthritis and multiple sclerosis, which are roughly dominated by a Th1/Th17 type response, often ameliorate during pregnancy followed by a “rebound” worsening *post partum* (Confavreux *et al.* 1998; Ostensen *et al.* 1983). These effects of pregnancy on autoimmune diseases likely result from the shift of the immune response from a Th1 type response towards a Th2 type immune response.

In accordance with the increased sensitivity of pregnant women to certain infections, pregnant individuals are also much more sensitive to pro-inflammatory stimuli than non-pregnant individuals (Bakker *et al.* 1989; Faas *et al.* 1992; Faas *et al.* 1995; Theiss and Beller 1972; Visscher *et al.* 1993). It has for instance been shown that pregnant animals are extremely sensitive to LPS or ATP, potent stimulators of innate immune responses: a very low dose of LPS or ATP infused into pregnant rats induced hypertension and/or proteinuria as well as a persistent inflammatory response whereas infusion of similar amounts of these substances into non-pregnant animals did not induce these effects (Faas *et al.* 1995; Faas *et al.* 2000; Faas *et al.* 2010a). The exact mechanism of this increased sensitivity to pro-inflammatory stimuli during pregnancy remains unknown. However, it seems to be related to the changes in the innate immune response during pregnancy, since infusion of pro-inflammatory stimuli in the pregnant rat induced a much more intense and persistent activation of the inflammatory response (Faas *et al.* 2004; Faas *et al.* 1995; Faas *et al.* 2000; Faas *et al.* 2010a). Moreover, vascular tissue of pregnant rats appeared more sensitive to the products of activated inflammatory cells, such as oxygen free radicals (Faas *et al.* 1999). Adverse effects on

pregnancy in experimental animals have also been described after infusion of other pro-inflammatory substances, also in low doses, such as poly I:C (Arad *et al.* 2005) and TNF (Alexander *et al.* 2002), as well as injection of type 1 cytokines (Athanasakis *et al.* 1996). Interestingly, the adverse effects of pro-inflammatory changes during pregnancy are counteracted by anti-inflammatory responses, in particular involving IL-10. In IL-10(-/-) mice, LPS (Murphy *et al.* 2005) or CpG-induced TLR9 stimulation (Thaxton *et al.* 2009) caused adverse pregnancy outcomes in lower amounts than in wild type mice, which could be rescued by administration of IL-10. Thus, not only hyper-inflammatory responses, but also failures of mounting an anti-inflammatory response may be key elements in the development of pregnancy complications.

Local adaptation of the immune system during pregnancy

The decidua, the site where maternal cells come into direct contact with fetally derived trophoblast cells, is a specialized tissue with a unique composition of immune cells (Fig 1). The majority of the decidual leukocytes are innate immune cells, with NK cells and macrophages encompassing ~90% of all leukocytes whereas only ~10% are T cells in early human pregnancy (King *et al.* 1991; Starkey *et al.* 1988). A few DCs are also present while granulocytes and B cells are practically absent (Bulmer and Johnson 1984; Gardner and Moffett 2003). This is in contrast to the situation in blood, where the dominating populations are granulocytes and T cells.

Cells of the innate immune system

Decidual NK cells

NK cells are the dominating population in the first trimester decidua (~70% of all leukocytes) (Bulmer *et al.* 1991; King *et al.* 1991), highlighting their central role at the time when the placenta is being established. The common view is that decidual NK (dNK) cell numbers decrease after the first trimester to become nearly absent at term (Moffett-King 2002). However, more recent data indicate that they are present during all stages of pregnancy (Rieger *et al.* 2009; Sanchez-Rodriguez *et al.* 2011; Williams *et al.* 2009b), implying that they also contribute to the regulation of pregnancy at later stages. The origin of dNK cells or the reason for their dramatic increase is not well established. However, several mechanisms (that may operate in parallel) have been suggested including: 1) the recruitment of peripheral blood NK cells that differentiate locally into dNK cells (Carlino *et al.* 2008; Keskin *et al.* 2007; Male *et al.* 2010), 2) the maturation from endometrial NK cells in response to pregnancy-associated factors (such as IL-15) (Manaster *et al.* 2008) and 3) the differentiation from hematopoietic precursors present in the decidua in response to decidual stromal factors (Vacca *et al.* 2011b).

In peripheral blood, NK cells can be divided into a major subset of CD56^{dim}CD16^{bright} (~90%) and a smaller population of CD56^{bright}CD16^{-/dim} cells (~10%) (Nagler *et al.* 1989). These populations differ in several aspects, including cytotoxic potential, cytokine production and expression of cell surface markers (Cooper *et al.* 2001b). The CD56^{bright}CD16^{-/dim} population is sometimes referred to as regulatory because of its reduced cytotoxic capacity and dominating potential to produce a range of cytokines influencing immune responses (Cooper *et al.* 2001a; Nagler *et al.* 1989). The vast majority of the dNK cells are CD56^{bright}CD16⁻ (King *et al.* 1991; Starkey *et al.* 1988), although they seem to be a unique subset, different from both CD56^{dim} and CD56^{bright} blood NK cells, as judged by their gene expression profile (Koopman *et al.* 2003). dNK cells share the ability of blood CD56^{bright} NK cells to produce cytokines (Engert *et al.* 2007; Lidstrom *et al.* 2003; Saito *et al.* 1993) and

they express immunosuppressive molecules (*e.g.* CD9, galectin 1 and glycodelin A) (Koopman *et al.* 2003). However, they also express NK cell activating receptors (*e.g.* 2B4, NKp30, NKp44, NKp46, NKG2D) (Hanna *et al.* 2006; Kopcow *et al.* 2005) and cytolytic granules (King *et al.* 1991; Koopman *et al.* 2003) making them potentially cytotoxic. Nevertheless, dNK cells have significantly reduced cytotoxic ability (Kopcow *et al.* 2005), which favors their close association with invading extravillous trophoblasts (EVTs) (Fig 1). The inefficient cytotoxic ability is believed to be due to the interaction of inhibitory receptors on dNK cells (*e.g.* ILT2, KIR2DL4 and CD94/NKG2A) and MHC class I molecules (HLA-C, -E, and -G) on trophoblasts (Manaster and Mandelboim 2010; Vacca *et al.* 2011a) (Fig 1).

Two major functions attributed to dNK cells are the ability to promote trophoblast invasion and angiogenesis (Fig 1). The recruitment of EVT has been shown to be mediated via secretion of CXCL8 (IL-8) and CXCL10, for which EVTs express the receptors CXCR1 and CXCR3, and by hepatocyte growth factor (De Oliveira *et al.* 2010; Fraser *et al.* 2012; Hanna *et al.* 2006). dNK cells, found in close association with remodeling vessels (Hazan *et al.* 2010; Smith *et al.* 2009), may also contribute to spiral artery remodeling through production of angiogenic factors such as vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and angiopoietins (Engert *et al.* 2007; Hanna *et al.* 2006; Kalkunte *et al.* 2009; Lash *et al.* 2006). The interactions between dNK cell receptors and HLA class I molecules on EVTs seem to play an important role in these processes. In particular, the ligation of activating dNK cell receptors by HLA-C molecules is favorable for the secretion of both chemotactic and angiogenic factors (Hanna *et al.* 2006). On the other hand, the presence of inhibitory receptors (*e.g.* KIRs) has been associated with an increased risk of complications, including preeclampsia (Hiby *et al.* 2004; Sanchez-Rodriguez *et al.* 2011), which is characterized by shallow trophoblast invasion and defective vascular remodeling (Fig 2). These data suggest

that proper dNK cell activation is a prerequisite for adequate trophoblast invasion and spiral artery remodeling, and thus a normal placentation.

Numerous studies have also addressed the numbers of dNK cells in complications although the results are inconsistent, for instance showing reduced, increased or unchanged dNK cell numbers in preeclampsia and fetal growth restriction (Bachmayer *et al.* 2006; Eide *et al.* 2006; Rieger *et al.* 2009; Sanchez-Rodriguez *et al.* 2011; Wilczynski *et al.* 2003; Williams *et al.* 2009a).

Decidual macrophages

Macrophages account for ~20% of decidual leukocytes and represent the most frequent antigen presenting cells throughout pregnancy (Bulmer and Johnson 1984; Starkey *et al.* 1988; Williams *et al.* 2009b). Tissue macrophages are commonly divided into pro-inflammatory M1, and immune regulatory M2 macrophages (Gordon 2003; Mantovani *et al.* 2004). The latter encompass a range of different macrophages with diverse functions including immune suppression, scavenging of apoptotic cells and tissue remodeling. Based on gene expression analysis, decidual macrophages can be classified as M2 macrophages, with functions particularly related to immune modulation and tissue remodeling (Gustafsson *et al.* 2008).

Decidual macrophages (CD14⁺HLA-DR⁺) express typical M2-associated receptors, including CD163 (scavenger receptor), CD206 (mannose receptor) and CD209 (DC-SIGN) (Kammerer *et al.* 2003; Laskarin *et al.* 2005; Repnik *et al.* 2008; Svensson *et al.* 2011), low levels of the co-stimulatory CD86 and M1-promoting transcription factor IRF5 (Heikkinen *et al.* 2003; Svensson *et al.* 2011) and produce IL-10 (Heikkinen *et al.* 2003; Lidstrom *et al.* 2003; Svensson *et al.* 2011) (Fig 1). In particular, decidual macrophages seem to belong to a subset of M2 macrophages with homeostatic and tolerogenic properties, regulated by M-CSF and IL-

10, rather than macrophages polarized by Th2-associated cytokines (Svensson *et al.* 2011). This is supported by their expression of CD163, a homeostatic receptor expressed by tissue resident macrophages (Akila *et al.* 2012), and the production of CCL18 (Gustafsson *et al.* 2008), associated with IL-10 and Treg cells (Ambarus *et al.* 2012; Svensson *et al.* 2011; Tiemessen *et al.* 2007). Although few studies have addressed the functional properties of decidual macrophages, they have indeed been shown to be suppressive (Mizuno *et al.* 1994) and were also recently demonstrated to induce Treg cells, in a dNK cell-dependent manner (Vacca *et al.* 2010). Nevertheless, the expression of pattern recognition receptors (CD163, CD206 and CD209) also suggests that decidual macrophages have the potential to clear infections, a function that was demonstrated by Singh *et al.* (Singh *et al.* 2005). Interestingly, M1-associated genes (*e.g.* IL1B, IL12RB2) appear to be hypermethylated while M2-associated genes (*e.g.* A2M, IL10) are hypomethylated, indicating that the anti-inflammatory machinery of macrophages at the fetal-maternal interface is kept more accessible by epigenetic regulation (Kim *et al.* 2012). Together, these characteristics may be essential for the ability of decidual macrophages to protect the fetus against invading pathogens while maintaining the homeostatic environment required for successful fetal development.

Decidual macrophages have also been attributed a role in spiral artery remodeling due to their production of molecules associated with tissue remodeling and angiogenesis (*e.g.* MMP9 and VEGF) (Engert *et al.* 2007; Gustafsson *et al.* 2008). MMP9⁺ macrophages with phagocytic activity were found to infiltrate remodeling decidual vessels suggesting that they are involved in the degradation of the extracellular matrix as well as the clearance of apoptotic cells, which are critical processes during vascular remodeling (Hazan *et al.* 2010). The role of decidual macrophages in the removal of apoptotic debris (Abrahams *et al.* 2004; Mor and Abrahams 2003), is consistent with their homeostatic properties. During this process, molecules such as fibronectin-1 and the complement component C1q (Gustafsson *et al.* 2008) could facilitate the

uptake of apoptotic cells that might otherwise create an unfavorable inflammatory environment in the uterus.

The contribution of decidual macrophages to pregnancy complications is not well characterized and the reported macrophage numbers associated with *e.g.* preeclampsia are inconsistent (Kim *et al.* 2007; Lockwood *et al.* 2006). However, the findings of elevated decidual GM-CSF levels (Huang *et al.* 2010), and reduced decidual and placental IL-10 in preeclamptic patients (Hennessy *et al.* 1999; Schonkeren *et al.* 2011) are consistent with decreased numbers of regulatory CD163⁺ macrophages in preeclamptic deciduas (Schonkeren *et al.* 2011). These data implicate a defect M2 macrophage polarization in the development of preeclampsia, and support a role for IL-10 in promoting, and for GM-CSF in counteracting, the induction of homeostatic decidual macrophages (Svensson *et al.* 2011). Supporting this, a recent study indicated that altered M2 macrophage polarization in early pregnancy may predispose to complications later in pregnancy (Prins *et al.* 2012).

Cells of the adaptive immune system

T cells in the decidua

As mentioned, there is a low frequency of T cells in the decidua relative to blood. Furthermore, the composition of T cell subsets is completely different. In blood, most T cells (>90%) are conventional CD4⁺ or CD8⁺ T cells expressing the alpha/beta T cell receptor, whereas in decidua there is enrichment of a population of CD4⁻/CD8⁻ (double negative) T cells expressing the gamma/delta T cell receptor (Fan *et al.* 2011; Mincheva-Nilsson *et al.* 1992). However, this very high frequency of gamma/delta T cells (30-50% of the T cells) has been challenged (Williams *et al.* 2009b). Gamma/delta T cells, which typically reside in mucosal sites, show a very restricted T cell repertoire and do not develop memory.

Consequently, gamma/delta T cells may be considered part of the innate immune system. In the decidua, the gamma/delta T cells are activated and despite their morphology with cytoplasmic granulae, they do not normally perform cytolytic activity (Mincheva-Nilsson *et al.* 1994). Instead they may contribute to fetal immune tolerance by secreting cytokines like IL-10 and TGF- β (Fan *et al.* 2011; Nagaeva *et al.* 2002) and by regulating trophoblast invasion and proliferation, at least *in vitro* (Fan *et al.* 2011).

While gamma/delta T cells respond to general danger signals, the conventional alpha/beta T cells of the adaptive immune system respond to specific antigens. Among these adaptive T cells, regulatory Foxp3⁺CD4⁺ T cells, which are enriched in the decidua, have drawn much attention (Ernerudh *et al.* 2011). Regarding the specificity of Treg cells there is growing evidence, in particular derived from mouse models, that they are induced by paternal antigens (reviewed in Guerin *et al.* 2009; Leber *et al.* 2010), with some supportive evidence from studies in humans regarding recognition of paternal antigens (Mjosberg *et al.* 2007) and enrichment of fetus-specific cells in decidua (Tilburgs *et al.* 2008). Interestingly, Treg cell induction is enhanced by exposure to semen, thus creating a specific immune tolerance that contributes to a successful subsequent pregnancy. These observations can explain that the risk for preeclampsia is lowered the more exposed the woman is to paternal antigens (Kho *et al.* 2009).

The Treg cells are not only enriched in the decidua, they also show a more pronounced suppressive phenotype than in blood with regards to expression of for example Foxp3, CTLA-4, CD25 and TGF- β (Dimova *et al.* 2011; Mjosberg *et al.* 2010). The recruitment of decidual T cells is governed by human chorionic gonadotropin (Schumacher *et al.* 2009) as well as by the expression of chemokine receptors and the production of corresponding ligands in the decidua, including CCR5/CCL4 (Kallikourdis *et al.* 2007) and CCR4/CCL17 (Mjosberg *et al.* 2010). In addition, Treg cells may proliferate (Mjosberg *et al.* 2010) and mature (Dimova *et*

al. 2011) locally. An intriguing mechanism for regulating T cell traffic was recently shown; stroma cells in the decidua downregulated, by epigenetic mechanisms, their expression of chemokines CXCL9-11, known to recruit Th1 cells (Nancy *et al.* 2012). Whether similar mechanisms exist for other populations, thereby creating the tolerogenic T cell setting, remains to be seen. In normal pregnancy, Treg and Th2 cells predominate over Th1 and Th17 cells, while a skewing of this balance seems to be involved in complications of pregnancy, as shown in part by mouse and *in vitro* studies (Ernerudh *et al.* 2011; Saito *et al.* 2010). In addition to perturbations of Treg cells, also other CD4⁺ cells, as well as CD8⁺ cells, can contribute to pregnancy complications for example based on mis-matches in HLA-C, which is expressed by trophoblast cells (for review see Scherjon *et al.* 2011).

Conclusions

Human pregnancy is associated with immune adaptations that are essential for successful fetal and placental development. Although changes occur both systemically and locally (mainly in the placental bed) the immune changes observed in the placental bed are more marked than in the maternal circulation. The decrease of certain populations in blood, *e.g.* Treg cells, NK cells and classical monocytes, may in part be explained by their recruitment to the fetal-maternal interface where these populations are enriched. Consequently, the enhanced activation of innate immunity in the circulation (*e.g.* increase in non-classical monocytes and granulocytes) might be a compensation mechanism necessary for maintaining a protective immunity against infections, and thus maternal health. Both systemic and local changes might be due to factors produced by the placenta and/or by direct contact of immune cells with placental trophoblast cells. Although there is still much to be settled, increasing evidence suggests that failure of the immune system to adapt adequately may contribute to pregnancy

complications. Maladaptation may be spontaneous or caused by interference of the immune response by toxic substances, which could disturb the pregnancy-specific immune balance, placentation, and thus the outcome or course of pregnancy. It would be of great value to increase our understanding of the intricate interplay between maternal immune cells in the decidua and the role of placental trophoblast cells in their regulation.

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Figure 1. The fetal-maternal interface. Fetally-derived trophoblast cells are in contact with maternal immune cells at two sites: 1) Villous trophoblasts (VTs) are surrounded by maternal immune cells in the intervillous space and 2) invading extravillous trophoblasts (EVTs) interact with decidual leukocytes. The VTs produce various cytokines and chemokines, and generate microparticles that may affect maternal blood leukocytes. The major decidual leukocyte populations are natural killer (NK) cells, macrophages (MΦ) and T cells (cytotoxic (Tc), helper (Th) and $\gamma\delta$ T cells). A small population of dendritic cells (DC) is also present. The main functions of NK cells, macrophages and regulatory T cells are summarized schematically. NK cells support spiral artery remodeling and EVT invasion via the production of angiogenic and chemotactic factors. The interaction between NK cell receptors and ligands on EVTs are important for adequate decidual NK cell function. The macrophages have immune regulatory properties which may support tissue homeostasis, *e.g.* by clearing infections and limiting T cell activation. They also support spiral artery remodeling via angiogenic factors and the clearance of apoptotic debris. Regulatory T cells (Tregs) are important immune suppressors and might contribute to fetal tolerance by inhibiting T cell activation and interacting with antigen presenting cells.

CTLA-4: cytotoxic T lymphocyte antigen 4, Foxp3: forkhead box p3, GM-CSF: granulocyte-macrophage colony-stimulating factor, IDO: indoleamine 2,3-dioxygenase, KIR: killer immunoglobulin-like receptor, M-CSF: macrophage colony-stimulating factor, MMP9: matrix metalloproteinase 9, PlGF: placental growth factor, VEGF: vascular endothelial growth factor.

Figure 2. Spiral arteries and trophoblast invasion in healthy and preeclamptic pregnancy. Preeclampsia is characterized by shallow trophoblast invasion and defective

vascular remodeling leading to reduced maternal blood flow, which in turn may compromise fetal growth. Failure of decidual NK cells and macrophages ($M\Phi$) to recruit trophoblasts and support angiogenesis may contribute to the development of the disease. EVT: extravillous trophoblast.

Figure 1

Figure 2