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## The Role of Macrophages in the Placenta

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### 1. Introduction

Placenta formation occurs through a complex and coordinated effort between the fetus's extraembryonic tissues and the gravid endometrial tissues. Many macrophages are present in the placenta throughout pregnancy and have been detected as early as day 10 of pregnancy (Chang et al., 1993). Placental macrophages include Hofbauer cells of the fetal chorionic villi and decidual macrophages of the maternal decidua basalis (Figure 1) (Bulmer & Johnson, 1984). Functions of placental macrophages include the production of substances that regulate local immune reactions (such as factors that regulate maternal immunological tolerance and fetal protection) and that promote angiogenesis in the placenta during its development (Mues et al., 1989; Sevala et al., 2007). Although they represent a significant presence on both the maternal and fetal sides of the placenta, placental macrophage functions have not been completely elucidated and still remain a significantly studied area of investigation.

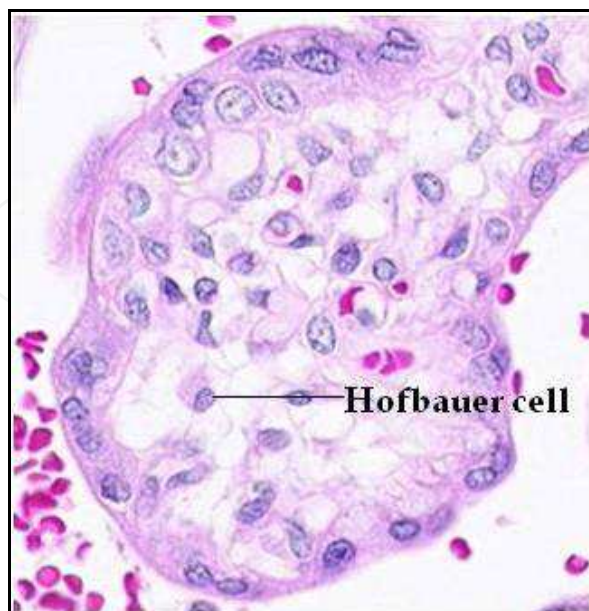


Fig. 1. The mesenchymal core of this fetal tertiary chorionic villus contains a Hofbauer cell, seen as a large, pale-staining placental macrophage with an eccentric nucleus.

## 2. General placenta function

The placenta provides an interface for communication between the maternal blood and the developing fetus. Substances are selectively transported between the separate but closely approximated maternal and fetal vascular systems. Substances exchanged between the fetal blood (contained within fetal vessels of chorionic villi) and maternal blood (located within sinusoids surrounding villi) include nutrients, waste products, oxygen, carbon dioxide, hormones, cytokines, immunoglobulins, drugs, and microbes.

Signaling molecules found in the placenta, such as hormones and cytokines, are important for the maintenance of pregnancy, maternal-fetal tolerance, parturition, lactation, and barrier to infection. Placental cells that produce these mediators include fetal trophoblasts and placental macrophages. This article is not intended to describe the placenta and all of its components in detail, but to discuss one facet of this complex, transient, vital organ of pregnancy, the placental macrophage.

## 3. Macrophage phenotypes overview

Human monocytes and macrophages are players in the innate immune system. Macrophages are bone marrow/hematopoietic-derived cells that migrate through the blood circulation to home to and take up residence within various tissues, where they play a pivotal role in coordinating processes such as development and host defense by secretion of cytokines, chemokines and growth factors and by phagocytosis. Furthermore, macrophages link the innate and adaptive immune systems through phagocytosis, digestion, and antigen presentation (macrophage expression of Human Leukocyte Antigen (HLA) class II molecules and co-stimulatory receptors such as CD80/CD86) to T lymphocytes. The role of macrophages in the orchestration of inflammation, immune responses, and wound healing/tissue remodeling has been a field of intensive study.

Macrophages exhibit great plasticity in their ability to transform between phenotypes. While unstimulated, resting macrophages are relatively quiescent and express low levels of cytokines and growth factors, activation by agents in the surrounding micro-environmental milieu polarizes macrophages to yield a broad spectrum of macrophage phenotypes based on location and micro-environmental influences. Environmental stimuli that influence the expression of inducible genes and polarization of macrophages may include activating agents, pathogens, cytokines, hypoxia, and ischemia (Pinhal-Enfield et al., 2003; Ramanathan et al., 2007).

Based on exposure to surrounding micro-environmental cues, macrophages are induced to alter their expression profiles. Two major classifications have been used in the past to describe activated macrophages as polarized towards pro-inflammatory or anti-inflammatory phenotypes. Although categorization of macrophage phenotypes can be muddled by assorted combinations of activating agents, macrophages respond to micro-environmental stimuli and are typically described as “classically”-activated (M1) pro-inflammatory macrophages or “alternatively”-activated (M2) wound healing macrophages. Based on exposure time and the types of activating stimuli, macrophages may switch activation state to other phenotypes (Martinez et al., 2009; Daley et al., 2010; Porcheray et al., 2005; Classen et al., 2009). M1 macrophages induce processes during injury and infection

that promote inflammatory processes and promote Th1- type immune responses leading to enhanced killing of microbes and tumor cells. M2 macrophages on the other hand, display immunosuppressive effects, resolve inflammation through production of anti-inflammatory cytokines, enhance phagocytosis and elimination of debris, secrete growth and angiogenic factors that stimulate tissue remodeling and repair, and stimulate Th2-type immune responses. M2 macrophages are characterized by a distinctive molecular repertoire and have been implicated in the down-regulation of inflammation, tissue remodeling, repair, parasite killing, allergic reactions, tumor promotion, and placenta formation (Pinhal-Enfield & Leibovich, 2011; Leibovich et al., 2010). These descriptions of polarized M1 macrophages or M2 macrophages only represent macrophages at the far ends of the spectrum. Macrophage phenotypes might be better described as a series of gradations and nuances within a broad spectrum between the pro-inflammatory macrophage at one far end and the anti-inflammatory macrophage at the other end.

### 3.1 Induction of macrophage phenotypes

Heterogenous macrophage populations are found within tissues. These differentiated macrophages are plastic and retain the capacity to transform their phenotype in response to micro-environmental cues and immune-specific influences. Activated macrophages switch phenotypes to develop phagocytic and secretory profiles directed for specific functional activities that are in alignment with the original stimuli (Daley et al., 2010).

Unstimulated macrophages express low levels of inflammatory cytokines and growth factors such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-12, and vascular endothelial growth factor (VEGF). Stimulation of macrophages with products of pathogenic agents such as Toll-like Receptor (TLR) agonists (e.g., lipopolysaccharide (LPS), flagellins, or lipoproteins), either with or without interferon- $\gamma$  (IFN- $\gamma$ ) induces "classical" M1 activation, characterized by production of TNF- $\alpha$  and IL-12.

M2 "alternatively activated" macrophages are commonly characterized as being induced in response to IL-4 and IL-13 through the IL-4 receptor- $\alpha$  (IL-4R $\alpha$ ), and up-regulating expression of IL-10, TGF- $\beta$ , and VEGF while down-regulating expression of TNF- $\alpha$  and IL-12 (Martinez et al., 2009; Classen et al., 2009). M2 macrophages also display a series of cell surface and intracellular markers (Table 1). Further investigation, however, has revealed that IL-4 and IL-13 are not necessary for induction of the M2-like phenotype and mouse wounds lacking IL-4/IL-13 still contain M1 and M2-like macrophages (Daley et al., 2010). The IL-4/IL-13-independent pathways of alternative macrophage activation that induce phenotypes resembling M2 macrophages represent an intense field of study as the functional traits of these M2 macrophages and the different activating agents that induce them are characterized.

#### 3.1.1 M1 macrophages

M1 macrophages promote and coordinate inflammatory processes during injury and infection that are essential for intracellular pathogen removal. These macrophages are induced by pathogen associated molecular patterns (PAMPs), such as the TLR4 agonist, LPS, damage associated molecular patterns (DAMPs), such as those derived from

mitochondria of apoptotic neutrophils, and IFN- $\gamma$  (produced by activated Th1, Tc1, NK cells). Pro-inflammatory and cytotoxic responses against intracellular pathogens and transformed cells (such as cancer cells) are directed through induction of cytokines (e.g., IL-1, IL-6, IL-12, TNF- $\alpha$ ) and inflammatory mediators (e.g., nitric oxide (NO) through inducible NO synthase (iNOS)), and increases in leukocyte recruitment and phagocytic and antigen presenting activity. These initial, pro-inflammatory M1 macrophages predominate in the early wound, which becomes a dynamic cauldron of mediators that are in constant flux as interactions, inductions, and adaptations take place (Martinez et al., 2009; Classen et al., 2009; VanGinderarchter et al., 2006). In contrast, M2 macrophages are critical for the resolution of inflammation and the induction of angiogenesis, tissue remodeling, and repair through production of anti-inflammatory cytokines, growth and angiogenic factors, and by phagocytosis and elimination of debris (Martinez et al., 2009; Daley et al., 2010; Classen et al., 2009; Taylor et al., 2005; Rubartelli et al., 2007; VanGinderarchter et al., 2006). M2 macrophages are discussed in further detail in the next section.

	M1	M2
INDUCING AGENTS	IFN- $\gamma$ , LPS	IL-4, IL-13 IC + IL-1 $\beta$ or TLR agonists GC, IL-10, TGF- $\beta$ TLR and AR agonists
UP-REGULATED EXPRESSION	TNF- $\alpha$ , IL-12, IL-1, IL-6, IL-23, IP-10, MIP-1 $\alpha$ , MHC II, CD80, collagenase?, A <sub>2A</sub> R, MMP9, NO/iNOS/respiratory burst	IL-10, IL-1R antagonist, TGF- $\beta$ , CD206, CD23, AMAC-1, CCL22, CCL17, CCL2/MCP-1, IL-1R $\alpha$ , IL-1 decoy receptor, TGF- $\beta$ , Arg-1, Fizz-1, Ym1/Ym2, dectin-1, VEGF
DOWN-REGULATED EXPRESSION		IL-12, TNF- $\alpha$
GENERAL PROFILE	pro-inflammatory	anti-inflammatory and wound healing

Table 1. Inducing agents involved in the regulation of gene expression contribute to the development of M1 and M2 macrophage phenotypes. (Martines et al., 2009; Leibovich et al., 2011).

### 3.1.2 M2 macrophages

Macrophage phenotypes can vary and switch based on temporal and environmental cues, making their investigation and classification complex. Wound macrophages in the early stages of healing have a more M1-like profile with elevated expression of TNF- $\alpha$  and IL-6 and less TGF- $\beta$ , while those in later stages of healing have a more M2-like profile, with less

pro-inflammatory cytokine expression, no induction of iNOS, and elevated markers of alternative activation, including CD206, dectin-1, arginase-1 (Arg-1), and chitinase 3-like 3 (Ym1) (Daley et al., 2010; Black et al., 2008). It is worth noting that the complexity of studying alternatively-activated M2 macrophage phenotypes is also attributed to divergent profiles in different animal models. While Arg-1 has become a reliable marker of murine M2 macrophages, it is not a good marker for human M2 macrophages.

The M2 macrophage phenotype describes a broad category of anti-inflammatory, wound healing macrophages that down-regulate effects of pro-inflammatory M1 macrophages and have often been described as IL-4/IL-13-dependent. However, both M1 and M2-like macrophage phenotypes were also observed in wounds in mice where IL-4 and IL-13 signaling is absent. Stimulation of macrophages with IL-4 and IL-13 (cytokines produced by induced CD4<sup>+</sup> Th2 and CD8<sup>+</sup> Tc2 cells, NK cells, basophils, mast cells, eosinophils) results in “alternative activation” of macrophages. These “alternatively activated” macrophages were first termed M2 macrophages, have anti-inflammatory and wound healing properties, and are associated with allergic and anti-parasitic responses (Martinez et al., 2009). M2 macrophages express low levels of pro-inflammatory mediators (such as TNF- $\alpha$ , IL-12, IL-1 $\beta$ , IL-8, and oxygen radicals) and express elevated levels of anti-inflammatory cytokines, growth factors, and phagocytic receptors such as IL-10, VEGF, TGF- $\beta$ , CD206 mannose receptor, and CD204 scavenger receptor (Martinez et al., 2009; Classen et al., 2009; Stein et al., 1992; Abramson and Gallin, 1990). M2 macrophages tailor immune responses by chemokine secretion patterns that attract specific sets of leukocytes (monocytes, basophils, memory T cells, Th2 cells, and eosinophils). See Table 1 for a list of some M2 macrophage markers (Martinez et al., 2009; Classen et al., 2009; Mantovani et al., 2006). In addition to their key role in resolving inflammation and mediating repair, M2 macrophages also play a role in some pathological processes. Up-regulation of alternative macrophage gene expression leads to stifling of Th1 responses and consequent vulnerability to infection, allergic responses in asthma, tumor progression assisted by tumor-associated M2-like macrophages (TAMs), and fibroproliferative complications of infection and inflammation (VanGinderachter et al., 2006; Mantovani et al., 2006; Luo et al., 2006; Yang et al., 2009; Porta et al., 2009; Macedo et al., 2007).

Further characterization of macrophages has led to the exploration of other types of alternatively activated M2-like macrophages involved in wound healing that are IL-4/IL-13-independent. The broad category of M2 macrophages has been subdivided based on inducing agents and subsequent expression patterns. M2 macrophage subtypes include the more commonly investigated M2a macrophage (activated by IL-4 or IL-13), M2b macrophage (induced by immune complexes (ICs) and IL-1 $\beta$  or TLR agonists), M2c macrophage (stimulated by IL-10, transforming growth factor- $\beta$  (TGF- $\beta$ ), or glucocorticoids), and M2d macrophage (a new subtype induced by switching M1 macrophages into M2-like macrophages through initial TLR activation followed by adenosine A<sub>2A</sub> receptor (A<sub>2A</sub>R) activation) (Figure 2) (Martinez et al., 2009; Leibovich et al., 2011).

In contrast to the relatively well-characterized M2a macrophage subtype, many M2 macrophages (M2b, M2c, M2d) are not associated with a Th2 immune response or IL-4/IL-13 activation. Evidence of this is seen with M2 macrophage activation in IL-4R $\alpha$  KO

mice and in the presence of the IL-13R $\alpha$ 2 decoy receptor in spite of inhibition of IL-13-dependent phosphorylation of downstream STAT6 and the absence of IL-4 or IL-13 in the wound environment. These IL-4/IL-13-deficient mice exhibit macrophages with both M1 and M2 overlapping and non-overlapping expression patterns (Daley et al., 2010; Leibovich et al., 2011).

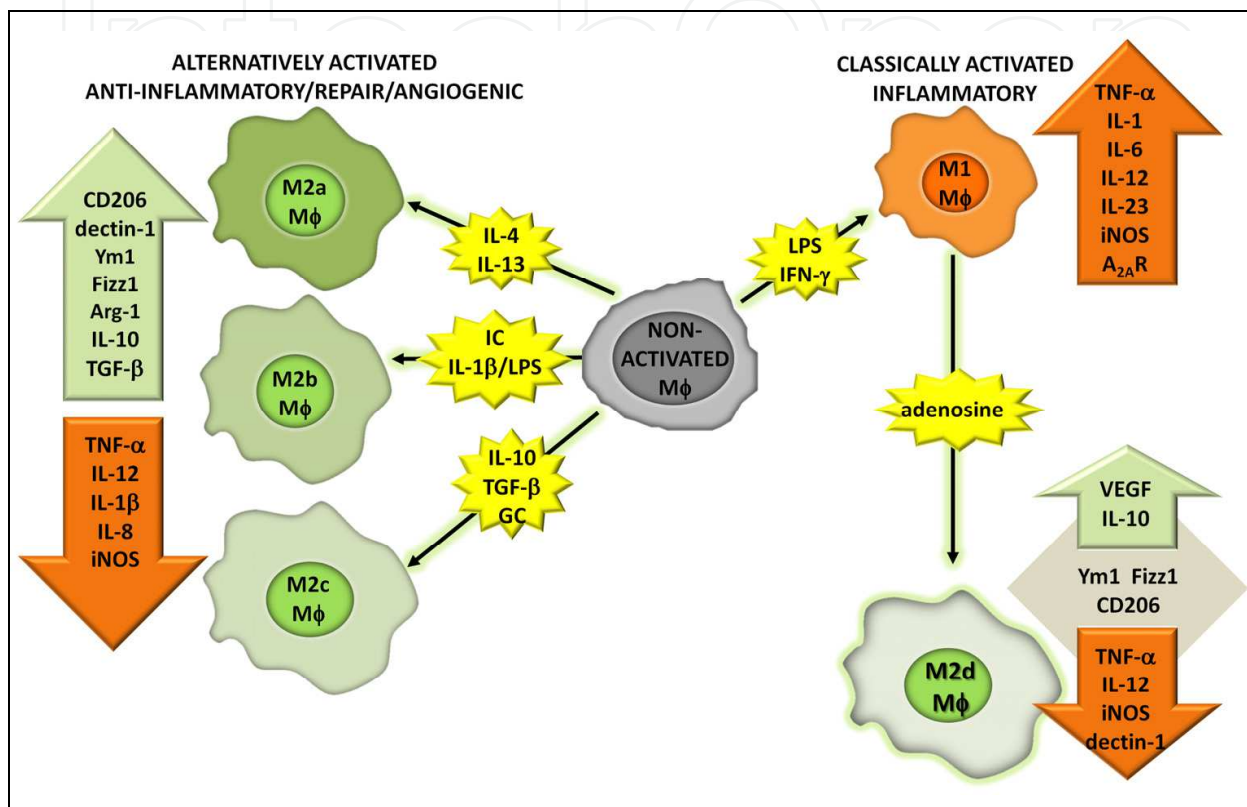


Fig. 2. The broad spectrum of macrophage phenotypes induced by various agents show overlapping and non-overlapping patterns of gene expression.

Investigation of the influence of multiple co-stimulators on macrophages allows for some insight on downstream signaling pathways and the resulting phenotypic macrophage profiles. For example, in the inflammatory environment, the retaliatory metabolite adenosine rapidly accumulates extracellularly as an ATP breakdown product and TLRs and A<sub>2A</sub>Rs are both expressed on macrophages as important regulators of inflammation and repair. Understanding the downstream effects from simultaneous activation of these receptors may provide insight on how to enhance or block M2 macrophage effects by regulating signaling mediators or using receptor agonists or antagonists. Characterization of M2 macrophages by simultaneous induction with multiple agents may more accurately mimic true micro-environmental conditions. Co-stimulation of TLRs and A<sub>2A</sub>Rs may be a more relevant reflection of macrophage surroundings, where adenosine accumulation occurs in inflammatory, ischemic, and hypoxic settings, leading to phenotype switching from TLR agonist-induced pro-inflammatory M1 macrophages (secrete TNF-α and IL-12) to adenosine-induced wound healing and anti-inflammatory M2d macrophages (down-regulation of TNF-α and IL-12 and secretion of IL-10 and VEGF) in a temporally defined

manner. This induction of A<sub>2A</sub>Rs plays a key role in switching pro-inflammatory M1 to an angiogenic M2-like phenotype. Solitary activation of these receptors may not show expression patterns seen in co-stimulation models. This example of receptor co-stimulation on macrophages shows that macrophage activation and its role in wound healing is a complex event involving multiple micro-environmental stimuli (Leibovich et al., 2011; Macedo et al., 2007). Knowledge of the processes leading to the phenotypic switches occurring in macrophages may yield potential therapeutic targets for promoting wound healing or for dampening abnormal wound healing. Although macrophages have varying and dynamic roles that adapt to activating agents in the surrounding milieu, a clear understanding of how to manipulate and switch these phenotypic profiles may provide potential benefits.

#### 4. Placental macrophages

In adult tissues, macrophages coordinate tissue homeostasis and orchestrate host defense and wound healing. During fetal development, macrophages are abundantly present in the pregnant uterus and placenta. These placental macrophages play a key role in synthesis of vital mediators involved in the establishment and maintenance of pregnancy, parturition, lactation, local immune reactions, and maternal-fetal tolerance.

Although they exhibit their own, unique phenotypic profile, placental macrophages display key features of many other tissue macrophages. These characteristics include growth with stimulation by colony-stimulating factor-1, expression of Fc receptors and phagocytosis, and expression of macrophage markers (CD14, CD11b/Cd18, and F4/80) (Chang et al., 1993).

##### 4.1 Origins of placental macrophages

In the human placenta, macrophages have been detected as early as day 10 of pregnancy and many of these macrophages are present throughout pregnancy (Chang et al., 1993). Placental macrophages reside within fetal chorionic villi and uterine decidua during pregnancy, where they assist placental development, manage host defense, and maintain pregnancy.

Fetal placental macrophages and maternal placental macrophages reach the placenta by different routes. During prenatal development, monocyte progenitors originate in the hypoblast-derived fetal yolk sac and migrate to the mesenchymal core of fetal chorionic villi in the placenta. These placental macrophages on the fetal side are called Hofbauer cells. Placental macrophages on the maternal side of the placenta are derived from hematopoietic pluripotent stem cells that differentiate into monocyte progenitors, which will mature and leave the bone marrow to enter the blood. After an approximate 8 hour migration through the circulatory system, the maturing monocyte will enter various organs and tissues, such as the uterine endometrial stroma, where tissue-specific factors will further induce differentiation to form a tissue macrophage (Figure 3) (McIntire et al., 2006).

##### 4.2 General functions of placental macrophages

Macrophages are present in many tissues, including the placenta, and make up a dynamic and heterogeneous population of cells. Macrophage phenotypes and functions, which are



induced at least in part by the surrounding micro-environmental milieu, are crucial in inflammation, immunity, and wound healing. Macrophages are induced by environmental cues to regulate expression of growth factors, chemokines, cytokines, proteolytic enzymes, adhesion molecules, and phagocytic receptors (Martinez et al., 2009).

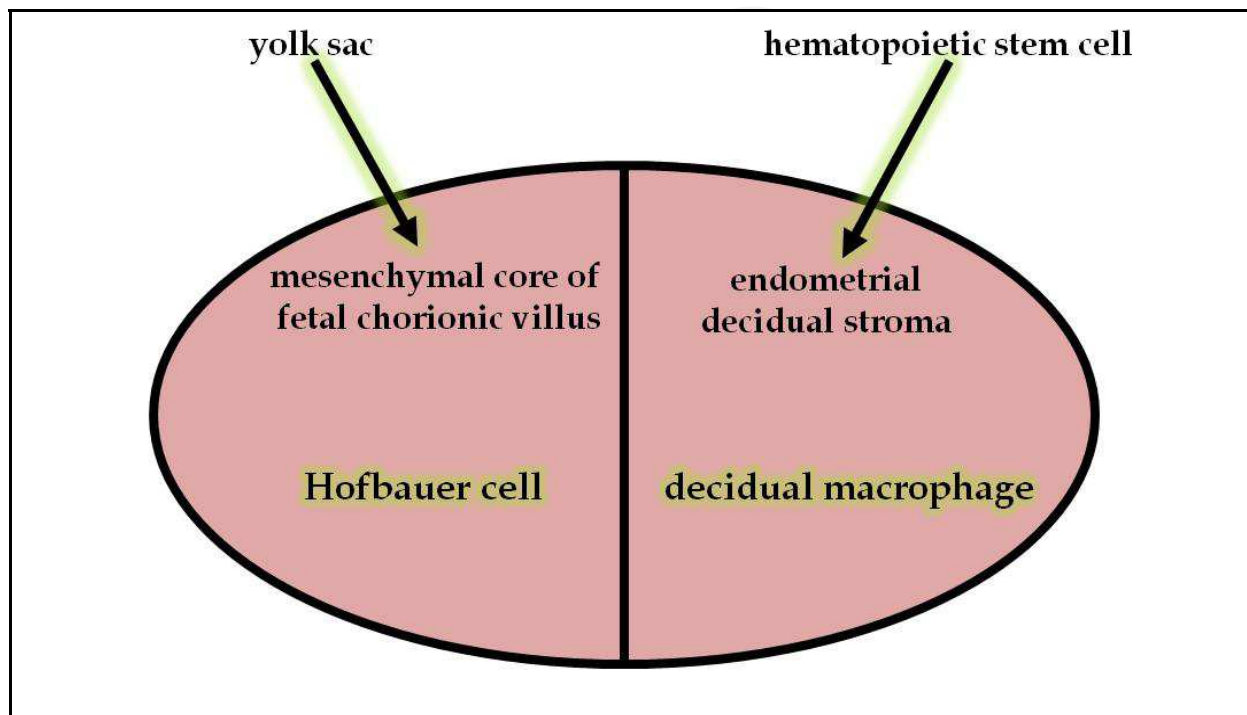


Fig. 3. Placental macrophages originate from two different sources. Hofbauer cells originate from yolk sac cells that migrate to ultimately take up residence in the fetal chorionic villi of the placenta. Decidual macrophages are bone marrow-derived and take up residence in the uterine endometrial stroma of the placenta's decidua basalis.

Macrophages are abundant in the placenta and play a large role through regulation of immune cells and tissue growth. Placental macrophages are influential in many processes, including implantation, trophoblast function, placenta construction (via induction of angiogenesis), maternal-fetal tolerance, fetal defense/protection from infection, and parturition (Bulmer & Johnson, 1984; Mues et al., 1989; Sevala et al., 2007; Kzhyshkowska et al., 2008; Nagamatsu & Schust, 2010; Garcia et al., 1993). Placental macrophages are pivotal to many of these processes because of their plasticity and ability to switch phenotypes based on surrounding micro-environmental cues. For example, decidual placental macrophages respond to placental stimuli that control maternal immune responses to the fetus (maternal-fetal tolerance) as well as to pathogenic stimuli during infections (host defense). During pregnancy, placental macrophages are exposed to local stimuli that induce an array of macrophage phenotypes with M1 and M2 characteristics. Placental macrophages express surface markers and secrete mediators such as cytokines, prostaglandins, proteolytic enzymes, and chemokines (McIntire et al., 2006). It should be noted that while microenvironmental modulation of placental macrophages plays an important role in regulating macrophage phenotype, it is also possible that selective recruitment of distinct

monocyte sub-populations might also play role. The nature of the monocyte precursor subpopulations that give rise to resident placental macrophages has not been studied in detail, and the role of local proliferation of macrophages in the placenta versus recruitment of new macrophages from the circulation in the maintenance of macrophage homeostasis is not yet clear.

#### 4.2.1 Placental macrophages in placenta construction

Macrophages are abundant in fetal tissue and these macrophages play a role in placental development. Angiogenesis is critical for vascular development in the fetal chorionic villi of the placenta and is induced by secretion of VEGF from nearby Hofbauer cells located in the chorionic villus mesenchyme. VEGF binds the fms-like tyrosine kinase 1 receptor (Flt-1), which is expressed on trophoblast and endothelial cells during early pregnancy (Clark et al., 1986). Interestingly, the placenta produces elevated levels of soluble Flt-1 receptor (sFlt-1) in women with pre-eclampsia. This sFlt-1 sequesters VEGF and results in dysfunctional angiogenesis in the placenta (Luttun and Carmeliet, 2003). Placental development progresses (apoptosis, phagocytosis, matrix degradation, remodeling) via continued interactions between maternal decidual placental macrophages and fetal-derived trophoblasts (Nagamatsu & Schust, 2010).

In addition to secretion of angiogenic factors by fetal Hofbauer cells that promote angiogenesis during placental development, maternal decidual placental macrophages also contribute to angiogenesis in the placenta by the abundant expression of the stabilin-1 scavenger receptor. Stabilin-1 is a receptor for placental lactogen (PL), a prolactin growth hormone secreted by trophoblasts. PL is important for placental angiogenesis, as seen by the correlation between low PL concentrations and aberrant placental angiogenesis leading to intrauterine growth restriction. PL is also involved in post-natal growth and mammogenesis/lactogenesis (Kzhyshkowska et al., 2008). Stabilin-1 binds and endocytoses PL. Internalized PL within the decidual macrophage can then proceed through a degradation pathway in which PL within lysosomes is enzymatically digested or through a storage pathway in which PL migrates to the *trans*-Golgi network for processing to be stored within secretory vesicles until called upon for release by low extracellular PL concentrations. Therefore, stabilin-1 expression on decidual M2 macrophages may serve as a sensor to regulate extracellular PL (through endocytosis and intracellular sorting of PL) and result in further regulation of factors involved in angiogenesis during placental growth (Kzhyshkowska et al., 2008).

#### 4.2.2 Placental macrophages in maternal-fetal tolerance

There are multiple factors involved in maternal-fetal tolerance. Decidual placental macrophages are an integral component for suppression of the maternal immunologic response to the allogenic placenta and fetus. Macrophages are antigen presenting cells (APCs) that often activate T cells to initiate an adaptive immune response. However, although decidual placental macrophages constitutively express major histocompatibility complex class II molecules, they have a decreased ability to present antigens to T cells. As a result, T lymphocyte activation does not occur and the allogenic fetus is protected from rejection by the maternal immune system. Another result of placental macrophage inability

to present antigen to T cells is the inability of placental macrophages to induce an efficient immune response against *Listeria monocytogene* despite expressing MHC class II molecules (Chang et al., 1993; Nagamatsu & Schust, 2010) Furthermore, placental macrophages express higher levels of indole 2,3 dioxygenase (IDO), which catabolizes L-tryptophan. L-tryptophan is required for T-cell activation and inhibition of IDO correlated to rejection of the fetus by the mother (Gordon et al., 2003).

#### 4.2.3 Placental macrophages as a barrier to infection

The main defenders against infection of the placenta are placental macrophages. Among many other functions, macrophages play a vital role in removal of nonself as well as unwanted self-ligands through expression of scavenger receptors and other receptors that are targeted for pathogen binding, such as Toll-like receptors (e.g., TLR4 bind LPS) and chemokine-CC motif-receptor 5 (CCR5) (HIV-1 infection requires surface expression of CCR5 and CD4). While sometimes displaying a weak barrier to some pathogens like *Listeria monocytogenes*, placental macrophages often show a strong barrier to numerous bacterial and viral infections (Garcia-Crespo et al., 2009; Kzhyshkowska et al., 2008).

Although macrophages are primary targets for viral infection, placental macrophages are less susceptible to HIV-1 infection (and other viral infections) than other macrophages. The placenta is often a barrier to viral replication, and viral transmission from mother to infant often occurs during the birth. Placental macrophages show lower levels of susceptibility to HIV-1 infection than monocyte-derived macrophages and this may be attributed to differing phenotypes. Although there are lower levels of CD4 and CCR5 expression in placental macrophages, it has been suggested that the mode of HIV-1 restriction in the placenta occurs at the transcriptional level. HIV-1-infected placental macrophages and monocyte-derived macrophages show different protein secretion profiles. Peroxiredoxins are antioxidant molecules that are more abundantly associated with placental macrophages compared to monocyte-derived macrophages. Also, some peroxiredoxins (1,2, and 4) have been shown to have anti-viral activity against HIV-1. Compared to monocyte-derived macrophages, placental macrophages show abundant secretion of peroxiredoxin 5. The up-regulated secretion of peroxiredoxin and its protective role against HIV-1 in the placenta may be through suppression of TNF- $\alpha$ -induced transcriptional activity of nuclear factor-kappa light chain enhancer of activated B cells (NF- $\kappa$ B). In fact, although there appears to be no difference in viral integration for placental macrophages versus other monocyte-derived macrophages, there is reduced viral protein expression in placental macrophages. Therefore, HIV-1 restriction appears to be occurring in placental macrophages at the transcriptional level. NF- $\kappa$ B is required for viral transcription of HIV-1 and suppression may lead to inefficient HIV-1 replication in placental macrophages. TNF- $\alpha$ -induced transcriptional activity of NF- $\kappa$ B may also be thwarted in placental macrophages by down-regulation of cystatin B. Cystatin B induces NO production in macrophages, which up-regulates TNF- $\alpha$ . Reduced HIV-1 replication has been correlated with cystatin B down-regulation and increased HIV-1 replication is enhanced by NO synthesis in macrophages. These factors (among others) distinguish placental macrophages from other macrophages and are influential in the restriction of HIV-1 (and perhaps other viruses) in the placenta (Garcia et al., 2009; Garcia-Crespo et al., 2010).

#### 4.2.4 Placental macrophages as a mediator in parturition and other signaling/hormone

Placental macrophages exhibit varying phenotypes throughout pregnancy. Placental macrophages are stimulated by changes in the micro-environment to switch phenotypes at varying stages of pregnancy as well as during aberrant activity. During late pregnancy, placental macrophage activity is switched to play a role in parturition through production of pro-inflammatory cytokines and prostaglandin E<sub>2</sub>. Altered placental macrophage phenotypes are also linked to the pathologic disease such as preeclampsia (Nagamatsu & Schust, 2010; Wetzka et al., 1997).

During most of pregnancy placental macrophages have an M2-like immunosuppressive gene expression profile (such as IL-10 secretion). However, decidual placental macrophages may express pro-inflammatory characteristics. For example, CD11c<sup>HI</sup> decidual placental macrophages may express pro-inflammatory and antigen-presenting genes while CD11c<sup>LO</sup> decidual placental macrophages express anti-inflammatory and tissue repair genes (Houser et al., 2011). It is important to recognize that classification of placental macrophages is complex and dependent on multiple factors, such as location within the placenta, stage of pregnancy, and factors (e.g., hormones and hypoxia) that change the surrounding micro-environment. Differential recruitment of distinct monocyte sub-populations may also play a role, as mentioned earlier.

#### 4.3 M2-type placental macrophages

Placental macrophages display polarization towards an alternatively-activated, M2 phenotype. Hofbauer cells (fetal placental macrophages within chorionic villi) and decidual macrophages (maternal placental macrophages within the endometrial decidual stroma) are both classified as M2/alternatively-activated macrophages (Joerink et al., 2011; Gustafsson et al., 2011). The role of IL-4 in polarization of placental macrophages is blurred by variable placental macrophage phenotypic profiles. Although IL-4 is involved in endocytosis of stabilin-1-bound PL by placental macrophages, decidual macrophages induced via IL-4-independent means display different expression profiles than those seen with IL-4 stimulation, indicating that a Th2 environment is not required for decidual macrophage development (Svensson et al., 2011; Kzhyshkowska et al., 2008).

As M2 macrophages, the placental macrophage profile typically shows down-regulation of pro-inflammatory mediators and up-regulation of mediators that are immunosuppressive and anti-inflammatory. However, as with many other tissue macrophages, a placental macrophage does not exhibit a phenotype extreme that solely expresses M2 markers. Placental macrophages may express M1 markers, but the representative expression pattern emphasizes many M2 markers. The placental macrophage M2 marker expression is regulated by activating agents (such as cytokines, hormones, and adenosine) in the surrounding micro-environment in a manner that yields an M2 phenotype profile that is distinct from other M2 macrophages. The exact nature and combinations of activating agents to achieve this unique profile has yet to be completely elucidated. Analyses of M2 placental macrophages from normal pregnancies and pregnancies with complications have shed some light on dysregulation. In fact, a correlation between dysregulation of some placental macrophage markers and

preeclampsia (MR, CCL2, IGF-1, MMP9), pre-term labor (CCL18) and intrauterine growth restriction (IGF-1) has been shown (Gustafsson et al., 2011).

Placental macrophages typically lack or exhibit low expression of pro-inflammatory M1 markers (such as CX3CR1, IL-7R and CCR7), as described in Hofbauer cells by Joerink et al. (2011). Many placental macrophage M2 markers have been investigated. A number of M2 markers have been identified in placental macrophages such as DC-SIGN (attachment factor for HIV-1 entry), CD163 and mannose receptor/CD206, stabilin-1, CCL18 (involved in tolerance of T lymphocytes with reduced expression after the onset of labor), CD209, fibronectin-1 and insulin-like growth factor-1 (IGF-1), TREM-2, alpha 2 macroglobulin (A2M), prostaglandin D<sub>2</sub> synthase (PGDS), and IL-10 (Kzhyshkowska et al., 2008; Gustafsson et al., 2011; Svensson et al., 2011; Joerink et al., 2011). Recall that placental macrophages are not at the extreme end of the M2 spectrum of macrophages and that M1-type markers can also be seen in placental macrophage populations. For example, placental macrophages express IL-6 and CCL2 (aka monocyte-chemoattractant protein-1, MCP-1), which are typically pro-inflammatory M1 markers. It has been suggested that CCL2 expression by placental macrophages may be important for monocyte recruitment to decidua and that macrophage phenotypes may then be altered by other environmental factors upon arrival (Svensson et al., 2011; Gustafsson et al., 2011). Furthermore, Houser et al. (2011) have characterized decidual placental macrophages as having profiles associated with M1 and M2 macrophages. As mentioned previously, CD11c<sup>HI</sup> decidual macrophages are pro-inflammatory while CD11c<sup>LO</sup> decidual macrophages are anti-inflammatory. Thus, decidual macrophages have varying phenotypes that help to coordinate maternal-fetal interactions at the placenta (Houser et al., 2011).

## 5. Research and clinical implications

### 5.1 Advantages and limitations of experimental models/materials

It should be emphasized that some limitations to consider in the characterization of alternatively-activated macrophages include species-species variation in gene expression patterns and variable effects of the micro-environment surrounding macrophages. Investigation of M1 and M2 marker expression has been focused more predominantly using murine macrophages and human homologs for some murine markers have not been found (Martinez et al., 2009).

Furthermore, agents in the environment surrounding macrophages influence macrophage activation, and while *in vitro* models are useful, analyses obtained from them are limited. Macrophages in culture enable investigators to highlight activation factors and gene expression patterns; however, absence of *in vivo* models may overlook interactions with other cell types and the mediators that they may elaborate. It is crucial to investigate interactions of expressed proteins that may be different in an *in vivo* context in which there may be interaction with other cells or mediators. Macrophage activation is a complex and dynamic enterprise with characteristics that vary with time to result in early and late markers. For example, expression of CCL18 in decidual macrophages declines with onset of labor (Gustafsson et al., 2011). Macrophage phenotype switching is driven in a temporally-orchestrated manner that depends upon alterations in the micro-environment, such as that seen in the changing placenta.

## 5.2 Clinical implications and future research

M1 and M2 macrophages are pivotal players in host defense, wound repair, and pregnancy and further investigation into these polarized activated macrophage phenotypes may elucidate normal and variant processes. Knowledge of processes associated with placental macrophages may shed light on pathological mechanisms and may present attractive potential pharmaceutical targets to regulate disease. For example, decidual macrophages with overexpression of M1 markers have been implicated in parturition and early embryo loss (Gustaffson et al., 2011). Thus, manipulation of placental macrophage phenotypes by modulating signaling pathways in macrophages or by altering the surrounding environment may provide therapeutic benefits. For example, because of their angiogenic effects, induction of M2 macrophages phenotypes may enhance placental growth.

## 6. Conclusion

Macrophages are found throughout the body and exhibit a broad spectrum of phenotypes based in large part on the surrounding microenvironment. Placental macrophages exhibit a phenotypic profile more characteristic of M2 macrophages that are immunomodulatory and promote angiogenesis for placental development. Investigation of placental macrophages may provide insight into normal development as well as possible causes of embryo loss.

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## 8. References

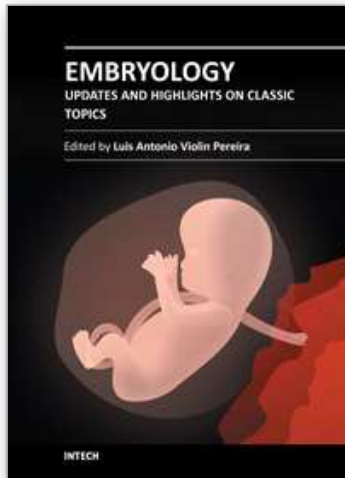
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