Decidual macrophages and Hofbauer cells in fetal growth restriction

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Placental macrophages, which include maternal decidual macrophages and fetal Hofbauer cells, display a high degree of phenotypical and functional plasticity. This provides these macrophages with a key role in immunologically driven events in pregnancy like host defense, establishing and maintaining maternal-fetal tolerance. Moreover, placental macrophages have an important role in placental development, including implantation of the conceptus and remodeling of the intrauterine vasculature. To facilitate these processes, it is crucial that placental macrophages adapt accordingly to the needs of each phase of pregnancy. Dysregulated functionalities of placental macrophages are related to placental malfunctioning and have been associated with several adverse pregnancy outcomes. Although fetal growth restriction is specifically associated with placental insufficiency, knowledge on the role of macrophages in fetal growth restriction remains limited. This review provides an overview of the distinct functionalities of decidual macrophages and Hofbauer cells in each trimester of a healthy pregnancy and aims to elucidate the mechanisms by which placental macrophages could be involved in the pathogenesis of fetal growth restriction. Additionally, potential immune targeted therapies for fetal growth restriction are discussed.

1 Introduction

Macrophages, one of the major immune cell subsets in the placenta, play a crucial role in placental development, maternal-fetal tolerance and host defense during pregnancy [1]. Macrophages of maternal origin are predominantly located in the decidua and are referred to as decidual macrophages (dMφs) [2, 2]. Hofbauer cells (HBCs), fetal macrophages, are the primary immune cell type in the villous stroma and are also present in the chorion and amnion [3]. Macrophages are capable of sensing changes in their microenvironment and

responding accordingly. This provides macrophages with a high degree of phenotypical and functional plasticity, making them key players in immunologically driven events in pregnancy (2).

For maternal macrophages, roughly three immunological states can be distinguished throughout gestation. These different states require specific adaptive capacity of maternal macrophages to facilitate the processes unique to each phase of pregnancy (4, 5). Early pregnancy is characterized by a pro-inflammatory phase that is needed for implantation of the conceptus and early placental development. This is followed by an immunoregulatory phase that starts around week 13 of gestation which is focused at maintaining maternal-fetal tolerance to ensure that the interaction between maternal and fetal tissue does not result in immunological rejection. The third phase is at the end of pregnancy and is marked by a pro-inflammatory response that initiates the onset of labor (4, 5). Fetal immune cells are naturally impaired in terms of pro-inflammatory cytokine and chemokine production, as well as eliciting cytotoxic responses (6, 7). Both the maternal and fetal immune system are thus inherently biased towards tolerance and the preservation of immune balance.

Maladaptation of the maternal or fetal immune system results in diverted immune functionalities, which can subsequently lead to placental developmental defects and disproportionate inflammatory responses against fetal and placental tissue. Dysregulation of dMφs and HBCs specifically have been associated with adverse pregnancy complications like infertility, preeclampsia, spontaneous preterm birth, (recurrent) pregnancy loss, and intrauterine infections (2, 3, 5, 8).

Fetal growth restriction (FGR) is a condition in which the fetus does not reach its intrinsic growth potential (9). The majority of FGR cases result from placental maldevelopment and subsequent placental insufficiency. However, our understanding of the contribution of placental macrophages to FGR remains limited. (10, 11). Given the many roles that macrophages play in placental development and proper functioning, it is likely that dysfunctional dMφs and HBCs contribute to various aspects of the pathogenesis of FGR. With this review, we aim to elucidate the various mechanisms through which placental macrophages could be involved in the pathophysiology of FGR. To do so, we will first address placental development and functioning under physiological conditions, followed by the pathophysiological aspects of the placenta in pregnancies complicated by FGR. Next, we will provide a comprehensive overview of the functionalities of dMφs and HBCs in healthy pregnancies and outline potential mechanisms by which placental macrophages could be involved in the pathogenesis of FGR. Additionally, potential therapies for fetal growth restriction will be discussed.

2 Placental development and anatomy

The placenta consists of a maternal part; the basal plate and decidua, and a fetal part; the chorionic and amniotic membranes, and villi (1). Placental development starts with the formation of the blastocyst, consisting of a trophoblast wall that surrounds the blastocystic cavity, which situates the embryoblast (1).

Implantation of the blastocyst in the uterus occurs by the formation of extensions from syncytriotrophoblast, the outer layer of the trophoblast wall, that deeply invade into the endometrium (1). After implantation, the endometrial stromal cells transform into decidual cells forming the decidua (12). Outgrows of the syncytriotrophoblast form the villi with its fetal capillaries, and the intervillous space in between (1).

Extravillous trophoblasts (EVT) further invade into the decidua where they destroy and replace the muscular wall of the maternal arteries, a process called spiral artery (SA) remodeling. This creates low resistance, high capacity and low velocity vessels (13, 14). Early in the first trimester, EVT plugs block the SAs in order to prevent a sudden high velocity blood flow into the placenta. The onset of the uteroplacental circulation is marked by the dissolvement of the EVT plugs at the end of the first trimester so that maternal blood can flow into the intervillous space. Hereafter, SA remodeling extends further into the decidua until the inner third of the my (15). Figure 1 gives a gross anatomical overview of the maternal-fetal interface once placental development is complete.

3 Pathophysiology of fetal growth restriction

FGR affects a considerable number of pregnancies, with a prevalence reaching up to ten percent (16). It is associated with a range of perinatal complications including respiratory distress syndrome, sepsis, thrombocytopenia and (iatrogenic) preterm birth (17, 18). Moreover, nearly one third of stillbirths is related to FGR (19). Also, long term consequences like neurodevelopmental disorders and an increased risk for cardiovascular and metabolic diseases later in life can result from restricted growth *in utero* (20–22). The etiology of FGR is multifactorial, encompassing fetal, maternal and placental factors. In the absence of fetal congenital abnormalities, placental malfunctioning is the most common underlying mechanism (10, 11). Typical placental lesions that are observed in the FGR placenta include maternal vascular malperfusion (MVM), fetal vascular malperfusion (FVM), and villitis of unknown etiology (VUE) (9, 23). Often, there is a combination of these lesions (11, 23, 24). MVM, resulting from significant impairments in SA remodeling, is the most common cause for deficits in the uteroplacental circulation (10, 11). It is marked by fewer extravillous trophoblasts (EVTs) that invade the SAs due to increased trophoblast apoptosis, resulting in insufficient disruption of the vascular smooth muscle cell (VSMC) layer with persistent muscularization of the SAs, less fibrinoid deposition in the SA vascular wall and an overall narrow lumen of the vessels with vasoactive capacity. As a consequence, a high velocity, low volume circulation arises which causes mechanical harm like vascular shear stress and damage to the villous structure. Moreover, the alterations in placental blood flow can lead to an increase in oxygen tension and ischemia-reperfusion injury due to intermittent blood flow. Reintroduction of oxygen after deprivation can cause an increase in the formation of reactive oxygen species (ROS), causing oxidative stress and inflammation (11, 25). Placental infarcts arise and villous development is impaired, resulting in villous hypoplasia (26–28).
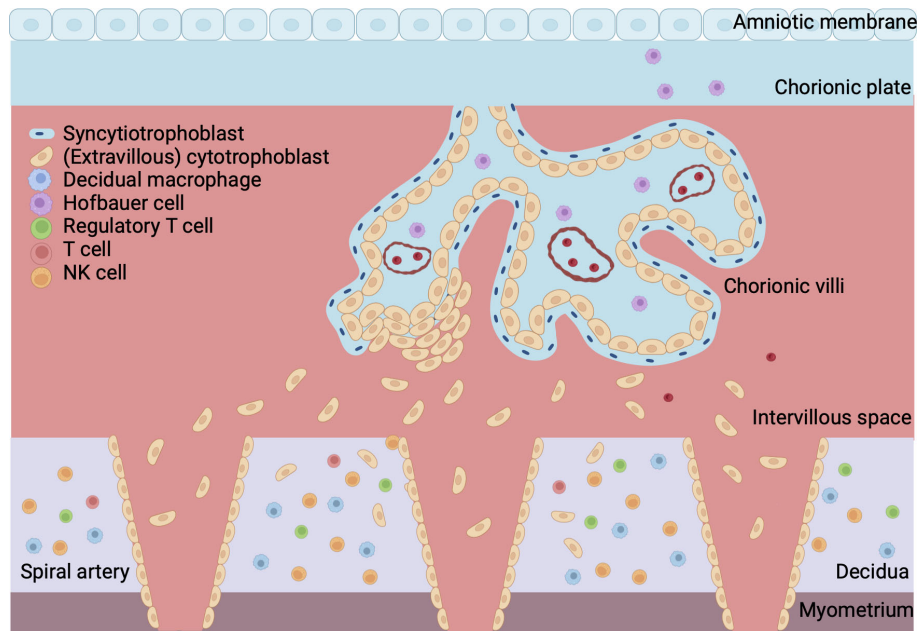
FIGURE 1

Overview of maternal-fetal interface. The fetal part of the placenta consists of the chorionic plate, amniotic membrane and villi. The cytotrophoblast forms the inner layer of the villi and is surrounded by a continuous outer layer of syncytiotrophoblast. Syncytiotrophoblast cells invade the endometrium during implantation of the conceptus. Extravillous trophoblast invades the maternal spiral arteries to facilitate remodeling of the vasculature. The maternal part of the placenta is formed by the decidua, which is attached to the myometrium, a layer of uterine smooth muscle cells. Blood from the maternal spiral arteries flows into the intervillous space where exchange of oxygen, nutrients and waste between mother and fetus takes place. Created with BioRender.com.

FVM is marked by impaired fetal blood flow, for example due to a high umbilical cord coiling index or vascular changes with fibromuscular muscular sclerosis, intramural fibrin deposition or calcifications leading to thrombosis, and avascular villi, or villous stromal vascular karyorrhexis. Histological features of FVM can be seen in the umbilical and chorionic vessels and stem and terminal villi (29, 30). FGR placentas exhibit characteristic features of oxidative stress and reduced antioxidant protection, resulting in further damage to the villi and impaired transport across the placental barrier (25, 31–33). Another placental lesion commonly found in FGR is VUE, a noninfectious chronic inflammatory lesion of the villi of which the etiology remains poorly understood (34). It has been hypothesized that VUE involves failed maternal-fetal tolerance as the inflammatory infiltrate constitutes of maternal T cells and macrophages that migrate into the intervillous space. In addition, numbers of intravillous HBCs are increased (35).
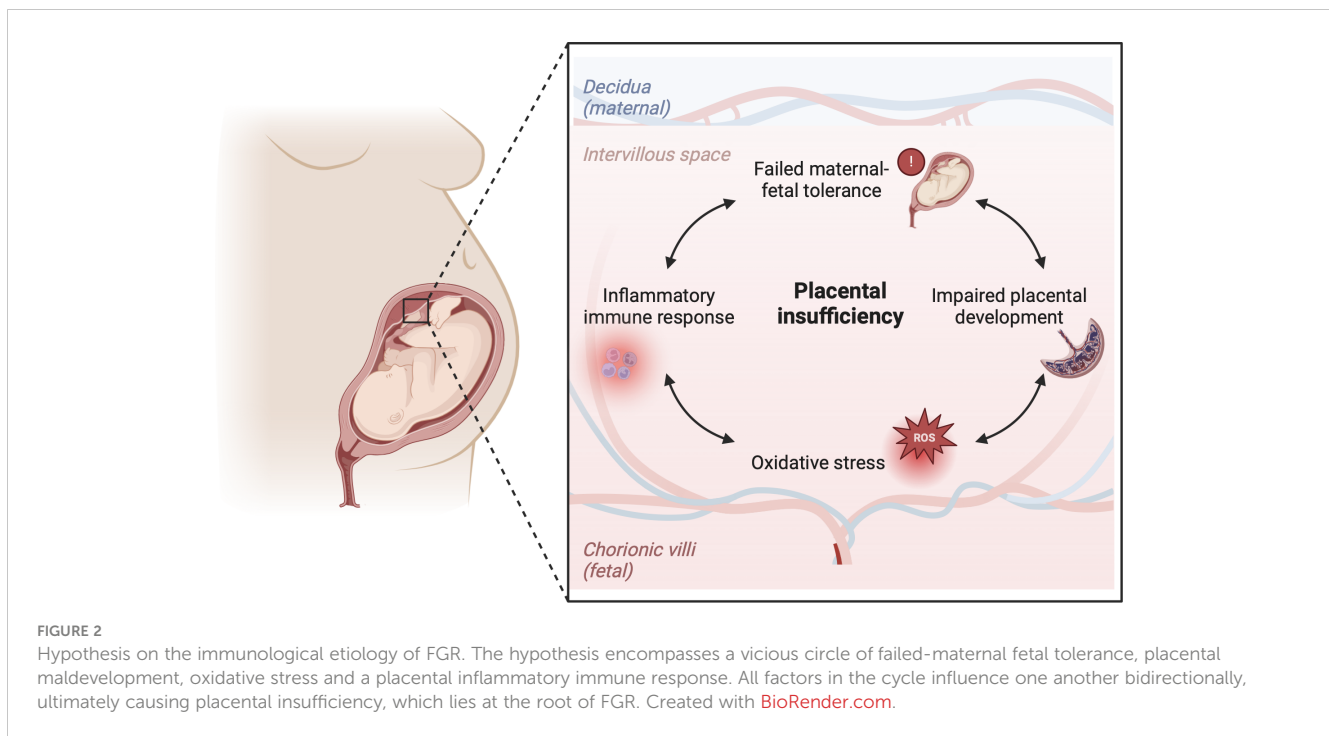
Failed maternal-fetal tolerance has been proposed as one of the causes of FGR (36). Indeed, several of the above mentioned placental pathophysiological mechanisms can result from, or initiate, a maternal inflammatory response. Multiple studies in FGR placentas have shown increased apoptosis of trophoblast cells as a result of inflammation, which leads to restricted trophoblast invasiveness during implantation of the conceptus and impaired remodeling of the SAs (37–40). On the other hand, maldevelopment of the uteroplacental circulation can cause oxidative stress, which has been implicated in cytokine signaling and regulation of the inflammatory immune response, including the proliferation and pro-inflammatory polarization of macrophages in the placenta (32, 33). Moreover, damage to the placental vascularization and villous tissue can directly elicit a pro-inflammatory response in the placenta. The release of trophoblast fragments into the maternal circulation provides opportunity for maternal immune cells to recognize these fetal antigens and elicit an inflammatory response which compromises maternal-fetal tolerance (37, 41, 42). This interplay can initiate a vicious cycle, as increased oxidative stress attracts more immune cells, potentially exacerbating inflammation and further impairing placental development and functioning (Figure 2). Indeed, increased levels of pro-inflammatory markers and damage-associated molecular patterns (DAMPs) can be found in the placenta and in the maternal blood in FGR pregnancies (36). Gene profiling studies on FGR placentas show differentially expressed genes associated with hypoxia, angiogenesis, vascular function, immune modulation and tissue growth in FGR (43–45).

In the next sections, we will outline the functionalities of dMφs and HBCs in healthy pregnancies, followed by reviewing what is currently known about the role of dMφs and HBCs in FGR.

4 Decidual macrophages in healthy pregnancy

In early pregnancy, dMφs form up to 20% of the decidual leukocyte population, being the second largest group of immune cells following natural killer (NK) cells (around 70%). T cells account for less than 20% of the decidual immune cell population

FIGURE 2
Hypothesis on the immunological etiology of FGR. The hypothesis encompasses a vicious circle of failed-maternal fetal tolerance, placental maldevelopment, oxidative stress and a placental inflammatory immune response. All factors in the cycle influence one another bidirectionally, ultimately causing placental insufficiency, which lies at the root of FGR. Created with BioRender.com.

(46, 47). As mentioned, dMφs have distinct functionalities in each trimester of pregnancy which are enabled by their phenotypical plasticity (4, 5). DMφs numbers increase as pregnancy progresses, highlighting the importance of dMφs throughout the entire course of gestation and making them an interesting cell population to study in healthy and pathological pregnancies (48).
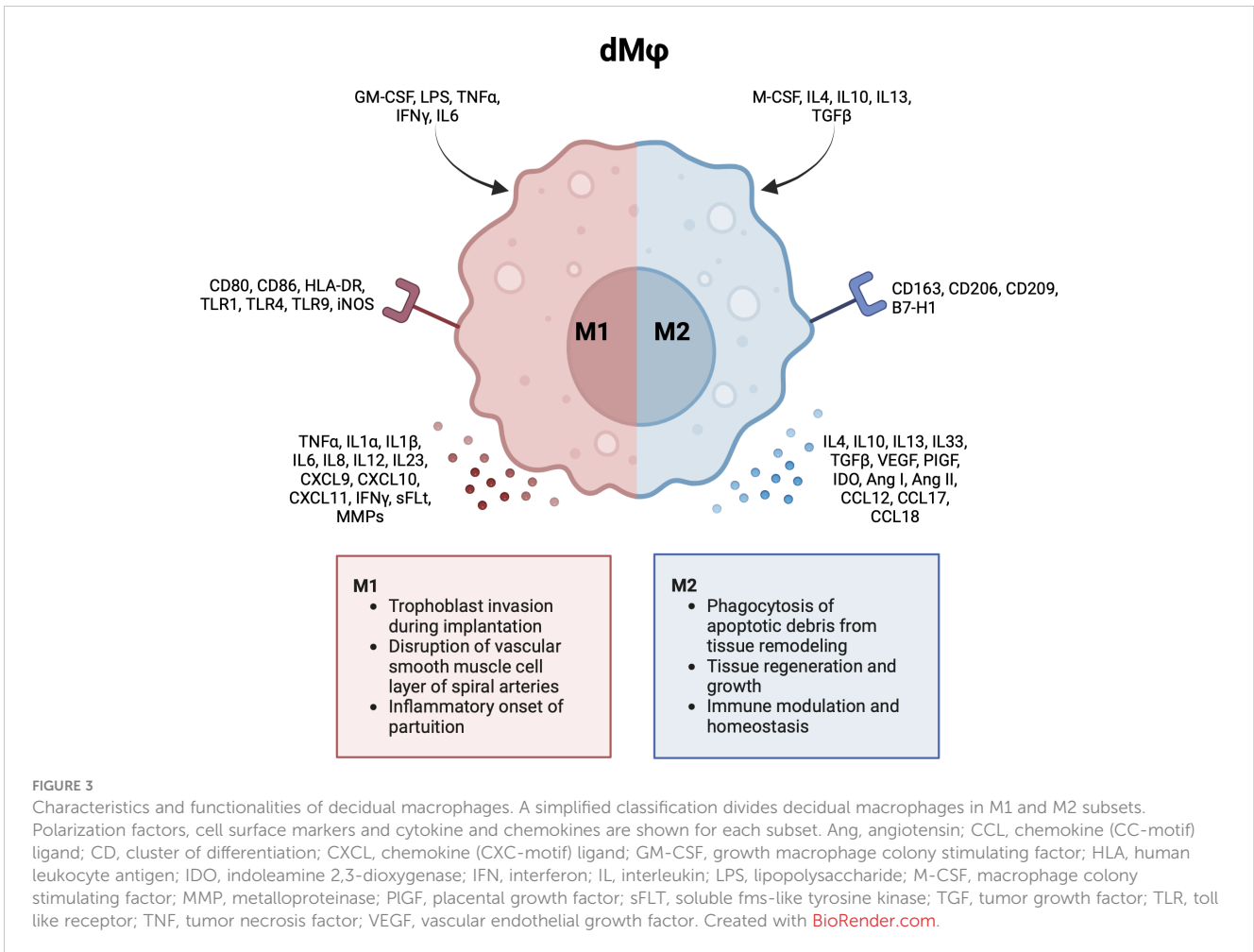
In general, Mφs can be grouped into classically activated, pro-inflammatory (M1) macrophages, and alternatively activated, anti-inflammatory (M2) macrophages (49). M1 macrophages can be characterized by cell surface markers like cluster of differentiation (CD) 80, CD86 and HLA-DR. M2 macrophages express markers like CD163, CD206 and CD209 (2, 5, 50, 51). Notably, this division represents the extreme ends of a broad dMφ polarization spectrum. It has been proposed to further group M2 dMφs into M2a, M2b and M2c based on the expression of CD11c and HLA-DR (46). However, this division is less often reported for dMφs compared to HBCs. As will be described in the next sections, the majority of dMφs express various phenotypes and functionalities and cannot be strictly classified as M1 or M2. Figure 3 summarizes the activation pathway, cell surface markers, cytokine and chemokine profiles, and main functionalities of dMφs during pregnancy (2, 5, 47, 50–53).

4.1 First trimester

Macrophages are already present in the uterus before pregnancy and play a crucial role in all phases of the menstrual cycle (54). Part of the dMφs are derived from maternal blood monocytes and are recruited to the uterus shortly after conception, which occurs in direct response to semen-derived immune regulatory proteins like MCP-1. Also the embryo itself produces MCP-1 to attract monocytes to the uterus (55). Monocytes migrate towards the syncytiotrophoblast that invades the endometrial stroma during implantation. This migration is guided by trophoblast cells that excrete several signaling molecules (e.g. IL8, MCP1 and MIPα) (56–59). As mentioned in the introduction section, a short pro-inflammatory phase is needed during implantation as this helps trophoblast cells to gain space in the endometrium and prepares the endometrium for implantation of the embryo (57, 58, 60). In response to trophoblast signals, recruited macrophages express pro-inflammatory cytokines like IL6, IL8, TNFα and IFNγ (58). DMφs actively prevent apoptosis of invading trophoblast, for instance by secreting TGFβ which can inhibit NK cells from inducing trophoblast apoptosis, and IL6 and IL10 which can downregulate Fas mediated apoptosis (61, 62). DMφs also secrete G-CSF and IL33, which promote trophoblast proliferation, migration and invasion (63, 64). Conversely, several *in vitro* studies have shown that pro-inflammatory monocytes can restrict trophoblast invasiveness and increase trophoblast apoptosis (65–68). This suggests that the pro-inflammatory macrophage response needs to be balanced and carefully timed to prevent trophoblast damage.

In mice, it has been clearly demonstrated that macrophages have pro-inflammatory characteristics early in the implantation phase, followed by a mixed pro-inflammatory and immunomodulatory response once trophoblast further invades the uterine stroma and placental development continues (55). In humans, a similar shift in dMφs phenotype can be expected in the first trimester. Indeed, some studies in humans describe both anti- and pro-inflammatory characteristics in first trimester dMφs (69–72). For example, macrophage activation markers like HLA-DR, CD80 and CD86, involved in macrophage T-cell interactions, can be found on first trimester dMφs (69, 70). Others found dMφs displaying immunomodulatory phenotypical markers (CD163, CD206 and

FIGURE 3

Characteristics and functionalities of decidual macrophages. A simplified classification divides decidual macrophages in M1 and M2 subsets. Polarization factors, cell surface markers and cytokine and chemokines are shown for each subset. Ang, angiotensin; CCL, chemokine (CC-motif) ligand; CD, cluster of differentiation; CXCL, chemokine (CXC-motif) ligand; GM-CSF, growth macrophage colony stimulating factor; HLA, human leukocyte antigen; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; M-CSF, macrophage colony stimulating factor; MMP, metalloproteinase; PlGF, placental growth factor; sFLT, soluble fms-like tyrosine kinase; TGF, tumor growth factor; TLR, toll like receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor. Created with BioRender.com.

CD209) to be capable of secreting both anti- and pro-inflammatory cytokines (71, 72). However, the majority of studies indicate dMφs in humans to be predominantly anti-inflammatory and immunomodulatory. Distinct observations on the phenotype of dMφs are likely due to differences in the specific time point in the first trimester at which the experiments are performed. Studies report expression of anti-inflammatory markers (e.g. CD163, CD206 and CD209) and the secretion of anti-inflammatory cytokines (e.g. IL10, CCL12, CCL17 and CCL18) (69, 72–75). Gene expression profiling studies show that genes related to immunomodulation and tissue remodeling are activated in first trimester dMφs (76). This is further observed when studying the response of dMφs to placental signals. Multiple studies have cultured dMφs with placental explants or with decidua or trophoblast derived cytokines and demonstrate that polarizes dMφs towards a more anti-inflammatory phenotype (73, 74, 77, 78). It was even shown that HLA-G expressed on invading trophoblast can directly bind to leukocyte immunoglobulin-like receptors on dMφs to induce a more anti-inflammatory phenotype in dMφs (79–82).

Besides implantation, dMφs participate in SA remodeling (83). The migration of dMφs towards the SAs relies on crosstalk between trophoblast cells and SA endothelial cells (ECs). Several factors expressed by EVT (e.g. CXCL8 and IL6) stimulate the ECs to secrete CCL14 and CXCL6, causing dMφs to home to the walls of the SAs.

At this site, dMφs secrete several cytokines (e.g. IL1β, IL6, IL8, IL10 and IFNγ) that are involved in remodeling of the SAs (84). DMφs cause disruption of the VSMC through the expression of several metalloproteinases (MMP) (85, 86) and induce apoptosis and phagocytosis of VSMCs (87, 88). The apoptotic cell bodies that are released into the maternal circulation during placental development are cleared by dMφs, which is accompanied by the release of anti-inflammatory cytokines (88). Furthermore, dMφs exert angiogenic properties via the production of VEGF and angiogenic growth factor (Ang) 1 and Ang2 (50, 89, 90). Similar to implantation of the embryo, remodeling of SAs seems to require a short inflammatory phase. In the SAs, this is characterized by foamy (lipid filled) macrophages in the intima of the vessel wall that resolve once SA remodeling is complete (90). It is important that this inflammatory response is transient as persistent inflammation can cause acute atherosis, marked by fibrinoid necrosis of the vessel wall, lymphocytic infiltration, and excessive accumulation of foamy macrophages, potentially resulting in MVM (84, 91). Besides alterations in dMφ activation, dysregulation in their numbers can cause defects in placental development. A macrophage surplus can lead to excessive apoptosis of EVT, limiting the invasion of trophoblast in the SAs and causing vessels to be (partly) unremodeled (88, 92). Mouse studies have shown that also reduced macrophage numbers can result in suboptimal

implantation, hypocellularity of the decidua and a thickened VSMC layer in decidual maternal blood vessels, indicating impaired decidualization and incomplete remodeling of the uterine vasculature (93, 94).

Next to their role in placental development, first trimester dMφs are involved in maintaining a tolerogenic intrauterine environment. DMφs are located in close proximity to decidual Treg cells and can induce Treg cell expansion through binding of CD80 and CD86 on dMφs to cytotoxic T-lymphocyte antigen 4 (CTLA4) on Treg cells. At the same time, this interaction increases the activity of indoleamine 2,3-dioxygenase (IDO) in dMφs, an enzyme known to induce immune tolerance in various immune cell subsets. In pregnancy, IDO is involved in preventing a harmful maternal T cell response towards the fetus (95). Another mechanism of preventing a maternal inflammatory response is through interaction of programmed death (PD)1 on T cells with its ligand (PDL1) on dMφs leading to downregulation of the IFNγ production by maternal T cells (96).

From the studies on first trimester dMφs we can conclude that their numbers and phenotypical characteristics must be carefully regulated to promote trophoblast viability and invasiveness, processes that are important for implantation of the embryo and remodeling of the SAs. Both implantation and SA remodeling are facilitated by a short inflammatory dMφ response important for trophoblast invasiveness, followed by dMφs that predominantly express immunomodulatory characteristics to further support placental development and maternal-fetal tolerance.

4.2 Second trimester to term

During the second trimester of pregnancy, numbers of dMφs continue to expand to nearly half of the maternal immune cell population at mid-pregnancy (46). Most of these second trimester dMφs are positive for anti-inflammatory, immunoregulatory cell surface markers like CD163, CD206, and CD209 (46, 97). Interestingly, HLA-DR, CD80 and CD86, co-stimulatory molecules for T cell activation, are expressed on second trimester dMφs. These molecules also have the ability to weaken T cell responses by binding to CTLA-4 expressed on decidual T cells, indicating that the expression of co-stimulatory molecules on dMφs might not cause T cell activation in pregnancy (97). Indeed, it was shown that dMφs co-cultured with allogeneic T cells were unable to induce T cell proliferation or activation, and instead caused upregulation of Treg cells (98). One mechanism for promoting Treg cell activity was found to be through the production of cellular retinal dehydrogenase by dMφs that can synthesize retinoic acid, which regulates Treg cells (99). In addition, dMφs suppress cytotoxic NK and T cell activity through TIM3 and Galectin9 expression (46).
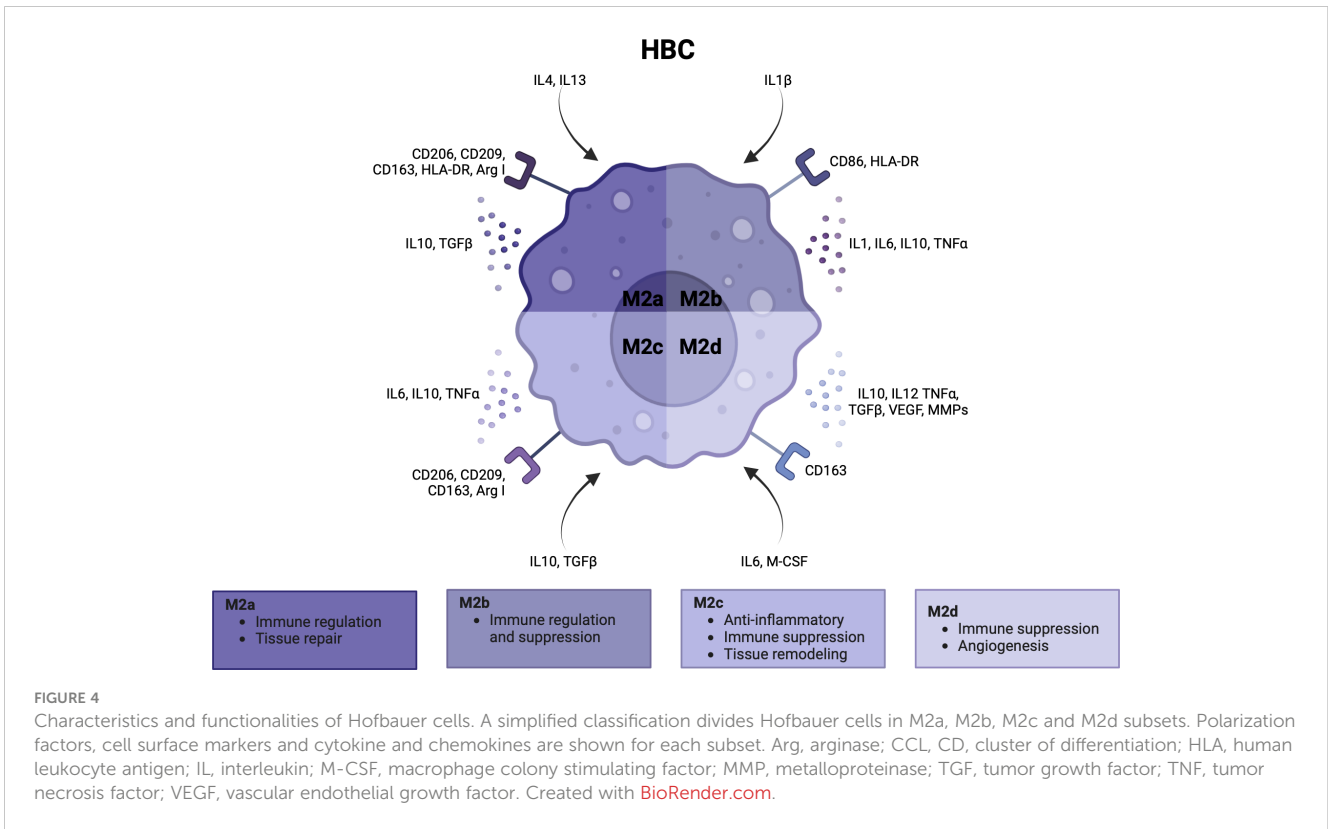
Third trimester dMφs are high in CD163 expression and relatively low in CD80, CD86 and HLA-DR (99, 100). In addition, genes encoding for a pro-inflammatory macrophage phenotype are silenced, while genes for a more regulatory, immunotolerant phenotype are activated (101). Similar to the first trimester, the decidua itself is key in promoting the

immunoregulatory macrophage phenotype. Upregulation of CD163 and CD209 and downregulation of CD86 has been observed after conditioning monocytes with cell medium from term decidual tissue (102). In mice, pro-inflammatory immune responses can be downregulated by decidua derived progesterone through inhibition of toll like receptor (TLR) 4 and TLR9 triggered release of IL6 and nitric oxide (NO) by dMφs (103). Another mechanism proposed to induce maternal-fetal tolerance is through exposure of maternal macrophages to trophoblast debris. Phagocytosis of trophoblast cells from term human placental explants by maternal blood monocytes resulted in reduced expression of CD80, CD86, CD40, MCP1, and B7-H3 (involved in suppressing T cell activation), increased secretion of cytokines like IL1β, IL12p70 and IL8, and upregulation of IDO (104).

Near birth, the immunoregulatory role of dMφs changes as dMφs participate in the inflammatory response that triggers the spontaneous onset of labor. This is marked by an increased level of inflammatory cytokines like IL1ß, IL8 and IL6 during labor, as well as elevated numbers of dMφs with pro-inflammatory character (105, 106). One study even reported macrophages to increase up to four times in laboring women (107). In rats, it could be demonstrated that macrophages infiltrate the decidua prior to the onset of labor, further suggesting that dMφs directly initiate parturition (107). To summarize, the second and third trimester of pregnancy, before the spontaneous onset of labor, are focused at immunotolerance and the downregulation of potential pro-inflammatory responses, which safeguards maternal-fetal tolerance and improves placental functioning, allowing for optimal fetal growth. At term, dMφs start to display pro-inflammatory characteristics that promote the onset of labor.

5 Hofbauer cells in healthy pregnancy

HBCs form over 90% of the fetal immune cell population in the placental villi. They appear at day 18 of pregnancy, increase up until 18 weeks and decrease as pregnancy progresses (1, 3, 108–110). Early in pregnancy, HBCs stem from primitive yolk sac macrophages that proliferate in the villi or from villous mesenchymal stem cells (1, 111, 112). Once the fetal circulation has been established, HBCs differentiate from fetal monocytes in the bone marrow that are recruited to the placenta (3) (113, 114). Similar to dMφs, the polarity of HBCs can be simplified as M1 and M2, although HBCs generally lack the M1 phenotype. Some studies subdivide M2 HBCs into M2a, M2b, M2c and M2d (8, 115). M2a macrophages are seen as 'alternatively activated' macrophages that are involved in immune regulation and tissue repair. M2b macrophages suppress and regulate inflammation as they can exert Th2 responses and produce a range of pro- and anti-inflammatory cytokines. M2c have more anti-inflammatory and tissue remodeling capacities, and M2d macrophages are involved in immune suppression and angiogenesis (49, 112, 115, 116). In Figure 4, the activation pathway, cell surface markers, cytokine and chemokine profiles, and main functionalities of HBCs in pregnancy are summarized (3, 5, 112, 117).

FIGURE 4
Characteristics and functionalities of Hofbauer cells. A simplified classification divides Hofbauer cells in M2a, M2b, M2c and M2d subsets. Polarization factors, cell surface markers and cytokine and chemokines are shown for each subset. Arg, arginase; CCL, CD, cluster of differentiation; HLA, human leukocyte antigen; IL, interleukin; M-CSF, macrophage colony stimulating factor; MMP, metalloproteinase; TGF, tumor growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor. Created with BioRender.com.

5.1 First trimester

In the first trimester, the large majority of HBCs are of the M2a (CD206$^+$CD209$^+$HLA-DR$^+$CD163$^+$) and M2c (CD206$^+$CD209$^+$HLA-DR$^-$CD163$^+$) subtype which are involved in tissue repair and immunoregulation. These cells express arginase (Arg) I and Arg II, enzymes involved in M2 polarization (110, 111). M1 markers like CD80, CD86 or iNOS, an enzyme involved in the M1 polarization pathway, are not expressed (110). Findings on the expression of HLA on first trimester HBCs are conflicting. While the majority of studies report HBCs to be typically low in HLA in the first trimester (111, 115, 118–120), some have found clusters of M1-like or M2a/M2b HBCs that do express the classical HLA-DR (110, 115).

Several alternative subdivisions of HBC types have been suggested. For example, classification into a group with an anti-inflammatory profile (IL11, IL17, TGFβ and VEGF) that formed the vast majority of HBCs, and a pro-inflammatory group that constituted only 5% of the population (IL1β, IL6 and TNFα) (121). Another division based on the expression of CD74 (gamma chain of HLA class II) revealed a CD74$^+$ HBC subset that expressed genes encoding for cytokine stimuli, inflammatory responses, antigen presentation and myeloid leukocyte activation. This subset was suggested to be involved in removal of cellular debris during early development of the placenta (122). Another mechanism for the removal of anti-fetal antibodies from the placenta is through the expression of three subtypes of the Fc receptors for IgG (FcγR), FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) on HBCs that can bind antigen-antibody complexes (123). These receptors are furthermore implicated in the transplacental transmission of maternal IgG antibodies into the fetal circulation,

providing HBCs with a crucial role in immunological protection of the fetus (8, 123–125). HBCs are furthermore involved in the development of the villi and its vasculature. They are found in abundance within the mesenchymal villous stroma that later develops into immature intermediate villi and are found in close association with fetal capillaries (108, 126, 127). Culturing trophoblast cells with HBC supernatant lead to enhanced trophoblast growth and differentiation (128). HBCs are the primary cells expressing sprouty proteins, important for branching villous and vascular morphogenesis and growth factor signaling (129). Moreover, HBCs produce VEGF and its receptors, and several other factors like fibroblast growth factor 2 (FGF-2) and osteopontin (OPN) that promote placental growth and angiogenesis, and tissue inhibitor metalloproteinase 9 (TIMP-9) and MMP-9 that facilitate trophoblast invasion (111, 130–135). Moreover, HBCs exhibit phagocytic capacities and bactericidal functions by producing reactive oxygen species (ROS) and glucose-6-phosphate dehydrogenase (G6PD) (111, 136).

To conclude, the largest share of the first trimester HBC population is involved in immune regulation and supporting a tolerogenic environment by removing tissue debris and fetal antibodies. In addition, HBCs have an important role in the development of the villous architecture and the fetal vasculature.

5.2 Second trimester to term

During the second and third trimester, HBCs are found in all types of villi where they are located close to the fetal capillaries, although they are rarely seen in terminal villi (109, 115, 137–139).

Almost no expression of M1 markers like CD40, CD11b and CD80 has been observed at term, while M2 markers like CD163, CD209, CD206, folate receptor (FR)-β are abundantly expressed (110, 115, 140). Genetic profiling of HBCs confirms this as M1 associated genes are silenced, and M2 related genes are activated (101).

In the third trimester, a notable shift in HBC phenotype is observed, characterized by a decrease in M2a and increase in M2c HBCs, which ultimately form the predominant subset (110, 115). M2c HBCs are engaged in anti-inflammatory reactions, tissue remodeling and immune suppression. M2b cells, mostly absent in the first and second trimester, emerge near term and constitute approximately one third of the HBC population (110, 115). They can be identified by CD86 expression, alongside the secretion of IL6 and TNFα. The M2b subset is stimulated by immune complexes and participates in antibody-mediated immunity (110, 115). Consistent with findings in the first trimester, studies report different results regarding HLA expression on HBCs. Either varying expression of HLA-DR between placentas, lack of HLA-DR expression or presence of HLA on the majority of HBCs have been reported (46, 110, 134, 140–143). One hypothesis proposed to explain these discrepancies is the mode of delivery, wherein placentas obtained after primary cesarean section exhibit lower HLA expression due to a lack of inflammatory responses that mark the onset of parturition (138). Alternatively, the presence of HLA on HBCs could indicate a matured third trimester subset that is capable of antigen presentation to T cells (142). This would be in line with the increase of M2b HBCs in the third trimester (110, 115).

Third trimester HBCs are involved in maintaining a state of immune tolerance. HBCs secrete IDO and IL10, which induce an anti-inflammatory phenotype in maternal immune cell subsets like macrophages and Tregs (73, 144). Mixed lymphocyte reactions show that third trimester HBCs inhibit the activation of lymphocytes, and the proliferation and production of cytotoxic cells (145). Limiting potentially harmful immune responses is also established through the expression of death-inducing TNF superfamily ligands and receptors that are implicated in autocrine programmed cell death, and therefore also involved in placental tissue remodeling processes (146). One study showed that third trimester HBCs might be more biased towards immune tolerance compared to HBCs earlier in pregnancy. It was demonstrated that first and second trimester HBCs could be polarized into M1-like cells with higher levels of inflammatory cytokine secretion (e.g. IL1β, IL12, IL6 and TNFα) using LPS, while HBCs at term kept an M1/M2 balance and did not upregulate M1 markers like CD80, CD86, TLR1 and TLR4 in response to LPS (110, 120). Although this response might be lower in the third trimester, studies consistently demonstrate that LPS stimulation can increase the number of HBCs and induce the secretion of inflammatory cytokines like TNFα, IL10, IL1α, IL1β, IL6 and IL12 (121, 139, 141). Term HBCs express low levels of NOS type II, which can implicate a surveilling function to detect pro-inflammatory events or maternal pathogens by being able to respond with a cytotoxic effect through NO secretion (147). DNA methylation studies of HBCs show enrichment of genes involved in immunological defense, inflammation, acute inflammatory response to stress, regulation of immune cell activation, complement activation, cell adhesion and angiogenesis (101).

Third trimester angiogenetic capacities of HBCs are mainly due to VEGF, which is produced at even higher levels in the third trimester compared to the first. VEGF producing HBCs are located near regions where Ang 1 and 2 is expressed within the mature intermediate and terminal villi (133, 134). Together with VEGF, HBCs secrete FGF2, which enhances endothelial branching and chemoattraction of fetal endothelial cells (115). HBCs furthermore contribute to the regulation of placental blood flow through thromboxane and prostaglandin E2, which are involved in vasoconstriction and vasodilatation respectively (148). HBC derived IL17 is likely involved in the upregulation of these angiogenic factors (149).

Taken together, second and third trimester HBCs have similar functionalities compared to the first trimester. From the aforementioned studies we can conclude that a more mature subset of HBCs emerges as pregnancy progresses, which is involved in immunosurveillance and downregulation of potential pro-inflammatory responses. HBCs furthermore continue to participate in the development of the placental vascularity throughout the second and third trimester.

6 Macrophages in fetal growth restriction

Numerous studies have emphasized the significance of dMφs and HBCs in placental functioning and the maintenance of an intrauterine environment that is tolerant to the conceptus. Given the diverse array of functions, it comes to no surprise that disturbances in numbers, phenotypes or functionalities of placental macrophages have been observed in various pregnancy complications that are characterized by defects in placental development. These include preeclampsia, spontaneous preterm birth, preterm premature rupture of membranes and (recurrent) pregnancy loss, among others (106, 150–152). For FGR specifically, the mechanisms through which imbalances in placental macrophages contribute to its pathogenesis remain largely unclear due to the limited number of studies on this topic.

We hypothesize the contribution of dMφs and HBCs to the pathophysiology of FGR to be multifaceted. Generally stated, dMφs work on the maternal side of the placenta where they are crucial for embryo implantation and SA remodeling, whereas HBCs are active in the villi, contributing to villous maturation and the formation and growth of the fetal vasculature. In addition, both dMφs and HBCs employ various mechanisms to maintain a tolerogenic intrauterine environment, such as promoting the differentiation of maternal T cells into Treg cells, inhibiting maternal T cell activation and clearing antibody-antigen complexes that could potentially trigger a maternal immune response against the fetus. Close regulation of the number and functionalities of placental macrophages is essential for adequate placentation and the maintenance of maternal-fetal tolerance.

Most likely, disturbances in dMφ and HBC activation could both be a cause and a consequence of placental insufficiency in FGR pregnancies. On one hand, it is possible that initial disruption of maternal-fetal tolerance skews dMφs and HBCs towards a more

pro-inflammatory subset, resulting in defects in placental development and disturbed functioning of the uteroplacental circulation throughout pregnancy. On the other hand, placental insufficiency can be caused by a variety of mechanisms. For example, maternal hypertensive disorders, diabetes mellitus, auto-immune diseases, obesity and smoking are all associated with maternal vasculopathy and an activated maternal immune system, which can cause placentation defects (16, 153). In these cases, it could be that the oxidative stress arising from impaired utero-placental perfusion and ischemia-reperfusion injury triggers an inflammatory response that results in pro-inflammatory macrophage polarization. Interestingly, a large number of pregnancies are complicated by placental insufficiency in the absence of factors that predispose to placental maldevelopment. It could be these pregnancies in which impaired maternal-fetal tolerance causes placentation defects in otherwise healthy women. The different etiologies of FGR makes that the interaction between placental functioning and placental macrophage populations in the context of FGR multifaceted and complex. Our overall theory is that placental insufficiency, oxidative stress and inflammation form a vicious circle that contributes to the pathogenesis of FGR, as depicted in Figure 2. Understanding this interaction in the pathophysiology of FGR is crucial for unraveling the immunological mechanisms at play. In the next section, the current knowledge on the role of dMφs and HBCs in human and animal pregnancies complicated by FGR will be discussed. Figure 5

summarizes the functionalities of dMφs and HBCs in healthy pregnancies and FGR.

6.1 Decidual macrophages in fetal growth restriction

Previously, we investigated decidual immune cell subsets in term pregnancies affected by FGR and showed a 50% increase in $CD68^+$ dMφ numbers in FGR compared to healthy pregnancies, accompanied by decrease of the $CD206^+/CD68^+$ dMφ ratio, suggesting a reduction of anti-inflammatory dMφs in FGR (24). The majority of FGR placentas in our cohort showed signs of VUE and a combination of multiple placental lesions (MVM, FMV and VUE) (24). Contrary to our results, another study showed no differences for dMφ numbers, defined as $CD14^+$, between FGR and healthy pregnancies at term. There was no assessment of placental pathology (154). Other studies do confirm our observations. Higher numbers of $CD68^+$ dMφ with increased HLA-DR staining were detected in the extravillous areas of term placentas. FGR cases with chronic villitis and VUE were excluded in this cohort (155). Another study showed elevated numbers of $CD68^+$ and $CD163^+$ macrophages in the intervillous space of FGR pregnancies, likely to be maternal macrophages that migrated there. Placentas showed signs of immaturity of the chorionic villi with sclerosis, intervillous fibrinoid deposition and
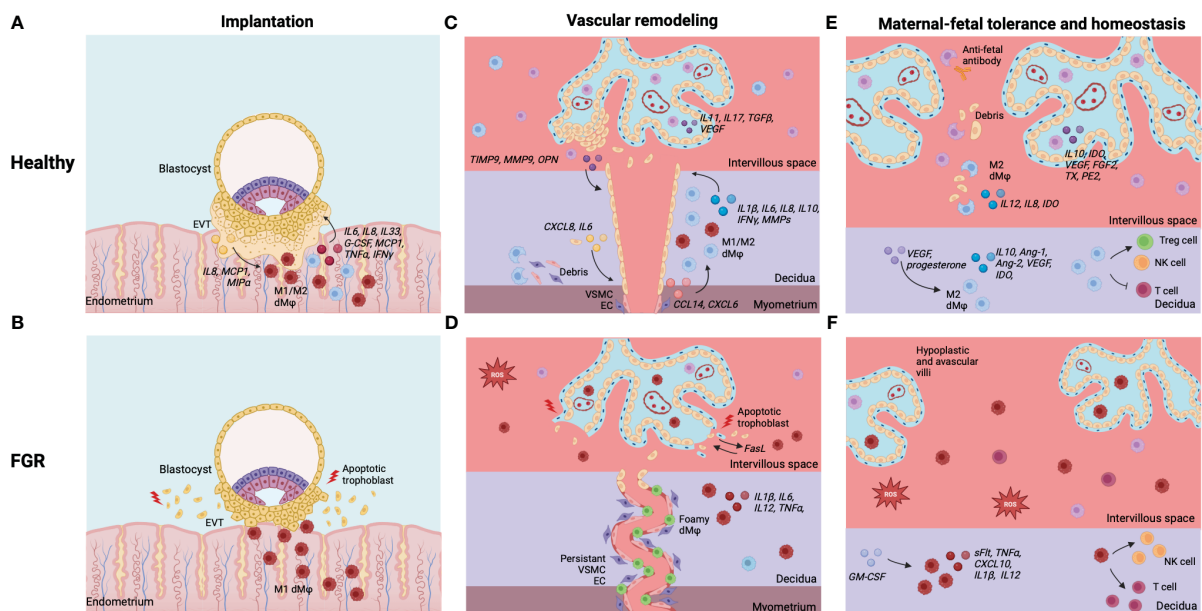

FIGURE 5
Summary of dMφs and HBCs in healthy pregnancies and FGR. **(A)** Implantation in healthy pregnancies: EVT and dMφs crosstalk facilitates deep invasion of EVT in endometrial stroma for implantation of the embryo. **(B)** Implantation in FGR: excess of inflammatory dMφs and EVT apoptosis, leading to shallow implantation of the embryo. **(C)** Vascular remodeling in healthy pregnancies: dMφs and HBCs cause disruption of VSCM and EC within maternal SA and stimulate EVT invasion, which causes remodeling of the SA into dilated, low-resistance vessels. **(D)** Vascular remodeling in FGR: excess of inflammatory (foamy) dMφs causes apoptosis of EVTs, leading to restricted EVT invasion. Disruption of the VSCM and EC layers is incomplete and the lumen of the SA remains narrow. **(E)** Tolerance and homeostasis in healthy pregnancies: dMφs and HBCs secrete anti-inflammatory cytokines and vascular growth factors, phagocytose placental debris and anti-fetal antibodies, and induce Treg cells while inhibiting potential inflammatory T cell and NK cell responses. **(F)** Tolerance and homeostasis in FGR: balance shifted towards dMφs and HBCs that secrete pro-inflammatory cytokines, lack vascular growth factors, and stimulate T cells and NK cells, leading to an inflammatory and hypoxic environment with impaired placental vascularization and damage to the villi. Created with BioRender.com.

calcification, resulting in afunctional villous areas. In addition, preterm FGR cases (born before 37 weeks) showed impaired vascularization and villous hypoplasia (156). Another study comparing gene expression between term FGR and control placentas showed more expression of genes for total macrophages and M1-like macrophages in FGR cases, which correlated with NEURL1 and ODF3B gene expression, both involved in immune cell proliferation, activation and inflammation (157).

One study investigated both second and third trimester dMφs in FGR pregnancies. In the second trimester, an increased number of CD68^{+} macrophages was detected in the microlymphatic vessels between decidual cells, in contrast to healthy pregnancies where macrophages are found in the decidual stroma or transforming vessels. This finding was associated with impaired SA remodeling. In third trimester FGR placentas, CD68^{+} macrophages were identified in leukocyte clusters close to apoptotic EVTs. There was a lack of EVT invasion in the SA, along with thickened VSMC layer and intact endothelium, suggesting that a macrophage excess was involved in trophoblast cell death and subsequent impaired SA remodeling (28).

Furthermore, it appears that macrophages from FGR pregnancies have impaired angiogenic capacities. Maternal monocytes differentiated into M2-like macrophages that were cultured with placental mesenchymal stromal cells from FGR pregnancies showed inhibited tube formation in *in vitro* assays, suggesting that macrophages from FGR pregnancies can cause impaired remodeling of the uterine vasculature (158). Although limited, the existing literature points towards an increase in macrophages of a more inflammatory character in FGR, which may have detrimental effects on implantation and the development of the placental vasculature.

6.2 Hofbauer cells in fetal growth restriction

One study found CD68^{+} HBCs to be increased in FGR cases compared to healthy pregnancies, while HBCs positive for CD206 were reduced. Placentas with VUE were excluded and no other placental pathology data was available (155). On the contrary, also lower numbers of CD68^{+} and CD163^{+} HBCs have been found in the terminal villi of term and preterm FGR compared to healthy controls. The FGR placentas showed signs of villous immaturity and afunctional villous areas (159). This decrease in CD68^{+} HBCs has also been reported by another study. However, when A1 antichymotrypsine was used as a marker for HBCs, more HBCs were found in FGR (160). The authors suggest that A1 antichymotrypsine, a protein involved in the inhibition of natural killing and antibody-dependent cell-mediated cytotoxicity, is more specific for HBCs than CD68, which could explain the different results. They proposed that the increase in HBCs could be a compensatory mechanism to prevent a maternal immune response towards fetal alloantigens. The placental morphology and histopathology was not assessed in this study (160). The limited availability of studies and the use of different markers makes it difficult to draw conclusions on the role of HBCs in FGR.

6.3 Animal studies on macrophages in fetal growth restriction

Different mouse models have been used to mimic FGR and investigate placental immune cell subsets. However, it must be considered that placentation in mice varies from placentation in humans. Mice placentas have a distinct trophoblast cell type that is less invasive compared to humans, and the placental labyrinth structure differs from the more open intervillous space in human placentas (161). Two mouse models, one based on the deficiency of FcR-γ and another one on complement factor C5a receptor deficiency, exhibit high rates of spontaneous pregnancy loss and severe FGR in the surviving fetuses, alongside morphological signs of impaired placental vascularization. There was an increased infiltration of monocytes and neutrophils, along with a functional deficiency of VEGF (162). Another mouse model based on PBX1-deficient decidual NK cells resulted in unexplained recurrent pregnancy loss and FGR. The placentas of these mice showed higher infiltration of dMφs and neutrophils compared to the wild type at mid gestation. No differences in placental morphology was found between FGR and control pregnancies (163). Also mice undergoing BCG-trained immunity show a higher incidence of FGR, but contrary to other studies, show decreased placental macrophages in the second week of pregnancy (similar to second trimester in humans) compared to controls. However, these macrophages did show a tendency towards a more inflammatory profile. The authors hypothesize that a lack of macrophages causes placental developmental defects, such as impaired EVT infiltration and reduced SA remodeling, causing FGR. Unfortunately, this hypothesis was not substantiated with placental morphology assessment at mid pregnancy. At term, no differences in placental morphology were found for FGR pregnancies and numbers of placental macrophages did not differ from control mice (159). Another way to induce FGR in mice is the administration of uric acid, which was found to induce placental inflammation and an increase of CD68^{+} macrophages in the junctional and labyrinth zone. No changes in placental gross histopathology were seen (164). Imbred mice mating models also demonstrate FGR, with a 1.8-fold increase in F4/80[+] macrophages, localized in the labyrinth and chorionic plate. At term, the macrophage number increased up to 11-fold. The placentas from FGR pregnancies were smaller, displayed signs of thrombosis and fibrosis and a reduced size of the placental labyrinth (165). Inflammation in mice has furthermore shown to impact fetal growth and placental macrophage levels in subsequent generations. First degree offspring of LPS treated female mice showed higher FGR rates compared to controls. Interestingly, their offspring (second generation) showed impaired fetal growth as well, together with higher levels of CD68^{+} macrophages and lower levels of CD163^{+} macrophages in the mesometrial triangle, junctional zone and labyrinth. Deficient trophoblast invasion and narrower SAs were observed and Doppler measurements confirmed inadequate SA perfusion (166). Another study used a mouse model based on maternal KIR2DL1 expression on NK cells and paternal HLA-C*050 expression on fetal trophoblast cells that lead to FGR with impaired remodeling of the SA and high resistance placental blood flow. FGR placentas were enriched in genes for macrophage recruitment and macrophages showed higher expression of genes

involved in macrophage invasion under hypoxic conditions, dendritic cell maturation, antigen presentation and inflammation (167). To summarize, these mouse models reveal that FGR placentas can be characterized by elevated numbers of placental macrophages skewed towards a pro-inflammatory phenotype. These immunological alterations likely contribute to the observed disturbances in placental development and functioning.

7 Current therapeutic approach for fetal growth restriction

At this point, there is no established and effective therapy to prevent or treat FGR. The current approach involves close monitoring with Doppler ultrasound and cardiotocography (CTG) and timely delivery of the baby (168). Although there are certain maternal serum biomarkers indicative for combined FGR and PE, no diagnostic biomarkers for FGR solely can be found that have additive value to biometry in predicting FGR (169–171).

Women with significant risk for developing FGR are often counseled before or early in pregnancy and monitored throughout (16). For pregnant women with previous pregnancies complicated by FGR and/or PE, or other specific risk factors, acetylsalicylic acid is often prescribed. Some studies have shown that starting acetylsalicylic acid before 16 weeks of pregnancy can significantly reduce the risk of FGR (172, 173). Acetylsalicylic acid has progangiogenic, vasodilatory and antithrombotic effects through upregulation of pro-angiogeneic factors like PIGF, prostacyclin and NO, and improves trophoblast invasion and SA remodeling (174). Moreover, acetylsalicylic acid-triggered lipoxins have anti-inflammatory, antioxidant and immune-modulatory functionalities by inhibiting NF-kB activation and TNF secretion by T cells, and upregulation of IL10 and NO (174, 175).

The combination of acetylsalicylic acid with low molecular weight heparin (LMWH) has also been suggested as a therapeutic option for FGR. LMWH has an anticoagulant effect on the placental vasculature and could furthermore improve endothelial function, increase NO and PlGF, and reduce pro-inflammatory cytokines like IL8, IL6 and TNFα (174–176). Statins too have been explored as an approach to improve placental functioning in FGR and PE. It has potential pro-angiogenic and antioxidant effects, and could aid in endothelial protection and reduction of placental inflammation. However, studies on the use of LMWH or statins are scarce and conflicting as to whether this has beneficial clinical effects (177, 178).

A drug that has been in trial for early-onset FGR is sildenafil, a phosphodiesterase-5 inhibitor suggested to enhance the function of NO and thereby promote vasodilatation of the maternal and fetal vasculature. Yet, no positive effects on placental functioning and the improvement of fetal growth have been observed (179).

7.1 Immune targeted therapies

Direct modulation of macrophage phenotype and functionality has not been investigated in the context of pregnancy complications. Potential strategies could be targeted at macrophage polarization, cytokine and chemokine secretion, or signaling pathways by which

macrophages interact with other immune cells. Naturally, these macrophage modulators must be safe to use in pregnancy. Several studies have demonstrated that culturing macrophages with medium derived from placental tissue can induce favorable M2-like characteristics in placental macrophages (58, 73, 102). Therefore, investigating the specific placental mediators at play could be promising in finding potential macrophage regulatory therapies. For example, vitamin D is produced by the placenta and can affect macrophage polarization by binding to intracellular vitamin D receptors (VDR) present in macrophages (180). Within the placenta, it stimulates the interaction between placental macrophages and Treg cells and skews the balance towards a Th2 milieu (181). Vitamin D supplementation reduces IL1, IL6 and IL12 secretion by macrophages and downregulates TLR2 and TLR4 expression, while it enhances phagocytotic capacities (180). Similar results have been found for medroxyprogesterone, which could polarize monocytes into an M2 phenotype with downregulation of CD11c, IL1β and TNFα and upregulation of CD163 and IL10. Moreover, medroxyprogesterone could reverse M1 polarization and promoted decidualization and trophoblast invasion (182). For VEGF derived from decidual stromal cells, it has been established that it can upregulate M2 markers like CD206, CD163 and CCL17, and can reverse M1 polarization. It furthermore enhances macrophage migration (74). Another example is IL6 secreted by trophoblast, which activates the STAT-3 pathway and increases CD206, CCL18, IL10 and TGFβ in macrophages. Furthermore, these IL6 activated macrophages promoted trophoblast invasion and migration (77). Also hyaluronan can activate STAT-3 and -6 by binding to CD44 on dMφs. This downregulates M1-like receptors like CD80 and CD86, and upregulates CD163 and CD206 (78). In mice, human chorionic gonadotropin (hCG) administration has been shown to reduce the number of dMφs and induce M2 polarization (183).

A drug that could potentially downregulate the maternal inflammatory response in FGR is hydroxychloroquine, an antimalarial drug by origin. In pregnant women, hydroxychloroquine has been shown to ameliorate autoimmune diseases like systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS), and it has been associated with lower PE and preterm birth rates, and an increased birth weight (184). Its immune modulatory effects include decreasing the production of prostaglandins and pro-inflammatory cytokines like TNFα, IFNγ and IL6, inhibiting MMPs and blocking TRL signaling pathways (185, 186). Furthermore, it impairs antigen processing in antigen presenting cells and reduces T cell activation (177). Hydroxychloroquine can have a direct anti-inflammatory effect on first trimester trophoblast tissue in pregnant women with APS and excessive placental inflammation and insufficiency (187). Another agent to might have favorable effects in FGR is curcumin. It has been established that it can promote trophoblast growth, and reduce oxidative stress and the secretion of pro-inflammatory cytokines (e.g. IL6, IL8) in the placental villi and fetal membranes (188, 189). In rats, it has been shown to improve defects in trophoblast invasion and SA remodeling, along with a decrease in NFkβ, IL6 and MCP1 (190). In a rat PE model, curcumin combined with aspirin resulted in a reduction in placental oxidative stress, with decreased sFlt-1 and pro-inflammatory cytokines, and increased PlGF expression (191). Although not investigated in FGR specifically, its mechanism of action might improve placental function in FGR as well.

Prior to exploring therapies that directly affect macrophages, acquiring a deeper understanding of the pathways through which dMφs and HBCs affect placental functioning in FGR and other immune-mediated pregnancy complications is crucial. Moreover, the use of potential macrophage modulating therapies requires adequate diagnostic tools to determine the pathophysiological mechanism in individual FGR cases and personalize the therapeutic approach accordingly.

8 Conclusion

Our comprehension of the role of maternal and fetal macrophages in ensuring a successful pregnancy has significantly advanced. DMφs and HBCs are important in several processes during gestation, such as implantation of the embryo, remodeling of the uterine arteries, and safeguarding maternal-fetal tolerance. To carry out these functionalities, macrophages exhibit a wide range of phenotypical variation on a spectrum with pro- and anti-inflammatory characteristics at its opposite ends. Notably, this requires temporal regulation and quick adaption when needed. Dysregulation of macrophage subsets has been implicated in impaired placental functioning and compromised maternal-fetal tolerance. Subsequently, this has been linked to the development of various common pregnancy complications. Given the pathophysiological features of FGR, with impaired development of the placental vascularity and villous structure, leading to oxidative stress and inflammation, the involvement of macrophages in the genesis of these placental deficits seems evident. Although studies on macrophages in FGR specifically are scarce, existing literature in humans and mice shows that macrophages of a more pro-inflammatory character predominate over macrophages with immunomodulatory properties, compromising placental functioning and thereby fetal growth. More research dedicated to this topic is essential to obtain a broader understanding of the immunological component of the pathophysiology of FGR and work towards investigating potential therapies targeted at modulating the phenotype and functionality of dMφs and HBCs.

Author contributions

RB: Data curation, Investigation, Writing – original draft. MF: Supervision, Writing – review & editing. HvG: Supervision, Writing – review & editing. SG: Supervision, Writing – review & editing. JP: Supervision, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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