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Placental macrophages: Origin, heterogeneity, function and role in pregnancy-associated infections

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ABSTRACT

Placental macrophages are a heterogenous population of immune cells present throughout pregnancy. They are essential for maintenance of the homeostatic placenta environment and host defense against infections. The characterization of placental macrophages as well as their activation have been limited for a long time by the lack of convenient tools. The emergence of unbiased methods makes it possible to reappraise the study of placental macrophages. In this review, we discuss the diversity and the functions of placental macrophages to better understand their dysfunctions during placental infections.

1. Revisit placental macrophages?

The placenta is a chimeric, rapidly growing organ in which fetal and maternal tissues are in close contact [1]. The maternal part of the placenta is composed of the decidua basalis which is directly related to the uterus; this constitutes an intimate connection between the mother and her developing fetus as fetal membranes that are composed of extravillous trophoblasts. The fetal part is covered by the amnion, which is involved in the secretion of the amniotic fluid. Under the amnion, the chorion, a membrane in continuity with the lining of the uterine wall, is required for supplying nutriments to the fetus and preventing fetus rejection by the maternal immune system [1–3]. The placenta is characterized by the presence of an immune system supporting the immune tolerance toward the fetus and the ability of mother to prevent infections. The placental immune system comprises natural killer (NK) cells, macrophages (~20%), T cells (~10–20%) and rarer cell types, such as dendritic cells, B cells, NKT cells and mast cells [3–5]. Although the NK population is the largest during the first trimester (~70%), its number steadily decreases to reach 20% of total immune cells at the end of the third trimester [6]. The number of macrophages follows same kinetics 50%–~20% before delivery based on immunohistochemical technique [7,8]. In contrast, the use of flow cytometry reveals that macrophage population remains stable during the first trimesters [8]. In contrast, T cell number gradually remains stable thought pregnancy [8].

Placental macrophages are composed of two distinct populations, i.e. decidual macrophages and Hofbauer cells; they are detected as early as day 10 of pregnancy and are present during the three trimesters of pregnancy [9]. The lack of convenient tools has limited the characterization of human placental macrophages as well as their functional studies. The diversity of these macrophages has been greatly underestimated, and most studies have been limited to immunohistochemical characterization and inferences from what has been reported in other resident macrophages. The application of single-cell RNA sequencing (scRNA-Seq) to placenta investigation has paved the way for a novel atlas of placental populations, including macrophages [10]. The analysis of placental macrophages publications revealed that the number of reports focusing on placental macrophages has increased steadily since 2000 but the number of publications on the role of placental macrophages and infection has not significantly increased (PubMed database).

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2. Placental macrophage investigation: breaking through the barriers

The study of tissue macrophages has been revolutionized by the development of unbiased methods such as multiparametric flow cytometry and mass cytometry. Surprisingly, placental macrophages have been overlooked for several reasons. First, placentation is distinct in humans and rodents, and human and mouse placental macrophages are phenotypically different, thus restricting the use of murine models [11]. Second, their location in a complex and rapidly evolving tissue has favored *in situ* investigations, which precluded functional studies. An *ex vivo* placental perfusion assay might be an alternative to study placental macrophages in their natural microenvironment [12,13]. However, despite its initial description some 50 years ago, placental perfusion assay has been mainly limited to pharmacological investigations [14]. In contrast, the combination of laser microdissection and scRNA-Seq has permitted the characterization of the signature of macrophage populations in their microenvironment [10].

The isolation of human macrophages from placenta has appeared as the most convenient approach for macrophage characterization and functional studies. Different methods have been used to isolate placental macrophages [15–18]. The location of macrophages in a complex tissue such as placenta and the lack of pertinent animal models make their study particularly challenging. The investigation of human placental macrophages has mostly been performed on immunohistochemical sections, which excludes functional studies. An *ex vivo* placental perfusion assay might be useful to study placental macrophages in their natural microenvironment in murine models and humans [12,13], but is to-day limited to pharmacological investigations [14]. Isolating macrophages from human placentas is the most convenient approach for functional studies. Different methods have been used: they vary in the use of enzymes (collagenase, DNase and/or trypsin), density gradient type (Ficoll or Percoll), positive or negative selection using anti-CD68, -CD10 or -CD14 antibodies or adhesive properties [15–18]. We have developed a method using Ficoll procedure and anti-CD14 antibodies to isolate human placental macrophages with high purity and yield [18], but this does not discriminate their fetal/maternal origin.

We recommend here a method to isolate human placental macrophages using CD14 antibodies with high purity and yield [17]. Placental cells obtained by CD14 positive selection exhibit typical macrophage features; despite the fact that they share CD14, the canonical marker of monocytes, they are morphologically, phenotypically and functionally distinct from circulating monocytes. They are of both maternal (30%) and fetal origin (70%) as determined by sex chromosome staining [19]. The introduction of scRNA-Seq on one hand and multicolor flow cytometry or mass cytometry on another hand would have a direct impact on the characterization of placental macrophages. Given the reported differences between Hofbauer cells and decidual macrophages, we will use the term “placental macrophages” for both Hofbauer cells and decidual macrophages, except when their origin is specified.

3. Ontogeny of placental macrophages: an emerging field

It is largely established that resident macrophages in most tissues appear during the pre-natal period, and self-renewal rather than replenishment with monocytes supports their maintain throughout life. In response to aggression, monocytes become the major source of tissue macrophages [20]. The ontogeny of placental macrophages must be analyzed according to the heterogeneity of their origin. Initially, Hofbauer cells were thought to be in the chorionic villi, suggesting a fetal origin, while deciduous macrophages were found in the decidua basalis in contact with the maternal myometrium, suggesting a maternal origin (**Graphical abstract**). The sex chromatin staining in the placenta of a newborn boy has shown that X and Y chromosomes are found in macrophages from the fetal part, but only X chromosomes in decidual macrophages [21]. We recently found that $CD14^{+}$ macrophages isolated from at term human placentas are of both maternal (30%) and fetal origin (70%) [19].

The lack of convenient animal models for genetic fate mapping methods does not permit to update the knowledge regarding placental macrophage ontogeny contrary to other tissue macrophages. Only fragmentary information concerning the ontogeny of human placental macrophages is available. Some authors proposed that Hofbauer cells originate from mesenchymal cells within stroma of developing chorionic villi at the early stage of gestation [22,23]. For others, Hofbauer cells were originate from monocyte progenitors of yolk sac and migrate to the chorionic villi, whereas decidual macrophages derive from hematopoietic pluripotent stem cells that differentiate into monocyte progenitors; these latter migrate from bone marrow to the bloodstream of maternal side of the placenta where they maturate [24]. It is noteworthy that transitional forms between monocytes and macrophages exist during the second and third trimesters, suggesting a differentiation of macrophages from fetal circulating monocytes [25]. The question of self-renewal of placental macrophages is warranted by placenta microenvironment. First, type 2 cytokines such as interleukin (IL)-4 and macrophage-colony-stimulating factor (M-CSF)-1 that are known to stimulate macrophage proliferation are over-represented in placenta [26]. Second, placental macrophages - and also extravascular trophoblasts - likely communicate with placental NK cells through interaction of M-CSF-1 produced by NK cells and M-CSF-1 receptor present on placental macrophages and extravascular trophoblasts [27]. Recently, the study of single cell transcriptomic atlas of maternal-fetal interface has shown that macrophages are able to self-renew [10]. In addition, placental macrophages proliferate in pathological conditions including Zika virus infection [28]. In contrast, Hofbauer cells did not exhibit mitotic activity or expression of Ki-67 marker [29]. In summary, placental macrophages represent a heterogeneous population of embryonic and hematopoietic origins but their ability to renew macrophage placental compartment is debated.

4. Placental macrophage heterogeneity: an increase in complexity

Besides ontogenic heterogeneity, placental macrophages change their phenotype with gestational age (Table 1). The phenotypic analysis of $CD14^{+}$ macrophages reveal that seventy percent of them express CD209 (dendritic cell-specific intercellular molecule adhesion (ICAM)-3-grabbing non-integrin or DC-SIGN) and CD206 (mannose receptor), considered as M2 markers (see below). The study of CD209 expression during first-trimester gestation has shown the existence of two subsets of decidual macrophages. The major subset expresses CD209 following CSF-1 stimulation [30] or combined action of CSF-1 and IL-10 [31]. These $CD209^{+}$ cells also express high levels of CD163, CD206, CD304 (neuropilin-1) and CD50 (ICAM-3), but low levels of CD11c, suggesting that they rather are M2 macrophages. The minor subset of decidual macrophages that does not express CD209, highly expresses CD11c and class II major histocompatibility complex (MHC) proteins, but not CD163, CD206, CD304 [32–34], suggesting that they are M1 macrophages. Although phenotypically distinct from blood monocytes, the transcriptional profile of these $CD209^{-}$ macrophages is close to that of circulating monocytes [35].

The recent use of scRNA-Seq has added an alternative degree in the heterogeneity of placental macrophage populations. Tsang et al. identified two clusters of macrophage-like cells that express activation markers of monocytes and Hofbauer cells. The monocyte signature varies during the pregnancy [36]. Vento-Torno et al. identified three new macrophage subsets. Two of them are discriminated by the level of expression of integrin subunit alpha X (ITGAX) [10]. Pique-Regi et al. identified different myeloid clusters, some of them matching with Hofbauer-type cells [37]. It is likely that the single cell transcriptome approach will allow to identify non-previously identified populations of placental macrophages beyond the classical dichotomy between

Table 1
Phenotype of placental macrophages during gestation.

Markers	Functions	1st trimester	2nd trimester	3rd trimester	Refs
CD1	•Antigen presentation to T lymphocytes	Yes	Yes	Nr	[105,106]
CD4	•Interacts with antigen-presenting cells	Yes	Yes	Yes	[71,105,107]
CD11c	•Antigen uptake and presentation	Yes	Yes	Nr	[105,106]
CD14	•Co-receptor for bacterial LPS detection •Cooperates with TLR-4 •Microbicidal functions	Yes	Nr	Yes	[86,105,108]
CD16	•Involved in phagocytosis •Degranulation •Oxidative burst: ROI production •Protective function against fetal antibodies	Yes	Yes	No	[105,109]
CD68	•Binds lectins and/or selectins •Crawling	Yes > than 3rd trimester	Yes > than 3rd trimester	Yes	[57,82,110,111]
CD80	•Co-stimulatory molecule •T cell priming	Yes > than 3rd trimester	Nr	Yes	[112–114]
CD86	•Co-stimulatory molecule •T cell priming	Yes > than 3rd trimester	Nr	Yes	[112–114]
CD163	•Recognizes and binds bacteria •Host defense •Immunosuppressive barrier between mother and fetus •Innate immune sensor for bacteria	Nr	Nr	Yes	[108,110,115,116]
CD206	•Pattern recognition receptor •Antigen processing •Endocytosis •Phagocytosis •Innate immune response	Yes	Yes	Yes	[112–114]
CD209	•Pattern recognition receptor •Binds CD50 (ICAM-3) •Bind microorganisms through envelope mannose •Viral receptor •Innate immune response through TLR modulation •Immune tolerance	Yes	Yes	Yes > than 1st trimester	[117]
CCR5	•Co-receptor for CD4 receptor •Used by macrophage-tropic (R5) HIV-1 for virus entry	Yes	Nr	Yes	[96,117–120]
CR3	•Pattern recognition receptor •Binds microorganisms •Phagocytosis •Destruction of cells/microorganisms	Yes	Yes	Nr	[105]
CXCR4	•Binds SDF-1 •Chemoattractant for T-lymphocytes •Receptor for HIV	Yes	Nr	Yes	[117]
HLA-DQ	•Binds and presents antigens to T cells •Immune tolerance •Its absence during the 1st trimester leads to the generation of cytotoxic cells •Cooperates with HLA-DR	No	Nr	Yes	[105,121–123]
HLA-DP	•Receptor for self-antigens	Yes (low expression)	Nr	Yes > than 1st trimester	[105,121,123]
HLA-DR	•Binds microorganism peptides •Antigen uptake and presentation to T cells •Cooperates with HLA-DQ	Yes (low expression)	Nr	Yes > than 1st trimester	[105,121,123]
TLR-2	•Microorganism recognition •Activation of innate immunity •Regulation of innate immune function at the maternal-fetal interface	Nr	Nr	Yes	[124]
TLR-4	•Microorganism recognition •Activation of innate immunity •Cooperates with CD14	Nr	Nr	Yes	[108,124,125]

CC and CXC: chemokines; CD: cluster of differentiation; CR3: complement receptor 32; HIV: human immunodeficiency virus; HLA: human leukocyte antigen; ICAM: intercellular adhesion molecule; LPS: lipopolysaccharide; Nr: not reported; ROI: reactive oxygen intermediates; SDF-1: stromal-derived-factor-1; TLR: toll-like receptor.

Hofbauer cells and decidual cells.

5. Placental macrophage polarization: the limits of such classification

Besides the different subsets of placental macrophages described above, the functional properties of placental macrophages also change during pregnancy. The M1/M2 dichotomy has been largely used to characterize activation changes of macrophages including placental macrophages. Macrophage polarization is crucial for maintaining tissue homeostasis, even in pathological conditions. These two polarization categories lead to the expression of specific surface markers and to the secretion of several key cytokines to respond to microenvironmental stimuli such as placenta tissue modulation throughout pregnancy. When macrophages are stimulated with inflammatory cytokines or bacterial ligands such as lipopolysaccharide, they acquire inflammatory and microbicidal properties. Immunoregulatory cytokines, such as IL-4, IL-10 or IL-13 render macrophages poorly inflammatory and microbicidal, but competent for healing [38]. These polarization profiles, called M1 and M2, respectively, correspond to specific transcriptional, epigenetic and proteomic signatures [38,39]. Throughout pregnancy, a balance of polarization between M1 and M2 placental macrophages is necessary for

the placenta plasticity and adaptation to the progression of gestation [40].

During the first and early second trimesters, placental macrophages exhibit an M1 profile characterized by the expression of pro-inflammatory cytokines including tumor necrosis factor (TNF), IL-12, IL-23, interferon (IFN)-γ and IL-18 [41]. However, Houser et al. showed that two subsets of placental macrophages, $CD11c^{low}$ and $CD11c^{high}$, do not fit a conventional M1/M2 categorization based on the secretion of both pro- and anti-inflammatory cytokines duting the first trimester [35]. At the end of the second trimester and during the early third trimester, placental macrophages exhibit an M2 profile characterized by the production of vascular endothelial growth factor (VEGF), IL-6 and IL-10 [42]. At the end of gestation, placental macrophages still exhibit an M1 profile. Indeed, at term, $CD14^{+}$ placental macrophages express a program including the transcriptional expression of several members of TNF superfamily, the expression of chemokine receptors and the secretion of immune cytokines (IL-6, IL-10 and IL-1) [17,41,42]. The M1/M2 dichotomy to characterize placental macrophage populations deserves some criticisms and requires to be rethought. M1/M2 markers were found vary according to the studies and are not enough robust to allow characterization of cell subsets. This is illustrated by conflicting results concerning macrophage polarization in preeclampsia, a major inflammatory disease of pregnancy in which we could expect an M1 signature [43]. We recommended to assess macrophage activation by considering agonist and cell types however with a combination of several markers [38]. When this latter approach was used, we were unable to detect M1/M2 polarization in at term placental macrophages [17].

6. Placental macrophages and multinucleated giant cells: a continuum

The originality of placental macrophages is to form multinuclear giant cells (MGCs). Their formation is associated with down-modulation of CD14 and up-regulation of CD68 and CD163, which suggests a maturation process [17]. Placental MGCs exhibit features reminiscent of osteoclasts and foreign body giant cells (FBGCs), other types of myeloid MGCs. Their cytoskeleton is reorganized with podosomes in peripheral ring as in osteoclasts and placental MGCs contain small number of nuclei randomly distributed as in osteoclasts and FBGCs [44]. The ability of placental macrophages to form MGCs may be related to the fusion of cytotrophoblasts into syncytiotrophoblasts, a step required for placenta function [45]. Although placental macrophages have an intrinsic ability to fuse, it is likely that trophoblasts create a microenvironment prone to favor macrophage fusion. Several molecules produced by trophoblasts are candidate to affect differentiation of placental macrophages into MGCs. They include syncytins [46,47], E-cadherin and IL-4, a cytokine that mediates macrophage fusion in an E-cadherin-dependent manner [44,48]. The functions of placental MGCs are still obscure relying on indirect evidence. They exhibit enrichment of genes implicated in cytoskeleton organization, adhesion and immune response, thus highlighting functional diversity [17]. They possess macrophage properties such as phagocytosis and production of reactive oxygen intermediates (ROIs). In the light of what is known in myeloid MGCs, it is likely that placental MGCs are involved in the engulfment of large particles resulting from remodeling of placenta during pregnancy [49]. The profile of cytokine and chemokine production by MGCs rules out a polarized phenotype, but they possess both inflammatory and anti-inflammatory features. We propose that placental MGCs are fully competent macrophages that play a role in host defense and placental homeostasis.

7. Placental macrophages and infections

Placental macrophages are armed to fight microbial pathogens. They express microbial sensors (Toll-like receptors, lectins, complement receptor of the immunoglobulin superfamily) [50–52]. They are competent to ingest inert particles and microorganisms, produce ROIs, but are poor antigen-presenting cells [17,53]. This latter point may be useful to limit deleterious effect of adaptive immunity on pregnancy. As a consequence, placental macrophages are likely involved in the occurrence of placental infections during pregnancy (Fig. 1), a major cause of obstetric complications, fetal pathologies and preterm deliveries [54].

7.1. Bacterial infection

Chorioamnionitis or intra-amniotic infection is an acute or chronic inflammation of fetal membranes [55] causing premature rupture of the membranes that allows the direct introduction of microorganisms during chorionic villi sampling or *via* amniocentesis or fetoscopy. The bacteria mainly found include *Escherichia coli*, group B *Streptococcus*, *Hemophilus* sp. and *Staphylococcus* sp. [56]. Conflicting results have been reported concerning the presence of placental macrophages in chorioamnionitis lesions: decreased number [17,57] and increased number [58] as compared to controls. The activation level of placental macrophages varies according to pregnancy trimesters. In the third trimester, they over-express T cell chemokines (CXCL9, CXCL10, CXCL11) associated with altered villous architecture [59,60]. We found that the balance between TNF and IL-10, as pro- and anti-inflammatory cytokines respectively, is reoriented toward inflammatory response in chorioamnionitis. The expression of CD163, an M2 marker, on placental macrophages is higher in grade III than in grade II chorioamnionitis. We also showed that $CD14^{+}$ placental macrophages from patients with chorioamnionitis are unable to form MGCs. This defect is partially corrected by incubating placental macrophages with control trophoblast supernatants [17], thus demonstrating the role of the placenta microenvironment in MGC formation. Placental macrophages may be also pathogenic through the release of extracellular traps (ET) in group B *Streptococcus* infection [61]. The release of placenta ET depends on actin polymerization and reactive oxygen species. Placenta ETs contain MMPs which are released during infection and lead to breakdown of extracellular matrix and placenta lesions [62]. This way of response of placenta macrophages to infection is shared with other placenta cells. Indeed, we reported the expression of cytonemes, actin-based structures, by placenta mast cells [63,64] in response to infection with anti-bacterial properties [5]. The role in infections by intracellular bacteria that present a placenta tropism is less well documented. During *Listeria monocytogenes* infection, a ubiquitous intracellular gram-positive bacterium responsible of listeriosis macrophages from placenta were found permissive [65,66]. It has been also provided evidence that during *Brucella* sp. Or *C. burnetii* infection, placental macrophages were found infected [19,67,68]. We recently showed that $CD14^{+}$ macrophages from at term healthy placentas infected *ex vivo* by *C. burnetii* eliminate bacteria within 9 days. This elimination is associated with their polarization in M1 cells and is related to the production of IFN-γ [19]. The microbicidal activity of macrophages from at term placentas may account for the fact that the transmission from mother to fetus mainly occurs during first and second trimesters, not during the third trimester. But to date, the factors that govern the mechanisms of infection, the resistance and/or the susceptibility to these bacteria in decidual macrophages are still unknown.

7.2. Viral infections

Viral infections increase the risk of pregnancy disorders and fetal pathologies, as demonstrated by attention of media for Zika outbreaks. The role of placental macrophages during viral infections has been extensively studied in human immunodeficiency virus (HIV) infection. In placenta from non-emitting mothers, the immune response is effective, but placenta from surrogate mothers have an inflammatory response associated with chorioamnionitis [69]. HIV is detected in

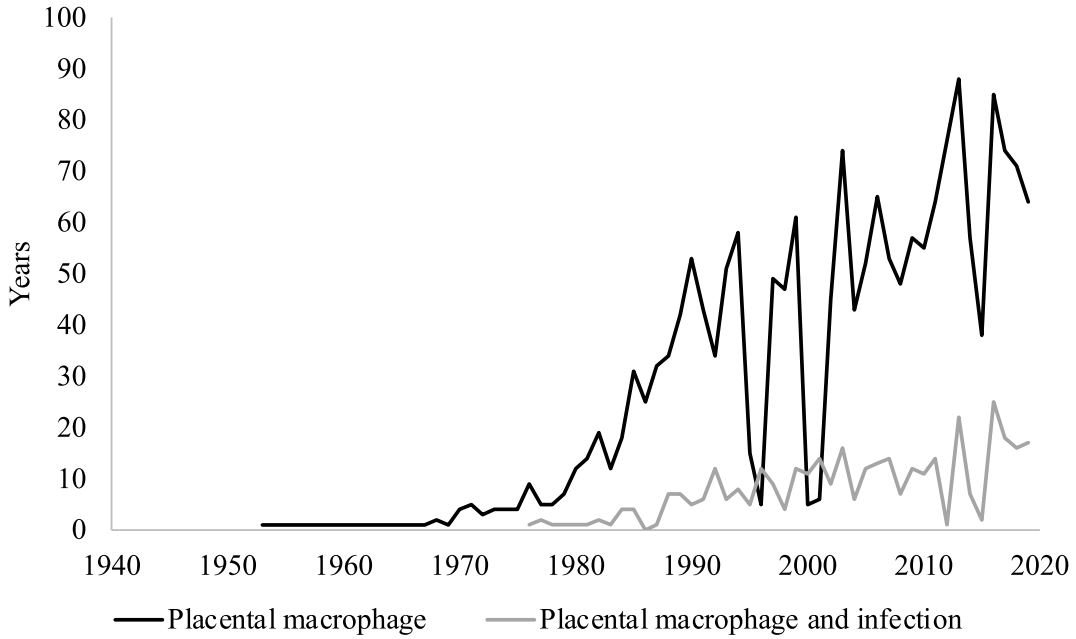

Fig. 1. Number of publications associated with "Placenta macrophage" an "placenta macrophage and infection" (Pubmed database).

placental macrophages that express receptors for HIV including CD4, CCR5, CXCR4 and CD209 [70,71]. $CD14^+$ placental macrophages are less permissive to HIV replication than monocyte-derived macrophages [72]. It has been shown that HIV transiently replicates within isolated $CD14^+/CD68^+$ placental macrophages [73]. In contrast, Johnson et *al.* showed that Hofbauer cells assemble and sequester HIV-1 without replication in compartments rich in endosomal/lysosomal markers, such as CD9, CD81, CD63 and lysosomal-associated membrane protein (LAMP)-1 [74]. Other placental partners are able to control placental macrophage permittivity to HIV. Hence, decidual NK cells inhibit infection of decidual macrophages by HIV through direct contact and IFN-γ release [75]. These findings highlight the key role of placental macrophages in the mediation of protection of placental tissue during HIV infection (Fig. 2). The spotlights have recently turned their attention to the ZIKA virus. The ZIKA virus is transmitted by mosquito bites (*Aedes*) that causes ZIKA fever in humans. During pregnancy, ZIKA virus is vertically transmitted from mother to fetus [76]. ZIKA infection in pregnant women is at the origin of adverse pregnancy and birth outcomes causing essentially severe brain malformations [77]. Pregnant women infected by ZIKA virus present a chronic placentitis with a chronic villous inflammation, edema and trophoblastic lesions [78]. There is evidence that ZIKA infection compromises mesenchymal and Hofbauer cells in human villi [79,80]. Immunohistochemistry approaches also reveal that ZIKA virus stimulates the proliferation of placental macrophages within the chorionic villous stroma [81]. CD163 or CD68 positive cells are colocalized with ZIKA virus antigens *in vivo* [82,83]. *Ex vivo* models show a higher permittivity of placental macrophages to ZIKA virus than trophoblasts [84]. Recently it has been shown that Abs directed against dengue virus increase ZIKA virus infection of Hofbauer cells, suggesting that pre-existing immunity to dengue affects host response to ZIKA virus [85]. The replication of ZIKA virus within Hofbauer cells induces the production of type I IFN, IL-6, CCL3 and inducible protein (IP)-10 [86]. The blockade of IFN production using Janus Kinase (JAK) inhibitors inhibits the production and the replication of the ZIKA virus in human Hofbauer cells *in vitro* [87]. Additionally, the expression of the co-stimulatory molecules CD80 and CD86 by Hofbauer cells is increased, suggesting that they are potentially APCs *in vivo*. All these studies suggest that the primary tropism of the ZIKA virus for placental macrophages enables the virus to cross the placental barrier and to eventually access to the fetal compartment. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection leading to the coronavirus disease in late 2019 (Covid-19) has been associated with a large debate concerning the occurrence of transplacental transmission. Although no transplacental transmission was reported from China, Hosier. H et al., presented for the first time the case of woman with Covid-19 with a SARS-CoV-2 invasion of the placenta and local tissue inflammation and fibrin deposition [88]. They reported an inflammatory infiltrate of immune cells composed of $CD3^+$ lymphocytes and $CD68^+$ macrophages with only infection of syncytiotrophoblast cells by SARS-CoV-2 virus. The infiltration of $CD68^+$ placenta macrophages in SARS-CoV-2 infected placenta was next confirmed [89,90] associated with a M2 phenotype in a case report of an asymptomatic Covid-19 positive woman [91]. Interestingly, Facchetti. F et al., reported a strong expression of S-protein in areas with dense monocytes-macrophage inflammation, which suggests a local activation of these cells [90]. Thus, to date, although the presence of placental macrophage into placenta lesions was clearly established, their role in SARS-CoV-2 infection of the placenta remains to elucidate.

7.3. Parasitic and fungal infections

The place of placental macrophages in pathophysiology of parasitic or fungal infections during pregnancy is less documented than bacterial and viral infections. Among them, *Plasmodium falciparum* represents the most virulent of the plasmodial species in pregnancy and is associated with poor birth outcomes and low birth weight [92]. Only *P. falciparum* among plasmodial species is found in decidual macrophages [93,94]. Intravital microscopy has shown that infected erythrocytes accumulate in maternal blood, interact with trophoblasts in a stable manner and are engulfed by placental macrophages [95]. The accumulation of infected erythrocytes in human placenta causes an inflammatory response characterized by the expression of CCR5 [96] and the release of CCL3 [97] by placental macrophages. If the role of placental macrophages in parasite clearance remains uncertain, their contribution to pathogenesis of inflammatory lesions is well admitted. Same conclusions were also attributed to *Tryposoma cruzi* infection an obligate intracellular parasite responsible for the Chagas disease. Chagasic villitis is characterized by inflammatory infiltrates in which $CD68^+$ macrophages and $CD8^+$ T cells are prominent [98]. In addition, there is evidence of the multiplication of *T. cruzi* in $CD68^+$ macrophages from placental chorionic villi [99,

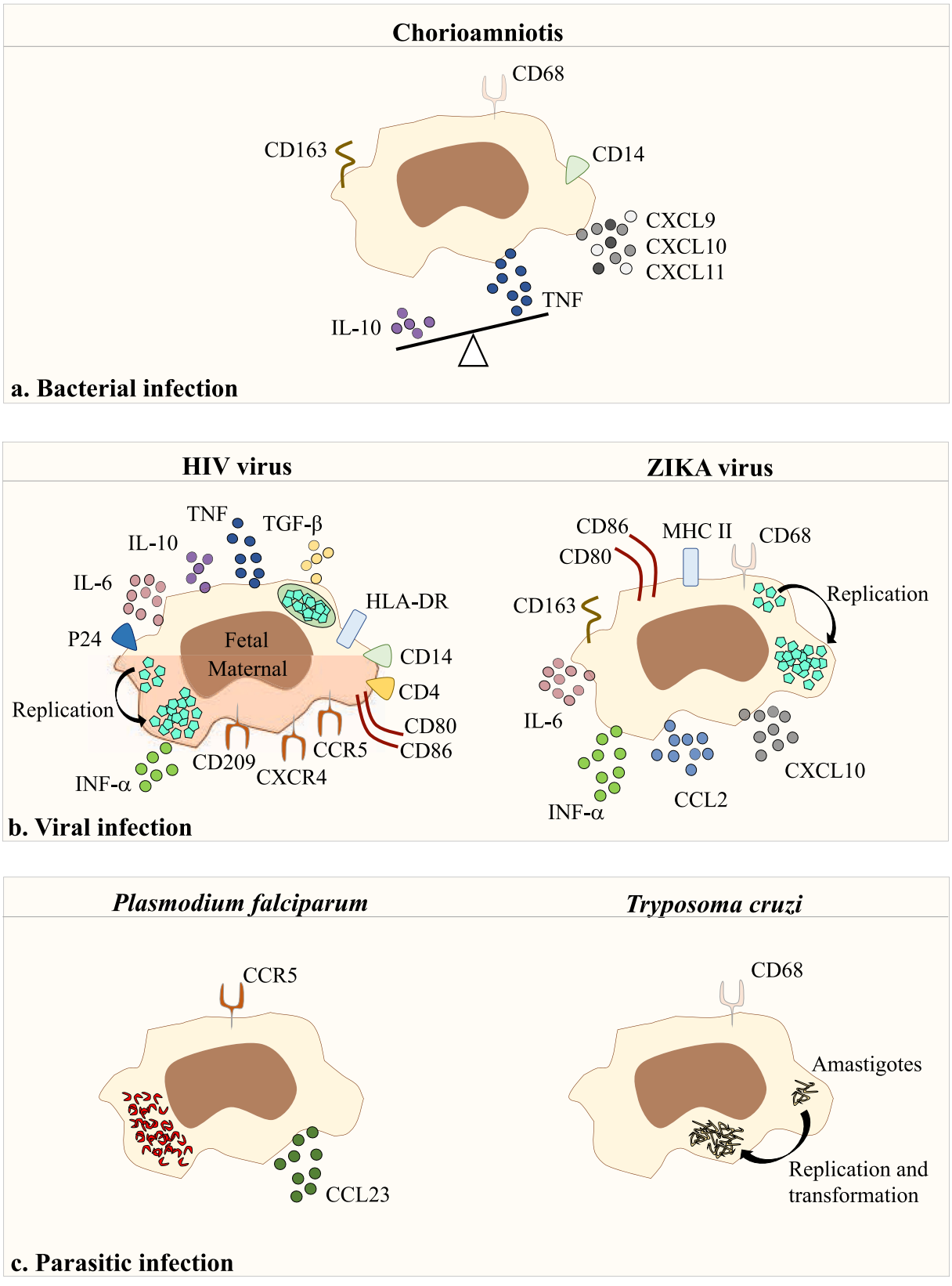

Fig. 2. Responses of placental macrophages to infection.

100]. However, the pattern of inflammatory reaction mediated by infected placental macrophages is so far unknown.

Although they represent a lower prevalence of chorioamnionitis with 0.3–0.5% [101], fungal infection are ascending infections that may severely compromise pregnancy rarely observed in neonates with preterm birth or neonatal death [102]. Congenital infections leading to preterm infants are mainly due to *Candida* species, including *C. albicans*, *C. grablata*, *C. kyfer* and *C. parapsilosis*. Immunohistological examination

of placentas infected by *C. guilliermondii* shows the presence of hypersegmented neutrophils and large macrophages with a filled cytoplasm by several 3–6 μm oval bodies [103]. In addition, these infected placentas present edema of the lamina propria and macrophage infiltration. Although the role of macrophages in *Candida* infection is well documented in host defense against deeply invasive candidiasis [104], their role in placental infection has not been investigated to date.

8. Concluding remarks

Through the focus on pregnancy-associated infections, this review pointed that it is necessary to break down the technical barriers that have long hindered the study of placental macrophages. The results of scRNA-Seq have questioned the dichotomy between Hofbauer cells and decidual macrophages and paved the way for a re-writing of placenta macrophage diversity. The study of placental macrophages in their tissue environment will require the development of *ex vivo* placenta perfusion coupled with intravital microscopy and multiplexed-single-molecule fluorescent *in situ* hybridization. The question of whether placental macrophages are competent to combat microbial pathogens or rather whether they are involved in pathogenicity has not been sufficiently studied. Similarly, the role of placental macrophages in pregnancy-associated infectious diseases is poorly understood and will be a major issue for future research. Nevertheless, placental macrophage characterization could help in prediction of obstetric complications. It might help obstetricians in tricky situations where prolongation of pregnancy generally improves neonatal outcomes but increase infection/inflammation materno-fetal risks.

Authorship

S.M and J.L.M conceived and wrote the manuscript. M.K., A.B.A and F.B provided critical revision of the manuscript.

Conflict of competing interest

The authors declare no competing interests.

Infection with (**a**) bacteria, (**b**) virus or (**c**) parasites results in the expression of membrane receptors and the secretion of several key proteins by placental macrophages. CC and CXC: chemokines; CD: Cluster of differentiation; HIV: Human Immunodeficiency virus; HLA: Human leukocyte antigen; IL: Interleukin; IFN: Interferon; MHC: Major Histocompatibility complex; TGF: Transforming growth factor; TNF: Tumor necrosis factor.

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