Chapter

Role of Cytotrophoblast Cells and Placenta-Derived Exosomes in Regulatory B Cell Differentiation and Function during Pregnancy

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Abstract

Pregnancy is a particular physiologic stage during which immune regulation is essential. A successful placentation and subsequent fetal development depend on the delicate balance between moderate pro-inflammatory response and immune tolerance. Findings have pointed out a crucial role for regulatory B cells (Bregs) in establishing an immunomodulatory (IM) environment relevant to pregnancy. In a steady state, Bregs represent 10% of B cells in peripheral blood, a proportion that increases during pregnancy, with the highest rate being observed in *post-partum*. In the context of pregnancy, Bregs seem to be well positioned to perform the mechanisms that accommodate the growing semi-allogenic fetus and also allow the adequate immune response to pathogen. This chapter discusses the mechanism of action of Bregs during human pregnancy. Also, we will evoke interactions between maternal immune cells and fetal annexes that result in hijacking the naïve B cells to educate and to differentiate them into Bregs.

Keywords: pregnancy, immunoregulation, exosomes, cytotrophoblast, Bregs

1. Introduction

Preeclampsia (PE) is a placental disorder affecting 2–8% of pregnancies with the highest burden observed in poor countries [1–4]. PE and severe PE are characterized by exacerbated pro-inflammatory (PI) responses, leading to significant maternal and perinatal morbidity and mortality [5–8]. Successful placentation and the subsequent fetal development depend on the delicate balance between moderate PI response and immune tolerance.

Recent data has shown that the B cell profile is changed during pregnancy to accommodate the growing fetus [9, 10]. Findings have pointed out a crucial role for regulatory B cells (Bregs) in establishing an immunomodulatory (IM) environment relevant for pregnancy [11]. In a steady state, Bregs represent 10% of B cells in peripheral blood, a proportion which increases during pregnancy, with the highest rate being observed in post-partum [12, 13]. Studies have demonstrated an IM function

for IL-10-producing Bregs against ongoing inflammatory events to both limits the infection [14] and promote a successful outcome of pregnancy [15, 16]. It was also shown that early transfer of Bregs from normal pregnant to abortion-prone mice prevented fetal rejection and restored pregnancy tolerance [17].

Indeed, the maternal immune system needs to recognize and accommodate a developing semi-allogeneic fetus. Regulatory T cells are shown to contribute to normal pregnancy, and considering the immune regulatory involvement of Bregs cells in the fields of autoimmunity, transplantation tolerance, and cancer biology, the mechanism underlying Bregs activities during pregnancy needs to be unraveled [18–20]. The immune regulatory function of Bregs consist of inhibition of the differentiation of effector T cells and dendritic cells (DCs), and activation of Tregs [21, 22].

Today, the consensus is not fully established on the characterization of Bregs with respect to cell surface markers. Recent studies have shown that the regulative roles of Bregs are due to the production of the antiinflammatory cytokine interleukin-10 (IL-10) [23–25]. However, recent data indicated that some B cell subsets perform regulatory functions without IL-10 involvement suggesting that other Bregs use multimechanistic to regulate immune responses.

In mice, multiple B cell subsets are identified to play regulatory function and include the marginal-zone B cells, the transitional 2 marginal-zone precursor cells, follicular B cells, $CD5^+CD178^+$ killer B cells, plasma cells, plasmablasts, $CD5^+CD1d^{hi}IL\text{-}10^+$ B cells, $CD5^+$B-1a cells, GIFT-15 B cells, TIM-1^+ B cells, and PD-L1hi B cells [26, 27]. The IL-10-producing Bregs, also called B10 cells have the $CD1dhiCD5^+$ phenotype [28].

In humans, immature B cells, IL-10^+ B cells (B10), GrB^+ B cells, Br1 cells, and plasmablasts are identified to have immunosuppressive functions [26]. Previous data in humans have described Bregs as $CD19^+CD24^{hi}CD27^+$ [29], which are analogous to the mouse B10 cells [26] and $CD19^+CD24^{hi}CD38^{hi}$ cells [26] with the ability to produce IL-10 and to express CD80 and CD86 costimulation molecules [30].

This disparity of these regulatory cells suggests that Bregs are not derived from one specific lineage; rather they may become Bregs following exposure to environmental stimuli such as placenta derived-exosomes.

As PE is PI disease and syncytiotrophoblast (STB)-derived exosomes (SDE) contribute to materno-foetal immuno-tolerance, it will be relevant to understand how STB cells and SDE contribute to PE by altering Bregs differentiation and function during human pregnancy. We will discuss whether a disrupted balance of Bregs could increase susceptibility to PE.

2. Cytotrophoblast cells and placenta-derived exosomes in successful placentation and fetal development

The successful pregnancy requires the suitable development of embryo and adequate placentation [31–33]. The appropriate placentation is based on the proper capacity of villous cytotrophoblasts to fuse and form the syncytiotrophoblast which contributes to development of placenta. Therefore, abnormal cytotrophoblast differentiation results in placental-related pregnancy diseases [34–36].

Many cytotrophoblast cell subtypes (cytotrophoblasts (CTBs), extravillous cytotrophoblasts (EVTs) and syncytiotrophoblast (STB)) with different structures and functions are involved in placentation [37, 38]. After implantation of the zygote, trophoblast cells develop from the outer cells which form the wall of the blastocyst,

and differentiate into either villous or extravillous trophoblast cells [38]. The STBs are the outer lining of the placenta; fulfill a vast range of role including gas and nutrient exchange between mother and fetus. The trophoblast cell subtypes in addition to secreting hormones and proteins, physically protect the fetus from pathogens [39, 40]. EVTs are invasive trophoblast cells that are important for implantation of the placenta and the development of the fetus [41–43]. Many pregnancy diseases such as PE and intrauterine growth retardation (IUGR) are the consequences of defective placentation [34–36]. Therefore, the normal development of the placenta is based on complex mechanisms of proliferation and differentiation of trophoblast cells [44, 45]. Many factors (e.g., interferon-induced transmembrane protein 1 (IFITM) and Storkhead box 1 (STOX1) SNPs, Syncytins, and factors released by placenta soluble fms-like tyrosine kinase-1 (sFlt-1), placenta growth factor (PlGF), transforming growth factor-β (TGF-β)) govern the regulation of cytotrophoblast cell differentiation showing their potential use as biomarker [46–48].

Placental syncytialization is maintained throughout pregnancy by the fusion of adjacent CTBs [49, 50] and is important for successful pregnancy [49, 51–54]. Syncytins are important players during syncytialization and *Vargas et al.* and Lokossou et al. indicated that insufficient Syncytin-2 (Syn-2) expression could be the potential cause of PE, shedding light on the correlation between Syn-2 level and cytotrophoblasts fusion [46, 50, 55, 56].

In recent years, the role of exosomes in the development of the placenta has become more and more precise [46, 56, 57]. These microvesicles with diameters of 20–130 nm are extracellular secreted vesicles and are involved in cell-to-cell communication [58]. They, therefore, affect cytotrophoblast differentiation and immune regulation, especially during pregnancy [58, 59]. Exosomes are secreted by most cells and embedded in various substances including proteins [46, 56, 60, 61], mRNA and miRNA [62], and DNA [63]. They can be transported to distant organs and are thought to modify various cells and organ functions [56, 64, 65]. SDE which embedded placenta-specific molecules, including Syn-2, were involved in CTB fusion [55], embryo implantation *via* the promotion of T regulatory cells, suppression of Nuclear Factor-kB signaling pathway [66] and thereby in immune reaction and inflammatory response [56]. Secreted exosomes from the placenta into the systemic circulation lead in multisystemic organ damage, in patients with PE [67]. Reduction of Syn-2 levels in exosomes is suggested to be an early biomarker of PE [46, 60]. Indeed, the identification of women at high risk of PE before its onset is especially a challenge. Exosomes miRNA pattern also appears to be used for early PE diagnosis [68]. SDE in preeclamptic placentas are thought to embed high concentrations of PE-specific contents, resulting in unfavorable microenvironments for the invasion of EVTs and the remodeling of spiral arteries for adequate placentation [46, 57, 63, 66–72].

Nowadays, evidence suggests that disruption of placentation characterizes the pathogenesis of PE [55, 73]. Indeed, STB-derived exosomes are found in maternal circulation [72], and affected endothelial function due to their abundant sFlt-1 and soluble endoglin (sEng) content [69]. These vesicles are also endowed with immune regulation capacities during pregnancy due to Syn-2 embedded in exosomes [56].

Placental exosomes are therefore able to deliver many molecules including proteins around CTB, inducing a particular environment that affects placenta and fetal growth.

The immunosuppressive protein derived from human endogenous retrovirus sequences, Sync-2. plays a leading role in placenta formation [49, 50]. For several years our knowledge has grown on PE and placental exosomes. We have demonstrated

the role of Sync-2 in placentation and in T cell immunosuppression [56, 60, 74] during normal pregnancy and PE, suggesting that Sync-2 could be used as an early biomarker of PE. Our recent data from Benin show a gradual diminution, between 7 and 10 weeks of pregnancy (WP), in the incorporation of Sync-2 in serum-derived exosomes from women who had developed a PE later during their pregnancy in comparison to samples from women with normal pregnancy [46]. Sync-2 through its immunosuppressive domain might contribute greatly to creating an immunosuppressive environment. This environment is reinforced and maintained by other factors such as Bregs. This immunosuppressive environment is essential at the beginning of pregnancy for the allograft tolerance constituted by the fetus [75]. As we demonstrated that Sync-2 generates an immunosuppression (IS) environment, Bregs should be important to maintain the IS environment and to prevent allograft rejection. Indeed, PE is a placental and inflammatory disease and syncytiotrophoblast-derived exosomes contribute to materno-fetal immuno-tolerance [56]. Such defective placentation is thought to be caused by an abnormal CTB fusion due to defective production of Sync-2 [46, 49, 55] but also to abnormal maternal immune regulation, involving Sync-2 [56]. Many immune cells, including T cell, macrophage, natural killer, regulatory B and T cells, are also affected during PE [76]. Therefore, it will be of great importance to understand how cytotrophoblast and/or syncytiotrophoblast cells and placenta-derived exosomes contribute to PE by altering Bregs differentiation and function during human pregnancy. By demonstrating that Bregs frequency and function increase susceptibility to PE, would lead to the immediate management of pregnant women predisposed to the development of severe PE and reduce the number of resulting morbidity and deaths.

3. Preeclampsia: state of knowledge

Preeclampsia (PE) is the most common placental disorder affecting pregnancy [77]. PE is associated with vascular dysfunction and deregulated inflammation, oxidative stress, and endothelial dysfunctions [78–80]. This chronic inflammation begins early in pregnancy as a result of stimulation of maternal immune response by trophoblasts and trophoblasts derived-products. It is associated with leukocyte activation, vascular activation and dysfunction and high serum levels of cytokines such as Tumor Necrosis Factor-α (TNF-α) [81–83]. Changes in the levels of immune factors (e.g., cytokines, chemokines) are followed by changes in blood coagulation factors, and apoptotic markers [78, 84–87]. Leukocyte activation is driven by TNF-α, and monocyte-derived cytotoxic protein, that also induces vascular endothelial adhesion molecules. Indeed, increased TNF-α levels in early pregnancy can increase the expression of intercellular adhesion molecule-1 (ICAM-1) on vascular endothelial cells (ECs) and trophoblasts, thereby activating them. Consequently, coagulation cascade, vascular tone, and permeability are disturbed. Moreover, chronic inflammation also activates lymphocyte function-associated antigen-1 (LFA-1) on leukocytes, resulting in the above consequences [84, 85, 88, 89]. This chronic inflammation is also associated with oxidative stress [90] which increases the adhesion of leukocytes to the vascular endothelium and the release of cytokines and anti-angiogenic molecules. Adhesion molecules (e.g., soluble E-selectin and soluble ICAM-1) and reactive oxygen species level are increased in blood collected early in pregnancy from women who develop later PE [83, 91]. During PE, abnormal levels of anti-vascular growth factors (e.g., sFlt-1 and sEng)) lead to maternal vascular inflammatory syndrome

characteristics [58]. These antiangiogenic factors induce the decrease of angiogenic placental growth factor (PlGF), poorly affecting angiogenesis during placentation [92]. Defective placenta secreted PlGF into the maternal circulation as a result of impaired endovascular invasion by trophoblast cells and is underlying by cellular oxidative or endoplasmic reticulum stress [93]. Therefore, the level of these factors in the maternal peripheral blood might enable an early diagnosis of PE. Nevertheless, these methods are questioned and did not allow an early prediction (i.e., during the first trimester) of the occurrence of PE [94, 95].

Commonly, PE results in multi-organ failure (e.g., renal insufficiency, liver dysfunction, neurological or hematological complications, uteroplacental dysfunction) in the mother and poor perinatal outcome. Thereby, PE results in significant maternal and perinatal morbidity and mortality [77]. PE affects 2 to 8% of pregnancies and low and middle-income countries (LMIC) are mostly affected [1–3]. PE is defined as new onset hypertension arising after 20 weeks' gestation, but can also occur at a later stage, i.e., 4–12 weeks postpartum [96, 97].

PE is a consequence of failure of paternal antigen-specific tolerance. Moreover, first pregnancy, first pregnancy after partner change, and long interval between pregnancies increase PE risk [98, 99]. The reduced opportunities for exposure to seminal plasma, and pregnancy by sperm or oocyte donation or pregnancies with donated greatly increase PE risk [100–103].

4. Immune regulation and immune tolerance during pregnancy

Contrary to what would be expected, the maternal immune system does not reject the semi-allogeneic fetus allowing the maintenance of the pregnancy [104], but still reacting with infectious agents. This is the result of interplay between maternal and fetal cells creating a tolerogenic microenvironment at the feto-maternal interface [105]. During PE, immunotolerance to fetal antigens, (e.g. trophoblast) is impaired, resulting in disrupted remodeling of the spiral artery and thereby poor placentation. Regulatory T (Tregs) cells play a crucial role in this tolerogenic microenvironment [106, 107] and the maintenance of pregnancy depends on the balance between Tregs and cytotoxic T cells. The ability of fetal cells to escape destruction by maternal immune cells is based on the expression of human leukocyte antigen (HLA)-C molecules by EVTs alone. Therefore, maternal T-cell reactivity to fetal cells is reduced [18]. The T cells immunosuppression during pregnancy is also mediated by HLA-G, E, F and programmed cell death ligand 1 (PD-L1), indoleamine 2,3-dioxygenase (IDO) expression by EVTs [18–20, 108–110]. EVTs also induce T cell suppression by expressing cytokines including IL-10, TGF-β, and IL-35 [12, 13]. At the beginning of pregnancy, $CD56^{bright}CD16^{-}$ decidual natural killer (NK) cells (dNK cells) represent more than 60% of decidual immune cell and express high level of immunosuppressive receptors [111, 112]. Immunotolerance is maintained at the feto-maternal interface by interaction between HLA-G expressed on EVTs and dNK cells and dNK derived-cytokines [57, 113, 114]. To maintain immunotolerance at the feto-maternal interface, between dendritic cells (DCs) and Tregs [115], the cross-presentation of paternal antigens to maternal cytotoxicity T CTLs and $CD4^{+}$ T cells [115, 116] is altered by fetal antigen-specific Tregs at the feto-maternal interface [117–121]. Seminal plasma components such as TGF-β, prostaglandins, MHCs, and minor antigens also functionally affect maternal antigen-presenting cells (APC). These functional changes were maintained by EVTs [103, 122]. In mice, these functional changes favor

fetal-antigen-specific Tregs cell expansion in the uterus and uterine drainage lymph nodes [123–125]. Moreover, in mice, PD-L2-expressing dendritic cells (DCs) increase during implantation in allogeneic pregnancy in mice [126]. These cells limit inflammation whereas, the decrease of M2 macrophages, which inhibit inflammation and promote tissue repair, results in implantation failure in mice [127]. Furthermore, *in vitro*, the close interaction between decidual macrophages and EVTs results in an increased number of Tregs after co-culture with peripheral blood-derived $CD4^+$ T cells [128, 129]. These results show clearly that EVTs oriented the differentiation of $CD4^+$ conventional T cells into antigen-specific peripheral Tregs [121].

Miscarriages, PE, and implantation failure are some characteristics of pregnancy complications. The dysfunction of Tregs is clearly involved [121, 130]. Indeed, in recurrent pregnancy loss and during PE, low level of Tregs in the peripheral blood and uterus has been reported [121]. A recent study has shown that during PE, clonally expanded effector Tregs were significantly decreased in the decidua compared with normal pregnancy, suggesting an insufficient Tregs antigen-specific tolerance [131].

In addition to this induced-immunosuppression, decidual CTLs were also suppressed by EVTs and other immune cells; to allow a good course of pregnancy, without suppression of CTLs functions against virus [117]. During late gestation, le level of PD-L1 on clonally expanded CTLs increases significantly compared to the beginning of pregnancy, showing that strong suppressive signals are necessary to inhibit the allo-reaction by CTLs in the late gestational period [119]. Moreover, during late onset of PE, the level of PD-L1 on clonal CTLs decreased compared with that in normal pregnancy [119], suggesting insufficient suppression of antigen-specific CTLs in PE.

Overall, during normal pregnancy although Tregs and CTLs recognize fetal antigens at the feto-maternal interface, antigen-specific Tregs induce tolerance, while the cytotoxic function of CTLs is suppressed. Therefore, the imbalance of suppressive role of Tregs and activation of CTLs is likely associated with PE.

In PE, type-1 T helper (Th1) cells numbers are also increased [73] and secrete pro-inflammatory cytokines, such as TNF-α, interferon-γ (IFN-γ), and interleukin (IL)-6. Increased Th17 cells secreting the pro-inflammatory cytokine IL-17 are also found during PE [83, 132].

5. Regulatory B cells establish immunomodulatory environment for pregnancy

With regard to their multi-faceted roles, B cells may participate in successful pregnancy [11]. A subtype of B cells named Bregs exhibits immunosuppressive function and therefore is considered an important players in immunological tolerance during pregnancy. During pregnancy, the change of the maternal immune response is governed by a range of cytokines that shape the type and abundance of leukocyte subsets in the decidua and placenta. In addition, these changes also include the reduction of antigen-presenting capacities of monocytes, macrophages, and DCs; inhibition of NK cells, T cells, and B cells; proliferation of dNK cells; maintenance of tolerogenic DCs; and the induction of Tregs [133].

Regulatory roles of Bregs were attributed exclusively to the production of the anti-inflammatory cytokine interleukin-10 [23, 29, 134], even recent data have identified other B cell subsets with regulatory functions without IL-10 production. Indeed, Bregs are cells that dampen ongoing inflammatory events in murine models [15]

and counteract excessive pro-inflammatory responses during infection [14]. IL-10 is crucial for optimal pregnancy outcomes, Therefore IL-10 deficiency is related to fetal resorption, growth restriction, and even death of mother and child [135, 136]. This antiinflammatory cytokine is found in high levels in the decidual and placenta during pregnancy and is involved in damping the pro-inflammatory cytokine response. Interesting fact, at the beginning of pregnancy, the inflammation induced by the recognition of paternal antigens is upset by the production of IL-10 [137].

Bregs proportion increases during pregnancy and *in postpartum* [138] and first-trimester peripheral blood-derived Bregs are shown to inhibit TNF-α secretion by activated T effector cells [139]. In the context of pregnancy, Bregs seem to be well positioned to perform the mechanisms that accommodate the growing semi-allogeneic fetus and also allow the adequate immune response to the pathogen [10]. However, the mechanism of action of Bregs during pregnancy remains curtailed even if their importance in pregnancy has been shown in mouse models [138].

In order to allow a successful placentation and suitable fetal development, the maternal immune system undergoes several changes while allowing the mother to defend herself against infections [10]. A German group has developed a PE mouse model by transferring activated Th1-like splenocytes into normal pregnant mice and has demonstrated that pregnancy-associated immuno-regulation involved a shift from inflammatory toward anti-inflammatory immune responses mainly controlled by T and B cells [140, 141]. They have reported that Bregs were active players in the maintenance of pregnancy by modulating T cell functions [142]. Indeed, they have shown that Bregs transfer from normal pregnant to abortion-prone mice early in pregnancy prevents fetal rejection and restores pregnancy tolerance in mice [17].

The action of Bregs during pregnancy is interconnected with that of Tregs and DCs, providing an appropriate environment for fetal growth.

As described above, both Tregs and DCs play critical roles in determining pregnancy outcomes. In normal pregnancy, fetal-tolerant involves decidual DCs that remain immature and knew as tolerogenic DCs [148]. Tregs are also crucial players in maintaining maternal-fetal immune tolerance. At the onset of embryo implantation, expansion of the Tregs population improves the outcome of pregnancy whereas deficiency or low numbers of Tregs in the uterus during pregnancy leads to pregnancy lost (i.e., abortion or miscarriage) [22, 149].

As anti-inflammatory signal such as IL-10 or TGF-β is crucial to maintain the DC immature phenotype that prevents the activation of T cells [150], IL-10 and or TGF-β must then be initially: 1- present in the uterus and placenta to either drive the induction of immune tolerant DCs followed by the induction of Tregs, or 2- directly drive the induction of Tregs.

Overall Breg's role in pregnancy seems to be upstream to that of Tregs [17] and therefore, Bregs seem to be the first sources of IL-10 and TGF-β [10, 17].

6. Impact of cytotrophoblast cells and placenta-derived exosomes on regulatory B cells differentiation and function

The pathophysiology of PE is poorly understood despite the evidences supporting a role of immune system in the development of PE [56]. B cells represent a dominant component in the pathogenesis of PE and studies focused on the number and functions of Bregs during PE are of great interest in understanding the pathophysiology of PE [139]. Harnessing Bregs functions may lead to the capacity of using Bregs as an

immunotherapeutic agent for averting and treating pregnancy pathologies such as PE. Perinatal cells including cells from term placenta and fetal annexes (amniotic membrane, chorionic membrane, umbilical cord, etc) are able to inhibit B cell proliferation, impair B cell differentiation and promote Bregs formation, frequently due to bioactive factors secreted by perinatal cells [11, 143]. These cells are considered as a promising tool for therapeutic approaches in PE [143]. Interactions between maternal immune cells and fetal annexes may result in hijacking naïve B cells and educating them to become Bregs. However, how cytotrophoblast (CT) and/or syncytiotrophoblast (ST) cells regulate Bregs differentiation and function during pregnancy is still unknown. Maybe in case of PE, CT and ST and their derived-vesicles (e.g. exosomes) will prevent adequate Bregs development and function, resulting in reduced and dysfunctional Bregs. This default of Bregs might result in an inflammatory environment, which will increase the susceptibility to PE.

Recent *in vitro* and *in vivo studies* have shown that perinatal cells and perinatal cells derived-vesicles interfere with the activation and differentiation of innate and adaptive immune system cells [11]. Poor knowledge is available about the impact of perinatal cells on B lymphocytes, even if some of the complex cross-talks between perinatal cells and B cells have been described. These studies demonstrated that perinatal cells have a strong antiproliferative capacity on B cells, but were not based on cell–cell contact. The demonstration is based on bioactive factors secreted by perinatal cells. For instance, co-cultured human mesenchymal stromal cells (MSC) isolated from umbilical cord (hUC-MSC) in a contact independent with mouse splenic B cells result in abrogation of the proliferation of activated B cells [144]. Likewise, human umbilical cord matrix cells co-cultured with a B cell cancer line (i.e. Burkitt's lymphoma cell line) [145], or with auto-reactive B cells from PBMC of immune thrombocytopenic patients results in inhibition of these B cells proliferation [146]. These observations were confirmed by using other perinatal cells (e.g., mesenchymal stromal cells (MSC)) purified from the amniotic membrane (hAMSC). This MSC supernatant is able to suppress $CD19^+$ B cell proliferation in PBMC or purified B cells from PBMC, confirming that cell-to-cell contact was not required and suggesting the role of soluble molecules and vesicles such as exosomes [11]. Similarly, human amniotic fluid stromal cells and their conditioned medium (CM) strongly suppress B cell activation and proliferation, and significantly inhibited the expression of CD80/CD86 costimulatory molecules on activated B lymphocytes [147].

However, some data contradict these observations and showed that human amniotic fluid stromal cells are able to suppress the apoptosis of B lymphocytes, favoring an increase in activated B cell survival. The mechanism underlying this inhibition is based on the decrease of the expression of the negative co-inhibitory molecules B7 homolog 4 (B7H4) and programmed death-ligand 1 (PD-L1) on activated B lymphocytes [147]. Moreover, an increase in B cell proliferation and a reduction in spontaneous apoptosis in the presence of human amniotic epithelial cells (hAEC) were also described [148]. Umbilical cord derived-MSC were not able to affect [149] or in other studies able to highly induce the *in vitro* growth of PBMC derived-B cells [150].

It is also demonstrated that human amniotic fluid stromal cells induce down-regulation of the proportion of B1 cells [147], resulting in the reduction of the B cell subset mainly involved in the production of autoantibodies in PE [151–153]. Many studies have shown that perinatal cell and their CM are able to block antibody-secreting cells $CD19^+CD27^+CD38^+$ and the differentiation of B cells into $CD138^+$ plasma cells, resulting in the reduction of secreted immunoglobulin [11, 144, 147]. However, co-culture

of purified B cells with human amniotic fluid stromal cells results in reduction of the proportion of $CD19^+CD20^+CD27^+$ memory B cells [147], whereas PBMC cultured in the presence of CM-hAMSC increases $CD19^+CD27^+CD38^-$ memory B cells [11]. These different results may be explained by the presence of other immune cells among PBMC instead purified B cells. Moreover, different conditions of stimulation were used to activate B cells, and the lack of consensus in the markers used to characterize the B cell population could also support the distinct results observed by different groups.

Perinatal cells not only modulate B cell function by favoring their differentiation toward plasma cells, but they also promote the formation of Bregs. Indeed, it was reported that hAEC induced the expansion of $CD19^+CD24^{hi}CD38^{hi}$ Bregs [148]. However, recent data suggested that IL-10^+ Bregs were inhibited by human amniotic fluid stromal cells [147]. These observations clearly showed that more knowledge is needed to understand the impact of perinatal cells and other related vesicles on Bregs differentiation and functions. Thereby, it's important to identify the signaling pathways involved in underlying how perinatal cells and derivatives affect B cell proliferation and differentiation. Two signaling pathways were identified to be suppressed through CpG oligodeoxynucleotides (CpG ODN) by hAMSC: 1-the Toll-like receptor 9 (TLR9)-myeloid differentiation primary response 88 (MyD88)-interleukin-1 receptor-associated kinase (IRAK)1/4 and 2- the TLR9-phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT) pathways [11]. This suppression results in a reduction of uptake of the CpG ODN by CD205, TLR9, and CD14. Consequently, IRAK-4, mitogen-activated protein kinases (MAPK) (c-Jun N-terminal Kinase (JNK), p38 MAPK, extracellular signal-regulated kinase (ERK)) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathways were inhibited. This induces an important reduction in the expression of phosphorylated AKT [11, 144]. The exact mechanism by which perinatal cells and derivatives induce Bregs differentiation is still unknown, and needs to be investigated [154]. Few data demonstrate that perinatal cells produce soluble factors including prostanoids (i.e., prostaglandin E2 (PGE2)), and maybe exosomes to immune regulate cells [155–157]. Therefore, we can speculate that Bregs differentiation is also induced by bioactive vesicles.

Based on *in vitro* results showing that perinatal cells have immunomodulatory properties, they were successfully tested in several inflammatory and immune-mediated diseases, including lung [158, 159] and liver [160] fibrosis, inflammatory bowel disease, collagen-induced arthritis, experimental autoimmune encephalomyelitis [161], multiple sclerosis, wound healing [157, 162], traumatic brain injury [163], cerebral ischemia [164], Huntington's disease [165], and diabetes [166].

The therapeutic using hAMSC in pathological conditions driven by B cells has demonstrated a reduced idiopathic pulmonary fibrosis progression [158]. This treatment allows low levels of B cells in alveolar spaces and reduced the amount of $CD138^+$ antibody-secreting cells in lung tissues, suggesting a decrease in B cell recruitment and an impairment of the maturation of B cells. Therapy using hAEC has also shown remarkable results in animal models of Hashimoto's thyroiditis and systemic lupus erythematosus (SLE) [167].

hAEC induced significant up-regulation of Bregs in experimental autoimmune thyroiditis mice. In this experiment, authors have shown that B10 cells are the major target of hAEC. In SLE mice, hAEC has shown the reduction in autoantibody production but without effect on B10 cells, suggesting that the mechanism of hAEC immunomodulation depends on the disease [167].

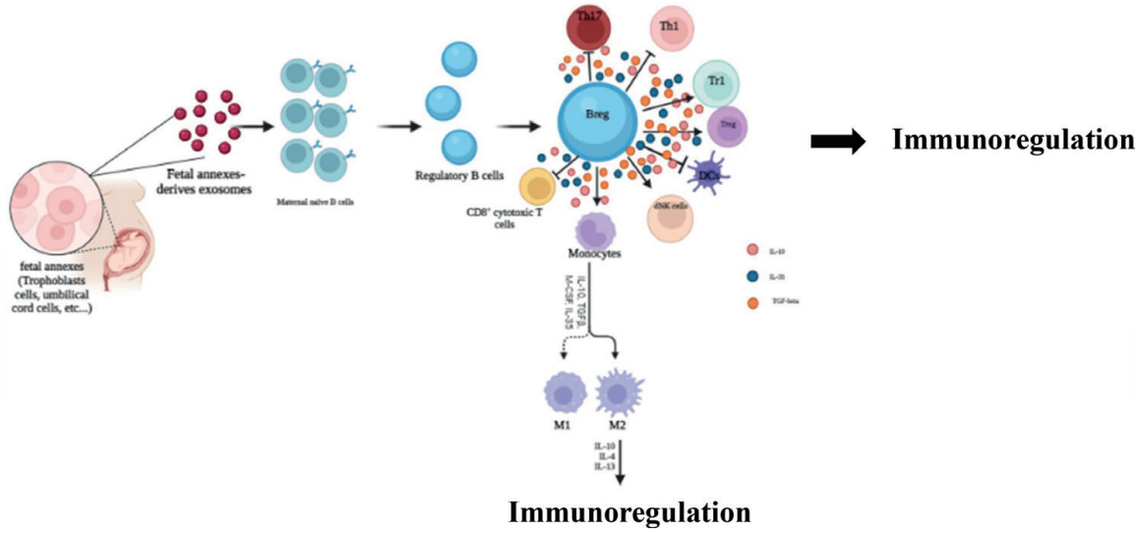

Figure 1.
Differentiation and functional properties of Bregs. Through the production of exosomes by fetal annexes including cytotrophoblast cells, naive B cells can differentiate into Bregs. By producing IL-10, TGF-b, and IL-35, Bregs can suppress tumor necrosis factor-a (TNF-a)-producing monocytes, IL-12-producing dendritic cells, Th17 cells, Th1 cells, and cytotoxic CD8+ T cells. Bregs can also induce the differentiation of immunosuppressive Tregs, T regulatory 1 (Tr1), and dNK cells. This figure was created using Biorender.com.

In the context of chronic graft-versus-host disease (cGVHD) prophylaxis repeated infusion of hUC-MSC seems to minimize the severity and the symptoms of cGVHD by increasing $CD27^{+}$ memory B cells [168].

The immune modulation properties of perinatal cells depend on the origin of these cells. Indeed, fetal-derived cells induce strong inhibition of T-cell proliferation, cytotoxicity, and switch to M2 macrophages, while maternal-derived cells were more strongly able to induce Tregs [169]. **Figure 1** describes the probable implication of exosomes in Bregs differentiation and function (**Figure 1**).

As PE is pro-inflammatory disease and syncytiotrophoblast-derived exosomes (SDE) contribute to materno-fetal immuno-tolerance, it will be useful to understand how STB cells and SDE contribute to PE by altering Bregs differentiation and function during human pregnancy. These mechanisms may be close to those that inhibit immune flares or chronic inflammation in autoimmune diseases and transplantation.

7. Conclusion

Gestation is a remarkable biological process in which the mother carries a fetus harboring half of a foreign genome belonging to the father. To allow the growth of the fetus, the maternal immune system needs to accommodate the semi-allogeneic fetus by dampening its immune responses. This results in a state of immunological tolerance throughout gravidity while maintaining the capacity to respond to pathogens properly. This paradoxical situation requires a perfect regulation of the balance between immune tolerance and immune activation.

To enable more accurate prediction and prevention of PE, its pathogenesis needs to be more understood. Increasing evidence suggests a consequence of the altered immune system in the development of PE. Today, it is clear that perinatal cells have capacity to regulate B cell response at different levels: by inhibiting B cell multiplication, impairing B cell differentiation, and inducing B regulatory cell formation.

Future research should focus on understanding how cytotrophoblast cells and placenta-derived exosomes act on B cells.

Overall, it is clear that cytotrophoblast cells and placenta-derived exosomes harbor the capacity of being a novel therapeutic approach for PE. However, the opposite results and the mainly small number of studies exploring the effect of cytotrophoblast cells and placenta-derived exosomes on the Bregs subset cannot allow deciding on a position. Further *in vitro* and *in vivo* studies are necessary to better decide the immunomodulatory potential of perinatal cells, leading to an important strategy for the treatment of PE.

Conflict of interest

The authors declare no conflict of interest.

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