Placental bed research: II. Functional and immunological investigations of the placental bed

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Research on the placenta as the interface between the mother and the fetus has been undertaken for some 150 years, and in 2 subsequent reviews, we attempted to summarize the situation. In the first part, we described the discovery of unique physiological modifications of the uteroplacental spiral arteries, enabling them to cope with a major increase in blood flow necessary to ensure proper growth of the fetus. These consist of an invasion of the arterial walls by trophoblast and a progressive disappearance of its normal structure. Researchers then turned to the pathophysiology of the placental bed and in particular to its maternal vascular tree. This yielded vital information for a better understanding of the so-called great obstetrical syndromes (preeclampsia, fetal growth restriction, premature labor and delivery, placenta accreta). Systematic morphological investigations of the uteroplacental vasculature showed that preeclampsia is associated with decreased or failed transformation of spiral arteries and the persistence of endothelial and smooth muscle cells in segments of their myometrial portion. Here we report on recent functional investigations of the placental bed, including in situ biophysical studies of uteroplacental blood flow and vascular resistance, and manipulation of uteroplacental perfusion. These new methodologies have provided a novel way of identifying pregnancies in which remodeling is impaired. In animals it is now possible to manipulate uteroplacental blood flow, leading to an enhancement of fetal growth; this opens the way to trials in abnormal human pregnancies. In this second part, we explored a new, extremely important area of research that deals with the role of specific subsets of leukocytes and macrophages in the placental bed. The human first-trimester decidua is rich in leukocytes called uterine natural killer cells. Both macrophages and uterine natural killer cells increase in number from the secretory endometrium to early pregnancy and play a critical role in mediating the process of spiral artery transformation by inducing initial structural changes. It seems therefore that vascular remodeling of spiral arteries is initiated independently of trophoblast invasion. Dysregulation of the immune system may lead to reproductive failure or pregnancy complications, and in this respect, recent studies have advanced our understanding of the mechanisms regulating immunological tolerance during pregnancy, with several mechanisms being proposed for the development of tolerance to the semiallogeneic fetus. In particular, these include several strategies by which the trophoblast avoids maternal recognition. Finally, an important new dimension is being explored: the likelihood that pregnancy syndromes and impaired uteroplacental vascular remodeling may be linked to future maternal and even the child's cardiovascular disease risk. The functional evidence underlying these observations will be discussed.

Key words: acute atherosis, cardiovascular disease, defective deep placentation, endothelial lining, immune cells, leukocytes, maternal-fetal interactions, natural killer cells, T cells, placental bed, preeclampsia, uteroplacental arteries

The term, placental bed, was introduced by Dixon and Robertson[1] in 1958 to identify the structure situated at the interface between the mother and the fetus, with its primary function to establish, maintain, and adapt an adequate maternal blood supply to the intervillous space of the placenta.

The pathophysiology of this structure and in particular the specific modifications of uteroplacental spiral arteries have been studied for more than a century (see part 1 of this review)[2] and have yielded vital information for a better understanding of the so-called great obstetrical syndromes.[3] More recently, to complete the picture, the morphological studies reported elsewhere[2] have been complemented with a series of diverse functional investigations, and these will be described in the present review.

In this connection, the extensive studies by Jauniaux et al[4] have shown that the human placenta is not hemochorial until the end of the first trimester. Intriguingly, until it has the enzyme battery necessary to metabolize the number of free radicals normally found in adult tissue, the placenta restricts the transfer of O_2 between the mother and the fetus but also to itself (Box). Clearly, the placental oxygen supply depends on the ability of uteroplacental arteries to convey a sufficient amount of blood, and this requires major modifications of the uterine spiral arteries, enabling them to accommodate the ever-increasing uteroplacental blood flow.[5]

The rheological and physiological consequences of this vital aspect of placental functional physiopathology have now been amply investigated, and

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BOX

The development of Roberts' hypothesis on the role of placental oxidative stress in the pathogenesis of preeclampsia

Roberts JM et al (1989)[129]

Inasmuch as endothelial cell injury reduces the synthesis of vaso-relaxing agents, increases the production of vasoconstrictors, impairs synthesis of endogenous anticoagulants, and increases procoagulant production, these cells are likely to be implicated in the pathophysiology of preeclampsia.

The fact is that the physiological changes of the decidual spiral arteries in normal pregnancy include the resorption of the endothelial cells in the placental bed arteries to be replaced by a fibro-fibrinoid vessel without endothelial cells. This process starts after the 10th week of gestation to be completed by midgestation.

On the other hand, in the absence of physiological changes, the spiral artery the endothelial lining is likely to persist and not only retain the capacity of vasoconstrictors production from the onset of pregnancy but also to increase with the growth of the uterine vascular capacity and cause the preeclamptic syndrome.

Roberts JM and Cooper DW (2001)[130]

Endothelial cell injury reduces the synthesis of vaso-relaxing agents, increases the production of vasoconstrictors, impairs synthesis of endogenous anticoagulants, and increases procoagulant production. The relative contributions of maternal and fetal genotypes are still unclear.

On the other hand, in the absence of physiological changes, the endothelial lining of uteroplacental spiral arteries is likely to persist and not only retain the capacity to produce vasoconstrictors from the onset of pregnancy but also for production to increase with the growth of the uterine vascular capacity and cause the preeclamptic syndrome.

Jauniaux E. et al (2000)[131]

A burst of oxidative stress occurs in the normal placenta as the maternal circulation is established. The authors speculate that this may serve a physiological role but may also be a factor in the pathogenesis of preeclampsia and early pregnancy failure if antioxidant defenses are depleted.

Chambers JC et al (2001)[132]

Endothelial function is impaired in women with previous preeclampsia and is not explained by established maternal risk factors but is reversed by antioxidant ascorbic acid administration.

Roberts JM and Lain KY (2002)[133]

The diversity of maternal factors argues that there will be no single gene to explain the disorder and no single magic bullet to treat the disorder.

Burton GJ and Jauniaux E (2004)[134]

Placental perfusion will be impaired to a greater or lesser extent, generating commensurate placental oxidative stress that is a major contributory factor to preeclampsia. Miscarriage, missed miscarriage, and early- and late-onset preeclampsia represent a spectrum of disorders secondary to deficient trophoblast invasion.

Jauniaux E et al (2006)[4]

Preeclampsia is a 3-stage disorder, with the primary pathology being an excessive or atypical maternal immune response. This would impair the placentation process, leading to chronic oxidative stress in the placenta and finally to diffuse maternal endothelial cell dysfunction.

Burton GJ et al (2009)[135]

Unexplained intrauterine growth restriction and early-onset preeclampsia are thought to share a common etiology in placental malperfusion secondary to deficient maternal spiral artery conversion. However, there is evidence of greater maternal vascular compromise of the placenta in preeclampsia, and it can be speculated that in cases complicated by preeclampsia, oxidative stress is further superimposed upon endoplasmic reticulum stress. The difference between these 2 conditions would then lie in the severity of the initiating deficit in spiral arterial conversion and the relative degrees of endoplasmic reticulum stress and oxidative stress induced in the placenta as a result.

Redman CW, Sargent IL, Staff AC (2014)[136]

Abnormal placental perfusion and syncytiotrophoblast stress both contribute to the pathogenesis of early- and late-onset preeclampsia. But the early variant is caused by an extrinsic cause, defective placentation, whereas the late variant is due to an intrinsic cause, microvillous overcrowding, impeding intervillous perfusion, and increasing intervillous hypoxia as placental growth reaches its functional limits.

the profound changes have been evaluated, both at a macro (sonographic) and a micro (molecular) level; their description will also be the object of the present review.

Biophysical studies of uteroplacental blood flow

Collins et al[6] have utilized color Doppler ultrasonography to functionally investigate developmental changes in spiral artery blood flow in the human placenta, and real-time ultrasound visualization of the dynamic vascular changes occurring during pregnancy has shown that the width and length of the jets of blood

emanating from the spiral arteries into the intervillous space increases with gestation, while the velocity decreases.

The length of the jets shows a bimodal frequency distribution; the width of the signals of longer ('mega') jets was significantly greater ($P = .001$) than that of regular ('normal') jets (mean, 4.3 mm [3.1−5.9] vs 3.8 mm [1.8−5.8], respectively) at 34 weeks of gestation. The bimodal distribution of jet lengths suggests that 'mega-jets' are a separate entity from 'normal-jets'. Because blood flow velocity of 'mega-jets' is the same of that of 'normal-jets', this phenomenon can be explained with the fact that 'mega-jets' are significantly wider and can carry a greater volume of blood.

Unfortunately, presently available 3-dimensional ultrasound instruments can capture the full placental bed only up to 16 weeks, and this limits the possibility of investigating global blood flow during the third trimester. Moreover, color Doppler studies of the placental bed spiral arteries should take into account potential differences in flow between spiral arteries in the central, paracentral, and peripheral zone of the placental bed.

Assessment of uteroplacental vascular resistance

A major barrier to investigating the pathology surrounding failed spiral artery remodeling is that the process of vascular transformation (or its failure) occurs many weeks before the presentation of obstetrical syndromes. Also, if first-trimester tissue is collected for analysis at pregnancy termination, there is no way of knowing whether those pregnancies would have ultimately been successful.

To identify pregnancies in which remodeling is impaired, Fraser et al[7] have developed a novel way of overcoming this problem by measuring uterine artery Doppler resistance index (RI) as a proxy measure of the extent of spiral artery transformation. Pregnancies with higher RI exhibit a number of features indicative of abnormal implantation, including altered decidual natural killer (dNK) cell interactions with trophoblast, mediated by decreased expression of HLA-binding cell surface receptors,[8] higher levels of placental apoptosis, reduced placental expression of insulin-like growth factor-2 expression, and altered antioxidant defenses.[9] Furthermore, dNK cells isolated from pregnancies with higher RI were less able to promote trophoblast migration and invasion, failed to induce vascular cell apoptosis, and secreted fewer proinvasive and proapoptotic factors.[7,10]

These findings suggest that both maternal (decidual) and fetal (placental) factors can contribute to the pathophysiology of impaired spiral artery transformation. Other factors can also influence placental vascular resistance. Bytautiene et al[11] measured placental vascular bed perfusion pressure and isometric tension in segments of chorionic plate artery and vein in response to potassium chloride, compound 48/80 (a polymer promoting histamine release), cromolyn (a mast cell stabilizer), and a thromboxane A2 mimetic. They observed that compound 48/80 significantly increased perfusion pressure in isolated human placental cotyledons and that this effect was significantly potentiated after induction of active vascular tone by the thromboxane A2 mimetic.

Cromolyn significantly attenuated responses to compound 48/80 in these preparations, whereas compound 48/80 significantly increased tone in isolated human chorionic artery and vein rings. These responses were abolished by cromolyn.

It therefore seems appropriate to conclude that degranulation of placental and intravascular mast cells and subsequent release of vasoconstrictive substances could alter normal placental vascular function. The availability of this technology raised the question of its application to identify at risk pregnancies. In 2010, Espinoza et al[12] presented evidence that bilateral uterine notching between 23 and 25 weeks' gestation is an independent risk factor for the development of early-onset preeclampsia (PE), gestational hypertension, and small-for-gestational-age newborns in the absence of PE. On this basis, they proposed that bilateral uterine artery notching be considered in the assessment of risk for the development of these pregnancy complications.

Subsequently, Polat et al[13] extended these observations to include pregnancies between 24 and 34 weeks' gestation. They confirmed that the presence of bilateral and double notches represented progressive deterioration in the uterine artery functionality and are predictive of adverse maternal outcomes. A further confirmation of the predictive role of various Doppler indices came from a study by Nagar et al[14] in a tertiary-level care center, after studying 500 women with high-risk pregnancies from rural and urban sites. They concluded that Doppler technology may be used for the prediction of PE and intrauterine growth restriction (IUGR), reducing maternal and perinatal morbidity and mortality.

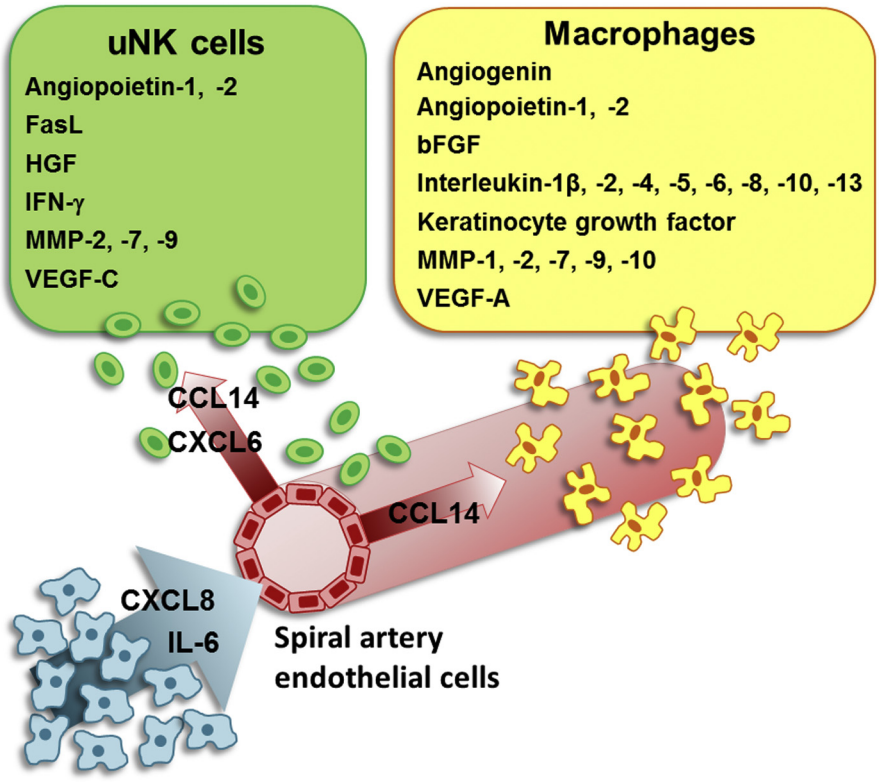
Finally, a recent trial in India[15] has indicated that pregnant women with laterally implanting placentas are at significant risk for development of PE. Furthermore, if the lateral placenta is associated with uterine artery Doppler abnormalities, the risk of developing PE increases significantly, as compared with lateral location alone. The study supports the opinion that unilateral placental location may predispose to the development of PE and IUGR by its effect on uterine artery resistance.[16]

Manipulation of uteroplacental perfusion during pregnancy

A number of recent studies have shown that it is possible to manipulate uteroplacental blood flow in animal models, leading to an enhancement of fetal growth. Endothelial nitric oxide synthase knock-out mice display impaired spiral artery remodeling and placental hypoxia, characterized by reduced uterine artery diameter, reduced spiral artery length, and retention of medial smooth muscle cells (SMCs).[17] Indeed, nanoparticle-mediated delivery of the vasodilator SE175 to the uterine spiral arteries increased mean spiral artery diameter in this model as well as reducing the expression of oxidative and inflammatory markers and enhancing fetal growth.[18]

Similarly, application of an adenoviral vector encoding vascular endothelial growth factor (VEGF) to the uterine arteries of pregnant sheep led to a sustained increase in uterine artery blood

FIGURE 1
Putative soluble mediators of leukocyte recruitment and uterine spiral artery remodeling



Data from ex vivo human tissue models suggests that EVT-derived IL-6 and CXCL8 stimulate release of CXCL6 and CCL14 from spiral artery endothelial cells. These chemokines attract decidual leukocytes into unremodeled vessels; macrophages and uNK cells secrete a diverse range of soluble mediators that regulate different aspects of vascular transformation. This figure is based on studies by Choudhury et al (2017),[38] Lash et al (2006),[34] and Lash et al (2016).[35]

EVT, extravillous trophoblast; *IL*, interleukin; *uNK*, uterine natural killer.

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flow, offering a means to treat growth restriction[19]; a clinical trial in humans is currently under development.[20] An early investigation by Kitzmiller and Benirschke[21] considering decidual vessels as a site of potential intimate maternal-fetal immunological contact suggested that the normal immunological relationship of the mother with the fetoplacental unit may be disturbed in women developing PE. In their opinion, immune reactions, such as acute atherosis, may occur in the uteroplacental spiral arteries, resulting in demonstrable lesions, which could impair placental blood flow. Thus, the immunopathological process in PE simulates closely that of the acute rejection of renal transplants in presensitized hosts and may contribute to impaired uteroplacental perfusion and subsequent pregnancy pathology. This phenomenon is discussed in more detail in the *Immunological tolerance in pregnancy* section.

The role of uterine natural killer cells and macrophages in the placental bed

The human first-trimester decidua is rich in leukocytes, in particular uterine NK (uNK) cells and macrophages. These cells increase in number from 23% of stromal cells (SCs) in the secretory endometrium to 32% in early pregnancy decidua.[22]

Uterine natural killer cells and macrophages during pregnancy

The majority of uNK cells are $CD56^{bright}$, $CD16^{-}$, and $CD3^{-}$, although a population of $CD56^{dim}$uNK is observed in the second trimester.[23] Decidual macrophages are predominantly $CD14^{+}$ $HLA\text{-}DR^{+}$ and decrease in number from the first to second trimester; however, a population of $CD163^{+}$ $CD206^{+}$ M2-like macrophages increases in number over this period.[24] In the first 20 weeks of pregnancy, uNK cells and macrophages in particular, play a critical role in mediating the process of spiral artery transformation by inducing initial structural changes, secreting a number of cytokines and chemokines, promoting the actions of extravillous trophoblast and contributing to tissue remodeling.[25]

In the first trimester of human pregnancy, 2 distinct populations of $CD14^{+}$ decidual macrophages have been described, identified by high or low CD11c expression.[26] The 2 subsets differ in their ability to process and present antigens and each secrete a repertoire that includes proinflammatory and antiinflammatory cytokines; thus, neither subset can be classified as having an M1 or M2 phenotype.

Later in gestation, Kim et al[26] compared the immunoreactivity and distribution patterns of placental $CD14^{+}$ and $CD68^{+}$ macrophages in PE and non-PE pregnancies at 25−35 weeks and identified a unique subset of $CD14^{-}$/$CD68^{+}$cells. These cells were present in greater numbers in the decidua than in the myometrium; however, the density and proportion of $CD14^{+}$/$CD68^{+}$ cells were significantly higher in the myometrium than in the basal plate. No differences in the density or distribution of macrophages were noted when comparing the basal plate, decidua, and myometrium of women with PE with gestationally age-matched tissues from women with spontaneous preterm labor.

These findings suggest that a gradient of $CD14^{+}$/$CD68^{+}$ macrophages exists

across the myometrium and basal plate, regardless of whether the pregnancy is complicated by PE or spontaneous preterm labor. In the third trimester, Xu et al[27] observed that onset of labor was associated with polarization of decidual macrophages toward an M1-like phenotype, regardless of whether labor was term or preterm; however, the number of M2-like macrophages was reduced in the decidua of women with preterm pregnancies, irrespective of whether they were laboring.

Role of uNK cells in uterine vascular remodeling

The concept of trophoblast-independent vascular remodeling was proposed in 1998 by Craven et al,[28] who noted subtle changes in spiral artery structure during the secretory phase of the menstrual cycle and in the first trimester of pregnancy, prior to the arrival of extravillous trophoblast. More recent investigations using first-trimester placental bed biopsies and ex vivo first-trimester tissue culture models have ascertained that the presence of uNK cells and macrophages in close proximity to the spiral arteries is associated with vasodilatation, swelling or loss of the endothelium, disruption of the medial vascular smooth muscle layers, and vascular cell vacuolation.[29,30] Both cell types are observed in pericellular and intramural locations, and their presence directly correlates with these changes; uNK cells associate with the spiral arteries for a limited period, departing prior to substantial vascular cell loss, whereas macrophages remain in and around the vessels for a longer time, consistent with their role in tissue remodeling and clearance of cellular debris.

In 2009, Smith et al[29] proposed a temporal sequence of events in which the early, trophoblast-independent phase of remodeling that occurs during the first trimester, is characterized by leukocyte infiltration of the spiral artery wall, matrix metalloproteinase (MMP) secretion, disruption of SMCs layers, and vascular cell detachment and loss, mediated at least in part by apoptosis. Significant loss of vascular extracellular matrix components, including laminin, elastin, fibrillin, and collagen types III, IV, and VI has also been observed.[31] Subsequent to these priming events, endovascular and interstitial extravillous trophoblast colonize and line the vessels, which become recovered with vascular endothelial cells (ECs) later in gestation.[32,33]

More detailed investigation of uNK cell phenotype in early pregnancy has identified a number of mechanisms by which they mediate vascular transformation. First trimester uNK secrete VEGF-C, angiopoietin-1 and -2, interferon-gamma (IFN-γ), and MMP-2, -7, and -9,[34] all of which induce vascular SMCs disruption in isolated spiral artery segments ex vivo, either when applied individually, or in the form of uNK-conditioned culture medium.[31]

Decidual macrophages secrete a similar, but not identical, repertoire of soluble factors, including MMP-1, -2, -7, -9, and -10, a number of interleukins, angiogenin, keratinocyte growth factor, fibroblast growth factor B, VEGF-A and angiopoietin-1 and -2.[35] First-trimester decidual macrophage-conditioned medium does not induce vascular cell disorganization ex vivo, but isolated primary first-trimester macrophages are capable of phagocytosing apoptotic vascular SMCs and initiating breakdown of laminin and fibronectin.[35]

Coculture experiments have shown that first-trimester uNK cells can induce apoptosis of vascular SMCs and ECs, via Fas-Fas ligand interactions.[36] uNK cells also promote trophoblast motility, invasion, and first-trimester extravillous trophoblast outgrowth, in part via secretion of hepatocyte growth factor.[36,37]

The complex regulatory cross talk that occurs between first-trimester spiral artery ECs, decidual leukocytes, and extravillous trophoblasts has recently been investigated by Choudhury et al,[38] who tested the hypothesis that extravillous trophoblast within the placental bed stimulates the vascular endothelium of unremodeled spiral arteries to secrete chemokines that attract leukocytes, initiating trophoblast-independent remodeling events. They showed that when exposed to first-trimester trophoblast-conditioned culture medium, ECs upregulate production of the chemokines C-C motif ligand 14 (CCL14) and C-X-C motif ligand 6 (CXCL6), which are chemotactic for first-trimester uNK cells and macrophages.[38]

Analysis of first-trimester decidua demonstrated that CCL14 and CXCL6 are expressed by the endothelium of remodeling spiral arteries and that the surrounding uNK cells and macrophages express the cognate chemokine receptors. First-trimester, trophoblast-derived interleukin (IL)-6 and CXCL8 were identified as the secreted factors responsible for inducing endothelial chemokine production. Although the triggers for leukocyte infiltration into the spiral arteries require further investigation, this study has identified one possible mechanism by which this could occur. The potential for spiral artery ECs to respond to soluble trophoblast-derived signals and begin to secrete chemokines that recruit uNK cells and macrophages is depicted in Figure 1.

Role of other leukocytes subtypes in the placental bed

Regarding the presence of other leukocyte subtypes within the placental bed, $CD8^+$ T lymphocytes are present in first trimester decidua, along with $CD3^+$ and $CD3^+CD4^+$ T cells, but in much lower numbers than macrophages and uNK cells. These cells exhibit a mixed profile of T cell dysfunction, activation and effector functions throughout gestation, proposed to promote both immune tolerance and immunity.[39] Dendritic cell (DCs) subpopulations are also observed, including $CD205^+$ intermediate cells, immature $CD209^+$ cells and mature $CD83^+$ DCs.[23] The role of T cells and DCs in spiral artery remodeling is as yet unknown, although reduced numbers of decidual $CD3^+$ T cells, $CD8^+$ T cells, $CD4^+$ T cells and Fox-$p3^+$-Regulatory T cells (Tregs) have been reported in PE.[40] The percentage of T cells is increased in term decidua compared to first trimester decidua,[41] but onset of labor is associated with a reduction in the number of decidual $CD3^+CD25^+$ and $CD3^+$HLA-DR^+ T cells[42] and an increase in the number of $CD3^+CD4^+CD45RO^+$ and $CD3^+$ $CD4^-CD8^-CD45RO^+$T cells.[43]

Given the ethical and technical challenges of studying early placental development and uterine spiral artery remodeling in humans, we believe that significant scientific developments will be reliant on further technological advances. Improved imaging systems, advanced 3-dimensional culture models, and better stratification of pregnancy pathologies will allow a deeper understanding of the complex mechanisms at play within the placental bed.

Immunological tolerance in pregnancy

To establish and maintain a successful pregnancy, a semiallograft must be tolerated by the maternal immune system; pregnancy involves mechanisms to prevent allograft rejection.[48] Dysregulation of the immune system may lead to reproductive failure or complications such as recurrent pregnancy loss,[44] implantation failure,[45] IUGR,[46] and PE.[47,48]

An interesting study evaluated the rate of placental complications including PE, gestational hypertension, and IUGR, occurring in women who conceived through in vitro fertilization using donor vs autologous oocytes.[49] The authors concluded that patients conceiving through oocyte donation, after adjustment for maternal age, gravidity, parity, and chronic hypertension had an increased risk of hypertensive disease. Impaired spiral artery remodeling has also been observed in women conceiving using donor oocytes, even in the absence of PE.[40] These findings support the theory of an immunological mechanism in the genesis of PE, suggesting that altered immunological tolerance between the mother and the fetus may play a role.

Several immunological mechanisms have been proposed for the development of tolerance to the semiallogeneic fetus. It has been suggested that miscarriage occurs when these tolerance mechanisms become dysregulated.[50] Because maternal alloreactive lymphocytes are not systemically depleted, it has been hypothesized that local mechanisms may act to avoid maternal immune attack.[51]

Maternal leukocytes, in particular T cells, NKs, and innate lymphoid cells (ILCs), are an important component of the decidua and several strategies have been identified by which the trophoblast avoids maternal recognition.[52-57] These include special features, such as low tryptophan levels, the expression of major histocompatibility complexes HLA-G/C inducing a lack of activation of uNK cells, the absence of classical HLA class I and class II trophoblast expression, high progesterone levels, and anti-idiotype network modulation, all playing an important role in immune tolerance of the fetus.

In addition, maternal T cell recognition of fetal antigens occurs in an indirect manner; this means that the fetal allograft is ignored by directly alloreactive T cells that cause acute transplant rejection.[58] ILCs, as reviewed by Vacca et al,[57] are a heterogeneous group of cells that lack genetically rearranged antigen receptors and derive from common lymphoid progenitors. Five major groups of ILCs have been defined based on their cytokine production pattern and developmental transcription factor requirements: NKs, ILC1s, ILC2s, ILC3s, and lymphoid tissue-inducer cells. ILCs produce cytokines involved in defense against pathogens, lymphoid organogenesis, and tissue remodeling. During the first trimester of pregnancy, decidual tissues contain high proportion of dNK cells, representing up to 50% of decidual lymphocytes, and ILC3s. They release peculiar cytokines and chemokines that contribute to successful pregnancy.

Despite the presence of various mechanisms of immune evasion, the maternal adaptive immune system can recognize paternal alloantigens,[59] and, as a study published in 2004 has reported, pregnancy induces cytotoxic T cells specific for minor histocompatibility antigens.[60] Fetal-specific T cell responses develop as a consequence of normal human pregnancy[61] and unlike in murine models, T cells specific for fetal alloantigens are not deleted during human pregnancy.

Fetal-specific cells demonstrated an effector memory phenotype, were broadly functional, and showed proliferation, IFN-γ secretion, and the ability to lyse target cells following recognition of processed male antigens.[62] T helper (Th)1/Th2 imbalance may contribute to placental bed pathology, given that the predominance of Th2 type immunity is believed to promote survival of the fetus in the uterus.[63] Women with recurrent pregnancy loss show an increased Th1 to Th2 cell ratio and increased IFN-γ production compared with normal healthy women.[64]

In addition, Th17 cells are positively associated with idiopathic recurrent pregnancy loss.[65] Increased Th2 polarization of maternal peripheral blood mononuclear cells and decreased IFN-γ secretion are associated with normal pregnancy as reviewed by Saito et al.[66] Tregs also play an important role in early pregnancy.[67] The role of Th2 cytokines in normal pregnancy has not yet been elucidated because studies on genetically deficient mice that lack the ability to secrete Th2 cytokines did not always lead to miscarriage.[68] Therefore, other mechanisms, such as the function of Tregs, may regulate alloreactive Th1 cells. Giving that progesterone promotes the differentiation of human cord blood fetal T cells into Tregs, but suppresses their differentiation into Th17 cells, a fine balance between Tregs and Th17 cell populations is associated with maternal tolerance to the fetus.[69] Increased numbers of Tregs are observed in normal pregnancy, and both reduced Tregs numbers and impaired Tregs function are associated with reproductive defects, such as recurrent miscarriage and PE as reviewed by Zenclussen.[56]

These observations have led to the suggestion that the suppressive activity of Tregs is impaired in inflammatory conditions.[70] Tregs are not the sole population of immunosuppressive cells in the decidua: recently other immune cell populations with regulatory functions have been found at the fetomaternal interface and play critical roles in pregnancy maintenance. It has recently been shown that regulatory B10 cells (B10regs) are active in pregnancy through secretion of IL-10.[71-73] IL-10 is a suppressive antiinflammatory cytokine

that correlates with pregnancy outcome and has been proposed as a therapeutic agent because it prevents naturally occurring pregnancy loss in a mouse model.[74]

Two main populations of B cells have been distinguished, based on cellular markers, function, and localization.[75] In the past, the role of B cells in pregnancy was confined solely to antibody production, because during normal pregnancies protective antibodies are increased compared with nonpregnant women.[76,77] In addition to antibody production, B cells have emerged as regulators of immune responses.[78] B10regs in particular are potent immunoregulatory cell subtypes with the ability to produce IL-10.[79] Indeed, most of the suppressive functions of regulatory B cells are directly attributed to their capacity to produce IL-10.[80]

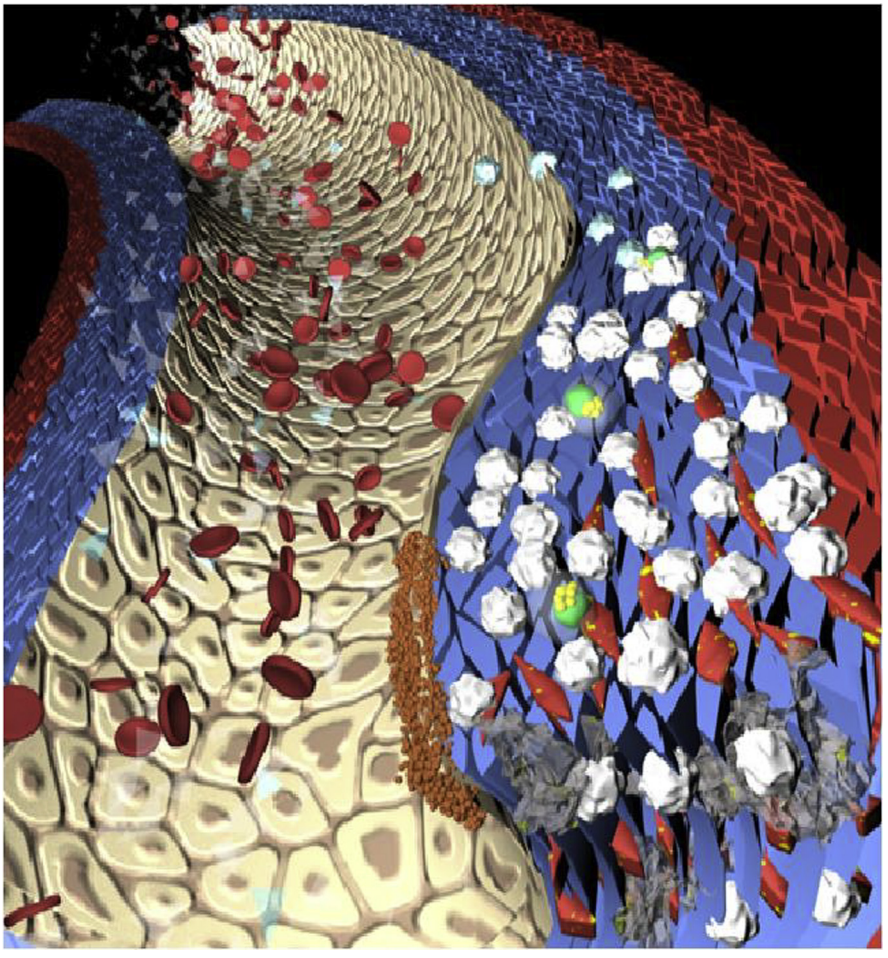
Recurrent spontaneous abortion is associated with elevated levels of the proinflammatory cytokines such as TNF-α, and blocking of TNF-α has been suggested as a possible treatment.[81] Regulatory B10 cells inhibit secretion of TNF-α by activated T cells in several pathological situations.[82] A second T cell lineage, named $\gamma\delta$T cells, is also present in the endometrium during pregnancy in all mammals.[83,84] The $\gamma\delta$T cells are increased in the peripheral blood and decidua of healthy pregnant women compared with nonpregnant women and are able to produce IL-10 and TGF-β at high levels.[85-87] These cells, in particular Vδ1-T cells, acting as type 1 immune response inhibitors, seem to contribute to Th2 bias at the maternal-fetal interface, thus favoring maternal tolerance.[88,89]

NKT cells are also detected in mouse and human decidua, and they are related to early[90] and late pregnancy complications.[91-93] There is increasing evidence that the interaction between dNK and Treg cells could be helpful during pregnancy.[94,95] Interestingly, 70% of all human decidual lymphocytes are NK cells, defined as uNK or dNK cells.[96] A description of their functions in pregnancy is provided elsewhere.[2]

It has been shown that dNK cells have the potential to promote Tregs cell proliferation by interacting with $CD14^+$ cells in decidua.[97] Cross talk between dNK cells and decidual myelomonocytic $CD14^+$ cells has been shown in some experiments, resulting in the production of IFN-γ that induces the expression of the immunoregulatory enzyme indoleamine 2,3-dioxygenase, a suppressor of T cell response and a promoter of immune tolerance in mammalian pregnancy and in decidual $CD14^+$ cells. These $CD14^+$ cells could induce Tregs generation via the effects of indoleamine 2,3-dioxygenase and TGF-β.[97] TGF-β is an important cytokine for the differentiation of extra thymic Tregs. Interestingly, dNK cells produce TGF-β at the feto-maternal interface, and this TGF-β-producing cell population decreases in miscarriage.

These findings suggest that dNK cells may play a role in regulating the

FIGURE 2
Inflammation of the vessel wall



Immune system triggering results in a radical change in the homeostasis of the vessel wall, regardless of the type of stimulus (antigenic, endothelial damage). Activated T lymphocytes (white cells) and macrophages, that turn into foam cells (green cells with lipid drops) are found within the intima and are able to secrete a broad range of cytokines, chemokines, and growth factors that produce profound changes in endothelial cell (*blue*) and smooth muscle cell (*red*) behavior, leading to the inflammatory process and eventually to thrombosis (platelets, *brown*). If inflammation continues unabated, its outcome can have various consequences, including acute atherosis, accelerated atherosclerosis, and chronic processes, all a result of this pathogenic inflammatory mechanism.

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differentiation of Tregs in decidua.[98,99] Conversely, both *in* vitro and in vivo findings show that cytotoxic activity, cytokine production, and proliferation of NK cells can be regulated by Treg cells.[100] TGF-β has also been described as a factor capable of converting peripheral blood NK cells into dNK cells,[101] which exhibit high proliferation rates in vitro.

Myeloid-derived suppressor cells are immature developing precursors of innate myeloid cells that are increased in pregnant women, implying their possible function during gestation.[102] The number of functionally suppressive myeloid-derived suppressor cells decreases in the blood and endometrium of miscarriage subjects compared with women undergoing successful pregnancies, especially in the first trimester.[103]

Role of human decidual stromal cells in immunological tolerance

Clinical and experimental studies have revealed that decidual stromal cells (DSCs) and placental stroma cells (PSCs) may contribute to a tolerogenic microenvironment crucial for the establishment and maintenance of a successful pregnancy.[104] In particular, it seems that DSCs are involved in fetomaternal tolerance, and they are particularly abundant during the first trimester of pregnancy.[104] PSCs can be isolated from placentas at term and despite their different origin, they share phenotypic and functional features.[105,106] Similar to mesenchymal stem cells, DSCs and PSCs can display stem cell properties such as multilineage differentiation into 3 embryonic layers.[107]

Moreover, they can have an immunomodulatory effect on innate and adaptive immunity, both in humans and animals.[108] It has been demonstrated that in mice, upon decidualization, DSCs are able to downregulate the expression of inflammatory chemokines that attract T lymphocytes, thus contributing to fetomaternal tolerance.[108] In humans, DSCs have been shown to suppress T cell function by inducing Tregs differentiation.[109] Furthermore, peripheral blood $CD14^+$ monocytes cultured in the presence of PSCs differentiate into tolerogenic DCs expressing low levels of CD83 and CD86 and producing IL-10 but not IL-12. These dendritic cells fail to induce T cell proliferation while promoting T cell differentiation into $IL\text{-}4^+$ Th2 rather than IFN-γ-producing Th1 cells.[110]

The decidual microenvironment has been shown to influence the phenotypic and functional features of leukocytes during pregnancy. DSCs play an important role in this modulatory function, in particular the number of dNK cells appears to correlate with the hormone-induced decidualization of SCs during pregnancy.[111] The hormonal environment is able to induce upregulation of CXCL10, CXCL12, CX3CL1, and CCL2 in DSCs, which are responsible for the recruitment of peripheral blood NK cells into the decidual tissue.[112] Furthermore, DSCs are able to produce TGF-β, which induces changes in expression of NK cell surface markers, such as CD69 and KIR.[113]

DSCs are capable of inhibiting the IL-15-mediated upregulation of important activating NK receptors in peripheral blood-derived NK cells, thus exerting a potent inhibitory effect on NK proliferation, cytotoxicity, and IFN-γ production.[114,115] Moreover, DSCs constitutively produce prostaglandin E_2, which increases when cocultured with NK cells.[106]

Further evidence demonstrates that SCs from term fetal membrane (FMSCs) and umbilical cords (UCSCs) significantly suppress proliferation in mixed lymphocyte reactions (MLR).[116] When added to MLR cultures, FMSCs suppressed the production of IFN-γ and IL-17. Secretion of IL-10 was increased after the addition of FMSCs and UCSCs. When analyzing the expression of adhesion markers, FMSCs expressed the highest levels of CD29, CD49d, and CD54 compared with the other types of SCs. Thus, these data indicate that SCs isolated from term fetal membranes have significant immunosuppressive capacity in terms of reducing both proliferation and production of proinflammatory cytokines from alloreactive T cells and also promoting antiinflammatory IL-10 production.

SCs express high levels of integrins, which may be of importance in homing to inflamed tissues.[112] Overall, DSCs together with PSCs, Tregs, FMSCs, NKs, and UCSCs represent a complex and fascinating cellular network crucial for pregnancy success and may represent a future target for new immunotherapeutic strategies.

Placental bed pathology and future cardiovascular disease risk

Evidence is accumulating that vascular pathology of the placental bed can increase the risk of cardiovascular disease (CVD) later in life for both the mother and the offspring.

As previously highlighted,[117] inflammation of the vascular bed is the primary cause of atherosclerosis, present in various forms like acute atherosis, accelerated atherosclerosis,[118,119] and chronic processes, which are all reflective of a single pathogenic inflammatory mechanism.[120] The main event leading to this inflamed state, which alters the homeostasis of the vessel walls that can be observed in various forms (Figure 2), is the triggering of the immune system by an antigenic stimulus or endothelial damage, which implies vessel walls remodeling.[43,121]

Subsequent cardiovascular risk for the mother

To properly evaluate the long-term risk of CVD in women with vascular pathology of the placental bed, the sometimes-confused terminology utilized by various authors needs to be clarified. As detailed in the first part of this review,[2] this involves the need to distinguish between acute atherosis of the basal arteries with diffuse lipid infiltration of the stroma and atherosis or atherosclerosis (also named arteriosclerosis) of uteroplacental arteries that is the phenomenon linked with major obstetrical syndromes.[117] Acute atherosis may be linked with a range of events inside and outside the placental bed, and the lipid findings confirm the association between acute atherosis and fetal distress conditions.

In view of this distinction, the increased risk of future maternal

cardiovascular complications is linked to atherosis or, at a later stage, to atherosclerosis of the uteroplacental arteries, rather than to the acute atherosis of the basal arteries.

In a small series of pregnancies with IUGR but with no or only a moderate and transient rise in blood pressure, De Wolf et al[122] found that vascular lesions in the placental bed spiral arteries exhibited fewer developed physiological and morphological changes, extensive intimal thickening with atherosis, and fibrinoid degeneration of the media; they argued that recurrent IUGR in these pregnancies may be the first clinical manifestation of an underlying renovascular disease.

Some 10 years ago, a systematic review and meta-analysis by Bellamy et al[123] found an association between PE and future CVD and hypothesized that it might reflect a common cause of both conditions, an effect of PE on CVD development, or both. There is good evidence that prepregnancy chronic hypertension, even in a mild form, is associated with a high risk of severe gestational hypertension, early-onset PE, and IUGR. The authors concluded that a history of PE should be considered when evaluating women for risk of CVD later in life.

Recently Veerbeek et al[124] investigated vascular and inflammatory lesions in placental bed biopsies, comparing healthy pregnant women with those with PE. As expected, they found a significantly lower proportion of completely remodeled spiral arteries in women with PE than in the control group (21% vs 68%; $P = .008$). Their findings included a correlation between acute atherosis and higher plasma triglyceride and low-density lipoprotein cholesterol levels, suggesting a correlation between vascular alterations in pregnancy (atherosclerosis of uteroplacental arteries and acute atherosis) and an increased risk of CVD later in life. This study provides the first valuable insight into the link between cardiovascular health and placental bed disorders.

Another recent retrospective analysis evaluated a population-based, clinically enhanced database of women in the state of Florida, including nulliparous women and girls aged 15−49 years experiencing their first delivery with no prepregnancy history of diabetes mellitus, hypertension, or heart or renal disease.[125] They compared the risk of subsequent CVD among women who experienced a placental syndrome during their first pregnancy vs those who did not. After reassessing the risk, they found that women who experienced placental syndromes and preterm labor or who delivered small-for-gestational-age infants were at increased risk of subsequent CVD in a short-term follow-up.

Nzelu et al[126] showed that the rate of development of severe CVD in pregnancy is related to antihypertensive medication use and blood pressure at the first hospital visit during the first trimester. The findings seemingly indicate that management of hypertension from early pregnancy can prevent severe complications.

Subsequent cardiovascular risk for the offspring

Bertagnolli et al[127] explored the evidence for developmental programming of the cardiovascular system in utero and the potential mechanisms linking preterm birth to the risk of hypertension and cardiovascular diseases into adulthood. The detection of elevated blood pressure in preterm individuals reaching adulthood has turned the attention to preterm birth-related complications and deleterious conditions as factors triggering early cardiovascular alterations, which may increase hypertension risk and associated CVD in this population. Furthermore, preterm birth is frequently associated with pregnancy complications, such as reduced placental perfusion, PE, and/or increased blood pressure in the mother, often resulting in IUGR. These conditions have a further impact on the risk of hypertension in the offspring, whether through inherited genetic factors or perpetuated pathophysiology leading to PE, preterm delivery, and chronic hypertension in adulthood.

As previously highlighted,[117] inflammation of the vascular bed is the primary cause of atherosclerosis, present in various forms like acute atherosis, accelerated atherosclerosis,[118,119] and chronic processes, which are all reflective of a single pathogenic inflammatory mechanism.[120] The main event leading to this inflamed state, which alters the homeostasis of the vessel walls (Figure 2) that can be observed in various forms, is the triggering of the immune system by an antigenic stimulus or endothelial damage, which implies vessel walls remodeling.[44,121]

As previously mentioned, the fetus is a semiallograft within the woman's body and a series of mechanisms result in tolerance of the graft by the maternal immune system. Nevertheless, in pathological pregnancies it could be hypothesized that a breakdown of tolerance may be the antigenic trigger that, together with other risk factors, can elicit the immunopathological processes leading to PE and CVD or an effect of PE on disease development or both. Taken together, these observations highlight that these immunopathological conditions can be responsible of future cardiovascular events.[128]

Summary

The anatomical and physiological changes that take place within the placental bed in early gestation establish the conditions for pregnancy success and, if suboptimal, can have an impact on both placental function and fetal growth for the duration of the pregnancy. Impaired uterine spiral artery remodeling can result in aberrant blood flow into the intervillous space, which can be detected as abnormal Doppler resistance indices and is considered an independent risk factor for pregnancy complications.

Maternal immune cells, in particular uNK cells and macrophages, play a key role in modulating vascular transformation, which is mediated both by direct cellular interactions and via secretion of a range of soluble factors. In addition, T cells, uNK cells, and ILCs facilitate immune evasion by the trophoblast, preventing recognition of paternal alloantigens and rejection of the conceptus; placental and decidual SCs also promote immune tolerance.

Finally, pregnancy is now being recognized as a biological stress test, whereby undiagnosed or subclinical levels of cardiovascular disease or altered immune responses may predispose pregnant women to complications; clinical management should therefore involve consideration of future cardiovascular disease risk for both affected mothers and their offspring. ■

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