Innate Immunity, Decidual Cells, and Preeclampsia

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Abstract

Preeclampsia (PE) manifested by hypertension and proteinuria complicates 3% to 8% of pregnancies and is a leading cause of fetal–maternal morbidity and mortality worldwide. It may lead to intrauterine growth restriction, preterm delivery, and long-term sequelae in women and fetuses, and consequently cause socioeconomic burden to the affected families and society as a whole. Balanced immune responses are required for the maintenance of successful pregnancy. Although not a focus of most studies, decidual cells, the major resident cell type at the fetal–maternal interface, have been shown to modulate the local immune balance by interacting with other cell types, such as bone marrow derived-immune cells, endothelial cells, and invading extravillous trophoblasts. Accumulating evidence suggests that an imbalanced innate immunity, facilitated by decidual cells, plays an important role in the pathogenesis of PE. Thus, this review will discuss the role of innate immunity and the potential contribution of decidual cells in the pathogenesis of PE.

Keywords

innate immunity, decidual cell, preeclampsia, immune cell

Introduction

Preeclampsia (PE) is characterized by the development of hypertension and proteinuria after 20 weeks of pregnancy and is a leading cause of fetal–maternal morbidity and mortality.^{1,2} PE is a systemic inflammatory disease that may lead to multiple maternal organ damage in liver, kidneys, lungs, and central nervous system.³ In addition, the long-term risks of cardiovascular, cerebrovascular, and renal diseases are increased in women with a history of PE.^{4,5} Fetuses in pregnancies complicated with PE are at risk of placenta abruption, intrauterine growth restriction, and preterm birth.⁶ Furthermore, an increasing body of evidence indicates that PE-related prematurity predisposes to chronic lung diseases, cardiovascular diseases, and intellectual and behavioral problems in the child's later life.^{7–9} Delivery of the placenta remains the only effective treatment for PE. Most of the symptoms disappear within a week after delivery. Thus, the decision to deliver warrants thoughtful consideration of the balance between maternal well-being and fetal maturity. A number of biological (fetal cells, cell-free fetal DNA, and RNA), biochemical (angiogenic factors, placental protein 13, pregnancy-associated plasma protein A, and pentraxin 3), and biophysical markers (uterine artery Doppler and arterial blood pressure) have been proposed to predict PE.¹⁰ However, none of these markers, alone or in combination, demonstrate satisfactory effectiveness for predicting PE.

To date, the pathogenesis of PE remains unknown. The prevailing hypotheses focus on abnormal placentation (shallow

trophoblast invasion and impaired vascular remodeling) in early pregnancy¹¹ that leads to the release of specific molecules (eg, soluble fms-like tyrosine kinase 1 [sFlt-1]) into circulation. Ultimately, symptomatic systemic inflammation and endothelial dysfunction develop.^{3,6} Balanced immune responses are required for tolerating the fetal semi-allograft yet defending against pathogens. Immune maladaptation at the fetal–maternal interface is suggested to play an important role in the pathogenesis of abnormal placentation.¹² Innate immunity, the first line of host defense, plays a pivotal role in recognizing and reacting to the invading signals. Aberrant infiltration of antigen-presenting cells ([APCs] ie, macrophages [MΦs] and dendritic cells [DCs]) has been demonstrated in preeclamptic decidua.¹³ Interestingly, decidual cells, the major resident cells at the fetal–maternal interface, are shown to secrete an array of

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cytokines and chemokines that are crucial in modulating local immune responses.¹⁴ In this review article, we will discuss the roles of various components in innate immunity in the pathogenesis of PE. The interactions between decidual cell and these components in the pathogenesis of PE will also be reviewed.

Immune System and Innate Immunity

The host defense against immune challenges requires coordination between innate and adaptive elements of immunity. As the first line of defense against exogenous challenges, the innate immune system launches an initial, nearly instantaneous, and relatively nonspecific response to potential pathogens at the site of invasion by granulocytes, MΦs, DCs, and natural killer (NK) cells. Phagocytosis, recognition of the pathogen by pattern-recognition receptors, and cytotoxicity play major roles in mediating the functions of these cells.¹⁵ The subsequent highly specific, albeit slower, adaptive immune response, composed of humoral and cellular arms, provides a far more efficient and long-term suppression of pathogens via B and T lymphocytes. While an innate immune response does not require a preceding or simultaneous immune response, an adaptive response does require a leading innate response. Both MΦs and DCs are the two primary APCs that play integral roles in synchronizing both elements of the immune system. The APCs, particularly DCs, are also inducers of immune tolerance by secreting anti-inflammatory cytokines, activating regulatory T cells (Treg cells), or inducing T cell anergy.^{16–18} Thus, MΦs and DCs are uniquely capable of modulating the balance between the innate and adaptive immune systems to provide protection against pathogens yet confer immune tolerance.^{19,20} Impaired cross talk among the different components in the immune system plays an important role in the pathogenic mechanisms and may affect the severity and outcome of diseases associated with immune maladaptation, such as PE.

Pregnancy and Immunity

Successful pregnancy requires coordination between a receptive endometrium and the implanting blastocyst. The human uterus is an immune-modulated site that segregates the implanted semi-allogeneic embryo from an aggressive maternal immune response defending against pathogens. The exact mechanism of maternal tolerance to the fetal semi-allograft remains unclear. The coordination of various mechanisms is required to ensure effective tolerance. Upon fetal antigen presentation by decidual APCs (mainly DCs), Treg cells are postulated to execute fetal protection^{21–23} by either cell-to-cell contact via inhibitory molecules, such as cytotoxic T lymphocyte antigen-4²⁴ and programmed cell death 1,²⁵ or secretion of such immunosuppressive cytokines as interleukin (IL)-10, leukemia inhibitory factor (LIF), transforming growth factor β (TGF-β), and heme oxygenase 1.²⁶ Moreover, galectin 1 secreted by Treg cells may facilitate activated T cell apoptosis and promote anti-inflammatory response.^{27–29} In addition, the unique expression of human leukocyte antigen (HLA)-G,

Figure 1. The Th1/Th2 paradigm and immune balance in pregnancy. The proinflammatory Th1 immune response is responsible for extravillous trophoblast invasion, parturition, and host defense. The anti-inflammatory Th2 reaction is crucial for tolerance to the fetus, decidualization, and remodeling. Imbalance of immune response will lead to adverse pregnancy outcomes such as preeclampsia and miscarriage. Th1 indicates type 1 CD4⁺ helper T.

a nonclassical major histocompatibility complex (MHC) class I molecule, by extravillous trophoblasts (EVTs) is demonstrated to be immunosuppressive via its interaction with decidual immune cells.³⁰ Furthermore, indoleamine 2,3-dioxygenase (IDO) expressed by EVT and decidual cells^{31,32} is known to catabolize tryptophan to produce kynurenine and picolinic acids that inhibits the cytotoxicity of T cells and NK cells.^{33,34} It is an imbalance of these immune responses that results in insufficient tolerogenesis to the fetus and leads to adverse pregnancy outcomes, such as miscarriage and PE.^{35–37}

A well-regulated cytokine network is crucial for normal immune reactions. Cytokines produced by type 1 CD4⁺ helper T (Th1) cells are primarily proinflammatory and promote protection against infectious pathogens while that of type 2 CD4⁺ helper T (Th2) cells are mainly anti-inflammatory and responsible for the regulation of humoral responses. During pregnancy, an array of cytokines are also shown to be expressed by both decidual cells and EVTs in addition to the immune cells.^{14,38} Pro- and anti-inflammatory immune responses are both postulated to be required for gestation.³⁹ For instance, maternal tolerance to the semi-allogeneic fetus is thought to be related to Th2 immune response, affected by IL-4, IL-5, IL-10, and TGF-β.⁴⁰ However, proinflammatory Th1 immune response, regulated by IL-1, IL-6, IL-12, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ), are crucial for trophoblast invasion, parturition, and defense against infections.^{41,42} Therefore, the “balance” between the Th1/Th2 immune responses is mandatory for normal pregnancy. Dysregulation of either arm may result in pathological conditions, such as PE (Figure 1).⁴³

Decidual Cells

Decidual cells are the main resident cell type at the fetal–maternal interface, composing 40% of all the cells.⁴⁴ They are derived

Figure 2. Decidual cells in normal pregnancy. As the major cell type encountering the invading extravillous trophoblasts, decidual cells play important roles in modulating implantation of the blastocyst, extravillous trophoblast (EVT) invasion, maintenance of oxidative stress resistance, tissue hemostasis, immune responses at the fetal–maternal interface, and their own decidualization by autocrine and paracrine effects.

from endometrial stromal cells in response to progesterone during the “window of implantation” in the secretory phase of the menstrual cycle and exist throughout pregnancy. The decidualization of endometrial stromal cells is mandatory for successful pregnancy.⁴⁵ Decidual cells are the major cell type encountered by the invading EVTs. Accumulating evidence shows that decidual cells play numerous important roles in modulating implantation of the blastocyst, EVT invasion, maintenance of oxidative stress resistance, tissue hemostasis, immune responses at the fetal–maternal interface, and their own decidualization by autocrine and paracrine effects (Figure 2). For instance, decidual cell-secreted proprotein convertase 6, LIF, and IL-11 are upregulated during decidualization and embryo implantation,^{46,47} while deficiency results in implantation failure in mice.^{48–50} Decidual cells express stimulatory IL-1 β ^{51,52} and inhibitory TGF- β and LIF^{52,53} to modulate the balance of metalloproteinase (MMP)/tissue inhibitor of metalloproteinase and urokinase plasminogen activator (uPA)/plasminogen activator inhibitor 1 (PAI-1) systems that are involved in the degradation and remodeling of extracellular matrix in decidua during EVT invasion.^{54,55} In addition, CX3CL1, CCL4, CCL14, CXCL12, and IL-11 secreted by decidual cells also play roles in controlling EVT invasion with unknown mechanisms.^{56–59} Decidual oxygen pressure suddenly increases during placentation and leads to the generation of harmful reactive oxygen species after tissue oxygen consumption.⁶⁰ Free radical scavengers, such as superoxide dismutase 2, antioxidative enzyme glutathione peroxidase 3, and GADD45 α protein expressed by decidual cells may counteract the oxidative stress.^{45,61} During decidual tissue and vascular remodeling, tissue factor is highly expressed by decidual cells which promotes

Figure 3. Decidual cells and the pathogenesis of adverse pregnancy outcomes. In the pathologic setting of inflammation and hemorrhage, decidual cells play important roles in mediating the pathogenesis of adverse pregnancy outcomes. PPROM indicates preterm premature rupture of membranes.

physiologic hemostasis.⁶² Moreover, decidual cells contribute to the development of maternal immune tolerance to fetus by expressing Fas ligand (FasL) that induces apoptosis of activated T cells and IDO which suppresses T cell-dependent inflammatory responses to the fetus.^{32,61} Cytokines produced by decidual cells are also important for the recruitment of immune cells which are mandatory for tissue remodeling and tolerogenesis.^{14,63}

Impaired decidual cell function results in adverse pregnancy outcomes (Figure 3).^{13,14,63–70} For instance, PE is associated with decidual hemorrhage in which excessive thrombin formation by binding of decidual cell-derived tissue factor to circulating factor VIIa leads to an increased anti-angiogenic sFlt-1 production.⁶⁴ The aberrant immune cell infiltration observed in PE may attribute to dysregulated chemoattractant production by decidual cells.¹⁴ The modulation of EVT invasion by decidual cells may indicate its potential role in the pathogenesis of PE. Excessive MMP-1 and MMP-3 production by decidual cells may predispose to preterm delivery in chorioamnionitis.⁶⁵

Natural Killer Cells

NK cells are the major immune cell type in the decidua in mid-secretory phase of menstruation cycle and early pregnancy (up to 70%–75%).⁷¹ The number of NK cells gradually decreases as pregnancy progresses.^{72–74} Decidual NK cells accumulate around uterine spiral arteries with proximity to EVTs,^{71,75} indicating their potential role in modulating trophoblast invasion and vascular remodeling. The origin of decidual NK cells remains uncertain. Accumulated evidence suggests that these decidual NK cells may be chemoattracted from peripheral blood and/or differentiated in situ.^{76–78} The proximity of decidual NK cell distribution to the spiral arteries suggests their recruitment from the circulation.⁷⁹ Such chemokines secreted

by EVT cells as CCL3, CCL4, and CXCL12 are potential chemoattractants for decidual NK cells.^{80–82} Recent work from our laboratory suggested that decidual cell-derived chemokines, CXCL10 and CXCL11, may also be involved in such chemoattraction.⁸³ However, decidual NK cells are also found in both premenstrual endometrium without trophoblasts and endometrium in ectopic pregnancy, suggesting in situ differentiation of decidual NK cells.⁸⁴ In murine pregnancy, local differentiation of decidual NK cells has been demonstrated.⁸⁵ Also, presence of CD34⁺CD56⁺ hematopoietic stem cell in human adult endometrium has been reported.⁸⁶ Interestingly, an in vitro study by Keskin et al illustrated the conversion of peripheral blood NK cell into decidual NK cell phenotype after incubation with TGF- β 1.⁸⁷

Decidual NK cells are unique and phenotypically different from peripheral NK cells. The phenotype of decidual NK cells is predominantly CD56^{bright}CD16[−] expressing low levels of perforin and high levels of CD94/NKG2 receptor and adhesion-mediating L-selectin⁸⁸ while the major peripheral NK cell population is CD56^{dim}CD16⁺ expressing high levels of killer cell immunoglobulin-like receptors (KIRs) and CD57.⁸⁹ Decidual NK cells display increased immunoregulatory cytokine production but decreased cytotoxic ability compared with peripheral NK cells.^{90–92} The dominance of cytotoxicity or cytokine production of NK cells is determined by a balance of signaling from different cell surface receptors in response to different stimuli.⁹³ The NK cell receptors, including KIR, the C-type lectin heterodimer family (CD94/NKG2), the Ly49 homodimers, the NK cytotoxicity receptors, and the immunoglobulin-like transcripts (ILTs), are either stimulatory or inhibitory or both.^{94,95}

Decidual NK cells are known to produce important cytokines and angiogenic factors, including vascular endothelial growth factor (VEGF), placental growth factor, angiopoietin 2 (Ang-2), TNF- α , IL-8, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1 β , TGF- β 1, macrophage colony-stimulating factor (M-CSF), LIF, and IFN- γ , which are essential for a successful pregnancy.^{96–99} Both IL-8 and CXCL10 expressed by NK cells are shown to stimulate trophoblast invasion.¹⁰⁰ In murine pregnancy, decidual NK cell-derived IFN- γ was suggested to promote vascular remodeling by modifying gene expression in uterine vasculature.¹⁰¹ Recent studies showed that the production of IL-1 β , GM-CSF, IL-6, IL-8, and IFN- γ by decidual NK cells was increased from 8 to 10 weeks to 12 to 14 weeks during gestation.^{99,102,103} By contrast, the expression of Ang-2 and VEGF-C by decidual NK cells are found to be lower in 12 to 14 weeks compared with that in 8 to 10 weeks.¹⁰⁴ These gestational age-dependent changes of cytokine profile reflect the variability of NK cell function during different periods of pregnancy.¹⁰⁵ Besides the cytokines and angiogenic factors, decidual NK cells also secrete MMP2, MMP9, and uPA, which are important for the breakdown of extracellular matrix required for trophoblast invasion and vascular remodeling.^{106–108}

EVTs express HLA-C, HLA-G, and HLA-E, rather than HLA-A, HLA-B and MHC class II molecules seen in other human cells.^{109,110} The HLA molecules expressed by EVT

may potentially be recognized by NK cell receptors. HLA-C binds to KIR2DL1, KIR2DL2, and KIR2DL3.¹¹¹ Both CD94-NKG2A and CD94-NKG2C are HLA-E receptors.¹¹² HLA-G has high affinity to ILT1 and can be a ligand for KIR2DL4.^{113,114} The proximity of NK cells and EVT in the decidua¹¹⁵ indicates that the recognition of HLAs on the invasive EVT by decidual NK cells may regulate NK cell cytotoxicity as well as the production of various cytokines and angiogenic factors essential for successful pregnancy.¹⁰⁰ EVTs are likely protected from cytotoxicity by decidual NK cells through the recognition of HLA-G on EVTs by the inhibitory receptors (either ILT2 or KIR2DL4 or both) on decidual NK cells.^{114,116–120} The binding of HLA-G and KIR receptor is thought to produce cytokines and angiogenic factors, including TNF- α , IL-8, IL-1 β , and IFN- γ , which facilitate trophoblast invasion and vascular remodeling.^{100,121–123} Moreover, the interaction between HLA-E on EVTs and CD94/NKG2A on decidual NK cells has been postulated to inhibit killing of trophoblasts.¹²⁴

Although the distribution of immune cells in the decidua has been extensively investigated in recent years, the association of decidual NK cell infiltration and PE remains controversial. The numbers of NK cells have been found to be either increased or decreased in the preeclamptic decidua.^{125–130} The conflicting results may be due to different methods of isolation and the location of sampling. While PE is believed to exhibit a Th1 immune profile, the Th1/Th2 paradigm has been extended to NK cells (NK1/NK2) with polarization of cytokine secretion.^{131,132} Compared with normal pregnancies, an increased NK1/NK2 ratio in peripheral NK cell populations was found in PE.¹³³ Also, women with PE were shown to have a significantly lower percentage of peripheral CD56^{bright}/Nkp46⁺ cells than women with normal pregnancy.¹³⁴ Despite our recent observations revealing an increased secretion of NK cell-recruiting chemokine, CXCL10 and CXCL11, by proinflammatory cytokine-stimulated first trimester decidual cells,⁸³ little is known about the interactions between decidual cell and NK cells. Decidual NK cells, with their interaction with EVTs and the sequential alterations in the effector functions, may play a role in the pathogenesis of PE. PE is found to be more prevalent in pregnancies where maternal NK cells express KIR receptors with the inhibitory AA genotype and HLA-C2-expressing EVTs,¹³⁵ indicating the association of PE and a relatively inhibited NK cell effector function. Also, low HLA-G expression by EVTs has been demonstrated in the placenta from severe PE.¹³⁶ Furthermore, increased numbers of CD56⁺ and CD94⁺ cells with decreased placental IL-12 expression were found in preeclamptic decidua at delivery compared with healthy controls.¹²⁶ In contrast, serum IL-12 level was significantly elevated in women with PE.¹²⁶ Further studies are needed to clarify the potential impact of this NK dysregulation on the pathogenesis of PE.

Macrophages

Second to NK cells in the first trimester decidua, CD14- and CD68-expressing M Φ s^{71,137,138} consist of 20% to 25% of

decidual leukocytes. Macrophages mediate both innate and adaptive immunity. Through its versatility in presenting antigens to defend against invading pathogens and the induction of immune tolerance, MΦ plays an important role in the maintenance of normal pregnancy.^{139,140} Throughout human pregnancy, substantial numbers of decidual MΦs are in the vicinity of invading EVT_s,^{12,141} suggesting their role in mediating both normal and abnormal placentations as well as modulating the placental response to infection.^{142–145} The invasion of EVT_s into the decidua and inner third of myometrium transforms the uterine spiral arteries and arterioles into low-resistance, high capacitance vessels that provide sufficient exchange of gas, waste, and nutrients required for fetal-placental development.^{11,146,147} This vascular remodeling process is characterized by the loss of vascular smooth muscle cells and endothelial cells of the uterine spiral arteries and relining the vessel wall with EVT_s.⁵⁵ With their influx to the implantation site in early pregnancy,¹⁴⁸ MΦs modulate vascular remodeling and angiogenesis by secreting VEGF, MMPs, TGF-β, fibroblast growth factor (FGF), fibronectin, osteopontin, and collagen.^{149,150} Macrophages are also proposed to involve in the degradation of extracellular matrix of local tissue, which promotes EVT invasion.^{62,151} Furthermore, MΦs phagocytose apoptotic cells in the decidua during the invasion and remodeling process.¹⁵² Ingestion of these apoptotic cells may elicit Th2 cytokine secretion by MΦs that plays an important role in the initiation of immune tolerance.¹⁵³ The subsequent remodeling of the decidua is postulated to facilitate further EVT invasion.^{152,154} However, incomplete removal of apoptotic cells will lead to the release of intracellular contents from apoptotic bodies and the induction of proinflammatory response of APCs by the secretion of proinflammatory cytokines, such as TNF-α, IL-1β, IL-12, and IFN-γ,^{155,156} which can cause further tissue damage.^{157,158}

Compared with normal pregnancies, shallow trophoblast invasion and impaired spiral artery remodeling were noted in pregnancies complicated by PE.^{159–161} Aberrant infiltration of MΦs was found in the preeclamptic decidua,¹³ which supports the postulation that PE is related to excess inflammation.⁶ Macrophages can be recruited to the decidua by decidual cell- and trophoblast-secreted CCL2, CCL7, CCL4, CCL5, and CXCL8^{14,66,162–164} which are increased in preeclamptic decidua.¹³ A recent study demonstrated that under the stimulation of proinflammatory cytokines (IL-1β and TNF-α), first trimester decidual cells produce an array of MΦ-recruiting chemokines through activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and mitogen-activated protein kinase pathways. Among these first trimester decidual cell-secreted chemokines, CCL2 was shown to be the most potent one in recruiting monocytes/MΦs.¹⁶⁴ In addition, the increased production of GM-CSF by decidual cells in preeclamptic decidua also contributes to the differentiation of MΦs and their secretion of proinflammatory cytokines.¹⁶⁵ In this setting of excessive inflammation, trophoblast invasion has been shown to be limited by TNF-α produced by MΦs, possibly through the induction of trophoblast apoptosis.^{13,166,167} Also,

Figure 4. The role of macrophage in normal and preeclamptic pregnancies. With its great versatility, macrophages are important in maintenance of a successful pregnancy. However, if dysregulated, macrophages also contribute to the pathogenesis of preeclampsia. Dashed line indicates the mechanism is not confirmed.

activated MΦ-produced PAI-1 and inducible nitric oxide synthase exhibit inhibitory effect on trophoblast invasion and spiral artery remodeling.^{168,169} Our recent study further demonstrated that proinflammatory cytokine-stimulated first trimester decidual cells enhance MΦ-induced EVT apoptosis, which may account for abnormal placentation.¹⁷⁰ Moreover, decidual MΦs regulate placental angiogenesis by secreting VEGF¹⁷¹ which binds to fms-like tyrosine kinase-1 (Flt-1).¹⁷² Zhou et al demonstrated that the expression of VEGF and Flt-1 was dysregulated in severe PE.¹⁷³ In addition, the circulating level of sFlt-1, a competitive antagonist of VEGF produced by alternative splicing of Flt-1,¹⁷⁴ is elevated in preeclamptic women.^{175,176} In recent studies, MΦs are suggested to be an additional source of sFlt-1 which is expressed in response to lipopolysaccharides (LPS) treatment.^{177,178} Thus, an imbalance of VEGF signaling induced by MΦs is thought to influence placental angiogenesis and the pathogenesis of PE.¹⁷⁹

Macrophage exhibits its dual role in both promoting normal pregnancy (such as promotion of vascular remodeling and placental angiogenesis) and in contributing to the development of pathological pregnancies (such as inhibition of trophoblast invasion; Figure 4). These observations suggest the importance of “balance” between cytokine production and intercellular reactions at the fetal-maternal interface that determines the success of uneventful pregnancy. Similar to the concept of Th1/Th2 polarization in effector T cell function, MΦs are proposed to be categorized into either classically activated macrophage (M1) or alternatively activated macrophage (M2) subgroups according to their effector phenotypes in response to specific stimuli.^{144,156,180} M1 MΦs produce TNF-α and IL-12 and promote inflammatory process after activation by LPS or IFN-γ. In response to IL-4 stimulation, M2 MΦs express IL-10 and IL-13 and engage in tissue repair and

Figure 5. The role of dendritic cells in pregnancy. Under the influence of cytokines and recruiting chemokines such as GM-CSF, dendritic cells modulate host defense, decidualization, angiogenesis, and development of immune tolerance during implantation and pregnancy. Increased dendritic cell-recruiting chemokine expression and aberrant dendritic cell infiltration in preeclamptic decidua suggest the important role of dendritic cell in the pathogenesis of preeclampsia. GM-CSF indicates granulocyte-macrophage colony-stimulating factor.

tolerogenesis.¹⁵⁴ Although the M1/M2 classification provides better understanding and explanation for MΦ function, additional evidence is required to confirm their existence in tissue or specific disease such as PE.⁸⁸ Recently, Schonkeren et al illustrated an increased number of CD14⁺ cells in preterm PE placentas compared with idiopathic preterm control placentas. Also, the observations demonstrating the lower CD163⁺/CD14⁺ cell ratio (M2) and the higher CD209⁺/CD14⁺ cell ratio (M1) in preeclamptic placentas compared with control placentas complement the M1/M2 paradigm.¹⁷⁸

Dendritic Cells

Complementary to MΦs, DCs are the most potent APCs, which mediate innate immune response and subsequent adaptive immune response. Although DCs consist of 1% to 2% of decidual leukocytes,⁷⁹ they are crucial for the modulation of the decidual tissue remodeling (including decidualization and angiogenesis), immunologic defense against possible pathogens, and the induction of immune tolerance at the fetal-maternal interface (Figure 5).

During implantation, the fetal semi-allograft induces the production of GM-CSF by endometrial epithelial cells^{181,182} that plays an important role in the development of uterine DCs.¹⁸³ Through the synthesis of an array of pro-angiogenic (VEGF,¹⁸⁴ FGF-2,¹⁸⁵ TNF-α,¹⁸⁶ IL-6,¹⁸⁷ TGF-β,¹⁸⁷ CXCL8,¹⁸⁵ CCL2,¹⁸⁸ and endothelin 1¹⁸⁵) and anti-angiogenic (sFlt-1,¹⁸⁹ IL-12,¹⁹⁰ IL-18,¹⁸⁸ thrombospondin 1,¹⁹¹ and pentraxin 3¹⁹¹) molecules, DCs are suggested to foster the decidualization and angiogenesis required for successful implantation and subsequent placentation.^{189,192,193} In addition, TGF-β expressed by uterine DCs may suppress cytotoxic CD8⁺ T cell function and

promote the development of Treg cells, which play major role in immune tolerance.¹⁹⁴ Furthermore, recognition of HLA-G on EVTs by inhibitory receptors ILT2 and ILT4 on decidual DCs¹⁹⁵ conveys immunosuppressive signals and modulates anti-inflammatory cytokine production.¹⁹⁶ The function of DCs differs based on their maturity. Specifically, immature DCs are postulated to induce T cell anergy, whereas semi-mature DCs promote the development of CD4⁺CD25⁺Foxp3⁺ Treg cells that are responsible for the immune tolerance. Mature DCs exhibit strong antigen-presenting activity that promotes T cell activation and may contribute to the Th1 immune response in preeclamptic decidua.^{197,198}

In normal pregnancy, decidual DCs were demonstrated to be mainly immature DCs.¹⁹⁹ Under the influence of cytokines, such as GM-CSF, DCs modulate decidualization, angiogenesis, and development of immune tolerance during implantation and pregnancy. Either excess or deficiency in DC function may lead to adverse pregnancy outcomes. Deficient GM-CSF expression was shown to result in impairment of T cell activation by uterine DCs in mice, which may be related to the immune maladaptation found in miscarriage.²⁰⁰ Ablation of uterine DCs had been demonstrated to result in failure of decidualization, impaired implantation, and increased embryo resorption in mice.²⁰¹

The number of DCs and the levels of their recruiting chemokines (CCL2, CCL4, CCL7, and CCL20) have been shown to be elevated in preeclamptic decidua compared with gestation age-matched controls.¹³ However, different from MΦs, DCs did not show effect in impeding trophoblast invasion *in vitro*.¹³ Interestingly, an increased expression of GM-CSF, a potent differentiation inducer and activator of DCs, in preeclamptic decidua was demonstrated in both *in vivo* animal study and *in situ* human tissue staining.¹⁶⁵ In addition, the aberrant first trimester decidual cell-derived GM-CSF induced by proinflammatory stimuli has been shown to enhance the development of DCs.¹⁶⁵ Together, these observations suggest that the increased recruitment and activation of DCs by decidual cell-secreted DC-recruiting chemokines and GM-CSF in decidua play a critical role in the pathogenesis of PE (Figure 5).

Toll-Like Receptors

Both MΦs and DCs recognize the pathogenic molecules and “danger signals” via toll-like receptors (TLRs) expressed on the cell surface and in the cytoplasm. In addition to APCs, TLR2 and TLR4 are found to be expressed in first trimester decidual cells,²⁰² Hofbauer cells, villous cytotrophoblasts, and EVTs, but not in syncytiotrophoblasts, indicating the role of placenta as a barrier in protecting the fetus from infectious molecules.^{145,202–204} The binding of TLRs and danger signals will trigger the host immune responses, including immune cell recruitment, cytokine production, and the activation of adaptive immune response.^{205,206} As members of pattern-recognition receptors, 10 TLRs²⁰⁷ selectively recognizing different pathogen-associated molecular patterns have been identified.²⁰⁸ Specifically, TLR4 recognizes paclitaxel and LPS from gram-

Table 1. Decidual Immune Cells and Their Roles in Normal Pregnancy and Preeclampsia.

	Physiological	Preeclampsia
Macrophage	Antigen presentation for defense against pathogens Remove apoptotic cells produced during trophoblast invasion and vascular remodeling Th2 cytokine production that facilitate fetal–maternal tolerance Secretion of angiogenic factors promoting angiogenesis	Aberrant MΦ infiltration with increased MΦ-recruiting chemokines in decidua Inhibit trophoblast invasiveness Induction of trophoblast apoptosis under proinflammatory stimuli
NK cell	Production of an array of cytokines promoting trophoblast invasion, vascular remodeling, and angiogenesis Interaction with trophoblast HLA may contribute to maternal–fetal tolerance	Controversial about its number in PE More prevalent in maternal NK cell expressing KIR AA genotype and fetal trophoblast expressing HLA-C2 Increased production of Th1 cytokines
Dendritic cell	Antigen presentation for defense against pathogens Activation of regulatory T cells promoting maternal–fetal tolerance Regulate angiogenesis by secretion of pro- and anti-angiogenic molecules	Aberrant DC infiltration with increased DC-recruiting chemokines in decidua

Abbreviations: DC, dendritic cell; KIR, killer cell immunoglobulin-like receptors; PE, preeclampsia; NK cell, natural killer cell; KIR, killer cell immunoglobulin-like receptors; HLA, human leukocyte antigen; Th1, type 1 CD4⁺ helper T.

negative bacteria;²⁰⁹ while TLR2 binds to bacterial lipoproteins, peptidoglycan from gram-positive bacteria, lipoteichoic acid, and fungal zymosan.^{210–212} Besides the pathogens, TLRs may also be activated by endogenous or noninfectious “danger”-associated molecular patterns such as apoptotic cells, extracellular matrix components including fibronectin, oligosaccharides of hyaluronic acid, and heat shock protein.^{213–216} After ligand recognition, most TLRs trigger intracellular signaling pathway via adapter protein myeloid differentiation factor 88 (MyD88), which in turn activates the NFκB pathway leading to the production of inflammatory cytokines.²¹⁷ Both TLR3 and TLR4 are able to signal in a MyD88-independent manner through adaptor protein Toll/IL-1 receptor-domain-containing adaptor inducing interferon β (TRIF) pathway not only to activate NFκB cascade but also to result in secretion of interferon.²¹⁸

The excessive proinflammatory response associated with PE is proposed to be mediated by TLRs via the recognition of danger signals, including infectious pathogens and anti-phospholipid antibodies.^{219–222} An increasing body of evidence shows that infectious agents may predispose the proinflammatory status and abnormal placentation observed in PE, in which the TLR is playing a role.²²³ For instance, in vivo animal studies revealed that injection of low-dose endotoxin to pregnant rats induced PE-like pathological changes.²²⁴ Also, sustained activation of TLR3 was shown to trigger PE-like symptoms in rats.²²⁵ Studies involving TLRs and the pathogenesis of PE are focused on trophoblasts. Although TLRs are expressed on decidual cells, their functions and interactions with other local immune cells during the pathogenesis of PE are still unclear. The association of PE and maternal infection has been revealed by epidemiological studies showing elevated antibody titers for *Chlamydia pneumonia* and cytomegalovirus in patients with PE.^{226–230} In vitro studies showed that with the binding of TLR3 by poly (I:C) or TLR4 by LPS, the cytokine secretion by trophoblast was significantly increased and

subsequently lead to monocyte chemotaxis.^{162,231,232} Poly (I:C) stimulation of TLR3 on trophoblast was also found to provoke the production of anti-angiogenic sFlt-1.²³³ The expression of TLR4 was demonstrated to be elevated in trophoblasts from patients with PE.²³⁴ Recently, the correlation between single-nucleotide polymorphisms (SNPs) of TLR has been described. Both TLR2 and TLR4 SNPs are postulated to alter susceptibility to developing PE.²³⁵ Common mutations in TLR4 (D299G and T399I) and NOD2 (R702W, G908R and L1007fs) were demonstrated in patients with history of PE.²³⁶ However, the presence of SNPs of the TLR4 gene: Asp299Gly (A896G) and Thr399Ile (C1196T) were not significantly related to PE in a Caucasian population.²³⁷ Further studies are required in exploring the effect of SNPs on PE.

Summary

PE, characterized by maternal hypertension and proteinuria after 20 weeks of gestation, remains a major threat to maternal and fetal health during pregnancy. The pathogenesis of PE is believed to be multifactorial involving abnormal placentation, excessive oxidative stress, impaired angiogenesis, and immunological maladaptation. Decidual cells, one of the major cell types at the fetal–maternal interface, yet least studied, have been shown to play potential key roles in modulating cell interaction and function in recent studies. Innate immunity, being the first direct contact with the fetal semi-allograft, plays a crucial role in maintaining successful pregnancy by keeping maternal–fetal immune tolerance and protecting against possible pathogens. Various mediators of the innate immune response, coordinately or independently, exert differential functions in normal pregnancy and PE by interacting with decidual cells (Table 1). Through secretion of cytokines, decidual cells are shown to be involved in the aberrant infiltration of MΦs and DCs in the proinflammatory preeclamptic decidua.¹³

Functional studies demonstrated that proinflammatory cytokine-stimulated first trimester decidual cells contribute to excess trophoblast apoptosis and the impediment of trophoblast invasion via interaction with MΦs. Also, excessive thrombin formation resulting from binding of decidual cell-secreted tissue factor to factor VIIa cause production of sFlt-1, which is an important anti-angiogenesis factor found in PE. However, researches attempting to demonstrate the interactions between decidual cells, NK cells, and TLRs in the pathogenesis of PE are limited. Further integrated studies are required.

Although PE is the leading complication of pregnancy, the research of PE is hindered by several factors: (1) PE only occurs naturally in humans due to the unique process of human implantation; (2) its symptoms generally appear only late in pregnancy (third trimester), whereas its pathology is usually initiated in early pregnancy (first trimester); (3) despite intense research efforts, there are currently no reliable and conclusive markers identifying those women who will go on to develop PE; (4) ethical proscriptions prevent investigators from using humans as participants to study the pathogenic development of this disorder in the early stage of pregnancy. The majority of the present studies have focused on mechanisms involving a single immune cell type. Investigation of multi-cell interactions including decidual cells or study integrating different pathological mechanisms will provide insight into the establishment of novel diagnostic, therapeutic, and preventative strategies.

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