Best Practice & Research Clinical Obstetrics and Gynaecology 60 (2019) 87–96

Contents lists available at ScienceDirect

Best Practice & Research Clinical Obstetrics and Gynaecology

journal homepage: www.elsevier.com/locate/bpobgyn

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Immunomodulation and preeclampsia

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Keywords:
Preeclampsia
Immune system
Inflammation
NK cell
VEGF
PLGF

A B S T R A C T

Preeclampsia (PE) is an enigmatic syndrome, still with unknown aetiology and multi-factorial pathogenesis. Our understanding of the role of the immune system in PE development has undergone a transformation over the years. From a model based on the alterations in cell-mediated immunity, research moved on to a vision centred on the alteration of the humoural immunity and on the systemic involvement of the inflammatory system. The first hypothesis was classically derived from the evidence that an adequate maternal immunological response is necessary in pregnancy to allow the survival of the foetus. An abnormal response of the maternal immune system against the placenta may be the first pathogenetic step of PE, followed by a systemic inflammatory reaction. Currently available treatments for PE are mainly preventative with aspirin. Treatment aims to modulate inflammation and the immune system before their changes become established.

Introduction

The aetiology of preeclampsia (PE) is still unknown, and various mechanisms have been proposed based on the alteration of one or more systems, such as cardiovascular, coagulative, genetic and immunological, but none of these mechanisms individually can explain the entire phenomenon. The

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https://doi.org/10.1016/j.bpobgyn.2019.06.005
1521-6934/

hypothesis that the immune system is involved in the development of PE originates from the evidence that a maternal immunological response in pregnancy is necessary to allow the survival of the foetus as a semi-allogeneic implant. An abnormal response of the maternal immune system against the placenta may represent the first pathogenic step of PE, followed by a systemic inflammatory response involving the endothelium [1–3] (Fig. 1). Supporting evidence comes from a greater incidence of PE in women with autoimmune diseases and in foetal–placental pathology, although the mechanism is probably more complex.

The maternal immune system, when altered by underlying autoimmune pathologies, may interfere with the immunological adaptations for optimal placentation, increasing the risk of PE and intrauterine growth restriction [4,5]. A higher incidence of PE has been reported in women who conceive through oocyte donation, where the foetus is genetically wholly allogeneic; the risk of PE is more than double when compared with other methods of assisted reproductive technologies and more than four-fold increase when compared with spontaneous conception [6,7].

The risk of PE is increased also in pregnancies where there has been reduced exposure to paternal antigens such as nulliparous women, and women who have different partners in different pregnancies have long inter-pregnancy intervals, use barrier contraception and conceive through intracytoplasmic sperm injection. Change in paternity can increase the risk of PE if the prior pregnancy was normotensive and, on the contrary, can decrease the risk if a prior pregnancy was complicated by PE [8]. A prior pregnancy with the same partner may protect against PE even if terminating in spontaneous or induced abortion whereas a previous miscarriage with a different partner does not [9].

Altered immunomodulation: the first pathogenic insult of PE

The first pathogenic insult of PE is an abnormal immune response to the allogeneic foetus and presents similar immunologic features to graft versus host disease [1,2] (Fig. 2). The maternal preparatory immunological response to pregnancy is typically characterised by a T-helper type-2 lymphocyte response (Th2) (suppressor T-helper), which increases in proportion with respect to T-helper type-1 lymphocytes (Th1) (pro-inflammatory T-helper). This shift facilitates maternal tolerance by lowering the activity of cytotoxic cytokines usually secreted by Th1 cells [10,11]. The normal shift to Th2 is altered in PE and is characterised by a higher ratio of circulating Th1/Th2 lymphocytes [10] and a total number of Th1 cells similar to those of non-pregnant women [12] with a cytokine profile towards the pro-inflammatory cytokines such as IFN ɣ and IL-4 [13].

Uterine natural killer (uNK) cells are thought to promote the induction of Treg cells and suppress Th17 cells to favour materno-foetal tolerance [1,2,14–16]. Tregs are decreased both in the circulation and decidua of women with PE, and their level is associated with the severity of the disease [17]. Additionally, Th17 cells are higher than in non-PE pregnancy, resulting in an imbalance between Treg/Th17 with a pro-inflammatory phenotype and increased secretion of inflammatory cytokines [15]. Natural killer (NK) cells are classified based on the expression of surface markers CD56 and CD16. The concentration of CD56 bright cells is decreased in serum from mothers with PE, and this difference can

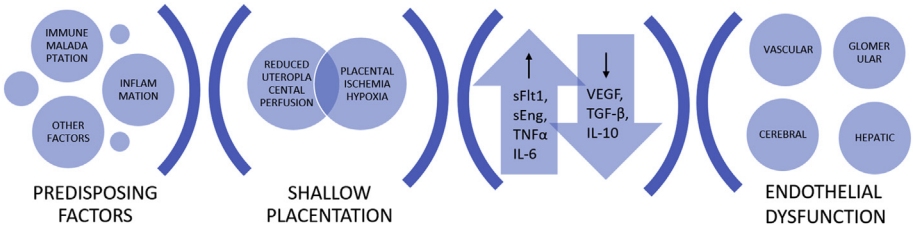

Fig. 1. The pathogenesis of PE originates from the presence of several predisposing factors, such as immune maladaptation, enhanced inflammation and others. These factors induce alterations of the placentation leading to reduced utero-placental perfusion, and placental ischemia and hypoxia. Placental damage leads to the alteration of cytokines and angiogenic factors that enter the maternal circulation and activate the endothelium. The endothelial systemic damage gives rise to PE clinical symptoms and can lead to multi-organ failure.

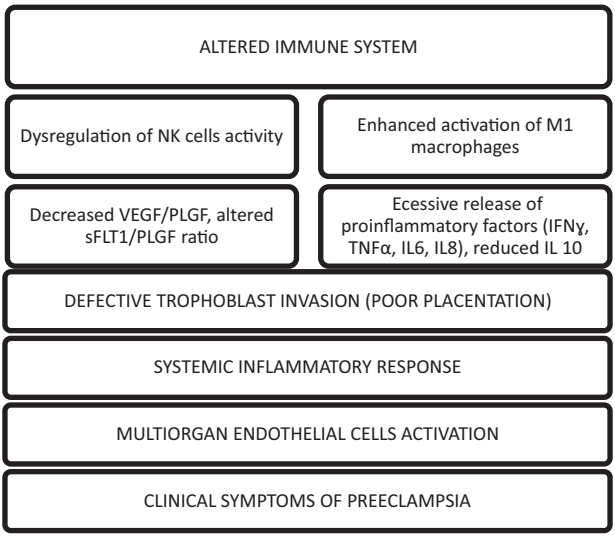

Fig. 2. The main executors of the immune system during pregnancy are NK cells and macrophages. Alterations in the function of these regulatory cells produce an imbalance in the production of angiogenic and anti-angiogenic factors, proinflammatory cytokines and anti-inflammatory cytokines. The resulting altered milieu induces defects in trophoblast invasion and placental damage that triggers a systemic inflammatory response with diffuse activation of the endothelium.

be found several months before the clinical diagnosis of the disease [18,19]. uNK cells are in close contact with extravillous trophoblast (EVT) at the foetal–maternal interface and regulate its invasion through the secretion of angiogenic factors such as vascular endothelial growth factor (VEGF) and placental growth factor (PLGF). In PE, the interaction between maternal and paternal genes is believed to induce abnormal placental implantation through increased NK cell activity. Patients with PE show a suboptimal interaction between uNK and the trophoblast, leading to an altered maternal spiral artery modification [20,21]. The action of uNK cells is regulated by the interaction of receptors that can be activators or inhibitors. Among these, the killer immunoglobulin-like receptor (KIR) has an important function inhibiting the production of cytotoxic cytokines and stimulating the production of angiogenic factors by uNK. The association between the polymorphisms KIR–AA in the KIR receptors on maternal NK cells and foetal HLA-C2 haplotype has been reported in pregnancies at increased risk of PE. This combination results in a strong inhibitor signal for the uNK function that would no longer be able to contribute to uterine vascular remodelling [22].

Macrophages are the second most abundant cells at the foetal–maternal interface. Macrophages surround the spiral arteries and promote remodelling by the secretion of pro-angiogenic cytokines such as VEGF and metalloproteinase and remove apoptotic cells [23]. It has been suggested that macrophage functional maturation is impaired in patients with PE, and a pro-inflammatory imbalance with a predominance of the phenotype M1 would be present. GM-CSF drives macrophage differentiation and the levels in decidual cells and plasma are higher in patients with PE than those with gestational-age matched controls [24].

The complement system is an essential component of innate humoural immunity. It is implicated in the clearance of pathogens, apoptotic cells, and immune complexes by forming a polymeric lytic pore that inserts into cells membranes, known as the membrane attack complex (MAC) [25]. Syncytiotrophoblast is able to regulate the complement system through the expression of regulatory proteins which interfere with MAC formation, avoiding cell lysis and preventing excessive complement activation [25]. Complement activation has been shown to stimulate monocytes to release anti-angiogenic factors [26]. A large body of evidence supports the hypothesis that complement dysregulation may be crucial in the pathogenesis of PE [27]. The association between PE, in particular early severe disease, and autoimmune diseases, especially systemic lupus erythematosus (SLE) and the anti-phospholipid

antibody syndrome (APS), supports this hypothesis [28,29]. Placental biopsies from pregnancies complicated by SLE and APS indeed show activation of classical complement activation [30,31]. Markers of alternative complement pathway activation have been found in maternal serum and urine in women with severe PE [32,33]. Even in the absence of pre-existing autoimmune disease, mutations in complement regulatory proteins have been associated with increased risk of PE [11,34].

The embryo also activates protective mechanisms against the mother's immune system. The EVT that migrates into the maternal decidua is poorly immunogenic and expresses an unusual combination of human leukocyte antigen (HLA) class I antigens. EVT hardly expresses the molecules of the main histocompatibility complex or the HLA-A or HLA-B that are primary stimulators of classical graft rejection and instead displays a unique pattern of non-classical HLA class IB antigens, the most prominent of which is HLA-G [1,2]. HLA-G is a smaller protein which has few alleles and shows no evidence of genetic imprinting [35,36]. HLA-G possesses immune-modulatory and tolerogenic functions, which protects the trophoblast during implantation. HLA-G and HLA-E mitigate the maternal immune reaction through a complex interaction with uNK, macrophages and $CD8^+$ cells, which lowers the killer effect of uNK cells and promote placentation [37,38]. In pre-eclamptic placentae, HLA-G expression was found to be absent or reduced [39]. Following placentation, during 8 and 18 weeks of pregnancy, the maternofoetal interface is characterised by the invasive cytotrophoblast, which expresses HLA-C that can signal paternal specificity [40]. This interface regresses in the second stage of pregnancy, before the clinical symptoms of PE arise [40].

Cytokines and systemic inflammatory response

The appropriate balance of cytokine and chemokine expression at the maternal–foetal interface influences the immune cell profile and function within the decidua. In PE, the regulation of immune responses is a result of aberrant activation of innate immune cells, leukocyte activation and imbalanced differentiation of T-helper cell subsets, which may account for elevated cytokine levels and the cytotoxic environment in utero [41]. In PE, the circulating levels of TNF-α and IL-6 increase, whereas the levels of IL-10 and IL-4 decrease [42]. The resulting imbalance leads to systemic inflammation and contributes to endothelial activation, which plays a role in the onset of the disease. TNF-α decreases the mRNA of nitric oxide synthase, reduces acetylcholine-induced vasodilatation and increases the production of the potent vasoconstrictor endothelin-1 (ET-1) from endothelial cells [43]. The levels of TNF-α, IL6 and ET-1 are increased by 2–3 fold in the circulation of women with PE compared with normotensive women or women affected by gestational hypertension and levels increase further with the progression of the disease [44,45]. Excessive TNF-α and IL-6 induce trophoblast apoptosis and increase endothelial activation [46]. Infusion of TNF-α or IL-6 in pregnant rats causes hypertension and reduced endothelium-dependent vascular relaxation [47]. IL-10 is an important anti-inflammatory cytokine involved in the regulation of the inflammatory response. Reduced levels of IL-10 have been found in the circulation and in the placenta of mothers affected by PE. IL-10 is secreted by Treg cells whose number is also decreased in PE [42,48]. Both Tregs and IL-10 are implicated in reducing the levels of Th1 cells, which produces TNF-α and IL-6.

The immune system also plays a role in the secretion of angiogenic factors. During normal pregnancy, uNK cells produce transforming growth factor beta (TGF β), which participates in immunoregulation and angiogenesis, together with angiogenic factors such as VEGF and PLGF; the increase in VEGF produced after the activation of uNK reacts on fms-like tyrosine kinase 1 (Flt1) receptors causing efficient EVT invasion. In PE, the total circulating level of VEGF is elevated, but the level of the soluble isoform of its receptor (sFLT1) increases more than the ligand [49]. The result is a reduction in free VEGF available for angiogenesis. VEGF is also important for endothelial stability so systemic inhibition can cause a generalised endothelial dysfunction [50,51]. PLGF is a pro-angiogenic factor of the VEGF family and binds with lower affinity to the same receptor FLT1. PLGF is overexpressed in pregnancy but is reduced in PE, and the reduction can precede the clinical onset of the disease, with an imbalance in the sFlt1/PLGF ratio. This altered balance due to the marked increase of sFlt1 neutralises the angiogenic activities of both VEGF and PLGF and may be responsible for defective EVT invasion in PE [52,53]. Plasma PLGF negatively correlates with total peripheral resistance and the uterine artery pulsatility index in PE [54]. Soluble endoglin (sEng) is an anti-angiogenic factor that inhibits TGF-β binding to its

receptors and downstream signalling including effects on activation of endothelial nitric oxide synthase and vasodilation [55,56]. SEng leads to dysregulated TGF-β signalling in the vasculature and may act in concert with sFlt1 to induce severe PE.

NK cells also produce IFNɣ that is specifically required for uterine artery remodelling [21]. In the second trimester, however, excessive production of pro-inflammatory cytokines such as IFN-ɣ and TNF-α, which are characteristic of PE inhibit trophoblastic migration, can be directly cytotoxic [57,58]. Of clinical interest, some of the agents described, especially PLGS and sFLT1, can be measured in the plasma of pregnant women during the long preclinical phase of PE, and can be used as biomarkers alone or in combination allowing the identification of women at risk or early diagnosis of the disease.

Assessment of the immune system in early pregnancy can be useful in predicting the risk of PE. The alternate complement pathway may be up-regulated and plasma levels of factor B-derived Bb fragment are higher in pregnancies with PE than in normotensive pregnancies [50]. Measurement of plasma levels of biomarkers such as VEGF, sFlt-1 and sEng might allow first trimester screening in asymptomatic pregnant women and may prevent PE onset. Later, in gestation, the same biomarkers can be useful for stratification of hypertensive patients in different categories according to the type of disease [59]. SEng and SFLt1/PLGF start to increase 2–3 months before the clinical onset of PE symptoms.

The possible predictive markers of PE are clinically relevant [60]. Markers associated with angiogenesis, both PlGF and sFlt-1, are consistently associated with the risk of PE. Serum levels of PlGF before 30 weeks have been shown to have an OR of 9.0 (95% confidence interval (CI) 5.6–14.5) in one large metanalysis [53] and OR 3.41 for early onset PE in a second study (95% CI 1.61–7.24) [59]. The sensitivity was 32% for a false positive rate of 5% [53]. For sFlt-1, the OR ranges from 1.3 (95% CI 1.02–1.65) to 6.6 (3.1–13.7) with a stronger association when tested later in pregnancy and a sensitivity of 26% for a false positive rate of 5% [53,61]. SEng and VEGF were not as consistently found to be associated with PE. The low sensitivity of each single marker led researchers to find a predictive model which could be more useful in clinical practice. Multiple investigators have used different combinations of variables in logistic regression analyses to create a tool to predict the individual risk of PE in early pregnancy [62]. Using a combination of more than two markers, the detection rate improves to between 38% and 100%. [60], whereas using a predicting model with a combination of risk factors, biophysical parameters, ultrasound measurement and biochemical markers, the sensitivity increased to 82% versus 41% using risk factors alone [62]. The best results (detection rate 100%, 95% CI 69–100%) were achieved with the combination of three biochemical markers (Inhibin A, PlGF and PAPP-A), uterine artery Doppler and maternal baseline clinical characteristics [60,63]. Despite the encouraging results, clinical data available are still too small and conflicting to be applied widely in clinical practice. Current recommendations do not support the routine use of these tools and recommend that the screening of women at risk of developing PE should be based on the assessment of clinical history and maternal parameters [64,65].

New treatments for PE

The most studied and used drugs to prevent PE are low dose aspirin (LDA) and low molecular weight heparin (LMWH). The use of LDA in PE prevention stems from its ability to reduce platelet thromboxane synthesis through the acetylation of platelet cyclooxygenase (COX) while maintaining vascular wall prostacyclin synthesis [66,67]. The function of LDA is probably also expressed also through the anti-inflammatory role of aspirin. Aspirin is able to trigger lipoxin, which is a bioactive metabolite of arachidonic acid able to promote the resolution of inflammation. Aspirin has antioxidant, anti-inflammatory and immuno-modulator functions [68,69]. Four large randomised trials have been published showing a reduction in the incidence of PE in high-risk patients treated with LDA prophylaxis [70–73]. The most recent of these are randomly assigned 1776 women to receive aspirin, or placebo from 11 to 14 weeks of gestation until 36 weeks of gestation. LDA reduced the incidence of PE in the treated group (OR 0.38; 95% confidence interval, 0.20 to 0.74; $P = 0.004$). LDA is more effective when started in the first or early-second trimester, and the benefit is higher for women at a higher risk of PE [74,75].

The mechanism of action of LMWH in preventing PE is based on its capacity to improve endothelial function more than the anti-coagulant effect. The evidence that most of the obstetrical complications

are different endpoints of the same underlying mechanism including inflammation, altered immunomodulation and shallow placentation suggests that there may be benefit from LMWH prophylaxis [76]. LMWH plays a role in inflammation modulating circulating levels of angiogenic factors such as PLGF and sFLT1 and inflammatory cytokines such as Il-8, Il-6 and TNF-α [77–81]. LMWH is able to inhibit leukocyte adhesion to damaged tissue and reduce complement activation [82,83]. It has been demonstrated that LMWH improves endothelium-dependent relaxation in pregnant women at risk of PE and increases circulating levels of PLGF [81]. The possible role of LMWH in preventing PE remains to be clarified. In 2016, an exhaustive meta-analysis of individual patient data from randomised controlled trials published before 2013 was conducted, which included 963 women from 8 studies. The result of this meta-analysis concluded that LMWH did not reduce the risk of recurrent placenta-mediated pregnancy complications (LMWH 14% versus LMWH 22% absolute difference −8%, 95% CI -17 · 3 to 1 · 4, p = 0 · 09, relative risk 0 · 64, 95% CI 0 · 36-1 · 11, p = 0 · 11). The authors noted significant heterogeneity between single-centre and multi-centre trials with single centre-based studies that reported positive results which were unconfirmed in the multi-centre studies [84]. Since the publication of these meta-analyses, two additional multi-centre, randomised trials have been published in which the authors concluded that LMWH prophylaxis is ineffective in preventing PE in high-risk women [85,86]. Although the evidence produced so far has not demonstrated the benefits of LMWH, concerns remain regarding the heterogeneity between single-centre and multi-centre studies, as noted by Rodger and co-authors; inclusion in trials of different phenotypes of PE; inclusion of different pathologies grouped under the umbrella of placental dysfunction; differences in the timing of initiation of therapy; the dosage and in the outcomes evaluated, which make it difficult to draw unambiguous conclusions.

Corticosteroids are the most widely used drugs for reducing inflammation. Steroid action is explicit both on innate immune cells and adaptive cells. The use of corticosteroid treatment has been evaluated in women with severe PE, especially in those affected by haemolysis, elevated liver enzyme and low platelet (HELLP) syndrome. The results of a Cochrane Database meta-analysis published in 2010 show that antenatal treatment with corticosteroids compared with a control group neither improve maternal outcomes nor infant outcomes [87]. The use of corticosteroid in the postpartum period showed improved liver function, with a faster recover of transaminase, a more rapid recover of kidney function, with a significant increase in urinary output, and a positive effect on platelet count which stabilised or increased in number, allowing more patients to receive regional anaesthesia [88–91].

Biologic agents may have a role in the treatment of immune dysfunction related to PE. The rationale for treatment is based on the hypothesis that PE is a systemic inflammatory disorder and the complement cascade is a key mediator. The evidence shows that biologic agents can be effective in the blockade of the complement system or TNFα on spiral artery remodelling that influences pregnancy outcome [11,92]. In 2013, a case report was published of pregnancy complicated by HELLP syndrome, successfully treated with eculizumab: clinical improvement was observed and blood tests completely recovered within 16 days [93]. Evidence is still limited and biological agents should be used with caution in pregnancy.

Intravenous immunoglobulin (IVIg) has immunomodulatory and anti-inflammatory effects. The mechanism of action is through different pathways in the innate and adaptive immune system. IVIg interacts with complement proteins and modulates the synthesis of cytokines and chemokines [94]. Data on the usefulness of IVIg in obstetric complications are still conflicting and are derived from studies on obstetric anti-phospholipid syndrome and recurrent miscarriages. A review published in 2016 on additional treatments for obstetric anti-phospholipid syndrome found 12 studies published and concluded that IVIg had a beneficial effect on pregnancy when used in addition to the standard protocol with LDA and LMWH but not when used as a single agent [95]. IVIg seems to be able to enhance placental function and has a positive effect on foetal growth, although the clinical usefulness is still unclear [96,97].

Conclusions

Our understanding of the immune system's involvement in pregnancies complicated by PE has undergone a transformation over the years. From a model based on the alterations of cell-mediated

immunity, we have moved on to a concept of altered humoural immunity and systemic involvement of the inflammatory system. The alterations of cytokines and angiogenic factors that trigger the onset of PE have been used to build predictive and diagnostic models useful in clinical practice. Understanding of the pathogenic mechanisms of PE made it possible to clarify the mechanism of action of drugs already used in the prophylaxis of women at risk for developing PE and to develop new drugs for the prevention and treatment of PE.

Future research should focus on clarifying the mechanisms that trigger PE, and differentiating between the alterations that give rise to different phenotypes of PE: placental PE associated with growth restriction, maternal PE associated with a chronic maternal inflammatory alteration and dysmetabolism associated with normal foetal growth.

The only definitive treatment of PE remains delivery of the foetus and removal of the placenta, which produces harmful cytokines. However, premature delivery in early onset PE is often associated with morbidity and even disability in the infant. The development of drugs capable of arresting immunological alterations and placental inflammation should be the goal of research in this area.

Conflicts of interest

The authors have no conflicts of interest.

Practice points

- Cytokines and angiogenic factors are involved in the pathogenesis of PE and their expression is altered long before the onset of clinical symptoms.
- Chemokines and angiogenic factors have a role as biomarkers for PE and may be helpful in the differential diagnosis of hypertensive disease.
- The therapeutic actions of current and future medical treatments should aim to interact on inflammation and the immune system.
- ASA is the treatment of choice for PE prevention in high-risk patients.

Research agenda

- The accurate identification of women at risk of developing PE remains a core issue for research.
- Future medical treatment can be studied on the basis of the known alteration of the immune system.

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