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Role of Innate Immunity in Preeclampsia: A Systematic Review

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Abstract

Innate immune system dysfunction has been known to be a key player in preeclampsia (PE). Activation of the maternal innate immunity may be triggered by invading microorganisms or endogenous ligands, which are detected by different pattern recognition receptors (PRRs). Although some studies have linked PRR activation to PE, it is still unclear if dysregulated PRR expression is associated with the development of this complication. Therefore, we conducted a systematic review of the literature, searching articles that evaluated associations of PRRs with PE. Twenty-six studies met the inclusion criteria: 20 of them analyzed PRR expressions and 6 studies investigated the association between PRR polymorphisms and PE. Among the PRRs, only few studies analyzed retinoic acid–inducible gene I-like helicase (RLH) and/or toll-like receptor (TLR)-1, 5, 6, 7, 8, and 9 expressions in immune cells or placentas from women with PE and controls; thus, it is inconclusive if these PRRs are involved in PE. Results from the 10 studies that analyzed *TLR-2* expressions in women with PE and controls are also contradictory. The majority of the studies that investigated *TLR-3* and *-4* expressions indicate that these PRRs are increased in placenta or immune cells from women with PE compared to pregnant control woman. To date, polymorphisms in *TLR-2*, *-3*, and *-4* and *nucleotide-binding oligomerization domain-like receptor 2* genes do not seem to be associated with PE development. No study has evaluated the association between polymorphisms in genes codifying other TLRs or RLHs genes. In conclusion, available data in literature support a role for *TLR-3* and *TLR-4* in the pathogenesis of PE.

Keywords

preeclampsia, pattern recognition receptors, innate immunity, toll-like receptors, gene expression

Introduction

Preeclampsia (PE) affects at least 3% to 5% of all pregnancies and is a rapidly progressive condition usually diagnosed by new onset high blood pressure (BP) and either proteinuria or end-organ dysfunction after 20 weeks of gestation in a previously normotensive woman.[1-3] Although the origins of PE are not fully understood, an activation of maternal immune system leading to inflammation and endothelial dysfunction may play an important role in the development of this condition.[4,5]

Innate immunity components are the first line of defense against microorganisms. Detection of invading microorganisms is carried out by pattern recognition receptors (PRRs), which recognize highly conserved pathogen-associated molecular patterns (PAMPs) derived from virus, bacteria, and fungi.[6,7] Three groups of PRRs are known: (1) retinoic acid–inducible gene I (RIG-I)-like helicases (RLHs) that recognize double-stranded RNA (dsRNA) derived from viral RNA, playing a role in the immune response triggered by viral infections[8,9]; (2) nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), including NOD1 and NOD2 that induce autophagy processes, and different NLRPs, which regulate inflammasome formation, leading to caspase-1 activation, and consequent cleavage of the cytokines interleukin (IL)-1β and IL-18 into their active forms[10]; (3) toll-like receptors (TLRs) that recognize different triggers derived from bacterial, viral, and fungal cell wall components, activating a variety of cellular responses including the production of type I interferon (IFN-I) and activation of nuclear factor KB (NF-κB) and mitogen-activated protein kinases (MAPK) pathways.[11-14]

Innate immunity dysfunction in women with PE may be triggered by invading microorganisms as well as endogenous danger-associated molecular patterns (DAMPs).[5,15] In this context, some studies have linked TLR expression to the development of PE.[15] The TLRs can be activated by DAMPs released

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during cellular damage due to poor placentation, oxidative stress, and endothelial dysfunction. Danger-associated molecular patterns that seem to activate TLRs leading to PE include fetal DNA, heat-shock proteins, hypoxia, fibrinogen, hyaluronic acid, and oxidized low-density lipoprotein.[15-17] However, it is still inconclusive whether differential expression of PRRs might lead to PE development. Therefore, as part of the ongoing effort to confirm the association of different PRRs with PE, we conducted a systematic review of the literature on the participant.

Materials and Methods

Search Strategy and Eligibility Criteria

This systematic literature search was designed and described in accordance with current guidelines.[18] PubMed and EMBASE repositories were searched to identify all studies that analyzed associations between PE and innate immunity receptors, including IFN-induced with helicase C domain 1 (IFIH1)/melanoma differentiation-associated protein 5 (MDA-5), RIG-I, TLRs, and NLRs. The following medical subject headings were searched: ("IFIH1 protein" OR "TLRs 1 to 10" OR "NLRP1 protein" OR "NLRP3 protein" OR "CIAS1 protein" "RARRES3 protein" OR "Robo3 protein" OR "NLRC5 protein" OR "NLRP10 protein"), AND ("Preeclampsia" OR "Hypertension, Pregnancy-Induced" OR "HELLP Syndrome" OR "eclampsia"). The search was not restricted to any period and was completed on March 2016. It was limited to English or Spanish language papers and included both human and animal studies. All articles identified were also searched manually to identify other important citations.

We included case–control studies that evaluated the association between PE and 1 or more of the above mentioned receptors, including studies reporting protein and gene expression data or polymorphism analyses. If data were duplicated and had been published more than once, the comprehensive study was chosen for inclusion in the systematic review. Two investigators (B.M.S. and A.P.B.) independently reviewed titles and abstracts of all articles selected in order to evaluate whether the studies were eligible for inclusion in the systematic review. Disagreements were resolved by discussion between them and when necessary a third reviewer (D.C.) was consulted.

Data Extraction

Data were independently extracted by 2 investigators (B.M.S. and A.P.B.) using a standardized abstraction form,[19,20] and consensus was sought in all extracted items. When consensus could not be achieved, differences in data extraction were resolved by a third reviewer (D.C.) and by referencing the original publication. The following data were extracted from each individual study according to the presence of PE: (1) characteristics of the studies (including name of first author, publication year, number of participants in case and control groups, age, gestational age, body mass index [BMI], systolic BP, diastolic BP, and ethnicity); (2) polymorphism frequencies (including genotype and allele distributions in case and control groups and odds ratio [OR; 95% confidence interval, CI]); and (3) protein and gene expressions.[19,20]

Results

Literature Search and Characteristics of Eligible Studies

The strategy used to identify and select studies for inclusion in the systematic review is shown in Figure 1. A total of 159 possibly relevant articles were retrieved by searching electronic databases; however, after full text analysis, only 26 articles fulfilled the eligibility criteria and were included in this review (Tables 1 and 2). Available characteristics for these 26 studies are described in Supplementary Table S1. Briefly, the mean age of analyzed women was 29.5 $\pm$ 5.0 years for PE groups and 30.1 $\pm$ 8.0 years for control groups. Mean gestational age was 34.7 $\pm$ 3.0 weeks in women with PE versus 37.9 $\pm$ 1.0 weeks in controls. Most studies were performed in European or North American populations (Supplementary Table S1).

Qualitative Analysis of Studies That Evaluated PRR Gene or Protein Expressions in Relation to PE

Twenty studies analyzed expressions of 1 or more genes codifying PRRs in humans or mice with PE (cases) compared to normal pregnant females (controls; Table 1). Ten studies were done in human placenta and 10 studies in immune cells. Most of them reported TLR-2 to -4 gene and/or protein expressions. No study evaluated NLR expression.

The RLH class mainly comprises RIG-I and IFIH1 cytoplasmic receptors. Only 1 study evaluated IFIH1 and RIG-I expressions in humans and mice,[15] showing that these proteins were increased in placenta from pregnant, poly I:C (PIC)-treated mice compared to normal pregnant mice. Poly I:C is a synthetic analog of viral dsRNA. Moreover, these helicases were increased in placenta from women with PE compared to controls.

Three studies investigated TLR-1,[21-23] TLR-8,[21,22,24] and/or TLR-9[21,22,25] expressions in placenta or immune cells from women with PE and control women, 10 studies reported TLR-2 expressions,[21,22,25-32] 2 studies evaluated TLR-5,[23,30] and only 1 study investigated TLR-6[30] or TLR-7[24] (Table 1). Nonetheless, results of these studies were inconclusive to point out the role of these PRRs in PE.

Six publications reported TLR-3 expressions in humans or mice. Five of them showed increased TLR-3 gene or protein expressions in placentas or dendritic cells from women with PE or mice compared to controls.[15,22,24,25,33] Sixteen articles investigated TLR-4 expressions in humans. Thirteen of them showed increased TLR-4 gene or protein expressions in maternal neutrophils,[31] PBMCs,[27,29] cord blood mononuclear cells,[34] monocytes,[28,35] dendritic cells,[22] or placenta[25,30,36-39] from women with PE compared to the control group. Only 1 study

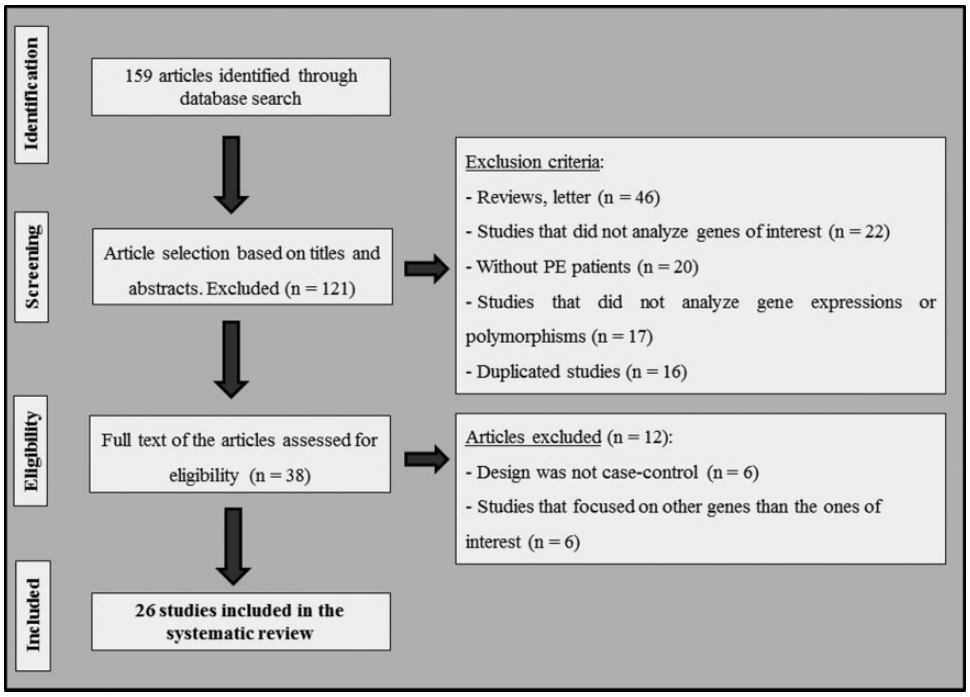

Figure 1. Flowchart illustrating the search strategy used to identify association studies of pattern recognition receptors (PRRs) genes and preeclampsia (PE) for inclusion in the systematic review.

demonstrated decreased *TLR-4* gene expression in neutrophils from women with PE compared to control women,[32] whereas 2 other studies were not able to find any differences in *TLR-4* gene expression between groups.[21,26]

Qualitative Analysis of Studies That Evaluated Associations Between Polymorphisms in Genes for PRRs and PE

Only 6 studies addressed the associations between polymorphisms in genes codifying PRRs and PE (Table 2). These studies focused in *TLR-2* to *-4* and *NOD2* genes. Three studies analyzed polymorphisms in the TLR-2 gene. Xie et al reported that presence of *TLR-2* Arg753Gln (G/A; rs5773708) polymorphism was associated with early-onset PE (<34 weeks' gestation; OR = 2.57; 95% CI: 1.31-5.05) but not late-onset PE (≥34 weeks' gestation) in Canadian women.[40] However, 2 other studies were not able to find any differences in frequencies of the *TLR-2* Arg753Gln polymorphism between women with PE and control women from Brazil[41] or United Kingdom.[42] Only 1 study evaluated polymorphisms in TLR-3 gene regarding PE, showing similar frequencies of the Leu412Phe (A/G; rs3775291) and −299698 (G/T; rs3775296) polymorphisms between case and control groups.[43] Four studies evaluated the association between polymorphisms in TLR-4 gene and PE. Van Rijn et al reported that positivity for ≥1 minor alleles of the *TLR-4* Asp299Gly (A/G; rs4986790) and Thr399Ile (C/T; rs4986791) polymorphisms was more common in women with PE than in controls from Germany (OR = 2.9; 95% CI: 1.2-6.7).[44] Nonetheless, 3 other studies, performed in Brazilian,[41] Hungarian,[45] and Canadian[40] populations, did not find any association between Asp299Gly and/or Thr399Ile polymorphisms and PE.

Only 1 study analyzed 2 polymorphisms in the NOD2 gene and development of PE, showing no association with the disease.[44] However, highest ORs for early-onset PE were observed for women carrying any of the 5 minor alleles of *TLR-4* (Asp299Gly/Thr399Ile) or *NOD2* (Arg702Trp/Gly908Arg/Leu1007 fs) polymorphisms within the highest tertiles for IL-6 and fibrinogen, with ORs of 6.9 (95% CI: 2.1-23.2) for IL-6 and 3.8 (95% CI: 1.2-11.8) for fibrinogen.[44]

Discussion

Immune system activation is known to be required for early stages of placental implantation. However, an excessive activation of the maternal immune system against the fetus may play an important role in the development of PE.[15,46,47] Different PRRs recognize specific PAMPs or endogenous DAMPs, leading to the activation of signaling cascades and production of pro-inflammatory cytokines, which coordinates local and systemic inflammatory responses.[13,48] Although some studies have linked excessive PRR activation to the development of PE, it is still inconclusive if PRRs are indeed involved in the development of this pregnancy complication. Thus, we performed a systematic review of 26 studies that analyzed the potential relationship between PRRs and PE.

IFIH1 and RIG-I are cytoplasmic PRRs that recognize different intracellular dsRNAs generated during viral replication.[8]

Table 1. Relationship Between Gene or Protein Expressions of Pattern Recognition Receptors and Preeclampsia.

First Author (Year)	Gene	Human or Animal	Tissue/Cells	Results	Method of Analysis
Al-ofi et al (2012)	*TLR-2* and *TLR-4*	Human	PBMCs	Both proteins were increased in cases (n = 17) compared to controls (n = 11)	FC
Bernardi et al (2012)	*TLR-4*	Human	Placenta	TLR-4 protein was higher in cases (n = 33) than controls (n = 33)	WB
Chatterjee et al (2011)	*RIG-1*, *IFIH1*, and *TLR-3*	Human and mouse	Placenta	Proteins of these 3 PRRs were increased in women with PE and PIC-treated mice compared to the respective control groups	WB and IMF
Chatterjee et al (2012)	*TLR-3*, *TLR-7*, and *TLR-8*	Human	Placenta	Expressions of these 3 genes were increased in cases (n = 17) compared to controls (n = 13)	GE and IHC
Chatterjee et al (2015)	*TLR-3*	Mouse	Placenta	TLR-3 protein was increased in placenta from PIC-treated pregnant mice compared to the control group (n = 3)	WB
Chen et al (2015)	*TLR-4*	Human	Monocytes	TLR-4 protein was increased in cases (n = 22) compared to controls (n = 23)	FC
Dabagh-Gorjani et al (2014)	*TLR-2*, *TLR-4*, *TLR-5*, and *TLR-6*	Human	Placenta	TLR-4, TLR-5, and TLR-6 were increased in cases (n = 15) compared to controls (n = 15) in both maternal and fetal portions of placenta. TLR-2 was increased only in the fetal part of placenta	GE
Holmlund et al (2007)	*TLR-2* and *TLR-4*	Human	Placenta	No differences in protein levels were observed between cases (n = 13) and controls (n = 12)	IHC
Kayisli et al (2013)[a]	*TLR-4*	Human	Placenta (DCs)	TLR-4 protein was increased in cases (271 ± 26; n = 7) compared to controls (202 ± 25; n = 8)	IHC
Medeiros et al (2014)	*TLR-2* and *TLR-4*	Human	Monocytes	TLR-2 protein was similar between cases (n = 85) and controls (n = 52); TLR-4 was increased in cases	FC
Nitsche et al (2011b)	*TLR-2* and *TLR-4*	Human	Neutrophils	*TLR-2* and *TLR-4* expressions were lower in cases than controls	GE
Nitsche et al (2011a)[a]	*TLR-1* and *TLR-5*	Human	Neutrophils	*TLR-1* and *TLR-5* expressions were lower in cases than controls	GE
Panda et al (2012b)	*TLR-1 a 4*, *TLR-8*, and *TLR-9*	Human	Dendritic cells	TLR-1, TLR-2, and TLR-8 proteins were similar between cases (n = 30) and controls (n = 30); TLR-3, TLR-4, and TLR-9 were increased in cases	FC
Panda et al (2012a)[a]	*TLR-1 a 4*, *TLR-8*, and *TLR-9*	Human	Dendritic cells	Gene expressions of these genes were similar between cases (n = 30) and controls (n = 30)	GE
Pineda et al (2011)	*TLR-2*, *TLR-3*, *TLR-4*, and *TLR-9*	Human	Placenta	Proteins concentrations of these 4 PRRs were higher in cases (n = 5) than controls (n = 5) in different cell types of placenta	IMF
Romao et al (2012)[a]	*TLR-2* and *TLR-4*	Human	Monocytes	TLR-2 protein was similar between cases (n = 30) and controls (n = 20); TLR-4 was increased in cases	FC
Semerci et al (2014)[a]	*TLR-4*	Human	Placenta (DCs and trophoblast cells)	TLR-4 protein was increased in cases (n = 7) compared to controls (n = 8)	IHC
Xia et al (2010)	*TLR-4*	Human	CBMCs	*TLR-4* gene and protein expressions were higher in cases than controls	GE and WB
Xie et al (2010)	*TLR-2* and *TLR-4*	Human	Neutrophils	*TLR-2* and *TLR-4* gene and protein expressions were higher in cases (n = 50) than controls (n = 75)	GE and FC
Zhang and Yang (2012)	*TLR-4*	Human	Placenta	TLR-4 protein was increased in cases with early-onset PE (n = 8) compared to controls (n = 8). No differences were observed between late-onset PE and the control group	WB

Abbreviations: CBMCs, cord-blood mononuclear cells; DCs, decidua cells; FC, flow cytometry; GE, gene expression; IFIH1, IFN-induced with helicase C domain 1; IHC, immunohistochemistry; IMF, immunofluorescence; PBMCs, peripheral blood mononuclear cells; PE, preeclampsia; PIC, poly I:C; PRRs, pattern recognition receptors; RIG, retinoic acid–inducible gene; TLR, toll-like receptor; WB, western blot.
[a]Data retrieved from abstracts from studies presented in International Congress.

Table 2. Association Between Polymorphisms in Genes for Pattern Recognition Receptors and Preeclampsia.[a]

First Author (Year)	Gene—Polymorphism	Cases	Controls	*P* Value/OR (95% CI)
Chen et al (2015)	*TLR-3* – Leu412Phe (A/G)	n = 989	n = 1227	
	GG/AG/AA	57.0/34.5/8.5	57.0/36.1/6.9	.346/NA
	G allele/A allele	74.2/25.8	75.0/25.0	.541/1.043 (0.91-1.19)
	TLR-3 – 299698 (5'-UTR) (G/T)	n = 989	n = 1227	
	GG/GT/TT	58.5/35.5/6.0	55.9/37.1/7.0	.329/NA
	G allele/T allele	76.2/23.7	74.3/25.6	.133/0.9 (0.78-1.03)
Franchim et al (2011)	*TLR-2* – Arg753Gln (G/A)	n = 91	n = 138	
	GG/GA/AA	84.6/15.4/0	84.8/15.2/0	.970/NA
	G allele/A allele	92.3/7.7	92.4/7.6	>.999/NA
	TLR-4 – Asp299Gly (G/A)	n = 109	n = 153	
	AA/AG/GG	93.6/6.4/0	87.6/11.7/0.7	.230/NA
	A allele/G allele	96.8/3.2	93.4/6.6	.110/NA
Fraser et al (2008)	*TLR-2* – (+2258)	n = 117	n = 146	
	GG/GA/AA	92.3/6.8/0.9	95.6/3.4/0	.200/NA
	G allele/A allele	95.7/4.3	98.3/1.7	NS
Molvarec et al (2008)	*TLR-4* – Asp299Gly (G/A)	n = 180	n = 172	
	AA/AG/GG	91.7/8.3/0	87.2/12.2/0.6	>.050/NA
	A allele/G allele	95.8/4.2	93.3/6.7	>.050/NA
	TLR-4 – Thr399Ile (C/T)	n = 180	n = 172	
	CC/CT/TT	91.7/8.3/0	86.6/12.8/0.6	>.050/NA
	C allele/T allele	95.8/4.2	93.0/7.0	>.050/NA
Van Rijn et al (2008)[b]	*TLR-4* – (Asp299Gly/Thr399Ile)	n = 340	n = 113	
	Positivity for the 1 or + minor alleles of the 2 polymorphisms	NA	NA	NA/2.9 (1.2-6.7)
	299Asp (G allele) = 7.4%	NA	NA	NA
	399Ile (T allele) = 7.2%			
	NOD2 – Arg702Trp (C/T)/Gly908Arg (G/C)/Leu1007Pro (-/C)	n = 340	n = 113	
	702Trp allele = 4.4%, 908Arg allele = 1.0%, and 1007Pro = 1.8%	NA	NA	NA
Xie et al (2010)[c]	*TLR-2* – Arg753Gln (G/A)	n = 94 (42 early-onset and 52 late-onset PE)	n = 176	
	GG/AG/AA	91.5/8.5/0	95.5/4.5/0	.094/1.95 (0.71-5.38)
	G allele/A allele	95.7/4.3	97.7/2.3	.098/1.91 (0.71-5.18)
	TLR-4 – Asp299Gly (A/G)	n = 94 (42 early-onset and 52 late-onset PE)	n = 176	
	AA/AG/GG	86.2/12.8/1.0	89.8/1.2/0	.310/NA
	A allele/G allele	92.6/7.4	94.8/5.2	.140/NA
	TLR-4 – Thr399Ile (C/T)	n = 94 (42 early-onset and 52 late-onset PE)	n = 176	
	CC/CT/TT	91.5/8.5/0	92.0/8.0/0	.440/NA
	C allele/T allele	95.7/4.3	96.0/4.0	.440/NA

Abbreviations: CI, confidence interval; IL, interleukin; NA, not available; NOD, nucleotide-binding oligomerization domain; NS, nonsignificant; OR, odds ratio; PE, preeclampsia; TLR, toll-like receptor.

[a]Data are expressed as percentage. Cases: women with preeclampsia. Controls: healthy pregnant women.

[b,c]These studies showed that individually the analyzed polymorphisms did not contribute to the development of PE.

[b]However, interaction analysis showed that highest ORs for early-onset PE were observed for women carrying any of the 5 minor alleles of the *TLR-4* (Asp299Gly/Thr399Ile) or *NOD2* (Arg702Trp/Gly908Arg/Leu1007 fs) polymorphisms, within the highest tertiles for IL-6 and fibrinogen, with ORs of 6.9 (95% CI: 2.1-23.2) for IL-6 and 3.8 (95% CI: 1.2-11.8) for fibrinogen, respectively.

[c]The *TLR-2* rs5773708 polymorphism was also associated with early-onset PE (OR = 2.57, 95% CI: 1.31-5.05). See results section for more details.

After binding with dsRNA, IFIH1 and RIG-I will activate signaling pathways leading to NF-κB and interferon regulatory factor (IRF)-3 activation, ultimately driving the production of pro-inflammatory cytokines, chemokines, and IFN-I. Then, IFN-I binds to its receptor and activates the JAK/STAT pathway to drive the expression of IFN-regulated genes and the innate immune response.[8,9,49] The role of IFIH1 and RIG-I in PE is still uncertain since only 1 study investigated these receptors in cases and controls[15] (Table 1). No study has evaluated the association between polymorphisms in *IFIH1* or *RIG-I* genes and PE.

Among the most important families of PRRs are TLRs, which selectively recognize several PAMPS and DAMPs.[11] For more details regarding which specific PAMP is recognized by each TLR, please refer to a review article from our group.[13] The TLR signaling proceeds through 2 pathways: the myeloid differentiation factor 88 (MyD88)-mediated pathway and the TIR domain-containing adaptor-inducing IFN-β (TRIF)-mediated pathway. The MyD88 pathway leads to activation of NF-κB and MAPK, triggering the expression of different genes related to inflammatory reactions. The TRIF pathway culminates in IFN-I production similarly as reported for IFIH1. The TLR-3 only activates the TRIF pathway; *TLR-4* activates both pathways, whereas all other TLRs activate exclusively the MyD88 pathway.[13,50,51]

Only few studies have analyzed *TLR-1* and *TLR-5* to *-9* expressions in immune cells or placentas from women with PE and healthy pregnant women (Table 1). Thus, it is still inconclusive if these TLRs are involved in the development of PE. Until this date, no study has evaluated the association between polymorphisms in *TLR-1* and *TLR-5* to *-9* genes and PE.

The TLR-2 recognizes PAMPs derived from bacteria, fungi, or parasites.[13] Although 10 studies have evaluated *TLR-2* expressions in humans, the results are inconclusive, as shown in Table 1. Three studies analyzed the association between the Arg753Gln polymorphism in the *TLR-2* gene and PE,[40-42] also with inconclusive results. The study by Xie et al was the only 1 to report an association of this polymorphism with increased risk for PE but only when comparing frequencies between women with early-onset PE and the control group.[40] Thus, the analyzed polymorphism might lower thresholds for early-onset and severe PE but not for late-onset or mild disease.

The TLR-3 endoplasmic receptor recognizes viral or endogenous dsRNA,[13] and it is the most abundant TLR in placenta.[52] Five studies[15,22,24,25,33] showed that TLR-3 expressions were increased in placenta or immune cells from women with PE or mice compared to control groups, strongly suggesting that this PRR is associated with PE. Interestingly, Chatterjee et al reported that treatment of human trophoblasts with specific agonists for TLR-3 (PIC) or TLR-7 to -8 (R837 and CLO97) increased their protein levels, leading to inflammation and immune cell activation.[24] Moreover, treatment of mice with PIC, R837, or CLO97 caused pregnancy-dependent hypertension, endothelial dysfunction, splenomegaly, placental inflammation, and increased incidence of fetal demise. Of note, fetal demise in mice probable results from placental dysfunction and decreased placental perfusion and somewhat mimics intrauterine growth restriction, usually seen in women with PE.[24] Other studies in rodent models also indicate a role of TLR-3 activation by PIC in detrimental pregnancy outcomes, such as increased systolic BP and urinary protein concentrations, increased rate of malformed pups/liter, embryo resorption, and induction of preterm labor.[53-55] Abrahams et al reported that first-trimester trophoblasts are able to modulate the maternal immune system by their ability to secrete cytokines and chemokines following TLR-3 activation by PIC treatment; thus, suggesting that trophoblasts recognize and specifically respond to viral products in a regulated manner.[56] The only study that evaluated TLR-3 polymorphisms was not able to find any association of them with PE.[43]

The TLR-4 plasma membrane receptor recognizes PAMPs derived from bacteria, fungi, or parasites.[13] The majority of the 16 studies[22,25,27-31,34-39] that evaluated *TLR-4* expressions in immune cells or placentas have demonstrated an increased expression of this PRR in women with PE compared to healthy pregnant women. An important role of TLR-4 in the development of PE is further supported by a study showing that TLR-4 activation by lipopolysaccharides (LPS) inhibits the migratory capacity of trophoblast, which might explain the impaired extravillous trophoblast invasion and remodeling of spiral arteries in the decidua from patients with PE.[57] Moreover, TLR-4 activation in pregnant rats led to the development of pathological changes similar to those observed in women with PE.[53,58] Holmlund et al demonstrated that HMGB1, a ligand for TLR-4, is highly expressed in decidua from women with PE.[26] Also, antiphospholipid antibodies, which are known to be involved in the pathology of recurrent miscarriage, PE, and preterm labor, were shown to induce a pro-inflammatory response in first-trimester trophoblasts via TLR-4 pathway.[59]

Four studies analyzed the association between *TLR-4* polymorphisms and PE.[40,41,44,45] Of them, only 1 study was able to find an association between the minor alleles of *TLR-4* Asp299Gly and Thre399Ile polymorphisms and early-onset PE.[44] In addition, *TLR-4* minor alleles interacted with *NOD2* polymorphisms (Table 2), further increasing the risk for early-onset PE.[44] Unfortunately, the above-mentioned study did not analyze women with late-onset PE. Probably the effect of polymorphisms in PRR genes might be different between early- or late-onset PE,[40] and it should be considered when analyzing the association between polymorphisms in these genes and the disease.

The PE is a multifactorial hypertensive disorder of pregnancy that can significantly impact maternal and fetal/neonatal morbidity and mortality. The etiology of PE remains uncertain, but gene and protein expression studies show that TLR receptors and other PRRs may be linked to PE development through either infection (PAMPs) or noninfection (DAMPs) inflammatory-associated processes,[25] as shown in this systematic review. Accordingly, clinical studies have shown an association between intrauterine bacterial or viral infections and pregnancy disorders such as PE, miscarriage, preterm labor,

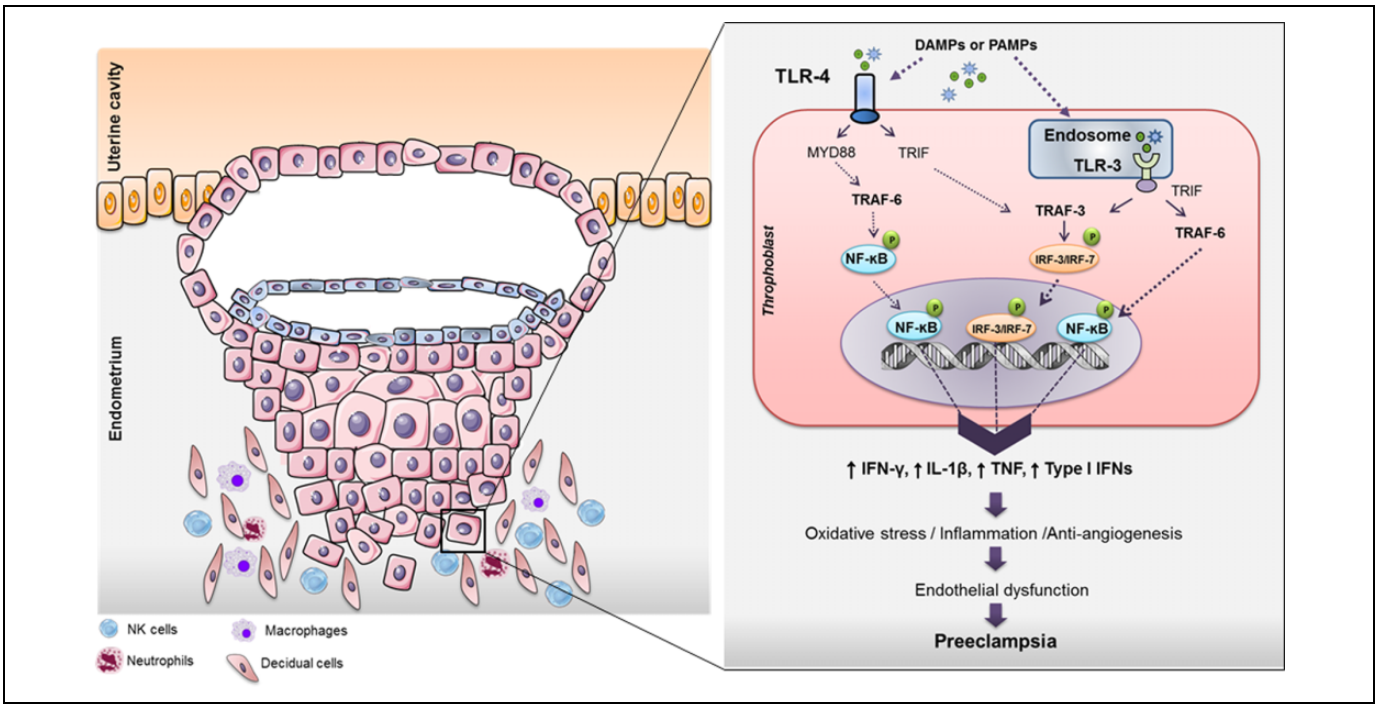

Figure 2. Proposed pathway by which activation of toll-like receptor (TLR)-3 and TLR-4 and possible other pattern-recognition receptors are involved in the development of preeclampsia. Trophoblasts or decidual cells recognize viral or bacterial components (pathogen-associated molecular patterns [PAMPs]) as well as endogenous danger signals (danger-associated molecular patterns [DAMPs]) through their TLR3-4 receptors. The TLR3 activates the TIR domain-containing adaptor-inducing IFN-β (TRIF) pathway while TLR4 activates both TRIF and myeloid differentiation factor 88 (MyD88)-dependent pathways. Both pathways will converge to nuclear factor KB (NF-κB) activation, which will upregulate the expression of several pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1β, and interferon (IFN)-γ. The TLR-3 also activates interferon regulatory factor (IRF)-3/7 transcription factors, which will trigger the production of type I IFNs. Cytokines produced by trophoblasts will also have a potent modulatory effect on maternal immune cells such macrophages, natural killer cells, and neutrophils, recruiting them to the placenta. Consequently, an excessive activation of the maternal immune system will cause inflammation, oxidative stress, and anti-angiogenesis, leading to endothelial dysfunction and preeclampsia (PE). Adapted from Chatterjee et al[15] and Koga et al.[16]

and intrauterine growth retardation.[16,60-62] Furthermore, excessive necrosis and apoptosis resulting from abnormal implantation, placentation, placental hypoxia, and/or trophoblast invasion can generate DAMPs, which will lead to increased placental expressions of various TLRs.[15] Even though studies only strongly support a role for TLR-3 and TLR-4 in the pathogenesis of PE, the activation of different TLR members might generate a local acute inflammatory reaction in placenta through both MyD88- or TRIF-mediated pathways (Figure 2).[13,50,51] Both pathways will converge to NF-κB activation, which will upregulate the expression of several pro-inflammatory cytokines such as IL-6, tumor necrosis factor, IL-1β, and IFN-γ by trophoblasts. The cytokines produced by trophoblasts have a potent modulatory effect on maternal immune cells such as macrophages, natural killer cells, and neutrophils. Thus, an excessive activation of the maternal immune system might result in the clinical manifestations of PE.[15,24,25]

In conclusion, this systematic review shows that available data in literature support a role of TLR-3 and TLR-4 in the pathogenesis of PE. However, additional research is still needed for a better understanding on how an excessive activation of these TLRs in response to exogenous or endogenous danger signals can lead to PE development. Moreover, further studies are needed to confirm whether other PRRs are indeed involved in PE pathogenesis and whether polymorphisms in their genes predispose to this pregnancy complication. Additional investigation on this field will allow a better understanding of the obscure PE etiology and possible emerge with potential targets for PE intervention.

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Supplementary Material

Supplementary material is available for this article online.

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