REPRODUCTION
REVIEW

Sterile inflammation and pregnancy complications: a review

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Abstract

Inflammation is essential for successful embryo implantation, pregnancy maintenance and delivery. In the last decade, important advances have been made in regard to endogenous, and therefore non-infectious, initiators of inflammation, which can act through the same receptors as pathogens. These molecules are referred to as damage-associated molecular patterns (DAMPs), and their involvement in reproduction has only recently been unraveled. Even though inflammation is necessary for successful reproduction, untimely activation of inflammatory processes can have devastating effect on pregnancy outcomes. Many DAMPs, such as uric acid, high-mobility group box 1 (HMGB1), interleukin (IL)-1 and cell-free fetal DNA, have been associated with pregnancy complications, such as miscarriages, preeclampsia and preterm birth in preclinical models and in humans. However, the specific contribution of alarmins to these conditions is still under debate, as currently there is lack of information on their mechanism of action. In this review, we discuss the role of sterile inflammation in reproduction, including early implantation and pregnancy complications. Particularly, we focus on major alarmins vastly implicated in numerous sterile inflammatory processes, such as uric acid, HMGB1, IL-1α and cell-free DNA (especially that of fetal origin) while giving an overview of the potential role of other candidate alarmins.
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Introduction

Inflammation is essential for successful female reproduction. Inflammatory processes are implicated in every step of fertility from menstrual cycle (ovulation and menses) to early pregnancy (implantation and decidualization) and later during labor (myometrial activation, cervical ripening and weakening of fetal membrane), whereas quiescence of these mechanisms is maintained by local immune cells during gestation to allow maternal tolerance of fetal antigen allograph. However, untimely inflammatory triggers shifting the immunological balance toward activation can lead to adverse pregnancy outcomes including preterm birth. Inversely, failure to mount a local inflammatory response in early or late gestation can also lead to adverse conditions, including miscarriages. Evidence shows that impaired inflammatory response is implicated in numerous female reproductive tract pathologies including menstrual disorders (Sales & Jabbour 2003), endometriosis-associated infertility (Gupta *et al*. 2008), recurrent miscarriage (von Wolff *et al*. 2000, Laird *et al*. 2003), intrauterine growth restriction (Heyborne *et al*. 1994), preeclampsia (Redman *et al*. 1999, Rinehart *et al*. 1999) and preterm labor (Romero *et al*. 2006, Christiaens *et al*. 2008). Infertility has an estimated global prevalence of 9% with >72 million infertile women worldwide (Boivin *et al*. 2007), whereas preterm birth and preeclampsia, the two leading causes of perinatal mortality and morbidity, have an estimated prevalence of >11% (Blencowe *et al*. 2013) and 3–5% (Ananth *et al*. 2013, Chaiworapongsa *et al*. 2014) respectively. Therefore, understanding the mechanisms by which inflammation is untimely triggered in the uterus is fundamental to developing effective therapeutics to improve fertility and decrease poor obstetrical outcomes.

Infection is not an essential component in reproductive disorders linked to inflammation. In animals, sterile inflammation is sufficient to recreate major features of common reproductive diseases (Romero *et al*. 1991, Scharfe-Nugent *et al*. 2012, Gomez-Lopez *et al*. 2016), whereas in humans, a significant part of patients suffering from preeclampsia, preterm labor or other inflammatory diseases during pregnancy display no clinical signs of infection. This has been extensively studied in the context of preterm birth; although observational, correlational and causal

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data accumulated for >30 years have linked infection to preterm labor, preterm birth without infection is more prevalent (Romero *et al*. 2001). Furthermore, antibiotics are inefficient to prevent preterm labor in women with infection (Olson *et al*. 2008), suggesting that infection-induced pro-labor effects arise from inflammation (self) rather than infection (non-self).

Pro-inflammatory stimuli can be classified as 'danger' (or damage) and 'stranger' signals; both types of signals are pharmacologically active via pattern recognition receptors (PRRs), a class of phylogenetically conserved receptors ubiquitously expressed by mammalian cells. These receptors act as sensors of damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns by operating a transduction cascade of intracellular and intranuclear signals leading to the mounting of cytokine-based inflammatory responses. PRRs include Toll-like receptors (TLRs) 1–11, scavenger receptors, C-type lectins, and NOD-like receptors and are expressed abundantly in decidua, placenta, membranes and myometrium throughout pregnancy, in immune and non-immune cells (Koga & Mor 2010, Lappas 2013, Zhang *et al*. 2014). Therefore, the uteroplacental compartment is a sensor of 'danger' and 'stranger' inflammatory stressors. We suggest that inflammatory processes implicated in physiological human reproduction are triggered mainly through sterile pathways (e.g. via tissue injury or cell death) compared to exogenous signals such as pathogens (bacteria or viruses, namely stranger signals), whereas pathological inflammatory events implicated in pregnancy complications can be triggered by both sterile and infectious pathways. This review focuses on the role of sterile inflammation in common pathologies of pregnancy.

Major players in sterile inflammation and their mechanism of action

Sterile inflammation is triggered when DAMPs activate PRRs (or other receptors including RAGE and IL-1R) to mount an acute immune response in order to solve the adverse condition that initially led to DAMP release. As DAMPs are endogenous intracellular molecules primarily released as a result of non-programmed cell death to convey danger cues in the first few hours of an injury, they are also referred to as alarmins (Matzinger 1994). Candidate alarmins include, but are not limited to, high-mobility group box 1 (HMGB1), uric acid, interleukin-1α (IL-1α) and cell-free DNA. A detailed description of these alarmins and their mechanism of action can be found below and in Fig. 1, whereas their roles in pathological conditions of pregnancy are presented in the next section.

HMGB1

HMGB1 is a highly conserved non-histone protein (25 kDa) with cytokine-like activity in the extracellular space. HMGB1 is abundantly and ubiquitously expressed in nucleus where it plays a role in DNA replication, transcription and repair, and nucleosome stabilization (Boonyaratanakornkit *et al*. 1998, Stros 2010, Celona *et al*. 2011). HMGB1 is structured into two DNA-binding domains, HMG box A and B and an aspartic and glutamic acid-rich C-terminal tail. Although originally discovered in the nucleus, HMGB1 is also found in cytosol, mitochondria, and on membrane surface and can be released to the extracellular milieu through active (secretion) and passive pathways (Erlandsson Harris & Andersson 2004). First, active pathways are triggered by

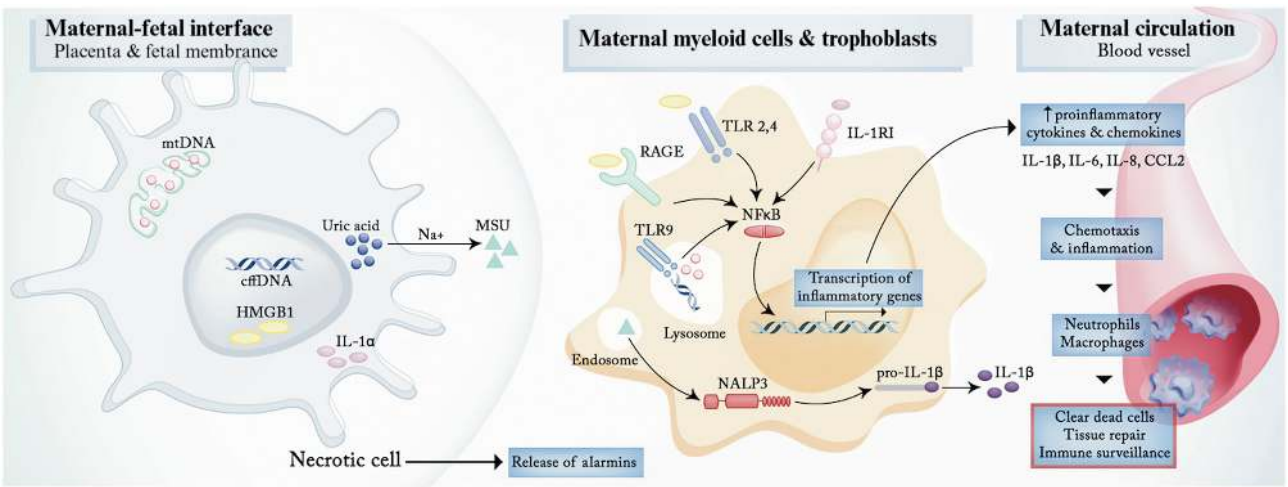

Figure 1 Inflammatory mechanism of action of HMGB1, uric acid, IL-1α and cell-free DNA. The general mechanism of action of alarmins at the maternal–fetal interface is shown, with alarmins (uric acid/MSU, HMGB1, cffDNA and IL-1α) being released from cells of the maternal–fetal interface (i.e., placenta and fetal membranes) following a stimulus or necrosis. They act on placental cells (primarily trophoblasts) and maternal myeloid cells to induce an inflammatory response. The inflammatory cascade leads to the secretion/release of cytokines/chemokines, which stimulate the recruitment of immune cells from the maternal circulation.

pathogenic products (e.g. bacteria and viruses) or other stressors (e.g. oxidative stress and cytokines), which have been shown in immune cells and non-immune cells (Tsung *et al*. 2007, Tang *et al*. 2011, Harris *et al*. 2012). Secondly, whereas (ii) passive release is observed following tissue injury and cell death, especially necrosis (Scaffidi *et al*. 2002) and in specific cases of apoptosis (Qin *et al*. 2006) – including when triggered by sterile injury events (e.g. hypoxia, senescence and autoimmune disease). The latter happens immediately (Scaffidi *et al*. 2002), whereas the former is a slower mechanism mediated by cellular signal transduction (Tsung *et al*. 2014). Once HMGB1 accumulates in the extracellular milieu, it conveys danger signals by triggering inflammatory pathways, including NF-κB, ERK and p38, in neighboring cells via numerous cell surface receptors such as TLRs 2, 4 and 9; RAGE; CD24; and others (Venereau *et al*. 2013). This leads to the activation of innate and adaptive immunity, cytokine, chemokine, and metalloprotease release and ensued pro-migration and pro-inflammatory outcomes (Andersson *et al*. 2000, Scaffidi *et al*. 2002, Rouhiainen *et al*. 2004, Andersson & Tracey 2011). Of interest, HMGB1 has also been shown to form complexes with many pro-inflammatory mediators and enhance their respective actions in a synergistic manner (Hreggvidsdottir *et al*. 2009). Furthermore, HMGB1 levels are elevated in multiple animal models of sterile injurious events (Tsung *et al*. 2014) and in humans with acute organ injury, autoimmune diseases or cancer (Tong *et al*. 2011, Wang *et al*. 2014). *In vitro* and *in vivo*, HMGB1 administration induces inflammation (Yang *et al*. 2005), and more importantly, HMGB1 antagonism protects against sepsis (Yang *et al*. 2004). This evidence highlights a critical alarmin role of HMGB1 as an endogenous sterile driver of inflammation.

Uric acid

Uric acid (160 Da) is a product of metabolic breakdown of purine nucleotides by xanthine oxidase, with normal blood concentration ranging between 40 and 60 μg/mL. Upon achieving concentrations of >70 μg/mL, uric acid forms needle-like, immunostimulatory monosodium urate (MSU) crystals, which cause acute inflammation of gout. In the last few years, uric acid has been vastly regarded as an alarmin of sterile inflammation because of the high cytosolic concentration (≈4 mg/mL) released upon cell death, which reacts with extracellular sodium to form MSU in the immediate vicinity of cellular injury (Shi *et al*. 2003). Transport of MSU inside antigen-presenting cells through phagocytosis promotes its interaction with NALP3 inflammasome and induces IL-1β maturation and release thereby triggering an inflammatory response (Martinon *et al*. 2006, Shi 2010). This is an important step in sterile inflammation that enables immune cells to sense injuries. Concordantly, administration of MSU causes acute inflammation (Faires & Mccarty 1962), and in mice, blocking uric acid is sufficient to inhibit the immunological and inflammatory responses associated with cellular death or injury in numerous cell types and tissues (Shi *et al*. 2003, Kono *et al*. 2010).

Interleukin-1

The interleukin-1 family comprises 11 cytokines that regulate inflammatory response to injuries and stressors. Two major members of the family are IL-1α and IL-1β, which bind to ubiquitous IL-1R1 to activate the translocation of transcription factors NF-κB and AP-1, thereby triggering the expression of numerous cytokines including itself and initiating or sustaining an inflammatory response (Di Paolo *et al*. 2009, Dinarello 2009). Although IL-1α and IL-1β bind to the same receptor and convey similar biological effect, the two cytokines are encoded by different genes and have distinct mode of action. Unlike IL-1β, IL-1α is not actively secreted but instead translocates to the nucleus to participate in the regulation of gene transcription (Werman *et al*. 2004). Furthermore, while IL-1β precursor requires exogenous (or endogenous in rare cases) signals to trigger its transcription and to initiate its inflammasome-dependent cleavage into a functional cytokine, IL-1α precursor is, on the other hand, ubiquitously expressed in the cytoplasm of healthy cells, in the form of a biologically active precursor. Consequently, only IL-1α is released in a functional form upon necrosis; therefore, IL-1α is regarded as an alarmin, whereas IL-1β is not (Eigenbrod *et al*. 2008, Lukens *et al*. 2012). Accordingly, sterile cell death-induced neutrophil inflammatory response in mice requires both IL-1α and IL-1R, but not IL-1β (Chen *et al*. 2007), suggesting that IL-1β is not essential for the mounting of a functional sterile inflammatory response to cell death. However, evidence shows that both IL-1α and IL-1β are implicated in sterile inflammation but have distinct timing of effect and roles (Rider *et al*. 2011), suggesting that IL-1β contributes to sterile inflammation, not as an initiator but as a redundant mechanism to amplify the initial trigger. Accordingly, it is documented that IL-1β can be produced to contribute to sterile inflammation in response to non-cytotoxic sterile stressors as those released upon necrosis (Lukens *et al*. 2012). This was also recently reported for IL-1α (Idan *et al*. 2015). Interestingly, the release of IL-1α is tightly regulated during programmed cell death via chromatin sequestration, which significantly reduces its pro-inflammatory effect during apoptosis; this is not observed during necrosis (Cohen *et al*. 2010). These data suggest a critical role of IL-1α in sterile inflammation, and a contributive, albeit non-essential, role of IL-1β. The major role of IL-1α in sterile inflammation has been reviewed elsewhere (Lukens *et al*. 2012).

Cell-free DNA

Circulating cell-free DNA refers to double-stranded, cell-unbound DNA fragments in the blood of humans. Cell-free DNA originates from genomic or mitochondrial DNA released subsequently to cell death. Cell-free DNA is present in small amounts in the blood of healthy individuals, but its concentration is increased in patients suffering from chronic diseases. In this context, studies suggest that it acts as a contributor to chronic diseases by inducing inflammation via TLR9, a PRR classically activated by unmethylated CpG motif-containing bacterial and viral DNA fragments (Chan & Lo 2002, Breitbach *et al*. 2012, Nishimoto *et al*. 2016). Mitochondrial DNA also triggers TLR9 to induce inflammation (Zhang *et al*. 2010).

Circulating blood of pregnant women contains an additional type of cell-free DNA, referred to as cell-free fetal DNA (cffDNA) that originates from the placenta. Evidence for the placental origin of cffDNA includes the following: (1) it is detected in anembryonic gestation (Alberry *et al*. 2007); (2) it is still detected after therapeutic abortion in which placenta is incompletely removed, albeit undetectable after normal delivery (Lo *et al*. 1999, Wataganara *et al*. 2005); (3) it is detected in cases of invasive placenta, a postpartum pregnancy complication in which trophoblasts invade myometrium (Sekizawa *et al*. 2002); and (4) it carries the placental genotype in patients with confined placental mosaicism (Masuzaki *et al*. 2004). In contrast to maternal cell-free DNA, of which 32% of the fragments are >356 bp, cffDNA are short hypomethylated fragments (<313 bp) and potent inducer of sterile inflammation (Chan *et al*. 2004, Scharfe-Nugent *et al*. 2012, Schroeder *et al*. 2013). The release of cffDNA is a physiological process present in all mammals, but its possible roles and implications in normal pregnancy (and more importantly parturition) remain poorly understood. Placental growth involves proliferation, differentiation and syncytial fusion of cytotrophoblasts, which is associated with significant release (grams per day) of microvesicles-encapsulated, cffDNA-containing apoptotic trophoblasts content into maternal circulation (Nelson 1996, Huppertz *et al*. 1998, Huppertz & Kingdom 2004, Bischoff *et al*. 2005, Taglauer *et al*. 2014). These microparticles, also referred to as syncytiotrophoblast microvesicles (SCTMs), were first described more than 100 years ago in lung capillaries of women who died from preeclampsia (Schmorl 1893) and were later described as a feature of normal pregnancy, although increased in preeclampsia (Johansen *et al*. 1999). SCTMs as well as cffDNA alone are pro-inflammatory (Redman *et al*. 1999, Redman & Sargent 2000, Phillippe 2015). Interestingly, once pregnancy is past 20 weeks, the levels of cffDNA in maternal circulation consistently increase 1% per additional week of gestation to abruptly rise (up to 13-folds) when gestation nears the end (Ariga *et al*. 2001, Majer *et al*. 2007, Wang *et al*. 2013). This evidence, along with the established pro-inflammatory effects of cffDNA, underpins the theory that cffDNA may represent a common trigger to parturition in mammals (Phillippe 2014). Furthermore, elevated cffDNA in the maternal circulation has been observed in pathological pregnancies (Levine *et al*. 2004, Alberry *et al*. 2009, Girard *et al*. 2014) in association with placental dysfunction and inflammation. For these reasons and others, cffDNA is increasingly used for diagnostic purposes to decrease the use of invasive amniocentesis.

Mechanistically, cffDNA can bind to TLR9 to induce a conformational change in the homodimers of the receptor resulting in the close apposition of the TIR signaling domains and downstream activation of NF-κB and transcription of inflammatory cytokine genes (Latz *et al*. 2007). Importantly, this TLR9, NF-κB-dependent pro-inflammatory effect of cffDNA was shown in pregnant mice and is characterized by IL-6 production in human peripheral blood mononuclear cells (Scharfe-Nugent *et al*. 2012). Classically, TLR9 is localized intracellularly in endoplasmic reticulum (ER), endosomes and lysosomes (Latz *et al*. 2004). Therefore, cffDNA must be transported by endocytosis inside immune cells in order to convey inflammatory effects via TLR9; this is likely occurring through phagocytosis of cffDNA-containing SCTMs by placental or circulating granulocytes. Given the half-life of cffDNA (16.3 min in humans) (Lo *et al*. 1999), this inflammatory stimulation is short-lived, but is likely sustained by unabated trophoblast turnover.

Others

Different levels of evidence have been accumulated, suggesting that many other intracellular factors can induce acute inflammation once released in their environment and therefore may represent potential alarmins. These include S100 proteins (Hofmann *et al*. 1999, Ryckman *et al*. 2003), nucleosomes (Decker *et al*. 2005), purines (Cronstein *et al*. 1990), heat-shock proteins (Basu *et al*. 2000), saturated fatty acids (Lee *et al*. 2001) and antimicrobial peptides (Yang *et al*. 1999, De *et al*. 2000, Zanetti 2004). Interestingly, possible alarmin activity has been reported for molecules of mitochondrial provenance such as mitochondrial DNA (Zhang *et al*. 2010), *N*-formylated mitochondrial peptides (Carp 1982) and others (Raoof *et al*. 2010), which could arise from their probable prokaryote origin. Noteworthy, the role and mode of action of the aforementioned candidates *in vivo* are mostly unknown. Along these lines, the possible alarmin activity of heat-shock proteins is still under debate. Early studies have shown that purified HSPs activate DCs *ex vivo* (Basu *et al*. 2000) and *in vivo* (Binder *et al*. 2000) to trigger an inflammatory response. This pro-inflammatory effect has latter been attributed to bacterial contaminant (Bausinger *et al*. 2002, Gao & Tsan 2003), and the

enthusiasm of a possible alarmin role of HSPs was consequently severely dampened.

By definition, any endogenous molecule physiologically expressed in low concentrations in the extracellular milieu, which is upregulated and released during pathological events, could be considered as an alarmin candidate. Therefore many other mediators could potentially be included in this category, such as glucose that has recently been shown to have pro-inflammatory actions in human trophoblast (Han *et al*. 2015).

A role for sterile inflammation in pathological conditions of pregnancy

Alarmin release, caused by tissue injury, hypoxia/ischemia, cellular senescence or other stressors, is implicated in pathologies of pregnancy independently of infection. In the next section, we will focus on the role of alarmins in miscarriages, recurrent pregnancy loss (RPL), intrauterine growth restriction (IUGR), preeclampsia and preterm labor. The proposed sites of release of alarmins in diseases of pregnancy are shown in Fig. 2.

Early pregnancy

Many inflammatory mediators (and immune cells) are implicated in the early events of pregnancy, primarily embryo implantation. A tight regulation of the immune system is required for proper invasion and remodeling of the spiral arteries by fetal trophoblasts, which has been recently reviewed (Erlebacher 2013). The involvement of DAMPs in RPL or miscarriages has only recently been addressed. Elevated HMGB1 levels in uterine fluids have been associated with pregnancy failure in rats and lower abundance of HMGB1 observed in the receptive phase of implantation in humans (Bhutada *et al*. 2014). Additionally, a genetic polymorphism of HMGB1 characterized by higher expression of HMGB1 in placenta has been associated with RPL (Jin *et al*. 2015). Furthermore, HMGB1 was shown to induce inflammation, characterized by an NF-κB- and reactive oxygen species-dependent increased secretion of IL-6, IL-8 and CCL2 in first-trimester trophoblast (Shirasuna *et al*. 2016). Other alarmins, including S100A8 (Nair *et al*. 2015) and cell-derived microparticles (Martinez-Zamora *et al*. 2016), were also found to be elevated in early pregnancy loss. Likewise, increased inflammatory cytokine levels (namely IL-18, LIF, MIF, IL-12, IFN-γ and ICAM-1) in the blood and endometrium were also associated with RPL (Comba *et al*. 2015). Correspondingly, increased expression of the NALP3 inflammasome and its products IL-1β and IL-18 was observed in the endometrium of patient with RPL (D'Ippolito *et al*. 2016). This evidence points toward a potential role of DAMPs in early pregnancy failure, although there are still many unresolved issues.

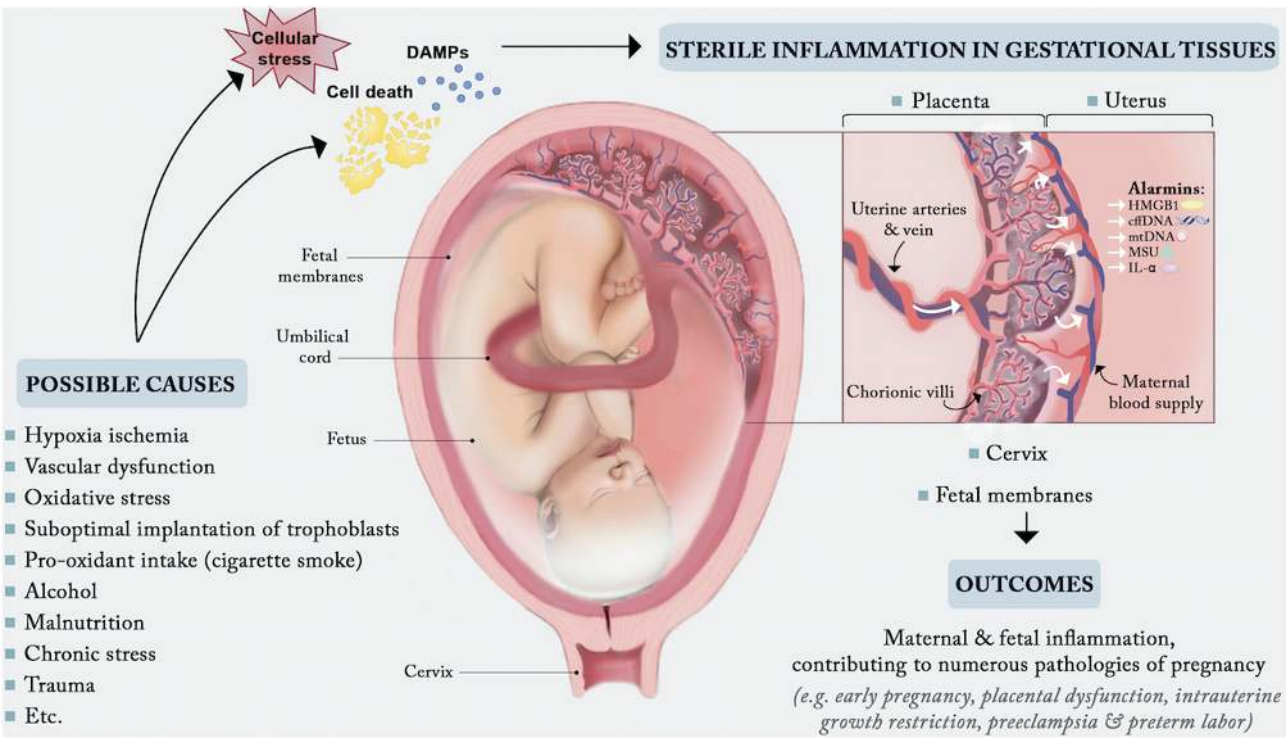

Figure 2 Principal sites of release and actions of alarmins in pathological pregnancy. Multiple causes of cellular stress will affect cell viability and lead to the release of DAMPs (alarmins) by the fetal membranes and the placenta. These DAMPs will then act not only on the placenta itself but also on the uterus, cervix and fetal membranes inducing inflammation and contributing to many complications of pregnancy.

Placental dysfunction

Placental dysfunction is an early event in both IUGR and preeclampsia (and represents a defined etiology of preterm labor) but will be addressed mainly in this subsection. Causes of placental dysfunction include (but are not limited to) hemodynamic conditions (hypertension and reduced blood viscosity (e.g., in anemia or as a result of using blood thinner drugs)), diabetes, placental abruption and improper implantation. These conditions induce hypoxia/ischemia, oxidative stress and/or inflammation in placenta, which subsequently induces increased trophoblast cell death (Maxwell *et al*. 2015), hence favoring the release of alarmins, and can be a cause or a consequence of abnormal placental function, which likely differs from one patient to another. Nonetheless, elevated alarmin levels have been associated with placental dysfunction, which leads to the amplification of the effects of placental inflammation and dysfunction. Accordingly, levels of HMGB1, uric acid and cffDNA are all elevated in pregnancies at high risk of developing complications associated with placental dysfunction such as growth restriction or preterm labor (Girard *et al*. 2014). Given that these alarmins have been reported to induce placental inflammation (Mulla *et al*. 2011, 2013, Phillippe 2015, Shirasuna *et al*. 2016), their upregulation in high-risk pregnancies may plausibly contribute to placental dysfunction. Additionally, elevated placental mitochondrial DNA concentrations were associated with placental abruption (Qiu *et al*. 2015).

Intrauterine growth restriction

IUGR is characterized by a suboptimal fetal growth and is associated with perinatal morbidity and mortality. Numerous causes have been suggested for IUGR, among which placental dysfunction is predominant. Hence, a role for sterile inflammation in its onset has already been established in the previous section. Furthermore, studies assessing the levels of alarmins in maternal plasma have found elevation of multiple candidates in IUGR. An increase of S100B proteins in maternal plasma was reported in women with IUGR, which correlated with brain morbidity outcomes in neonates (intraventricular hemorrhage) (Gazzolo *et al*. 2006). Interestingly, amniotic fluid concentration of S100B was found to sensitively and specifically predict spontaneous intrauterine fetal death (Florio *et al*. 2004). Although an early study conducted by Sekisawa et al. found no significant difference in maternal plasma levels of cffDNA in nine women with IUGR compared with 20 gestation age-matched controls (Sekizawa *et al*. 2003), more recent studies using larger cohorts found significant increases in women with IUGR (Smid *et al*. 2006, Al Nakib *et al*. 2009, Alberry *et al*. 2009). Similarly, circulating mitochondrial DNA was also found to be increased in patients with IUGR (Colleoni *et al*. 2010). Furthermore, a study by Mert *et al*. found a marginal increase in maternal plasma uric acid levels in pregnancies complicated with IUGR; this elevation was more pronounced in women with preeclampsia (PE) (Mert *et al*. 2012).

Preeclampsia

PE represents a leading cause of maternal and fetal mortality and morbidity and is characterized by maternal hypertension and proteinuria; additionally, it is often associated with fetal growth restriction (Srinivas *et al*. 2009). PE is mainly a consequence of deficient uteroplacental blood flow, and removal of placenta is the only known curative treatment. Along these lines, a major feature of the pathophysiology of PE is the failure of fetal trophoblasts to invade uterine arteries, resulting in reduced placental perfusion and ensued hypoxia/ischemia. This localized oxygen and nutrient deprivation is associated with exaggerated trophoblast cell death (Jones & Fox 1980, Chua *et al*. 1991, Knight *et al*. 1998, Johansen *et al*. 1999, Leung *et al*. 2001, Wu *et al*. 2012), which has been suggested to directly contribute to the disease by releasing mediators of inflammation that promote endothelial activation and systemic maternal inflammation (Smarason *et al*. 1993, Knight *et al*. 1998, Redman & Sargent 2003). Although the etiology of PE is mostly unknown, a major hallmark is a generalized inflammatory response characterized by high cytokine levels, such as IL-1β, IL-6, IL-8 and tumor necrosis factor-α (Vince *et al*. 1995, Mellembakken *et al*. 2001, Laresgoiti-Servitje 2013, Harmon *et al*. 2016). Because this inflammatory response usually occurs in the absence of microbial infection, PE primarily represents a sterile inflammation.

Trophoblasts contain numerous alarmins, including uric acid, cffDNA, HMGB1 and IL-1α, which can mount a sterile inflammatory response and whose contributions to PE are increasingly reported. These alarmins are released in maternal circulation as free mediators upon trophoblast necrosis, or as part of SCTMs consequently to trophoblast apoptosis; the latter form is subsequently prone to engulfment and clearance by phagocytes, wherein it can interact with intracellular targets including TLRs. Studies by Huppertz et al. and Chen et al. respectively reported that placental hypoxia, as observed in PE, favors trophoblast necrosis vs apoptosis (Huppertz *et al*. 2003), and that once cleared by phagocytes, necrotic (but not apoptotic) trophoblasts can induce an inflammatory response characterized by increased adhesion of monocytes to endothelial cells (Chen *et al*. 2006). This suggests that pathological trophoblast necrosis, as observed in PE, induces distinct inflammatory mechanisms than those observed with normal pregnancy, which may contribute to the pathophysiology. Complementary evidence by Aly et al. suggests that SCTMs from pregnancy complicated with preeclampsia exhibit different contents and induce

the generation of significantly increased amount of superoxide radicals from neutrophils, implying increased toxicity (Aly *et al*. 2004).

cffDNA

The most studied alarmin in PE is cffDNA. cffDNA is elevated in pregnancies complicated by preeclampsia (Levine *et al*. 2004, Lazar *et al*. 2009, Vlkova *et al*. 2015) and its levels correlate with the degree of impairment in placental perfusion (Sifakis *et al*. 2009). For these reasons, it is broadly investigated as a biomarker of PE (Martin *et al*. 2014). As mentioned above, cffDNA can trigger inflammation and adverse pregnancy outcomes in mice via TLR9 (Scharfe-Nugent *et al*. 2012). Similarly, mitochondrial DNA (mtDNA) is released by trophoblasts upon death and its levels in maternal circulation are increased in PE (and IUGR) (Lattuada *et al*. 2008, Goulopoulou *et al*. 2012, Qiu *et al*. 2012); the immunostimulatory and pro-inflammatory role of mitochondrial DNA in PE has been reviewed elsewhere (McCarthy & Kenny 2016). Activation of TLR9 by fetal or mitochondrial cell-free DNA, or other mediators (including HMGB1), has been suggested to lead to vascular dysfunction and hypertension (Goulopoulou *et al*. 2012). Interestingly, women with preeclampsia express higher levels of placental TLR9 (Pineda *et al*. 2011). Self DNA is becoming increasingly considered as a major immunostimulatory molecule responsible for adverse pregnancy outcomes (Hartley *et al*. 2015, Sifakis *et al*. 2015).

Uric acid

Hyperuricemia is commonly observed in pregnancies complicated by PE (Laughon *et al*. 2011). More importantly, plasmatic levels of uric acid positively correlate with PE severity (Voto *et al*. 1988), and hyperuricemia (serum uric acid >4–5.8 mg/dL) generally heralds poor maternal and fetal outcomes (Hawkins *et al*. 2012). This hyperuricemia may represent a consequence of the symptoms associated with PE, such as loss in renal function, tissue injury, acidosis and increased activity of xanthine oxidase (Johnson *et al*. 2003). However, whether uric acid is an inactive, dead-end consequence of PE or a contributor to the disease is controversial (Kang *et al*. 2004, Martin & Brown 2010). Evidence for a causal role of uric acid includes the following: (1) uric acid exerts pro-inflammatory and vasoconstrictive effects in rats (Kang *et al*. 2004); furthermore, rats rendered hyperuricemic with uric acid, and uricase inhibitor administration develop hypertension and many other major features of preeclampsia, which is reversed by the xanthine oxidase inhibitor allopurinol (Mazzali *et al*. 2001, Kang *et al*. 2004); (2) elevation of uric acid levels in maternal plasma often precedes hypertension and proteinuria in humans (Powers *et al*. 2006, Laughon *et al*. 2011); (3) mechanistically, uric acid induces a pro-inflammatory, pro-contractile phenotype in vascular smooth muscle cells by activating NF-κB, AP-1 and MAPK (p38, ERK p42/p44) pathways and downstream expression of Cox-2, MCP-1, CRP and thromboxane A2, which plausibly contributes to many features of PE including inflammation (Kang *et al*. 2002, 2005, Kanellis *et al*. 2003); (4) hyperuricemic rats have decreased placental activity of eNOS (Kang *et al*. 2005), an enzyme catalyzing nitric oxide generation, which is a critical vasodilator for efficient placental perfusion given the absence of autonomic innervation in placenta; and (5) uric acid promotes the release of pro-inflammatory cytokines in rats challenged with LPS, and oppositely treatment of hyperuricemic rats with allopurinol or sodium bicarbonate, which decreases uric acid concentrations, decreases this inflammation (Netea *et al*. 1997). This suggests that uric acid, once released by trophoblasts upon necrosis, may play a role in the pathogenesis of PE. Interestingly, as PE is associated with decreased levels of ascorbate (Mikhail *et al*. 1994), an antioxidant enzyme responsible for reducing urate anions back into uric acid, it is possible that uric acid, via its oxidized form, induces oxidative stress specifically during PE. Accordingly, it was demonstrated that the pro-inflammatory effects of uric acid are attenuated in the presence of antioxidants, which suggests that urate anions are an important part of uric acid action (Kanellis *et al*. 2003).

HMGB1

Similar to cffDNA and uric acid, maternal plasma levels of HMGB1 have been found to increase during pregnancy, and more importantly to reach exaggerated levels in women with PE (Pradervand *et al*. 2014). This finding was corroborated in a study by Zhu *et al*., with further evidence revealing increased levels of HMGB1 and RAGE in the placenta of women with severe PE, especially in the cytoplasmic compartment of trophoblasts (Zhu *et al*. 2015). Additionally, RAGE and TLR4, two receptors with affinity for HMGB1, were found to have higher expression in placentas from pregnancies complicated with PE (Kim *et al*. 2005, Chekir *et al*. 2006). Pro-inflammatory effects of HMGB1 via TLR9 have been suggested to contribute to the pathophysiology of PE (Scharfe-Nugent *et al*. 2012).

IL-1α

An important role of IL-1 as a contributor to hypertension has been proposed (Voelkel *et al*. 1994, Zou *et al*. 2001, Krishnan *et al*. 2014). Additionally, IL-1 is known to exacerbate inflammation in placenta (Baergen *et al*. 1994, Nadeau-Vallee *et al*. 2015*b*), the key organ in PE. Accordingly, variation in the IL-1α and IL-1Ra genes has been associated with increased risk of PE

(Hefler *et al*. 2001, Li *et al*. 2014, Ghasemi *et al*. 2015). Interestingly, antagonism of IL-1 dose dependently restores adequate placental perfusion following endotoxin exposure (Girard *et al*. 2010).

Overall, there is accumulating evidence that alarmins (1) are released in maternal circulation in numerous conditions, but mainly as a result of trophoblast death; (2) are elevated in pregnancies complicated with PE, systemically and locally in the placenta; (3) can initiate an inflammatory response via PRRs abundantly expressed in the uteroplacental compartment especially in women with PE; and (4) can directly contribute to the pathogenesis of the disease by mounting a sterile inflammatory response to placental hypoxia/ischemia. The establishment of animal models of preeclampsia is needed to test novel therapeutics to block alarmin activity and therefore achieve a greater knowledge of the importance of alarmins in PE.

Preterm labor

Labor is the culmination of an inflammatory cascade wherein leukocytes invade the uterus to produce a broad range of uterotrophins (cytokines, chemokines and others) leading to uterine activation (cervical ripening, weakening of fetal membranes and myometrial contractility intensification). Once prematurely triggered by pathological inflammation, this pathway can lead to preterm labor, independent of infection (Romero *et al*. 2006, Christiaens *et al*. 2008). Efforts are underway to identify the most early or upstream event in this cascade to develop effective preventive treatments. Alarmins are interesting candidate as their release represents an initiating step in sterile inflammation following an injury, and because most preterm births happen without any evidence of infection, which raises interrogations about what factor (or group of factors) initiates pro-labor pathways in women with preterm labor and no infection. Accordingly, it is reported that sterile intra-amniotic inflammation is observed significantly more often than microbial-associated intra-amniotic inflammation in patients with preterm labor and intact membranes (Romero *et al*. 2014), and recent advances have found increased expressions of alarmins, namely cffDNA (Leung *et al*. 1998, Farina *et al*. 2005, Jakobsen *et al*. 2012), HMGB1 (Bredeson *et al*. 2014), interleukin-1 (Romero *et al*. 1989, Puchner *et al*. 2011), uric acid (Roberts *et al*. 2005, Homer *et al*. 2008) and S100B (Friel *et al*. 2007), in maternal serum or gestational tissue of women at risk of preterm labor or having delivered preterm. This increase in alarmins could be a link between numerous causes of preterm labor wherein tissue injury and cell death are implicated, and the initiation of a pro-inflammatory, pro-labor response. These causes include placental and uterine senescence, breakdown of maternal/fetal tolerance, uterine and cervical structural insufficiency, hemorrhage, multiple pregnancy, vascular disorders and hypoxia/ischemia. Establishment of such a link could convey major implications for the development of effective therapeutics and diagnosis. Along these lines, cell-free DNA fraction ≥95th percentile as screened between 14 and 20 weeks' gestation has been suggested as an effective biomarker to assess the risk of preterm birth (Dugoff *et al*. 2016).

Although most of the data linking a rise in alarmin levels and the onset of preterm labor are correlational, causal and mechanistic data have also been documented, especially for cffDNA and HMGB1. First, administration of cffDNA was found to induce placental inflammation and fetal resorption via TLR9 when injected i.p. to pregnant mice, contrastingly with the lack of effects of adult DNA (Scharfe-Nugent *et al*. 2012). This suggests that high levels of circulating cffDNA, as achieved in women with preterm or term labor (referenced above), can trigger pathological inflammation in gestational tissue via TLR9. Accordingly, hypomethylated CpG fragments, the TLR9-responsive element in cffDNA, have been found to induce prompt (24–48 h) leukocyte migration to uterus, TNFα production and preterm labor/birth in IL-10-deficient mice (Thaxton *et al*. 2009). Secondly, recent evidence shows that stimulation with HMGB1 induces the expression of uterine activation genes including *Tnfa*, *Il6* and *Pghs2* in human myocytes (Menon *et al*. 2016), and labor in mice when administered intra-amnion (Gomez-Lopez *et al*. 2016); and correspondingly, an association between high HMGB1 amniotic levels and earlier deliveries in patients with intra-amniotic sterile inflammation has been reported (Romero *et al*. 2014, Baumbusch *et al*. 2016). Furthermore, HMGB1 administration *ex vivo* in human fetal membranes induces p38-mediated IL-6 and IL-8 production (Bredeson *et al*. 2014). In this setting, a potential role in labor for the HMGB1 pathway was reported using transcriptomics and bioinformatics analysis (Stephen *et al*. 2015, Menon *et al*. 2016). Additionally, HMGB1 and its receptors RAGE, TLR2 and TLR4 are found in cervix and extranuclear fraction of HMGB1 increases with labor onset at term and preterm (Dubicke *et al*. 2010), suggesting that HMGB1 may play a role in cervical ripening. Interestingly, stimulation with endotoxins triggers HMGB1 expression and release *in vitro* in human fetal membranes (Bredeson *et al*. 2014) and *in vivo* in murine fetuses when endotoxins are administered in dams (i.p.) (Buhimschi *et al*. 2009); concordantly, women with intra-amniotic infection/inflammation and women with chorioamnionitis have higher amniotic fluid levels of HMGB1 (Romero *et al*. 2011, Romero *et al*. 2012). The latter suggests that HMGB1 may also have an implication in the infectious etiology of preterm birth.

IL-1α was the first alarmin to be associated with preterm labor and labor at term (Romero *et al*. 1989). However, as IL-1β is released in response to infection by immune and non-immune cells in the uteroplacental

Table 1 Therapeutic molecules targeting the alarmin activity of HMGB1, uric acid, IL-1α and cell-free DNA.

Target	Therapeutic molecule	Description	Mode of action	References
HMGB1/RAGE	Recombinant box A	Truncated N-terminal domain of HMGB1 (~10 kDa)	Competitive antagonist of the receptor RAGE	Kokkola *et al*. (2003), Li *et al*. (2003)
	S100P-derived RAGE antagonistic peptides	Small peptide inhibitors derived from S100P, a RAGE ligand (~1 kDa)	Binds with RAGE and inhibits HMGB1-mediated NF-κB activation	Arumugam *et al*. (2012)
	Ethyl pyruvate	Derivative of pyruvate (~116 Da)	Downregulates the HMGB1–RAGE axis *in vitro* and *in vivo*	Dave *et al*. (2009), Li *et al*. (2012)
	Methotrexate	Antimetabolite and anti-folate drug used in the treatment of cancer and autoimmune diseases (~454 Da)	Binds to HMGB1 and prevents its interaction with RAGE	Kuroiwa *et al*. (2013)
	Neutralizing HMGB1 antibody	Polyclonal antibody against the B box domain of HMGB1	Binds to HMGB1 and prevents its interaction with RAGE	Kokkola *et al*. (2003)
	Anti-HMGB1 mAB	Monoclonal antibody against HMGB1 (IgG2b 2G7)	Binds to HMGB1 and prevents its interaction with RAGE	Schierbeck *et al*. (2011)
	Glycyrrhizin	Natural anti-inflammatory and antiviral triterpene in clinical use (~822 Da)	Binds to HMGB1 and reduces its activity	Mollica *et al*. (2007)
	Quercetin	Plant-derived flavonoid (302 Da)	Inhibits the cytokine activity of HMGB1	Tang *et al*. (2009)
	Lycopene	Natural carotenoid (~536 Da)	Inhibits the cytokine activity of HMGB1	Lee *et al*. (2012)
	Vasoactive intestinal peptide (VIP) and urocortin	Endogenous neuropeptides	Inhibits HMGB1 secretion	Chorny and Delgado (2008)
	Pituitary adenylate cyclase-activating polypeptide (PACAP)	Endogenous neuropeptide (~4.5 kDa)	Inhibits HMGB1-induced cytokine release *in vitro* and *in vivo*	Tang *et al*. (2008)
	Chim 2A	Kinked oligonucleotide duplexes (18 bp)	Interacts with HMGB1; potently blocks a number of HMGB1 extracellular effects	Musumeci *et al*. (2011)
Uric acid	Allopurinol	Uric acid analog (~136 Da)	Reduces uric acid production by xanthine oxidase	Shi *et al*. (2003), Kono *et al*. (2010)
	Uricase, also known as urate oxidase	Homotetrameric enzyme specific to uric acid (~33 kDa)	Breaks down uric acid to allantoin	Shi *et al*. (2003), Kono *et al*. (2010)
	Sodium bicarbonate	Salt composed of sodium ions and bicarbonate ions (~84 Da)	Increases urine pH, thus increasing the dissolution and excretion of uric acid and decreasing its plasma concentrations	Netea *et al*. (1997)
	Benzbromarone	Small organic molecule (~424 Da)	Uricosuric agent and non-competitive inhibitor of xanthine oxidase	Hanvivadhanakul *et al*. (2002)
IL-1α/IL-1R	Anakinra	Recombinant version of the interleukin-1 receptor antagonist (IL1-Ra) (~17 kDa)	IL-1R competitive antagonist	Dinarello *et al*. (2012)
	Rilonacept also known as IL-1 Trap	Dimeric fusion protein (~251 kDa)	Soluble decoy IL-1R; competitive antagonist	Dinarello *et al*. (2012)
	101.10, also known as rytvela	Small peptide (all-d) (~850 Da)	Non-competitive IL-1R antagonist, negative allosteric modulator of IL-1R-associated JNK, p38, c-jun and Rho/ROCK activity	Quiniou *et al*. (2008), Nadeau-Vallee *et al*. (2015*b*)
Cell-free DNA including cffDNA and mtDNA/TLR9	ODN TTAGGG (A151)	Synthetic oligonucleotide	Interacts with TLR9; neutralizes the stimulatory effect of CpG-containing oligonucleotides	Kaminski *et al*. (2013)
	Chloroquine	Small organic molecule (~320 Da), diprotic weak base	Reduces NF-κB and AP-1 activation induced by CpG oligonucleotides; also exerts other anti-inflammatory effects	Hong *et al*. (2004)
	AT791 and E6446	Small organic molecules	Inhibit DNA–TLR9 interaction and TLR9 signaling *in vitro*; *in vivo* efficacy also reported	Lamphier *et al*. (2014)

compartment, its role has been primarily investigated rather than IL-1α. As mentioned previously, IL-1β and IL-1α bind to the same receptor and have similar effects. Evidence linking IL-1α to preterm labor are as follows: (1) stimulation of IL-1R induces the transcription of numerous pro-labor genes via MAPK p38, JNK, c-jun and small GTPase Rho in myometrial smooth muscle cells, which results in increased myometrial contractility

(Tribe *et al*. 2003, Chevillard *et al*. 2007, Nadeau-Vallee *et al*. 2015*b*); and preterm labor in mice and non-human primates (Romero *et al*. 1991, Sadowsky *et al*. 2006); (2) antagonism of IL-1R prevents all of these events (Romero & Tartakovsky 1992, Nadeau-Vallee *et al*. 2015*b*); (3) IL-1α amniotic fluid levels are elevated in women that deliver preterm (Figueroa *et al*. 2005); (4) preterm labor is associated with increased IL-1α activity in amniotic fluids (Romero *et al*. 1989); and (5) maternal polymorphisms and haplotypes in the IL-1α gene (Sata *et al*. 2009), as well as fetal polymorphism in the endogenous IL-1R antagonist (Witkin *et al*. 2003), have been associated with increased risk of preterm birth. The critical role of IL-1 in preterm labor has been reviewed elsewhere (Nadeau-Vallee *et al*. 2015*a*).

Interestingly, sterile inflammation has been suggested to induce a common inflammatory pathway leading to labor at term in normal pregnancies (Kobayashi 2012, Phillippe 2014). Accordingly, transcriptomic analysis of choriodecidual tissue collected at term predicted HMGB1 as a potential upstream regulator of parturition (Stephen *et al*. 2015). As mentioned previously, cffDNA is another alarmin candidate initiator of labor at term (Phillippe 2014).

Perspectives

Inflammatory processes are key determinants of pregnancy outcomes, independent of infection. We hereby presented a body of evidence pointing to an important role of alarmins in numerous complications of pregnancy. Alarmins are released as a result of sterile tissue stress and trigger an inflammatory cascade via PRRs (and other receptors), which are abundantly expressed in gestational tissue in immune and non-immune cells. Of all candidate alarmins, HMGB1, uric acid, IL-1α and cffDNA have been predominantly studied for their effect in pathological pregnancy events, particularly miscarriages, RPL, placental dysfunction, IUGR, preeclampsia and preterm labor. Data accumulated to date converge toward a deleterious contribution of alarmins to the pathophysiology of these diseases. Hence, effectively blocking alarmins could potentially result in favorable outcomes. This strategy has yielded positive outcomes in other inflammatory diseases, as it applies to HMGB1 (Gong *et al*. 2009), uric acid (Netea *et al*. 1997), IL-1 (Thaler *et al*. 2009) and cffDNA (Scharfe-Nugent *et al*. 2012). Therapies effective to block HMGB1, uric acid, IL-1α and cffDNA are summarized in Table 1. Furthermore, the development of suitable animal models of IUGR and preeclampsia is an unavoidable step in assessing the efficacy and innocuousness of such therapy. Although many models have been described, none of them reproduce the complete spectrum of pathophysiological changes associated with IUGR or preeclampsia (McCarthy *et al*. 2011, Swanson & David 2015). Further efforts are also needed to develop specific and potent antagonists of uric acid and cffDNA to gain better knowledge in their role during physiological and pathological pregnancy.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

Al Nakib M, Desbriere R, Bonello N, Bretelle F, Boubli L, Gabert J & Levy-Mozziconacci A 2009 Total and fetal cell-free DNA analysis in maternal blood as markers of placental insufficiency in intrauterine growth restriction. *Fetal Diagnosis and Therapy* **26** 24–28. (doi:10.1159/000236355)

Alberry M, Maddocks D, Jones M, Abdel Hadi M, Abdel-Fattah S, Avent N & Soothill PW 2007 Free fetal DNA in maternal plasma in anembryonic pregnancies: confirmation that the origin is the trophoblast. *Prenatal Diagnosis* **27** 415–418. (doi:10.1002/pd.1700)

Alberry MS, Maddocks DG, Hadi MA, Metawi H, Hunt LP, Abdel-Fattah SA, Avent ND & Soothill PW 2009 Quantification of cell free fetal DNA in maternal plasma in normal pregnancies and in pregnancies with placental dysfunction. *American Journal of Obstetrics and Gynecology* **200** 98.e91–96. (doi:10.1016/j.ajog.2008.07.063)

Aly AS, Khandelwal M, Zhao J, Mehmet AH, Sammel MD & Parry S 2004 Neutrophils are stimulated by syncytiotrophoblast microvillous membranes to generate superoxide radicals in women with preeclampsia. *American Journal of Obstetrics and Gynecology* **190** 252–258. (doi:10.1016/j.ajog.2003.07.003)

Ananth CV, Keyes KM & Wapner RJ 2013 Pre-eclampsia rates in the United States, 1980–2010:age-period-cohort analysis. *BMJ* **347** f6564. (doi:10.1136/bmj.f6564)

Andersson U & Tracey KJ 2011 HMGB1 is a therapeutic target for sterile inflammation and infection. *Annual Review of Immunology* **29** 139–162. (doi:10.1146/annurev-immunol-030409-101323)

Andersson U, Wang H, Palmblad K, Aveberger AC, Bloom O, Erlandsson-Harris H, Janson A, Kokkola R, Zhang M, Yang H *et al*. 2000 High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. *Journal of Experimental Medicine* **192** 565–570. (doi:10.1084/jem.192.4.565)

Ariga H, Ohto H, Busch MP, Imamura S, Watson R, Reed W & Lee TH 2001 Kinetics of fetal cellular and cell-free DNA in the maternal circulation during and after pregnancy: implications for noninvasive prenatal diagnosis. *Transfusion* **41** 1524–1530. (doi:10.1046/j.1537-2995.2001.41121524.x)

Arumugam T, Ramachandran V, Gomez SB, Schmidt AM & Logsdon CD 2012 S100P-derived RAGE antagonistic peptide reduces tumor growth and metastasis. *Clinical Cancer Research* **18** 4356–4364. (doi:10.1158/1078-0432.CCR-12-0221)

Baergen R, Benirschke K & Ulich TR 1994 Cytokine expression in the placenta. The role of interleukin 1 and interleukin 1 receptor antagonist expression in chorioamnionitis and parturition. *Archives of Pathology and Laboratory Medicine* **118** 52–55.

Basu S, Binder RJ, Suto R, Anderson KM & Srivastava PK 2000 Necrotic but not apoptotic cell death releases heat shock proteins, which deliver

a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. *International Immunology* **12** 1539–1546. (doi:10.1093/intimm/12.11.1539)

Baumbusch MA, Buhimschi CS, Oliver EA, Zhao G, Thung S, Rood K & Buhimschi IA 2016 High Mobility Group-Box 1 (HMGB1) levels are increased in amniotic fluid of women with intra-amniotic inflammation-determined preterm birth, and the source may be the damaged fetal membranes. *Cytokine* **81** 82–87. (doi:10.1016/j.cyto.2016.02.013)

Bausinger H, Lipsker D, Ziylan U, Manie S, Briand JP, Cazenave JP, Muller S, Haeuw JF, Ravanat C, de la Salle H *et al*. 2002 Endotoxin-free heat-shock protein 70 fails to induce APC activation. *European Journal of Immunology* **32** 3708–3713. (doi:10.1002/1521-4141(200212)32:12<3708::AID-IMMU3708>3.0.CO;2-C)

Bhutada S, Basak T, Savardekar L, Katkam RR, Jadhav G, Metkari SM, Chaudhari UK, Kumari D, Kholkute SD, Sengupta S *et al*. 2014 High mobility group box 1 (HMGB1) protein in human uterine fluid and its relevance in implantation. *Human Reproduction* **29** 763–780. (doi:10.1093/humrep/det461)

Binder RJ, Anderson KM, Basu S & Srivastava PK 2000 Cutting edge: heat shock protein gp96 induces maturation and migration of CD11c+ cells in vivo. *Journal of Immunology* **165** 6029–6035. (doi:10.4049/jimmunol.165.11.6029)

Bischoff FZ, Lewis DE & Simpson JL 2005 Cell-free fetal DNA in maternal blood: kinetics, source and structure. *Human Reproduction Update* **11** 59–67. (doi:10.1093/humupd/dmh053)

Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller AB, Kinney M & Lawn J 2013 Born too soon: the global epidemiology of 15 million preterm births. *Reproductive Health* **10** (Supplement 1) S2. (doi:10.1186/1742-4755-10-S1-S2)

Boivin J, Bunting L, Collins JA & Nygren KG 2007 International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Human Reproduction* **22** 1506–1512. (doi:10.1093/humrep/dem046)

Boonyaratanakornkit V, Melvin V, Prendergast P, Altmann M, Ronfani L, Bianchi ME, Taraseviciene L, Nordeen SK, Allegretto EA & Edwards DP 1998 High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding in vitro and transcriptional activity in mammalian cells. *Molecular and Cellular Biology* **18** 4471–4487. (doi:10.1128/MCB.18.8.4471)

Bredeson S, Papaconstantinou J, Deford JH, Kechichian T, Syed TA, Saade GR & Menon R 2014 HMGB1 promotes a p38MAPK associated non-infectious inflammatory response pathway in human fetal membranes. *PLoS ONE* **9** e113799. (doi:10.1371/journal.pone.0113799)

Breitbach S, Tug S & Simon P 2012 Circulating cell-free DNA: an up-coming molecular marker in exercise physiology. *Sports Medicine* **42** 565–586. (doi:10.2165/11631380-000000000-00000)

Buhimschi CS, Baumbusch MA, Dulay AT, Oliver EA, Lee S, Zhao G, Bhandari V, Ehrenkranz RA, Weiner CP, Madri JA *et al*. 2009 Characterization of RAGE, HMGB1, and S100beta in inflammation-induced preterm birth and fetal tissue injury. *American Journal of Pathology* **175** 958–975. (doi:10.2353/ajpath.2009.090156)

Carp H 1982 Mitochondrial N-formylmethionyl proteins as chemoattractants for neutrophils. *Journal of Experimental Medicine* **155** 264–275. (doi:10.1084/jem.155.1.264)

Celona B, Weiner A, Di Felice F, Mancuso FM, Cesarini E, Rossi RL, Gregory L, Baban D, Rossetti G, Grianti P *et al*. 2011 Substantial histone reduction modulates genomewide nucleosomal occupancy and global transcriptional output. *PLoS Biology* **9** e1001086. (doi:10.1371/journal.pbio.1001086)

Chaiworapongsa T, Chaemsaithong P, Yeo L & Romero R 2014 Pre-eclampsia part 1: current understanding of its pathophysiology. *Nature Reviews Nephrology* **10** 466–480. (doi:10.1038/nrneph.2014.102)

Chan KC & Lo YM 2002 Circulating nucleic acids as a tumor marker. *Histology and Histopathology* **17** 937–943.

Chan KC, Zhang J, Hui AB, Wong N, Lau TK, Leung TN, Lo KW, Huang DW & Lo YM 2004 Size distributions of maternal and fetal DNA in maternal plasma. *Clinical Chemistry* **50** 88–92. (doi:10.1373/clinchem.2003.024893)

Chekir C, Nakatsuka M, Noguchi S, Konishi H, Kamada Y, Sasaki A, Hao L & Hiramatsu Y 2006 Accumulation of advanced glycation end products in women with preeclampsia: possible involvement of placental oxidative and nitrative stress. *Placenta* **27** 225–233. (doi:10.1016/j.placenta.2005.02.016)

Chen CJ, Kono H, Golenbock D, Reed G, Akira S & Rock KL 2007 Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nature Medicine* **13** 851–856. (doi:10.1038/nm1603)

Chen Q, Stone PR, McCowan LM & Chamley LW 2006 Phagocytosis of necrotic but not apoptotic trophoblasts induces endothelial cell activation. *Hypertension* **47** 116–121. (doi:10.1161/01.HYP.0000196731.56062.7c)

Chevillard G, Derjuga A, Devost D, Zingg HH & Blank V 2007 Identification of interleukin-1beta regulated genes in uterine smooth muscle cells. *Reproduction* **134** 811–822. (doi:10.1530/REP-07-0289)

Chorny A & Delgado M 2008 Neuropeptides rescue mice from lethal sepsis by down-regulating secretion of the late-acting inflammatory mediator high mobility group box 1. *American Journal of Pathology* **172** 1297–1307. (doi:10.2353/ajpath.2008.070969)

Christiaens I, Zaragoza DB, Guilbert L, Robertson SA, Mitchell BF & Olson DM 2008 Inflammatory processes in preterm and term parturition. *Journal of Reproductive Immunology* **79** 50–57. (doi:10.1016/j.jri.2008.04.002)

Chua S, Wilkins T, Sargent I & Redman C 1991 Trophoblast deportation in pre-eclamptic pregnancy. *British Journal of Obstetrics and Gynaecology* **98** 973–979. (doi:10.1111/j.1471-0528.1991.tb15334.x)

Cohen I, Rider P, Carmi Y, Braiman A, Dotan S, White MR, Voronov E, Martin MU, Dinarello CA & Apte RN 2010 Differential release of chromatin-bound IL-1alpha discriminates between necrotic and apoptotic cell death by the ability to induce sterile inflammation. *PNAS* **107** 2574–2579. (doi:10.1073/pnas.0915018107)

Colleoni F, Lattuada D, Garretto A, Massari M, Mando C, Somigliana E & Cetin I 2010 Maternal blood mitochondrial DNA content during normal and intrauterine growth restricted (IUGR) pregnancy. *American Journal of Obstetrics and Gynecology* **203** 365.e361–366. (doi:10.1016/j.ajog.2010.05.027)

Comba C, Bastu E, Dural O, Yasa C, Keskin G, Ozsurmeli M, Buyru F & Serdaroglu H 2015 Role of inflammatory mediators in patients with recurrent pregnancy loss. *Fertility and Sterility* **104** 1467–1474.e1461. (doi:10.1016/j.fertnstert.2015.08.011)

Cronstein BN, Daguma L, Nichols D, Hutchison AJ & Williams M 1990 The adenosine/neutrophil paradox resolved: human neutrophils possess both A1 and A2 receptors that promote chemotaxis and inhibit O2 generation, respectively. *Journal of Clinical Investigation* **85** 1150–1157. (doi:10.1172/JCI114547)

D'Ippolito S, Tersigni C, Marana R, Di Nicuolo F, Gaglione R, Rossi ED, Castellani R, Scambia G & Di Simone N 2016 Inflammosome in the human endometrium: further step in the evaluation of the 'maternal side'. *Fertility and Sterility* **105** 111–118.e111–114. (doi:10.1016/j.fertnstert.2015.09.027)

Dave SH, Tilstra JS, Matsuoka K, Li F, DeMarco RA, Beer-Stolz D, Sepulveda AR, Fink MP, Lotze MT & Plevy SE 2009 Ethyl pyruvate decreases HMGB1 release and ameliorates murine colitis. *Journal of Leukocyte Biology* **86** 633–643. (doi:10.1189/jlb.1008662)

De Y, Chen Q, Schmidt AP, Anderson GM, Wang JM, Wooters J, Oppenheim JJ & Chertov O 2000 LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. *Journal of Experimental Medicine* **192** 1069–1074. (doi:10.1084/jem.192.7.1069)

Decker P, Singh-Jasuja H, Haager S, Kotter I & Rammensee HG 2005 Nucleosome, the main autoantigen in systemic lupus erythematosus, induces direct dendritic cell activation via a MyD88-independent pathway: consequences on inflammation. *Journal of Immunology* **174** 3326–3334. (doi:10.4049/jimmunol.174.6.3326)

Di Paolo NC, Miao EA, Iwakura Y, Murali-Krishna K, Aderem A, Flavell RA, Papayannopoulou T & Shayakhmetov DM 2009 Virus binding to a plasma membrane receptor triggers interleukin-1 alpha-mediated proinflammatory macrophage response in vivo. *Immunity* **31** 110–121. (doi:10.1016/j.immuni.2009.04.015)

Dinarello CA 2009 Immunological and inflammatory functions of the interleukin-1 family. *Annual Review of Immunology* **27** 519–550. (doi:10.1146/annurev.immunol.021908.132612)

Dinarello CA, Simon A & van der Meer JW 2012 Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nature Reviews Drug Discovery* **11** 633–652. (doi:10.1038/nrd3800)

Dubicke A, Andersson P, Fransson E, Andersson E, Sioutas A, Malmstrom A, Sverremark-Ekstrom E & Ekman-Ordeberg G 2010 High-mobility group box protein 1 and its signalling receptors in human preterm and term cervix. *Journal of Reproductive Immunology* **84** 86–94. (doi:10.1016/j.jri.2009.09.010)

Dugoff L, Barberio A, Whittaker PG, Schwartz N, Sehdev H & Bastek JA 2016 Cell-free DNA fetal fraction and preterm birth. *American Journal of Obstetrics and Gynecology* **215** 231.e1–231.e7. (doi:10.1016/j.ajog.2016.02.009)

Eigenbrod T, Park JH, Harder J, Iwakura Y & Nunez G 2008 Cutting edge: critical role for mesothelial cells in necrosis-induced inflammation through the recognition of IL-1 alpha released from dying cells. *Journal of Immunology* **181** 8194–8198. (doi:10.4049/jimmunol.181.12.8194)

Erlandsson Harris H & Andersson U 2004 Mini-review: the nuclear protein HMGB1 as a proinflammatory mediator. *European Journal of Immunology* **34** 1503–1512. (doi:10.1002/eji.200424916)

Erlebacher A 2013 Immunology of the maternal-fetal interface. *Annual Review of Immunology* **31** 387–411. (doi:10.1146/annurev-immunol-032712-100003)

Faires JS & Mccarty DJJ 1962 Acute arthritis in man and dog after intrasynovial injection of sodium urate crystals. *Lancet* **2** 682–685. (doi:10.1016/S0140-6736(62)90501-9)

Farina A, LeShane ES, Romero R, Gomez R, Chaiworapongsa T, Rizzo N & Bianchi DW 2005 High levels of fetal cell-free DNA in maternal serum: a risk factor for spontaneous preterm delivery. *American Journal of Obstetrics and Gynecology* **193** 421–425. (doi:10.1016/j.ajog.2004.12.023)

Figueroa R, Garry D, Elimian A, Patel K, Sehgal PB & Tejani N 2005 Evaluation of amniotic fluid cytokines in preterm labor and intact membranes. *Journal of Maternal-Fetal and Neonatal Medicine* **18** 241–247. (doi:10.1080/13506120500223241)

Florio P, Michetti F, Bruschettini M, Lituania M, Bruschettini P, Severi FM, Petraglia F & Gazzolo D 2004 Amniotic fluid S100B protein in mid-gestation and intrauterine fetal death. *Lancet* **364** 270–272. (doi:10.1016/S0140-6736(04)16677-4)

Friel LA, Romero R, Edwin S, Nien JK, Gomez R, Chaiworapongsa T, Kusanovic JP, Tolosa JE, Hassan SS & Espinoza J 2007 The calcium binding protein, S100B, is increased in the amniotic fluid of women with intra-amniotic infection/inflammation and preterm labor with intact or ruptured membranes. *Journal of Perinatal Medicine* **35** 385–393. (doi:10.1515/jpm.2007.101)

Gao B & Tsan MF 2003 Endotoxin contamination in recombinant human heat shock protein 70 (Hsp70) preparation is responsible for the induction of tumor necrosis factor alpha release by murine macrophages. *Journal of Biological Chemistry* **278** 174–179. (doi:10.1074/jbc.M208742200)

Gazzolo D, Marinoni E, Di Iorio R, Lituania M, Marras M, Bruschettini M, Bruschettini P, Frulio R, Michetti F, Petraglia F *et al*. 2006 High maternal blood S100B concentrations in pregnancies complicated by intrauterine growth restriction and intraventricular hemorrhage. *Clinical Chemistry* **52** 819–826. (doi:10.1373/clinchem.2005.060665)

Ghasemi M, Kashani E, Fayyaz A, Attar M & Shahbazi M 2015 Interleukin-1 alpha variation is associated with the risk of developing preeclampsia. *European Journal of Obstetrics and Gynecology and Reproductive Biology* **193** 75–78. (doi:10.1016/j.ejogrb.2015.06.020)

Girard S, Heazell AE, Derricott H, Allan SM, Sibley CP, Abrahams VM & Jones RL 2014 Circulating cytokines and alarmins associated with placental inflammation in high-risk pregnancies. *American Journal of Reproductive Immunology* **72** 422–434. (doi:10.1111/aji.12274)

Girard S, Tremblay L, Lepage M & Sebire G 2010 IL-1 receptor antagonist protects against placental and neurodevelopmental defects induced by maternal inflammation. *Journal of Immunology* **184** 3997–4005. (doi:10.4049/jimmunol.0903349)

Gomez-Lopez N, Romero R, Plazyo O, Panaitescu B, Furcron AE, Miller D, Roumayah T, Flom E & Hassan SS 2016 Intra-amniotic administration of HMGB1 induces spontaneous preterm labor and birth. *American Journal of Reproductive Immunology* **75** 3–7. (doi:10.1111/aji.12443)

Gong Q, Xu JF, Yin H, Liu SF, Duan LH & Bian ZL 2009 Protective effect of antagonist of high-mobility group box 1 on lipopolysaccharide-induced acute lung injury in mice. *Scandinavian Journal of Immunology* **69** 29–35. (doi:10.1111/j.1365-3083.2008.02194.x)

Goulopoulou S, Matsumoto T, Bomfim GF & Webb RC 2012 Toll-like receptor 9 activation: a novel mechanism linking placenta-derived mitochondrial DNA and vascular dysfunction in pre-eclampsia. *Clinical Science* **123** 429–435. (doi:10.1042/CS20120130)

Gupta S, Goldberg JM, Aziz N, Goldberg E, Krajcir N & Agarwal A 2008 Pathogenic mechanisms in endometriosis-associated infertility. *Fertility and Sterility* **90** 247–257. (doi:10.1016/j.fertnstert.2008.02.093)

Han CS, Herrin MA, Pitruzzello MC, Mulla MJ, Werner EF, Pettker CM, Flannery CA & Abrahams VM 2015 Glucose and metformin modulate human first trimester trophoblast function: a model and potential therapy for diabetes-associated uteroplacental insufficiency. *American Journal of Reproductive Immunology* **73** 362–371. (doi:10.1111/aji.12339)

Hanvivadhanakul P, Akkasilpa S & Deesomchok U 2002 Efficacy of benzbromarone compared to allopurinol in lowering serum uric acid level in hyperuricemic patients. *Journal of the Medical Association of Thailand* **85** (Supplement 1) S40–S47.

Harmon AC, Cornelius DC, Amaral LM, Faulkner JL, Cunningham MW Jr, Wallace K & LaMarca B 2016 The role of inflammation in the pathology of preeclampsia. *Clinical Science* **130** 409–419. (doi:10.1042/CS20150702)

Harris HE, Andersson U & Pisetsky DS 2012 HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease. *Nature Reviews Rheumatology* **8** 195–202. (doi:10.1038/nrrheum.2011.222)

Hartley JD, Ferguson BJ & Moffett A 2015 The role of shed placental DNA in the systemic inflammatory syndrome of preeclampsia. *American Journal of Obstetrics and Gynecology* **213** 268–277. (doi:10.1016/j.ajog.2015.03.026)

Hawkins TL, Roberts JM, Mangos GJ, Davis GK, Roberts LM & Brown MA 2012 Plasma uric acid remains a marker of poor outcome in hypertensive pregnancy: a retrospective cohort study. *BJOG* **119** 484–492. (doi:10.1111/j.1471-0528.2011.03232.x)

Hefler LA, Tempfer CB & Gregg AR 2001 Polymorphisms within the interleukin-1 beta gene cluster and preeclampsia. *Obstetrics and Gynecology* **97** 664–668. (doi:10.1016/s0029-7844(01)01128-0)

Heyborne KD, McGregor JA, Henry G, Witkin SS & Abrams JS 1994 Interleukin-10 in amniotic fluid at midtrimester: immune activation and suppression in relation to fetal growth. *American Journal of Obstetrics and Gynecology* **171** 55–59. (doi:10.1016/S0002-9378(94)70077-X)

Hofmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y, Avila C, Kambham N, Bierhaus A, Nawroth P *et al*. 1999 RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell* **97** 889–901. (doi:10.1016/S0092-8674(00)80801-6)

Homer CS, Brown MA, Mangos G & Davis GK 2008 Non-proteinuric pre-eclampsia: a novel risk indicator in women with gestational hypertension. *Journal of Hypertension* **26** 295–302. (doi:10.1097/HJH.0b013e3282f1a953)

Hong Z, Jiang Z, Liangxi W, Guofu D, Ping L, Yongling L, Wendong P & Minghai W 2004 Chloroquine protects mice from challenge with CpG ODN and LPS by decreasing proinflammatory cytokine release. *International Immunopharmacology* **4** 223–234. (doi:10.1016/j.intimp.2003.12.006)

Hreggvidsdottir HS, Ostberg T, Wahamaa H, Schierbeck H, Aveberger AC, Klevenvall L, Palmblad K, Ottosson L, Andersson U & Harris HE 2009 The alarmin HMGB1 acts in synergy with endogenous and exogenous danger signals to promote inflammation. *Journal of Leukocyte Biology* **86** 655–662. (doi:10.1189/jlb.0908548)

Huppertz B, Frank HG, Kingdom JC, Reister F & Kaufmann P 1998 Villous cytotrophoblast regulation of the syncytial apoptotic cascade in the human placenta. *Histochemistry and Cell Biology* **110** 495–508. (doi:10.1007/s004180050311)

Huppertz B, Kingdom J, Caniggia I, Desoye G, Black S, Korr H & Kaufmann P 2003 Hypoxia favours necrotic vs apoptotic shedding of placental syncytiotrophoblast into the maternal circulation. *Placenta* **24** 181–190. (doi:10.1053/plac.2002.0903)

Huppertz B & Kingdom JC 2004 Apoptosis in the trophoblast – role of apoptosis in placental morphogenesis. *Journal of the Society for Gynecologic Investigation* **11** 353–362. (doi:10.1016/j.jsgi.2004.06.002)

Idan C, Peleg R, Elena V, Martin T, Cicerone T, Mareike W, Lydia B, Marina F, Gerhard M, Elisa FM *et al*. 2015 IL-1alpha is a DNA damage

sensor linking genotoxic stress signaling to sterile inflammation and innate immunity. *Scientific Reports* **5** 14756. (doi:10.1038/srep14756)

Jakobsen TR, Clausen FB, Rode L, Dziegiel MH & Tabor A 2012 High levels of fetal DNA are associated with increased risk of spontaneous preterm delivery. *Prenatal Diagnosis* **32** 840–845. (doi:10.1002/pd.3917)

Jin H, Wu J, Yang Q, Cai Y, He W & Liu C 2015 High mobility group box 1 protein polymorphism affects susceptibility to recurrent pregnancy loss by up-regulating gene expression in chorionic villi. *Journal of Assisted Reproduction and Genetics* **32** 1123–1128. (doi:10.1007/s10815-015-0493-3)

Johansen M, Redman CW, Wilkins T & Sargent IL 1999 Trophoblast deportation in human pregnancy – its relevance for pre-eclampsia. *Placenta* **20** 531–539. (doi:10.1053/plac.1999.0422)

Johnson RJ, Kang DH, Feig D, Kivlighn S, Kanellis J, Watanabe S, Tuttle KR, Rodriguez-Iturbe B, Herrera-Acosta J & Mazzali M 2003 Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? *Hypertension* **41** 1183–1190. (doi:10.1161/01.HYP.0000069700.62727.C5)

Jones CJ & Fox H 1980 An ultrastructural and ultrahistochemical study of the human placenta in maternal pre-eclampsia. *Placenta* **1** 61–76. (doi:10.1016/S0143-4004(80)80016-6)

Kaminski JJ, Schattgen SA, Tzeng TC, Bode C, Klinman DM & Fitzgerald KA 2013 Synthetic oligodeoxynucleotides containing suppressive TTAGGG motifs inhibit AIM2 inflammasome activation. *Journal of Immunology* **191** 3876–3883. (doi:10.4049/jimmunol.1300530)

Kanellis J, Watanabe S, Li JH, Kang DH, Li P, Nakagawa T, Wamsley A, Sheikh-Hamad D, Lan HY, Feng L *et al*. 2003 Uric acid stimulates monocyte chemoattractant protein-1 production in vascular smooth muscle cells via mitogen-activated protein kinase and cyclooxygenase-2. *Hypertension* **41** 1287–1293. (doi:10.1161/01.HYP.0000072820.07472.3B)

Kang DH, Finch J, Nakagawa T, Karumanchi SA, Kanellis J, Granger J & Johnson RJ 2004 Uric acid, endothelial dysfunction and pre-eclampsia: searching for a pathogenetic link. *Journal of Hypertension* **22** 229–235. (doi:10.1097/00004872-200402000-00001)

Kang DH, Nakagawa T, Feng L, Watanabe S, Han L, Mazzali M, Truong L, Harris R & Johnson RJ 2002 A role for uric acid in the progression of renal disease. *Journal of the American Society of Nephrology* **13** 2888–2897. (doi:10.1097/01.ASN.0000034910.58454.FD)

Kang DH, Park SK, Lee IK & Johnson RJ 2005 Uric acid-induced C-reactive protein expression: implication on cell proliferation and nitric oxide production of human vascular cells. *Journal of the American Society of Nephrology* **16** 3553–3562. (doi:10.1681/ASN.2005050572)

Kim YM, Romero R, Oh SY, Kim CJ, Kilburn BA, Armant DR, Nien JK, Gomez R, Mazor M, Saito S *et al*. 2005 Toll-like receptor 4: a potential link between 'danger signals,' the innate immune system, and preeclampsia? *American Journal of Obstetrics and Gynecology* **193** 921–927. (doi:10.1016/j.ajog.2005.07.076)

Knight M, Redman CW, Linton EA & Sargent IL 1998 Shedding of syncytiotrophoblast microvilli into the maternal circulation in pre-eclamptic pregnancies. *British Journal of Obstetrics and Gynaecology* **105** 632–640. (doi:10.1111/j.1471-0528.1998.tb10178.x)

Kobayashi H 2012 The entry of fetal and amniotic fluid components into the uterine vessel circulation leads to sterile inflammatory processes during parturition. *Frontiers in Immunology* **3** 321. (doi:10.3389/fimmu.2012.00321)

Koga K & Mor G 2010 Toll-like receptors at the maternal-fetal interface in normal pregnancy and pregnancy disorders. *American Journal of Reproductive Immunology* **63** 587–600. (doi:10.1111/j.1600-0897.2010.00848.x)

Kokkola R, Li J, Sundberg E, Aveberger AC, Palmblad K, Yang H, Tracey KJ, Andersson U & Harris HE 2003 Successful treatment of collagen-induced arthritis in mice and rats by targeting extracellular high mobility group box chromosomal protein 1 activity. *Arthritis and Rheumatism* **48** 2052–2058. (doi:10.1002/art.11161)

Kono H, Chen CJ, Ontiveros F & Rock KL 2010 Uric acid promotes an acute inflammatory response to sterile cell death in mice. *Journal of Clinical Investigation* **120** 1939–1949. (doi:10.1172/JCI40124)

Krishnan SM, Sobey CG, Latz E, Mansell A & Drummond GR 2014 IL-1beta and IL-18: inflammatory markers or mediators of hypertension? *British Journal of Pharmacology* **171** 5589–5602. (doi:10.1111/bph.12876)

Kuroiwa Y, Takakusagi Y, Kusayanagi T, Kuramochi K, Imai T, Hirayama T, Ito I, Yoshida M, Sakaguchi K & Sugawara F 2013 Identification and characterization of the direct interaction between methotrexate (MTX) and high-mobility group box 1 (HMGB1) protein. *PLoS ONE* **8** e63073. (doi:10.1371/journal.pone.0063073)

Laird SM, Tuckerman EM, Cork BA, Linjawi S, Blakemore AI & Li TC 2003 A review of immune cells and molecules in women with recurrent miscarriage. *Human Reproduction Update* **9** 163–174. (doi:10.1093/humupd/dmg013)

Lamphier M, Zheng W, Latz E, Spyvee M, Hansen H, Rose J, Genest M, Yang H, Shaffer C, Zhao Y *et al*. 2014 Novel small molecule inhibitors of TLR7 and TLR9: mechanism of action and efficacy in vivo. *Molecular Pharmacology* **85** 429–440. (doi:10.1124/mol.113.089821)

Lappas M 2013 NOD1 and NOD2 regulate proinflammatory and prolabor mediators in human fetal membranes and myometrium via nuclear factor-kappa B. *Biology of Reproduction* **89** 14. (doi:10.1095/biolreprod.113.110056)

Laresgoiti-Servitje E 2013 A leading role for the immune system in the pathophysiology of preeclampsia. *Journal of Leukocyte Biology* **94** 247–257. (doi:10.1189/jlb.1112603)

Lattuada D, Colleoni F, Martinelli A, Garretto A, Magni R, Radaelli T & Cetin I 2008 Higher mitochondrial DNA content in human IUGR placenta. *Placenta* **29** 1029–1033. (doi:10.1016/j.placenta.2008.09.012)

Latz E, Schoenemeyer A, Visintin A, Fitzgerald KA, Monks BG, Knetter CF, Lien E, Nilsen NJ, Espevik T & Golenbock DT 2004 TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nature Immunology* **5** 190–198. (doi:10.1038/ni1028)

Latz E, Verma A, Visintin A, Gong M, Sirois CM, Klein DC, Monks BG, McKnight CJ, Lamphier MS, Duprex WP *et al*. 2007 Ligand-induced conformational changes allosterically activate Toll-like receptor 9. *Nature Immunology* **8** 772–779. (doi:10.1038/ni1479)

Laughon SK, Catov J, Powers RW, Roberts JM & Gandley RE 2011 First trimester uric acid and adverse pregnancy outcomes. *American Journal of Hypertension* **24** 489–495. (doi:10.1038/ajh.2010.262)

Lazar L, Rigo J Jr, Nagy B, Balogh K, Mako V, Cervenak L, Mezes M, Prohaszka Z & Molvarec A 2009 Relationship of circulating cell-free DNA levels to cell-free fetal DNA levels, clinical characteristics and laboratory parameters in preeclampsia. *BMC Medical Genetics* **10** 120. (doi:10.1186/1471-2350-10-120)

Lee JY, Sohn KH, Rhee SH & Hwang D 2001 Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. *Journal of Biological Chemistry* **276** 16683–16689. (doi:10.1074/jbc.M011695200)

Lee W, Ku SK, Bae JW & Bae JS 2012 Inhibitory effects of lycopene on HMGB1-mediated pro-inflammatory responses in both cellular and animal models. *Food and Chemical Toxicology* **50** 1826–1833. (doi:10.1016/j.fct.2012.03.003)

Leung DN, Smith SC, To KF, Sahota DS & Baker PN 2001 Increased placental apoptosis in pregnancies complicated by preeclampsia. *American Journal of Obstetrics and Gynecology* **184** 1249–1250. (doi:10.1067/mob.2001.112906)

Leung TN, Zhang J, Lau TK, Hjelm NM & Lo YM 1998 Maternal plasma fetal DNA as a marker for preterm labour. *Lancet* **352** 1904–1905. (doi:10.1016/S0140-6736(05)60395-9)

Levine RJ, Qian C, Leshane ES, Yu KF, England LJ, Schisterman EF, Wataganara T, Romero R & Bianchi DW 2004 Two-stage elevation of cell-free fetal DNA in maternal sera before onset of preeclampsia. *American Journal of Obstetrics and Gynecology* **190** 707–713. (doi:10.1016/j.ajog.2003.12.019)

Li J, Kokkola R, Tabibzadeh S, Yang R, Ochani M, Qiang X, Harris HE, Czura CJ, Wang H, Ulloa L *et al*. 2003 Structural basis for the proinflammatory cytokine activity of high mobility group box 1. *Molecular Medicine* **9** 37–45.

Li J, Liu M, Zong J, Tan P, Wang J, Wang X, Ye Y, Liu S & Liu X 2014 Genetic variations in IL1A and IL1RN are associated with the risk of preeclampsia in Chinese Han population. *Scientific Reports* **4** 5250. (doi:10.1038/srep05250)

Li ML, Wang XF, Tan ZJ, Dong P, Gu J, Lu JH, Wu XS, Zhang L, Ding QC, Wu WG *et al*. 2012 Ethyl pyruvate administration suppresses growth and invasion of gallbladder cancer cells via downregulation of HMGB1-RAGE axis. *International Journal of Immunopathology and Pharmacology* **25** 955–965.

Lo YM, Zhang J, Leung TN, Lau TK, Chang AM & Hjelm NM 1999 Rapid clearance of fetal DNA from maternal plasma. *American Journal of Human Genetics* **64** 218–224. (doi:10.1086/302205)

Lukens JR, Gross JM & Kanneganti TD 2012 IL-1 family cytokines trigger sterile inflammatory disease. *Frontiers in Immunology* **3** 315. (doi:10.3389/fimmu.2012.00315)

Majer S, Bauer M, Magnet E, Strele A, Giegerl E, Eder M, Lang U & Pertl B 2007 Maternal urine for prenatal diagnosis – an analysis of cell-free fetal DNA in maternal urine and plasma in the third trimester. *Prenatal Diagnosis* **27** 1219–1223. (doi:10.1002/pd.1875)

Martin A, Krishna I, Badell M & Samuel A 2014 Can the quantity of cell-free fetal DNA predict preeclampsia: a systematic review. *Prenatal Diagnosis* **34** 685–691. (doi:10.1002/pd.4416)

Martin AC & Brown MA 2010 Could uric acid have a pathogenic role in pre-eclampsia? *Nature Reviews Nephrology* **6** 744–748. (doi:10.1038/nrneph.2010.125)

Martinez-Zamora MA, Tassies D, Reverter JC, Creus M, Casals G, Civico S, Carmona F & Balasch J 2016 Increased circulating cell-derived microparticle count is associated with recurrent implantation failure after IVF and embryo transfer. *Reproductive BioMedicine Online* **33** 168–173. (doi:10.1016/j.rbmo.2016.05.005)

Martinon F, Petrilli V, Mayor A, Tardivel A & Tschopp J 2006 Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* **440** 237–241. (doi:10.1038/nature04516)

Masuzaki H, Miura K, Yoshiura KI, Yoshimura S, Niikawa N & Ishimaru T 2004 Detection of cell free placental DNA in maternal plasma: direct evidence from three cases of confined placental mosaicism. *Journal of Medical Genetics* **41** 289–292. (doi:10.1136/jmg.2003.015784)

Matzinger P 1994 Tolerance, danger, and the extended family. *Annual Review of Immunology* **12** 991–1045. (doi:10.1146/annurev.iy.12.040194.005015)

Maxwell JR, Denson JL, Joste NE, Robinson S & Jantzie LL 2015 Combined in utero hypoxia-ischemia and lipopolysaccharide administration in rats induces chorioamnionitis and a fetal inflammatory response syndrome. *Placenta* **36** 1378–1384. (doi:10.1016/j.placenta.2015.10.009)

Mazzali M, Hughes J, Kim YG, Jefferson JA, Kang DH, Gordon KL, Lan HY, Kivlighn S & Johnson RJ 2001 Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. *Hypertension* **38** 1101–1106. (doi:10.1161/hy1101.092839)

McCarthy CM & Kenny LC 2016 Immunostimulatory role of mitochondrial DAMPs: alarming for pre-eclampsia? *American Journal of Reproductive Immunology* **76** 341–347. (doi:10.1111/aji.12526)

McCarthy FP, Kingdom JC, Kenny LC & Walsh SK 2011 Animal models of preeclampsia; uses and limitations. *Placenta* **32** 413–419. (doi:10.1016/j.placenta.2011.03.010)

Mellembakken JR, Aukrust P, Hestdal K, Ueland T, Abyholm T & Videm V 2001 Chemokines and leukocyte activation in the fetal circulation during preeclampsia. *Hypertension* **38** 394–398. (doi:10.1161/01.HYP.38.3.394)

Menon R, Behnia F, Polettini J, Saade GR, Campisi J & Velarde M 2016 Placental membrane aging and HMGB1 signaling associated with human parturition. *Aging* **8** 216–230. (doi:10.18632/aging.100891)

Mert I, Oruc AS, Yuksel S, Cakar ES, Buyukkagnici U, Karaer A & Danisman N 2012 Role of oxidative stress in preeclampsia and intrauterine growth restriction. *Journal of Obstetrics and Gynaecology Research* **38** 658–664. (doi:10.1111/j.1447-0756.2011.01771.x)

Mikhail MS, Anyaegbunam A, Garfinkel D, Palan PR, Basu J & Romney SL 1994 Preeclampsia and antioxidant nutrients: decreased plasma levels of reduced ascorbic acid, alpha-tocopherol, and beta-carotene in women with preeclampsia. *American Journal of Obstetrics and Gynecology* **171** 150–157. (doi:10.1016/0002-9378(94)90462-6)

Mollica L, De Marchis F, Spitaleri A, Dallacosta C, Pennacchini D, Zamai M, Agresti A, Trisciuoglio L, Musco G & Bianchi ME 2007 Glycyrrhizin binds to high-mobility group box 1 protein and inhibits its cytokine activities. *Chemistry and Biology* **14** 431–441. (doi:10.1016/j.chembiol.2007.03.007)

Mulla MJ, Myrtolli K, Potter J, Boeras C, Kavathas PB, Sfakianaki AK, Tadesse S, Norwitz ER, Guller S & Abrahams VM 2011 Uric acid induces trophoblast IL-1beta production via the inflammasome: implications for the pathogenesis of preeclampsia. *American Journal of Reproductive Immunology* **65** 542–548. (doi:10.1111/j.1600-0897.2010.00960.x)

Mulla MJ, Salmon JE, Chamley LW, Brosens JJ, Boeras CM, Kavathas PB & Abrahams VM 2013 A role for uric acid and the Nalp3 inflammasome in antiphospholipid antibody-induced IL-1beta production by human first trimester trophoblast. *PLoS ONE* **8** e65237. (doi:10.1371/journal.pone.0065237)

Musumeci D, Bucci EM, Roviello GN, Sapio R, Valente M, Moccia M, Bianchi ME & Pedone C 2011 DNA-based strategies for blocking HMGB1 cytokine activity: design, synthesis and preliminary in vitro/in vivo assays of DNA and DNA-like duplexes. *Molecular BioSystems* **7** 1742–1752. (doi:10.1039/c1mb05009e)

Musumeci D, Roviello GN & Montesarchio D 2014 An overview on HMGB1 inhibitors as potential therapeutic agents in HMGB1-related pathologies. *Pharmacology and Therapeutics* **141** 347–357. (doi:10.1016/j.pharmthera.2013.11.001)

Nadeau-Vallee M, Obari D, Quiniou C, Lubell WD, Olson DM, Girard S & Chemtob S 2015*a* A critical role of interleukin-1 in preterm labor. *Cytokine and Growth Factor Reviews* **28** 37–51. (doi:10.1016/j.cytogfr.2015.11.001)

Nadeau-Vallee M, Quiniou C, Palacios J, Hou X, Erfani A, Madaan A, Sanchez M, Leimert K, Boudreault A, Duhamel F *et al*. 2015*b* Novel noncompetitive IL-1 receptor-biased ligand prevents infection- and inflammation-induced preterm birth. *Journal of Immunology* **195** 3402–3415. (doi:10.4049/jimmunol.1500758)

Nair RR, Khanna A & Singh K 2015 Association of increased S100A8 serum protein with early pregnancy loss. *American Journal of Reproductive Immunology* **73** 91–94. (doi:10.1111/aji.12318)

Nelson DM 1996 Apoptotic changes occur in syncytiotrophoblast of human placental villi where fibrin type fibrinoid is deposited at discontinuities in the villous trophoblast. *Placenta* **17** 387–391. (doi:10.1016/S0143-4004(96)90019-3)

Netea MG, Kullberg BJ, Blok WL, Netea RT & van der Meer JW 1997 The role of hyperuricemia in the increased cytokine production after lipopolysaccharide challenge in neutropenic mice. *Blood* **89** 577–582.

Nishimoto S, Fukuda D, Higashikuni Y, Tanaka K, Hirata Y, Murata C, Kim-Kaneyama JR, Sato F, Bando M, Yagi S *et al*. 2016 Obesity-induced DNA released from adipocytes stimulates chronic adipose tissue inflammation and insulin resistance. *Science Advances* **2** e1501332. (doi:10.1126/sciadv.1501332)

Olson DM, Christiaens I, Gracie S, Yamamoto Y & Mitchell BF 2008 Emerging tocolytics: challenges in designing and testing drugs to delay preterm delivery and prolong pregnancy. *Expert Opinion on Emerging Drugs* **13** 695–707. (doi:10.1517/14728210802568764)

Phillippe M 2014 Cell-free fetal DNA – a trigger for parturition. *New England Journal of Medicine* **370** 2534–2536. (doi:10.1056/NEJMcibr1404324)

Phillippe M 2015 Cell-free fetal DNA, telomeres, and the spontaneous onset of parturition. *Reproductive Sciences* **22** 1186–1201. (doi:10.1177/1933719115592714)

Pineda A, Verdin-Teran SL, Camacho A & Moreno-Fierros L 2011 Expression of toll-like receptor TLR-2, TLR-3, TLR-4 and TLR-9 is increased in placentas from patients with preeclampsia. *Archives of Medical Research* **42** 382–391. (doi:10.1016/j.arcmed.2011.08.003)

Powers RW, Bodnar LM, Ness RB, Cooper KM, Gallaher MJ, Frank MP, Daftary AR & Roberts JM 2006 Uric acid concentrations in early pregnancy among preeclamptic women with gestational hyperuricemia at delivery. *American Journal of Obstetrics and Gynecology* **194** 160. (doi:10.1016/j.ajog.2005.06.066)

Pradervand PA, Clerc S, Frantz J, Rotaru C, Bardy D, Waeber B, Liaudet L, Vial Y & Feihl F 2014 High mobility group box 1 protein (HMGB-1): a pathogenic role in preeclampsia? *Placenta* **35** 784–786. (doi:10.1016/j.placenta.2014.06.370)

Puchner K, Iavazzo C, Gourgiotis D, Boutsikou M, Baka S, Hassiakos D, Kouskouni E, Economou E, Malamitsi-Puchner A & Creatsas G 2011 Mid-trimester amniotic fluid interleukins (IL-1beta, IL-10 and IL-18) as possible predictors of preterm delivery. *In Vivo* **25** 141–148.

Qin S, Wang H, Yuan R, Li H, Ochani M, Ochani K, Rosas-Ballina M, Czura CJ, Huston JM, Miller E *et al*. 2006 Role of HMGB1 in apoptosis-mediated sepsis lethality. *Journal of Experimental Medicine* **203** 1637–1642. (doi:10.1084/jem.20052203)

Qiu C, Hevner K, Enquobahrie DA & Williams MA 2012 A case-control study of maternal blood mitochondrial DNA copy number and preeclampsia risk. *International Journal of Molecular Epidemiology and Genetics* **3** 237–244.

Qiu C, Sanchez SE, Hevner K, Enquobahrie DA & Williams MA 2015 Placental mitochondrial DNA content and placental abruption: a pilot study. *BMC Research Notes* **8** 447. (doi:10.1186/s13104-015-1340-4)

Quiniou C, Sapieha P, Lahaie I, Hou X, Brault S, Beauchamp M, Leduc M, Rihakova L, Joyal JS, Nadeau S *et al*. 2008 Development of a novel noncompetitive antagonist of IL-1 receptor. *Journal of Immunology* **180** 6977–6987. (doi:10.4049/jimmunol.180.10.6977)

Raoof M, Zhang Q, Itagaki K & Hauser CJ 2010 Mitochondrial peptides are potent immune activators that activate human neutrophils via FPR-1. *Journal of Trauma* **68** 1328–1332; discussion 1332–1324. (doi:10.1097/TA.0b013e3181dcd28d)

Redman CW, Sacks GP & Sargent IL 1999 Preeclampsia: an excessive maternal inflammatory response to pregnancy. *American Journal of Obstetrics and Gynecology* **180** 499–506. (doi:10.1016/S0002-9378(99)70239-5)

Redman CW & Sargent IL 2000 Placental debris, oxidative stress and pre-eclampsia. *Placenta* **21** 597–602. (doi:10.1053/plac.2000.0560)

Redman CW & Sargent IL 2003 Pre-eclampsia, the placenta and the maternal systemic inflammatory response – a review. *Placenta* **24** (Supplement A) S21–S27. (doi:10.1053/plac.2002.0930)

Rider P, Carmi Y, Guttman O, Braiman A, Cohen I, Voronov E, White MR, Dinarello CA & Apte RN 2011 IL-1alpha and IL-1beta recruit different myeloid cells and promote different stages of sterile inflammation. *Journal of Immunology* **187** 4835–4843. (doi:10.4049/jimmunol.1102048)

Rinehart BK, Terrone DA, Lagoo-Deenadayalan S, Barber WH, Hale EA, Martin JN Jr & Bennett WA 1999 Expression of the placental cytokines tumor necrosis factor alpha, interleukin 1beta, and interleukin 10 is increased in preeclampsia. *American Journal of Obstetrics and Gynecology* **181** 915–920. (doi:10.1016/S0002-9378(99)70325-X)

Roberts JM, Bodnar LM, Lain KY, Hubel CA, Markovic N, Ness RB & Powers RW 2005 Uric acid is as important as proteinuria in identifying fetal risk in women with gestational hypertension. *Hypertension* **46** 1263–1269. (doi:10.1161/01.HYP.0000188703.27002.14)

Romero R, Brody DT, Oyarzun E, Mazor M, Wu YK, Hobbins JC & Durum SK 1989 Infection and labor. III. Interleukin-1: a signal for the onset of parturition. *American Journal of Obstetrics and Gynecology* **160** 1117–1123. (doi:10.1016/0002-9378(89)90172-5)

Romero R, Chaiworapongsa T, Alpay Savasan Z, Xu Y, Hussein Y, Dong Z, Kusanovic JP, Kim CJ & Hassan SS 2011 Damage-associated molecular patterns (DAMPs) in preterm labor with intact membranes and preterm PROM: a study of the alarmin HMGB1. *Journal of Maternal-Fetal and Neonatal Medicine* **24** 1444–1455. (doi:10.3109/14767058.2011.591460)

Romero R, Chaiworapongsa T, Savasan ZA, Hussein Y, Dong Z, Kusanovic JP, Kim CJ & Hassan SS 2012 Clinical chorioamnionitis is characterized by changes in the expression of the alarmin HMGB1 and one of its receptors, sRAGE. *Journal of Maternal-Fetal and Neonatal Medicine* **25** 558–567. (doi:10.3109/14767058.2011.599083)

Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel LA & Nien JK 2006 Inflammation in preterm and term labour and delivery. *Seminars in Fetal and Neonatal Medicine* **11** 317–326. (doi:10.1016/j.siny.2006.05.001)

Romero R, Gomez R, Chaiworapongsa T, Conoscenti G, Kim JC & Kim YM 2001 The role of infection in preterm labour and delivery. *Paediatric and Perinatal Epidemiology* **15** (Supplement 2) 41–56. (doi:10.1046/j.1365-3016.2001.00007.x)

Romero R, Mazor M & Tartakovsky B 1991 Systemic administration of interleukin-1 induces preterm parturition in mice. *American Journal of Obstetrics and Gynecology* **165** 969–971. (doi:10.1016/0002-9378(91)90450-6)

Romero R, Miranda J, Chaiworapongsa T, Korzeniewski SJ, Chaemsaithong P, Gotsch F, Dong Z, Ahmed AI, Yoon BH, Hassan SS *et al*. 2014 Prevalence and clinical significance of sterile intra-amniotic inflammation in patients with preterm labor and intact membranes. *American Journal of Reproductive Immunology* **72** 458–474. (doi:10.1111/aji.12296)

Romero R & Tartakovsky B 1992 The natural interleukin-1 receptor antagonist prevents interleukin-1-induced preterm delivery in mice. *American Journal of Obstetrics and Gynecology* **167** 1041–1045. (doi:10.1016/S0002-9378(12)80035-4)

Rouhiainen A, Kuja-Panula J, Wilkman E, Pakkanen J, Stenfors J, Tuominen RK, Lepantalo M, Carpen O, Parkkinen J & Rauvala H 2004 Regulation of monocyte migration by amphoterin (HMGB1). *Blood* **104** 1174–1182. (doi:10.1182/blood-2003-10-3536)

Ryckman C, Vandal K, Rouleau P, Talbot M & Tessier PA 2003 Proinflammatory activities of S100: proteins S100A8, S100A9, and S100A8/A9 induce neutrophil chemotaxis and adhesion. *Journal of Immunology* **170** 3233–3242. (doi:10.4049/jimmunol.170.6.3233)

Sadowsky DW, Adams KM, Gravett MG, Witkin SS & Novy MJ 2006 Preterm labor is induced by intraamniotic infusions of interleukin-1beta and tumor necrosis factor-alpha but not by interleukin-6 or interleukin-8 in a nonhuman primate model. *American Journal of Obstetrics and Gynecology* **195** 1578–1589. (doi:10.1016/j.ajog.2006.06.072)

Sales KJ & Jabbour HN 2003 Cyclooxygenase enzymes and prostaglandins in pathology of the endometrium. *Reproduction* **126** 559–567. (doi:10.1530/rep.0.1260559)

Sata F, Toya S, Yamada H, Suzuki K, Saijo Y, Yamazaki A, Minakami H & Kishi R 2009 Proinflammatory cytokine polymorphisms and the risk of preterm birth and low birthweight in a Japanese population. *Molecular Human Reproduction* **15** 121–130. (doi:10.1093/molehr/gan078)

Scaffidi P, Misteli T & Bianchi ME 2002 Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* **418** 191–195. (doi:10.1038/nature00858)

Scharfe-Nugent A, Corr SC, Carpenter SB, Keogh L, Doyle B, Martin C, Fitzgerald KA, Daly S, O'Leary JJ & O'Neill LA 2012 TLR9 provokes inflammation in response to fetal DNA: mechanism for fetal loss in preterm birth and preeclampsia. *Journal of Immunology* **188** 5706–5712. (doi:10.4049/jimmunol.1103454)

Schierbeck H, Lundback P, Palmblad K, Klevenvall L, Erlandsson-Harris H, Andersson U & Ottosson L 2011 Monoclonal anti-HMGB1 (high mobility group box chromosomal protein 1) antibody protection in two experimental arthritis models. *Molecular Medicine* **17** 1039–1044. (doi:10.2119/molmed.2010.00264)

Schmorl G 1893 *Pathologisch-Anatomische Untersuchungen Uber Puerperal-Eklampsie*. Leipzig, Germany: Verlag von FC Vogel.

Schroeder DI, Blair JD, Lott P, Yu HO, Hong D, Crary F, Ashwood P, Walker C, Korf I, Robinson WP *et al*. 2013 The human placenta methylome. *PNAS* **110** 6037–6042. (doi:10.1073/pnas.1215145110)

Sekizawa A, Jimbo M, Saito H, Iwasaki M, Matsuoka R, Okai T & Farina A 2003 Cell-free fetal DNA in the plasma of pregnant women with severe fetal growth restriction. *American Journal of Obstetrics and Gynecology* **188** 480–484. (doi:10.1067/mob.2003.27)

Sekizawa A, Jimbo M, Saito H, Iwasaki M, Sugito Y, Yukimoto Y, Otsuka J & Okai T 2002 Increased cell-free fetal DNA in plasma of two women with invasive placenta. *Clinical Chemistry* **48** 353–354.

Shi Y 2010 Caught red-handed: uric acid is an agent of inflammation. *Journal of Clinical Investigation* **120** 1809–1811. (doi:10.1172/JCI43132)

Shi Y, Evans JE & Rock KL 2003 Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* **425** 516–521. (doi:10.1038/nature01991)

Shirasuna K, Seno K, Ohtsu A, Shiratsuki S, Ohkuchi A, Suzuki H, Matsubara S, Nagayama S, Iwata H & Kuwayama T 2016 AGEs and HMGB1 increase inflammatory cytokine production from human placental cells, resulting in an enhancement of monocyte migration. *American Journal of Reproductive Immunology* **75** 557–568. (doi:10.1111/aji.12506)

Sifakis S, Koukou Z & Spandidos DA 2015 Cell-free fetal DNA and pregnancy-related complications (review). *Molecular Medicine Reports* **11** 2367–2372. (doi:10.3892/mmr.2014.3118)

Sifakis S, Zaravinos A, Maiz N, Spandidos DA & Nicolaides KH 2009 First-trimester maternal plasma cell-free fetal DNA and preeclampsia. *American Journal of Obstetrics and Gynecology* **201** 472.e471–477. e471. (doi:10.1016/j.ajog.2009.05.025)

Smarason AK, Sargent IL, Starkey PM & Redman CW 1993 The effect of placental syncytiotrophoblast microvillous membranes from normal and pre-eclamptic women on the growth of endothelial cells in vitro. *British Journal of Obstetrics and Gynaecology* **100** 943–949. (doi:10.1111/j.1471-0528.1993.tb15114.x)

Smid M, Galbiati S, Lojacono A, Valsecchi L, Platto C, Cavoretto P, Calza S, Ferrari A, Ferrari M & Cremonesi L 2006 Correlation of fetal DNA levels in maternal plasma with Doppler status in pathological pregnancies. *Prenatal Diagnosis* **26** 785–790. (doi:10.1002/pd.1504)

Srinivas SK, Edlow AG, Neff PM, Sammel MD, Andrela CM & Elovitz MA 2009 Rethinking IUGR in preeclampsia: dependent or independent of maternal hypertension? *Journal of Perinatology* **29** 680–684. (doi:10.1038/jp.2009.83)

Stephen GL, Lui S, Hamilton SA, Tower CL, Harris LK, Stevens A & Jones RL 2015 Transcriptomic profiling of human choriodecidua during

term labor: inflammation as a key driver of labor. *American Journal of Reproductive Immunology* **73** 36–55. (doi:10.1111/aji.12328)

Stros M 2010 HMGB proteins: interactions with DNA and chromatin. *Biochimica et Biophysica Acta* **1799** 101–113. (doi:10.1016/j.bbagrm.2009.09.008)

Swanson AM & David AL 2015 Animal models of fetal growth restriction: considerations for translational medicine. *Placenta* **36** 623–630. (doi:10.1016/j.placenta.2015.03.003)

Taglauer ES, Wilkins-Haug L & Bianchi DW 2014 Review: cell-free fetal DNA in the maternal circulation as an indication of placental health and disease. *Placenta* **35** (Supplement) S64–S68. (doi:10.1016/j.placenta.2013.11.014)

Tang D, Kang R, Xiao W, Zhang H, Lotze MT, Wang H & Xiao X 2009 Quercetin prevents LPS-induced high-mobility group box 1 release and proinflammatory function. *American Journal of Respiratory Cell and Molecular Biology* **41** 651–660. (doi:10.1165/rcmb.2008-0119OC)

Tang D, Kang R, Zeh HJ 3rd & Lotze MT 2011 High-mobility group box 1, oxidative stress, and disease. *Antioxidants and Redox Signaling* **14** 1315–1335. (doi:10.1089/ars.2010.3356)

Tang Y, Lv B, Wang H, Xiao X & Zuo X 2008 PACAP inhibit the release and cytokine activity of HMGB1 and improve the survival during lethal endotoxemia. *International Immunopharmacology* **8** 1646–1651. (doi:10.1016/j.intimp.2008.07.014)

Thaler K, Chandiramani DV, Hansen RA & Gartlehner G 2009 Efficacy and safety of anakinra for the treatment of rheumatoid arthritis: an update of the Oregon Drug Effectiveness Review Project. *Biologics* **3** 485–498. (doi:10.1016/j.ajog.2009.05.025)

Thaxton JE, Romero R & Sharma S 2009 TLR9 activation coupled to IL-10 deficiency induces adverse pregnancy outcomes. *Journal of Immunology* **183** 1144–1154. (doi:10.4049/jimmunol.0900788)

Tong HS, Tang YQ, Chen Y, Qiu JM, Wen Q & Su L 2011 Early elevated HMGB1 level predicting the outcome in exertional heatstroke. *Journal of Trauma* **71** 808–814. (doi:10.1097/TA.0b013e318220b957)

Tribe RM, Moriarty P, Dalrymple A, Hassoni AA & Poston L 2003 Interleukin-1beta induces calcium transients and enhances basal and store operated calcium entry in human myometrial smooth muscle. *Biology of Reproduction* **68** 1842–1849. (doi:10.1095/biolreprod.102.011403)

Tsung A, Klune JR, Zhang X, Jeyabalan G, Cao Z, Peng X, Stolz DB, Geller DA, Rosengart MR & Billiar TR 2007 HMGB1 release induced by liver ischemia involves Toll-like receptor 4 dependent reactive oxygen species production and calcium-mediated signaling. *Journal of Experimental Medicine* **204** 2913–2923. (doi:10.1084/jem.20070247)

Tsung A, Tohme S & Billiar TR 2014 High-mobility group box-1 in sterile inflammation. *Journal of Internal Medicine* **276** 425–443. (doi:10.1111/joim.12276)

Venereau E, Schiraldi M, Uguccioni M & Bianchi ME 2013 HMGB1 and leukocyte migration during trauma and sterile inflammation. *Molecular Immunology* **55** 76–82. (doi:10.1016/j.molimm.2012.10.037)

Vince GS, Starkey PM, Austgulen R, Kwiatkowski D & Redman CW 1995 Interleukin-6, tumour necrosis factor and soluble tumour necrosis factor receptors in women with pre-eclampsia. *British Journal of Obstetrics and Gynaecology* **102** 20–25. (doi:10.1111/j.1471-0528.1995.tb09020.x)

Vlkova B, Turna J & Celec P 2015 Fetal DNA in maternal plasma in preeclamptic pregnancies. *Hypertension in Pregnancy* **34** 36–49. (doi:10.3109/10641955.2014.954722)

Voelkel NF, Tuder RM, Bridges J & Arend WP 1994 Interleukin-1 receptor antagonist treatment reduces pulmonary hypertension generated in rats by monocrotaline. *American Journal of Respiratory Cell and Molecular Biology* **11** 664–675. (doi:10.1165/ajrcmb.11.6.7946395)

von Wolff M, Thaler CJ, Strowitzki T, Broome J, Stolz W & Tabibzadeh S 2000 Regulated expression of cytokines in human endometrium throughout the menstrual cycle: dysregulation in habitual abortion. *Molecular Human Reproduction* **6** 627–634. (doi:10.1093/molehr/6.7.627)

Voto LS, Illia R, Darbon-Grosso HA, Imaz FU & Margulies M 1988 Uric acid levels: a useful index of the severity of preeclampsia and perinatal prognosis. *Journal of Perinatal Medicine* **16** 123–126. (doi:10.1515/jpme.1988.16.2.123)

Wang E, Batey A, Struble C, Musci T, Song K & Oliphant A 2013 Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. *Prenatal Diagnosis* **33** 662–666. (doi:10.1002/pd.4119)

Wang XW, Karki A, Zhao XJ, Xiang XY & Lu ZQ 2014 High plasma levels of high mobility group box 1 is associated with the risk of sepsis in severe blunt chest trauma patients: a prospective cohort study. *Journal of Cardiothoracic Surgery* **9** 133. (doi:10.1186/s13019-014-0133-5)

Wataganara T, Chen AY, LeShane ES, Sullivan LM, Borgatta L, Bianchi DW & Johnson KL 2005 Changes of cell-free fetal DNA in maternal plasma after elective termination of pregnancy. *Clinical Chemistry* **51** 217–219. (doi:10.1373/clinchem.2004.042135)

Werman A, Werman-Venkert R, White R, Lee JK, Werman B, Krelin Y, Voronov E, Dinarello CA & Apte RN 2004 The precursor form of IL-1alpha is an intracrine proinflammatory activator of transcription. *PNAS* **101** 2434–2439. (doi:10.1073/pnas.0308705101)

Witkin SS, Vardhana S, Yih M, Doh K, Bongiovanni AM & Gerber S 2003 Polymorphism in intron 2 of the fetal interleukin-1 receptor antagonist genotype influences midtrimester amniotic fluid concentrations of interleukin-1beta and interleukin-1 receptor antagonist and pregnancy outcome. *American Journal of Obstetrics and Gynecology* **189** 1413–1417. (doi:10.1067/S0002-9378(03)00630-6)

Wu ZM, Yang H, Li M, Yeh CC, Schatz F, Lockwood CJ, Di W & Huang SJ 2012 Pro-inflammatory cytokine-stimulated first trimester decidual cells enhance macrophage-induced apoptosis of extravillous trophoblasts. *Placenta* **33** 188–194. (doi:10.1016/j.placenta.2011.12.007)

Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, Anderson M, Schroder JM, Wang JM, Howard OM *et al*. 1999 Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. *Science* **286** 525–528. (doi:10.1126/science.286.5439.525)

Yang H, Ochani M, Li J, Qiang X, Tanovic M, Harris HE, Susarla SM, Ulloa L, Wang H, DiRaimo R *et al*. 2004 Reversing established sepsis with antagonists of endogenous high-mobility group box 1. *PNAS* **101** 296–301. (doi:10.1073/pnas.2434651100)

Yang H, Wang H, Czura CJ & Tracey KJ 2005 The cytokine activity of HMGB1. *Journal of Leukocyte Biology* **78** 1–8. (doi:10.1189/jlb.1104648)

Zanetti M 2004 Cathelicidins, multifunctional peptides of the innate immunity. *Journal of Leukocyte Biology* **75** 39–48. (doi:10.1189/jlb.0403147)

Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K & Hauser CJ 2010 Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* **464** 104–107. (doi:10.1038/nature08780)

Zhang YY, Chen H, Sun C, Wang HZ, Liu ML, Li YY, Nie XL, Du MR, Li DJ & Zhang JP 2014 Expression and functional characterization of NOD2 in decidual stromal cells isolated during the first trimester of pregnancy. *PLoS ONE* **9** e99612. (doi:10.1371/journal.pone.0099612)

Zhu L, Zhang Z, Zhang L, Shi Y, Qi J, Chang A, Gao J, Feng Y & Yang X 2015 HMGB1-RAGE signaling pathway in severe preeclampsia. *Placenta* **36** 1148–1152. (doi:10.1016/j.placenta.2015.08.006)

Zou CJ, Liu JD & Zhou YC 2001 Roles of central interleukin-1 on stress-induced-hypertension and footshock-induced-analgesia in rats. *Neuroscience Letters* **311** 41–44. (doi:10.1016/S0304-3940(01)02140-1)

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