A clear and present danger: inflammasomes DAMPing down disorders of pregnancy

Raheela N. Khan* and Daniel P. Hay

Division of Medical Sciences & Graduate Entry Medicine, School of Medicine, University of Nottingham, Royal Derby Hospital Centre, Uttoxeter Road, Derby DE22 3DT, UK

*Correspondence address. Tel: +44-1332-724664; E-mail: raheela.khan@nottingham.ac.uk, http://www.nottingham.ac.uk/research/groups/reproductivephysiology/index.aspx

Submitted on July 15, 2014; resubmitted on October 12, 2014; accepted on October 27, 2014

TABLE OF CONTENTS

- Introduction
- Methods
- Inflammation in pregnancy
- Pregnancy pathophysiology
- Clear and present danger
  - Pattern and danger-associated molecular patterns (PAMPs)
  - Danger (Damage)-associated molecular patterns (DAMPs)
  - Interleukin-1 cytokines
  - Caspase-1
- Inflammasomes
  - Inflammasome subtypes and organization
  - Inflammasome assembly
- Mechanisms of inflammasome activation
  - Potassium (K+) efflux and purinergic P2X7 receptor
  - ROS pathway
  - Lysosomal rupture pathway
- Inhibition and termination of inflammasome activity
- Inflammasomes: insights from disease
- Inflammasomes in pregnancy
  - Early pregnancy
  - Term pregnancy and parturition
  - Preterm labour
  - Pre-eclampsia
  - Metabolic dysfunction
  - The neonate
- Future perspectives

BACKGROUND: When the normal progression of pregnancy is threatened, inflammatory processes are often amplified in order to minimize detrimental effects and eliminate noxious agents. Inflammasomes are unique, intracellular, multiprotein assemblies that enable caspase-1 mediated proteolytic processing of the proinflammatory cytokine interleukin-1β, levels of which are elevated in some forms of preterm birth and maternal metabolic disorders.
Inflammasomes and disorders of pregnancy


**Results:** In the decade since the discovery of the inflammasome, between January 2002 and June 2014 over 2200 articles have been published. Articles in the reproductive field are scarce but there is clear evidence for a role of the inflammasome axis in pregnancy, preterm birth and the maternal metabolic syndrome.

**Conclusion:** Further investigations on the inflammasome in pregnancy are needed in order to elucidate the biology of this unique structure in reproduction. Coordination of maternal, fetal and placental aspects of inflammasome function will potentially yield new information on the detection and transduction of host and non-host signals in the inflammatory response.

**Key words:** inflammasome / pregnancy / preterm birth / pre-eclampsia / inflammation

---

**Introduction**

Advances in innate immunity have highlighted the concept of danger as a proinflammatory trigger with damage- (DAMPs) and pathogen-associated molecular patterns (PAMPs) linked to activation of inflammasomes. Inflammasomes are high molecular weight, intracellular multi-protein complexes that form a scaffold enabling caspase-mediated processing of the proinflammatory cytokines of the interleukin (IL)-1 family (Martinon et al., 2002). The specific sequence of protein interactions initiated in assembling the inflammasome follows receipt and decoding of an appropriate trigger conveyed via ligand binding to a target pattern recognition receptor. Inflammasomes typically form from the induced assembly of caspase-1, a NOD-like receptor or NLR (NOD mean ing nucleotide-binding oligomerization domain), and an adaptor protein, ASC (apoptosis-associated speck-like protein with CARD domain) (Martinon et al., 2002; Mariathasan et al., 2006). Inflammasome activation is triggered by a diverse array of microbes, microbial toxins or inappropriate activity of host molecules including glucose (Zhou et al., 2010; Stienstra et al., 2012), urates (Martinon et al., 2006), β-amyloid (Halle et al., 2008) and cholesterol (Duewell et al., 2010; Rajamaki et al., 2010). Following inflammasome assembly, the resultant oligomeric construction yields a transient platform that enables a sequence of enzymatic reactions exemplified by autocatalysis of the inflammasome caspases (caspase-1) and its proteolysis of proforms of the interleukins IL-1β, IL-18 and IL-33 to generate their respective mature, secretory forms. To date, four main types of inflammasomes have been characterized of which the NLRP3 family is the most studied. A variety of autoinflammatory diseases characterized by periodic fever and exacerbated inflammation have been mapped to mutations in genes encoding proteins comprising the inflammasome (Aganna et al., 2002; Goldbach-Mansky et al., 2006).

While the majority of pregnancies end in the timely delivery of a healthy newborn, preterm birth (PTB) remains a leading cause of perinatal morbidity and mortality (Goldenberg et al., 2002; Lawn et al., 2014). Spontaneous PTB is initiated predominantly by bacterial infection via the female genital tract with latent or subclinical infection also linked to preterm delivery. In addition, in developed countries, the increased incidence of maternal metabolic disorders warns of generational shifts in reproductive life. Thus the newborn is primarily reliant upon suboptimal innate and adaptive mechanisms for immune protection.

Both implantation and parturition resemble inflammatory processes. Inflammation itself is the host’s response to injury, stress and infection in which a sequence of coordinated immunological events serve to restore normal tissue homeostasis and resolve infection. Implantation and invasion of the endometrium and spiral arteries utilize cytokines and chemokines from immune cells (natural killer cells, macrophages, dendritic cells) for tissue remodelling and placentaion (Moffett-King, 2002; Sargent et al., 2006; Mor et al., 2011). Similarly, normal labour as an inflammatory event is predicated on observations that demonstrate an increased leukocytic infiltrate in the uterus (Thomson et al., 1999; Young et al., 2002), and increased mRNA and protein expression for...
proinflammatory cytokines in cervix, myometrium and fetal membranes (Osman et al., 2003; Sakamoto et al., 2005) at term parturition. Levels of cytokines that include IL-1β and IL-6 are also elevated with normal labour (Opsjøn et al., 1993; Ammala et al., 1997; Keelan et al., 1999). Inflammatory processes are clearly of benefit to mother and fetus during pregnancy, not least by countering infection and limiting tissue injury, however an exacerbated inflammatory response is of course detrimental to many organ systems thereby affecting neonatal and maternal outcomes.

**Pregnancy pathophysiology**

Inflammation is a major component of some pregnancy disorders. The largest category is that of PTB, a syndrome with a multifactorial aetiology. Defined as delivery before 37 completed weeks of gestation, PTB affects 5–12% pregnancies worldwide (Goldenberg et al., 2008) and 1 million deaths annually (Lawn et al., 2014). In the UK, PTB is estimated to cost the UK public sector ~£2.9 billion (Mangham et al., 2009). Some 75% of all PTBs are spontaneous in onset (sPTB) comprising preterm labour (PTL) and preterm premature rupture of membranes (PPROM), of which nearly half are causally linked to intrauterine bacterial infection (Goldenberg et al., 2008). Babies are at greatest risk of adverse outcomes the earlier they are born (Goldenberg, 2002; Marlow et al., 2005; Costeloe et al., 2012) but some born at late preterm gestations (34–36 weeks), are compromised compared with term infants (Petritini et al., 2009) with negative outcomes that extend beyond childhood (Saigal and Doyle, 2008). In low to middle income countries, malaria and syphilis are significant causes of PTB (Simmons et al., 2014).

Microbial invasion of the amniotic cavity is a key factor in sPTB and is present in approximately one-fifth of women with intact membranes (Romero et al., 2006, 2007). In women with PPROM, histological evidence of infection is much greater with approximately one-third of women testing positive at admission, rising to 75% with labour (Romero et al., 2007) and 50–60% delivering within a week of membrane rupture (Mercer, 2003) indicative of the loss of barrier function provided by the fetal membranes.

The main route of infection of the uterine cavity and amniotic fluid is ascent of microbes from the genital tract via the cervix and vagina, by iatrogenic means, retrograde transmission through the Fallopian tubes or by haematogenous spread (a route for systemic infection) (Goldenberg et al., 2000; Romero et al., 2007). Having traversed the cervix, bacteria then infect the decidua, fetal membranes and finally the amniotic fluid and fetus. The cervix offers protection from ascending microbial infection as a physical barrier but also due to its abundance of antimicrobial peptides (Hein et al., 2002). Histological changes in the placenta and the microbes that accompany cervical insufficiency reflect those seen in PTL and PPROM (McElrath et al., 2008). In a recent study using whole-genome shotgun metagenomics, a unique placental microbiome niche was described that bears greatest similarity to the human oral microbiome over vaginal, gut, skin and airway habitats. This finding provides a basis for the documented links between periodontal disease and PTB (Aagaard et al., 2014). Both gram-positive and gram-negative, commensals and pathogens have been implicated in triggering PTL with the most common isolated from amniotic fluid belonging to Mycoplasma species, including Ureaplasma urealyticum and Mycoplasma hominis (see Table I for a list of some micro-organisms). This is further supported by the presence of gram-positive and gram-negative microbes detected at the maternal-fetal interface (Aagaard et al., 2014). A fetal inflammatory response is observed in pregnancies complicated by PTL (Romero et al., 1998) with affected infants at increased risk of severe neonatal morbidities including sepsis, periventricular malacia and respiratory distress (Gomez et al., 1998). One of the main mechanisms connecting infection with PTL is the up-regulation of the cyclooxygenase enzymes by IL-1β which promotes prostaglandin (PG)E2 and PGF2α production with consequent activation of myometrial contractility (Romero et al., 1989, 2006); however this is a complex area with very few definitive signalling pathways identified.

Inflammation is also a factor in pre-eclampsia (Redman et al., 1999; Redman and Sargent, 2003; Luppi et al., 2006) which presents with de novo hypertension and proteinuria (Brown et al., 2001). Pre-eclampsia affects ~2–7% of pregnancies (Roberts and Cooper, 2001; Duley, 2009) and accounts for a significant proportion of the 30% of iatrogenic preterm deliveries (Goldenberg and Culhane, 2003). While the origins of pre-eclampsia resulting from impaired trophoblast invasion and spiral artery transformation in early pregnancy (Khong et al., 1986) are widely accepted, numerous hypotheses have been proposed to explain the aetiology and progression of this disease. Currently favoured are maternal endothelial dysfunction, inflammation and oxidative stress (Redman and Sargent, 2003; Chaiworapongsa et al., 2014). Pre-eclampsia exhibits many of the classical features of inflammation and these are exaggerated in late pregnancy (Redman et al., 1999; Redman and Sargent, 2003).

### Table I Some examples of PAMPs and DAMPs.

<table>
<thead>
<tr>
<th>Pathogens producing PAMPs</th>
<th>DAMPs</th>
<th>Endogenous</th>
<th>Exogenous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus anthracis Lethal toxin</td>
<td>Extracellular ATP</td>
<td>Silica</td>
<td></td>
</tr>
<tr>
<td>Muramyldipeptide</td>
<td>Glucose</td>
<td>Alum</td>
<td></td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>Cholesterol</td>
<td>Asbestos</td>
<td></td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>Hyaluronan</td>
<td>Haemozoin</td>
<td></td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>Monosodium urate</td>
<td>Imidazoquinolines (adjuvants)</td>
<td></td>
</tr>
<tr>
<td>Francisella tularensis</td>
<td>HMGBl</td>
<td>Trinitrophenol chloride</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>ß-amyloid</td>
<td>Dinitrofluorobenzene</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Calcium pyrophosphate</td>
<td>Trinitrochlorobenzene</td>
<td></td>
</tr>
<tr>
<td>Flagellins</td>
<td>Double-stranded DNA</td>
<td>Double-stranded DNA</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Serum amyloid A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>Heat shock protein 72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Heparan sulphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sendai virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Encephalomyocarditis virus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*PAMPs – pathogen-associated molecular pattern.

*DAMPs – damage/danger-associated molecular pattern.

‡HMGBl – High mobility group box 1.
Inflammations and disorders of pregnancy

Other current foci of pregnancy research are conditions of a metabolic nature with maternal obesity (Ramsey et al., 2002) and gestation diabetes mellitus (GDM) (Wolf et al., 2004; Winzer et al., 2004) displaying evidence of subclinical inflammation. This reflects findings in nonpregnant individuals that both obesity and diabetes are associated with a chronic inflammatory response characterized by abnormal cytokine production, increased acute-phase reactants, and activation of inflammatory signaling pathways (Hotamisligil, 2003). The prevalence of obesity in pregnancy in the UK has risen from 9–10% in the early 1990s to 16–19% in the 2000s (Heslehurst et al., 2007). In addition, maternal obesity is associated with negative outcomes for the mother and fetus including increased risk for GDM, pre-eclampsia, stillbirth and miscarriage (Sebire et al., 2003; Bodnar et al., 2005; Catalano and Ehrenberg, 2006). Moreover adult offspring of obese mothers are also at increased risk of premature death (Reynolds et al., 2013). Since inflammation may be triggered by microbial and non-microbial causes of fetal and/or maternal derivation, an understanding of inflammatory mechanisms and metabolic disorders of pregnancy may inform new directions in managing the spectra of these diseases.

Elaboration of inflammatory cascades is mediated by structures termed ‘inflammasomes’ (Martinon et al., 2002). Originally discovered in 2002, inflammasomes are transient (~700 kDa) multiprotein cytosolic complexes that act as conduits through which multiple signals are efficiently construed. Resembling the apotopsome, that deciphers death signals by procaspase-9, with apoptosis as the end-point (Riedl and Salvesen, 2007), inflammasomes facilitate a series of catalytic reactions involving procaspase-1 and terminating with the maturation and release of key cytokines of the IL-1 family (Martinon et al., 2002). Inflammasomes have been most extensively studied in cells of the myeloid lineage, typically macrophages and monocytes. Their relevance to pregnancy is discussed after detailing the biology of these remarkable structures.

Clear and present danger

Pattern and danger-associated molecular patterns (PAMPS)

Traditionally, the main challenge to host defences is from microorganisms that express unique motifs, absent in eukaryotes, termed pathogen-associated molecular patterns (PAMPS) (Medzhitov and Janeway, 2000; Takeda and Akira, 2005). Combating such threats from a continual, ever-changing assault from hostile microbes is achieved through an elaborate array of pattern recognition receptors (PRRs) or pattern recognition molecules that recognize PAMPS and are expressed on cells, in intracellular compartments or secreted (Janeway and Medzhitov, 2002). Of these, the human Toll-like receptor (TLR) family (TLR1–10) has evolved to recognize a panoply of molecules including bacterial flagellin (Hayashi et al., 2001), lipopolysaccharide (Medzhitov et al., 1997), peptidoglycan, lipoteichoic acid (Schwandner et al., 1999) and bacterial unmethylated CpG (Henmi et al., 2000), amongst others. Having breached the barrier afforded by the plasma membrane, microbes are confronted with a second line of defence in the form of NOD-like receptors (NLRs) (Martinon and Tschopp, 2005; Franchi et al., 2006b). For the purpose of this review, we will focus on the NLRs.

The family of NLRs encode cytosolic proteins for which 22 human NLR genes have been identified (Ting et al., 2008). Structurally, each NLR is a trio of core components: a central NOD-like nucleotide-binding domain also called NACHT (for domain found in NAIP, CIITA, HET-E and TP-1), with a region of leucine-rich repeats (LRR) at the C-terminus and an N-terminal effector domain that mediates interactions with other protein partners (Tschopp et al., 2003). In 2008, the nomenclature pertaining to NLRs was revised and simplified based on the moiety of the N-terminus effector (Ting et al., 2008). Thus NLRB denotes an acidic activation domain; NLRB has a baculovirus inhibitor of apoptosis domain; NLRC contains a caspase activation and recruitment domain (CARD) while NLRP hosts a pyrin (PYD) domain (Ting et al., 2008). Both CARD and PYD are members of the death-fold superfamily which enables homotypic interactions between members of the same family (Martinon et al., 2001). The NACHT domain is essential for receptor activation via ATP-dependent dimerization and oligomerization mediated by an AAA+ cassette (Neuwald et al., 1999). Detection and ligand-binding, in common with TLRs, is a function fulfilled by the LRR domain. Activation of NLRs is suppressed in resting conditions by interactions between the carboxy terminus of LRRs and the AAA+ cassette, preventing oligomerization (Martinon and Tschopp, 2007). As seen later, PAMP detection sets into play inflammatory signalling cascades organized through intracellular platforms termed ‘inflammasomes’ (Martinon et al., 2002). Of those characterized to date, three of the four main inflammasome types contain an NLR; however not all NLR proteins form inflammasomes.

Danger (Damage)-associated molecular patterns (DAMPS)

In 1994, a cogent argument on an alternative to the long-held view of the immune system as the sine qua non for discriminating self from non-self was propounded; the concept of ‘danger’ as a signal driving adaptive immunity (Matzinger, 1994). Although controversial, experimental evidence since accrued supports the phenomenon that host-derived molecules are also potent initiators of innate immune defences and inflammation (Gallucci and Matzinger, 2001). Referred to as damage-associated molecular patterns (DAMPS or alarmins) (Bianchi, 2007) to distinguish them from PAMPs, these non-microbial ‘danger’ or ‘damage’ molecules signify escape from their normal intracellular compartments due to cellular stress or injury (Gallucci and Matzinger, 2001; Bianchi, 2007; Kono and Rock, 2008) and cause sterile inflammation by deploying inflammasomes (Chen and Nunez, 2010). A point worth noting is that the term DAMPS is used interchangeably with the ‘D’ denoting ‘damage’ or ‘danger’ but ‘danger’ may be deemed too vague a term to convey much information on the nature of the trigger (Pradeu and Cooper, 2012). The earliest described DAMPS cross a spectrum of biochemical and physicochemical complexity and include extracellular ATP (Bours et al., 2006), high mobility group box-1 (HMGB1) nuclear protein (Lotze and Tracey, 2005) and crystalline matter, for example, monosodium urate (Shi et al., 2003); further examples of DAMPS and PAMPS are provided in Table I. Inflammatory cascades initiated by DAMPS and PAMPS lead to the release of proinflammatory mediators by deploying inflammasomes. As IL-1 processing by caspase-1 is a core function of inflammasomes, key aspects of these two pivotal proteins are summarized below.

Interleukin-1 cytokines

Belonging to the IL-1 family of cytokines, three members, IL-1α, IL-1β and IL-33, are synthesized as precursors lacking a signal peptide sequence that prevents their extrusion from the cell via classical exocytosis
IL-1 exists as either IL-1α or IL-1β and biological actions follow its binding to the IL-1 receptor (IL-1R) with signalling via the intracytoplasmic Toll/IL-1 receptor (TIR) domain (Martin and Wesche, 2002). This results in translocation to the nucleus of the transcription factor nuclear factor κB (NF-κB) which is then translated on free ribosomes, primed for release (Auron et al., 1997) unlike IL-33 (Fuentes-Prior and Salvesen, 2004) which is not IFN-inducible, nuclear localization and length of 200 amino acid

Caspase-1

Caspases are cysteinyl aspartate proteases that are classed as initiator, effector or inflammatory subtypes based on their physiological roles (Fuentes-Prior and Salvesen, 2004). Their main function is to cleave proteins at sites at aspartic acid residues (Martinon and Tschopp, 2007). Human inflammatory caspases include caspase-1, -4 and -5 and -12. Caspase-1 is synthesized as a 45 kDa zymogen (Thornberry et al., 1992) possessing a CARD at the N-terminus (Martinon and Tschopp, 2007). Functional proteolytic activity is conferred by monomers of procaspase-1 undergoing homodimerization and autocatalysis generating two active subunits of 10 kDa (p10) and 20 kDa (p20) (Thornberry et al., 1992) that then assemble as a heterodimer (p10/p20). In a similar manner, IL-18 in its proform is also a substrate for caspase-1 (Gu et al., 1997) unlike IL-33 (Fuentes-Prior and Salvesen, 2004) which is not (Luthi et al., 2009) and is, paradoxically, inactivated by caspase-1 (Cayrol and Girard, 2009). While IL-1 and IL-18 as substrates for caspase-1 have been intensely studied, another eighty possible candidates are suggested based on a proteomic and bioinformatics evaluation (Agard et al., 2010) but their physiological relevance remains to be clarified. Interestingly, caspase-1 elicits a specific form of cell death termed pyroptosis (Swanson and Molofsky, 2005; Fink et al., 2008). Originally reported for Salmonella and Shigella and distinct from apoptosis and necrosis, this inflammatory form of cell death results in osmotic lysis, DNA fragmentation and proinflammatory cytokine release (Fink and Cookson, 2005). Pyroptosis occurs on pyrptosomes, a supramolecular complex assembled following ASC oligomerization (Fernandes-Alnemri et al., 2007) and provides a novel angle on host-pathogen interactions and implications for infection.

Inflammasomes

All known inflammasomes share the same elementary structural template which consists of an integral PRR (typically an NLR), procaspase-1 and the apoptosis-associated speck-like protein containing a CARD (ASC; also known as PYCARD) which harbours an N-terminal pyrin (PYD) domain and a CARD at the C-terminus (Srinivasula et al., 2002). This configuration of ASC facilitates homotypic associations with other PYD- and CARD containing proteins required for inflammasome assembly by linking the NLR with procaspase-1 (Srinivasula et al., 2002). The structure of the four known inflammasomes and key proteins is illustrated in Fig. 1.

Inflammasome subtypes and organization

The NLRP1 (NALP1) inflammasome is the original oligomeric complex discovered consisting of caspase-1, caspase-5, NLRP1 and ASC (Martinon et al., 2002). It is presently the only known inflammasome containing a FIIND (function to find) domain which undergoes autocleavage to confer functionality (Finger et al., 2012). Both anthrax lethal factor from Bacillus anthracis (Boyden and Dietrich, 2006) and muramyl dipeptide (Faustin et al., 2007) initiate its activation.

The NLRC4 (IPAF) inflammasome is characterized by the presence of an intrinsic CARD domain that interacts directly with procaspase-1 (Mariathasan et al., 2004). The main triggers for this inflammasome are bacterial flagellins of gram-negative bacteria with type III or IV secretion systems exemplified by Salmonella typhimurium and Shigella flexneri (Mariathasan et al., 2004; Amer et al., 2006; Miao et al., 2006; Franchi et al., 2006a). Activation has also been shown to be flagellin-independent (Suzuki et al., 2007) indicating that other, as yet unknown, bacterial ligands are also sensed by NLRC4.

The NLRP3 (CIAS, NALP3, cryopyrin) inflammasome is unique amongst inflammasomes in being responsive not only to microbial cues but also a plethora of chemically diverse non-microbial signals arising from, for example, monosodium urate (Martinon et al., 2006), glucose (Zhou et al., 2010) extracellular ATP (Mariathasan et al., 2006) as well as PAMPs (Mariathasan et al., 2006; Craven et al., 2009). The NLRP3 couples directly to the PYD of ASC via its PYD domain to execute function.

The non-NLR AIM2 (absent in melanoma 2 (AIM2)) inflammasome belongs to the HIN200 (haematopoietic expression, IFN-inducible, nuclear localization and length of 200 aminoacid
Inflammasomes and disorders of pregnancy

**Mechanisms of inflammasome activation**

Once assembled, the actual signal(s) whereby the inflammasome is activated has engendered much curiosity; specifically the ‘switch’ that turns on the inflammasome. Quite how such structurally and chemically diverse molecules activate the NLRP3 inflammasome remains perplexing but is not thought to require direct contact between the PAMP/DAMP and sensing domains of the inflammasome. Research on this aspect has emerged mostly from in vitro studies of the NLRP3 inflammasome and three main mechanisms have been postulated. Figure 2 depicts the intracellular machinery that comprises the inflammasome and its linkage to the three main activation pathways that include the P2X7 receptor, reactive oxygen species (ROS) and lysosomal destabilization.

**Potassium (K⁺) efflux and purinergic P2X7 receptor**

Physiologically, cytosolic K⁺ levels in all cells at rest are high with a substantial exit of intracellular K⁺ ions warning the cell of danger. A change in K⁺ ion concentration induces cleavage of immature IL-1β and IL-18 into their corresponding processed entities (Perregaux and Gabel, 1994) through the purinergic P2X7 receptor (Falzone et al., 1995; Ferrari et al., 1997). Abundant in hematopoietic cells (Collo et al., 1997; Ferrari et al., 2006), the P2X7 receptor is an ion channel gated by extracellular ATP which generates an initial current on binding of the nucleotide (Surprenant et al., 1996) and has since been shown to link with the NLRP3 inflammasome (Mariathasan et al., 2004; Duncan et al., 2007). Low cytosolic K⁺ concentrations also initiate activation of NLRP1 (Petrilli et al., 2007), NLRC4 (Arlehamn et al., 2010) and AIM2 (Petrilli et al., 2007; Fink et al., 2008; Muruve et al., 2008). Intriguingly, activation of caspase-1 by intracellular bacteria does not require ATP or intracellular K⁺ depletion (Franchi et al., 2007). A distinct second phase of the P2X7 ion current, indicative of large pore formation, is also observed in the presence of ATP (Di Virgilio, 1995; Falzone et al., 1995; Surprenant et al., 1996) and in macrophages this pore activity has since been attributed to the pannexin-1 hemichannel. Research on this aspect has emerged mostly from in vitro studies of the NLRP3 inflammasome and three main mechanisms have been postulated. Figure 2 depicts the intracellular machinery that comprises the inflammasome and its linkage to the three main activation pathways that include the P2X7 receptor, reactive oxygen species (ROS) and lysosomal destabilization.

**Inflammasome assembly**

The formative steps in assembly of all inflammasomes occur on receipt of an appropriate trigger which induces a conformational change that signals ATP-dependent self-oligomerization (Martinon et al., 2002). Once activated, the PYD domains of NLRP1, NLRP3 and AIM-2 (all lacking CARD) associate with ASC and recruit procaspase-1 monomers by clustering (Schröder and Tschopp, 2010). Through this induced proximity, CARD-CARD associations ensue resulting in autoproteolysis of procaspase-1 into its active subunits thus catalysing the processing of pro-IL-1β into active IL-1β (Martinon and Tschopp, 2006; Schröder and Tschopp, 2010). Recently, a common mechanism of assembly involving nucleation of PYD domains of ASC, CARD and caspase-1 has been proposed for the NLRP3 and AIM2 inflammasomes (Lu et al., 2014). Near atomic resolution imaging depicts the PYD-containing inflammasomes as filamentous (Lu et al., 2014).

**ROS pathway**

Molecules involved in sterile inflammation are associated with transient ROS generation (Fubini and Hubbard, 2003). It is not clear how ROS trigger such activation but one proposed mechanism suggests that dissociation of constitutively-expressed thioredoxin-interacting protein from thioredoxin allows the former to bind to the LRR domain of the inflammasome (Zhou et al., 2010). Another route of inflammasome activation by ROS is postulated as being due to NADPH oxidase (Nox) activity (Dostert et al., 2008). However, contradictory effects were reported in patients with chronic granulomatous disease who carry a mutation in the p22phox gene and in whom IL-1β secretion as a measure of inflammasome activity was Nox-independent (van Bruggen et al., 2010). A role for ROS signals emanating from damaged mitochondria has also
None of the three mechanisms highlighted above serve as the sole trigger for inflammasome activation nor do they fully explain activation of the NLRP3 inflammasome or indeed the three others. It is probable that NLRP1, NLRC4 and AIM2 will be triggered by alternative stimuli; hence new pathways and molecules will certainly emerge as we discover more about the control of these unique scaffolds.

### Inhibition and termination of inflammasome activity

With the formidable potential of the inflammasome to cause damage by aggravated inflammatory responses, normal cellular processes must exist to regulate and terminate inflammasome activity. Crucially, the multimeric nature of the inflammasome and the sequential processing of both caspases and proinflammatory cytokines introduces a series of checkpoints that prevent inappropriate inflammation or cell death by regulating expression and activity of constituent proteins. Thus procaspase-1 (Thornberry et al., 1992) and ASC are expressed at rest but are inactive (Bryan et al., 2009). While procaspase-1 levels are similar to those for other caspases studied, the activity of the mature form, caspase-1, is restricted by its lability and substantially shorter half-life (Walsh et al., 2011) mediated by autoproteolysis or dissociation into catalytically inactive p20 and p10 subunits (Talanian et al., 1996). In the case of ASC, the protein resides within nuclei of resting macrophages and monocytes but is rapidly mobilized to the cytosol following stimulation (Bryan et al., 2009). ASC then forms 4–6 μm aggregates in the cytosol and colocalises with NLRP3 in intracellular vesicles (Mariathasan et al., 2004; Bryan et al., 2009). Interestingly, NLRP3 expression is either induced following a priming signal by cytokines or PRR ligands (O’Connor et al., 2003; Bauernfeind et al., 2009; Franchi et al., 2009), is suppressed by chaperone proteins (Poyet et al., 2001; Mayor et al., 2007) or relocalizes to the perinuclear space on stimulation with DAMPs (Zhou et al., 2011).

In terminating inflammasome activity, autophagy as the cell’s intrinsic mechanism for removing damaged proteins and organelles has been suggested (Shi et al., 2012). Alternative mechanisms include inactivation of caspase-1 (Druilhe et al., 2001) or proteins that interfere with caspase-1 oligomerization such as the CARD-only proteins (Kersse et al., 2007) and ICEBERG (Humke et al., 2000). The effector protein ASC may also be blocked by the decoy ASC pyrin-only protein (POP2), competing with it to inhibit inflammasome formation (Bedoya et al., 2007; Atianand and Harton, 2011). Nitric oxide has been shown to inhibit the NLRP3 inflammasome likely by S-nitrosylation (Hernandez-Cuellar et al., 2012). Endogenous inhibitors also include antiapoptotic Bcl-2 and Bcl-x proteins that prevent NLRP1 activation by interfering with ATP binding to the NACHT domain (Bruey et al., 2007). Recently, CRID3, a synthetic cytokine release inhibitory drug, inhibited ASC oligomerization in NLRP3 and AIM2 but not NLRC4 inflammasomes (Coll and O’Neill, 2011). The sulphhydrylurea glibenclamide has also been shown to inhibit the NLRP3 inflammasome downstream of the P2X7 receptor (Lamkanfi et al., 2009).
Inflammasomes: insights from disease

The genetic basis for certain debilitating autoinflammatory syndromes emerged from the discovery of the gene named CIAS1 (cold-induced autoinflammatory syndrome-1 encoding the cryopyrin protein) present in cryopyrin-associated periodic syndromes (CAPS) (Hoffman et al., 2001a). Characterized by dominant, heterozygous, gain-of-function mutations in the CIAS (NLRP3) gene, these inherited disorders include Muckle–Wells syndrome (MSW), familial cold autoinflammatory syndrome (FCAS) and neonatal-onset multisystem inflammatory disease (Hoffman et al., 2001a, b). Phenotypically, they manifest with symptoms of relapsing fever, an urticaria-like rash and joint problems (Hoffman et al., 2001a, b). Hyperactivity of inflammasomes is also linked to gout (Martinon et al., 2002, 2011). Interestingly, familial or recurrent hydatidiform mole (HM), a form of gestational trophoblastic disease characterized by a lack of embryonic growth, has been linked with at least 5–13 mutations of the NLRP7 (NALP7) gene located on chromosome 19q13.42 (Murdoch et al., 2006; Wang et al., 2009).

Resolution of symptoms in CAPS is achieved using a recombinant form of the IL-1R antagonist anakinra (Goldbach-Mansky et al., 2004; Ryan and Goldbach-Mansky, 2008).

Mutations in the NLRP1 gene have been identified in generalized vitiligo (Jin et al., 2007a, b) and suggested in Crohn’s disease (Cummings et al., 2010) with the adverse effects of autoinflammation probably due to the direct association between the CARD of NLRP1 and procaspase-1. Recent studies suggest that defective NLRP3 signalling in the gut contributes to inflammatory bowel disease through increased permeability across the epithelial barrier and the induction of an impaired immune response against invading commensals (Zaki et al., 2011). Hyperactivity of inflammasomes is also linked to gout (Martinon et al., 2006), pseudogout (Martinon et al., 2006) and type II diabetes (Larsen et al., 2007; Grant and Dixit, 2013). Glucotoxicity in pancreatic β-cells is induced by IL-1β in response to high glucose (Maedler et al., 2002) and mice with a gene knockout for NLRP3 and ASC exhibited improved insulin signalling in adipose and other insulin-sensitive tissues (Vandamgat et al., 2011). Interestingly, type II diabetic patients demonstrated improved glycaemic control on treatment with anakinra (Larsen et al., 2007) supporting diabetes as an autoinflammatory disease (McGonagle and McDermott, 2006).

Inflammasomes in pregnancy

Many facets of potential inflammasome involvement are reflected in normal pregnancy and pregnancy-specific disorders. Here we explore the evidence for, and implications that arise via, aggravated or inappropriate inflammatory responses in gestation.

Early pregnancy

The role of the inflammasome in early pregnancy and associated complications has received scant attention to date. Establishing a pregnancy requires several hurdles to be circumvented beginning with fertilization.

Term pregnancy and parturition

As stated earlier, proinflammatory cytokines are spatially and temporally expressed in fetoplacental compartments with impending labour (Thomson et al., 1999; Young et al., 2002). Cytokines are not thought to cross the placenta (Aaltonen et al., 2005) thus their detection in amniotic fluid points to local fetoplacental production and in the case of IL-1β and IL-18, the cellular machinery to generate bioactive product.

In studies conducted using fetal membranes obtained prior to and after the spontaneous onset of labour, a potential role for the inflammasome was inferred from increased gene transcription of caspase-1 (Lappas, 2014b). This observation was also mirrored in myometrium (Lappas, 2014b). Moreover, in explant models of fetal membranes and myometrium, LPS-stimulated bioactive IL-1β was detectable in culture supernatant with release potentiated by ATP and nigericin and sensitive to pharmacological blockade using a P2X7 inhibitor and the pannexin-1...
cordance with its role as a ‘danger’ signal, high (mM concentration) extracellular ATP promotes secretion of IL-1β from labouring and non-labouring women in line with activation of the P2X7 receptor (Warren et al., 2008). Levels of the natural ligand, ATP, for the P2X7 receptor, are tightly regulated in vivo yet in accordance with its role as a ‘danger’ signal, high (mM concentration) extracellular ATP levels are required to activate the P2X7 receptor. In the pregnant human uterus, elevated ATP levels could result from (i) its release from endothelial cells in cord blood vessels (Yegutkin et al., 2000) and (ii) mechanical trauma (Pedersen et al., 1999) possibly from uterine stretch or contractions. Expression of the rat myometrial P2X7 receptor increases prior to labour and in an animal model of PTB, P2X7 mRNA and protein increased a further 18- to 22-fold at Day 19 of gestation indicating a distinct activation of inflammatory pathways in PTB (Urabe et al., 2009). This group also reported an ATP-activated conductance in rat myometrium with a pharmacological profile consistent with P2X7 stimulation (Miyoshi et al., 2010).

Unstimulated fetal membranes with intact chorion and amnion, expressed mRNA for all ten human TLRs, NLRP1, NLRP3, ASC and caspase-1 (Hoang et al., 2014). In this study, it was also shown that active IL-1β levels were higher in fetal membranes after stimulation with PAMPs typified by LPS, peptidoglycan and flagellin; caspase-1 dependent secretion of IL-1β was only observed in response to TLR-2, -4 and -5, in contrast to NOD1 and NOD2 ligands where IL-1β release was caspase-1 independent (Hoang et al., 2014). While the NLRs NOD1 and NOD2 are not known to form inflammasomes, both of these NLRs can interact indirectly to influence inflammasome activation (Hsu et al., 2008). Moreover, following infection with Chlamydia trachomatis, IL-1β release from trophoblasts was shown to be ASC-independent, mediated instead by NOD1 (Kavathas et al., 2013). Nevertheless, from this limited data, evidently gestational tissues express components of inflammasomes as well as the upstream signalling pathways and the corresponding effector cascades leading to IL-1β release. The ontogeny of inflammasome expression in the various cells and tissues of the materno/fetal/placental axis may be an important factor in combatting infection.

**Pre-eclampsia**

A potential involvement of the inflammasome in pre-eclampsia is hypothesized since metabolites such as uric acid and ROS could be considered danger signals driving inflammation. Recently Xie et al. reported raised mRNA levels of TLR2, TLR4 and IL-1β with elevated protein expression for cryopyrin (NLRP3) in neutrophils of women with pre-eclampsia compared with normal pregnancy (Xie et al., 2010). The increase in cryopyrin was even more striking in the early onset pre-eclamptic group but IL-1β levels were not measured in serum to either confirm or refute an association with the higher levels in pre-eclampsia. In fact, neutrophils have been little-studied with respect to the inflammasome compared with mononuclear cells. However, they secrete significant amounts of IL-1β on stimulation with LPS and Nigericin, a K⁺ ionophore (Mankan et al., 2012). In addition, resting neutrophils purified from whole blood, expressed ASC, caspase-1, NLRP3 and AIM2 with lower expression of NLRC4 and NLRP1b leading the authors to conclude that the NLRP3/ASC/caspase-1 axis in neutrophils is indeed functional (Bakele et al., 2014). This finding was confirmed for both IL-1β and IL-18 (but not IL-1α and IL-13) in later studies where a highly pure neutrophil preparation was employed (Bakele et al., 2014).

In terms of a direct association between DAMPs and pre-eclampsia, raised plasma levels of uric acid are apparent before clinical signs of the disease (Redman and Bonnar, 1978; Koopmans et al., 2009; Gowri and Al-Zakwani, 2010) but controversy exists on their clinical utility. In human first trimester villous trophoblast and two trophoblast cell lines (Sw.71 and HTR-8/SVneo), monosodium urate (MSU) promoted IL-1β secretion via NLRP3-inflammasome activation indicative of its proinflammatory effects (Mulla et al., 2011). The fact that the Sw.71 trophoblast cell line expresses constitutively high levels of pro-IL-1β removes the need for the first hit in the IL-1β production pathway (Mulla et al., 2011). A previous study highlighted mRNA expression for NLRP1, NLRP3 and ASC mRNA and translation to protein in human syncytiotrophoblast, cytotrophoblast and the Sw.71 cell line (Abrahams, 2011) but the functional significance of this observation has not been

blocker carbenoxolone (Lappas, 2014b). Our own data from human cord blood leukocytes demonstrate that extracellular ATP promotes secretion of IL-1β from labouring and non-labouring women in line with activation of the P2X7 receptor (Warren et al., 2008). Levels of the natural ligand, ATP, for the P2X7 receptor, are tightly regulated in vivo yet in accordance with its role as a ‘danger’ signal, high (mM concentration) extracellular ATP levels are required to activate the P2X7 receptor. In the pregnant human uterus, elevated ATP levels could result from (i) its release from endothelial cells in cord blood vessels (Yegutkin et al., 2000) and (ii) mechanical trauma (Pedersen et al., 1999) possibly from uterine stretch or contractions. Expression of the rat myometrial P2X7 receptor increases prior to labour and in an animal model of PTB, P2X7 mRNA and protein increased a further 18- to 22-fold at Day 19 of gestation indicating a distinct activation of inflammatory pathways in PTB (Urabe et al., 2009). This group also reported an ATP-activated conductance in rat myometrium with a pharmacological profile consistent with P2X7 stimulation (Miyoshi et al., 2010).

Unstimulated fetal membranes with intact chorion and amnion, expressed mRNA for all ten human TLRs, NLRP1, NLRP3, ASC and caspase-1 (Hoang et al., 2014). In this study, it was also shown that active IL-1β levels were higher in fetal membranes after stimulation with PAMPs typified by LPS, peptidoglycan and flagellin; caspase-1 dependent secretion of IL-1β was only observed in response to TLR-2, -4 and -5, in contrast to NOD1 and NOD2 ligands where IL-1β release was caspase-1 independent (Hoang et al., 2014). While the NLRs NOD1 and NOD2 are not known to form inflammasomes, both of these NLRs can interact indirectly to influence inflammasome activation (Hsu et al., 2008). Moreover, following infection with Chlamydia trachomatis, IL-1β release from trophoblasts was shown to be ASC-independent, mediated instead by NOD1 (Kavathas et al., 2013). Nevertheless, from this limited data, evidently gestational tissues express components of inflammasomes as well as the upstream signalling pathways and the corresponding effector cascades leading to IL-1β release. The ontogeny of inflammasome expression in the various cells and tissues of the materno/fetal/placental axis may be an important factor in combatting infection.

**Pre-eclampsia**

A potential involvement of the inflammasome in pre-eclampsia is hypothesized since metabolites such as uric acid and ROS could be considered danger signals driving inflammation. Recently Xie et al. reported raised mRNA levels of TLR2, TLR4 and IL-1β with elevated protein expression for cryopyrin (NLRP3) in neutrophils of women with pre-eclampsia compared with normal pregnancy (Xie et al., 2010). The increase in cryopyrin was even more striking in the early onset pre-eclamptic group but IL-1β levels were not measured in serum to either confirm or refute an association with the higher levels in pre-eclampsia. In fact, neutrophils have been little-studied with respect to the inflammasome compared with mononuclear cells. However, they secrete significant amounts of IL-1β on stimulation with LPS and Nigericin, a K⁺ ionophore (Mankan et al., 2012). In addition, resting neutrophils purified from whole blood, expressed ASC, caspase-1, NLRP3 and AIM2 with lower expression of NLRC4 and NLRP1b leading the authors to conclude that the NLRP3/ASC/caspase-1 axis in neutrophils is indeed functional (Bakele et al., 2014). This finding was confirmed for both IL-1β and IL-18 (but not IL-1α and IL-13) in later studies where a highly pure neutrophil preparation was employed (Bakele et al., 2014).

In terms of a direct association between DAMPs and pre-eclampsia, raised plasma levels of uric acid are apparent before clinical signs of the disease (Redman and Bonnar, 1978; Koopmans et al., 2009; Gowri and Al-Zakwani, 2010) but controversy exists on their clinical utility. In human first trimester villous trophoblast and two trophoblast cell lines (Sw.71 and HTR-8/SVneo), monosodium urate (MSU) promoted IL-1β secretion via NLRP3-inflammasome activation indicative of its proinflammatory effects (Mulla et al., 2011). The fact that the Sw.71 trophoblast cell line expresses constitutively high levels of pro-IL-1β removes the need for the first hit in the IL-1β production pathway (Mulla et al., 2011). A previous study highlighted mRNA expression for NLRP1, NLRP3 and ASC mRNA and translation to protein in human syncytiotrophoblast, cytotrophoblast and the Sw.71 cell line (Abrahams, 2011) but the functional significance of this observation has not been
tested further. In a recent study, the NLRP3 inflammasome was also implicated in the effects of antiphospholipid syndrome in recurrent miscarriage, fetal demise (Abrahams, 2011) and placental dysfunction in antiphospholipid syndrome-induced pre-eclampsia (Mulla et al., 2013). An intriguing aspect of a potential disease mechanism in pre-eclampsia is the shedding of syncytiotrophoblast microparticles and their export into the maternal circulation which has been linked to a maternal inflammatory response (Sargent et al., 2003). It is conceivable that these microparticles can act as DAMPs and that the exaggerated inflammatory state in pre-eclampsia originates from hyperactive inflammasome activity.

**Metabolic dysfunction**

During pregnancy, through the actions of human placental lactogen, maternal insulin resistance develops in order to ensure the ready availability of glucose to the fetus as its main energy substrate (Buchanan and Xiang, 2005). In cases of GDM, the fetus utilizes copious amounts of glucose underlying the macrosomia associated with this disorder. In insulin resistance, the NLRP3 inflammasome mediates the release of IL-1β via a caspase-1 dependent mechanism (Vandanmagsar et al., 2011). IL-1β may itself inhibit both tyrosine phosphorylation and gene expression of insulin receptor substrate, IRS-1 (Jager et al., 2007). On comparing adipose tissue of women with GDM with a normal glucose tolerant cohort, several lines of evidence emerge in favour of the involvement of the inflammasome (Lappas, 2014a). Although mRNA levels for IL-1β were unchanged in adipose tissue of the two groups, IL-1β release was greater in the GDM group, as were levels of active caspase-1 (p35 and p10) (Lappas, 2014a). Furthermore, adipose tissue explants from women with GDM, treated with IL-1β had reduced expression of glut-4 mRNA and protein in addition to reduced glucose uptake (Lappas, 2014a). Finally ATP pre-incubation stimulated IL-1β production while levels of IL-6 and IL-8 were unaffected (Lappas, 2014a).

### The neonate

The switch to an extraterine existence necessarily presents immune challenges to the neonate. For the first weeks of life, infants are particularly susceptible to infections even from commensal microbes. They have little memory cell-mediated or antibody-mediated immunity as a consequence of their anatomically-isolated early life (Adkins et al., 2004). Postnatally, additional adaptations are vital as the neonate’s mucosal surfaces are challenged by combining the need to accommodate commensal microbes yet protect itself from pathogens. Using a fetal rat model Kempster et al. showed that mRNA transcripts of the inflammasome components PYCARD, caspase-1 and nlrp6 were higher in intestinal epithelia but not in lung at late gestation (Kempster et al., 2011). Of interest is also the observation that monocyte-derived dendritic cells (MoDCs) of newborns express a functional inflammasome, based on caspase-dependent mediated IL-1β release although levels were significantly lower than adult MoDCs (Philbin et al., 2012). The authors attributed this to reduced generation of the active 10 kDa caspase subunit (Philbin et al., 2012). The NLRP3 inflammasome has also been implicated in hypoxic-ischaemic encephalopathy based on the presence of metabolic acidosis, elevated uric acid (Hoffman and Wanderer, 2010) and raised IL-1β levels in cerebrospinal fluid from newborns (Wanderer, 2009). Since extremely preterm (<28 weeks) neonates are susceptible to hypoxic brain injury and neonatal sepsis, animal studies using IL-1β targeted therapies (Girard et al., 2008, 2010; Leitner et al., 2014) would provide proof of concept in manipulating inflammasome function to limit brain injury. Many of the components involved in the inflammasome are candidates in mediating this form of brain injury.

---

**Table II** Common pathogens implicated in preterm birth and their links to inflammasomes.

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Link to preterm birth</th>
<th>Link to inflammasome</th>
<th>Inflammasome type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Yes1</td>
<td>Yes2</td>
<td>NLRP3</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Yes3</td>
<td>Yes4</td>
<td>NLRP3</td>
</tr>
<tr>
<td>Encephalomyocarditis virus</td>
<td>No</td>
<td>Yes5, 6</td>
<td>NLRP3</td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
<td>Yes7</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>Yes7</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>Herpes virus</td>
<td>Yes8</td>
<td>Yes9</td>
<td>NLRP3</td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>No</td>
<td>Yes10</td>
<td>NLRP3</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Yes7, 11</td>
<td>Yes12, 13</td>
<td>NLRP3</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>Yes14</td>
<td>Yes15</td>
<td>NLRP3</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>No</td>
<td>Yes16, 17</td>
<td>NLRC4</td>
</tr>
<tr>
<td>Sendai virus</td>
<td>No</td>
<td>Yes18</td>
<td>NLRP3</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>No</td>
<td>Yes19</td>
<td>NLRC4</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>Yes7, 20, 21</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>Toxoplasma gondii</td>
<td>Yes22</td>
<td>Yes23</td>
<td>NLRP1</td>
</tr>
<tr>
<td>Ureaplasma urealyticum</td>
<td>Yes7, 24, 25</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>Mycoplasma hominis</td>
<td>Yes7, 26</td>
<td>No</td>
<td>–</td>
</tr>
</tbody>
</table>

*Details of references 1–26 can be found in Supplementary Data.*
In summary, Fig. 3 depicts the main pregnancy syndromes discussed, the causal factors and associated disease outcomes.

**Future perspectives**

The unremitting nature of PTB and the escalation of births associated with maternal metabolic disorders that tend to result in post-term pregnancies (Heslehurst et al., 2008; Arrowsmith et al., 2011) necessitate new approaches to the prediction, diagnosis and treatment of these syndromes. Combined, these conditions account for a sizable proportion of feto-maternal morbidity and mortality. If the mechanisms of IL-1β release within the context of pregnancy for eventual screening and therapy are to be realized, then an appreciation of how release of this cytokine is controlled is necessary.

In most cases of PTL, tocolysis provides only limited respite. Few markers of impending PTB are used in the clinic and in predicting PTB in an asymptomatic population, there are few tools at present that are of use. A recent review article suggests that fetal fibronectin (FFN) is most promising with a likelihood ratio of 4 for PTB before 34 weeks (Chan, 2014). FFN has high specificity but a mixed sensitivity that diminishes with gestational age (Goldenberg et al., 1996). Another possibility is the transvaginal measurement of cervical length which is reported to be predictive of PTB in all populations studied (Grimes-Dennis and Berghella, 2007). Of note, for this article, inflammatory markers including cytokines and C-reactive protein were not useful in this population (Grimes-Dennis and Berghella, 2007). However, we would argue that recent developments merit a further evaluation of these parameters.

With respect to infection and PTB, many of the pathways utilized by pathogens to evade host defences have not been fully explored. Bacteria have developed ingenious methods for evading host defences and a number of bacterial products including muramyl dipeptide and flagellins activate inflammasomes as does malarial hemozoin (Dostert et al., 2009). The latter is interesting in light of the burden of PTB attributable to malaria (Simmons et al., 2010). In contrast to bacteria, we know very little about the link between viral infections and PTB. Tsekoura et al. reported that adenoviral infection of the placenta was associated with PTB and histological evidence of chorioamnionitis (Tsekoura et al., 2010). Interestingly, production of IL-1β from fetal membranes stimulated with viral single-stranded RNA was reduced by over half in the presence of a caspase-1 inhibitor (Bakaysa et al., 2014). In terms of outcomes, women with hepatitis B or hepatitis
Neither has inflammation in pregnancy been targeted with anti-inflammatory agents to manage or treat PTB. Drugs with anti-inflammatory actions have been used in women with PTB; high dose dexamethasone by default, as it is principally used to mature the fetal lung but notably is used in association with conventional tocolytics so its effect on labour is hard to assess; it is true to say that at the point of being used, in established PTL, it seems to make little difference. Anti-biotic prophylaxis shows no improvement in neonatal outcomes in women with PTL with intact membranes (Flenady et al., 2013). Anti-microbial peptides are a potential alternative to antibiotics with their potent microbicidal activity. Indeed, the cathelicidin-derived peptide LL37 induces IL-1β processing and also activates the P2X7 receptor (Eisner et al., 2004); hence the actions of defensins and other groups of antimicrobials are worth exploring further. The prospect of a possible intervention to reduce the incidence of PTB is tantalizing and is ultimately the rationale for research into inflammatory processes in pregnancy. The ideal is a situation whereby we can reliably predict, early on, asymptomatic pregnant women who are likely to go into PTB and have a safe intervention to prevent that happening.

The pathogenesis of PE is one of the most studied and scrutinized topics in obstetrics and is largely considered a disease of defective implantation. It is pertinent to consider new factors and review existing targets, such as urates and ROS, in light of their associations with the inflammasome. A potentially exciting area would be the role of microparticles and the possibility that inflammasome (possibly NLRP3) activation exaggerates maternal endothelial dysfunction by adverse endothelial-leukocyte interactions. Our presumption is that NLRP3 is the most likely target for shed microparticles transporting fetal DNA given the diversity of molecular signals that activate NLRP3.

The predicted increase in maternal obesity and GDM is likely to constitute an even greater disease burden over the next decade. The source of elevated proinflammatory cytokines typified by IL-1β in any of these pregnancy disorders is either the result of activation of the fetal inflammasome response syndrome or damage to the maternal-placental axis by DAMPs. This raises the unanswered question of whether the apparent inflammation arises from the maternal or fetal compartment. In the case of the maternal circulation, ‘damage’ may have multiple origins, be it PAMPs or DAMPs. For example, high extracellular glucose, or MSU are DAMPs and may explain the inflammation that accompanies both acute and chronic inflammation by engaging inflammasomes. Importantly, elevated levels of circulating free fatty acids as observed in obesity and diabetes have been proposed as the ‘second hit’ (instead of ATP) required for IL-1β processing. Via this mechanism, palmitate activated the NLRP3 inflammasome in macrophages via an adenosine monophosphate-activated protein kinase (AMPK) -ROS pathway which led to IL-1β-mediated insulin resistance in target tissues (Wen et al., 2011). This overnutrition hypothesis could potentially be a mechanism whereby fetal exposure to high glucose or circulating free fatty acid levels in the placenta can reprogramme metabolic pathways that alter risk to adult chronic diseases such as Type II diabetes and cardiovascular disorders. The same is true for maternal obesity and GDM where we now have clues regarding cholesterol interactions with the NLRP3 inflammasomes (Dewell et al., 2010; Rajamaki et al., 2010) that will allow us to probe these interactions further in pregnancy. Indeed, the endocrine nature of pregnancy provides an opportunity to investigate potential hormonal influences on the inflammasome in leukocytes given anti-inflammatory effects of progesterone.

The lack of biomarkers and drugs to treat the syndromes described herein stem from a paucity of knowledge regarding the immunopathology underlying these complex diseases, thus alternative therapeutic approaches should incorporate strategies that target pathways earlier in the inflammatory cascade. The rapidly burgeoning field of inflammasome biology with new molecules and pathways is entering the foray. While IL-1β is considered the end product of inflammasome activation, recent findings indicate that this may not be the case always; inflammasome activation in vivo has been shown to trigger an eicosanoid storm (von Moltke et al., 2012). Many of the metabolites produced through this ‘storm’ are eicosanoids including PGE2, 12-hydroxyeicosatetraenoic acid (12-HETE) and thromboxanes—all three are demonstrated to have roles in reproduction. Indeed, raised placental 12-HETE levels have been measured in pre-eclamptic placentas (Pearson et al., 2010). Thus these observations are of direct relevance to pregnancy and justify exploring such phenomena during gestation.

The decade following the discovery of the inflammasome has produced fascinating insight into the intricacies of enzymes and cytokines in the amplification of inflammatory responses. Initially identified as a vehicle for IL-1β release, inflammasomes appear to have the capacity to generate alternative signalling mediators as end-points thus novel pathways may emerge from future studies. One must also caution against attributing all of IL-1β activities and effects to be via the inflammasome (Netea et al., 2010; Kono et al., 2012). It is also noteworthy that the majority of the findings of inflammasome activity have used *in vitro* assays and models. It is probable that *in vivo* mechanisms will differ and as our knowledge of these develops, more inflammasome complexity is likely to be unravelled. Finally, the genetics of autoinflammatory disorders has been instrumental in manipulating the inflammasome for therapeutic benefit and is a probable route whereby the genetics of PTB and may be further explored in order to identify women at risk. Moreover, IL-1 targeted therapies that have been successfully administered to treat hereditary autoinflammatory disorders and also hold promise for type II diabetes, raise the possibility of utilizing versions of these molecules as possible treatments for PTB, neonatal brain injury and sepsis. Understanding the role of inflammasome processes within the major morbidities of pregnancies needs further elucidation with the hope that some benefit to mother and child may result.

### Supplementary data

Supplementary data are available at http://humupd.oxfordjournals.org/.

### Authors’ roles

Both authors (R.N.K. and D.P.H.) made substantial contributions to conception and design or acquisition of data; analysis and interpretation of
data, drafted and revised the article for important intellectual content and approved the final version to be published.

**Funding**

No funding was received for this study.

**Conflict of interest**

None declared.

**References**


Boyden ED, Dietrich WF. Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. Nat Genet 2006;38:240–244.


Catalano PM, Ehrenberg HM. The short- and long-term implications of maternal obesity on the mother and her offspring. BJOG 2006;113:1126–1133.

Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. Proc Natl Acad Sci USA 2009;106:9021–9026.


Duley L. The global impact of pre-eclampsia and eclampsia. Semin Perinatol 2009; 33:130–137.


Elssner A, Duncan M, Gavrilin M, Wewers MD. A novel P2X7 receptor activator, the receptor and intracellular K(RNS) generation by silica in inflammation and fibrosis.


Fubini B, Hubbard A. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis. Free Radic Biol Med 2003; 34:1507–1516.


Inflammasomes and disorders of pregnancy


Redman CW, Sargent IL. Pre-eclampsia, the placenta and the maternal systemic inflammatory response—a review. Placenta 2003; 24(Suppl A):S21–S27.


