10

Cytokines and the Innate Immune Response at the Materno-Fetal Interface

Aled H. Bryant and Catherine A. Thornton
Institute of Life Science, Swansea University, UK

1. Introduction

Labour is the climax of pregnancy resulting in the expulsion of the fetus from the uterus. It is a complex process and the mechanisms involved in the initiation of labour are poorly understood despite decades of investigation. Generally, labour is not a sudden occurrence but one for which the body prepares: numerous physiological, biochemical and immunological events take place at the materno-fetal interface, and in both mother and fetus in the lead up to parturition (Hendricks, Brenner et al. 1970). These same processes might be accelerated or other pathways brought into play when labour occurs prematurely. The bulk of perinatal morbidity and mortality is associated with premature labour and delivery of a preterm infant (Tucker and McGuire 2004; Steer 2005). Understanding the mechanisms of labour in health and in adverse obstetric outcomes should provide insight into the pathogenesis of preterm birth.

2. Labour, inflammation and cytokines

Human labour and delivery have been compared to an inflammatory response (Aboussahoud, Bruce et al. 2010; Liggins, Fairclough et al. 1973) of at least three physiologically interdependent processes: remodelling of the cervix to allow it to stretch open to the width of the reproductive tract, weakening and rupture of the membranes in the region that overlies the cervix, and the initiation of rhythmic contractions of increasing amplitude and frequency that ultimately force the fetus and placenta from the uterus. Pro-inflammatory cytokines have a role in most of these processes, suggesting that the immune privileges that the fetal-placental unit has enjoyed during pregnancy might be revoked at the time of labour (Aboussahoud, Bruce et al. 2010; Bayraktar, Peltier et al. 2009). Other beneficial effects of strong pro-inflammatory activity during labour could include removal of placental fragments and a heightened innate immune response in the postpartum uterus to combat the pathogens undoubtedly encountered at this time.

2.1 Cervical ripening

Re-modelling of the extracellular matrix, including decreased collagen concentration and the dispersion of collagen fibrils, is a feature of cervical ripening (Leppert 1995; Winkler and Rath 1999). This is facilitated by increased local production of many pro-inflammatory

cytokines and chemokines, such as interleukin-1β (IL-1β), IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1; CCL2) and tumour necrosis factor α (TNFα) (Osman, Young et al. 2003; Tornblom, Klimaviciute et al. 2005), and an influx of neutrophils and macrophages that also secrete cytokine and chemokines and amplify the inflammatory response via the recruitment of other inflammatory cells to the cervix (Rodney W; Sennstrom, Brauner et al. 1997; Sakamoto, Moran et al. 2005). Cytokines such as TNFα and IL-1β activate the nuclear factor (NF)κB pathway, leading to increased production of proteases, cathepsins and matrix metalloproteinases (MMPs), that allow for the digestion of collagen (Watari, Watari et al. 1999). Inhibitors of MMP are down-regulated by IL-1β, and IL-1β also increases production of cyclooxygenase (COX)-2 and prostaglandin E_2 (PGE_2) which further increases production of proteinases and modulates leukocyte trafficking (Sato, Michizu et al. 2001). Murine models show that mechanisms regulating cervical ripening might differ in preterm and term birth, and with underlying cause of labour: preterm birth subsequent to progesterone withdrawal in the absence of infection was comparable to term cervical ripening but preterm ripening in response to infection (using lipopolysaccharide (LPS)) was associated with a robust pro-inflammatory response including neutrophil influx and activation of the prostaglandin synthesis cascade (Holt, Timmons et al. 2011).

2.2 Membrane rupture

Membrane rupture is also characterised by extracellular matrix remodeling: fibronectin is degraded by MMPs and other proteases (Lockwood, Senyei et al. 1991; Vadillo-Ortega, Hernandez et al. 1996) facilitating separation of the previously fused chorioamniontic membranes and deciduas. Similar to the cervix, production of MMPs is increased in response to the augmented production of pro-inflammatory cytokines such as IL-8, IL-6, TNFα and IL-1β (Laham, Brennecke et al. 1996a, 1996b, 1999.). Inflammatory cytokines decrease rupture strength of fetal membranes. This is achieved by induction of prostaglandin and reactive oxygen species production, and collagen remodelling via the release of proteases such as MMP9 (Moore, Novak et al. 2009).

2.3 Myometrial contractions

A similar pattern of cytokine activity is observed in the myometrium: increased levels of IL-6, IL-1β, TNFα and IL-8 result in increased production of COX-2 and PGE_2 (Pollard and Mitchell 1996; Young, Thomson et al. 2002). IL-6 in particular promotes increased production of oxytocin and expression of the oxytocin receptor by myometrial cells (Friebe-Hoffmann, Chiao et al. 2001). PGE_2 and oxytocin in combination are responsible for intracellular calcium increase which are essential for contractions (Thornton, Gillespie et al. 1992).

3. Preterm birth

Birth prior to 37 weeks of gestation is the leading cause of perinatal morbidity and mortality in the Western world; 75% of perinatal mortality and nearly 50% of long-term neurological morbidity are associated with preterm birth (PTB) (Goldenberg, Culhane et al. 2008). Preterm infants are prone to higher rates of complications of the gastrointestinal, renal and

respiratory systems (Saigal and Doyle 2008). Increasing PTB rates over the past 30 years in industrialised countries reflects increased indicated preterm births for reasons such as preeclampsia and intrauterine growth restriction (IUGR), and the fallout of multiple gestations associated with assisted reproductive technologies (ART). Other risk factors for preterm birth include ethnicity, previous preterm birth, low maternal body mass index (BMI), genetic variation, infection including periodontal disease, and adverse behaviours. Annual estimates currently suggest that 12.9 million (about 10%) infants worldwide are born prematurely (Beck, Wojdyla et al. 2010). The prevention and management of PTB is one of the major challenges of contemporary obstetrics and gynaecology. PTB also has a large economic impact. The cost of preterm birth to the public sector in England and Wales is approximately £3 billion; the average preterm baby is one and a half times more costly than a full term neonate with the estimated incremental cost per PTB infant at around £30,000 in England and Wales (Mangham, Petrou et al. 2009). Recent decades have seen improvements in the survival rates of preterm infants due to advances in neonatal care and the increased use of antenatal steroids but little change in the ability to prevent preterm birth (Elovitz and Mrinalini 2004).

Preterm delivery for maternal and/or fetal indications accounts for around 30% of preterm births (Hamilton, Martin et al. 2006). Other obstetric precursors of preterm birth are spontaneous preterm labour (sPTL) with intact membranes and preterm premature rupture of the membranes (PPROM). Spontaneous preterm labour, defined as regular uterine contractions with accompanying cervical changes before 37 weeks gestation accounts for 45%, while PPROM, defined as spontaneous membrane rupture accounts for 15% (Goldenberg, Culhane et al. 2008). Both sPTL and PPROM are the outcome of maternal and/or fetal inflammatory responses that can have a systemic component or remain localised to the reproductive tract.

Intrauterine infection is a common mechanism of preterm labour accounting for 25 – 40% of all sPTL cases, although limitations in microbiological culture techniques might make this a conservative estimate (Goldenberg, Hauth et al. 2000). Several routes have been suggested for microbial invasion into the intrauterine cavity. The most common route is the ascension of microorganisms from the vagina through the cervix and into the uterus. The infection can ultimately gain access to the amniotic fluid thereby exposing the fetus to infection (Kim, Romero et al. 2009). Other routes of infection include hematogenous spread through the placenta of non-genital tract infections such as those from the oral cavity (Bearfield, Davenport et al. 2002). Irrespective of the route, microbial invasion results in infection at various sites including the placenta, the fetal membranes, the amniotic fluid, the umbilical cord, and the fetus itself. Many microorganisms have been associated with PPROM, sPTL and PTB. Common microorganisms resulting in intrauterine infection include *Escherichia coli* (Naeye 1979), *Ureaplasma urealyticum* (Yoon, Romero et al. 1998), *Streptococcus agalactiae* (Gibbs, Romero et al. 1992) and *Chlamydia trachomatis* (Gibbs, Romero et al. 1992) among many others. For example, genital mycoplasmas have been associated with a higher maternal white blood count and C-reactive protein (CRP), and more leukocytes in the amniotic fluid (Oh, Lee et al. 2010).

4. Inflammation and cytokines in healthy and adverse pregnancy outcomes

A wealth of evidence indicates that labour is an inflammatory process (Bowen, Chamley et al. 2002). Inflammation is a primary response mechanism resulting from the biological

activity of cytokines and other mediators produced in response to harmful stimuli of both infectious and non-infectious origins. Cytokines are small proteins secreted by various cells that function as extracellular signalling molecules to facilitate communication between various cells of the body, a response induced by binding to specific receptors expressed by target cells. Cytokines including IL-6, IL-8 and TNFα are key immunological and inflammatory mediators which can act in an autocrine, paracrine or endocrine manner.

The pathophysiological mechanisms underlying preterm birth are largely unknown but might relate to premature activation of the normal labour process or the response to an insult. Proposed triggers of preterm birth include: uterine over-distension, stress, infection and inflammation, and other immunologically-mediated processes. Irrespective of the triggering event, local and systemic inflammation tends to be a feature of preterm labour and delivery. Understanding the inflammatory pathways that contribute to the initiation and maintenance of preterm (and term) labour could be used to develop strategies to: (i) identify those women most at risk of preterm labour and birth, and (ii) prevent preterm birth. Whilst infection-associated PTB is the focus of this chapter, clinical studies have revealed an association between infection and other pregnancy complications such as preeclampsia and IUGR (Hsu and Witter 1995; Arechavaleta-Velasco, Koi et al. 2002).

A burgeoning body of literature implicates numerous cytokines in the normal physiological processes of pregnancy (e.g. implantation, placental function, parturition) and in the inflammatory response during infection associated preterm labour. The analysis of changes in candidate cytokines has proved worthwhile in identifying potential underlying mechanisms of PTB. IL-1, the first cytokine implicated, is up-regulated in response to microbial products in the human decidua, resulting in the production of prostaglandins by the amnion and deciduas (Romero, Brody et al. 1989a; Romero, Durum et al. 1989b; Romero, Wu et al. 1989c). Mid-trimester amniotic fluid levels of IL-1β levels are associated positively with preterm delivery (Puchner, Iavazzo et al. 2011), and IL-6 concentrations in amniotic fluid are considered a marker for infection (Romero, Avila et al. 1990; Yoon, Romero et al. 1995). Other cytokines including IL-10 (Gotsch, Romero et al. 2008), TNFα (Romero, Manogue et al. 1989d), colony stimulating factor (CSF) (Saito, Kato et al. 1992) and IL-18 (Pacora, Romero et al. 2000) among others have been linked to infection associated preterm labour. There are now a number of groups developing strategies to identify cytokine and other protein signatures that might rapidly identify those women most at risk of delivering prematurely especially in the setting of intrauterine infection. Mass spectrometry-based proteomic profiling of amniotic fluid from women with PTL or PPROM found an inverse relationship between time to delivery and severity of intra-amniotic inflammation as determined by measurement of 4 biomarkers. While minimal inflammation was also associated with preterm birth, the extent of intra-amniotic inflammation correlated with negative outcomes for the neonate (Buhimschi, Bhandari et al. 2007). The potential of proteomic profiling of cervico-vaginal fluid also has been evaluated in a non-human primate model of intra-amniotic infection. Differential expression of proteins was observed in control versus infected samples and this might offer a relatively non-invasive strategy for detection of infection via signatures created by specific biomarkers (Gravett, Thomas et al. 2007).

The cytokines of interest are made by cells normally present in the gestation-associated tissues such as trophoblast cells and macrophages, and by leukocytes that infiltrate these

tissues in response to inflammatory stimuli. An accumulation of leukocytes evident upon histological analysis of the placental membranes, so-called chorioamnionitis, occurs in around one-third of preterm deliveries. Chorioamnionitis was once considered a hallmark of infection but it was soon recognised to occur in the absence of any detectable signs of infection. Now, two types of chorioamnionitis have been classified: acute chorioamnionitis (ACA) associated with infection, and chronic chorioamnionitis (CCA) of immunologic origin related to maternal anti-fetal allograft rejection and graft-versus-host disease in the placenta (Lee, Romero et al. 2011). The local cytokine profile differs with the type of chorioamnionitis: IL-6 is the prototypic cytokine elevated in amniotic fluid in ACA whereas for CCA amniotic fluid levels of CXCL10 (IP-10 – interferon-inducible protein-10) are increased and there is elevated gene expression of CXCL9 (MIG – monokine induced by interferon gamma), CXCL10 and CXCL11 (I-TAC - interferon-inducible T-cell alpha chemoattractant) (Kim, Romero et al. 2010).

While chorioamnionitis generally is associated with adverse pregnancy outcomes, around 9 - 20% of term deliveries have evidence of histologic chorioamnionitis and the duration of labour might impact on the occurrence of this (Lee 2011, Romero et al.; Park, Romero et al. 2010). Notably chorioamnionitis is also increased in spontaneous versus induced preterm birth (Kim, Romero et al. 2010). Preterm chorioamnionitis is accompanied by villitis in around 40% of cases (Kim, Romero et al. 2010). Placental villous macrophages (Hofbauer cells) in particular increase when there is evidence of chorioamnionitis. It has been suggested that fibroblast production of MCP-1 in response to bacterial products such as LPS or inflammatory cytokines such as IL-1β or TNFα might drive the accumulation of macrophages within placental villi in this setting (Toti, Arcuri et al. 2011).

5. The innate immune response and inflammation: pattern recognition receptors and cytokine production

Changes in cytokine production at the maternal-fetal interface are a feature of both term and preterm labour. This has generated much interest in the mechanisms of cytokine production in the placenta and attached membranes, and whether such changes precede labour or are simply a consequence of it. Signalling pathways of the innate immune system which produce a defined cytokine output in response to microbial stimuli have been postulated as central to this. Several studies have found a link between the treatment of various gestation-associated tissues with microbial stimuli and cytokine outputs (Holmlund, Cebers et al. 2002; Wang and Hirsch 2003; Elovitz and Mrinalini 2004; Kim, Romero et al. 2004; Kumazaki, Nakayama et al. 2004). These studies have shown that microbial products such as LPS can trigger key molecular events ultimately leading to the production of relevant cytokines.

The innate immune system uses evolutionary conserved germline encoded receptors, termed pattern recognition receptors (PRRs), to recognise and respond to a variety of pathogenic and non-pathogenic microorganisms. Identification of PRRs was initially demonstrated by work on the *Drosophila* protein Toll, a protein involved in development of dorsoventral polarity during embryonic development. However, Hoffman and colleagues demonstrated that Toll was also required for an effective immune response to *Aspergillus fumigates* in the fly (Lemaitre, Nicolas et al. 1996). This realisation inspired a search for mammalian homologues of Toll, which lead to the discovery initially of Toll-like receptor 4

(TLR4), and consequently the remainder of the Toll-like receptor family (TLRs) (Medzhitov, Preston-Hurlburt et al. 1997). As increasing research attention has been placed on the mechanisms of innate immune recognition and signalling, other pattern recognition families have been discovered. These include; RIG-I (retinoic acid-inducible gene I)-like receptors (RLRs) (Yoneyama and Fujita 2007), NOD (nucleotide oligomerisation domain)-like receptors (NLRs) (Mathews, Sprakes et al. 2008), C-type lectin receptors (CLRs) (Netea, Ferwerda et al. 2005) and DAI (DNA dependent activator of IFN regulatory factors)-like receptors (DLRs) (Takaoka, Wang et al. 2007). Each family of PRRs has numerous members.

PRRs detect conserved molecular patterns called pathogen associated molecular patterns (PAMPs) on a wide range of pathogens. Detection of a PAMP by its specific PRR, activates intracellular signalling, leading to cytokine gene expression and eventual activation of inflammatory and antimicrobial responses (Ishii, Koyama et al. 2008). Examples of PAMPs include lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, and peptidoglycan (PGN), a component of Gram-positive bacteria cell walls (Janeway and Medzhitov 2002). PRRs have also been shown to recognise damage associated molecular pattern patterns (DAMPs) released by the host (Bianchi 2007).

Below is an overview of each PRR family including some detail about cellular expression, signalling pathways, and the exogenous and endogenous ligands that might initiate activity via each pathway. This will be too much detail for some readers but for others will provide some insight into the constantly growing complexity of the innate immune response. Following this we review the current knowledge of expression and activity of components of each of these pathways/families in gestation-associated tissues.

5.1 Toll-like Receptors

The first characterised mammalian TLR was TLR4, and a further 12 mammalian TLRs have since been identified (Akira 2004). Only TLRs 1 – 10 are expressed and functional in humans. TLRs 1-9 are functional in both human and mice, however TLR10 is likely non-functional in mice due to substitution of the C-terminal half of the mouse TLR10 gene with a non-productive sequence. In contrast, a stop codon in the human TLR11 gene, results in the gene not being expressed, while mouse TLR11 is functional and has a role in urogenital tract infections in particular (Zhang, Zhang et al. 2004). Stimulation of TLRs by their specific PAMPs initiates an intracellular signalling cascade involving numerous proteins, most notably MyD88. Activation of these signalling molecules ultimately leads to the activation of NFκB and other transcription factors to induce the production of inflammatory cytokines.

TLRs are type I transmembrane glycoproteins. The extracellular N-terminal end of all TLRs is composed of leucine-rich repeats (LRRs), which mediate PAMP binding and receptor dimerisation (Akira 2004). The LRR domain is composed of 19-25 tandem LRR motifs, of 24-29 amino acids in length (Matsushima, Tanaka et al. 2007). The mechanisms by which TLRs can differentiate between PAMPs or how any one TLR can respond to multiple PAMPs are only now being revealed. It has been suggested that specific ligand binding sites are created in each TLR by specific insertions of the PAMP into the LRR (Bell, Mullen et al. 2003). The conserved cytoplasmic region of each TLR is termed the Toll/IL-1 receptor (TIR) domain due its similarity to the cytoplasmic domains of the interleukin-1 receptor family. The TIR domain varies between 135 and 160 amino acids in length and functions as a binding site for downstream adaptor molecules (Akira and Takeda 2004).

TLRs can be characterised into two groups based on their cellular location and ligand specificity: plasma membrane localised TLRs - 1, 2, 4, 5 and 6 – that generally recognise lipid based PAMPs; TLRs localised to intracellular endosomes - 3, 7, 8 and 9 – that recognise nucleic acid based PAMPs. While the ligand(s) for TLR10 are unknown its localisation to the plasma membrane suggests these would be lipid based.

The most extensively studied member of the TLR family is TLR4. TLR4 is expressed on various haematopoietic cells including monocytes, macrophages, polymorphonuclear (PMN) cells, dendritic cells, and B cells. It is also expressed by non-haematopoietic cells including epithelial cells and fibroblasts (Ospelt and Gay 2010). TLR4 predominantly recognises lipopolysaccaride (LPS), a cell wall component of Gram-negative bacteria, composed of O-antigen, lipid A (endotoxin) and an oligosaccharide core (Hoshino, Takeuchi et al. 1999). TLR4 recognition of LPS requires formation of a complex with CD14 and MD2 (Shimazu, Akashi et al. 1999). MD2 binds to the extracellular region of TLR4, enabling it to bind to the lipid A component of LPS. CD14, a glycosyl phosphatidylinositol (GPI)-anchored, high affinity membrane protein, binds LPS in the presence of LPS-binding protein (LBP). LBP exchanges monomers of LPS for other lipids bound in its lipid binding site prior to transferring the LPS monomers to CD14. This enables CD14 to concentrate the LPS which is released from the bacterium in small amounts prior to presenting the LPS to the TLR4-MD2 complex (Lu, Yeh et al. 2008). TLR4 also has been implicated in the detection of fungal PAMPs including glucuronoxylomannan of *Cryptococcus neoformans,* and mannan derived from *Saccharomyces cerevisiae* and *Candida albicans* (Netea, Van Der Graaf et al. 2002).

TLR2, which has a similar expression profile to TLR4, recognises a variety of PAMPs from both Gram-positive and Gram-negative bacteria including lipoproteins/lipopeptides and peptidoglycan, glycolipids, lipoteichoic acid and non-endobacterial LPS (Takeuchi, Hoshino et al. 1999). TLR2 also recognises fungal PAMPs including cell surface phospholipomannan of *C. albicans,* and *S. cerevisiae* derived zymosan (Netea, Van Der Graaf et al. 2002; Netea, Van der Graaf et al. 2004). TLR2 forms a heterodimer with its structural relatives, TLR1 or TLR6 (Takeuchi and Akira; Takeuchi, Kawai et al. 1999). These heterodimers - TLR2/TLR1 and TLR2/TLR6 - can detect subtle variations in the lipid component of lipoproteins: tri-acetylated lipopeptides by TLR2/TLR1 and di-acetylated lipopeptides by TLR2/TLR6 (Takeuchi and Akira). Recognition of di-acetylated lipopeptides by TLR2/TLR6, is facilitated by the co-receptor CD36, a class II scavenger protein (Hoebe, Georgel et al. 2005). Expression patterns of TLR1 and TLR6 are similar to that of TLR2, although it has been shown that they are both highly expressed on B cells, while TLR2 is not (Ospelt and Gay 2010). However the regulation of TLR2 expression differs between various cell types depending on their specific function, which is not the case for TLR1 and TLR6 (Ospelt and Gay 2010).

TLR5 recognises bacterial flagellin, a structural protein which is the major component of flagella of Gram-negative bacteria (Hayashi, Smith et al. 2001). Recognition of flagellin by TLR5 is possibly via highly conserved regions in the flagellin protein (Hawn, Verbon et al. 2003). A common stop codon in TLR5 is associated with loss of signalling to flagellin and increased susceptibility to pneumonia caused by *Legionella pneumophila* (Hawn, Verbon et al. 2003). In addition to the conventional expression of TLR5 by haematopoietic cells, TLR5 is expressed on the basolateral surface of healthy human intestinal epithelium, suggesting that bacterial recognition by TLR5 only occurs as the bacteria invade across the epithelium (Gewirtz, Navas et al. 2001).

TLR3 is involved in the recognition of double-stranded RNA (dsRNA) from double-stranded viruses, such as reovirus, or that are generated during viral replication of single stranded viruses (Alexopoulou, Holt et al. 2001; Wang, Town et al. 2004). Multiple haematopoietic cells, such as dendritic cells, and non-haematopoietic cells, such as epithelial cells, express TLR3 which is located within endosomes. Recognition of dsRNA by TLR3 was first demonstrated using TLR3 deficient mice, which showed susceptibility to mouse cytomegalovirus (Tabeta, Georgel et al. 2004). The type I interferon (IFN) inducing dsRNA synthetic analog, polyinosine-deoxycytidylic acid (poly I:C) is often used *in vitro* to induce TLR3 activity (Alexopoulou, Holt et al. 2001).

TLR7 and TLR8 are structurally highly conserved and recognise uridine or guanosine-rich single stranded RNAs from a variety of viruses, including the influenza virus and human immunodeficienty virus (HIV) (Heil, Ahmad-Nejad et al. 2003; Heil, Hemmi et al. 2004; Lund, Alexopoulou et al. 2004). Additionally, they can recognise several synthetic imidazoquinoline-like molecules, such as resiquimod, which have potent antiviral activities due to their structural similarity to ribonucleic acids. While TLR7 and TLR8 recognise viral nucleic acid structures (Diebold, Kaisho et al. 2004; Heil, Hemmi et al. 2004) their expression within endosomes prohibits, under normal circumstances, interaction with host derived ssRNA.

TLR9 recognises the commonly found unmethylated CpG motifs of single-stranded DNA present in the genomes of many viruses and bacteria (Lund, Sato et al. 2003; Hochrein, Schlatter et al. 2004; Krug, Luker et al. 2004). DNA viruses shown to induce inflammatory cytokine and type 1 IFN production via TLR9, include Herpes simplex virus-1 (HSV-1), HSV-2 and murine cytomegalovirus (MCM). Two structurally different forms of CpG exist: A-type CpG oligodeoxynucleotides (ODNs) which stimulate plasmacytoid DCs (pDCs) to produce IL-12 and IFNα, and B-type CpG ODNs, which induce IL-6, IL-12 and TNFα production by pDCs. In addition B-type CpG ODNs also up-regulate expression of MHC Class II and the costimulatory molecules CD80 and CD86 on B cells and pDCs (Verthelyi, Ishii et al. 2001; Krug, Luker et al. 2004). TLR9 might also recognise host derived CpGs but these are weak inducers of activation due to the presence of highly methylated cytosine bases.

5.1.1 TLRs: recognition and response

Binding of PAMPs leads to the dimerisation of TLRs, which triggers the activation of the TLR mediated signalling pathways and the expression of various genes involved in the immune response. As previously described TLR2 forms a heterodimer with either TLR1 or TLR6, the remaining TLRs form homodimers (Saitoh, Akashi et al. 2004). TLR signalling originates from the TIR domain of the receptor, which associates with an adaptor containing a TIR domain. Various adaptors have been characterised including MyD88, TRIF, TRAM and TIRAP; each are involved in slightly different signalling pathways, dependent on which TLR is activated (Watters, Kenny et al. 2007). Two principle TLR signalling pathways have been described: the MyD88-dependent pathway and the MyD88-independent pathway also known as the TRIF-dependent pathway.

The adaptor MyD88, which has a C-terminal TIR domain in addition to an N-terminal death domain, associates with the TIR domain of the TLR. Upon activation of a TLR, MyD88

recruits IRAK-4, a member of the IL-1 receptor associated kinase (IRAK) family, via interactions between the death domains of both molecules. This enables activation of IRAK-1 by IRAK-4 mediated phosphorylation. IRAK-1 then associates with TRAF6 (TNF receptor associated factor 6), resulting in the eventual activation of the IκB kinase (IKK) complex, which consists of IKKα, IKKβ and NEMO/IKKγ. The IKK complex induces the phosphorylation of IκB, resulting in translocation of the nuclear transcription factor NF-κB, from the cytosol to the nucleus. Once in the nucleus NF-κB can induce expression of multiple inflammatory cytokines (Chen, Bhatia et al. 2006; Adhikari, Xu et al. 2007; Kawagoe, Sato et al. 2008). Alternatively, activation of TRAF6 can lead to the activation of MAP kinases, resulting in AP-1 transcription factor activation. As shown in MyD88-deficient mice, MyD88 is vital for signalling via this pathway. A second adaptor, TIRAP (TIR domain-containing adaptor protein)/Mal (MyD88-adaptor-like) has been shown to be associated with the MyD88-dependent pathway for signalling via TLR2 and TLR4 (Yamamoto, Sato et al. 2002).

Evidence for a My88-independent pathway was first shown in MyD88-deficient macrophages, in which NFκB activation was not observed but production of inflammatory cytokines was (Kawai, Adachi et al. 1999). Further investigation noted that TLR4 stimulation results in IRF3 activation, a transcription factor involved in the production of IFNα (Kawai, Takeuchi et al. 2001). Additionally IRF3 was activated in response to dsRNA or viral infection, via TLR3, suggesting that both TLR3 and TLR4 can utilise a common MyD88-independent pathway (Yoneyama, Suhara et al. 1998).

In addition to TIRAP/Mal, two other main adaptors have been identified as involved in the MyD88-independent pathway: TRIF (TIR domain containing adaptor inducing IFNα) also known as TICAM-1 (TIR domain containing molecule), and TRAM (TRIF-related adaptor molecule) or TICAM-2. TRIF has been associated with MyD88-independent signalling via TLR3, while TRIF and TRAM are associated with MyD88-independent signalling via TLR4 (Oshiumi, Matsumoto et al. 2003; Oshiumi, Sasai et al. 2003; Yamamoto, Sato et al. 2003). The TIR domain of TRIF is located in the centre of the molecule and the flanking N-terminal and C-terminal regions can both mediate the activation of NFκB but by two different actions: the N-terminal region associates with TRAF6, while the C-terminal region associates with RIP1 (receptor interacting protein) (Sato, Sugiyama et al. 2003; Meylan, Burns et al. 2004). Activation of the IFNα promoter is exclusively via the N-terminal region in association with the non-canonical IKKs, TBK1 and IKKi/IKKε, which mediate the phosphorylation and nuclear translocation of IRF3, resulting in the induction of IRF3 dependent IFNβ production.

5.1.2 TLRs: negative regulation

Excessive production of inflammatory cytokines in response to PAMPs by TLRs can lead to detrimental outcomes including sepsis and autoimmune disease. Thus negative regulatory mechanisms have developed in response to TLR-mediated signalling. Once such mechanism is the phenomenon known as tolerance, in which a subsequent challenge by a PAMP results in a reduced response. This was first documented in relation to LPS, however the exact mechanisms are not yet fully understood. A number of inhibitors of TLR signalling have also been described. One such molecule is IRAK-M. IRAK-M lacks kinase activity and prevents the dissociation of IRAK-1/IRAK4 from MyD88 which in turn prevents the IRAK-1/TRAF6 complex forming (Wesche, Gao et al. 1999; Kobayashi, Inohara et al. 2002).

Another inhibitor SOCS1 (suppressor of cytokine synthesis) has been shown to directly modulate TLR signalling (Yasukawa, Sasaki et al. 2000).

5.2 C-type Lectin Receptors (CLRs)

CLRs are a family of receptors that recognise and bind carbohydrates in a calcium-dependent manner. Binding is mediated via a conserved carbohydrate recognition domain (CRD) first identified on circulating mannose binding lectin (MBL). Other than MBL, CLRs are primarily expressed on antigen presenting cells (APCs) such as macrophages and dendritic cells, and are involved particularly in fungal recognition and modulation of the anti-fungal innate immune response. CLR intracellular signalling is activated via immunoreceptor tyrosine-based activation (ITAM)-like motifs present in the cytoplasmic tail of the receptor or on ITAM containing adaptors. Examples of such CLRs include; Dectin-1, Dectin-2, macrophage-inducible C-type lectin (MINCLE), DC-SIGN and the mannose receptor (Netea, Ferwerda et al. 2005).

Mannose binding lectin has two to six clusters of CRDs which facilitate the identification of and attachment to repetitive mannose and fucose residues found on various microorganisms. The fixed orientation of the CRDs requires not only the presence of these residues but their specific spatial arrangement to initiate interaction between MBL and the target micro-organism. Recognition of mannose and fucose by MBL leads to the activation of the lectin complement pathway and enhanced polymorphonuclear cell uptake (Ji, Gewurz et al. 2005; Ji, Olinger et al. 2005; van Asbeck, Hoepelman et al. 2008).

The mannose receptor (MR) has similar recognition strategies to MBL. Primarily expressed by macrophages and dendritic cells, the mannose receptor is a type-1 membrane protein with eight tandemly arranged CRD domains. These allow the recognition of various carbohydrates terminating in L-fucose, D-mannose and N-acetyl glucosamine (Taylor, Gordon et al. 2005). In addition to CRDs at the extracellular region, MR has two additional domains, an N-terminal cysteine-rich domain and a fibronectin II domain, these are involved in calcium-independent binding to sulphated sugars and collagen, respectively. Unlike the CRDs, these additional domains are only involved in the recognition of endogenous ligands and not those of microbial origin (Taylor, Gordon et al. 2005; Napper, Drickamer et al. 2006). The role of the MR in host defence remains a mystery: animal knockout models do not demonstrate an increased susceptibility to pathogens such as *Candida albicans* and mycobacteria which are known to contain MR ligands (Wojcikiewicz, Zhang et al. 2003; Appelmelk, den Dunnen et al. 2008).

The most widely studied CLR is Dectin-1. Dectin-1, also known as C-type lectin domain family 7 member A (CLEC7A), is a small (33kDa) type II glycosylated transmembrane receptor with an extracellular CRD connected to a cytoplasmic ITAM-like motif by a stalk domain. Two functional isoforms exist - Dectin-1A, and Dectin-1B which lacks the stalk domain, due to alternative splicing (Herre, Gordon et al. 2004). Dectin-1 expression in humans can be found on myeloid cells, including neutrophils, monocytes/macrophages and dendritic cells, however limited expression has been noted on other cell types (Herre, Gordon et al. 2004). Dectin-1 recognises specifically the glucose polymers β-1-3 and/or β-1-6-glucans primarily found in the cell walls of fungi, including *Candida albicans* and *Saccharomyces cerevisiae,* but which can also be found in the cell walls of some plants (Brown

and Gordon 2001). Upon binding of the appropriate PAMP, phosphorylation of Dectin-1 occurs by a non-receptor tyrosine kinase, Src, via interaction with its ITAM motif. This leads to the activation of another kinase, Syk, which induces the activation of the CARD9-Bc110-Malt1 adaptor complex leading ultimately to the activation of NFκB and the production of pro-inflammatory cytokines (Gross, Gewies et al. 2006). In addition, activation of Dectin-1 mediates the production of reactive oxygen species (ROS) and further modulation of cytokine expression via the NFAT pathway (Underhill, Rossnagle et al. 2005; Goodridge, Simmons et al. 2007). Attenuation of the Dectin-1 mediated pro-inflammatory response has been linked to internalisation of the receptor (Hernanz-Falcon, Joffre et al. 2009). It has been shown that signalling via Dectin-1 and TLR2/TLR6 combined enhances the response triggered by each receptor alone (Gantner, Simmons et al. 2003).

Similarly to Dectin-1, ITAM-dependent signalling via MINCLE leads to the activation of NFκB and the NFAT pathway, via recognition of numerous exogenous PAMPs and endogenous $DAMP_S$, including mycobacteria, *C. albicans* and necrotic cells (Yamasaki, Ishikawa et al. 2008). The identification of necrotic and damaged cells is mediated via recognition of splicesome-associated protein 130 (SAP130), secreted by these cells. MINCLE was the first known example of a CLR that can interact with PAMPs and DAMPs (Yamasaki, Ishikawa et al. 2008). MINCLE and another CLR, Galectin-3, have a role in anti-*Candida* defence, by recognition of β-mannose, resulting in MINCLE interacting with the Fc receptor common γ-chain (FcRγ), leading to Syk and CARD9-depedent activation of NFκB (Jouault, El Abed-El Behi et al. 2006; Yamasaki, Ishikawa et al. 2008). MINCLE also recognises the immunostimulatroy component of *Mycobacterium tuberculosis* - trehalose-6'6'-dimycolate better known as cord factor (Ishikawa, Ishikawa et al. 2009).

Other CLRs, such as DC-SIGN and Dectin-2, are involved in the recognition and uptake of *Candida albicans* by detecting highly mannose structures (McGreal, Rosas et al. 2006). DC-SIGN, expressed primarily on monocyte-derived dendritic cells, is a type II transmembrane receptor with only one C-type lectin domain. DC-SIGN also interacts with *Leishmania* but has gained increasing interest because of its involvement in the recognition of several viruses including human immunodeficiency virus (HIV)(Geijtenbeek, van Vliet et al. 2001; Cambi, Gijzen et al. 2003; Koppel, van Gisbergen et al. 2005). Dectin-2 which can be found on myeloid cells, has been suggested to have a role in hyphal recognition (Netea, Ferwerda et al. 2005). In response to *Candida albicans*, Dectin-2 induces the production of TNF, via interaction with FcγR (Nakahara, Nakagawa et al. 2006). Additionally, Dectin-2 has been implicated in Th17 inducing activity in response to *Candida* (Robinson, Osorio et al. 2009).

5.3 Nod-like Receptors (NLRs)

NLRs (also known as CATERPILLERs) consist of a large family of 23 human intracellular (cytosolic) PRRs, which recognise both PAMPs and/or DAMPs. Acitvation via NLRs leads to cytokine production via NFκB, or the inflammasome (see section 5.3.1) (Ting and Davis 2005; Kanneganti, Lamkanfi et al. 2007; Shaw, Reimer et al. 2008). NLRs have also been implicated in autophagy, a lysosomal degradation and cell death pathway that follows infection (Ting, Willingham et al. 2008). Structurally, NLRs are characterised by the presence of a trimodular structure: a central nucleotide binding domain (NBD) flanked by leucine rich repeats (LRRs) at the C-terminal and a protein binding domain - caspase activation and recruitment domain (CARD), baculovirus inhibitor of apoptosis protein repeat (BIR), death

effector domain (DED) or pyrin domain (PYD) - at the N-terminal. These N-terminal domains, also termed the effector region, are responsible for the protein-protein interactions needed to activate downstream signal transduction. NLRs can be categorised into subfamilies based on this effector domain including: NLRC (Nod-like receptor containing a CARD domain) and CIITA or NLRA (class II, major histocompatibility complex, transactivator) all contain CARD effector domains, while NLRPs contain a pyrin effector domain, and NAIPs or NLRBs contain three BIR domains (Kanneganti, Lamkanfi et al. 2007; Kumar, Kawai et al. 2009).

The first identified and most widely studied NLRs are NOD1 and NOD2. These belong to the NLRC subfamily and are highly expressed in monocytes, macrophages and dendritic cells, in addition to other hematopoietic cells and epithelial cells (Fritz, Ferrero et al. 2006). Both NOD1 and NOD2 recognise peptidoglycan (PGN) an essential building block of Gram-positive bacterial cell walls and to a lesser extent Gram-negative bacteria. PGN consists of glycan chains cross-linked via short peptides (Girardin, Travassos et al. 2003), NOD1 and NOD2 recognise different motifs in this structure: NOD2 recognises the conserved muramyl dipeptide (MDP) motif found in all PGNs (McDonald, Inohara et al. 2005), whereas NOD1 recognises D-γ-glutamyl-meso-DAP dipeptide (iE-DAP), which is present in all Gram-negative, but only some Gram-positive PGNs (Chamaillard, Hashimoto et al. 2003; Benko, Philpott et al. 2008). NOD2 can also recognise viral ssRNA and mycobacterial N-glycolylmuramyl dipeptides (Coulombe, Divangahi et al. 2009; Sabbah, Chang et al. 2009). Thus both NOD1 and NOD2 are involved in the recognition of a variety of pathogenic bacteria including: *E. coli*, *Chlamydia spp*, *Haemophilus influenza* by NOD1, and *M. tuberculosis* and *Streptococcus pneumonia* by NOD2 (Kumar, Kawai et al. 2009). Since the majority of these bacteria replicate outside of the cytoplasm where NOD1 and NOD2 are located, a mechanism by which PGN can cross the cell membrane to activate them is required. Although numerous transport proteins including PepT1, PepT2, and pannexin have been identified to facilitate MDP passage into the cytoplasm, an exact mechanism is not fully understood (Vavricka, Musch et al. 2004; Lee, Tattoli et al. 2009; Marina-Garcia, Franchi et al. 2009).

In general, activation of NOD1 and NOD2 by their respective ligands results in a conformational change allowing interaction with CARD domain containing receptor–interacting serine-threonine kinase 2 (RIP2) in a homophilic CARD-CARD manner. Cellular inhibitors of apoptosis 1 and 2 (clAP1 and 2) are also involved (Kobayashi, Inohara et al. 2002). RIP2 can then mediate the ubiquitination of NFκB essential modulator (NEMO)/IKKγ, subsequently leading to the activation of NFκB and the production of pro-inflammatory cytokines and antimicrobial peptides (Inohara, Koseki et al. 2000). Activation of NOD2 by MDP also can result in the activation of the mitogen activated protein kinase (MAPK) pathways via the adapter CARD9 (Kobayashi, Chamaillard et al. 2005).

5.3.1 The inflammasome

Certain members of the NLR family detect microbial components in the cytosol and trigger the assembly of a large caspase 1 activating complex termed the inflammasome. This complex supports the autocatalytic cleavage of caspase-1 which enables the processing and secretion of IL-1β and IL-18. NLRP1, NLRP3, NLRC4 and the adapter apoptosis-associated speck-like protein containing a CARD (ASC) are critical components of the inflammasome but emerging components include NLRP6 (Bauernfeind 2011, Ablasser et al.; Kersse 2011,

Bertrand et al.; Franchi, Eigenbrod et al. 2009). While much of the focus has been on the caspase-1 inflammasome, other caspases are also associated with an inflammasome-triggered response in a caspase-1-independent manner (Kayagaki, Warming et al. 2011).

The NLRP3 inflammasome is the most extensively studied: it comprises NLRP3, ASC, and caspase-1 (Martinon and Tschopp 2004). Like other NLRP family members, NLRP3 is composed of a C-terminal LRR domain, a central NOD domain and an N-terminal PYD domain. NLRP3 is expressed in many types of hematopoietic cells, in addition to osteoblasts, skin keratinocytes and transitional epithelium of the urinary tract (Elinav, Strowig et al. 2011). A wide variety of pathogens of bacterial, fungal and viral origin can initiate NLRP3 inflammasome formation: *Listeria monocytogenes*, *Staphylococcus aureus*, *C. albicans, Saccharomyces cerevisiae*, and adenovirus and influenza viruses. A number of host-derived DAMPs indicative of cellular injury, including extracellular ATP and uric acid among others, have been shown to activate the inflammasome (Mariathasan, Weiss et al. 2006; Gasse, Riteau et al. 2009). The mechanisms involved in the recognition of these stimuli by NLRP3 are currently not fully understood. Interaction of cellular mediators with a variety of PAMPs and DAMPs, ultimately relaying signals to NLRP3 has been postulated but physical interaction between NLRP3 and the PAMPs/DAMPs has not been ruled out (Marina-Garcia, Franchi et al. 2008). Following activation, NLRP3 oligomerisation leads to the clustering of PYD domains which can then recruit the CARD containing adaptor ASC which by a CARD-CARD interaction can then recruit pro-caspase-1. The clustering of pro-capase-1 results in its autocleavage to the active caspase-1 p10/p20 tetramer enabling the processing of cytokine proforms to yield mature molecules for secretion. Production of these proforms depends on NFκB driven transcriptional activity resulting from signalling from other PRRs such as NOD2 and TLRs (Schroder and Tschopp 2010).

Less is known about the NLRP1 inflammasome, which is comprised of ASC, caspase-1, caspase-5, and NLRP1 (Martinon and Tschopp 2004). NLRP1 is expressed in various haematopoietic cells including: T and B cells, monocytes, dendritic cells and granulocytes, on non-haematopoietic cells within the testes, and neurons (Elinav, Strowig et al. 2011). The structure of NLRP1 is different to that of NLRP3, as NLRP1 contains a C-terminal CARD domain, allowing NLRP1 to interact directly with pro-caspase-1. However ASC forms part of the inflammasome complex, allowing for the recruitment of caspase-5 providing additional inflammasome activity (Martinon and Tschopp 2004).

NLRC4 (also known as IPAF) is expressed primarily in lymphoid tissue. Like NOD1 and NOD2 it has a C-terminal LRR domain, a central NOD domain, and an N-terminal CARD domain (Hu, Elinav et al. 2010). Activation of the NLRC4 by microbial flagellin leads to the activation of caspase-1, IL-1β secretion and pyroptosis, a rapid form of cell death (Lightfield, Persson et al. 2008; Miao, Ernst et al. 2008). Similar to NLRP1, NLRC4 can interact directly with pro-caspase-1 via its CARD domain. The role of ASC in the NLRC4 inflammasome remains inconclusive: ASC cannot interact with NLRC4 which lacks a PYD domain but a role for ASC in regulation of this inflammasome has been suggested (Schroder and Tschopp 2010).

5.4 RIG-I like Receptors (RLRs)

The cytoplasmic RNA helicases that comprise the RIG-I like family of receptors play a major role in host anti-viral defence. RLRs include the highly characterised RIG-1 (retinoic acid-inducible gene I), MDA5 (melanoma differentiation associated factor 5) and LGP2

(laboratory of genetics and physiology 2). These are able to detect a variety of viral RNA ligands present in the cytoplasm and triggering the activation of transcription factors resulting in the production of type 1 interferons (IFN) in addition to expression of other anti-viral genes (Yoneyama and Fujita 2007). Viral recognition also occurs via TLR3. It seems that RIG-1 and MDA5 play a greater role in viral recognition by fibroblasts, macrophages and myeloid dendritic cells whereas TLR3 plays a more important role in viral recognition by plasmacytoid dendritic cells (Kato, Sato et al. 2005).

Both RIG-1 and MDA5 share a number of structural similarities. They contain a central DExD/H box RNA helicase domain flanked by an N-terminal of tandem CARD domains and a C-terminal domain (CTD). In the case of RIG-1 the CTD also contains a repressor domain (RD), involved in autoregulation, which is not present in the CTD of MDA5. Despite their similar structures, RIG-1 and MDA5 are able to detect distinct viral species: RIG-1 is involved in the recognition of Paramyxoviridae, Filoviridae and Rhabdoviridae among others, whereas MDA5 is important in the recognition of Picornaviruses (Loo and Gale 2011). More is known about RLR signalling in regard to RIG-1, however its believed that both RIG-1 and MDA5 share a common signalling pathway, involving the adaptor ISP-1 (Kawai, Takahashi et al. 2005). Prior to recognition of RNA by the RD or CTD region, RIG-1 is inactive in a ”closed” conformation, where the CARD domain is bound to the RD. Activation of RIG-1 results in a conformational change whereby CARD is released from the RD, allowing the CARD domain to interact with the adaptor ISP-1 (Loo and Gale 2011). ISP-1 can then initiate two distinct signalling routes resulting in the activation of various transcription factors including NFκB which induces the production of pro-inflammatory cytokines, and IRF3 and IRF7 which are responsible for the expression of type 1 IFNs (Kawai, Takahashi et al. 2005). Unlike RIG-1, MDA5 does not contain a RD to regulate its activation and when expressed ectopically signalling occurs in the absence of RNA recognition (Terness, Kallikourdis et al. 2007). Unlike RIG-I and MDA5, LGP2 lacks the N-terminal CARD domain, consisting only of the RNA helicase domain and the C-terminal domain containing an RD. It has been suggested that LGP2 is involved in the negative regulation of RIG-1 and possibly MDA5 (Loo and Gale 2011). This negative feedback is thought to take place on many levels including acting as a competitor for dsRNA, interaction with ISP-1, and maybe direct binding to RIG-1 via RD interactions (Terness, Kallikourdis et al. 2007).

5.5 Other pattern recognition receptors

Other less well characterised groups of pattern recognition receptors have also been described, including DAI (DNA dependent activator of IFN regulatory factors)-like receptors (DLRs) and scavenger receptors (SR). DAI (also known as DLM-1 and ZBP1) is the only DLR receptor to be described to date. It was first identified in mouse macrophages and mouse tumour stromal cells. DAI was up-regulated by exposure to cytosolic DNA or interferon gamma (IFNγ) suggesting that DAI functions as a DNA sensor (Fu, Comella et al. 1999; Ishii, Coban et al. 2006). A human homologue containing two N-terminal Zα domains and a C-terminal domain has since been described but it is of unknown function (Rothenburg, Schwartz et al. 2002). Activation of DAI by either microbial or host derived DNA, results in the activation of both the NFκB pathway and the IRF pathway (Takaoka and Taniguchi 2008).

Scavenger receptors (SRs) are a group of cell surface transmembrane receptors that are important in the clearance of several pathogens, host modified molecules, and apoptotic cells by endocytotic internalisation. These receptors also play a role in lipid metabolism. Expressed primarily on myeloid cells and some endothelial cells, both their expression and structure are regulated by various cytokines. Numerous SRs have been shown to play a role in innate immunity, including SR-A I, SR-A II, CD36, LOX-1, MARCO, SR-CL I, SR-CL II, SCARF1 and DSR-C1 (Peiser, Mukhopadhyay et al. 2002; Means, Mylonakis et al. 2009).

6. Pattern recognition receptors at the maternal-fetal interface

The discovery of an association between intrauterine infection, inflammation and certain adverse pregnancy outcomes has lead to increased interest in the innate immune response at the maternal-fetal interface (Goncalves, Chaiworapongsa et al. 2002). The production by gestation-associated tissues of cytokines in response to microbial products has been well documented. Since pattern recognition receptors are a key component of the innate immune response, linking infection by various microorganisms to the production of inflammatory mediators, a role for PRRs at the maternal–fetal interface has been postulated. To date studies have principally focused on the role of TLRs and most recently NLRs, primarily in the placenta and the trophoblast.

6.1 Placenta

Expression of transcripts for TLR 1-10 in the term placenta has been demonstrated with all but TLR9 shown to be functional (note that TLR10 function has not been studied due to the lack of an identified ligand). Changes were observed in both mRNA expression and functional cytokine outputs in response to labour at term (Patni, Wynen et al. 2009). TLR2 and TLR4 protein has been localised to term syncytiotrophoblast and intermediate trophoblast cells and both receptors are highly expressed in first trimester placental tissue (Holmlund, Cebers et al. 2002; Abrahams and Mor 2005). First trimester trophoblast cells also express TLR6. TLR6 blocks apoptosis induced by PGN via TLR1 and TLR2 and mediates NFκB activation and secretion of IL-6 and IL-8 leading to the postulate that TLR6 might regulate the balance of apoptosis and inflammation in response to Gram-positive infection (Abrahams, Aldo et al. 2008).

The NLRs, NOD1 and NOD2 are both expressed in the first trimester placenta where they are localised to the syncytiotrophoblast and cytotrophoblast. In contrast only NOD1 is expressed in term trophoblast cells. This corresponds to the functional outputs of first versus third trimester trophoblast cells; first trimester cells respond to both MDP and iE-DAP, and third trimester cells only respond to iE-DAP (Abrahams 2011; Cardenas, Mulla et al. 2011). The NOD1 ligand, iEDAP, can induce preterm delivery in a murine model. When lower doses that did not induce preterm delivery were used there was heightened inflammation at the materno-fetal interface and in the fetus itself (Cardenas, Mulla et al. 2011). Immunohistochemical studies have shown that the CLR DC-SIGN is expressed by fetal macrophages (Hofbauer cells) within the chorionic villi of the term placenta (Geijtenbeek, van Vliet et al. 2001). Viral ssRNA also activates cytokine, chemokine, and type I interferon production by primary first trimester trophoblast cells. This ligand also induces apoptosis in trophoblast cells in an IFNβ-dependent fashion (Aldo, Mulla et al. 2010).

6.2 Amnion

Amniotic epithelial cells represent the first line of defence against intra-amniotic infection. Earlier studies were restricted to TLR2 and TLR4 and found that both of these receptors are up-regulated in the amnion from women with chorioamnionitis compared to those without (Kim, Romero et al. 2004) although not all studies support this finding (Choi, Jung et al. 2011). Transcripts for TLRs 1-10 have been detected in human amniotic epithelial cells. However only TLR2/6, TLR4 and TLR5 have been reported to be functional: activation of TLR2/6 and TLR5 resulted in increased production of IL-6 and IL-8, while activation of TLR4 reduced cell viability via apoptosis (Gillaux, Mehats et al. 2011). Immunohistochemical studies of human fetal membranes have shown that the expression of TLR4 is greater in the chorion than the amnion, that expression decreases with gestational age but that expression does not differ by anatomic location within the uterus (Choi, Jung et al. 2011).

6.3 Decidua

Despite its juxtaposition to the myometrium the expression and function of PRRs within the maternally derived decidua is not studied extensively. Transcripts for TLRs 1-6 have been detected in term decidual cells but only TLR1, TLR2, TLR4 and TLR6 where shown to be functional via production of IL-8 in response to stimulation with LPS or PGN (Canavan and Simhan 2007). Immunohistochemical studies have demonstrated the expression of NOD1 and NOD2 in first trimester decidualised stroma (King, Horne et al. 2009). DC-SIGN has also been detected on decidual macrophages (Repnik, Tilburgs et al. 2008).

7. PRRs and adverse pregnancy outcomes

PRRs expressed at the maternal-fetal interface could play an important role in the pathogenesis of infection-associated preterm birth and other adverse pregnancy outcomes (Abrahams 2005a). This possibility has been studied mostly with regards to TLRs, especially TLR4. LPS from Gram-negative bacteria has been implicated in infection associated preterm birth and there have been a number of studies exploring the possible role of TLR4 in preterm labour. Functional TLR4 has been implicated in preterm labour triggered by administration of heat killed *E.coli* in mice (Wang and Hirsch 2003) . Evidence for a role for TLRs in infection-associated preterm birth also comes from genetic studies. A polymorphism (Asp299Gly) known to be associated with impaired TLR4 function and an increased likelihood of Gram-negative sepsis (Agnese DM 2002) was carried more often by preterm infants than term infants or by mothers delivering preterm than at term (Vacca, Cantoni et al. 2010). Genetic variation in TLRs is also associated with other adverse obstetric outcomes: *TLR4*, *TLR9* and *TLR1* but not *TLR2* variants are associated with placental malaria (low birth weight and maternal anaemia; no difference in parasite densities) (Hamann, Bedu-Addo et al. 2010). Other genetic variants are also associated with risk of PTB: maternal genetic variants in extracellular matrix metabolism with risk of PPROM and fetal genetic variation (e.g. IL-6R1) are associated with risk of PTB (Romero, Friel et al. 2010).

While LPS has been used as the model for many years it is worth noting that LPS from different species of bacteria might differentially regulate inflammatory responses from gestation-associated tissues such as the amniochorion (Chang, Jain et al. 2010). Also tissue

processing for ex vivo investigations can impact on cytokine measurements. For example, in a comparison of punch biopsies of amnion or choriodecidua versus dual compartment transwells, the punch biopsies typically made greater amounts of cytokines (Miller 2010. and Loch-Caruso). Such observations highlight the need to take these factors into account when designing studies and when comparing data from different studies.

In addition to the direct pro-inflammatory effects initiated by exposure to infection, disturbances in the regulation of apoptosis might also be associated with sub-optimal pregnancy outcome (Jerzak and Bischof 2002). Increased trophoblast apoptosis is seen during the first trimester of pregnancies complicated with IUGR or preeclampsia (Saito, Kato et al. 1992; Smith, Baker et al. 1997), and elevated trophoblast apoptosis has been observed in preterm births (Kakinuma, Kuwayama et al. 1997; Balkundi, Ziegler et al. 2003). It has been suggested that the direct or indirect effects of infectious microorganisms upon trophoblast cell survival might depend upon which TLRs are activated with TLR2-mediated events presumably favouring apoptosis.

Recently there has been growing interest in how a viral infection might itself cause preterm birth and also how it might increase the risk of pregnancy failure during subsequent or concurrent bacterial infection. In a murine model, intraperitoneal injection of a synthetic TLR3 ligand, poly I:C, caused preterm delivery within 24 hours. This was associated with inflammation in multiple gestation-associated tissues (polymorphonuclear cell infiltrate, necrosis and haemorrhage), infiltration of NK cells and macrophages into the placenta, and placental cytokine (e.g. IL-6) and chemokine (e.g. MCP-1) production that could also be detected systemically. This did not occur in TLR3 knock out animals. The cytokine response could be replicated *in vitro* by polyI:C treatment of primary murine trophoblast and a human trophoblast cell line and involved activation of NFκB (Koga, Cardenas et al. 2009). In contrast, intra-peritoneal injection of murine herpes virus was associated with evidence of inflammation in the placenta and spleen but no adverse pregnancy outcomes. Evidence of viral infection in the placenta and decidua but not the fetus led the investigators to postulate that these tissues act as a barrier to capture virus and prevent infection of the fetus. However, this might not prevent developmental impacts on the fetus. Human primary first trimester trophoblast also can be infected with herpes virus *in vitro* but unlike the response to poly I:C treatment, cytokine and chemokine production tended to be down-regulated. Viral infection but not polyI:C induced increased expression of TLR2 and TLR4 in human trophoblast cells and in the accompanying mouse model, viral infection sensitised for a response – preterm delivery in less than 24hrs in all mice accompanied by 100% fetal death - to intraperitoneal infection of LPS (Cardenas 2010, 2011 respectively, Means et al.; Cardenas 2010, 2011 respectively, Mor et al.).

These observations have highlighted a need for better understanding of the expression and activity of viral detecting PRRs at the materno-fetal interface. Although the focus of this chapter is the placenta and attached membranes it is also worth noting that there is incredible interest in how anti-viral responses by women are affected by pregnancy.

Preeclampsia, a pregnancy specific hypertensive disorder, also is characterised by inflammation. Pathways related to stress, inflammation (including TLR signalling pathways), growth, tissue remodelling, and metabolism are all altered during preeclampsia (Sado, Naruse et al. 2011). The differences might reflect acute inflammation secondary to microbial infection versus chronic inflammation secondary to oxidative stress. Possible

involvement of the inflammasome in preeclampsia has been suggested. Uric acid is known to activate the inflammasome: circulating uric acid levels increase prior to clinical manifestations of preeclampsia and levels relate to disease severity. Components of the inflammasome, including ASC, are expressed in first and third trimester trophoblasts and monosodium urate up-regulates IL-1β production in an inflammasome-dependent manner (Mulla, Myrtolli et al. 2011). There is also interest in the potential role of viral PAMPs or related DAMPs from necrotic cells in preeclampsia. Activation via TLR3 or the RLRs RIG-1 and MDA-5 leading to downstream inflammation, anti-angiogenesis and oxidative stress converging on endothelial dysfunction has been postulated (Chatterjee, Weaver et al. 2011).

8. Regulating the inflammatory response at the materno-fetal interface

The resolution of inflammation is essential for immune homeostasis. It has become apparent that there are intracellular stress proteins that have extracellular properties related to the regulation of the innate immune response and inflammation. These so-called RAMPs (resolution-associated molecular patterns) (Shields, Panayi et al. 2011) have anti-inflammatory activity or the ability to resolve inflammation and counterbalance the activity of PAMPs and DAMPs. There is keen interest in the potential therapeutic use of these.

There are also a number of cytokines well recognised for their anti-inflammatory activity. These include IL-4, IL-10 and IL-13. IL-10 can down-regulate LPS- and LTA-induced cytokine/chemokine responses by the healthy term placenta (Bayraktar, Peltier et al. 2009). Paradoxically, IL-10 is increased in amniotic fluid from women in term labour and women with intra-amniotic infection at term and preterm, and is also increased in those without infection who delivered preterm rather than term (Gotsch, Romero et al. 2008). Elevated IL-10 in these circumstances might represent a compensatory mechanism that has failed. Similarly, an anti-inflammatory cytokine (IL-4, IL-10 and IL-13) bias within the cervix prior to 16 weeks of gestation might identify those women most likely to suffer microbial invasion of the utero-placental unit and then spontaneous preterm labour and delivery (Simhan, Bodnar et al. 2011). Whether the greater anti-inflammatory milieu permits ascending infection or is a compensatory response to a pro-inflammatory response to existing infection that when no longer controlled tips in favour of the pro-inflammatory response and the initiation of labour remains to be determined.

There is also a need to better understand the bioactivity of key cytokines at the materno-fetal interface. The biological effect of any cytokine depends on the expression of cognate receptors on target cells and this can be modified by the presence of soluble receptors, macromolecules that bind the cytokine, and the relative abundance of other cytokines. IL-6 provides a relevant example. IL-6 is a multi-functional cytokine: while its pro- and anti-inflammatory properties are of particular interest in the context of this review it is also involved in the acute phase reaction and regulates haematopoiesis. Classic signaling by IL-6 is mediated via interaction with the non-signaling transmembrane IL-6 receptor followed by homodimerisation of signalling gp130. IL-6R-negative cells can utilize IL-6 *trans*-signaling via IL-6 binding to soluble IL-6R (sIL-6R) for interaction with membrane expressed gp130. The presence of soluble gp130 (sgp130) inhibits *trans*-signaling by offering an alternative binding site for IL-6/sIL-6R thereby prohibiting interaction with the membrane bound form of gp130 and downstream intracellular signalling. Although sgp130 cannot bind directly to IL-6 it has been shown recently to also inhibit classic signaling depending on the ratio of IL-

6 and sIL-6R (Garbers, Thaiss et al. 2011). Human amniotic fluid contains IL-6, sIL-6R and sgp130 with levels of sgp130 declining with increasing gestational age. Amniotic fluid IL-6 and sIL-6R are increased when there is evidence of intraamniotic infection: the fetal membranes are a key source of these mediators. Increased IL-6R, either membrane or soluble, combined with decreased sgp130 in intraamniotic infection would favour IL-6 pro-inflammatory signalling. Indeed, sgp130 but not sIL-6R modulates LPS-mediated MMP9 production by fetal membranes (Lee, Buhimschi et al. 2011). Reduced IL-6 *trans*-signalling mainly due to reduced circulating soluble gp130 also has been implicated in recurrent spontaneous abortion via a postulated role in modulating activity of regulatory T ell at materno-fetal interface (Arruvito, Billordo et al. 2009).

9. Conclusion

The study of PRR-mediated inflammation at the maternal-fetal interface has only just begun. A better understanding of these receptors and the signal transduction cascades they initiate might explain why some pregnancies are complicated by PTL and PPROM whereas others are only affected by PPROM. Moreover, investigations into the endogenous activators of PRRs might explain how PTL and PPROM can occur in the absence of infection (e.g. preeclampsia, multiple gestation, teenage pregnancy, or excessive tobacco and alcohol consumption). These molecules (either the receptors or their signalling molecules) might therefore be excellent targets for therapeutic strategies because they are upstream effectors of the pro-inflammatory cascade that ultimately results in premature labour and preterm birth.

10. References

Aboussahoud, W., C. Bruce, et al. (2010). "Activation of Toll-like receptor 5 decreases the attachment of human trophoblast cells to endometrial cells in vitro." *Hum Reprod* 25(9): 2217-2228.

Abrahams, V. M. (2005a). "Toll-like receptors in the cycling female reproductive tract and during pregnancy." *Current Women's Health Review* 1: 35-42.

Abrahams, V. M. (2011). "The role of the Nod-like receptor family in trophoblast innate immune responses." *J Reprod Immunol* 88(2): 112-7.

Abrahams, V. M., P. B. Aldo, et al. (2008). "TLR6 Modulates First Trimester Trophoblast Responses to Peptidoglycan." *J Immunol* 180(9): 6035-6043.

Abrahams, V. M. and G. Mor (2005). "Toll-like receptors and their role in the trophoblast." *Placenta* 26(7): 540-7.

Adhikari, A., M. Xu, et al. (2007). "Ubiquitin-mediated activation of TAK1 and IKK." *Oncogene* 26(22): 3214-26.

Agnese DM, C. J., Hahm SJ, Coyle SM, Corbett SA, Calvano SE, Lowry SF. (2002). "Human toll-like receptor 4 mutations but not CD14 polymorphisms are associated with an increased risk of gram-negative infections." *J Infect Dis.* Nov 15;186(10):1522-5.

Akira, S. (2004). "Toll receptor families: structure and function." *Semin Immunol* 16(1): 1-2.

Akira, S. and K. Takeda (2004). "Toll-like receptor signalling." *Nat Rev Immunol* 4(7): 499-511.

Aldo, P. B., M. J. Mulla, et al. (2010). "Viral ssRNA Induces First Trimester Trophoblast Apoptosis through an Inflammatory Mechanism." *Am J Reprod Immunol* 64(1): 27-37.

Alexopoulou, L., A. C. Holt, et al. (2001). "Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3." *Nature* 413(6857): 732-8.

Appelmelk, B. J., J. den Dunnen, et al. (2008). "The mannose cap of mycobacterial lipoarabinomannan does not dominate the Mycobacterium-host interaction." *Cell Microbiol* 10(4): 930-44.

Arechavaleta-Velasco, F., H. Koi, et al. (2002). "Viral infection of the trophoblast: time to take a serious look at its role in abnormal implantation and placentation?" *J Reprod Immunol* 55(1-2): 113-21.

Arruvito, L., A. Billordo, et al. (2009). "IL-6 trans-signaling and the frequency of CD4+FOXP3+ cells in women with reproductive failure." *J Reprod Immunol* 82(2): 158-165.

Balkundi, D. R., J. A. Ziegler, et al. (2003). "Regulation of FasL/Fas in human trophoblasts: possible implications for chorioamnionitis." *Biol Reprod* 69(2): 718-24.

Bauernfeind, F., A. Ablasser, et al. (2011). "Inflammasomes: current understanding and open questions." *Cell Mol Life Sci* 68(5): 765-783.

Bayraktar, M., M. Peltier, et al. (2009). "IL-10 Modulates Placental Responses to TLR Ligands." *Am J Reprod Immunol* 62(6): 390-399.

Bearfield, C., E. S. Davenport, et al. (2002). "Possible association between amniotic fluid micro-organism infection and microflora in the mouth." *BJOG* 109(5): 527-33.

Beck, S., D. Wojdyla, et al. (2010). "The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity." *Bull World Health Organ* 88(1): 31-8.

Bell, J. K., G. E. Mullen, et al. (2003). "Leucine-rich repeats and pathogen recognition in Toll-like receptors." *Trends Immunol* 24(10): 528-33.

Benko, S., D. J. Philpott, et al. (2008). "The microbial and danger signals that activate Nod-like receptors." *Cytokine* 43(3): 368-73.

Bianchi, M. E. (2007). "DAMPs, PAMPs and alarmins: all we need to know about danger." *J Leukoc Biol* 81(1): 1-5.

Bowen, J. M., L. Chamley, et al. (2002). "Cytokines of the placenta and extra-placental membranes: roles and regulation during human pregnancy and parturition." *Placenta* 23(4): 257-73.

Brown, G. D. and S. Gordon (2001). "Immune recognition. A new receptor for beta-glucans." *Nature* 413(6851): 36-7.

Buhimschi, C. S., V. Bhandari, et al. (2007). "Proteomic Profiling of the Amniotic Fluid to Detect Inflammation, Infection, and Neonatal Sepsis." *PLoS Med* 4(1): e18.

Cambi, A., K. Gijzen, et al. (2003). "The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for Candida albicans on dendritic cells." *Eur J Immunol* 33(2): 532-8.

Canavan, T. P. and H. N. Simhan (2007). "Innate immune function of the human decidual cell at the maternal-fetal interface." *J Reprod Immunol* 74(1-2): 46-52.

Cardenas, I., R. E. Means, et al. (2010). "Viral Infection of the Placenta Leads to Fetal Inflammation and Sensitization to Bacterial Products Predisposing to Preterm Labor." *J Immunol* 185(2): 1248-1257.

Cardenas, I., G. Mor, et al. (2011). "Placental Viral Infection Sensitizes to Endotoxin-Induced Pre-Term Labor: A Double Hit Hypothesis." *Am J Reprod Immunol* 65(2): 110-117.

Cardenas, I., M. J. Mulla, et al. (2011). "Nod1 activation by bacterial iE-DAP induces maternal-fetal inflammation and preterm labor." *J Immunol* 187(2): 980-6.

Chamaillard, M., M. Hashimoto, et al. (2003). "An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid." *Nat Immunol* 4(7): 702-7.

Chang, J., S. Jain, et al. (2010). "Differential host response to LPS variants in amniochorion and the TLR4/MD-2 system in Macaca nemestrina." *Placenta* 31(9): 811-817.

Chatterjee, P., L. E. Weaver, et al. (2011). "Do double-stranded RNA receptors play a role in preeclampsia?" *Placenta* 32(3): 201-205.

Chen, F., D. Bhatia, et al. (2006). "Finding NEMO by K63-linked polyubiquitin chain." *Cell Death Differ* 13(11): 1835-8.

Choi, S. J., S. H. Jung, et al. (2011). "Immunohistochemical distribution of toll-like receptor 4 in preterm human fetal membrane." *J Obstet Gynaecol Res*.

Coulombe, F., M. Divangahi, et al. (2009). "Increased NOD2-mediated recognition of N-glycolyl muramyl dipeptide." *J Exp Med* 206(8): 1709-16.

Diebold, S. S., T. Kaisho, et al. (2004). "Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA." *Science* 303(5663): 1529-31.

Elinav, E., T. Strowig, et al. (2011). "Regulation of the antimicrobial response by NLR proteins." *Immunity* 34(5): 665-79.

Elovitz, M. A. and C. Mrinalini (2004). "Animal models of preterm birth." *Trends Endocrinol Metab* 15(10): 479-87.

Franchi, L., T. Eigenbrod, et al. (2009). "The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis." *Nat Immunol* 10(3): 241-247.

Friebe-Hoffmann, U., J. P. Chiao, et al. (2001). "Effect of IL-1beta and IL-6 on oxytocin secretion in human uterine smooth muscle cells." *Am J Reprod Immunol* 46(3): 226-31.

Fritz, J. H., R. L. Ferrero, et al. (2006). "Nod-like proteins in immunity, inflammation and disease." *Nat Immunol* 7(12): 1250-7.

Fu, Y., N. Comella, et al. (1999). "Cloning of DLM-1, a novel gene that is up-regulated in activated macrophages, using RNA differential display." *Gene* 240(1): 157-63.

Gantner, B. N., R. M. Simmons, et al. (2003). "Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2." *J Exp Med* 197(9): 1107-17.

Garbers, C., W. Thaiss, et al. (2011). "Inhibition of classic signaling is a novel function of soluble GP130 which is controlled by the ratio of interleukin 6 and soluble interleukin 6 receptor." *J Biol Chem* 286(50):42959-70.

Gasse, P., N. Riteau, et al. (2009). "Uric acid is a danger signal activating NALP3 inflammasome in lung injury inflammation and fibrosis." *Am J Respir Crit Care Med* 179(10): 903-13.

Geijtenbeek, T. B., S. J. van Vliet, et al. (2001). "DC-SIGN, a dentritic cell-specific HIV-1 receptor present in placenta that infects T cells in trans-a review." *Placenta* 22 Suppl A: S19-23.

Gewirtz, A. T., T. A. Navas, et al. (2001). "Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression." *J Immunol* 167(4): 1882-5.

Gibbs, R. S., R. Romero, et al. (1992). "A review of premature birth and subclinical infection." *Am J Obstet Gynecol* 166(5): 1515-28.

Gillaux, C., C. Mehats, et al. (2011). "Functional screening of TLRs in human amniotic epithelial cells." *J Immunol* 187(5): 2766-74.

Girardin, S. E., L. H. Travassos, et al. (2003). "Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2." *J Biol Chem* 278(43): 41702-8.

Goldenberg, R. L., J. F. Culhane, et al. (2008). "Epidemiology and causes of preterm birth." *Lancet* 371(9606): 75-84.

Goldenberg, R. L., J. C. Hauth, et al. (2000). "Intrauterine infection and preterm delivery." *N Engl J Med* 342(20): 1500-7.

Goncalves, L. F., T. Chaiworapongsa, et al. (2002). "Intrauterine infection and prematurity." *Ment Retard Dev Disabil Res Rev* 8(1): 3-13.

Goodridge, H. S., R. M. Simmons, et al. (2007). "Dectin-1 stimulation by Candida albicans yeast or zymosan triggers NFAT activation in macrophages and dendritic cells." *J Immunol* 178(5): 3107-15.

Gotsch, F., R. Romero, et al. (2008). "The anti-inflammatory limb of the immune response in preterm labor, intra-amniotic infection/inflammation, and spontaneous parturition at term: A role for interleukin-10." *J Matern Fetal Neonatal Med* 21(8): 529-547.

Gotsch, F., R. Romero, et al. (2008). "The anti-inflammatory limb of the immune response in preterm labor, intra-amniotic infection/inflammation, and spontaneous parturition at term: a role for interleukin-10." *J Matern Fetal Neonatal Med* 21(8): 529-47.

Gravett, M. G., A. Thomas, et al. (2007). "Proteomic analysis of cervical-vaginal fluid: identification of novel biomarkers for detection of intra-amniotic infection." *J Proteome Res* 6(1): 89-96.

Gross, O., A. Gewies, et al. (2006). "Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity." *Nature* 442(7103): 651-6.

Hamann, L., G. Bedu-Addo, et al. (2010). "The toll-like receptor 1 variant S248N influences placental malaria." *Infection, Genetics and Evolution* 10(6): 785-789.

Hamilton, B. E., J. A. Martin, et al. (2006). "Births: preliminary data for 2005." *Natl Vital Stat Rep* 55(11): 1-18.

Hawn, T. R., A. Verbon, et al. (2003). "A Common Dominant TLR5 Stop Codon Polymorphism Abolishes Flagellin Signaling and Is Associated with Susceptibility to Legionnaires' Disease." *J Exp Med* 198(10): 1563-1572.

Hayashi, F., K. D. Smith, et al. (2001). "The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5." *Nature* 410(6832): 1099-103.

Heil, F., P. Ahmad-Nejad, et al. (2003). "The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily." *Eur J Immunol* 33(11): 2987-97.

Heil, F., H. Hemmi, et al. (2004). "Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8." *Science* 303(5663): 1526-9.

Hendricks, C. H., W. E. Brenner, et al. (1970). "Normal cervical dilatation pattern in late pregnancy and labor." *Am J Obstet Gynecol* 106(7): 1065-82.

Hernanz-Falcon, P., O. Joffre, et al. (2009). "Internalization of Dectin-1 terminates induction of inflammatory responses." *Eur J Immunol* 39(2): 507-13.

Herre, J., S. Gordon, et al. (2004). "Dectin-1 and its role in the recognition of beta-glucans by macrophages." *Mol Immunol* 40(12): 869-76.

Hochrein, H., B. Schlatter, et al. (2004). "Herpes simplex virus type-1 induces IFN-alpha production via Toll-like receptor 9-dependent and -independent pathways." *Proc Natl Acad Sci U S A* 101(31): 11416-21.

Hoebe, K., P. Georgel, et al. (2005). "CD36 is a sensor of diacylglycerides." *Nature* 433(7025): 523-7.

Holmlund, U., G. Cebers, et al. (2002). "Expression and regulation of the pattern recognition receptors Toll-like receptor-2 and Toll-like receptor-4 in the human placenta." *Immunology* 107(1): 145-51.

Holt, R., B. C. Timmons, et al. (2011). "The Molecular Mechanisms of Cervical Ripening Differ between Term and Preterm Birth." *Endocrinology* 152(3): 1036-1046.

Hoshino, K., O. Takeuchi, et al. (1999). "Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product." *J Immunol* 162(7): 3749-52.

Hsu, C. D. and F. R. Witter (1995). "Urogenital infection in preeclampsia." *Int J Gynaecol Obstet* 49(3): 271-5.

Hu, B., E. Elinav, et al. (2010). "Inflammation-induced tumorigenesis in the colon is regulated by caspase-1 and NLRC4." *Proc Natl Acad Sci U S A* 107(50): 21635-40.

Inohara, N., T. Koseki, et al. (2000). "An induced proximity model for NF-kappa B activation in the Nod1/RICK and RIP signaling pathways." *J Biol Chem* 275(36): 27823-31.

Ishii, K. J., C. Coban, et al. (2006). "A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA." *Nat Immunol* 7(1): 40-8.

Ishii, K. J., S. Koyama, et al. (2008). "Host innate immune receptors and beyond: making sense of microbial infections." *Cell Host Microbe* 3(6): 352-63.

Ishikawa, E., T. Ishikawa, et al. (2009). "Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle." *J Exp Med* 206(13): 2879-88.

Janeway, C. A., Jr. and R. Medzhitov (2002). "Innate immune recognition." *Annu Rev Immunol* 20: 197-216.

Jerzak, M. and P. Bischof (2002). "Apoptosis in the first trimester human placenta: the role in maintaining immune privilege at the maternal-foetal interface and in the trophoblast remodelling." *Eur J Obstet Gynecol Reprod Biol* 100(2): 138-42.

Ji, X., H. Gewurz, et al. (2005). "Mannose binding lectin (MBL) and HIV." *Mol Immunol* 42(2): 145-52.

Ji, X., G. G. Olinger, et al. (2005). "Mannose-binding lectin binds to Ebola and Marburg envelope glycoproteins, resulting in blocking of virus interaction with DC-SIGN and complement-mediated virus neutralization." *J Gen Virol* 86(Pt 9): 2535-42.

Jouault, T., M. El Abed-El Behi, et al. (2006). "Specific recognition of Candida albicans by macrophages requires galectin-3 to discriminate Saccharomyces cerevisiae and needs association with TLR2 for signaling." *J Immunol* 177(7): 4679-87.

Kakinuma, C., C. Kuwayama, et al. (1997). "Trophoblastic apoptosis in mice with preterm delivery and its suppression by urinary trypsin inhibitor." *Obstet Gynecol* 90(1): 117-24.

Kanneganti, T. D., M. Lamkanfi, et al. (2007). "Intracellular NOD-like receptors in host defense and disease." *Immunity* 27(4): 549-59.

Kato, H., S. Sato, et al. (2005). "Cell type-specific involvement of RIG-I in antiviral response." *Immunity* 23(1): 19-28.

Kawagoe, T., S. Sato, et al. (2008). "Sequential control of Toll-like receptor-dependent responses by IRAK1 and IRAK2." *Nat Immunol* 9(6): 684-91.

Kawai, T., O. Adachi, et al. (1999). "Unresponsiveness of MyD88-deficient mice to endotoxin." *Immunity* 11(1): 115-22.

Kawai, T., K. Takahashi, et al. (2005). "IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction." *Nat Immunol* 6(10): 981-8.

Kawai, T., O. Takeuchi, et al. (2001). "Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes." *J Immunol* 167(10): 5887-94.

Kayagaki, N., S. Warming, et al. (2011). "Non-canonical inflammasome activation targets caspase-11." *Nature* 479(7371): 117-121.

Kersse, K., M. J. M. Bertrand, et al. (2011). "NOD-like receptors and the innate immune system: Coping with danger, damage and death." *Cytokine Growth Factor Rev 22(5-6):257-76*.

Kim, C. J., R. Romero, et al. (2010). "The frequency, clinical significance, and pathological features of chronic chorioamnionitis: a lesion associated with spontaneous preterm birth." *Mod Pathol* 23(7): 1000-1011.

Kim, M. J., R. Romero, et al. (2009). "Widespread microbial invasion of the chorioamniotic membranes is a consequence and not a cause of intra-amniotic infection." *Lab Invest* 89(8): 924-36.

Kim, Y. M., R. Romero, et al. (2004). "Toll-like receptor-2 and -4 in the chorioamniotic membranes in spontaneous labor at term and in preterm parturition that are associated with chorioamnionitis." *Am J Obstet Gynecol* 191(4): 1346-55.

King, A. E., A. W. Horne, et al. (2009). "Differential expression and regulation of nuclear oligomerization domain proteins NOD1 and NOD2 in human endometrium: a potential role in innate immune protection and menstruation." *Mol Hum Reprod* 15(5): 311-9.

Kobayashi, K., N. Inohara, et al. (2002). "RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems." *Nature* 416(6877): 194-9.

Kobayashi, K. S., M. Chamaillard, et al. (2005). "Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract." *Science* 307(5710): 731-4.

Koga, K., I. Cardenas, et al. (2009). "Activation of TLR3 in the Trophoblast is Associated with Preterm Delivery." *Am J Reprod Immunol* 61(3): 196-212.

Koppel, E. A., K. P. van Gisbergen, et al. (2005). "Distinct functions of DC-SIGN and its homologues L-SIGN (DC-SIGNR) and mSIGNR1 in pathogen recognition and immune regulation." *Cell Microbiol* 7(2): 157-65.

Krug, A., G. D. Luker, et al. (2004). "Herpes simplex virus type 1 activates murine natural interferon-producing cells through toll-like receptor 9." *Blood* 103(4): 1433-7.

Kumar, H., T. Kawai, et al. (2009). "Pathogen recognition in the innate immune response." *Biochem J* 420(1): 1-16.

Kumazaki, K., M. Nakayama, et al. (2004). "Immunohistochemical distribution of Toll-like receptor 4 in term and preterm human placentas from normal and complicated pregnancy including chorioamnionitis." *Hum Pathol* 35(1): 47-54.

Laham, N., S. P. Brennecke, et al. (1996a). "Differential release of interleukin-6 from human gestational tissues in association with labour and in vitro endotoxin treatment." *J Endocrinol* 149(3): 431-9.

Laham, N., S. P. Brennecke, et al. (1996b). "Labour-associated increase in interleukin-1 alpha release in vitro by human gestational tissues." *J Endocrinol* 150(3): 515-22.

Laham, N., S. P. Brennecke, et al. (1999). "Interleukin-8 release from human gestational tissue explants: effects of gestation, labor, and chorioamnionitis." *Biol Reprod* 61(3): 823-7.

Lee, J., R. Romero, et al. (2011). "A Signature of Maternal Anti-Fetal Rejection in Spontaneous Preterm Birth: Chronic Chorioamnionitis, Anti-Human Leukocyte Antigen Antibodies, and C4d." *PLoS ONE* 6(2): e16806.

Lee, J., I. Tattoli, et al. (2009). "pH-dependent internalization of muramyl peptides from early endosomes enables Nod1 and Nod2 signaling." *J Biol Chem* 284(35): 23818-29.

Lee, S. Y., I. A. Buhimschi, et al. (2011) "IL-6 Trans-Signaling System in Intra-Amniotic Inflammation, Preterm Birth, and Preterm Premature Rupture of the Membranes." *J Immunol* 186(5): 3226-3236.

Lemaitre, B., E. Nicolas, et al. (1996). "The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults." *Cell* 86(6): 973-83.

Leppert, P. C. (1995). "Anatomy and physiology of cervical ripening." *Clin Obstet Gynecol* 38(2): 267-79.

Liggins, G. C., R. J. Fairclough, et al. (1973). "The mechanism of initiation of parturition in the ewe." *Recent Prog Horm Res* 29: 111-59.

Lightfield, K. L., J. Persson, et al. (2008). "Critical function for Naip5 in inflammasome activation by a conserved carboxy-terminal domain of flagellin." *Nat Immunol* 9(10): 1171-8.

Lockwood, C. J., A. E. Senyei, et al. (1991). "Fetal fibronectin in cervical and vaginal secretions as a predictor of preterm delivery." *N Engl J Med* 325(10): 669-74.

Loo, Y. M. and M. Gale, Jr. (2011). "Immune signaling by RIG-I-like receptors." *Immunity* 34(5): 680-92.

Lu, Y.-C., W.-C. Yeh, et al. (2008). "LPS/TLR4 signal transduction pathway." *Cytokine* 42(2): 145-151.

Lund, J., A. Sato, et al. (2003). "Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells." *J Exp Med* 198(3): 513-20.

Lund, J. M., L. Alexopoulou, et al. (2004). "Recognition of single-stranded RNA viruses by Toll-like receptor 7." *Proc Natl Acad Sci U S A* 101(15): 5598-603.

Mangham, L. J., S. Petrou, et al. (2009). "The cost of preterm birth throughout childhood in England and Wales." *Pediatrics* 123(2): e312-27.

Mariathasan, S., D. S. Weiss, et al. (2006). "Cryopyrin activates the inflammasome in response to toxins and ATP." *Nature* 440(7081): 228-32.

Marina-Garcia, N., L. Franchi, et al. (2009). "Clathrin- and dynamin-dependent endocytic pathway regulates muramyl dipeptide internalization and NOD2 activation." *J Immunol* 182(7): 4321-7.

Marina-Garcia, N., L. Franchi, et al. (2008). "Pannexin-1-mediated intracellular delivery of muramyl dipeptide induces caspase-1 activation via cryopyrin/NLRP3 independently of Nod2." *J Immunol* 180(6): 4050-7.

Martinon, F. and J. Tschopp (2004). "Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases." *Cell* 117(5): 561-74.

Mathews, R. J., M. B. Sprakes, et al. (2008). "NOD-like receptors and inflammation." *Arthritis Res Ther* 10(6): 228.

Matsushima, N., T. Tanaka, et al. (2007). "Comparative sequence analysis of leucine-rich repeats (LRRs) within vertebrate toll-like receptors." *BMC Genomics* 8: 124.

McDonald, C., N. Inohara, et al. (2005). "Peptidoglycan signaling in innate immunity and inflammatory disease." *J Biol Chem* 280(21): 20177-80.

McGreal, E. P., M. Rosas, et al. (2006). "The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose." *Glycobiology* 16(5): 422-30.

Means, T. K., E. Mylonakis, et al. (2009). "Evolutionarily conserved recognition and innate immunity to fungal pathogens by the scavenger receptors SCARF1 and CD36." *J Exp Med* 206(3): 637-53.

Medzhitov, R., P. Preston-Hurlburt, et al. (1997). "A human homologue of the Drosophila Toll protein signals activation of adaptive immunity." *Nature* 388(6640): 394-7.

Meylan, E., K. Burns, et al. (2004). "RIP1 is an essential mediator of Toll-like receptor 3-induced NF-kappa B activation." *Nat Immunol* 5(5): 503-7.

Miao, E. A., R. K. Ernst, et al. (2008). "Pseudomonas aeruginosa activates caspase 1 through Ipaf." *Proc Natl Acad Sci U S A* 105(7): 2562-7.

Miller, M. F. and R. Loch-Caruso (2010) "Comparison of LPS-stimulated release of cytokines in punch versus transwell tissue culture systems of human gestational membranes." *Reprod Biol Endocrinol* 8: 121.

Moore, R. M., J. B. Novak, et al. (2009). "Alpha-Lipoic Acid Inhibits Tumor Necrosis Factor-Induced Remodeling and Weakening of Human Fetal Membranes." *Biology of Reproduction* 80(4): 781-787.

Mulla, M. J., K. Myrtolli, et al. (2011) "Uric Acid Induces Trophoblast IL-1β Production Via the Inflammasome: Implications for the Pathogenesis of Preeclampsia." *Am J Reprod Immunol* 65(6): 542-548.

Naeye, R. L. (1979). "Causes of the excessive rates of perinatal mortality and prematurity in pregnancies complicated by maternal urinary-tract infections." *N Engl J Med* 300(15): 819-23.

Nakahara, K., M. Nakagawa, et al. (2006). "Maternal ghrelin plays an important role in rat fetal development during pregnancy." *Endocrinology* 147(3): 1333-42.

Napper, C. E., K. Drickamer, et al. (2006). "Collagen binding by the mannose receptor mediated through the fibronectin type II domain." *Biochem J* 395(3): 579-86.

Netea, M. G., G. Ferwerda, et al. (2005). "The Frameshift Mutation in Nod2 Results in Unresponsiveness Not Only to Nod2- but Also Nod1-activating Peptidoglycan Agonists." *JJ Biol Chem* 280(43): 35859-35867.

Netea, M. G., C. Van der Graaf, et al. (2004). "Recognition of fungal pathogens by Toll-like receptors." *Eur J Clin Microbiol Infect Dis* 23(9): 672-6.

Netea, M. G., C. A. Van Der Graaf, et al. (2002). "The role of toll-like receptor (TLR) 2 and TLR4 in the host defense against disseminated candidiasis." *J Infect Dis* 185(10): 1483-9.

Oh, K. J., K. A. Lee, et al. (2010) "Intraamniotic infection with genital mycoplasmas exhibits a more intense inflammatory response than intraamniotic infection with other

microorganisms in patients with preterm premature rupture of membranes." *Am J Obstet Gynecol* 203(3): 211.e1-211.e8.

Oshiumi, H., M. Matsumoto, et al. (2003). "TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction." *Nat Immunol* 4(2): 161-7.

Oshiumi, H., M. Sasai, et al. (2003). "TIR-containing adapter molecule (TICAM)-2, a bridging adapter recruiting to toll-like receptor 4 TICAM-1 that induces interferon-beta." *J Biol Chem* 278(50): 49751-62.

Osman, I., A. Young, et al. (2003). "Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term." *Mol Hum Reprod* 9(1): 41-5.

Ospelt, C. and S. Gay (2010) "TLRs and chronic inflammation." *Int J Biochem Cell Biol* 42(4): 495-505.

Pacora, P., R. Romero, et al. (2000). "Participation of the novel cytokine interleukin 18 in the host response to intra-amniotic infection." *Am J Obstet Gynecol* 183(5): 1138-43.

Park, H. S., R. Romero, et al. (2010) "Histologic Chorioamnionitis is More Common after Spontaneous Labor than after Induced Labor at Term." *Placenta* 31(9): 792-795.

Patni, S., L. P. Wynen, et al. (2009). "Expression and activity of Toll-like receptors 1-9 in the human term placenta and changes associated with labor at term." *Biol Reprod* 80(2): 243-8.

Peiser, L., S. Mukhopadhyay, et al. (2002). "Scavenger receptors in innate immunity." *Curr Opin Immunol* 14(1): 123-8.

Pollard, J. K. and M. D. Mitchell (1996). "Intrauterine infection and the effects of inflammatory mediators on prostaglandin production by myometrial cells from pregnant women." *Am J Obstet Gynecol* 174(2): 682-6.

Puchner, K., C. Iavazzo, et al. (2011) "Mid-trimester amniotic fluid interleukins (IL-1beta, IL-10 and IL-18) as possible predictors of preterm delivery." *In Vivo* 25(1): 141-8.

Repnik, U., T. Tilburgs, et al. (2008). "Comparison of macrophage phenotype between decidua basalis and decidua parietalis by flow cytometry." *Placenta* 29(5): 405-12.

Robinson, M. J., F. Osorio, et al. (2009). "Dectin-2 is a Syk-coupled pattern recognition receptor crucial for Th17 responses to fungal infection." *J Exp Med* 206(9): 2037-51.

Kelly, R.W. (2002) "Inflammatory mediators and cervical ripening." *J Reprod Immunol* 57(1-2): 217-224.

Romero, R., C. Avila, et al. (1990). "Amniotic fluid interleukin 6 in preterm labor. Association with infection." *J Clin Invest* 85(5): 1392-400.

Romero, R., D. T. Brody, et al. (1989a). "Infection and labor. III. Interleukin-1: a signal for the onset of parturition." *Am J Obstet Gynecol* 160(5 Pt 1): 1117-23.

Romero, R., S. Durum, et al. (1989b). "Interleukin-1 stimulates prostaglandin biosynthesis by human amnion." *Prostaglandins* 37(1): 13-22.

Romero, R., L. A. Friel, et al. (2010) "A genetic association study of maternal and fetal candidate genes that predispose to preterm prelabor rupture of membranes (PROM)." *Am J Obstet Gynecol* 203(4): 361.e1-361.e30.

Romero, R., K. R. Manogue, et al. (1989d). "Infection and labor. IV. Cachectin-tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor." *Am J Obstet Gynecol* 161(2): 336-41.

Romero, R., Y. K. Wu, et al. (1989c). "Human decidua: a source of interleukin-1." *Obstet Gynecol* 73(1): 31-4.

Rothenburg, S., T. Schwartz, et al. (2002). "Complex regulation of the human gene for the Z-DNA binding protein DLM-1." *Nucleic Acids Res* 30(4): 993-1000.

Sabbah, A., T. H. Chang, et al. (2009). "Activation of innate immune antiviral responses by Nod2." *Nat Immunol* 10(10): 1073-80.

Sado, T., K. Naruse, et al. (2011) "Inflammatory pattern recognition receptors and their ligands: factors contributing to the pathogenesis of preeclampsia." *Inflamm Res* 60(6): 509-520.

Saigal, S. and L. W. Doyle (2008). "An overview of mortality and sequelae of preterm birth from infancy to adulthood." *Lancet* 371(9608): 261-9.

Saito, S., Y. Kato, et al. (1992). "Amniotic fluid granulocyte colony-stimulating factor in preterm and term labor." *Clin Chim Acta* 208(1-2): 105-9.

Saitoh, S., S. Akashi, et al. (2004). "Lipid A antagonist, lipid IVa, is distinct from lipid A in interaction with Toll-like receptor 4 (TLR4)-MD-2 and ligand-induced TLR4 oligomerization." *Int Immunol* 16(7): 961-9.

Sakamoto, Y., P. Moran, et al. (2005). "Macrophages and not granulocytes are involved in cervical ripening." *J Reprod Immunol* 66(2): 161-73.

Sato, S., M. Sugiyama, et al. (2003). "Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF) associates with TNF receptor-associated factor 6 and TANK-binding kinase 1, and activates two distinct transcription factors, NF-kappa B and IFN-regulatory factor-3, in the Toll-like receptor signaling." *J Immunol* 171(8): 4304-10.

Sato, T., H. Michizu, et al. (2001). "Hormonal regulation of PGE2 and COX-2 production in rabbit uterine cervical fibroblasts." *J Appl Physiol* 90(4): 1227-1231.

Schroder, K. and J. Tschopp (2010). "The inflammasomes." *Cell* 140(6): 821-32.

Sennstrom, M. K., A. Brauner, et al. (1997). "Interleukin-8 is a mediator of the final cervical ripening in humans." *Eur J Obstet Gynecol Reprod Biol* 74(1): 89-92.

Shaw, M. H., T. Reimer, et al. (2008). "NOD-like receptors (NLRs): bona fide intracellular microbial sensors." *Curr Opin Immunol* 20(4): 377-82.

Shields, A. M., G. S. Panayi, et al. (2011) "Resolution-associated molecular patterns (RAMP): RAMParts defending immunological homeostasis?" *Clin Exp Immunol* 165(3): 292-300.

Shimazu, R., S. Akashi, et al. (1999). "MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4." *J Exp Med* 189(11): 1777-82.

Simhan, H. N., L. M. Bodnar, et al. (2011) "Lower genital tract inflammatory milieu and the risk of subsequent preterm birth: an exploratory factor analysis." *Paediatr Perinat Epidermiol* 25(3): 277-282.

Smith, S. C., P. N. Baker, et al. (1997). "Placental apoptosis in normal human pregnancy." *Am J Obstet Gynecol* 177(1): 57-65.

Steer, P. (2005). "The epidemiology of preterm labor--a global perspective." *J Perinat Med* 33(4): 273-6.

Tabeta, K., P. Georgel, et al. (2004). "Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection." *Proc Natl Acad Sci U S A* 101(10): 3516-21.

Takaoka, A. and T. Taniguchi (2008). "Cytosolic DNA recognition for triggering innate immune responses." *Adv Drug Deliv Rev* 60(7): 847-57.

Takaoka, A., Z. Wang, et al. (2007). "DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response." *Nature* 448(7152): 501-5.

Takeuchi, O. and S. Akira "Pattern recognition receptors and inflammation." *Cell* 140(6): 805-20.

Takeuchi, O., K. Hoshino, et al. (1999). "Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components." *Immunity* 11(4): 443-51.

Takeuchi, O., T. Kawai, et al. (1999). "TLR6: A novel member of an expanding toll-like receptor family." *Gene* 231(1-2): 59-65.

Taylor, P. R., S. Gordon, et al. (2005). "The mannose receptor: linking homeostasis and immunity through sugar recognition." *Trends Immunol* 26(2): 104-10.

Terness, P., M. Kallikourdis, et al. (2007). "Tolerance signaling molecules and pregnancy: IDO, galectins, and the renaissance of regulatory T cells." *Am J Reprod Immunol* 58(3): 238-54.

Thornton, S., J. I. Gillespie, et al. (1992). "Mobilization of calcium by the brief application of oxytocin and prostaglandin E2 in single cultured human myometrial cells." *Exp Physiol* 77(2): 293-305.

Ting, J. P. and B. K. Davis (2005). "CATERPILLER: a novel gene family important in immunity, cell death, and diseases." *Annu Rev Immunol* 23: 387-414.

Ting, J. P., S. B. Willingham, et al. (2008). "NLRs at the intersection of cell death and immunity." *Nat Rev Immunol* 8(5): 372-9.

Tornblom, S. A., A. Klimaviciute, et al. (2005). "Non-infected preterm parturition is related to increased concentrations of IL-6, IL-8 and MCP-1 in human cervix." *Reprod Biol Endocrinol* 3: 39.

Toti, P., F. Arcuri, et al. (2011) "Focal Increases of Fetal Macrophages in Placentas from Pregnancies with Histological Chorioamnionitis: Potential Role of Fibroblast Monocyte Chemotactic Protein-1." *Am J Reprod Immunol* 65(5): 470-479.

Tucker, J. and W. McGuire (2004). "Epidemiology of preterm birth." *Bmj* 329(7467): 675-8.

Underhill, D. M., E. Rossnagle, et al. (2005). "Dectin-1 activates Syk tyrosine kinase in a dynamic subset of macrophages for reactive oxygen production." *Blood* 106(7): 2543-50.

Vacca, P., C. Cantoni, et al. (2010) "Crosstalk between decidual NK and CD14+ myelomonocytic cells results in induction of Tregs and immunosuppression." *Proceedings of the Proc Natil Acad Sci USA* 107(26): 11918-11923.

Vadillo-Ortega, F., A. Hernandez, et al. (1996). "Increased matrix metalloproteinase activity and reduced tissue inhibitor of metalloproteinases-1 levels in amniotic fluids from pregnancies complicated by premature rupture of membranes." *Am J Obstet Gynecol* 174(4): 1371-6.

van Asbeck, E. C., A. I. Hoepelman, et al. (2008). "Mannose binding lectin plays a crucial role in innate immunity against yeast by enhanced complement activation and enhanced uptake of polymorphonuclear cells." *BMC Microbiol* 8: 229.

Vavricka, S. R., M. W. Musch, et al. (2004). "hPepT1 transports muramyl dipeptide, activating NF-kappaB and stimulating IL-8 secretion in human colonic Caco2/bbe cells." *Gastroenterology* 127(5): 1401-9.

Verthelyi, D., K. J. Ishii, et al. (2001). "Human peripheral blood cells differentially recognize and respond to two distinct CPG motifs." *J Immunol* 166(4): 2372-7.

Wang, H. and E. Hirsch (2003). "Bacterially-induced preterm labor and regulation of prostaglandin-metabolizing enzyme expression in mice: the role of toll-like receptor 4." *Biol Reprod* 69(6): 1957-63.

Wang, T., T. Town, et al. (2004). "Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis." *Nat Med* 10(12): 1366-73.

Watari, M., H. Watari, et al. (1999). "Pro-inflammatory cytokines induce expression of matrix-metabolizing enzymes in human cervical smooth muscle cells." *Am J Pathol* 154(6): 1755-62.

Watters, T. M., E. F. Kenny, et al. (2007). "Structure, function and regulation of the Toll/IL-1 receptor adaptor proteins." *Immunol Cell Biol* 85(6): 411-9.

Wesche, H., X. Gao, et al. (1999). "IRAK-M is a novel member of the Pelle/interleukin-1 receptor-associated kinase (IRAK) family." *J Biol Chem* 274(27): 19403-10.

Winkler, M. and W. Rath (1999). "Changes in the cervical extracellular matrix during pregnancy and parturition." *J Perinat Med* 27(1): 45-60.

Wojcikiewicz, E. P., X. Zhang, et al. (2003). "Contributions of molecular binding events and cellular compliance to the modulation of leukocyte adhesion." *J Cell Sci* 116(12): 2531-2539.

Yamamoto, M., S. Sato, et al. (2003). "TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway." *Nat Immunol* 4(11): 1144-50.

Yamamoto, M., S. Sato, et al. (2002). "Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-beta promoter in the Toll-like receptor signaling." *J Immunol* 169(12): 6668-72.

Yamasaki, S., E. Ishikawa, et al. (2008). "Mincle is an ITAM-coupled activating receptor that senses damaged cells." *Nat Immunol* 9(10): 1179-88.

Yasukawa, H., A. Sasaki, et al. (2000). "Negative Regulation of Cytokine Signaling Pathways." *Ann Rev Immunol* 18(1): 143-164.

Yoneyama, M. and T. Fujita (2007). "Function of RIG-I-like receptors in antiviral innate immunity." *J Biol Chem* 282(21): 15315-8.

Yoneyama, M. and T. Fujita (2007). "RIG-I family RNA helicases: cytoplasmic sensor for antiviral innate immunity." *Cytokine Growth Factor Rev* 18(5-6): 545-51.

Yoneyama, M., W. Suhara, et al. (1998). "Direct triggering of the type I interferon system by virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300." *Embo J* 17(4): 1087-95.

Yoon, B. H., R. Romero, et al. (1995). "Amniotic fluid interleukin-6: a sensitive test for antenatal diagnosis of acute inflammatory lesions of preterm placenta and prediction of perinatal morbidity." *Am J Obstet Gynecol* 172(3): 960-70.

Yoon, B. H., R. Romero, et al. (1998). "Microbial invasion of the amniotic cavity with Ureaplasma urealyticum is associated with a robust host response in fetal, amniotic, and maternal compartments." *Am J Obstet Gynecol* 179(5): 1254-60.

Young, A., A. J. Thomson, et al. (2002). "Immunolocalization of proinflammatory cytokines in myometrium, cervix, and fetal membranes during human parturition at term." *Biol Reprod* 66(2): 445-9.

Zhang, D., G. Zhang, et al. (2004). "A toll-like receptor that prevents infection by uropathogenic bacteria." *Science* 303(5663): 1522-6.

Recent Advances in Research on the Human Placenta

Edited by Dr. Jing Zheng

ISBN 978-953-51-0194-9
Hard cover, 428 pages
Publisher InTech
Published online 07, March, 2012
Published in print edition March, 2012

This book contains the total of 19 chapters, each of which is written by one or several experts in the corresponding field. The objective of this book is to provide a comprehensive and most updated overview of the human placenta, including current advances and future directions in the early detection, recognition, and management of placental abnormalities as well as the most common placental structure and functions, abnormalities, toxicology, infections, and pathologies. It also includes a highly controversial topic, therapeutic applications of the human placenta. A collection of articles presented by active investigators provides a clear update in the area of placental research for medical students, nurse practitioners, practicing clinicians, and biomedical researchers in the fields of obstetrics, pediatrics, family practice, genetics, and others who may be interested in human placentas.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen