Published in final edited form as:

Am J Reprod Immunol. 2017 March ; 77(3): . doi:10.1111/aji.12623.

Current concepts in maternal-fetal immunology: Recognition and response to microbial pathogens by decidual stromal cells

Anjali P. Anders, MD¹, Jennifer A. Gaddy, PhD^{2,3}, Ryan S. Doster, MD³, and David M. Aronoff, MD^{3,4,*}

¹Division of Neonatology, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN

²Tennessee Valley Healthcare Systems, Department of Veterans Affairs, Nashville, TN

³Division of Infectious Diseases, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN

⁴Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN

Abstract

Chorioamnionitis is an acute inflammation of the gestational (extraplacental) membranes, most commonly caused by ascending microbial infection. It is associated with adverse neonatal outcomes including preterm birth, neonatal sepsis and cerebral palsy. The decidua is the outermost layer of the gestational membranes and is likely an important initial site of contact with microbes during ascending infection. However, little is known about how decidual stromal cells (DSCs) respond to microbial threat. Defining the contributions of individual cell types to the complex medley of inflammatory signals during chorioamnionitis could lead to improved interventions aimed at halting this disease. We review available published data supporting the role for DSCs in responding to microbial infection, with a special focus on their expression of pattern recognition receptors and evidence of their responsiveness to pathogen sensing. While DSCs likely play an important role in sensing and responding to infection during the pathogenesis of chorioamnionitis, important knowledge gaps and areas for future research are highlighted.

Keywords

chorioamnionitis; decidual stromal cells; infection; microbes; pathogen recognition

Introduction

Prematurity is a leading cause of death in children less than 5 years old [1]. A common cause of prematurity is chorioamnionitis, in which acute inflammation of the gestational membranes ensues, often as a result of bacteria ascending from the vagina [2].

Chorioamnionitis occurs in 1–4% of all US births and complicates as many as 40–70% of

*Corresponding author: David M. Aronoff, MD, 1161 21st Avenue South, A-2200 MCN Nashville, TN 37232-2582, Ph: 615-322-8972, Fax: 615-343-6160 d.aronoff@vanderbilt.edu.

preterm births with premature rupture of membranes or spontaneous labor [2]. Adverse neonatal outcomes secondary to chorioamnionitis include neonatal sepsis, necrotizing enterocolitis, bronchopulmonary dysplasia, cerebral palsy and retinopathy of prematurity [3]. A major challenge in this field is our limited understanding of how pathogens evade host defenses to establish chorioamnionitis and fetal inflammation, crippling efforts to improve preventive and therapeutic options.

The gestational membranes, also referred to as extraplacental or fetal membranes, are a critical protective barrier during normal pregnancy that can serve as a nidus for inflammation (chorioamnionitis). In simplified terms, these membranes are composed of three layers, including the fetally-derived amnion and chorion and the maternally-derived decidua (Figure 1). The decidual layer is composed mainly of decidual stromal cells (DSC), along with macrophages and lymphocytes [4]. Previous studies have shown that DSCs are important in pregnancy to foster tolerance of the semi-allogeneic fetus [5–7], but few studies have been performed to determine the immune response of DSCs against pathogens, such as viruses or bacteria. Given their anatomical position, it is likely that DSCs are among the first cells of the gestational membrane to confront potential microbial threats ascending from the cervix and vagina (Figure 1).

During chorioamnionitis, bacterial pathogen-associated molecular patterns (PAMPs) can be recognized by host cell pattern recognition receptors (PRRs). This immune activation leads to a proinflammatory cascade, including the activation of transcription factor, NF- κ B and the expression of cytokines such as interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , interleukin (IL)-8, as well as the release of prostaglandins and matrix metalloproteases (MMPs). This inflammatory response can provoke uterine contractions, infiltration of inflammatory cells into gestational tissues, structural changes in the cervix, and weakening of the gestational membranes [8–15]. Elucidating the contributions of individual cell types to the complex medley of inflammatory signals during chorioamnionitis could lead to novel chemotherapeutics which target the proinflammatory signaling cascade, thereby altering the host response to infection.

Existing evidence suggests that DSCs respond to microbes or PAMPs to produce an inflammatory response and that such responses occur because DSCs express PRRs, including NOD-like receptors (NLRs) and Toll-like receptors (TLRs) (Figure 2) [16–20]. The present work seeks to review published data supporting the role for DSCs in responding to microbial threat. While DSCs likely play an important role in sensing and responding to infection during the pathogenesis of chorioamnionitis, important knowledge gaps and areas for future research will be highlighted in this review.

Methods

References cited in this review were obtained by searching the MEDLINE database for English language articles using PubMed (United States National Library of Medicine (Bethesda, MD)) for all years available. The following search terms or combination of terms were used: “AIM2-like receptor”, “ALR”, “C-type lectin receptor”, “CLR”, “chorioamnionitis”, “decidua”, “decidual cell/s”, “decidual stromal cell/s”, “*E. coli*”,

“*Escherichia coli*”, “*Fusobacteria*”, “*Fusobacterium*”, “*Gardnerella*”, “GBS”, “Group B *Streptococcus*”, “lipopolysaccharide”, “lipoteichoic acid”, “LPS”, “LTA”, “*Mycoplasma*”, “nod-like receptor”, “nod receptor”, “nucleotide-binding oligomerization domain receptors”, “PAMP receptor”, “pathogen recognition receptor”, “RIG-I-like receptor”, “RLR”, “Strep”, “*Streptococcus*”, “TLR”, “toll receptor”, “toll-like receptor”, “*Ureaplasma*” and “virus”.

Additional references were obtained through bibliographies cited in manuscripts.

References were included in this review if they assessed PRR expression by highly purified DSCs (both primary cells and cell lines), used specific tissue staining to document PRR expression by DSCs, or provided experimental evidence of PRR function in response to pathogens or PAMPs. References were excluded if they addressed tissue-level responses (because decidual tissue is a heterogeneous mix of different cell types), if data regarding the purity of primary DSCs was not presented or difficult to infer from the data provided, or if there was evidence of significant contamination ($\geq 5\%$) by non-DSC cell types. Literature was reviewed through March 1, 2016.

Pattern Recognition Receptors (PRRs) are expressed by decidual stromal cells

Toll-like receptors—Most PRRs recognize PAMPs or products of cell damage or stress (danger-associated molecular patterns, DAMPs) [21]. The TLRs are one of the major and best characterized classes of PRRs [18]. TLRs are expressed in innate immune cells and non-immune cells to allow for recognition of infection and initiation of immune response [18, 22]. It has been postulated that TLR stimulation during pregnancy may lead to a cascade of events culminating in myometrium contractions, cervical ripening, and membrane rupture [23]. Several TLRs have been discovered, 10 of which are present in humans [18, 24]. TLRs have specificity for different types of microbial ligands (Table I). TLR2 binds to Gram-positive peptidoglycans, lipopeptides, lipoteichoic acids, and zymosan. TLR6 is also activated by peptidoglycan [16]. TLR4 binds to Gram-negative lipopolysaccharide [18, 22, 24, 25]. TLR 3, 7 and 8 are important in viral immunity (Table I) [18, 22, 24, 25].

Through use of immunohistochemistry (IHC) of term human DSCs (obtained in the absence of active labor), Canavan *et al.* discovered that TLR 1–6 were expressed by DSCs and by reverse transcription (RT)-PCR found gene expression of TLR 1, 2, 4 and 6 (Figure 2) [16]. These investigators also examined functions of TLRs through stimulation by microbial ligands discussed below [16]. Krikun *et al.* utilized first trimester and term human decidual cells and discovered expression of TLRs 1–10 by RT-PCR [17]. Western blot confirmed presence of TLR2 and TLR4 peptide expression by first trimester DSCs [17]. Krikun *et al.* assessed for downstream signaling proteins from the TLR pathway, myeloid differentiation primary response gene 88 (MyD88) and TIR-domain-containing adapter-inducing interferon- β (TRIF), in first and third trimester DSCs and detected both signaling proteins [17]. Schatz *et al.* identified the presence of TLR-4 on human DSCs. Taken together this data suggests that DSCs possess TLRs and may be capable of activating inflammatory responses during pregnancy [19].

NOD-like Receptors—NOD-like receptors (NLRs) are another major group of PRRs that, unlike most TLRs, are expressed and function within cells and sense PAMPs once they are internalized into the host cell [20, 26]. NOD1 and NOD2 are involved in recognition of intracellular pathogens and are expressed mainly in monocytes, macrophages, dendritic cells, and epithelial cells [20]. NOD1 recognizes Gram-negative bacteria while NOD2 recognizes Gram-positive and negative bacteria (Table I) [20]. Binding of NOD receptors to their ligands leads to inflammatory cytokine response. Both NOD1 and NOD2 are expressed in human gestational membranes and appear to be induced by infection or inflammation [9, 27]. Zhang *et al.* utilized human first trimester decidua and found NOD2 expression by immunohistochemistry in DSCs. Purified DSCs expressed NOD2 mRNA and protein [20]. Interestingly, their studies discovered that both IL-1 β and TNF- α stimulation increased the protein expression of NOD2 in DSCs [20], suggesting infection might enhance tissue inflammation by inducing inflammasome proteins [20].

Other PRRs—Our search of available literature did not identify studies documenting DSC expression of other well-characterized PRRs such as AIM2-like receptor [28], C-type lectin receptor [29], and RIG-I-like receptor [30]. These are topics in need of more investigation. Current evidence of PRR expression by DSCs is summarized in Table I.

Decidual stromal cell response to PAMPs

Expression of PRRs does not speak to function. A number of studies have addressed the extent to which DSCs exhibit a functional response either to whole pathogens or PAMPs (or PAMP mimics). DSCs respond to bacterial and viral microbial ligands, including LPS, peptidoglycan, and poly (I:C). The majority of studies performed on DSCs utilized LPS.

Lipopolysaccharide produces a significant proinflammatory response by DSCs, as measured by changes in either gene or protein expression. NF- κ B is an essential transcription factor in TLR pathway leading to proinflammatory cytokine secretion, and when activated translocates to the nucleus [17, 24]. Krikun *et al.* reported that LPS and other microbial ligands induced NF- κ B translocation to the nucleus in DSCs, supporting a functional role for TLR 4 in these cells [17]. With regards to additional transcriptional effects, studies using primary human DSCs have illustrated that LPS increases the expression of a number of genes in the NF- κ B pathway, including monocyte chemoattractant protein-1 (MCP-1) [31], IL-1 β [31, 32], colony stimulating factor-2 (CSF-2) [31] and IL-8 [16, 31]. Other studies utilizing primary human DSCs observed increased gene expression of TNF- α [32, 33], IL-6 [17, 32, 33], lipid metabolism genes (*e.g.*, those involved in prostaglandin production) [32], and decreased solute carrier family 40 member 1 (SLC40A1), which is key in modulating iron levels [32]. Utilizing an immortalized line of human decidualized endometrial stromal cells, Grasso and colleagues assessed responses to LPS, which also elicited increased gene expression of regulated on activation, normal T cell expressed and secreted (RANTES), MCP-1 and IL-8 [34].

At the protein level, MCP-1 production by primary human DSCs was increased after LPS stimulation, which may be important in recruitment of monocytes to areas of inflammation [20, 35]. Zhang *et al.* illustrated increased protein expression of IL-1 β by primary human

DSCs with no significant change in TNF- α or IL-12 after LPS stimulation [20]. LPS has also been found to increase protein expression of IL-6 [36] and IL-8 [16, 36] by primary human DSCs, which are important in neutrophil chemotaxis. Periodontitis, an inflammatory disease of the tissues surrounding the teeth, provoked by supra and subgingival microorganisms, has been associated with preterm labor and other adverse pregnancy outcomes, possibly due to oral bacteria or inflammatory mediators directly stimulating an immune response in the gravid uterus [37]. Keelan *et al.* assessed the response of term human DSCs to LPS molecules produced from various periodontopathic bacteria and *Escherichia coli* [33]. They observed increased proinflammatory cytokine protein production of TNF- α and IL-6 [33]. LPS has also been found to significantly increase prostaglandin E2 (PGE2) secretion from primary human DSCs [38].

With LPS eliciting significant proinflammatory stimulation of DSCs, studies have been performed to determine if the inflammatory response can be dampened to help prevent inflammation-associated adverse effects during pregnancy. After co-incubation with IL-4, an anti-inflammatory cytokine, it was found there was significant decrease in LPS-stimulated PGE2 response by primary human DSCs while the anti-inflammatory cytokine IL-10 induced minimal change [38]. Endogenously produced progesterone has anti-inflammatory actions that are thought to be important in maintaining uterine quiescence and preventing the onset of labor until term [39]. Synthetic progestins are sometimes used to prevent prematurity [40]. Interestingly, pretreatment of primary human DSCs with various progestins (progesterone, 17- α hydroxyprogesterone, or 17- α hydroxyprogesterone caproate) prior to LPS stimulation did not lead to suppression of TLR pathway gene expression [31].

Cha *et al.* assessed effects of LPS on DSCs and the hypothesis that increased mammalian target of rapamycin complex 1 (mTORC1) activity and cyclooxygenase (COX) signaling are associated with preterm birth [36]. They found LPS increased expression of the COX-2 protein. Preincubation with rapamycin (mTORC1 inhibitor) and progesterone prior to LPS exposure resulted in decreased IL-6 and IL-8 [36].

Previous studies have shown LPS leading to increased TNF- α production in mixed decidual cell cultures [41] and from amnion and choriodecidua explants [42]. TNF- α is believed to increase apoptosis in cells of the gestational membrane [43]. Yu *et al.* demonstrated LPS can lead to DSC death *in vitro*, then assessed the effect of soluble TNF receptor (TNFR1) on primary human DSC proliferation after LPS exposure [43]. Soluble TNFR1 increased DSC proliferation after LPS stimulation possibly by neutralizing TNF- α [43]. Yu *et al.* concluded soluble TNFR1 could aid in protection against LPS [43].

As noted above, DSCs respond to other PAMPs although fewer studies have been performed on these other microbial ligands. Krikun *et al.* found after exposure to microbial ligands peptidoglycan and Poly (I:C), NF- κ B translocated to the nucleus in DSCs, supporting a functional role for TLRs 2 and 3 in these cells [17]. Canavan *et al.* assessed peptidoglycan effects on gene expression of IL-8 and found increased expression after exposure in primary human DSCs [16]. Krikun *et al.* assessed gene expression of IL-6 and IL-8 after peptidoglycan exposure in primary human DSCs and did not observe increased mRNA

expression, although noted increased gene expression of both cytokines after exposure to Poly (I:C) [17]. The conflicting IL-8 gene expression obtained from these two researchers could be related to different concentrations of peptidoglycan utilized in their experiments. Zhang *et al.* stimulated primary human DSCs with muramyl dipeptide (MDP; ligand present in Gram-positive and negative bacteria) and found increased IL-1 β and MCP-1 protein expression, no significant change in IL-12 or TNF- α [20]. MDP also up-regulated DSC apoptosis [20].

Decidual stromal cell response to intact pathogens

As indicated above, although DSCs are not considered typical immune cells, they are capable of mounting an innate immune response to PAMPs. Intact bacteria can engage multiple different PRRs and may stimulate cellular responses in a different manner than individual PAMPs. The extent to which DSCs respond to intact pathogens has also received attention and the results of such studies are reviewed here.

Responses of DSCs to bacteria in vitro—A small number of studies have examined how DSCs in pure culture respond to microbial infection. Bacteria including *E. coli* and Group B *Streptococcus* (GBS) can adhere to DSCs such as decidualized telomerase-immortalized human endometrial stromal cells (dTHESCs) *in vitro* as determined by high resolution scanning electron microscopy analyses (Figure 3). Keelan *et al.* utilized primary human DSCs and assessed their response to periodontal bacteria to assess the hypothesis that periodontal disease may be associated with preterm birth [33]. Bacteria used included *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Peptoniphilus asaccharolyticus*, and *Peptostreptococcus anaerobius* in comparison to *E. coli* [33]. In the same study, they also assessed their response to aqueous bacterial LPS suspensions from the above bacteria, which is reviewed above [33]. The LPS was a more potent stimulus and produced a proinflammatory response with increased TNF- α and IL-6 protein production [33]. Bacteria, on the other hand, elicited a weaker inflammatory response, which varied based on the bacterial inoculum size and the particular organism [33]. *E. coli* was the most potent organism they tested [33]. Korir *et al.* utilized decidualized human endometrial stromal cells to study attachment and invasion by various strains of GBS [44], finding strain-dependent differences and a proclivity for invasive GBS strains to be the most capable at adhering and invading [44].

Responses to bacteria in situ—Several studies have characterized DSC responses *in situ* using tissues obtained from cases of chorioamnionitis. Such studies are difficult to interpret because changes in gene or protein expression in DSCs might result from microbial sensing *per se* or might be induced through paracrine signaling by cytokines or other inflammatory mediators. Thus, these studies provide only circumstantial evidence for microbe sensing by DSCs.

Oner *et al.* utilized human decidual tissue to identify that tissues obtained from cases of chorioamnionitis showed enhanced decidual cell expression of matrix metalloprotease enzymes compared to uninfected tissues [45]. These MMPs may promote preterm delivery via decidual, gestational membrane, and cervical extracellular matrix degradation [45].

Castro-Leyva *et al.* used preterm gestational membranes from patients with no clinical signs of infection, microbiologically tested the decidua and cultured DSCs from these tissues [46]. They assessed for subclinical infection, which was found in ~ 29% of women, by GBS, *E. coli*, *U. urealyticum* and *Gardnerella vaginalis* [46]. Castro-Leyva *et al.* found significant increase in proinflammatory cytokines (IL-8, IL-6, IL-1 β and TNF- α) and decrease in anti-inflammatory cytokines (IL-2 and IL-10) in women with subclinical infection compared to women without infection [46]. They also noted increase in MMP-1, MMP-8, MMP-9 and PGE2 in supernatants from DSCs with subclinical infection [46].

Arcuri *et al.* utilized human placenta from patients diagnosed with chorioamnionitis and controls, and isolated primary human DSCs [47]. They assessed for the presence of colony stimulating factor 2 (CSF2), a potent chemoattractant and activator of neutrophils and macrophages, in chorioamnionitis-affected tissues, as compared to controls, and reported markedly increased staining for CSF2 in the DSCs affected by chorioamnionitis [47].

Tadesse *et al.* utilized term human placental tissue from control patients and patients with chorioamnionitis to assess for neutrophil gelatinase-associated lipocalin (NGAL) [48]. NGAL is a host protein that participates in nutritional immunity as well as transport of various substances including iron, prostaglandins and MMPs [48]. NGAL was occasionally observed in DSCs without significant increase during infection (less frequent than trophoblasts) [48]. No significant change was detected in DSC NGAL expression after exposure to LPS, TNF- α or IL-1 β [48].

We identified few studies regarding the interaction between DSCs and viruses. For instance, cytomegalovirus (CMV), a leading cause of congenital infection in developed countries, has been found in various cells at the maternal-fetal interface including cytotrophoblasts, macrophages, endothelial cells, decidual stromal cells, and dendritic cells [49, 50]. Although the initial steps of CMV congenital infection seem to occur in the decidua, little is known of the response of DSCs to infection by CMV [49]. Although DSCs express PRRs that recognize viral RNA [17], this literature search failed to identify relevant studies of virus-DSC interactions.

Conclusions

Chorioamnionitis is an acute inflammation of the gestational membranes, most commonly caused by ascending bacterial infection. The decidua of the gestational membranes is the initial cellular layer to come into contact with microbes during ascending infection. This review defines what is known about the contributions of DSCs to the innate immune response. We limited our review to studies where primary DSCs (or cell lines) were highly purified and studied *in vitro* or where tissue studies clearly defined DSC responses *in situ*.

In summary, DSCs, although considered to be non-immune cells, express pathogen-recognizing receptors including TLRs and NLRs. These receptors are functional and respond to diverse microbial ligands, including LPS, peptidoglycans, and viral analogs. DSC responses to microbial threats are subject to autocrine or paracrine regulation by cytokines and hormones. DSCs have also been shown to respond to intact, living bacteria by the

production of a proinflammatory response. While DSCs likely play an important role in sensing and responding to infection during the pathogenesis of chorioamnionitis, important knowledge gaps exist and are open areas for future research. There is a compelling need to better define the response of DSCs to the most common microbial pathogens associated with chorioamnionitis. Research is also currently lacking in the paracrine signals involved during chorioamnionitis at the cellular level prior to the development of preterm labor and premature rupture of membranes. A better understanding of the contribution of individual cell types, such as DSCs, to the proinflammatory signal cascade and disease progression could illuminate new biomarkers and/or chemotherapeutic targets to beneficially modulate host immune responses and improve pregnancy outcomes.

Acknowledgments

Funded by a grant from the Global Alliance to Prevent Prematurity and Stillbirth (GAPPS; to DMA), a T32 grant from the National Institutes of Health (HD068256 to APA), a Career Development Award from the Dept. of Veterans Affairs (IK2BX001701 to JAG), National Institutes of Health grants T32-AI007474 and 2T32HD060554-06A2 (to RSD). Core Services including use of the Cell Imaging Shared Resource were performed through support from Vanderbilt Institute for Clinical and Translational Research program supported by the National Center for Research Resources, Grant UL1 RR024975-01, and the National Center for Advancing Translational Sciences, Grant 2 UL1 TR000445-06 and by U.S. EPA Grant #83573601. Its contents are solely the responsibility of the grantee and do not necessarily represent the official views of the U.S. EPA. Further, U.S. EPA does not endorse the purchase of any commercial products or services mentioned in the publication.

References

1. Liu L, et al. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet*. 2015; 385(9966):430–40. [PubMed: 25280870]
2. Tita AT, Andrews WW. Diagnosis and management of clinical chorioamnionitis. *Clin Perinatol*. 2010; 37(2):339–54. [PubMed: 20569811]
3. Gantert M, et al. Chorioamnionitis: a multiorgan disease of the fetus? *J Perinatol*. 2010; 30(Suppl):S21–30. [PubMed: 20877404]
4. Vince GS, et al. Flow cytometric characterisation of cell populations in human pregnancy decidua and isolation of decidual macrophages. *J Immunol Methods*. 1990; 132(2):181–9. [PubMed: 2145368]
5. Vinketova K, Mourdjeva M, Oreshkova T. Human Decidual Stromal Cells as a Component of the Implantation Niche and a Modulator of Maternal Immunity. *J Pregnancy*. 2016; 2016:8689436. [PubMed: 27239344]
6. Vacca P, et al. MSC and innate immune cell interactions: A lesson from human decidua. *Immunol Lett*. 2015
7. Wagner GP, et al. Evolution of mammalian pregnancy and the origin of the decidual stromal cell. *Int J Dev Biol*. 2014; 58(2–4):117–26. [PubMed: 25023677]
8. Agrawal V, Hirsch E. Intrauterine infection and preterm labor. *Semin Fetal Neonatal Med*. 2012; 17(1):12–9. [PubMed: 21944863]
9. Lappas M. NOD1 and NOD2 regulate proinflammatory and prolabor mediators in human fetal membranes and myometrium via nuclear factor-kappa B. *Biol Reprod*. 2013; 89(1):14. [PubMed: 23740944]
10. Romero R, et al. The role of inflammation and infection in preterm birth. *Semin Reprod Med*. 2007; 25(1):21–39. [PubMed: 17205421]
11. Romero R, et al. Human decidua: a source of interleukin-1. *Obstet Gynecol*. 1989; 73(1):31–4. [PubMed: 2642326]

12. Casey ML, et al. Cachectin/tumor necrosis factor- α formation in human decidua. Potential role of cytokines in infection-induced preterm labor. *J Clin Invest.* 1989; 83(2):430–6. [PubMed: 2913048]
13. Goldenberg RL, Andrews WW, Hauth JC. Choriodecidual infection and preterm birth. *Nutr Rev.* 2002; 60(5 Pt 2):S19–25. [PubMed: 12035853]
14. Strauss JF 3rd. Extracellular matrix dynamics and fetal membrane rupture. *Reprod Sci.* 2013; 20(2):140–53. [PubMed: 22267536]
15. Menon R, et al. Diversity in cytokine response to bacteria associated with preterm birth by fetal membranes. *Am J Obstet Gynecol.* 2009; 201(3):306.e1–6. [PubMed: 19733282]
16. Canavan TP, Simhan HN. Innate immune function of the human decidual cell at the maternal-fetal interface. *J Reprod Immunol.* 2007; 74(1–2):46–52. [PubMed: 17196257]
17. Krikun G, et al. Expression of Toll-like receptors in the human decidua. *Histol Histopathol.* 2007; 22(8):847–54. [PubMed: 17503341]
18. Patni S, et al. An introduction to Toll-like receptors and their possible role in the initiation of labour. *BJOG.* 2007; 114(11):1326–34. [PubMed: 17903232]
19. Schatz F, et al. Toll-like receptor 4 expression in decidual cells and interstitial trophoblasts across human pregnancy. *Am J Reprod Immunol.* 2012; 68(2):146–53. [PubMed: 22564191]
20. Zhang YY, et al. Expression and functional characterization of NOD2 in decidual stromal cells isolated during the first trimester of pregnancy. *PLoS One.* 2014; 9(6):e99612. [PubMed: 24932916]
21. Wiersinga WJ, et al. Host innate immune responses to sepsis. *Virulence.* 2014; 5(1):36–44. [PubMed: 23774844]
22. Koga K, Mor G. Toll-like receptors at the maternal-fetal interface in normal pregnancy and pregnancy disorders. *Am J Reprod Immunol.* 2010; 63(6):587–600. [PubMed: 20367625]
23. Romero R, Dey SK, Fisher S. Preterm Labor: One Syndrome, Many Causes. *Science.* 2014; 345(6198):760–765. [PubMed: 25124429]
24. Riley JK, Nelson DM. Toll-like receptors in pregnancy disorders and placental dysfunction. *Clin Rev Allergy Immunol.* 2010; 39(3):185–93. [PubMed: 19866377]
25. Koga K, et al. Toll-like receptors at the maternal-fetal interface in normal pregnancy and pregnancy complications. *Am J Reprod Immunol.* 2014; 72(2):192–205. [PubMed: 24754320]
26. Brubaker SW, et al. Innate immune pattern recognition: a cell biological perspective. *Annu Rev Immunol.* 2015; 33:257–90. [PubMed: 25581309]
27. Romero R, et al. A Role for the Inflammasome in Spontaneous Labor at Term. *Am J Reprod Immunol.* 2016
28. Gray EE, et al. The AIM2-like Receptors Are Dispensable for the Interferon Response to Intracellular DNA. *Immunity.* 2016; 45(2):255–66. [PubMed: 27496731]
29. Drickamer K, Taylor ME. Recent insights into structures and functions of C-type lectins in the immune system. *Curr Opin Struct Biol.* 2015; 34:26–34. [PubMed: 26163333]
30. Barik S. What Really Rigs Up RIG-I? *J Innate Immun.* 2016; 8(5):429–36. [PubMed: 27438016]
31. Simhan HN, et al. Human decidual cell Toll-like receptor signaling in response to endotoxin: the effect of progestins. *Am J Obstet Gynecol.* 2008; 198(1):119.e1–4. [PubMed: 17936235]
32. Himes KP, et al. Comprehensive analysis of the transcriptional response of human decidual cells to lipopolysaccharide stimulation. *J Reprod Immunol.* 2012; 93(1):17–27. [PubMed: 22196106]
33. Keelan JA, et al. Innate inflammatory responses of human decidual cells to periodontopathic bacteria. *Am J Obstet Gynecol.* 2010; 202(5):471.e1–11. [PubMed: 20452492]
34. Grasso E, et al. VIP contribution to the decidualization program: regulatory T cell recruitment. *J Endocrinol.* 2014; 221(1):121–31. [PubMed: 24492467]
35. Xu X, et al. Monocyte chemoattractant protein-1 secreted by decidual stromal cells inhibits NK cells cytotoxicity by up-regulating expression of SOCS3. *PLoS One.* 2012; 7(7):e41869. [PubMed: 22848642]
36. Cha J, et al. Combinatory approaches prevent preterm birth profoundly exacerbated by gene-environment interactions. *J Clin Invest.* 2013; 123(9):4063–75. [PubMed: 23979163]

37. Zi MY, et al. Mechanisms Involved in the Association between Periodontitis and Complications in Pregnancy. *Front Public Health*. 2014; 2:290. [PubMed: 25688342]
38. Simhan HN, Chura JC, Rauk PN. The effect of the anti-inflammatory cytokines interleukin-4 and interleukin-10 on lipopolysaccharide-stimulated production of prostaglandin E2 by cultured human decidual cells. *J Reprod Immunol*. 2004; 64(1–2):1–7. [PubMed: 15596223]
39. Sfakianaki AK, Norwitz ER. Mechanisms of progesterone action in inhibiting prematurity. *J Matern Fetal Neonatal Med*. 2006; 19(12):763–72. [PubMed: 17190686]
40. O'Brien JM, Lewis DF. Prevention of preterm birth with vaginal progesterone or 17-alpha-hydroxyprogesterone caproate: a critical examination of efficacy and safety. *Am J Obstet Gynecol*. 2016; 214(1):45–56. [PubMed: 26558340]
41. Arntzen KJ, et al. LPS mediated production of IL-1, PGE2 and PGF2alpha from term decidua involves tumour necrosis factor and tumour necrosis factor receptor p55. *J Reprod Immunol*. 1999; 45(2):113–25. [PubMed: 10674980]
42. Leroy MJ, et al. Inflammation of choriodecidua induces tumor necrosis factor alpha-mediated apoptosis of human myometrial cells. *Biol Reprod*. 2007; 76(5):769–76. [PubMed: 17215489]
43. Yu XW, Zhang XW, Li X. Soluble tumor necrosis factor receptor mediates cell proliferation on lipopolysaccharide-stimulated cultured human decidual stromal cells. *Int J Mol Sci*. 2009; 10(5):2010–8. [PubMed: 19564935]
44. Korir ML, et al. Association and virulence gene expression vary among serotype III group B *Streptococcus* isolates following exposure to decidual and lung epithelial cells. *Infect Immun*. 2014; 82(11):4587–95. [PubMed: 25135682]
45. Oner C, et al. Progesterin-inflammatory cytokine interactions affect matrix metalloproteinase-1 and -3 expression in term decidual cells: implications for treatment of chorioamnionitis-induced preterm delivery. *J Clin Endocrinol Metab*. 2008; 93(1):252–9. [PubMed: 17940116]
46. Castro-Leyva V, et al. Preserved ex vivo inflammatory status in decidual cells from women with preterm labor and subclinical intrauterine infection. *PLoS One*. 2012; 7(8):e43605. [PubMed: 22928002]
47. Arcuri F, et al. Mechanisms of leukocyte accumulation and activation in chorioamnionitis: interleukin 1 beta and tumor necrosis factor alpha enhance colony stimulating factor 2 expression in term decidua. *Reprod Sci*. 2009; 16(5):453–61. [PubMed: 19164476]
48. Tadesse S, et al. Intra-amniotic infection upregulates neutrophil gelatinase-associated lipocalin (NGAL) expression at the maternal-fetal interface at term: implications for infection-related preterm birth. *Reprod Sci*. 2011; 18(8):713–22. [PubMed: 21421891]
49. Weisblum Y, et al. Modeling of human cytomegalovirus maternal-fetal transmission in a novel decidual organ culture. *J Virol*. 2011; 85(24):13204–13. [PubMed: 21976654]
50. Pereira L, et al. Human cytomegalovirus transmission from the uterus to the placenta correlates with the presence of pathogenic bacteria and maternal immunity. *J Virol*. 2003; 77(24):13301–14. [PubMed: 14645586]

Figure 1.

A). H & E stain of uninfected gestational membrane at 100×, 200× and 400× magnification illustrating amnion, chorion and decidua (top row). B). H & E stain of gestational membrane infected with USA300 strain of *S. aureus* at 100×, 200× and 400× magnification reveal *S. aureus* adhering to, and forming biofilms on decidua (2nd row). C). Scanning electron microscope (SEM) image at 1000× magnification of uninfected gestational membranes. D). SEM image at 1000× magnification (20,000× magnification-inset) of gestational membrane infected by *S. aureus* forming biofilms. White arrows indicate *S. aureus* microcolonies. E). Depiction of cells in the gestational membrane.

Figure 2.

Decidual stromal cell (DSC) inflammatory response to pathogen-associated molecular patterns (PAMPs). Pattern recognition receptors (PRRs) expressed by DSCs include toll-like receptors (TLR 1, 2, 3, 4 and 6) and nucleotide-binding oligomerization domain receptor 2 (NOD2). After binding of PAMPs to PRRs, transcription and translation of proinflammatory cytokines are increased including MCP-1 (monocyte-chemoattractant protein-1), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interleukin-8 (IL-8), and some studies found increased tumor necrosis factor- α (TNF- α). DSCs have increased production of prostaglandin E2 (PGE2) and matrix metalloproteinases (MMPs) upon infection. LPS, lipopolysaccharide; LTA, lipoteichoic acid; MDP, muramyl dipeptide; PG, peptidoglycan; poly (I:C), Polyinosinic: polycytidylic acid.

Figure 3.

A). Scanning electron microscope (SEM) image of decidualized telomerase-immortalized human endometrial stromal cells (dTHESCs) at 1000 $\times$, 5000 $\times$ and 30,000 $\times$ magnification (top row). B). SEM image of *E. coli* infected dTHESCs at 1500 $\times$, 4000 $\times$ and 35,000 $\times$ magnification (middle row). C). SEM image of GBS infected dTHESCs at 1500 $\times$, 4000 $\times$, and 70,000 $\times$ magnification (bottom row).

Table I

Pattern recognition receptor mRNA and protein expression by decidual stromal cells (DSCs). Several TLRs are expressed by DSCs along with NOD2. There are still several PRRs including C-type lectin receptor (CLR), RIG-I-like receptor (RLR) and AIM-2 like receptor (ALR) that have not yet been studied in DSCs.

Abbreviations: iE-DAP, g-D-glutamyl-meso-diaminopimelic acid; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MDP, muramyl dipeptide; ND, not determined; PG, peptidoglycan; poly (I:C), Polyinosinic: polycytidylic acid.

PATTERN RECOGNITION RECEPTORS				
Toll-like receptor	Microbial ligands	mRNA expression	Protein expression	References
TLR 1	Lipoproteins (with TLR2), glycolipids	+	+	[16, 17]
TLR 2	Gram-positive PG, lipopeptides, LTA, zymosan	+	+	[16, 17]
TLR 3	dsRNA (virus), poly (I:C)	+	+	[16, 17]
TLR 4	Gram negative LPS	+	+	[16, 17, 19]
TLR 5	Bacterial flagellin	+	+	[16, 17]
TLR 6	Zymosan, lipoproteins (with TLR2)	+	+	[16, 17]
TLR 7	ssRNA (virus)	+	ND	[17]
TLR 8	ssRNA (virus)	+	ND	[17]
TLR 9	Unmethylated CpG DNA (bacteria)	+	ND	[17]
TLR 10	Unknown	+	ND	[17]
Nucleotide-binding oligomerization domain receptor	Microbial ligands	mRNA expression	Protein expression	References
NOD1	IE-DAP (gram-negative bacterial outer membrane)	ND	ND	
NOD2	MDP (gram-negative & positive bacteria)	+	+	[20]
C-type lectin receptor (CLR), RIG-I-like receptor (RLR) & AIM-2 like receptor (ALR)-not determined				