Inflammation and preterm birth

Monica Cappelletti, Silvia Della Bella, Enrico Ferrazzi, Domenico Mavilio, and Senad Divanovic

Division of Immunobiology, Cincinnati Children’s Hospital Research Foundation, and the University of Cincinnati College of Medicine, Cincinnati, Ohio, USA; 1Unit of Clinical and Experimental Immunology, Humanitas Clinical and Research Center, Rozzano, Italy; and 2Department of Woman, Mother and Neonate, Buzzi Children’s Hospital, Biomedical and Clinical Sciences School of Medicine, University of Milan, Italy

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ABSTRACT

Preterm birth is the leading cause of neonatal morbidity and mortality. Although the underlying causes of pregnancy-associated complication are numerous, it is well established that infection and inflammation represent a highly significant risk factor in preterm birth. However, despite the clinical and public health significance, infectious agents, molecular trigger(s), and immune pathways underlying the pathogenesis of preterm birth remain underdefined and represent a major gap in knowledge. Here, we provide an overview of recent clinical and animal model data focused on the interplay between infection-driven inflammation and induction of preterm birth. Furthermore, here, we highlight the critical gaps in knowledge that warrant future investigations into the interplay between immune responses and induction of preterm birth. J. Leukoc. Biol. 99: 000–000; 2016.

Introduction

PTB is the leading cause of neonatal morbidity and mortality worldwide. Annually, 15 million babies are born prematurely, resulting in an excess of 1 million deaths. Common health problems associated with PTB include motor and cognitive neurodevelopmental disabilities (e.g., cerebral palsy, blindness, deafness, mental retardation, and learning disabilities), chronic lung disease, gastrointestinal problems, and vision and hearing loss [1]. Based on gestational age, PTB can be divided into several subcategories: extreme preterm (<28 wk), very preterm (28 to <32 wk), and moderate-to-late preterm (32 to <37 wk). As expected, the severity of adverse outcomes inversely correlates with gestational age, and therefore, extremely preterm babies have the highest risk of long-term complications [2].

PTB is a global problem [3]. The highest burden of PTB, in excess of 60% of overall cases and 18% of all live births, is seen in African and South Asian countries—statistics presumably linked with limited medical resources, increased risk, and/or prevalence of infectious diseases and malnutrition [4]. Notably, PTB also affects western world countries, where most severe infections and other causes of neonatal death have been markedly resolved. Indeed, the United States surprisingly is one of the 10 countries with the highest numbers of PTBs, approaching 12% of all live births [5]. This is in striking contrast to European countries, where the rate of PTB is significantly lower (approaching 6% of all live births) [4]. Despite the clinical and public health significance, the etiology of PTB remains largely enigmatic. Notably, multiple causes, including environmental exposure, fetal and/or maternal genetics, stress, and immune/inflammatory conditions, have all been associated with induction of premature delivery.

Infection and infection-driven activation of inflammatory responses are thought to be the leading risk factor of “spontaneous” PTB [6–8]. Consequently, increased production of proinflammatory cytokines has been associated with uterine activation and PTB, whereas production of anti-inflammatory cytokines has been shown to play an essential role in uterine quiescence during gestation [9, 10]. However, the molecular triggers and mechanisms underlying the activation of immune pathways associated with induction of PTB remain poorly understood. In fact, several critical questions that remain unanswered include the following: the identification of a causative infectious agent(s), the role of polymicrobial infection, the critical locus of infection, and the central immune cells and immune pathways. Thus, herein, we aim to summarize the current understandings of the innate immune response in orchestrating infection- and/or inflammation-driven PTB. Importantly, a better understanding of the immune mechanism(s) involved in preterm labor may allow for development of novel therapeutic strategies to improve pregnancy outcomes.

Abbreviations: AIM-2 = absence in melanoma 2; CLR = C-type lectin receptor, Cox-2 = cyclooxygenase-2; DC = dendritic cell; dDC = decidual dendritic cell; dNK = decidual NK; HMGB1 = high-mobility group box-1, i.a. = intra-amniotic, i.p. = intraperitoneal, i.u. = intrauterine, LPS = lipopolysaccharide, LTA = lipoteichoic acid, mDC = myeloid dendritic cell, MMP = matrix metalloproteinase, NLR = nucleotide-binding oligomerization domain-like

1. Correspondence: Division of Immunobiology, Cincinnati Children’s Hospital Medical Center, TCHRF—Location S, Room #S.5.405, 3333 Burnet Ave., Cincinnati, OH 45229-3039, USA. E-mail: senad.divanovic@cchmc.org

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INFECTION, INFLAMMATION, AND PTB IN HUMANS

Infection and inflammation are well-defined risk factors of prematurity [1]. In fact, infection is detected in at least 25% of all PTB cases. Notably, 79% of patients with extreme PTB tested positive for an infectious insult, suggesting that infection and infection-associated inflammation regulate the timing of parturition [11, 12]. Multiple loci of infection, including systemic (e.g., influenza, sepsis, listeriosis, pneumonia) and i.u. (e.g., i.a. and extra-amniotic) infections, have been associated with PTB [13, 14]. Although the ability of systemic infections to drive PTB is highly significant, the overall occurrence of systemic infections during pregnancy remains relatively rare, likely associated with timely clinical diagnosis and improved prophylaxis. In contrast, i.u. infections, including chorioamnionitis, occur more commonly and thus, have been largely considered to play a central role in the induction of PTB [15]. Notably, i.u. infections have been significantly better investigated than systemic infections, with the majority of studies focused on the role of bacterial infections in induction of PTB. Although of interest, the contribution of viral and fungal/yeast i.u. infections or the interplay of multiple microbes/pathogens in i.u. infection-driven induction of PTB has been largely undefined—something that represents a major gap in knowledge.

Infection

Although the critical locus/loci of infection remain under-defined, the ability of pathogens to gain access to the i.u. compartment via ascending the cervix and vagina or by hematogenous dissemination through the placenta is well established [13]. Whether a microbe’s ability to disseminate systemically or throughout the reproductive sites represents a central component for induction of PTB remains to be formally defined. Published reports certainly argue in favor of such an observation. Specifically, a common periodontal infectious agent, *Fusobacterium nucleatum*, is also one of the most prevalent species found in amniotic fluid in patients experiencing PTB [16]. Likewise, *Listeria monocytogenes*, a common contaminant of a variety of raw foods, in addition to systemic infection, also has tropism for the placenta and is known to cause PTB [14]. Furthermore, *Toxoplasma gondii*, transmitted by food and domestic animal feces, can cause adverse pregnancy outcome—a fact related to the ability of the parasite to cross the placental barrier [17]. Given this variety of PTB, inducing infectious agents, identification of the critical locus/loci of infection and the biologic process that regulate microbe/host interplay represents a daunting but necessary task toward improving intervention and prevention of PTB.

In contrast to the unknowns about the specifics of a systemic infection, i.u. infections, in the context of PTB, are better defined. Through the use of standard clinical testing (e.g., amniotic fluid culture and PCR), the most common microorganisms identified in amniotic fluid from women with PTB include the following: *Ureaplasma urealyticum, Bacteroides ureolyticus, Streptococcus sanguinis, Ureaplasma parvum, Streptococcus agalactiae, Mycoplasma hominis, Gardnerella vaginalis, Peptostreptococcus sp., Enterococcus sp., Streptococcus sp, *N. gonorrhoeae*, Leptotrichia sp., *S. sanguinis*, *Haemophilus influenzae*, and *Escherichia coli* [18, 19]. Noted of infection with such pathogens has been directly correlated with increased production of i.a. proinflammatory cytokine levels and in particular, IL-6 [18]. However, despite obvious colonization, the ability of these microbes to drive PTB is not well defined. Although *U. urealyticum* is the most frequently identified microorganism in women with PTB [20], it is also known to colonize the amniotic cavity of women with regular parturition [21]. Thus, whether the *U. urealyticum*, or other aforementioned microbes, can induce pregnancy complications alone or in association with other microorganisms needs to be formally examined [22].

Given the association between bacterial infections with PTB, the use of antibiotic therapy to prevent PTB has been investigated [23]. However, clinical trials evaluating the therapeutic efficacy of antibiotic treatment in PTB showed lack of significant association with the reduction of PTB [24]. Possible reasons for the lack of antibiotic therapy effectiveness in PTB may, in part, be a result of the following: 1) the timing of therapeutic application that may be initiated long after infection is established [25] or 2) the difficulties in identifying the specific pathogens and consequent prescribing of the pathogen-specific therapy. Furthermore, recent reports have suggested that antibiotic therapy during pregnancy might, in fact, have deleterious effects on neonatal morbidity—a theory that invokes concerns not only in regard to efficacy but also regarding safety of such therapeutic approaches in PTB [23, 26, 27].

In addition to bacterial pathogens, i.u. and systemic infections with viruses have been associated with PTB. Specifically, the presence of an adenoviral genome in the amniotic fluid is reported in 40% of PTB cases [28]—a fact associated with chorioamnionitis and correlated with adverse pregnancy outcome [29]. However, despite the significance, the role of i.u. viral infections in context of PTB has not been well defined. In contrast, systemic viral infections have gained notoriety in induction of PTB—something directly linked to viral pandemic/epidemic outbreaks. Of note, direct vertical transmission of the influenza virus to the fetus, although extremely rare, has been demonstrated [30]. Thus, the adverse consequences on the fetus in mothers infected by systemic viral pathogens are likely to represent a complex interplay of the mother’s immune response to the pathogen and fetal sensing of local microbial milieu.

Pandemic/epidemic viral outbreaks are well known to increase the risk of severe illness and mortality in pregnant women [14]. A highly pathogenic/pandemic strain of influenza (e.g., 1918 and 2009 pandemics and 2005 avian influenza) [14] and severe acute respiratory syndrome [31] strongly associate with incidence of PTB and induction of adverse pregnancy outcomes [32, 33]. Specifically, during the 2009 H1N1 influenza A pandemic, pregnant women were at an increased risk for hospitalization and death compared with the general population [14]. Thus,
pregnant women represent a population for which special considerations for prevention and treatment for influenza infection have been recommended [34]. Of note, vaccines represent one of the most effective interventions, with recent reports strongly suggesting that vaccination against influenza virus protects from PTB [35, 36]. However, whether viral infection-driven immune activation is directly associated with induction of PTB has not been formally defined. Therefore, a better understanding of the role of viral infection in the context of pregnancy is required for the discovery of new strategies for overall protection of maternal and neonatal health. Although prenatal care guidelines for a diagnosis of viral infections (e.g., rubella, cytomegalovirus, and HSV) during pregnancy are in existence, it is well established that conventional viral therapies are inadequate for pregnant women. Furthermore, to date, no treatments focused on prevention of virus-induced adverse consequences during pregnancy have been defined.

In addition to bacterial and viral infections, the role of fungal infections in the context of PTB has not been well investigated. The limiting factor is represented by the poor reference databases with specific sequences for fungi/yeast identification [18]. The knowledge about the consequences of the fungi/yeast in the uterine cavity appears to be limited to Candida species. Specifically, the presence of Candida albicans has been detected in amniotic fluid from i.u. infection samples [29].

Overall, there is a growing evidence of an association among pathogens, including bacteria, virus and fungi, and adverse pregnancy outcome. In particular, intracellular pathogens (both facultative and obligate) are poorly studied, as a result of technical difficulties of their detection [18, 37, 38]. Further studies and new technologies to detect the presence of pathogens are thus clearly needed to better define the role of infections in induction of PTB.

**Inflammation**

Chronic inflammatory and complex immunologic abnormalities, occurring in the absence of well-defined infectious triggers, have similarly been correlated with PTB. Notably, the rate of PTB is significantly more frequent in women affected by obesity [39] and autoimmune diseases, including SLE [40], multiple sclerosis [41], and Type 1 diabetes [42]. Furthermore, the maternal race has been associated with both adverse pregnancy outcomes and development of autoimmune diseases. In fact, PTB and SLE are known to occur with higher frequency among African-American women compared with Caucasian women [1, 43]. Despite the strong correlation, however, the role of maternal race is still an open debate. Previous studies suggest that the measures of neighborhood socioeconomic deprivation were associated with poor newborn outcome independently from race (white, African-American, Hispanic, and Asian) [44]. This idea has been reverted by another study showing that genetic diversity per se in humans possibly accounts for a fraction of PTB, even in some macro-ethnicities, such as African-American [45]. Thus, whether maternal race and/or ethnicity and genetic components play a role in induction of PTB requires further examination.

In addition to chronic inflammation, breakdown of the maternal/fetal tolerance, similar to an allograft rejection, can lead to adverse pregnancy outcome and PTB—evidence observed in chronic chorioamnionitis [46]. Exposure to fetal DNA that arises by the maternal immune system-driven elimination of fetal cells or from spontaneous fetal cell apoptosis has been correlated with adverse pregnancy outcomes, including PTB [47]. Furthermore, endogenous triggers, uncovered during tissue injury and inflammation, have been correlated with adverse pregnancy outcomes. Specifically, high levels of the HMGB1 protein have been reported in patients with i.u. infection/inflammation [48]. Whether endogenous triggers can induce robust inflammation sufficient for induction of PTB and whether they sensitize the host to subsequent microbial insult or work in parallel with an infectious/inflammatory insult have not been examined.

In summary, a complex interplay between infection/inflammation (both systemic and i.u.) and pathogen/host biologic processes appears to play a central role in defining pregnancy outcomes. Further studies are clearly needed to better define the immune mechanisms underlying infection and/or inflammation-driven PTB.

**ANIMAL MODELS FOR PTB**

Despite significant differences among mammalian species in the biology of pregnancy and parturition, animal models have been highly useful for understanding the molecular mechanisms involved in induction and regulation of birth [49]. Current, popular animal models of preterm parturition include nonhuman primates, sheep, and lower mammalian species (e.g., mouse, rat, guinea pig, rabbit). Despite obvious limitations, each model can provide strong advantages specific to the experimental question being examined.

Here, we provide a brief description of the major features of each animal model. Of note, a detailed comparison between humans and animal models is beyond the purview of this manuscript and has been discussed in detail elsewhere [50, 51].

**Nonhuman primates**

The similarity of reproductive biology and biologic processes, in general, between humans and nonhuman primates makes this the ideal model to study PTB. However, despite the obvious advantages, the use of nonhuman primates to study PTB has clear limitations. Specifically, the availability of large numbers of nonhuman primates and specific research reagents is somewhat limited. Furthermore, a long gestation period compared with other experimental animal models and high cost associated with executing well-structured investigations present difficulties in the use of nonhuman primates for studying PTB.

**Sheep**

The similarity in gestation length to humans and number of offspring makes sheep a well-established animal model for studying fetal inflammation [52]. However, divergence in 1) anatomy, 2) reproduction, and 3) placentaion between humans and sheep is obvious. In particular, compared with humans that have a unicorneate uterus and a hemochorial placenta, sheep have a bicornuate uterus and an epitheliochorial placenta. Moreover, parturition in sheep is preceded by progesterone
withdrawal, a consequence not observed in human and non-human primates [53]. Lastly, in humans, the fetus invades into the uterine lining, whereas in sheep, there are numerous discrete attachment sites [54]. Overall, these differences have all significantly impacted the use of sheep as a model for studying PTB [51, 54].

**Mice**

Despite the obvious limitations, mice represent the most common animal model of PTB. The major concerns for the use of mice as a model of PTB include the following: 1) significant difference in the length of gestation period; 2) difference in biologic processes regulating parturition (as in sheep, parturition in mice involves progesterone withdrawal); 3) different locus of progesterone production (ovary through the gestation in mice; placenta after early stage in humans); and 4) significant differences in placentation (in particular, trophoblast invasion of uterine arteries, transformation of uterine arteries, and placental exchange area) [50, 55, 56]. Indeed, mice are not humans; however, the pathways regulating immune responses are highly conserved between mice and humans, indicating that mice are a useful experimental tool for the interrogation of the role of infection/inflammation in induction of PTB [57, 58]. Furthermore, low cost, ability of rapid and complex genetic manipulation, availability of research reagents, and short gestational period have all led to mice being the most popular animal model for studying PTB. In addition to mice, rats, guinea pigs, and rabbits are tractable, small animal systems that have all contributed to PTB studies. However, unlike mice, their fully sequenced genome is not yet completed. Therefore, fewer research reagents and experimental protocols have been developed so far. The summary of various animal models and their characteristics in PTB is depicted in Table 1.

**INNATE IMMUNE RESPONSE RECEPTORS**

TLRs, NLRs, cytosolic RLRs, surface expressed CLR, and AIM-2-like receptors, as well as a family of intracellular enzymes that sense nucleic acids, including 2'-5' oligo-adenylate synthase proteins and cyclic GMP-AMP synthase, are the major innate immune sensors that provide immediate responses against pathogenic infection or tissue injury [59–62]. Members of different innate immune receptors are able to recognize the same ligand. For example, TLRs and RLRs can sense the presence of viral component dsRNA [63], or TLRs and NLRs recognize PGN [64, 65]. These findings suggest that innate immune system is equipped with several levels of surveillance against the infectious agents. Of note, detailed review of various innate immune receptors/sensors and their role in shaping subsequent immune response is beyond the purview of this manuscript and has been discussed in detail elsewhere [59, 60].

Unlike TLRs, NLRs, RLRs, CLRs, and AIM-2-like receptors have not been investigated extensively in PTB. Thus, here, we will discuss the role of TLRs in the context of PTB. TLRs are a family of evolutionarily conserved innate immune receptors (10 in humans and 13 in mice). Activation of TLR signaling by conserved microbial molecular structures, called -pathogen-associated molecular patterns, and endogenous molecules uncovered upon tissue injury, called damage-associated molecular patterns, drive proinflammatory cytokine production, chemokines, PGs, and other molecules (e.g., Cox-2 and Connexin 43) and the induction of innate and adaptive immune responses [61, 66]. Of note, these molecules play a major role in maternal/fetal tissues as mediators of cell signaling [67, 68].

In particular, TLR4 and TLR2 are critical for recognition of Gram-negative (e.g., LPS) and Gram-positive (e.g., LPs) bacterial components, respectively. TLR5 senses the presence of flagellin from Gram-positive and -negative bacteria. A subfamily consisting of TLR3, TLR7/8, and TLR9 specifically recognize nucleic acid motifs [Poly (I:C), ssRNA, and CpG, respectively] [69, 70]. TLRs also signal the presence of nonmicrobial products, including a variety of endogenous molecular structures (e.g., fibronectin extra domain A, heat shock proteins, HMGB1, and minimally oxidized LDL), generated or unmasked during tissue injury and inflammation [71–74].

Ligand-induced activation leads to TLR homo- and heteromultimerization. Subsequent signaling depends on constitutively associated or recruited adapter proteins that interact with TLRs through structurally conserved Toll-IL-1R domains present in both TLRs and adapters. In turn, the TLR adapter molecules recruit a variety of kinases and substrates, leading to the activation of distinct signaling pathways that result in the activation of pathway-specific transcription factors [75]. Although of significant interest, the role of signaling pathways in induction of inflammatory cascade is beyond the purview of this manuscript.

TLRs are widely expressed by diverse immune cells and nonimmune cells [60, 61]. Of note, TLRs are also expressed at

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**TABLE 1. Characteristics of animal models compared with human**

<table>
<thead>
<tr>
<th>Species</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Rhesus macaques</td>
<td>Reproductive biology; biologic processes similar to human</td>
<td>Limited number of animals per experiment; high cost</td>
</tr>
<tr>
<td>Sheep</td>
<td>Gestation length; number of fetuses similar to human</td>
<td>Placentation and uterus anatomy; limited number of animals per experiment; progesterone withdrawal before labor; high cost</td>
</tr>
<tr>
<td>Mice</td>
<td>Rapid genetic manipulation; high number of animals per experiment; low cost</td>
<td>Gestation length, number of fetuses, and placentation; progesterone withdrawal before labor; different source of progesterone</td>
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the maternal-fetal interface by the pregnant uterus, placenta, and amniotic membranes and in cytotrophoblast and syncytiotrophoblast [76–78]. Moreover, their expression can vary according to the stage of pregnancy [79]. Further human studies have linked genetic components, in particular, polymorphisms of TLR pathway genes, with PTB [80, 81]. Such findings provide evidence that TLRs may play an important role in regulation of parturition via sensing of infectious and endogenous molecular patterns, induction of inflammation, and orchestration of the subsequent immune responses.

In mice and nonhuman primates, challenge with diverse TLR ligands, via i.p., i.u., or i.a. routes, increases proinflammatory cytokine release in the uterus and fetal membranes [82–84], recruits immune cells into the cervix [85], and induces PTB [84, 86]. Specifically, in nonhuman primates, administration of a TLR4 antagonist before LPS challenge inhibited uterine contractility and reduced proinflammatory cytokine production [86]. In mice, TLR4 stimulation induces PTB [87], whereas its blockade reduced LPS-induced PTB [88, 89]. Moreover, TLR4 mutant mice are protected from LPS-induced PTB [88, 90]. Furthermore, systemic or i.u. challenge with bacterial cell wall component PGN, and LTA have been shown to induce PTB in mice [84, 91]—events assumed to occur via TLR2 activation. However, PGN and LTA preparations are often contaminated with lipoproteins and activate other receptors [64]. In contrast to the proinflammatory immune mediators, anti-inflammatory cytokines (e.g., IL-10) have been shown to regulate the inflammatory response and limit inflammation-induced tissue damage. IL-10 is highly expressed in the uterus and placenta during pregnancy and has been implicated directly in regulation of inflammation-induced human pregnancy complications [104]. In particular, decreased IL-10 expression in human PTB has been demonstrated, suggesting the relevance of IL-10 in down-regulating inflammatory responses in the gestational tissues [105].

Importantly, animal models have been central to the definition of the role of immune mediators and cytokines, in particular, in induction of PTB, as well as the locus of infection critical to induce PTB. Infusion of IL-1 or TNF into the amniotic fluid of Rhesus macaques leads to marked increases in i.a. proinflammatory cytokines levels [106] or chorioamnionitis [107]. Whether the presence of such immune mediators alone in the amniotic cavity is sufficient to drive PTB remains somewhat controversial [106, 107]. However, systemic infusion of IL-1 and TNF was shown to be sufficient for induction of PTB in mice [108, 109]—evidence blocked by preadministration of the IL-1R antagonist before LPS challenge inhibited uterine contractility and reduced proinflammatory cytokine production [86]. In mice, TLR4 stimulation induces PTB [87], whereas its blockade reduced LPS-induced PTB [88, 89]. Moreover, TLR4 mutant mice are protected from LPS-induced PTB [88, 90]. Furthermore, systemic or i.u. challenge with bacterial cell wall component PGN, and LTA have been shown to induce PTB in mice [84, 91]—events assumed to occur via TLR2 activation. However, PGN and LTA preparations are often contaminated with lipoproteins and activate other receptors [64].

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**Cytokines**

Human studies support the association between elevated levels of circulating proinflammatory cytokines and PTB [96, 97]. Specifically, human studies have implicated IL-1, TNF, and IL-6 as major players in the onset of PTB [98, 99]. Furthermore, polymorphisms in pro- and anti-inflammatory genes, including the above-mentioned mediators, have been associated with PTB [100, 101]. Recently, IL-6 was identified as a critical marker of i.a. inflammation [102] and a predictor of PTB; increased amniotic fluid IL-6 levels from the second trimester were associated with the timing and initiation of PTB [103].

<table>
<thead>
<tr>
<th>TLR</th>
<th>Ligand</th>
<th>Route of challenge</th>
<th>Mouse genotype</th>
<th>Pregnancy outcome</th>
</tr>
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<tbody>
<tr>
<td>TLR4</td>
<td>LPS</td>
<td>i.p. [88] i.u. [87]</td>
<td>WT</td>
<td>PTB</td>
</tr>
<tr>
<td>TLR4</td>
<td>LPS</td>
<td>i.p. [88] i.u. [90]</td>
<td>TLR4−/−</td>
<td>Normal birth</td>
</tr>
<tr>
<td>TLR3</td>
<td>Poly (I:C)</td>
<td>i.p. [92] i.u. [84]</td>
<td>WT</td>
<td>PTB</td>
</tr>
<tr>
<td>TLR2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>PGN, LTA</td>
<td>i.u. [84]</td>
<td>WT</td>
<td>PTB</td>
</tr>
<tr>
<td>TLR9</td>
<td>CpG</td>
<td>i.p. [95]</td>
<td>IL-10−/−</td>
<td>PTB</td>
</tr>
<tr>
<td>TLR9</td>
<td>CpG/fetal DNA</td>
<td>i.p. [47]</td>
<td>WT</td>
<td>Fetal resorption or PTB</td>
</tr>
<tr>
<td>TLR9</td>
<td>CpG/fetal DNA</td>
<td>i.p. [95]</td>
<td>TLR9−/−</td>
<td>Normal birth</td>
</tr>
</tbody>
</table>

<sup>a</sup>PGN and LTA preparations are often contaminated with lipoproteins and activate other receptors [64, 65, 93].
antagonist [110]. Furthermore, IL-6-deficient mice exhibit delayed, spontaneous parturition (24 h later than WT mice) and have been shown to be resistant to LPS-induced PTB [111]. In contrast, IL-10-deficient mice are more susceptible to low-dose, LPS-driven PTB compared with WT mice [112]. Mechanistically, it has been proposed that IL-10-dependent silencing of TNF, IL-6, and IL-1 attenuate the overall inflammatory response and thus, reduce infection/inflammation-induced PTB [113]. Therefore, these findings indicate that interplay between pro- and anti-inflammatory cytokines plays an important role in parturition and in PTB [111]. The summary of various immune mediators and TLR ligands in induction of PTB is depicted in Table 3.

### INNATE IMMUNE CELLS AND PTB

The maternal innate immune system plays a pivotal role in all phases of human pregnancy. Furthermore, innate immune cells predominate in the decidua, where they interact with trophoblasts in supporting fetal growth [114, 115]. At the site of implantation neutrophils, NK cells, macrophages, and DCs provide the proangiogenic and proinflammatory environment that is necessary for decidual and trophoblast development [116–118]. During the pregnancy progression, at the interface between maternal and fetal tissues, decidual innate immune cells also actively collaborate with the adaptive branch of the immune system to tolerate fetal alloantigens and allow the fetus to grow and develop in the uterus in spite of being recognized by maternal immune cells [119]. Additionally, innate immune cells actively participate in the labor process; in fact, as discussed above, labor is an inflammatory process [120]. Of note, the decidua is ideally placed to coordinate inflammatory events via paracrine cross-talk between the adjacent myometrium and fetal membranes [121]. Decidual activation (high PG and proinflammatory cytokines levels) is thought to be a critical, early event in the induction of parturition [122, 123]. Thus, the activation of immune cells from infectious or noninfectious triggers can lead to a disruption of the homeostatic balance, which may play an important role in promoting PTB. The possible roles of various innate immune cells in regulation of PTB are discussed below.

#### Neutrophils

Neutrophils are the most abundant leukocytes in the peripheral blood. Endowed with several antimicrobial effector mechanisms, neutrophils represent the first line of innate immune defense against most bacterial agents and as such, are considered to be central effectors of acute inflammation [124]. Specifically, to date, 2 distinct populations of neutrophils have been described, termed N1 and N2. N1 neutrophils are highly cytotoxic, immunostimulatory, and inflammatory cells. In contrast, N2 neutrophils are known to exert strong immunosuppressive activity, lack cytotoxic potential, and exhibit proangiogenic and vascular remodeling properties [125]. Of note, the presence of proangiogenic neutrophils in human and murine decidua during the second trimester of normal pregnancy has been reported [126]. In addition to their role in the maintenance of pregnancy, the role for neutrophils in the induction of parturition is still under debate: studies suggest that decidual neutrophils contribute to labor by producing several inflammatory mediators and MMPs that favor the rupture of membranes [127]. However, there are studies in animal models that neutrophil depletion does not alter the onset of normal labor [128] and infection/inflammation-driven PTB [129, 130]. Thus, further studies are required to elucidate the role of neutrophils in the context of PTB.

In addition, as neutrophils are important in establishment of antibacterial immune defenses, they are, by far, the most predominant leukocyte population in acute chorioamnionitis, as well as other infectious conditions associated with PTB. However, the mechanisms regulating neutrophil recruitment to mucosal sites, myometrium, and fetal membranes and their role in promotion of PTB are not well understood and currently represent an area of active investigation [119, 122].

#### NK cells

Besides their ability to exert natural and antibody-dependent cell cytotoxicity, NK cells deeply affect innate and adaptive immune responses through the secretion of variety of cytokines and chemokines. NK cells can be divided into different subsets based on the intensity of CD56 and CD16 surface expression: CD56<sup>bright</sup>/CD16<sup>dim</sup> subset (immature NK cells), CD56<sup>dim</sup>/CD16<sup>hi</sup> subset (highly cytotoxic mature effector NK cells), and CD56<sup>seg</sup>/CD16<sup>pos</sup> subset (minor subset of NK cells with impaired effector functions) [131–133]. Of note, NK cells are the dominant leukocyte population in the uterine decidua, representing ~70% of total leukocytes. Contrary to peripheral blood NK cells that are mostly mature CD56<sup>dim</sup>/CD16<sup>pos</sup> cells, dNK cells mostly belong to the noncytotoxic, immature CD56<sup>bright</sup>/CD16<sup>seg</sup> subset. Likely, as a consequence of the local environmental stimulation, these CD56<sup>bright</sup>/CD16<sup>seg</sup> dNK cells differ from their circulating counterpart in their transcriptional profile, characterized by the expression of high levels of cytokines, chemokines, and angiogenic factors [119]. Accordingly, increasing evidence suggests that the pre-eminent role of dNK cells is to promote adequate placentalation. In humans, dNKs first appear in the secretory endometrium before implantation.

### TABLE 3. Cytokines and PTB

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Animal model</th>
<th>Route of challenge</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>Mouse (C3H/HeJ)</td>
<td>Subcutaneous injection [108]</td>
<td>PTB</td>
</tr>
<tr>
<td>TNF</td>
<td>Mouse (C3H/HeJ)</td>
<td>i.p. [109]</td>
<td>PTB</td>
</tr>
<tr>
<td>IL-1 or TNF</td>
<td>Rhesus macaques</td>
<td>i.a. [106]</td>
<td>↑ Proinflammatory cytokines</td>
</tr>
<tr>
<td>IL-1</td>
<td>Rhesus macaques</td>
<td>i.a. [107]</td>
<td>Chorioamnionitis</td>
</tr>
<tr>
<td>LPS</td>
<td>Mouse (IL-10&lt;sup&gt;−/−&lt;/sup&gt;)</td>
<td>i.p. [112]</td>
<td>↑ PTB</td>
</tr>
<tr>
<td>LPS</td>
<td>Mouse (IL-6&lt;sup&gt;−/−&lt;/sup&gt;)</td>
<td>i.p. [111]</td>
<td>↓ PTB</td>
</tr>
</tbody>
</table>
suggesting a role during the peri-implantation period. As pregnancy progresses, dNK cells promote the uterine vascular changes needed for maximizing placental vascularization by crucially participating in the process of spiral arteriolar remodeling and trophoblast invasion. Notably, the mechanisms involved in this proangiogenic activity of dNK cells mainly depend on killer cell Ig-like receptor-mediated interactions with HLA molecules expressed on extravillous trophoblast. In particular, the interaction of dNK cells with polymorphic HLA-C molecules, as well as with nonclassical HLA-E and HLA-G molecules, altogether influences trophoblast invasion and vascular remodeling [119, 134–137]. Furthermore, the interaction of dNK cells with HLA-E and HLA-G molecules expressed by extravillous trophoblast has been shown to be critically involved in the inhibition of dNK cell cytolytic functions during normal pregnancy [119, 136]. Notably, the recent findings that human trophoblast cells promote the activation and cytotoxicity of dNK cells upon hepatitis C virus sensing [138] clearly indicate that NK cells at the feto-maternal interface can switch their phenotype in case of infection and suggest a possible involvement of these cells in the pathogenesis of PTB.

Macrophages

Macrophages are versatile innate immune cells that participate in a wide variety of biologic processes. In the uterine decidua, they represent a major leukocyte subset throughout pregnancy, representing ~20% of total leukocytes. Because of their high plasticity, macrophages respond to tissue environmental stimuli by acquiring distinct functional phenotypes [139]. Specifically, the M1 phenotype is characterized by strong microbicidal and tumoricidal activity, high production of reactive nitrogen and oxygen species, high production of proinflammatory cytokines, and promotion of Th1 response. In contrast, the M2 phenotype is characterized by efficient phagocytic activity, promotion of tissue remodeling, parasite containment, tumor progression containment, and immunoregulatory functions. In fact, these M1–M2 phenotypes mirror the Th1–Th2 polarization of T cells [140, 141]. In addition to immunologic processes, macrophages actively participate in the pregnancy, as transient inflammatory phase is required for successful implantation. Notably, during the peri-implantation period, the polarization pattern of decidual macrophages is skewed toward M1. As trophoblasts attach to the endometrium and invade the uterine stroma, decidual macrophages undergo a transition to a mixed M1–M2 profile [142]. These mixed M1–M2 macrophages persist until midpregnancy and cooperate with trophoblast cells to support an extensive remodeling of uterine vasculature needed for an adequate placental-fetal blood supply [143]. Following the completion of placental development, decidual macrophages have been shown to undergo M2 polarization, characterized by abundant production of IL-10 and indoleamine 2,3-dioxygenase. These M2 features promote maternal immune tolerance to semiallogenic fetus and fetal growth until parturition [144]. Finally, decidual macrophages are among the primary innate immune cells that contribute to parturition [145]. The role of decidual macrophages in the regulation of cervical ripening and the initiation of labor mainly relies on their secretion of the proinflammatory cytokines IL-1, IL-6, and TNF, as well as MMPs and NO [127].

Increasing evidence indicates that an aberrant activity of uterine macrophages is likely involved in the etiology of PTB. In fact, similarly to term labor, idiopathic PTB is preceded by selective accumulation of decidual macrophages [145]. Moreover, augmented levels of chemokines in the amniotic fluid of women undergoing idiopathic and infection-associated PTB correlate with an increased monocyte/macrophage recruitment [127]. Furthermore, macrophage depletion protects pregnant mice from LPS-induced PTB [146, 147].

As these cells play a central role in the maintenance of fetal-maternal tolerance and in the initiation of labor, regulation of macrophage trafficking and activation/polarization during normal parturition and PTB need further investigation.

DCs

DCs are APCs that bridge innate and adaptive immunity and thus, play a central role in the maintenance of immune homeostasis during normal pregnancy. DCs are divided into 2 main subsets: mDCs and pDCs, which in turn, are characterized by distinct phenotype, cytokine profile, and function. Specifically, mDCs orchestrate proinflammatory responses upon activation by microbes, danger signals, and other immune cells. In contrast, pDCs have different functions based on their maturation status: tolerance maintenance in their immature status and antiviral defense in their mature status [148–153].

Notably, environmental, tissue-specific signals are central to shaping DC behavior [154]. Accordingly, environmental factors coming from the specialized uterine stroma, together with the hormonal milieu of pregnancy, strongly affect dDCs. In humans and in mice, dDCs are characterized by features that agree with their role in fetal tolerance. Furthermore, in normal pregnancy, dDCs have been shown to participate in decidua tissue remodeling, thus playing a crucial role in the formation of decidua during embryo implantation [155]. The DCs present within the decidua mainly belong to the mDC subset [134] and are characterized by low expression of costimulatory molecules, low capacity for antigen presentation, and low production of the Th1-skewing cytokines, suggesting that dDCs regulate the Th1/Th2 balance to maintain a Th2-polarized decidua [156–158]. Moreover, an increase of DC-derived IL-10 has been described in dDCs during most of the gestation period [159].

In addition to the maternal-decidual interface, pregnancy is known to alter pbDCs as well. We and others [158, 157] have demonstrated that during normal pregnancy, circulating mDCs and pDCs undergo profound changes that likely reflect the maternal systemic reaction to the fetus, an indication of potential relevance to the maintenance of fetal tolerance and the favorable outcome of healthy pregnancy. In particular, mild variations in the number of pbDCs during pregnancy result in an increased mDC/pDC ratio that is consistent with the predominance of mDCs occurring in the decidua. Importantly, mDCs and pDCs undergo a progressive state of incomplete activation, characterized by increased expression of costimulatory molecules and cytokine production but lacking HLA-DR up-regulation [159]. This peculiar pattern of incomplete activation may reveal a further mechanism operated by the immune system to maintain fetal tolerance. Our recent demonstration that pbDCs from
pregnant women with i.u. growth restriction lack this state of activation supports the involvement of DCs in sustaining the normal growth of the fetus [160]. Because of the role played by DCs in maternal immune adaptation to pregnancy and fetal health, an incoming, premature activation of DCs may likely participate in the etiology of PTB [127, 156]. However, studies specifically demonstrating this aspect of DC function have not been reported.

An overview of the interplay among infection, innate immune cells, and immune mediators at the maternal/fetal interface is shown in Fig. 1.

CONCLUSIONS

Infection and inflammation are major risk factors for PTB. However, the molecular triggers and mechanisms underlying the activation of immune pathways associated with induction of PTB remain poorly understood. Here, we reviewed the critical involvement of the innate immune sensors/receptors (TLRs, in particular), cytokines, and innate immune cells in the immunopathogenesis of PTB. Furthermore, we discussed the features of various animal models commonly used to dissect the mechanisms underlying infection/inflammation-driven PTB and have alluded to critical gaps of knowledge in the field.

Several types of pathogens (including bacteria, viruses, and fungi) that disseminate systemically or through the placenta play an important role in induction of PTB. The sensing of pathogen or endogenous ligand (uncovered during tissue injury and/or inflammation) by innate immune receptors and subsequent induction of immune mediators play an important role for shaping the phenotype and activity of various innate immune cells that predominate in the decidua and are known to participate in the labor process. Furthermore, such studies imply that a disruption of homeostasis, either systemically or at the maternal/fetal interface, by an infection and/or inflammatory triggers, contributes to adverse pregnancy outcomes.

However, numerous critical questions pertaining to inflammation-driven PTB remain unanswered, including the following: 1) the identification of a causative infectious agent(s) (e.g., bacterial, viral, and fungal); 2) the role of polymicrobial infection; 3) the critical locus/loci of infection; 4) the critical immune cell(s); and 5) the critical inflammatory and/or immunoregulatory pathways. Of note, adverse pregnancy outcomes have been well correlated with bacterial infections, including chorioamnionitis. However, the sequelae of viral or fungal infections remain poorly understood and warrant further investigations. The newly proposed “double-hit hypothesis” [161] suggests that PTB is a consequence of polymicrobial infection: viral infection can sensitize to a subsequent bacterial infection. However, the mechanisms underlying such processes remain underdefined. Additional medical screening of pregnant women for signs of infections and infection-associated immune mediators thus may lead to the discovery of novel biomarkers, identify

Figure 1. Overview of the interplay among infection, innate immune cells, and immune mediators at the maternal/fetal interface. Innate immune cells and trophoblasts at the maternal/fetal interface robustly express innate immune receptors, including TLRs, and respond to pathogens via production of a wide range of chemokines and proinflammatory cytokines. In turn, such milieu results in recruitment and activation of innate immune cells, amplification of inflammation, and induction of PTB. Despite the available information, numerous gaps in knowledge need to be addressed, including the following: 1) the identification of a causative infectious agent(s); 2) the critical locus/loci of infection and of immune response (maternal or fetal side); 3) the critical immune cell(s); and 4) the critical inflammatory and/or immunoregulatory pathways.
possible at-risk pregnancies, and help to define specific drugs (e.g., specific inhibitors and antibiotics, respectively) for an effective intervention.

Furthermore, to study these complex relationships, additional effort should be placed on interdisciplinary approaches spanning the disciplines of reproductive biology, infectious diseases, and immunology. Such combined approaches would allow for a detailed mechanistic interrogation of the biologic processes at the interface of pregnancy, infection/inflammation, immunology, and their intertwined and clinically relevant complications. Moreover, translation of basic scientific findings into therapeutic interventions for patients should be encouraged. In fact, use of such approaches toward in-depth analysis of immunologic processes and cellular and molecular mechanisms underlying induction of parturition (term or preterm) may lead to discovery of novel therapeutics aimed at reduction of the morbidity and mortality associated with PTB.

AUTHORSHIP
M.C. and S.D. contributed to the scope and setup of and edited and critically reviewed the manuscript. M.C., S.D.B., E.F., D.M., and S.D. wrote the review. All authors approved the final version.

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The authors declare no conflicts of interest.

REFERENCES
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51. VG24.
54. S20.
infection/inflammation is associated with induction of preterm birth


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