REVIEW

Pathogenesis of preterm birth: bidirectional inflammation in mother and fetus

Ella Shana Green[1] · Petra Clara Arck[1] 📷

Abstract

Preterm birth (PTB) complicates 5–18% of pregnancies globally and is a leading cause of maternal and fetal morbidity and mortality. Most PTB is spontaneous and idiopathic, with largely undefined causes. To increase understanding of PTB, much research in recent years has focused on using animal models to recapitulate the pathophysiology of PTB. Dysfunctions of maternal immune adaptations have been implicated in a range of pregnancy pathologies, including PTB. A wealth of evidence arising from mouse models as well as human studies is now available to support that PTB results from a breakdown in fetal-maternal tolerance, along with excessive, premature inflammation. In this review, we examine the current knowledge of the bidirectional communication between fetal and maternal systems and its role in the immunopathogenesis of PTB. These recent insights significantly advance our understanding of the pathogenesis of PTB, which is essential to ultimately designing more effective strategies for early prediction and subsequent prevention of PTB.

Keywords Preterm birth · Labor · Mouse models · Regulatory T cells · Inflammatory signaling pathways · Microbiome · Fetal signals

Introduction

Preterm birth (PTB), defined as birth before the 37th week of gestation, affects up to 15 million pregnancies each year globally [1]. Being born too soon can lead to neonatal death, but also a high risk for early-life infections and neurodevelopmental, cardiometabolic, and inflammatory disorders later in the life of surviving infants [2–6]. A current understanding of the complex pathogenesis of PTB is still poor, which also explains the limited availability of targeted and effective strategies for PTB prevention.

Several risk factors for PTB are well recognized, such as twin pregnancies, chorioamnionitis, pre-existing maternal

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✉ Petra Clara Arck
p.arck@uke.de

[1] Department of Obstetrics and Fetal Medicine, Laboratory for Experimental Feto-Maternal Medicine, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20251 Hamburg, Germany

diseases, genetic factors, previous PTB, and uterine abnormalities [7–10]. However, a large proportion of PTBs have no identifiable cause. These spontaneous PTBs present as pathological inflammation, premature rupture of membranes (PROM), and onset of preterm labor.

Interestingly, inflammation is also a key trigger of the physiological onset of labor at term [11]. Thus, the onset of inflammation seems to be a common denominator initiating term as well as preterm labor. Here, we comprehensively review why inflammation may be prematurely initiated in some pregnancies, which then leads to PTB.

A brief overview of maternal immune adaptation during normally progressing pregnancies

In order to understand the onset of inflammation during pregnancy, it is pivotal to summarize milestones of pregnancy (Fig. 1), including key features of feto-maternal immune tolerance, as the breakdown of such immune tolerance is associated with a number of pregnancy complications [14–17].

As covered by a large number of reviews [14, 15, 17–20], the need for feto-maternal immune tolerance arises from the
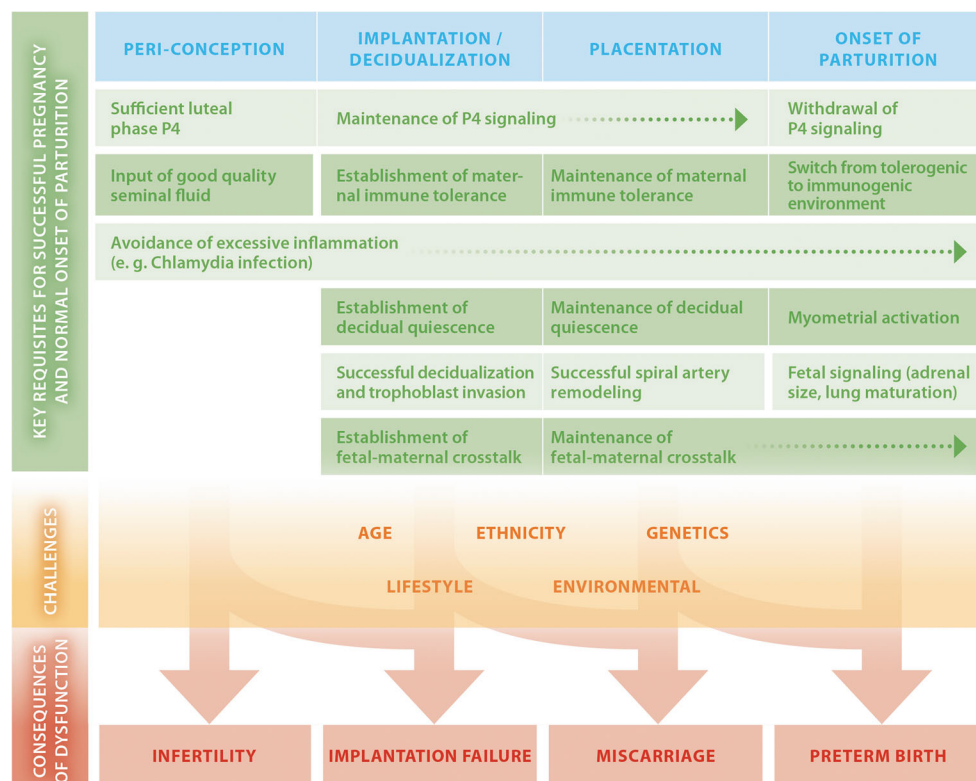
Fig. 1 Milestones of pregnancy and related immune and endocrine adaptations. Normally progressing pregnancies result from the successful orchestration of processes involved in peri-conception, implantation, decidualization, and placentation. Hormone signaling, in particular, progesterone (P4), establishes a receptive endometrium and decidual quiescence; maternal immune tolerance mitigates the risk for fetal rejection and supports spiral artery remodeling and placentation. The onset of parturition is characterized by a switch in the local immune environment from a tolerogenic state to an activation state, a reversal of decidual quiescence in favor of myometrial activation, and a withdrawal of P4 signaling. Maternal and paternal lifestyle factors (such as stress and nutrition), environmental factors, age, ethnicity, and genetics contribute to the success or failure of pregnancy, including the risk for PTB [9, 12, 13]

expression of paternal antigens on fetal tissues. The maternal immune system recognizes and responds to these fetal/ paternal antigens firstly at coitus, when the reproductive tract comes into contact with seminal fluid, followed by continuous paternal antigen contact throughout pregnancy upon the invasion of fetal trophoblast cells into the endometrium. Notably, the process of implantation itself is an inflammatory process, during which cytokines, chemokines, and prostaglandins contained in seminal fluid elicit pro-inflammatory cytokine production and leukocyte recruitment within the uterus [14]. Around the time of implantation, this inflammation needs to be resolved and a tolerogenic environment induced. Numerous mechanisms are mounted in the induction of such tolerogenic environment, including the release of anti-inflammatory molecules such as TGFβ [21] and the induction of specialized, anti-inflammatory T cells known as CD4+FOXP3+ regulatory T (Treg) cells, which constrain anti-fetal inflammatory immune responses [22–26]. Tolerogenic dendritic cells (DCs) uniquely present in the decidua cross-present fetal antigens to maternal CD4+ T cells, thereby also activating the generation of Treg cells [27]. Treg cells reciprocally interact with DCs and macrophages, modifying their phenotypic states to be pro-tolerogenic [28]. Importantly, Treg cells protect against anti-fetal immune responses by limiting existing T effector (Teff) cell responses and maintaining anergy in the T conventional (Tcon) cell population that would otherwise become Teff cells [25, 29]. Placental cells also contribute to the generation of a tolerogenic environment, e.g., via trophoblast-derived colony-stimulating factor (CSF)1 (formerly M-CSF) and interleukin (IL)10, which induces decidual macrophages with a regulatory "M2" phenotype, expands functionally suppressive CD4+FOXP3+ Treg cells, and limits activation of T helper (Th)1-, Th17-, and Th2-cytokine-producing Teff cells [30].

Maternal-fetal crosstalk and reproductive success

In normal pregnancy, there is extensive crosstalk between maternal and fetal systems due to the sharing of circulatory systems in the placenta. Nutrients and metabolic waste products are exchanged from mother to fetus and vice versa, along with cells, signaling molecules, extracellular vesicles (ECVs),

and nucleic acids. The exchange of cells results in microchimerism in mother or fetus, whereby maternal cells transferred to the fetus are referred to as maternal microchimerism (MMC). Reciprocally, fetal cells transferred to the mother are termed fetal microchimerism (FMC). Both, MMC and FMC can persist for decades after pregnancy in a broad range of tissues in the body, including the brain, skin, heart, lung, bone marrow, spleen, and lymph nodes [31–34].

MMC cells promote tolerance to maternal antigens in fetal immune cells during pregnancy through the induction of non-inherited maternal antigen (NIMA)–specific CD4$^+$FOXP3$^+$ Treg cells [35]. Furthermore, they may enhance reproductive fitness in the next generation through sustained tolerance to NIMA. However, these insights are solely based on a mouse model to date and limited to settings where the paternal antigens and NIMA were shared [36].

In addition, microvesicles, exosomes, and cell-free (cf) proteins and nucleic acids such and cfRNA and cfDNA are exchanged via the placenta. In general, the concentration of cfRNA in maternal circulation increases over the course of gestation [37, 38], and fetal cfDNA and RNA transcripts also increase in maternal circulation late in gestation [39–42]. Some fetal cfDNA and RNA are contained within apoptotic fetal cells [39, 41], but immunosuppressive CD71$^+$ fetal erythroid cells are also an abundant source of fetal DNA in maternal circulation [37]. The cfDNA and RNA transcripts could be transferred passively, or via ECVs. Exosomes are a type of ECV released by exocytosis into the extracellular environment. Notably, the placenta releases exosomes into maternal circulation during pregnancy [43], which increase with increasing gestational age peaking at term [44, 45]. The functional importance of various ECVs in pregnancy is largely unknown, but they are suspected to mediate fetal-maternal communication at key stages of pregnancy including implantation and parturition [43, 46]. Human villous trophoblasts secrete exosomes containing placenta-specific miRNAs into maternal circulation, which play key roles in regulating immune signaling [47, 48]. Maternal-derived exosomes are also enriched during pregnancy, and the transfer of exosomes between the fetus and mother is bidirectional [49].

Immune tolerance versus inflammation in the development of preterm birth

As mentioned above, inflammation has been linked to the pathogenesis of idiopathic PTB, and also other pregnancy complications in mice and humans [7, 50–55]. Furthermore, PTB is considered to have early pregnancy origins, prior to placental development. Thus, it has been proposed that PTB is a disorder that results from defective deep placentation [56, 57], similar to preeclampsia.

As the maternal immune adaptation is involved in the processes sustaining pregnancy success throughout the entire period of gestation, poor immune adaptation from the outset of pregnancy and the failure to mount immune tolerance or to dampen excessive inflammation could be implicated in the cause of PTB (Fig. 2). This theory extends the possibility that early immune perturbations can have a lasting, detrimental effect on the pregnancy, as proposed in [17].

Early immune perturbations caused by maternal stress perception, infection, and diet, may result in blunted establishment of immune tolerance and excess inflammation [14, 16, 17, 58, 59]. In addition, the vaginal microbiota composition may be of relevance, as a differential vaginal microbial composition has been associated with an increased risk of PTB in women with African ancestry [60, 61].

Challenges occurring later during gestation, such as maternal infection, endocrine dysfunction, microbiota composition, or metabolic dysregulation, can also further exacerbate the inflammatory burden and lead to PTB [7, 10, 62, 63].

Immune cells involved in term and preterm labor

Considering the importance of inflammation in the onset of labor at term or preterm, it is key to understand the mechanisms behind its origin. In this context, mouse models have become useful tools to provide insights into the complexity of PTB (Table 1). There are various ways to induce preterm labor (PTL) and birth in mice, including immune, hormonal, and environmental agents. Immunomodulatory agents activate or exacerbate the physiologically occurring inflammatory pathways involved in labor induction and recapitulate the clinical manifestations of labor associated with infection and/or inflammation (Table 1). The most common mouse models of PTB involve the administration of lipopolysaccharide (LPS), a bacterial cell wall component of gram-negative bacteria, or whole heat-killed bacteria such as *Escherichia coli* (*E. coli*) via intrauterine or intraperitoneal routes of administration. Importantly, correlations made between these studies are limited due to differences in dosage, route, and timing of the compound administered, as well as the outcomes measured. For an extensive list of other models of PTB, we refer to the recent study by McCarthy et al. [88]. The use of mouse strains with specific genetic mutations has further revealed the importance of numerous proteins and signaling pathways in normal and/or preterm labor (Table 1). However, most of these studies carry certain limitations and care should be taken to interpret the data. Firstly, the use of global knockout mice does not allow for analysis of the effect of cell-specific mutations and can complicate interpretations if littermate controls are not used and if pre-pregnancy immune and endocrine status is not considered. Furthermore, as most studies also mate to the same genotype male, maternal versus fetal effects can be difficult to determine. Finally, the background strain of the mutant mice used is important to note, since genetic background is
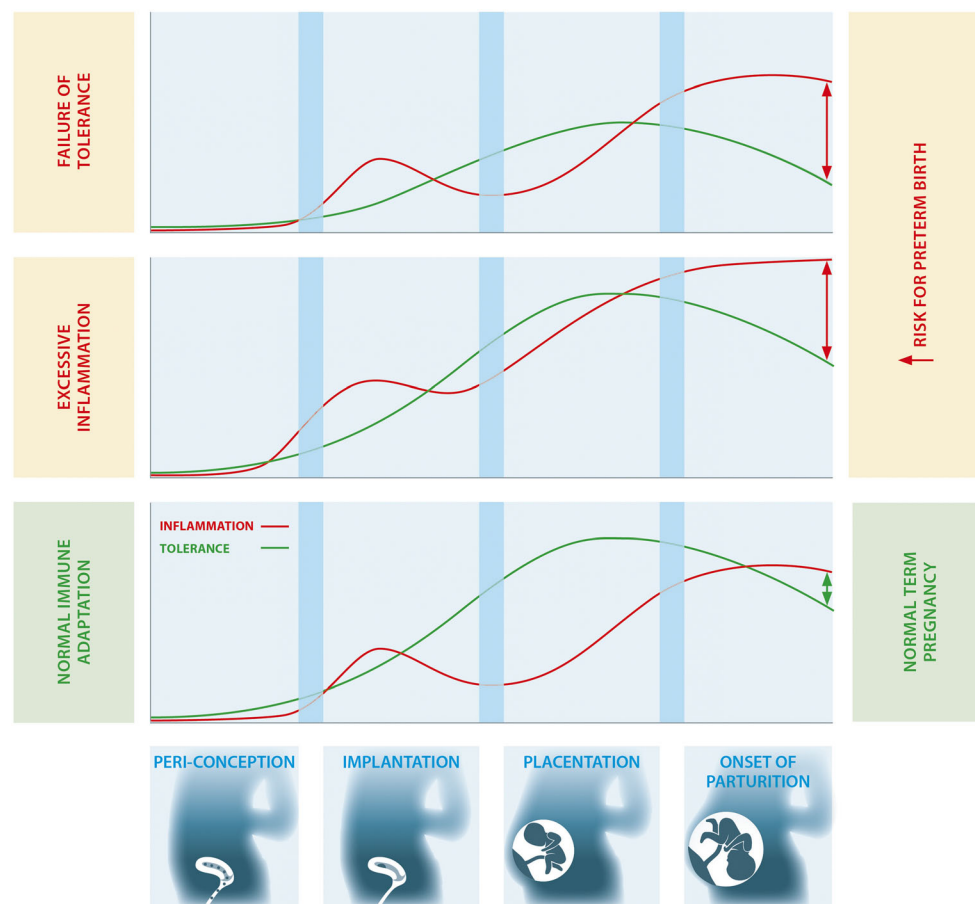
Fig. 2 Trajectories of maternal immune tolerance versus inflammation determines the susceptibility to PTB. A balanced tolerogenic and inflammatory immune response underlies the normal progressing of pregnancy and onset of birth at term. Failure to mount sufficient levels of tolerance (top panel) or excess inflammation (middle panel) creates an immune imbalance in favor of inflammation, thereby increasing the risk for PTB

a determinant of gestation length in mice [89]. Despite their limitations, studies using either interventions or mutant mouse lines to cause or delay preterm or term labor provide valuable insights into the inflammatory mechanisms of these processes and highlight the balance of factors that work in concert to produce controlled inflammation in normal term labor. Importantly, dysregulation of many essential components of these inflammatory pathways either prevents or increases susceptibility to preterm labor in mice.

Using mouse models of PTB, macrophages were identified as major contributors to the induction of term and preterm labor (PTL). Depletion of macrophages using anti-F4/80 administration prior to experimental LPS-induced onset of PTB eliminates susceptibility to PTB [51]. Macrophages likely contribute to the pathogenesis of PTB via secretion of inflammatory cytokines, such as tumor necrosis factor (TNF)α, IL1, IL6, and IL8 and uterine contractility genes such as matrix metalloproteinases (MMPs) [90]. In particular, IL1 is known to critically contribute to inflammation-induced PTB. As shown in mice, IL1 administration induces PTL, and inhibition of its receptor prevents labor [72], likely via activation of

NF-κb signaling pathways [64]. Similarly, IL6 is another cytokine important in the timing of parturition and the pathogenesis of PTB, since IL6-deficient mice have delayed parturition and are also resistant to LPS-induced PTB [65].

Moreover, complement activation plays an important role in PTB. This has been observed in infection-induced PTB in humans, where women with spontaneous PTL showed increased concentrations of complement products C3a, C4a, and C5a [51]. In mice, complement receptor C5aR-deficient mice are protected against LPS- and RU486-induced PTB. In this study, increased complement deposition was present in the cervical epithelium of PTB mice, along with complement C5a activation–dependent MMP9 release from macrophages and cervical remodeling [77].

Besides these examples of the involvement of the innate immune system in modulating the risk for PTB, the adaptive immune response is also critical. A subset of PTB is postulated to result from a failure of maternal tolerance to fetal antigens [7]. Indeed, Treg and Teff cells exhibit distinct changes in frequency and phenotype in PTB. Treg cells in peripheral blood of women in PTL are reported to have differential activation and diminished suppressive capacity compared with term controls [52, 91,

Table 1 Interventions and mutant mouse strains used to study the pathophysiology of preterm birth

Interventions used to induce PTL in wild-type mice	
Lipopolysaccharide (LPS)	[63–70]
E. coli	[71]
Poly(I:C)	[63]
IL1β	[72]
Anti-CD3e	[73]
Anti-galactosylceramide (a-GalCer)	[74]
Plasma exosomes	[75]
Recombinant IL6	[65]
In vitro activated neonatal CD4$^+$ T cells	[53]
HMGB1	[76]
IL10 neutralizing antibody	[67]
Low dose influenza virus (PR8)	[63]
Lymphocytic choriomeningitis virus (LCMV)	[63]
Interventions used to prevent PTL in wild-type mice	
Anti-F480	[77]
(+)-Naloxone (toll-like receptor-4 antagonist)	[78]
101.10 (IL-1R-biased ligand)	[64]
Mutant mouse strains used to study term and preterm labor	
Recombination activating gene (Rag) 1	[79]
Interleukin 6 (IL6)	[65]
Type I IFN receptor	[63, 64]
Myeloid differentiation primary-response gene 88 (MyD88)	[71]
TIR domain-containing adaptor protein-inducing IFNβ (TRIF)	[71]
Myd88 and TRIF	[71]
PAF acetylhydrolase (PAF-AH)	[80]
Complement (anaphylatoxin C5a)	[77]
Steroid receptor coactivase 1 (SRC-1)	[81]
Steroid receptor coactivase 2 (SRC-2)	[81]
Heterozygous mutation in SRC-1 and 2	[81]
Toll-like receptor 3 (TLR2)	[82]
Surfactant protein (SP)-A	[82]
Surfactant protein (SP)-D	[82]
SP-A and SP-D	[82]
CXC3 chemokine receptor 1 (CXC3CR1)	[83]
Toll-like receptor 4 (TLR4)	[70]
TLR4 deletion in progesterone receptor (Pgr)–expressing cells	[84]
TLR4 deletion in Tie2-expressing cells	[84]
p53 deletion in progesterone receptor (Pgr)–expressing cells	[84]
Trp53 deletion in Pgr-expressing cells	[85, 86]
Prostaglandin F2 alpha receptor	[87]
Oxytocin and PGF2α receptor	[87]
Oxytocin receptor and PGF2α receptor	[87]

92]. Normal human labor is associated with a decline in Treg cell function and reciprocal activation of Teff cells which are normally controlled by Treg cells [92].

A decrease in Treg cell number and/or function in pregnancy pathologies is typically associated with an increase in Tcon/Teff

activation. This holds true for labor, as T cells with an activated memory phenotype are increased in the choriodecidua (fetal membranes) of women with spontaneous labor at term [93]. Effector memory CD4$^+$ and CD8$^+$ T cells are also enriched in the decidua in spontaneous PTL, suggesting the inflammatory potential of these cells is greater in PTL women [73]. CD4$^+$ and CD8$^+$ T cells expressing "exhausted" (PD-1$^+$TIM-3$^+$CTLA4$^-$LAG-3$^-$) and "senescent" (KLRG-1$^+$CD57$^+$) memory and effector phenotypes were identified in the decidua of women with term and preterm labor [66]. Notably, the proportions of exhausted CD4$^+$ T cells increased in the decidua parietalis with increasing gestational age but declined in the decidua basalis of women who underwent PTL with placental inflammation. As TNFα and interferon (IFN)γ production could be induced *ex vivo* in the exhausted T cells, these cells may restore their effector function upon placental inflammation [66].

T and B cell–deficient Rag1$^{-/-}$ mice have provided important clues for the role of T cells in PTB. These mice show an increased susceptibility to LPS-induced PTB, which may be mediated by macrophage activation. However, adoptive transfer of CD4$^+$ T cells at mid-gestation conferred resistance to LPS-induced PTB, as these cells seem to rapidly differentiate into Treg cells [79]. Conversely, activation of Teff cells using anti-CD3 causes PTB via upregulation of local and systemic pro-inflammatory responses such as IL6 and IFNγ [73]. Interestingly, in utero adoptive transfer of CD4$^+$ and CD8$^+$ Teff cells causes late fetal resorption dependent on TNFα and IFNγ. These cytokines also cause uterine contractility in vitro. However, it is unclear whether these Teff cells cause PTB if administered later or systemically [54].

Together, these studies suggest that activated memory T cells and Teff cells may participate in mediating inflammatory processes in normal term labor, whereas Treg cells may act to minimize the inflammatory potential or premature activation of exhausted and memory Teff cells. However, the molecular details of these maternal T cell interactions implicated in normal and PTL are yet to be defined. One potential pathway could involve IL10, as Treg cells are capable of producing this anti-inflammatory cytokine. IL10 has been shown to prevent PTB, as IL10 deficiency or administration of IL10-neutralizing antibodies increases susceptibility to PTB in mice [67]. As global IL10 deficiency led to a decrease in inflammatory cytokine gene expression in the uterus and placenta, IL10 may act to regulate excessive inflammatory responses implicated in labor. Recent work demonstrates that TLR4 signaling in decidual endothelial cells at term may cause IL10 induction in perivascular stromal cells via NF-κb activation of IL6 and STAT3. This could be a mechanism to preserve the homeostatic immune balance under inflammation, which may be perturbed in PTB [84].

Emerging data arising from humans and mice indicate that the fetal environment in PTB is also characterized by inflammation, along with the priming of fetal T cells against

maternal antigens [54]. Future studies are now needed to confirm if fetal inflammation is causal to maternal inflammation and related PTB, or a consequence of the maternal spill-over of inflammatory cytokines.

NKT cells, an innate-like subset of T cells with specialized functions, are also implicated in PTB. Maternal NKT cells recognize CD1-restricted lipid antigens, which are expressed by fetal trophoblast cells, and are thought to play an immunoregulatory role in pregnancy [94]. Interestingly, depletion of invariant NKT cells reduces the rate of LPS-induced PTB in mice and administration of an antibody specific for NKT cells (α-GalCer) in late gestation causes PTB via activation of CD4^{+} T cells, macrophages, neutrophils, and DCs in the myometrium/decidua [68, 74, 95].

Inflammatory signaling pathways in term and preterm labor

A crucial component of inflammatory immune responses is the engagement of toll-like receptors (TLRs) which trigger signaling pathways leading to secretion of cytokines and chemokines by innate immune cells. TLRs are an evolutionarily conserved class of pattern recognition receptor (PRR) that recognize pathogen-associated molecular patterns (PAMPS) derived from microorganisms, and damage-associated molecular patterns (DAMPS) released from immune cells and stressed and dying cells. TLR activation is an essential initiating component of the inflammatory pathway that leads to both normal and preterm labor (Fig. 3).

Most research on the role of TLRs in PTB has focused on TLR4 which binds to a specific range of PAMP and DAMP ligands including bacterial LPS. TLR4 is activated in the uterus in both normal and PTB and is essential for on-time parturition in mice, controlling uterine activation and onset of labor [70]. Pregnant TLR4^{−/−} mice deliver an average of 13 h later than controls and their offspring exhibit reduced viability [70]. Furthermore, inhibition of TLR4 signaling with the TLR4-antagonist (+)-naloxone following intrauterine administration of E. coli suppressed the inflammatory cascade and effectively prevented against PTB [78]. Recently, TLR4 expression by decidual endothelial cells, and not immune cells, was identified to be a key for initiating this response, since mice with endothelial-specific TLR4 deletion are resistant to LPS-induced PTB [84]. In humans, maternal single nucleotide
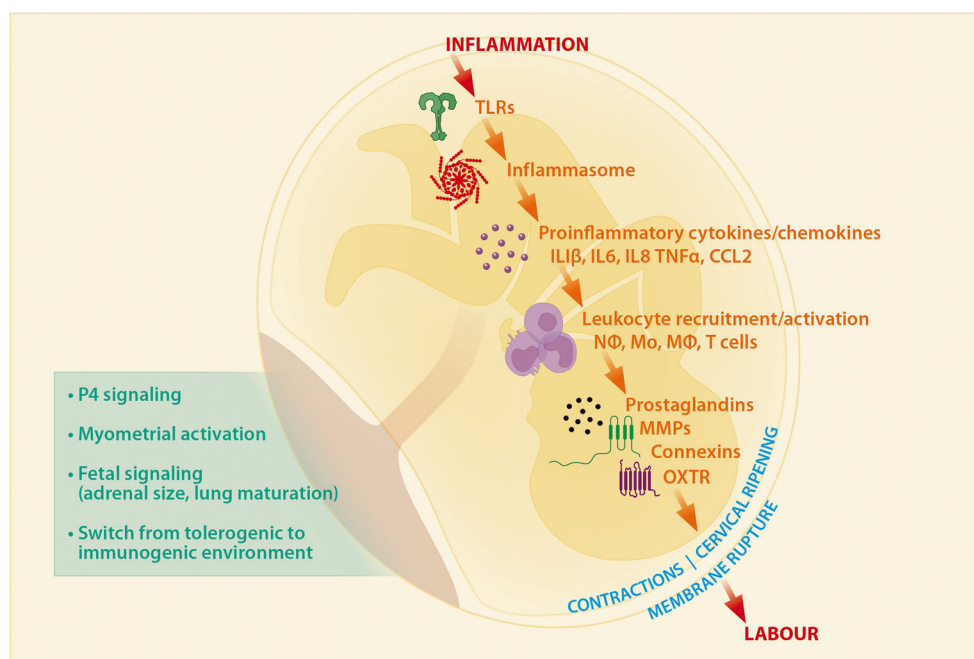
Fig. 3 Current understanding of the inflammatory pathways of parturition. In pregnancy, toll-like receptors (TLRs) expressed on immune and endothelial cells at the fetal-maternal-interface are activated during sterile or active inflammation, via a range of DAMPs and PAMPs. TLR activation causes a cascade of intracellular signaling events leading to inflammasome activation and release of pro-inflammatory cytokines and chemokines. This leads to further recruitment of pro-inflammatory leukocytes such as macrophages, monocytes, neutrophils, and T cells to the decidua, placenta, and amniotic cavity. In macrophages, NF-κb activation controls expression of genes encoding uterine contractility and cervical ripening proteins such as prostaglandins F2α receptor, connexin-43, oxytocin receptor, and cyclooxygenase 2 (COX-2), as well as genes encoding pro-inflammatory cytokines TNFα, IL1β, IL6, and IL8. Uterine contractility and cervical ripening lead to membrane rupture and subsequently, the onset of labor. Withdrawal of P4 signaling, myometrial activation, fetal signals, and a switch in maternal immune phenotypes from tolerance to inflammation all contribute to the activation of the main inflammatory pathway. In the case of preterm birth, studies in mice show that various perturbations in this inflammatory pathway can occur, leading to premature activation of essential pathways components, resulting in preterm labor

polymorphisms (SNPs) in the *TLR4* gene are associated with early preterm delivery before gestational week 32 [96]. Downstream of TLR4 and its coreceptor CD14 is MyD88-dependent and independent (TRIF-dependent) signaling pathways, that each control specific inflammatory gene expression. In mice, PTB is dependent on MyD88, since MyD88$^{-/-}$ mice are completely protected against *E. coli*–induced PTB [71].

In addition to TLR4, other TLRs likely mediate timing of birth, such as TLR2 [82, 97]. In addition to maternal TLR expression, fetal TLR expression may have involvement in the timing of birth as polymorphic fetal TLR4 and TLR2 alleles were associated with prematurity [98, 99].

TLR activation subsequently leads to the activation of the inflammasome and the perpetuation of additional cytokine and chemokine production in the placenta and decidua, such as IL8, CCL2, IL1β, and IL6 [100]. TLR activation also initiates the recruitment of immune cells, production of prostaglandins, and MMPs, leading to the activation of cervical ripening and uterine contractions (Fig. 3). Also, the influx of immune cells such as macrophages to the amniotic cavity is pivotal for the onset of labor, as it induces the production of inflammatory mediators such as NF-κb. NF-κb is a key mediator of the inflammatory cascade leading to labor, since it directly binds to the promoters of genes that cause uterine contractility such as *PTGFR* (prostaglandin F2α receptor), *GJA1* (connexin 43), *OXTR* (oxytocin receptor), and *PTGS2* (cyclooxygenase 2; COX-2), as well as genes encoding proinflammatory cytokines TNFα, IL1β, IL6, and IL8 [90]. Taken together, PRRs, and specifically TLRs and their downstream signaling molecules, are key players in triggering the timing of birth at term and preterm.

The DAMPs and PAMPs which bind to PRRs also play essential roles as the "messengers" to initiate the inflammatory cascade leading to term and preterm labor. TLRs in pregnancy are activated during sterile inflammation, and in the absence of active infection, via DAMPs [101]. The most studied DAMPs in preterm and term labor are high-mobility group box 1 (HMGB1), fetal cfDNA, and platelet-activating factor (PAF). Oxidative stress and cellular senescence of the fetal amnion and chorion may trigger human parturition through the release of DAMPs. In senescent cells, DAMPs relocate from the nucleus to the cytosol where they can be secreted as alarmins and drive the inflammatory parturition cascade [102]. Intra-amniotic administration of HMGB1, an alarmin that induces inflammation upon tissue damage, causes preterm labor in mice [76] and is elevated in the amniotic fluid of women who underwent PTL, independent of intra-amniotic infection status [103]. HMGB1 was found to be primarily expressed by amnion epithelial cells, myofibroblasts, neutrophils, and macrophages [103], and incubation of chorioamniotic membranes with HMGB1 leads to release of pro-inflammatory IL1β and IL6 [104]. cfDNA, a common

activator of TLRs, was found to be released upon cell death to cause TLR signaling in the placenta [105]. The inflammatory phospholipid PAF is another DAMP critical for labor. It is increased in the amniotic fluid of women who deliver preterm [106–108], and its administration induces PTB in mice via TLR signaling in macrophages [80, 108, 109]. Alarmins released from cells upon tissue stress can also bind to PRRs, and concentrations of alarmins IL1α and S11 family proteins calgranulin A and calgranulin C were increased in amniotic fluid during sterile intra-amniotic inflammation [110]. Other PRRs such as NRLP3 and NOD2 activate inflammasomes, which likely play a role in initiating spontaneous labor at term and preterm labor, since the activation of components of this pathway causes the secretion of IL1β in chorioamniotic membranes in normal labor at term [100]. Placental PRR expression mediates their functions as a key barrier capable of recognizing and responding to microbes and stress.

Perturbations affecting the placenta could therefore affect the placental response to PAMPs and DAMPs and have an impact on pregnancy outcome. In summary, DAMPs play key roles in initiating labor at term and are implicated in the cause of PTL; however, their origin (fetal versus maternal), triggers, and regulation are not well understood.

Fetal-placental and vaginal microbiomes in PTB

Recent research endeavors aimed to identify the impact of the microbiome in modulating the risk for PTB [111]. Features of the vaginal microbiome have been shown to determine the risk for PTB. For example, in women of African ancestry, dramatic changes in the diversity of the microbiota were observed in early pregnancy, with an increased prevalence of multiple vagitypes and dysbiosis [61]. Several dysbiotic vagitypes could be associated with PTB, likely caused by an early pregnancy increase in pro-inflammatory cytokines in vaginal fluid, such as the chemoattractant CXCL10 [60]. Interestingly, preconception administration of antibiotics to women with previous preterm delivery did not reduce preterm birth rate and may be associated with earlier delivery and decreased birth weight [112], suggesting that complex host-microbiome dynamics from the outset of pregnancy are likely essential for optimal pregnancy success [113]. The molecular mechanisms of these interactions will be important to uncover in future research efforts. In contrast to the vaginal microbiota, there is still a great deal of ambiguity as to the question of whether the intrauterine environment is sterile or non-sterile. 16S rRNA sequencing and culture of bacterial species isolated from the female reproductive tract revealed microbial communities in the cervix, uterus, fallopian tubes, and peritoneal fluid that were diverse and distinct compared with the vagina and cervix, which were Lactobacillus dominated [114].

Examination of microbiota from the endometrial fluid of infertile women undergoing *in vitro* fertilization (IVF) revealed an association between non-Lactobacillus-dominated endometrial microbiota and decreases in implantation, pregnancy, and live birth rates [115]. Several mechanisms have been proposed to limit bacterial invasion to the uterus from the lower genital tract, and these may operate during pregnancy to ensure a tolerogenic environment is maintained for pregnancy success. Bacterial invasion of the uterus and amniotic cavity found in subsets of pregnant women could theoretically cause inflammation and disruption of the immune adaptation to pregnancy, predisposing to PTB [113]. Microorganisms similar to the vaginal microbiota can also be isolated from the placenta [116]. However, others have rejected this concept, showing contaminants were responsible for the detection of most bacterial species in the placenta [117]. Given these findings, there is still debate as to whether the uterus and placenta harbor a microbiome or not. Hence, it is still unknown if a skew of the intrauterine microbiome—if it indeed exists—may modulate the risk for PTB.

The role of maternal progesterone in PTB

Hormone levels have also been extensively studied in order to understand the pathogenesis of PTB. Adequate progesterone (P4) levels are known to be essential for the establishment and progression of normal pregnancy. Hence, it is not surprising that labor is associated with a P4 withdrawal in mice and functional progesterone withdrawal in women [118, 119]. The P4 decline appears essential for labor to occur, as the administration of P4 to mice in late gestation extends the gestation length and prevents labor. Similarly, mice lacking the P4-metabolizing enzyme 20αHSD show prolonged gestation [120]. Conversely, treatment with the progesterone antagonist RU486 causes PTB in mice and is associated with increased decidual PGE2 and IL6 concentrations [121]. P4 application is also protective against LPS-induced PTB in mice via prevention of inflammation-induced cervical remodeling, cervical macrophage infiltration, and MMP9 expression that precedes PTB, possibly through the disruption of complement signaling on macrophages [77]. P4 is known as a strong immunomodulator, it induces stable Treg cells, arrests DC in a tolerogenic state, and suppresses inflammatory responses [122–124]. P4 signaling withdrawal may therefore be one mechanism of immune modulation leading to PTB. The P4 decline in late gestation may be triggered by immune pathways, as macrophages have been shown to regulate P4 production in the ovary in mice [125]. However, it should be noted that PTB in mice can also occur in the absence of P4 withdrawal, for instance, with intrauterine administration of bacteria [126]; multiple, independent immune and hormonal mechanisms are likely in place.

Myometrial and decidual clocks in PTB

The decline in P4 signaling induces a switch of the myometrium from a quiescent to a contractile state at labor [127]. However, evidence places the establishment of myometrial quiescence also at the start of gestation, as it may be regulated by estrogen and P4 hormone receptor signaling [128]. Besides the myometrium, the endometrium and decidua are also proposed to be responsible for the timing of birth via induction of a decidual "clock." This decidual clock mediates mechanisms of fetal-maternal tolerance in early pregnancy and wanes over time with advancing gestational age, leading to a withdrawal of active suppression or induction of inflammatory signals [10]. The induction of decidual quiescence is an active process occurring in early pregnancy, caused by transcriptional silencing in decidual stromal cells (DSCs) which recruit the repressive histone mark H3K27me3 for epigenetic regulation of hundreds of genes, causing suppression of type 1 immunity and wound healing response [129]. Again, P4 signaling from the outset of gestation may be essential to decidual quiescence and maintenance of an anti-inflammatory immune environment needed to last for the duration of pregnancy, until labor at term.

Fetal origins of the timing of birth

There are several lines of evidence to support that the fetus itself is involved in initiating signals of parturition, such as the secretion of hormones (cortisol, estrogen, placental corticotropin-releasing hormone (CRH)) and surfactant protein A (SP-A) [127]. The fetal adrenal gland is responsible for fetal cortisol production. Notably, placental CRH secretion may provide the first signal for cortisol stimulation [130]. Dysregulation or early activation of these processes may be implicated in the pathogenesis of PTB. Interestingly, SP-A, a major lung surfactant protein secreted by the maturing fetal lung when the capacity to sustain air breathing is developed, was identified in mice as a signal for parturition through its action on fetal macrophages [131]. SP-A binds to TLR4 on human and mouse macrophages and causes TLR4-dependent inflammatory cytokine expression [132]. SP-A was detected in the amniotic fluid at term pregnancy and stimulated IL1β and NF-κb expression in amniotic fluid–derived macrophages in vitro. Intra-amniotic injection of SP-A in mice additionally caused preterm delivery within 24 h [131]. Expression of steroid receptor coactivators SRC-1 and SRC-2, regulators of SP-A transcription, was determined to be key for the initiation of labor, and additionally key for the expression of PAF and lysophosphatidylcholine acyltransferase-1 (LPCAT1), which catalyzes the synthesis of PAF, in the fetal lung. As discussed, PAF initiates TLR signaling in mice and administration of PAF causes PTB [80, 109]. However, whether pathological

activation of PAF signaling in PTB arises from the fetus is currently unknown.

In addition to fetal hormone- and SP-A-dependent modulation of labor and PTB, the fetal immune system has long been recognized to also have involvement in preterm birth [133]. The theory that the fetal immune system is immature has been robustly disproven, as neonatal T cells have the potential for activation [31, 54, 134]. Additionally, qualitative distinctions of immune responses between neonates and adults are in place. These include CD71^{+} erythroid cells, which are uniquely present in neonates and have been proposed to suppress microbial colonization-related inflammation. Also, IL8 (CXCL8) drives major T cell effector function in human newborns, as it allows for the activation of antimicrobial neutrophils and γδ T cells [135]. Importantly, these mechanisms may already instruct fetal immunity prior to birth during pregnancy and their dysregulation could be involved in the pathogenesis of PTB [53, 54, 134].

Interestingly, fetal macrophages appear to play a key role in initiating inflammatory cascade at parturition, as amniotic fluid fetal macrophages were shown to migrate to the uterus at term in response to increasing SP-A levels and engage with via TLR2 signaling [82, 131]. In neonates, polymorphic TLR4 and TLR2 alleles are associated with prematurity [98, 99]. Therefore, TLR signaling in fetal macrophages may be important in the pathogenesis of PTB. However, the phenotype of fetal macrophages seems to be tightly controlled to avoid inappropriate inflammation that may cause early activation of the inflammatory cascade leading to parturition [134] and failure of this control may result in an increased risk for PTB.

Insights arising from comparative cord blood analyses revealed distinct differences between term and preterm born infants [54]. Preterm cord blood was characterized by higher concentrations of inflammatory cytokines, increased activation of dendritic cells, and central memory (CCR7^{+}CD45RA^{−}) T cells with a Th1 phenotype. However, it needs to be confirmed if these differences are involved in the pathogenesis of PTB, or merely result from the less advanced immune ontogeny in preterm born neonates.

Emerging data arising from humans and mice also indicate that the fetal environment in PTB is also characterized by inflammation, along with the priming of fetal T cells against maternal antigens [54]. Activated fetal T cells are also found in the amniotic cavity and are increased in cases with chorioamnionitis [53]. Future studies are needed to confirm if fetal inflammation is the cause of the maternal inflammation and related PTB, or the consequence of the maternal spill-over of inflammatory cytokines.

The above-mentioned population of immunosuppressive CD71^{+} erythroid cells, which allows for microbial colonization upon birth, may play a functional role before birth through constraining fetal T cell activation. This is supported by observations upon depletion of CD71^{+} cells in splenocytes of neonatal mice, which caused robust cytokine production upon stimulation in vitro [134, 136].

The potential role of FMC and MMC in PTB

The earlier introduced population of FMC cells may too represent a pathway through which the fetus modulates the time of labor. FMC, which increase over the course of gestation and peaking at term [32, 34], may be a key source of antigen that drives the accumulation of maternal-fetal antigen-specific Treg cells in pregnancy, hereby mediating fetal-maternal tolerance and maintenance of pregnancy [31]. Whether dysregulated FMC, e.g., resulting from fetal inflammation, are implicated in PTB is currently unknown. When FMCs were measured in maternal blood of term and PTL pregnancies, no quantitative differences were found [54], and insight on altered functions are still unknown.

As detectable amounts of MMC cells are transferred across the placenta to the fetus during pregnancy, there is a possibility for these cells to play a role in initiating the timing of birth. Indeed, increased MMC with T or B cells or myeloid cell phenotypes were found in mouse fetuses from mothers mated allogeneically and treated with LPS in late gestation [69]. In a human study, increased fetal DC and T cell activation in cord blood of preterm infants was associated with an increase in MMCs compared with term cord blood. A correlation between MMCs and central memory (CM) T cells was observed, leading to the hypothesis that the MMCs could be the source of maternal antigens to prime the alloreactive fetal T cells that are implicated in the induction of PTL [54]. However, as causality has not been shown, it needs to be addressed in future studies whether maternal cells trafficking to the fetus are the actual drivers of preterm birth and which mechanism is driving this phenomenon. One study addressed the impact of MMC cells on reproductive outcome in mice and describes that T cells specific for the NIMA on MMC mitigate reproductive fitness in the next generation [36]. Therefore, MMC cell transmission during gestation may play a role in the health and pregnancy trajectory of the subsequent offspring, including modulating their susceptibility to pregnancy complications such as PTB. However, this benefit may be restricted to pregnancies of the female filial generation where the paternal antigen specificity is overlapping between mating partner and father and future studies are needed to confirm this reproductive advantage irrespective of the parental antigen specificity. A study in humans also addressed the topic of MMC-mediated reproductive outcome and could identify that the presence of MMC cells from a woman's own mother is associated with healthy pregnancy outcome, whereas no MMC cells could be detected in women diagnosed with preeclampsia, albeit specificity to NIMA could not be addressed [137].

To date, the role of cross-generational MMC has not yet been measured in women with PTL. However, this is an interesting prospect since women born preterm have an increased risk for PTB themselves [138].

Non-cellular mediators and their potential role in PTB: fetal cfRNA, cfDNA, and exosomes

Fetal cell-free (cf)DNA has been investigated as a potential trigger of PTB. It is well known that fetal cfDNA, which increases steadily throughout normal pregnancy, is associated with labor [139]. cfDNA originates from the placenta via cell death and may act as a DAMP to initiate signals of labor [105]. Fetal cfDNA binds to TLR9 and induces NF-κb-dependent inflammatory effects [140]. Recently, several cfRNA transcripts, which have also been associated with immune, placental, or developmental function, can predict gestational age with comparable accuracy to ultrasound. Interestingly, cfRNA transcripts are enriched in women who delivered preterm [38]. The functional significance of these RNA transcripts in the

pathogenesis of PTB is currently unknown. While they may represent novel biomarkers for the detection of PTB, understanding the molecular basis for their upregulation will be important for understanding the root causes of PTB.

Similarly, placental exosomes are detected at increased concentrations and have distinct compositions in pregnancy complications such as preeclampsia, IUGR, and PTB [43, 141]. Exosomes may mediate inflammatory signaling between fetal and maternal compartments in response to signals such as infection and stress. Exosomes are also detected in amniotic fluid and may play a role in labor induction, providing inflammatory signaling molecules [141]. Exosomes identified in human amniotic fluid showed distinct protein profiles in spontaneous PTB [141] and were shown to be enriched for inflammatory cytokines in women with intra-amniotic inflammation [50, 110]. Notably, DAMPs contained in fetal exosomes are released into maternal tissues and can induce inflammatory signaling pathways [102, 142].

Maternal exosomes must also be considered in initiating parturition. Human trophoblast explants internalize macrophage-derived exosomes, inducing secretion of pro-
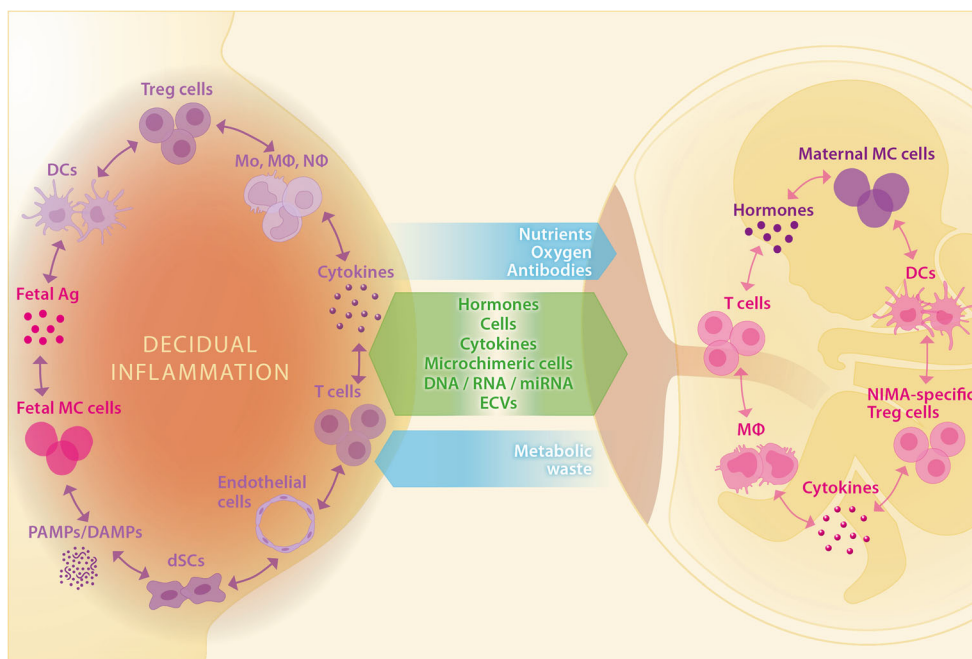
Fig. 4 Fetal-maternal crosstalk is dysregulated in preterm versus normal pregnancy. Fetal-maternal crosstalk is essential for the progression of normal pregnancy. Via the placenta, nutrients, oxygen, and maternal antibodies are transferred to the fetus, and fetal metabolic waste is transferred to the mother. In addition, bidirectional transfer of key signaling molecules and cells occurs, such as the transfer of hormones, cytokines, nucleic acids, extracellular vesicles (ECVs), and microchimeric fetal and maternal cells. These molecules and cells influence both maternal and fetal immune environments. In the fetus, interactions between fetal immune cells and maternal MC cells induce fetal T cell tolerance to maternal antigens. As the fetus matures, the fetal lung and adrenal gland signal for parturition through fetal macrophages, which drive inflammation in the amniotic cavity. Approaching

parturition, the decidual immune environment reflects interactions between various cells including macrophages, monocytes and T cells, and decidual endothelial and stromal cells. Decreased Treg cell activity may result in activation of fetal antigen-specific Teff cells. Fetal MC cells may provide a source of fetal antigen in the decidua. These cells respond to increasing inflammatory signals via PAMPs, DAMPs, and cytokines drive an inflammatory immune response that leads to the induction of labor. In preterm labor, various components of fetal-maternal crosstalk can be dysregulated, leading to premature activation of the inflammatory pathways off labor. This may occur due to a breakdown in fetal-maternal tolerance, whereby maternal T cells are activated towards fetal antigens, or fetal T cells are activated against maternal antigens

inflammatory cytokines IL6, IL8, IL10, and IL12 [49]. As macrophages infected with intracellular bacteria release PAMP-containing exosomes, which activate naïve T cells and macrophages [143, 144], this may provide a mechanism for the induction of decidual inflammation in infection-related PTB. Thus, macrophage-derived exosomes may traffic to and be taken up by the placenta to deliver key signals facilitating immunomodulatory pathways of parturition. Whether exosomes containing DAMPs are also delivered to the placenta in the case of sterile inflammation in term and preterm pregnancies is currently unknown.

Clinical developments aiming to identify biomarker and therapeutic avenues

PTB is the leading cause of morbidity and mortality worldwide. Despite decades of research on the topic, the underlying etiology of PTB is still largely unknown. Multiple fetal-maternal signaling pathways exist and provide key communication between mother and fetus during pregnancy. This complexity clearly hampers the identification of the multilayered processes implicated in the immunopathogenesis of PTB (Fig. 4).

To date, therapeutic options for PTB are sparse. Current treatments to prevent suspected preterm birth include P4 supplementation and progestin prophylaxis. Other studies aimed at PTB prevention by targeting immune mediators. Here, the TLR4-antagonist (+)-naloxone, which is the non-opioid isomer of the opioid receptor antagonist (−)-naloxone, was tested in mice, where it suppressed the inflammatory cascade and prevent preterm birth [78]. Another immune-based therapeutic approach preventing PTB in mice targeted IL1β by using a novel IL1 receptor biased ligand [64]. Given the importance of IL10 mediating anti-inflammatory actions in the decidua, boosting IL10 in the decidua may represent one future strategy to combat PTB [67, 84]. The development of therapies aiming to increase Treg cells is also considered [145].

Conclusion

Despite its essential nature, a detailed understanding of the molecular mechanisms underlying normal labor induction is only slowly emerging. PTB is a syndrome hypothesized to result from pathological activation of the normal physiological processes that lead to labor [7]. Yet most of our understanding of the pathways involved in labor originates from studies investigating single pathways or mediators by comparing preterm and term pregnancies in humans and various animal models. As multiomic assessments are now underway to evaluate the plausibility of immunological findings [146], the power of biological signatures predictive of preterm birth

may soon be within reach and will inform novel approaches of early prediction and therapeutic interventions. Hence, understanding of molecular mechanisms will likely experience rapid developments in the near future, hereby enabling the identification of women at risk and the development of early prevention strategies of PTB.

Compliance with ethical standards

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