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REVIEW

# Immune cells in term and preterm labor

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**Labor resembles an inflammatory response that includes secretion of cytokines/chemokines by resident and infiltrating immune cells into reproductive tissues and the maternal/fetal interface. Untimely activation of these inflammatory pathways leads to preterm labor, which can result in preterm birth. Preterm birth is a major determinant of neonatal mortality and morbidity; therefore, the elucidation of the process of labor at a cellular and molecular level is essential for understanding the pathophysiology of preterm labor. Here, we summarize the role of innate and adaptive immune cells in the physiological or pathological activation of labor. We review published literature regarding the role of innate and adaptive immune cells in the cervix, myometrium, fetal membranes, decidua and the fetus in late pregnancy and labor at term and preterm. Accumulating evidence suggests that innate immune cells (neutrophils, macrophages and mast cells) mediate the process of labor by releasing pro-inflammatory factors such as cytokines, chemokines and matrix metalloproteinases. Adaptive immune cells (T-cell subsets and B cells) participate in the maintenance of fetomaternal tolerance during pregnancy, and an alteration in their function or abundance may lead to labor at term or preterm. Also, immune cells that bridge the innate and adaptive immune systems (natural killer T (NKT) cells and dendritic cells (DCs)) seem to participate in the pathophysiology of preterm labor. In conclusion, a balance between innate and adaptive immune cells is required in order to sustain pregnancy; an alteration of this balance will lead to labor at term or preterm.**
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## INTRODUCTION

Pregnancy demonstrates the capabilities of the human immune system. The fetus, a semi-allogeneic graft, grows and develops within the mother without succumbing to immunological rejection, a process which depends on the proper establishment of fetomaternal tolerance.[1] This tolerance is initiated by the presentation of the paternal–fetal antigen from semen and is facilitated by seminal plasma factors.[2–4] Antigen is processed by dendritic cells (DCs) and then presented to T cells in the uterine draining lymph nodes.[3,5] As a result, antigen-specific regulatory T cells (Tregs) proliferate in order to create peripheral tolerance towards fetal antigens and allow conceptus implantation.[6,7] Treg numbers are maintained through pregnancy, creating a tolerogenic anti-inflammatory state or hyporesponsiveness towards paternal antigens until late gestation.[7–10] During late pregnancy, we have proposed that circulating maternal leukocytes (innate and adaptive) are recruited into reproductive tissues (cervix and myometrium) and to the maternal/fetal interface (decidual tissues) by chemotactic processes,[11–16] where a pro-inflammatory state develops and leads to labor and delivery of the baby.[17–19] It is thought that the premature activation of this pro-inflammatory pathway can lead to a breakdown of fetomaternal tolerance and play a role in the induction of labor, which subsequently can result in preterm birth.[20–22]

Preterm birth is a major determinant of neonatal mortality and morbidity.[23] In 2011, 11.7% of all births in the United States were diagnosed as preterm.[23] Among problems occurring after preterm birth are chronic respiratory illnesses, neurodevelopmental disorders and long-term cognitive impairment.[24–26] However, the mechanisms that lead to preterm birth/labor are poorly understood. The main goal of this review is to summarize the innate and adaptive immune cell components that participate in term and in preterm labor, clarifying the contributions of resident, adaptive leukocytes to the physiological or pathological

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activation of parturition. Achieving a deeper understanding of the innate and adaptive immune cell components involved in preterm labor might allow us to develop strategies to prolong pregnancy and thereby improve pregnancy outcomes.

## INNATE IMMUNE CELLS IN TERM AND PRETERM LABOR

Labor is an inflammatory process.[27,28] A number of studies in humans and mice have reported the presence of inflammatory neutrophils and macrophages in the uterus, decidua, cervix and fetal membranes during labor.[27,29–34] The spreading and homing of these granulocytes is facilitated by chemokines and cellular adhesion molecules.[16] Additionally, mast cells are present in the uterus and cervix during late gestation and may contribute to the process of labor.[35–38] Uterine contractions, cervical ripening and dilation, and rupture of the fetal membranes (ROM) are processes which must occur in a timely fashion for a successful delivery.[39] Innate immune cells have been linked to these processes by various studies, and this section aims to discuss the possible roles for these cells in the processes of term and preterm labor.

### Neutrophils

Neutrophil numbers are higher in the circulation of women who undergo labor than in those who do not undergo labor.[40] Labor-related granulocytes are activated since they exhibit an increased ability to migrate.[40] The presence of neutrophils in reproductive tissues at term and their ability to migrate to this region during labor have been well documented in both humans and rodents.[11,27,29,41] Neutrophils participate in the process of labor by releasing pro-inflammatory cytokines and secreting matrix metalloproteinases (MMPs);[42–47] however, their role in each anatomical compartment seems to be unique and will be discussed further below.

In the myometrium, mRNA levels of CXCL8, a neutrophil chemoattractant, are higher in women at term during labor than in women without labor,[28] suggesting that neutrophils are more abundant in the myometrium during labor. However, it was recently demonstrated in a murine model of infection-induced preterm birth (intrauterine administration of lipopolysaccharide (LPS)) that there is no increase in the neutrophil numbers (Gr-1$^+$ cells) in the myometrium 6 h after LPS administration.[48] Preliminary studies in our laboratory put forward evidence on the role of myometrial neutrophils during LPS-induced preterm birth. We found that LPS administration in the peritoneum causes high rates of preterm birth and this is associated with increases in proportion and in absolute numbers of neutrophils in the myometrium (NGL, unpublished data).[49] This discrepancy between studies may be due to the fact that we collected tissues prior to delivery (24 h after LPS injection) instead of 6 h after the intrauterine administration of LPS. Nonetheless, the potential role of myometrial neutrophils in the process of labor needs continuing exploration.

Neutrophils are proposed to play a central role in the cervical ripening process,[42,43,46,50] although recent studies have indicated that this is likely not the case.[51,52] In mice, neutrophil numbers in the cervix do not vary from 15 days postcoitum (dpc) to the time of cervical ripening (late 18 dpc).[52] It has been consistently reported that there are no differences in the number of cervical neutrophils between women without labor with cervical ripening and women who had not undergone the ripening process.[51] However, the number of neutrophils is higher in the cervixes of women who have just completed a vaginal delivery following spontaneous labor at term than in women who were in the first trimester of pregnancy.[51] This finding supports the new hypothesis that neutrophil function is required shortly after parturition, in the phase of postpartum tissue repair.[52,53]

There have been several studies in mice implicating decidual neutrophils in the process of infection-induced preterm birth.[34,48] A large influx of neutrophils into the decidua and myometrium is observed during LPS-induced preterm labor and during term labor; however, this increment was not seen in a non-infectious model of preterm birth (caused by mifepristone).[41,54] Another study reported a sevenfold increase in neutrophils in the decidua after 6 h of intrauterine LPS administration.[48] Despite these findings, the role of neutrophils as a causative agent of preterm labor is questioned since the depletion of these cells does not alter the timing or success of labor and does not prevent LPS-induced preterm birth.[48,52] Neutrophil depletion prior to LPS administration did, however, reduce the amount of a key pro-inflammatory cytokine, IL-1β, in the uteroplacental tissues.[48] This finding is relevant since systemic administration of IL-1β leads to preterm birth in mice.[55] These results suggest that neutrophils are not a necessary component in infection-induced preterm birth, yet they may be required in inflammation-induced preterm birth.

In human decidual tissues, the number of neutrophils was higher in women with preterm labor associated with chorioamnionitis than in women with term gestations (with and without labor) and in women with spontaneous preterm labor/birth without chorioamnionitis.[34] The maternal origin of these decidual leukocytes (e.g., neutrophils) in preterm labor/birth associated with acute chorioamnionitis was proven by FISH.[56] Maternal cells could be recruited into this maternal/fetal interface by decidual-derived chemokines, such as CXCL8.[11,12] Human decidual neutrophils release several inflammatory mediators and MMPs, which degrade the extracellular matrix of the fetal membranes during both term and preterm labor.[44,47,57–60] Taken together, these data suggest that decidual neutrophils contribute to the physiological ROM and pathological preterm premature rupture of membranes (PPROM) during term and preterm labor.

### Macrophages

Macrophages are among the primary innate immune cells that contribute to the processes of term and preterm labor, and their roles have been studied in humans, mice and rats. Macrophages are significant during late gestation primarily due to their secretory products, which include MMPs, IL-1β, IL-6, TNF-α and nitric oxide (NO).[61–63] These versatile leukocytes are being extensively studied to deepen our understanding of the

parturition process. We discuss below the possible effector actions of macrophages in term and preterm labor.

Macrophages play a relevant role in the uterus during parturition. In mice, the number of uterine macrophages at 15 dpc (4 days prior to birth) was significantly higher than in non-pregnant controls though these numbers dropped to non-pregnant levels one day prior to birth.[33] This trend for macrophages to decrease immediately prior to labor correlates with another study, performed in rats, which found that NO synthesis in the uterus was elevated during pregnancy but reduced during term labor.[64] NO, which can be produced by macrophages,[61] has been demonstrated to inhibit myometrial contractions.[65] Altogether, these results suggest that a decrease in macrophages, and the resultant reduction in NO, is required for the onset of labor.

Although the aforementioned studies indicate that macrophage numbers decrease in the uterus prior to labor, a study in rats found concentrations of CCL2, a monocyte/macrophage chemoattractant, increased in the myometrium near the time of delivery in comparison to earlier points of gestation and during RU486-induced preterm labor.[66] Additionally, macrophages may exert effects on the uterus during parturition through the release of pro-inflammatory cytokines, such as TNFA, which are able to upregulate uterine activation proteins,[67] allowing the uterus to prepare for labor. These findings suggest that macrophages are instead recruited into the uterus during labor.

Ripening and dilation of the cervix are the next steps in parturition after the initiation of uterine contractions;[39,53] an inflammatory response has been associated with these processes.[31,51] During pregnancy at term, but before the onset of labor, women with a ripened cervix were found to have greater numbers of cervical macrophages in comparison to women who were not undergoing cervical ripening.[51] A murine model similarly found an increased proportion of macrophages in the cervix the day before birth (18 dpc) in comparison to mid/late gestation (15 dpc).[31] A large number of cervical macrophages was also found in antepartum and in LPS-induced preterm labor.[21,68] These data suggest the possible involvement of macrophages in cervical remodeling.

Although their presence suggests that macrophages play a role in the cervix during the process of labor, the characterization of these cells also supports this theory. Murine cervical macrophages expressing markers associated with adhesion ($CD11b^{high}$) and migration (CD54) were lower prior to birth (18 dpc) than in mid/late gestation(15 dpc).[31] However, macrophages that express markers associated with MMP activation (CD147) and cell matrix remodeling (CD169) are significantly higher at 18 dpc than at 15 dpc.[31] These results suggest that cervical macrophages are probably not migrating or binding to vessels prior to birth, but are instead remodeling and degrading the extracellular matrix,[31] which are important processes in ripening of the human cervix.[69,70] The fact that cervical leukocytes (e.g., macrophages) secrete MMP-9 at term pregnancy[71] and that macrophage depletion prevents LPS-induced preterm birth in mice,[21] suggests that macrophages are a main source of MMP-9 and contribute to the process of labor at both term and preterm stages. Moreover, macrophage-derived cytokines IL-1β and TNF-α increase the levels of MMP-1, MMP-3 and MMP-9,[72] which may be another pathway whereby macrophages participate in the cervical ripening process. Despite the evidence above, it is important to point out that several studies have contrarily suggested that macrophages are not necessary for cervical ripening in mice,[52,68,73,74] but play a role in postpartum repair.[52,53] Further research on the human cervix during labor and preterm labor must be performed in order to come to a definitive conclusion.

Macrophages could also play a role in the rupture of the fetal membranes since macrophages are recruited by these tissues[11] and produce MMP-9.[63] MMP-9 concentrations are significantly increased in the fetal membranes during labor, preterm labor and PPROM,[58,75–77] which directly links this enzyme to physiological ROM and pathological PPROM. Additionally, pro-inflammatory cytokines released by macrophages during labor can regulate the further release of MMPs[78] by the fetal membranes, suggesting another mechanism whereby macrophages may contribute to ROM and PPROM.

Macrophages also reside in the decidua near or during the time of labor.[14,34] In human decidual tissues, macrophage proportions are higher at term than in preterm gestations without labor.[14] Macrophage tissue density is even greater in decidua from women who delivered term and preterm with labor in comparison to women who delivered at term without labor.[34] In mice, the proportion of decidual macrophages increases prior to birth (18 dpc) in comparison to mid/late gestation (15 dpc).[41] Altogether, these results suggest that decidual macrophages have a role prior to the onset of labor.

Macrophages are also implicated in the etiology of preterm labor since CCL2 concentrations are increased in the amniotic fluid of women delivering preterm, both in the presence and absence of intra-amniotic infection, in comparison to women delivering at term.[79] One of the most significant indicators of the role of macrophages in preterm labor was the demonstration that the depletion of macrophages in pregnant mice protected these animals from LPS-induced preterm birth.[21] Ultimately, macrophages are potentially involved in several processes during parturition. The precise role of this cell type in labor remains disputed, yet much evidence gives credibility to their putative roles. Further studies are required to fully elucidate the roles of macrophages in the physiological process of labor and the pathological induction of preterm labor.

Currently, we are investigating the role of macrophages during preterm birth using animal models. Our preliminary data suggest that the plasticity of these cells at the maternal/fetal interface is unique, and that besides participating in the process of labor, macrophages play a central role in the maintenance of fetomaternal tolerance during late pregnancy.

### Mast cells

Mast cells (MCs) are also important innate immune effector cells during late gestation and labor due to their secretion of

mediators.[16,80] Fast-acting MC mediators are histamine, serotonin, heparin, proteoglycans, proteases, prostaglandins and leukotrienes.[81] MCs also secrete the long-term modulators IL-1β, IL-3, IL-5, IL-6 and TNF-α.[36] Moreover, human MCs induce the expression of endothelial adhesion molecules,[82] and express several chemokine receptors.[83] This combination of MC recruitment and up-regulation of cellular adhesion molecules allows MCs to localize within the uterus and cervix, where they may play a role in the development of a pro-inflammatory environment. Due to their presence and actions in cervical tissue during late gestation, MCs and histamine have been associated with the stimulation of cervical contractility;[38] however, MCs have been detected in higher proportions in postpartum than during late gestational cervix which indicates a greater role for this cell type in postpartum uterine cervical repair than during labor.[84] We therefore focus the section below on discussing the role of MCs and their mediators in the uterus during term and preterm labor.

MC degranulation releases mediators which likely play major roles in the process of labor by remodeling uterine smooth muscle cells and stimulating uterine contractions.[35–37,85,86] The release of histamine and serotonin has been linked to uterine contractility since MCs reside adjacent to smooth muscle in the myometrium.[35,87] Indeed, during murine pregnancy MCs are more abundant in the myometrium than in the endometrium.[88] The degranulation of MCs *in vitro*, utilizing compound 48/80, induces greater uterine contractility in tissue from late gestation than in non-pregnant uterine tissue.[36] In addition, the tissue density of human uterine MCs is greater during pregnancy than in the non-pregnant state, which also suggests that uterine MCs modulate myometrial contractility during late pregnancy.[89] A link between MCs and allergy has been suggested as a mechanism of preterm labor/birth[37,90] since MCs are one of the cells effecting immediate hypersensitivity reactions and allergic disease[81] and allergies play a central role in uterine contractions.[37,90] Furthermore, pre-treatment of guinea pigs with a histamine $H_1$ receptor antagonist decreased the rate of preterm birth induced by an allergic reaction.[91] This finding suggests a vital role for histamine, and therefore MCs, in the processes of term and preterm labor.

A recent study contradicts the notion of MC involvement in labor. This study found that, in human myometrial tissues, the abundance of MCs was similar at mid-pregnancy and during labor.[86] In MC deficient $Kit^{W\text{-}sh}$ mice, labor still occurs and leukocyte recruitment into the myometrium is not different from wild-type controls.[86] A possible explanation is that MCs are not the sole leukocyte recruiters,[16] and the pro-inflammatory cascade can be upregulated by other leukocyte subpopulations even in the absence of MCs. Further research is needed in order to clarify the role of mast cells during term and preterm labor.

## ADAPTIVE IMMUNE CELLS IN TERM AND PRETERM LABOR

The adaptive immune system creates memory and responds to specific antigens. During pregnancy, the adaptive immune limbs of both the mother and the fetus must tolerate each other in order to maintain pregnancy until term. A breakdown of this fetomaternal tolerance may lead to labor. In term pregnancy, lack of the tolerogenic state results in physiologic labor. However, a premature retreat of this tolerogenic state might lead to preterm labor.

### T cells

During pregnancy, maternal T cells recognize fetal antigens through interactions with antigen-presenting cells.[92] Fetal antigen-specific T cells maintain fetomaternal immune tolerance across pregnancy.[7] We previously proposed that maternal circulating T cells infiltrate into the maternal/fetal interface prior to delivery and during labor at term.[11,93] Decidual T cells are activated and have both a regulatory and an effector phenotype.[94–97] The next section further addresses the putative roles of specific T-cell subsets during late pregnancy and in term and preterm labor.

*Effector T cells.* We recently provided evidence that decidual $CD4^+$ T cells are involved in term parturition.[14] Specifically, we demonstrated that decidual $CD4^+$ T cells are more abundant in term than in preterm gestations without labor. These T cells express CD45RO, but not CD45RA, which suggests that they are memory cells that were generated early in pregnancy when fetal–antigen presentation occurs.[7,14,92] We also demonstrated that decidual $CD4^+$ T cells express IL-1β, TNF-α and MMP-9 during spontaneous labor at term.[14] The fact that decidual T cells express activation markers such as CD25[98] and labor mediators implicated in both term and preterm labor[17,29,55,58,75,77,99–102] suggests that the adaptive limb of the immune system participates during labor.

Additionally, we demonstrated that during term labor T cells are preferentially recruited into the rupture zone of the fetal membranes by chemotactic processes facilitated by CXCL10 and CCL5.[13,14,93] However, T-cell attraction to the rupture zone was significantly diminished in premature ROM cases.[13] These data suggest that T-cell recruitment into the maternal/fetal interface is required for term pregnancy, and the dysregulation of this recruitment may lead to pathological rupture of membranes.

Th17 cells ($CD3^+CD4^+IL\text{-}17A^+$) also congregate in human decidua,[103] and their tissue density is higher in cases of chorioamnionitis than in cases without chorioamnionitis.[104] This finding further supports the idea that pro-inflammatory adaptive immune cells at the maternal/fetal interface are associated with chorioamnionitis, which can lead to preterm labor/birth. Studies in our laboratory are currently exploring the potential role for this T-cell subset in preterm labor using LPS-induced and RU486-induced preterm birth models.

Fetal T cells might also play a role during preterm labor. Memory fetal T cells ($CD45RO^+RA^-$) are present in higher proportions in cord blood from cases of preterm labor compared to term labor.[105] Fetal T cells are also activated ($CD25^+CD69^+$) during preterm labor.[106] Indeed, acute chorioamnionitis, a leading cause of preterm deliveries, is associated with an increase in cord blood T-cell chemokines

(CXCL9, -10 and -11).[107] These results suggest that fetal T cells can contribute to the pathophysiology of preterm labor.

Cytotoxic T cells (CTLs) are present at the maternal/fetal interface in term gestations in the absence of labor, where they express perforin and granzyme B.[95,97,108] In placenta, CTLs are abundant in cases with villitis of unknown etiology and express T-cell chemokine receptors (CXCR3 and CCR5).[107] In peripheral circulation, $CD300a^+$ CTLs have an effector memory phenotype, and their proportion is higher in women with chronic chorioamnionitis than in women without this lesion.[109] Taken together, these data suggest that CTLs may participate in pathological inflammation associated with preterm birth, but their role during spontaneous labor at term and preterm requires further exploration.

### Tregs

There are two main Treg subsets: thymic Tregs (tTregs) and extrathymic or peripheral Tregs (pTregs). During pregnancy, $CD4^+$ pTregs have been categorized into four subsets: $DR^{high+}CD45RA^-$, $DR^{low+}CD45RA^-$, $DR^-CD45RA^-$ and naïve $DR^-CD45RA^+$.[110] The proportion of each subset seems to be relevant in the pathophysiology of pregnancy complications such as preterm labor. Women with preterm labor have a reduced proportion of naïve $DR^-CD45RA^+$ Tregs, accompanied by higher proportions of $DR^-CD45RA^-$ and $DR^{low+}CD45RA^-$ Tregs within their total pTreg pool.[110,111] Indeed, the suppressive activity of pTregs is strongly reduced in term and preterm labor,[111] which is correlated with a reduction in the expression of HLA-DR in preterm cases.[112] This suggests that the lack of suppressive function during late pregnancy could trigger the onset of parturition at term and preterm gestations.[113]

At term pregnancy, Tregs are found at the maternal/fetal interface, have a unique phenotype ($CD4^+CD25^{bright}FoxP3^+CD69^+HLA\text{-}DR^+CTLA\text{-}4^+$), and exhibit suppressive function *in vitro*.[108,114] However, the role of decidual Tregs remains undetermined. Currently, we are investigating the function and phenotypic characteristics of these cells during term and preterm labor.

Mechanistic studies have successfully demonstrated that the systemic ablation of Tregs by targeting $FoxP3^+$ cells leads to pregnancy failure during early gestation.[6,7,115] A separate study suggested that regulatory T cells are not required in late pregnancy;[116] however, the targeted depletion of $CD25^+$ cells is not specific for Tregs. Preliminary data from our laboratory demonstrates that systemic depletion of $FoxP3^+$ cells during late gestation does lead to pregnancy complications (NGL, unpublished data).

Additional unpublished data from our laboratory demonstrate that LPS-induced preterm labor causes an expansion of $CD4^+$ Tregs in the spleen and thymus but a reduction of uterine $CD4^+$ Tregs (NGL, unpublished data).[117] We also found that the administration of vaginal progesterone, a clinical strategy to prevent preterm birth in women with a short cervix,[118] increases the proportion of decidual $CD4^+$ Tregs (NGL, unpublished data).[119] Altogether, these data suggest that preterm birth is characterized by altered proportions of $CD4^+$ Tregs at the maternal/fetal interface and that natural progesterone can restore the number of these cells during late pregnancy, preventing preterm birth.

### B cells

A few years ago, we suggested a role for B cells during term labor since the fetal membranes from laboring women who delivered at term exhibit B-cell attraction *in vitro*.[12,16]Current preliminary data demonstrate that B cells are indeed present in the decidua and cord blood at term and preterm stages (NGL, unpublished data). However, the role of B cells in the processes of term and preterm labor is still under investigation.

Several studies have linked various B cell subsets to pregnancy. B1 cells are present in lower proportions in maternal blood during pregnancy and return to non-pregnant proportions postpartum.[120] However, B2 cell frequencies are unchanged by pregnancy in the peripheral blood of women.[120] Regulatory B cells exist during early and late gestation and release IL-10.[121,122] Regulatory B cells are potential immune players in the development of immunological tolerance, and their presence during mid-gestation may be important in sustaining pregnancy until labor. B10 cells suppress TNF-$\alpha$ secretion by $CD4^+$ T cells during pregnancy,[122] and this may regulate the inflammatory state prior to labor. Furthermore, B cells isolated from term placentas produce increased amounts of asymmetric IgG upon stimulation by IL-6, IL-10 and IL-4.[123] Therefore, an abnormal disruption of B cell-derived cytokine and asymmetric antibody production could play a part in disturbing fetal tolerance and possibly in eliciting preterm labor.

## BRIDGES BETWEEN THE INNATE AND ADAPTIVE IMMUNE SYSTEMS IN TERM AND PRETERM LABOR

Immune tolerance involves both the innate and adaptive immune systems. Therefore, fetomaternal tolerance must involve the participation of immune cells that bridge the innate and adaptive immune systems, such as DCs and natural killer T (NKT) cells. The roles of these cells during late gestation, labor and preterm labor are discussed below.

### NKT cells in term and preterm labor

NKT cells are a unique lymphocyte subpopulation that express markers and characteristics of both the adaptive and innate limbs of the immune system. NKT cells recognize lipid antigens presented by the non-polymorphic CD1D molecule,[124] which is expressed by trophoblast cells, placenta, and choriocarcinoma cell lines.[125,126] There are two types of NKT cells, type I and type II.[127] Type I NKT or invariant NKT (iNKT) cells can be activated by the marine-derived glycolipid $\alpha$-galactosylceramide,[124] and this molecule has been utilized to explore the role of iNKT cell activation during pregnancy *in vivo*.[128–130] Therefore, this section will primarily focus on iNKT cells.

During murine pregnancy, $NK1.1^+TCR\alpha\beta^+$ NKT cells have been observed in the decidua and uterus primarily in early gestation, although they are still present near term (14–18 dpc).[129,131] Murine $NK1.1^+CD3^+$ NKT cell proportions were higher in the livers of pregnant mice during late gestation (16 dpc) than in non-pregnant controls.[132] iNKT cells can

secrete large quantities of IL-4 (Th2) and IFN-γ (Th1) upon TCR activation,[133] and their activation has been shown to have roles in activating NK cells, B cells and T cells.[134] As a result of their immunological effects and their presence during gestation, iNKT cells may participate in pathological or physiological responses during late gestation.

iNKT cells have been involved in the induction of an increased cytotoxic state during human pregnancy complications such as preeclampsia.[135] The proportion of iNKT cells expressing activation markers ($CD69^+$), perforin and IFN-γ is increased in the blood of pre-eclamptic women in comparison to pregnant women without this pathology.[135] Although the aforementioned study did not address preterm labor, these results indicate that pro-inflammatory iNKT cells are increased during late gestational pregnancy complications; this Th1-like environment could potentially disrupt fetomaternal tolerance and lead to preterm labor.

The role of iNKT cells in the induction of LPS-induced preterm birth has been studied in iNKT cell deficient ($J\alpha18^{-/-}$) mice.[136] The injection of LPS at 15 dpc caused preterm birth in wild-type mice but not in iNKT cell-deficient mice,[136] suggesting that iNKT cells modulate the process of labor induced by microbial products. Conversely, the stimulation of iNKT cells *in vivo* by injection of α-galactosylceramide during late gestation (16 dpc) induced early preterm birth (17 dpc),[128] which may be due to an expansion of $NK1.1^+TCR\alpha\beta^+$ NKT cells in the uterus.[129] In contrast, studies conducted in our laboratory found that the activation of iNKT cells during late gestation (16 dpc) through α-galactosylceramide administration induced late preterm birth (birth at 18 dpc, 28 h post-injection), which is relevant since 70% of all preterm births in woman fall under this category (NGL, unpublished data).[130] Current experiments in our laboratory are addressing the immune mechanisms whereby iNKT cell activation leads to late preterm birth.

### DCs in term and preterm labor

DCs are specialized in antigen recognition and presentation. DCs exhibit properties that include induction of antigen-specific T-cell activation, T-cell suppression, Treg generation and peripheral tolerance.[137,138] Lymphoid $CD8\alpha^+$ DCs (DCs1) induce a Th1 response whereas myeloid CD8α DCs (DCs2) elicit a Th2 response.[139,140] A third type of DCs is the inflammatory DCs which initiates a Th1 response as well.[141] Due to their immunomodulatory properties, these three subsets of DCs are relevant in the study of fetomaternal tolerance and inflammation during labor and preterm labor.

DCs contribute to fetomaternal tolerance during early pregnancy.[142] In mice, uterine DCs have a DC2 phenotype at 15 dpc,[143] which suggests that these cells contribute to the tolerogenic state by inducing a local anti-inflammatory (Th2) response during late gestation. Later in pregnancy (17.5 dpc), the predominant DC subset in the uterus is $CD11c^+CD8\alpha^-MHCII^-$ (immature phenotype).[144] The fact that immature DCs express the anti-inflammatory cytokine IL-10,[144] a potential early biomarker of preterm birth,[145] suggests that these cells may participate in the etiology of preterm labor. Moreover, in T and B cell-deficient mice ($Rag1^{-/-}$) injected with LPS to induce preterm birth, uterine DC activation was observed,[146] suggesting the participation of DCs in the induction of labor. Further research is needed in order to establish a role for DCs during late gestation, labor and preterm labor.

## CONCLUSIONS

During late pregnancy, paternal–fetal antigen-specific memory T cells (including Tregs) participate in the maintenance of fetomaternal peripheral tolerance. Collectively, these cells create an anti-inflammatory environment which will sustain pregnancy. We suggest the following pathway could lead to labor: (1) activation of innate and adaptive immune cells increases their migratory ability; (2) reproductive tissues and the maternal/fetal interface actively recruit the activated cells through the release of chemokines such as CXCL10, CXCL8, CCL2 and CCL5; and (3) infiltrating leukocytes amplify the pro-inflammatory microenvironment at the maternal/fetal interface leading to labor. A triggered stimulus (e.g., infection/inflammation, sterile inflammation, stress, *etc.*) can cause the premature activation of this pathway, eliciting a shift from an anti-inflammatory to a pro-inflammatory microenvironment and consequently preterm labor (Figure 1).

An overview of the innate and adaptive immune cells in reproductive tissues and at the maternal/fetal interface during term and preterm labor is shown in Figure 2. Neutrophils are present in the cervix, myometrium, fetal membranes and decidua at term pregnancy; however, their density increases in the myometrium and decidua in term labor and infection-associated preterm labor. Neutrophils are present in the cervix and participate in the repair process during the postpartum period. Macrophages are present in the cervix, myometrium, fetal membranes and decidua at term pregnancy and their density increases in all these tissues, except the cervix, during term and preterm labor. Cervical macrophages also seem to participate in postpartum repair processes. Mast cells are found in cervical and myometrial tissues during late gestation; however, their roles during term and preterm labor are unclear. Effector $CD4^+$ T cells are present in decidual tissues during term labor, and decidual Th17 cells also seem to be involved in the pathology of preterm labor. CTLs are found in term pregnancy and in placental tissues in cases with villitis of unknown etiology; however, their role during labor is unknown. The fetal membranes exhibit B-cell recruitment during term labor, and B cells are found in decidual tissues and cord blood; however, their role in preterm labor is still under investigation. Finally, in myometrial tissues, NKT cell and DC activation seem to be involved in the pathophysiology of preterm labor.

Overall, collaboration between the innate and adaptive limbs of the immune system is required to sustain pregnancy until term. A disruption of either limb at term may lead to physiological labor, and an untimely disruption could result in pathological preterm labor. Research targeting the immune cells involved in the process of labor might reveal new strategies to prevent preterm labor and consequently preterm birth.

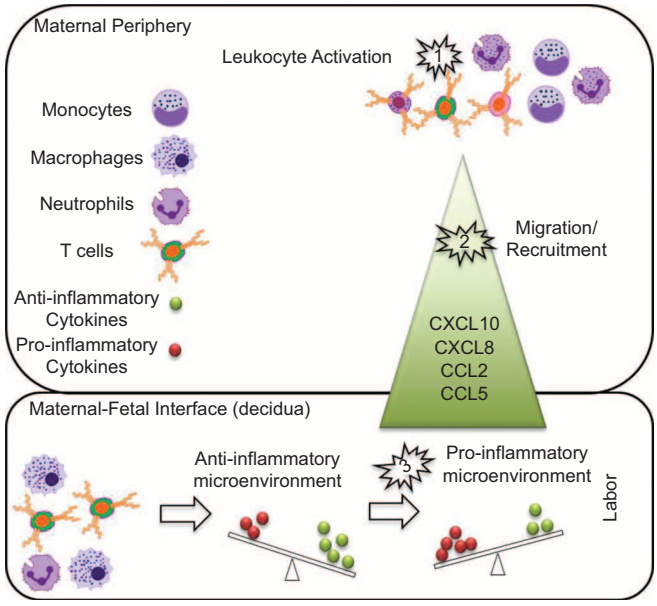


**Figure 1** A suggested pathway that leads to term or preterm labor. The following pathway could lead to labor: (1) activation of innate and adaptive immune cells increases their migratory ability; and (2) the maternal/fetal interface actively recruits the activated cells through the release of chemokines such as CXCL10, CXCL8, CCL2 and CCL5; (3) infiltrating leukocytes amplify the pro-inflammatory microenvironment at the maternal/fetal interface leading to labor. A triggered stimulus (e.g., infection/inflammation, sterile inflammation, stress, *etc.*) can cause the premature activation of this pathway, eliciting a shift from an anti-inflammatory to a pro-inflammatory microenvironment and consequently preterm labor.

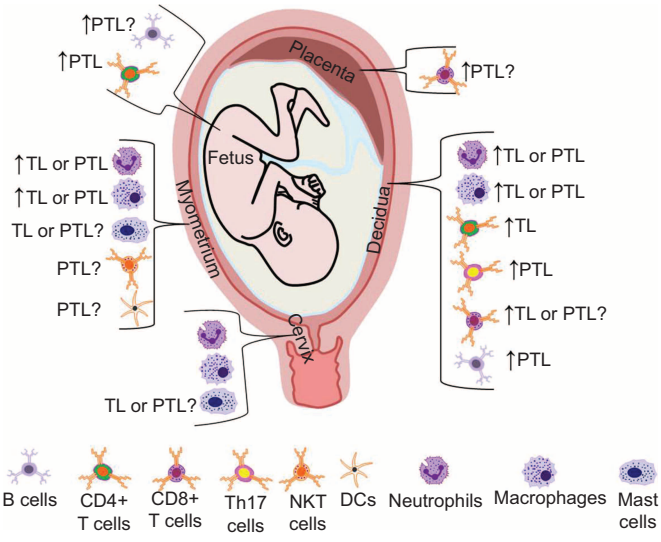


**Figure 2** Immune cells in term and preterm labor. Schematic representation of innate and adaptive immune cells in reproductive tissues and at the maternal/fetal interface in term and preterm labor.

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