REVIEW ARTICLE

Maternal and fetal T cells in term pregnancy and preterm labor

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Pregnancy is a state of immunological balance during which the mother and the developing fetus must tolerate each other while maintaining sufficient immunocompetence to ward off potential threats. The site of closest contact between the mother and fetus is the decidua, which represents the maternal–fetal interface. Many of the immune cell subsets present at the maternal–fetal interface have been well described; however, the importance of the maternal T cells in this compartment during late gestation and its complications, such as preterm labor and birth, has only recently been established. Moreover, pioneer and recent studies have indicated that fetal T cells are activated in different subsets of preterm labor and may elicit distinct inflammatory responses in the amniotic cavity, leading to preterm birth. In this review, we describe the established and proposed roles for maternal T cells at the maternal–fetal interface in normal term parturition, as well as the demonstrated contributions of such cells to the pathological process of preterm labor and birth. We also summarize the current knowledge of and proposed roles for fetal T cells in the pathophysiology of the preterm labor syndrome. It is our hope that this review provides a solid conceptual framework highlighting the importance of maternal and fetal T cells in late gestation and catalyzes new research questions that can further scientific understanding of these cells and their role in preterm labor and birth, the leading cause of neonatal mortality and morbidity worldwide.

Keywords: maternal-fetal interface; decidua; amniotic fluid; adaptive immunity; parturition

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INTRODUCTION

Pregnancy, a state in which the mother must maintain a synergetic relationship with the developing fetus, depends on constant, effective crosstalk between these two genetically dissimilar individuals.[1–12] Thus, pregnancy represents a complex state of balance in which the maternal immune system must tolerate the fetus[3,13–17] (and vice versa[18–21]) and concurrently maintain a certain level of immunocompetence to protect against potential threats.[22–24] In particular, such distinction necessitates systemic and local immune interactions throughout pregnancy, in which the most intimate site of contact is the maternal–fetal interface (i.e., decidua).[25,26] In humans, the maternal–fetal interface comprises the decidualized endometrium, named according to the specific adjacent maternal and fetal tissues: the decidua basalis forms the junction between the uterine endometrium and the placenta, and the decidua parietalis connects the fetal membranes to the uterine wall.[26,27] Together, these two unique tissues form the site of specific mechanisms through which maternal and fetal immunity are bridged to promote the maintenance of pregnancy.[12,22,25,28–32]

The cellular immune repertoire of the maternal–fetal interface comprises numerous innate subsets, including natural killer (NK) cells[33–40] and macrophages[41–47] as well as adaptive immune cells such as effector T cells,[48–56] regulatory T cells (Tregs),[3,45,57–60] and a fraction of B cells,[38,39,49,61–64] all of which vary throughout gestation.[65–68] Cell types that bridge innate and adaptive immune responses, such as invariant NK T cells (iNKT cells)[69–71] and innate lymphoid cells (ILCs)[67,72–75] have also been described at the maternal–fetal interface. Each of these subsets performs specific roles throughout pregnancy such as supporting placental development,[35,68,76] maintaining maternal–fetal tolerance,[1–12,45] and participating in the inflammatory processes that accompany labor.[46,49,55,64,70,77–82] Consequently, a disruption of these different immune cell subsets is often associated with adverse pregnancy outcome.[22,31,68,83] In particular, the premature influx of activated effector T cells[55,84–87] or alterations in the functions or proportions of Tregs[88–93] in the decidual tissues have been associated with pregnancy complications. Thus, a clearer understanding of the roles that maternal T cells play at the maternal–fetal interface in late gestation, as well as in the physiological and pathological processes of labor, is warranted.

To further explore the cellular immune repertoire of the maternal–fetal interface, recent cutting-edge molecular surveys utilizing next-generation sequencing technologies have provided a deep characterization of the many leukocyte subsets present in the decidual tissues as well as their interaction networks.[94–98] Importantly, placental and decidual cell type-specific gene signatures can be monitored in the maternal circulation, providing a noninvasive means of evaluating maternal–fetal crosstalk.[95,98] Pertinent to the topic discussed herein, we have shown that

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activated T cell-specific transcriptional signatures derived from the maternal–fetal interface are modulated during pregnancy, in term labor, and in preterm labor.[98–100] This evidence further supports a role for maternal T cells in normal and complicated pregnancies.

Fetal immunity must also be considered in the context of pregnancy and its complications, as the fetus displays an established and functional T-cell repertoire beginning early in gestation.[18,101–103] Indeed, pioneer studies showed that the fetal immune system is activated during the onset of preterm labor, which precedes preterm birth.[104,105] Recently, this concept has been revisited to establish the contribution of fetal T cells to preterm labor and birth as well as to elucidate the stimuli that might drive such fetal T-cell activation;[21,106] however, additional investigation is required in this research area.

In this review, we highlight the established and proposed roles for maternal T cells at the maternal–fetal interface in the process of normal parturition at term. Moreover, we describe the known contributions of maternal T cells to the pathological process of preterm labor and birth. Finally, we summarize current knowledge on the presence and functional status of fetal T cells in the context of preterm labor and birth. We aim to provide a clear summary of the importance of both maternal and fetal T cells in the mechanisms of physiological and pathological parturition, which can shed light on the gaps in knowledge that still remain in order to guide future research.

MATERNAL T CELLS AT THE MATERNAL–FETAL INTERFACE

Generally speaking, naïve T cells (T_N) are present in the circulation, awaiting presentation of their cognate antigen by dendritic cells in secondary lymphoid tissues,[107] after which the T cells will proliferate and differentiate to carry out effector functions.[108] Once the threat has been cleared, a fraction of antigen-specific T cells will persist in the circulation as memory T cells to provide more rapid responses should the same antigen be detected again.[108] Memory T cells can be further divided into central (T_{CM}), effector (T_{EM}), and terminally differentiated effector memory (T_{EMRA}) T cells based on immediate or latent function and the expression of migratory homing receptors.[108] In addition, CD4+ T cells (and CD8+ T cells, to some extent) can differentiate into one of several effector subsets including T helper type 1 (Th1), Th2, Th9, and Th17 cells based on the surrounding microenvironment.[108,109] Various reports described below have indicated the participation of multiple T-cell subsets in both the normal physiological processes of pregnancy and in its complications such as preterm labor and birth.

Conventional effector T cells had typically been considered a relatively minor component of the cellular immune repertoire at the maternal–fetal interface prior to term parturition, with their increased presence or activation indicating inflammation-related pregnancy pathologies. Indeed, early reports indicated that suppressive mechanisms exist in the decidua that are targeted specifically toward IL-2-dependent cells,[110] likely to be driven at least partially by Tregs. Seminal studies have shown that tissue-specific gene silencing mechanisms exist to prevent uncontrolled invasion of effector T cells into the decidua during early pregnancy, thereby preventing premature fetal loss.[111] Thus, together with the presence of Tregs and other homeostatic cells such as macrophages,[41–46] the decidual tissues represent a tightly controlled microenvironment. More recently, we and others showed that a proportion of the maternal T cells found at the maternal–fetal interface display an exhausted or dysfunctional phenotype at term pregnancy,[24,82] demonstrating other mechanisms in which effector T cells are precluded from disrupting normal gestation, which is further described below.

In addition to the abovementioned mechanisms of regulation, the fetal tissues themselves contribute to the modulation of the maternal immune environment through the expression of specific HLA antigens, most notably HLA-G.[112] First described as being solely expressed by extravillous trophoblast cells,[113–115] soluble isoforms of this protein are found in the placenta, chorion, decidua, and even maternal blood during pregnancy.[116] HLA-G interacts with several members of the immunoglobulin-like transcript (ILT) receptor family[117–121] [now termed leukocyte Ig-like receptor subfamily B (LILRB)[122]] that are expressed by multiple subsets of immune cells,[117,123] including T cells.[124–128] LILRB receptors exhibit immunosuppressive behavior upon their activation and have thus been considered as immune checkpoints for T cells.[129] Accordingly, studies have indicated that the interaction of fetal HLA-G with maternal T cells may promote a more tolerant state.[130–135] On the other hand, several studies have suggested that altered levels of HLA-G at the maternal–fetal interface or in the maternal circulation could be associated with adverse pregnancy outcomes including preterm birth,[136–140] and increased concentrations of soluble HLA-G have been detected in amniotic fluid of women with intra-amniotic infection.[141] Yet, the participation of HLA-G in adverse pregnancy outcomes remains unclear.[142,143] Interestingly, HLA-G expression may not be exclusive to fetal tissues during pregnancy, as recent reports have shown that subsets of immunosuppressive regulatory-like T cells can express HLA-G themselves[144–146] or in some cases can acquire the expression of this molecule through trogocytosis from antigen presenting cells (APCs).[147,148] The investigation of whether the latter method of HLA-G "transfer" from APCs to T cells occurs during pregnancy could provide important insight into the mechanisms of maternal–fetal tolerance during this critical period.

T cells at the maternal–fetal interface in term pregnancy

As the end of gestation approaches, conventional T cells constitute an obvious proportion of the immune cellular repertoire at the maternal–fetal interface.[48,51,55,62,78,79,81,98,149] A comparative immunohistochemistry-based study reported that the proportion of CD45+CD3+ cells was significantly increased in the human term decidua compared to that in the first trimester, and such an increase was reflected by the elevated numbers of T cells expressing CD4, CD8, TCRαβ, or TCRγδ,[48] suggesting that a general increase in decidual T cells occurs in late pregnancy. The comparison of specific T-cell subsets between the two main sites of the maternal–fetal interface at term indicated that the frequencies of TCRγδ+ and CD8+ T cells were greater in the decidua parietalis versus the decidua basalis, whereas the opposite was true for CD4+ T cells.[62] In addition to the above observations, several studies have also indirectly confirmed the presence of conventional T cells at the maternal–fetal interface using immunofluorescence microscopy[150] or immunophenotyping[151] to detect CD4+ and CD8+ T cells in the murine placenta during late pregnancy. These studies indicate that T cells are residents of the maternal–fetal interface in term pregnancy, even before the onset of labor, the coordinated timing of which is critical.

The migration of conventional T cells to the maternal–fetal interface prior to term parturition is controlled at least partially through tissue-specific mechanisms: a series of studies revealed that T-cell chemotactic pathways were upregulated in the decidual tissues from cases of term labor.[78–80] Moreover, decidual T cells express activation markers such as CD38 and CD69 at term pregnancy,[149] diminishing the possibility of such cells being mere bystanders at the maternal–fetal interface. More recently, in support of the previously described general residence of T cells at the maternal–fetal interface, high-dimensional immunophenotyping studies showed that term decidual tissues host a heterogeneous population of CD4+ and CD8+ T_N, T_{CM}, and T_{EM} subsets.[55] In addition, decidual effector T cells expressed enhanced levels of effector molecules such as perforin and granzyme B, cytotoxic molecules that function in a cooperative manner to perforate the target cell membrane and initiate

apoptosis,[152] in women who underwent the physiological process of labor at term.[55] This suggests that T-cell activation at the maternal–fetal interface is an intrinsic mechanism associated with the inflammatory process of labor at term, which is in line with in vitro observations showing that the stimulation of decidual T cells results in upregulation of perforin and granzymes.[24] Single-cell analyses of the maternal–fetal interface complemented these prior studies by showing upregulation of the cell signature specific to activated T cells in the decidua basalis in cases of term labor compared to term deliveries without labor.[98] Together, these data support a role for activated effector maternal T cells at the maternal–fetal interface in physiological parturition at term.

Effector T cells at the maternal–fetal interface in preterm labor and birth

As the presence of functional maternal effector T cells at the maternal–fetal interface is amplified toward the end of pregnancy, a premature increase in the frequency and/or activation of such cells has thus been associated with pathological pregnancy outcomes such as preterm labor and birth. Initial studies established that the invasion of cytotoxic T cells into the decidual tissues, a placental lesion classified as chronic histological chorioamnionitis, can occur in a subset of term deliveries but is more frequently observed in cases of preterm labor and birth, preterm prelabor rupture of membranes (pPROM), and fetal death.[84–86] Such associations have been confirmed using flow cytometry[55] and single-cell transcriptomic[98] approaches to demonstrate the increased presence of effector and activated T cells at the maternal–fetal interface of women with preterm labor and birth. Indeed, these pathological cases are characterized by an influx of T_{EM} expressing perforin and granzyme B into the maternal–fetal interface, which is even greater than that observed in labor at term.[55] Further, murine studies have established that the systemic activation of T cells through the administration of an anti-CD3 antibody is sufficient to induce preterm birth[55,87] through inflammatory mechanisms that are distinct from those observed in conventional models of preterm birth [e.g., administration of endotoxin (LPS) or progesterone receptor antagonist (RU486)].[55] Together, these observations have caused a paradigm shift in regard to how the adaptive immune system is viewed in the context of pregnancy complications.

IL-17-producing T cells were shown to be present at the maternal–fetal interface in early pregnancy,[153–157] and a disruption of the balance between Th17 cells and Tregs has been implicated in early pregnancy complications (e.g., spontaneous abortion).[158–164] Yet, studies have also suggested that Th17 cells are implicated in late pregnancy disorders such as preeclampsia[162,165–170] or preterm labor.[171] Cases of preterm labor with chronic inflammation of the placenta showed higher amounts of IL-17 at the maternal–fetal interface and in amniotic fluid compared to preterm labor cases without this lesion, which may be released by the Th17 cells infiltrating the chorioamniotic membranes.[171] Moreover, a recent transcriptomic study demonstrated increased expression of Th1- and Th17-related genes together with downregulation of Foxp3 at the maternal–fetal interface of women with preterm labor and pPROM.[172] Together, these findings provide a possible link between dysregulated Th17 responses and preterm labor and birth.

Besides dedicated adaptive lymphocytes such as T cells, cells that bridge innate and adaptive immunity [e.g., iNKT cells (discussed below) and ILCs (reviewed in ref. [67])] are also present at the maternal–fetal interface in early and late gestation.[173,174] Pregnant mice deficient in iNKT cells were less susceptible to endotoxin-induced preterm birth, indicating that iNKT-cell activation could be detrimental to pregnancy outcomes.[69,175] Accordingly, the systemic activation of iNKT cells using α-galactosylceramide resulted in preterm delivery and increased neonatal mortality.[70] Such systemic activation of iNKT cells resulted in their expansion at the maternal–fetal interface as well as concomitant reductions in the local numbers of total T cells and Tregs,[71] suggesting an inverse relationship between the Tregs and iNKT cells in this compartment. Moreover, such expansion of iNKT cells at the maternal–fetal interface was accompanied by increased numbers of Th17 cells.[71] In humans, transcriptomic analysis of decidual lymphocytes revealed elevated expression of CD1d (an established iNKT-cell receptor) in cases of preterm labor and birth compared to labor at term.[39] Moreover, immunophenotyping consistently revealed an increased proportion of activated iNKT-like cells in the decidual basalis of women with preterm labor and birth.[70] Given that iNKT cells are present at the murine maternal–fetal interface throughout pregnancy, further investigation of this unique decidual subset is required to establish the different roles of iNKT cells in early and late gestation.

Exhausted and senescent T cells at the maternal–fetal interface during pregnancy

Several physiological mechanisms exist to reduce the severity of effector T-cell functions in situations where such responses may cause more harm than good. Prime examples of these mechanisms include T-cell suppression and the more recently described phenomena of T-cell exhaustion and senescence.[176,177] Although the endpoints of T-cell exhaustion and senescence are similar (i.e., the control of effector T-cell responses), these two cell fates have been demonstrated to be distinct[176] and are the topic of current investigation.[178–181]

T-cell exhaustion typically occurs in the context of chronic antigen exposure/stimulation that results in a progressive loss of function accompanied by upregulated expression of multiple inhibitory receptors such as TIM-3, PD-1, CTLA-4, and LAG-3.[182–184] Clinically, this phenomenon has been described in the context of chronic viral infections and cancers;[182–185] yet, recent reports have suggested that this process occurs in pregnancy as well.[24,52,82]

In contrast to exhaustion, senescent T cells maintain effector functions in the absence of inhibitory receptor expression while losing the ability to proliferate,[176] thus reaching a terminal state. Senescence is indicated by the upregulation of markers such as CD57, KLRG-1, and CD45RA together with the downregulation of CD27 and CD28.[186–190] Among T cells, recent studies have indicated that the T_{EMRA} subset is at least partially comprised of senescent T cells based on phenotypic and functional data.[191] To date, few reports have investigated the presence of senescent T cells during pregnancy,[82] evidence that is further discussed below.

Animal studies have revealed that the decidua hosts a population of CD8+PD-1+TIM-3+ T cells in early and mid-pregnancy that display enhanced inflammatory capacity upon blockade of either inhibitory marker.[52] In addition, the in vivo blockade of PD-1 or TIM-3 resulted in increased rates of fetal loss, indicating that the expression of inhibitory receptors is important for pregnancy maintenance.[52] This was further supported by the observation that CD8+PD-1+TIM-3+ T cells were impaired in decidual tissues from women with miscarriage.[52] In the third trimester, decidual cytotoxic T cells expressed a transcriptomic signature indicative of a combination of dysfunction and activation that could be reversed by in vitro stimulation,[24] and detailed immunophenotypic analysis revealed that a large proportion of CD4+ and CD8+ T_{EM} expressed an exhausted-like PD-1+TIM-3+ phenotype at the maternal–fetal interface.[82] Notably, the proportion of exhausted CD4+ T cells in the decidua parietalis increased with advancing gestational age,[82] suggesting that the prolonged exposure to antigens (fetal, microbial, or even self) that occurs during pregnancy necessitates increasing regulation of these cells to avoid aberrant T-cell activation. The process of physiological labor affected exhausted T cells in the decidua basalis and decidua parietalis differently, as a significant reduction in the proportions of exhausted CD4+ and CD8+ T cells was observed in the decidua basalis of women who underwent term parturition compared to non-labor controls.[82] These studies

confirm that T-cell exhaustion is a physiological phenomenon at the maternal–fetal interface in late gestation; yet, further investigation into the clinical implications of exacerbated T-cell exhaustion in term gestation (delayed parturition, prolonged labor, dystocia, etc.) is needed. In line with this concept, a recent report showed that mice of advanced maternal age undergo prolonged labor and dystocia that is associated with a reduced number of pro-inflammatory T cells at the maternal–fetal interface; yet, the exhausted phenotype of such cells was not investigated.[192]

Besides exhaustion, a fraction of T cells at the maternal–fetal interface was shown to express surface markers consistent with a senescent phenotype (KLRG-1 and CD57) in late gestation.[82] Among both CD4+ and CD8+ populations, a large proportion of senescent T cells was of the T_{EMRA} subset,[82] which is in line with previous reports.[191] Unlike their exhausted counterparts, senescent T cells at the maternal–fetal interface did not significantly vary with gestational age or with the presence of labor.[82] Taken together, these findings suggest that both exhaustion and senescence play a role in regulating the function of effector T cells at the maternal–fetal interface, which future mechanistic studies may demonstrate.

Exhausted and senescent T cells at the maternal–fetal interface in preterm labor and birth

Mechanistic studies demonstrated that the blockade of PD-1 and TIM-3 in early to mid-pregnancy results in fetal loss.[52] However, whether a reduction in the number or function of these cells is associated with late pregnancy complications such as preterm labor and birth is still unclear. Among women with preterm labor and birth, those with acute inflammatory lesions of the placenta displayed reduced proportions of exhausted T cells in the decidua basalis.[82] These observations suggest that, although T-cell exhaustion is a physiological process in late gestation, adverse events such as pathological inflammation can reactivate these cells. Such a concept is supported by the fact that the in vitro stimulation of exhausted decidual T cells results in the restoration of effector functions.[24,82]

Investigation focused on senescent T cells is relevant to pregnancy complications given that the process of decidual senescence has been proposed as a new mechanism of disease for preterm labor and birth (reviewed in refs. [193,194]). In line with this concept, the presence of acute placental inflammation in preterm gestations, but not at term, was associated with a significantly reduced proportion of senescent T cells in the decidua basalis.[82] Senescence has been demonstrated to be reversible in T cells in vitro;[195–198] thus, it is possible that the pathological inflammation accompanying specific subsets of preterm labor cases will cause reversal of senescent T cells in the decidual tissues, further propagating inflammation.

Collectively, these studies suggest that the inflammatory responses accompanying preterm labor may reactivate exhausted and senescent T cells at the maternal–fetal interface, leading to an aberrant effector T-cell response that can trigger preterm labor and birth. However, further investigation into how the various etiologies of preterm labor differentially affect exhausted and/or senescent T cells at the maternal–fetal interface is warranted in order to find novel strategies to prevent the adverse neonatal outcomes associated with this syndrome.

Regulatory T cells at the maternal–fetal interface during pregnancy

Regulatory T cells (Tregs) are well known for their critical functions in preventing autoimmunity, driving successful transplantation, and modulating tumor–immune interactions (reviewed in refs. [199,200]). Importantly, Tregs are also highly relevant for maternal–fetal tolerance.[1–12,45,57–60] Regulatory T cells are classically described as CD4+CD25+Foxp3+ cells that display potent immunosuppressive functions.[200] In particular, expression of the transcription factor Foxp3 is essential for the development, differentiation, and suppressive function of Tregs, and is thus a key phenotypic marker for the identification of such cells.[201–204] Regulatory T cells are further subdivided into thymic/natural Tregs and peripheral/induced Tregs, which arise through different developmental processes.[205,206] Natural Tregs are thought to be mainly involved in the control of autoimmune responses,[207–209] while peripheral Tregs appear to be significant in mucosal immunity,[210–214] including maternal–fetal tolerance.[8,9,215–217] Regulatory T cells exert their suppressive function primarily by direct cell-cell interactions and through the release of anti-inflammatory cytokines such as IL-10,[218,219] TGFβ,[220,221] and IL-35.[222] Such regulatory functions are especially critical for pregnancy establishment and maintenance, as the systemic depletion of Tregs prior to implantation or in early gestation leads to implantation failure or pregnancy loss.[4,6–9,223–225] Yet, due to their importance in the establishment and maintenance of maternal–fetal tolerance, few studies have investigated the function of Tregs at the maternal–fetal interface in the third trimester of pregnancy, a central period for the onset of obstetrical disease.[226]

A role for decidual Tregs late in term gestation was first suggested when it was found that the proportions of CD3+CD4+CD25+ T cells in the decidua basalis and decidua parietalis were reduced in women with spontaneous term labor compared to those with term cesarean sections.[49] The authors proposed that this subset may include decidual Tregs that "disappeared" prior to the onset of labor.[49] A later study further investigated the CD4+CD25bright and CD4+CD25dim populations in decidual tissues from the second trimester and term deliveries (both cesarean section and vaginal), and showed that these populations did not change with the length of gestation.[50] Subsequently, the same CD4+CD25+ population was further characterized by the expression of Foxp3 and other cellular markers associated with Tregs, revealing that the CD4+CD25bright decidual population was largely composed of Tregs with high proportions of Foxp3, CTLA-4, and HLA-DR expression, whereas the CD4+CD25dim population displayed an activated phenotype with high percentages of CD69+ cells.[227] Together, these studies provided an initial overview of Tregs at the maternal–fetal interface in normal term deliveries, prompting further discovery.

A subsequent in-depth investigation included decidual samples from women undergoing cesarean section either without any signs of labor, in early labor, or in advanced labor, and confirmed that Tregs are present at the maternal–fetal interface at term.[228] Notably, the authors found that the proportions of decidual Tregs declined as labor progressed, suggesting that the process of labor is associated with alterations in Treg populations at the maternal–fetal interface.[228] A recent report described three distinct Treg subsets in the decidua defined by the high expression of CD25, PD-1, or TIGIT.[60] The authors compared Treg populations in the decidua basalis and decidua parietalis and showed that there are no significant differences in Treg populations between these tissues, although the proportions of the CD25+ and TIGIT+ Treg subsets tended to be greater in the decidua parietalis.[60] Moreover, the ability of decidual Tregs to suppress CD4+ effector T-cell proliferation was significantly reduced at term compared to those from the first trimester decidua, suggesting that the functional capacity of decidual Tregs declines, but is not completely reduced, toward the end of gestation.[60]

Several investigations in mice have also consistently found a Treg population in the decidual tissues prior to term delivery.[229–231] Specifically, two of these reports investigating the anti-inflammatory properties of vaginal progesterone[229] or human chorionic gonadotropin (hCG)[231] showed that both treatments significantly increased the proportion of Tregs at the maternal–fetal interface in the third week of murine pregnancy, suggesting that the observed anti-inflammatory properties of these hormones were at least partially due to modulation of immune cell populations in this compartment (the immune modulatory functions of progesterone

and hCG during pregnancy have recently been reviewed in refs. [232,233]). Together, human and animal studies support the presence and functionality of Tregs at the maternal–fetal interface in term gestations.

Is there a role for Tregs at the maternal–fetal interface in preterm birth?

The importance of Tregs throughout gestation is underscored by the fact that systemic and local alterations in these cells are consistently observed in women with preeclampsia (as reviewed in refs. [91,93]), a clinical condition for which the only current effective treatment is delivery of the placenta.[234,235] Indeed, animal studies have demonstrated a preeclamptic-like phenotype resulting from the depletion of Tregs in early gestation.[92] Such alterations hold true in late pregnancy, as preeclampsia impacts Treg numbers and function at the human maternal–fetal interface.[59,88,89,170,236] Importantly, preeclampsia can have adverse effects on neonatal Treg populations, as evidenced by studies of umbilical cord blood,[237,238] further extending the negative outcomes associated with this obstetric disease.

While dysfunctional or reduced Tregs have been implicated in the pathogenesis of preeclampsia, which could secondarily result in preterm delivery, the concept that Tregs could directly contribute to the onset of preterm labor has largely been ignored. Yet, a pioneer study indicated that the depletion of CD25+ cells in late pregnancy did not cause preterm birth.[7] However, given that CD25 is a less specific marker for Tregs than Foxp3,[239–241] the question of whether alterations in the number or function of decidual Tregs are implicated in the onset of preterm labor is still unclear. Importantly, human and murine data from our laboratory show that Tregs play a central role in the pathophysiology of preterm labor and birth as well as in neonatal well-being, highlighting the importance of these regulatory cells in the last period of pregnancy (Gomez-Lopez N, 2020, unpublished data).

FETAL T CELLS DURING PREGNANCY AND PRETERM LABOR

Fetal T cells during early and mid-pregnancy

The fetal immune system is exposed to maternal antigens, thus necessitating the development of a tolerogenic state to avoid any potential adverse reactions.[18–21] Accordingly, the cellular immunity of the fetus undergoes continuous development, in some cases starting as early as 6 weeks of gestation.[18–20,101–103] Notably, maternal microchimerism (i.e., the presence of maternal cells in fetal circulation,[242–244] which can persist long after delivery[245]), drives the induction of fetal Tregs that serve to suppress immune responses against maternal antigens.[18,101] Indeed, the large naïve T-cell populations found in the fetus are predisposed to differentiate into Tregs when exposed to maternal antigens, likely driven by the enhanced TGFβ signaling.[246] Genetic surveys of fetal naïve T cells showed that multiple epigenetic and transcriptional programs are expressed, similar to those expressed by committed Tregs, confirming this underlying predisposition for naïve T-cell differentiation into Tregs in the fetus.[247] The transcription factor Helios was demonstrated to be important for such fetal-specific predisposition, as the ablation of this molecule reduced the expression of genes associated with immunosuppression and increased that of inflammatory mediators.[247]

Recent studies have made the remarkable observation that fetal T cells display a memory phenotype as early as in the first trimester.[102,103] Single-cell and mass cytometry surveys of the first trimester fetal intestine described multiple CD4+ T-cell subsets with a memory-like gene expression profile.[102] Moreover, TCR analysis and imaging cytometry revealed CD4+ T-cell clonal expansion and localization with APCs in the fetal intestinal tissues, suggestive of exposure to foreign antigens.[102] Another report identified effector memory CD4+ T cells with a PLZF+CD161+ phenotype that displayed effector functions (such as IFNγ production) in the fetal intestine, and such cells were enriched in the cord blood of infants with gastroschisis.[103] Moreover, new evidence suggests that, in a limited number of cases, viable bacteria in the fetal intestine at mid-gestation modulate the activation of fetal T cells;[248] yet, there is no consistent evidence for a fetal microbiome in late gestation.[249] Together, these studies demonstrate the presence and functionality of antigen-experienced fetal T cells during early and mid-pregnancy.

Fetal T cells in preterm labor and birth

The participation of the fetal immune system in the pathogenesis of preterm labor and birth was first indicated by a seminal study showing enhanced activation of leukocytes in the umbilical cord blood of fetuses who were ultimately delivered after preterm labor compared to those who were delivered at term, even in the absence of intra-amniotic infection.[104] This study was unique in that the cord blood sampling was performed prior to delivery (obtained via cordocentesis), thus allowing for a rare view of in vivo fetal immunity in humans.[104] Importantly, this report also gave rise to the concept that the fetus itself was responding to foreign antigens, including those derived from microbes in the context of intra-amniotic infection-associated preterm labor.[105] More recently, the concept that the fetal immune system participates in the host defense mechanisms against microbial invasion of the amniotic cavity was further expanded by showing that women with intra-amniotic infection have abundant innate and adaptive immune cells that are highly functional in amniotic fluid, including T cells.[250–256]

In recent times, the concept that the fetus responds to maternal alloantigens was revisited by implementing a multifaceted approach that combined human samples (umbilical cord blood obtained at the time of delivery) and animal models.[21] This study demonstrated that not only does the cord blood of preterm neonates contain a significant fraction of functional central memory Th1 cells that is largely absent in term neonates, but such T cells also specifically respond to maternal alloantigens in vitro.[21] Yet, given the fact that some of these neonates were born to women who presented with pPROM (a clinical condition highly associated with intra-amniotic infection[257,258]), whether a subset of these neonatal T cells were activated against microbes and/or their products should also be considered. These preterm neonatal T cells also induced myometrial contractility in vitro through the release of Th1 cytokines.[21] Finally, the adoptive transfer of activated T cells into murine fetuses resulted in pregnancy loss, providing in vivo confirmation that the presence of activated T cells in the fetus is associated with adverse pregnancy outcome. Similar phenomena were observed in the context of placental malaria infection, in which a large proportion of functional effector memory T cells was found in the cord blood of infants born to infected mothers.[259] These fetal T cells responded to malarial antigens in vitro and, importantly, the strength of the proliferative response to such antigens correlated with prospective protection from this infectious disease during childhood.[259] Each of these studies provided novel contributions to the understanding of fetal adaptive immunity; yet, both featured T cells that were isolated from the umbilical cord blood, thus providing only indirect measurements of fetal T-cell responses in utero.

In light of these limitations, a recent study investigated the presence and role of fetal T cells in the amniotic cavity.[106] Amniotic fluid, which is in direct contact with the fetus throughout pregnancy, contains a notable fraction of immune cells.[253] Multiple studies have established that neutrophils and monocytes found in amniotic fluid in the context of local (i.e., intra-amniotic) inflammation/infection are of fetal origin in preterm gestations.[260–262] In addition, a recent report indicated that functional ILCs of fetal origin are present in amniotic fluid in the absence of intra-amniotic inflammation/infection.[263] Amniotic fluid ILCs

expressed a phenotype consistent with that of intra-epithelial localization,[263] which suggested that amniotic fluid lymphocytes could originate directly from fetal tissues such as the intestine. In line with these observations, we reported that T cells present in amniotic fluid in preterm gestations are of fetal origin and express surface markers such as CD103 and CD161 that are indicative of a

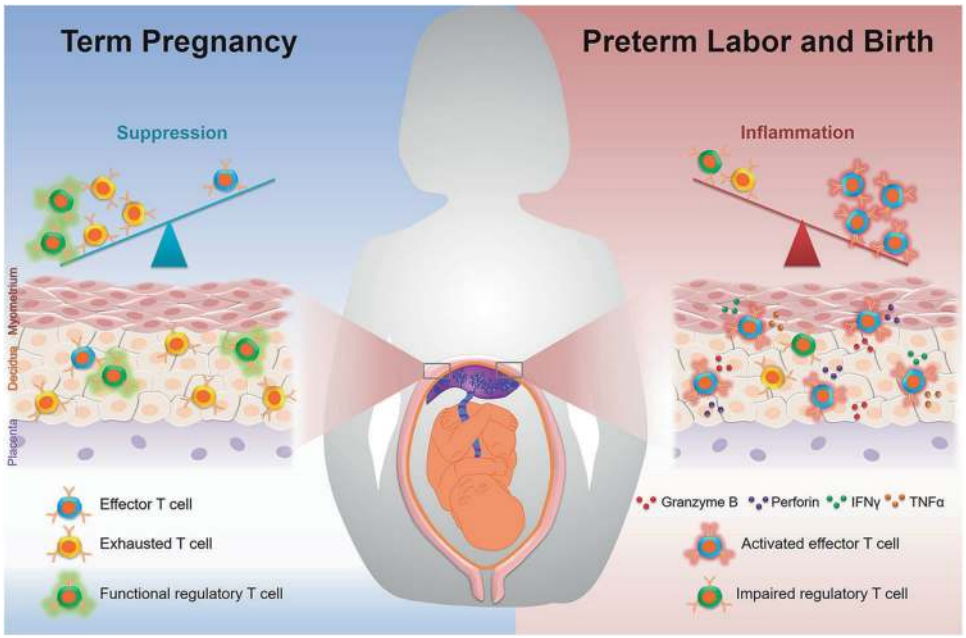

Fig. 1 Maternal T cells at the maternal–fetal interface in term pregnancy and preterm labor/birth. (Left panel) During normal pregnancy, a suppressive microenvironment exists at the maternal–fetal interface to prevent aberrant maternal immune responses against foreign antigens. This suppressive microenvironment is mainly driven by regulatory T cells and exhausted T cells. (Right panel) Preterm labor is accompanied by inflammation at the maternal–fetal interface, which can be driven or exacerbated by the invasion of activated effector T cells that release pro-inflammatory mediators such as perforin and granzyme B. Moreover, local inflammation may lead to the reactivation of exhausted T cells, restoring effector functions such as the release of pro-inflammatory cytokines and thereby further propagating T-cell responses

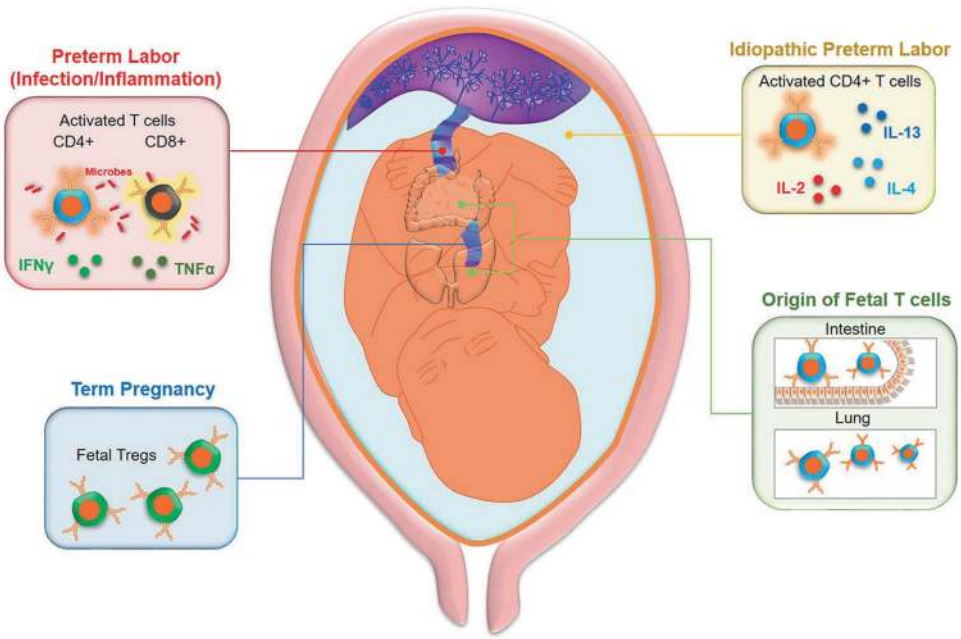

Fig. 2 Fetal T cells in preterm labor subsets and term gestation. (Upper left panel) Intra-amniotic infection/inflammation-associated preterm labor and birth are accompanied by the activation of conventional CD4+ and CD8+ T cells by microbial products, resulting in the release of pro-inflammatory cytokines. (Upper right panel) A subset of idiopathic preterm labor cases (preterm labor occurring in the absence of clinical intra-amniotic infection/inflammation) are associated with an increased number of activated fetal CD4+ T cells in amniotic fluid together with elevated concentrations of T-cell cytokines. (Lower left panel) In term pregnancy, fetal immunity is skewed toward a tolerant state, as evidenced by an elevated propensity for fetal naïve T cells to differentiate into regulatory T cells (Tregs). (Lower right panel) Fetal T cells in the amniotic cavity express a phenotype similar to that of intra-epithelial lymphocytes in the intestines, suggesting that some T cells in this compartment are derived from the mucosal organs that are in contact with amniotic fluid

mucosal origin.[106] Together, these studies provide evidence that fetal T cells in the amniotic cavity are largely derived from the mucosal tissues, thereby providing clues that can shape future investigations of these cells.

A notable finding of the latter study was that, among the major immune cell populations, only fetal CD4+ T cells were increased in the amniotic fluid of women with preterm labor occurring in the absence of a demonstrable clinical cause (intra-amniotic inflammation/infection), which is commonly known as idiopathic preterm labor.[106] The influx of fetal CD4+ T cells into the amniotic cavity was accompanied by modest increases in T-cell specific cytokines, providing further evidence that some cases of idiopathic preterm labor are accompanied by a mild and distinct fetal T-cell response.[106] This represents novel evidence that, in cases of idiopathic preterm labor, fetal T-cell activation is not directed against microbial antigens given that the patients included in this study were confirmed to have no viable microbes in the amniotic cavity.[106] In support of these findings, umbilical cord blood T cells from neonates born after idiopathic preterm labor displayed enhanced in vitro functionality compared to those from women who delivered at term.[106] Finally, the ultrasound-guided intra-amniotic injection of activated neonatal T cells induced preterm birth in mice,[106] providing a causal in vivo demonstration of a new mechanism of disease for preterm labor and birth, which is mediated by activated fetal T cells. Yet, it is worth mentioning that the mechanisms leading to premature activation of amniotic fluid fetal T cells in idiopathic preterm labor require further exploration.

CONCLUSION

In summary, the maternal–fetal interface contains a diverse population of maternal T cells at term, which includes effector T cells, exhausted and senescent T cells, and Tregs. Maternal Tregs may serve to prevent fetus-specific immune responses and control the presence of effector T cells as well as prevent their premature activation. Moreover, the presence of exhausted and senescent T cells at the maternal–fetal interface indicates additional safeguards that exist to further control T-cell responses. Failure of such mechanisms, either through the excessive invasion of effector T cells into the maternal–fetal interface or the dysfunction or reduced presence of Tregs, may lead to the premature activation of the common pathway of parturition resulting in preterm birth (Fig. 1). Importantly, recent studies have now provided firm evidence that fetal T cells, most likely derived from the mucosal tissues, undergo premature activation in a subset of preterm labor cases, most notably in cases that would otherwise be considered idiopathic. Fetal T cells also participate in the host immune defense mechanisms involved in the better-characterized clinical scenario of intra-amniotic infection-associated preterm labor (Fig. 2). Together, these findings implicate the fetus as an entity that can contribute to adverse pregnancy outcome, thus adding a new layer of complexity to the syndrome of preterm labor. It is our hope that this review provides a solid conceptual framework highlighting the importance of maternal and fetal T cells in pregnancy and catalyzes new research questions that can further scientific understanding of these cells and their role in preterm labor and birth.

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ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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