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Research Article

Exhausted and Senescent T Cells at the Maternal-Fetal Interface in Preterm and Term Labor

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Successful pregnancy requires a tightly-regulated equilibrium of immune cell interactions at the maternal-fetal interface (i.e., the decidual tissues), which plays a central role in the inflammatory process of labor. Most of the innate immune cells in this compartment have been well characterized; however, adaptive immune cells are still under investigation. Herein, we performed immunophenotyping of the decidua basalis and decidua parietalis to determine whether exhausted and senescent T cells are present at the maternal-fetal interface and whether the presence of pathological (i.e., preterm) or physiological (i.e., term) labor and/or placental inflammation alter such adaptive immune cells. In addition, decidual exhausted T cells were sorted to test their functional status. We found that (1) exhausted and senescent T cells were present at the maternal-fetal interface and predominantly expressed an effector memory phenotype, (2) exhausted $CD4^+$ T cells increased in the decidua parietalis as gestational age progressed, (3) exhausted $CD4^+$ and $CD8^+$ T cells decreased in the decidua basalis of women who underwent labor at term compared to those without labor, (4) exhausted $CD4^+$ T cells declined with the presence of placental inflammation in the decidua basalis of women with preterm labor, (5) exhausted $CD8^+$ T cells decreased with the presence of placental inflammation in the decidua basalis of women who underwent labor at term, (6) both senescent $CD4^+$ and $CD8^+$ T cells declined with the presence of placental inflammation in the decidua basalis of women who underwent preterm labor, and (7) decidual exhausted T cells produced IFNγ and TNFα upon *in vitro* stimulation. Collectively, these findings indicate that exhausted and senescent T cells are present at the human maternal-fetal interface and undergo alterations in a subset of women either with labor at term or preterm labor and placental inflammation. Importantly, decidual T cell function can be restored upon stimulation.

1. Introduction

Successful pregnancy requires that the mother and semiallogeneic fetus coexist, which involves systemic and local (i.e., maternal-fetal interface) immune interactions [1–9]. The maternal-fetal interface (i.e., the decidua) is formed after the endometrium undergoes morphological and functional changes ("decidualization"), allowing for invasion of fetal

trophoblast and forming the area of contact between the endometrium and the placenta (decidua basalis) or chorioamniotic membranes (decidua parietalis) [10, 11]. The major immune cell types present at the maternal-fetal interface [7, 12] include components of the innate limb such as natural killer (NK) cells [13–17], macrophages [18–27], neutrophils [28, 29], and the recently described innate lymphoid cells [30–35]. The adaptive immune cells, T cells [36–50] and B cells [51–54], are also present at the maternal-fetal interface. A tightly-regulated equilibrium between these immune cells is required for pregnancy maintenance [6, 7], and a disruption of this balance may lead to pregnancy complications such as preterm labor and birth [55, 56], the leading cause of neonatal mortality and morbidity worldwide [57–59]. Specifically, we have recently shown that a pool of effector and activated decidual T cells leads to pathological inflammation resulting in spontaneous preterm labor and birth [60, 61]. However, whether decidual T cells undergo a process of exhaustion (exhausted T cells [62–69]) or senescence (senescent T cells [70–72]), which leads to a loss of function, is unknown. To date, there is no evidence of exhausted or senescent T cells at the human maternal-fetal interface.

T cell exhaustion results from continuous exposure to antigen and occurs as a progressive loss of function, characterized by increased coexpression of multiple inhibitory receptors (e.g., TIM-3, PD-1, CTLA-4, and LAG-3), changes in the expression of transcription factors, distinctive patterns of cytokine receptors, loss of effector cytokine secretion, and metabolic alterations [68, 69, 73]. A key hallmark of exhausted T cells is the lack of canonical memory T cell properties and maintenance [73]. In humans, T cell exhaustion was described during chronic viral infections [e.g., human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV)] as well as in cancer [68, 69, 73, 74]. T cell exhaustion has also been implicated in the mechanisms of allograft or transplant tolerance [75–77]. However, whether T cell exhaustion is implicated in pregnancy complications such as preterm labor and birth is unknown.

T cell exhaustion has been related to T cell senescence as both processes involve cell dysfunction [78]. However, it is now clear that these cell fates are distinct and regulated independently of each other [78]. Senescent T cells lose their proliferative capacity while maintaining effector functions (i.e., cytokine production and cytotoxicity) [78], whereas exhausted T cells have typically lost both proliferative capacity and the majority of their functions [65]. In addition, senescent T cells express high levels of CD57 and KLRG-1 [79, 80], while expression of these markers is low on exhausted T cells [65, 68]. Moreover, exhausted T cells have high expression of inhibitory receptors, whereas senescent cells do not [73]. Given that T cell exhaustion is being investigated herein, we also determined whether senescent T cells are present at the maternal-fetal interface and whether such cells are associated with preterm labor and birth.

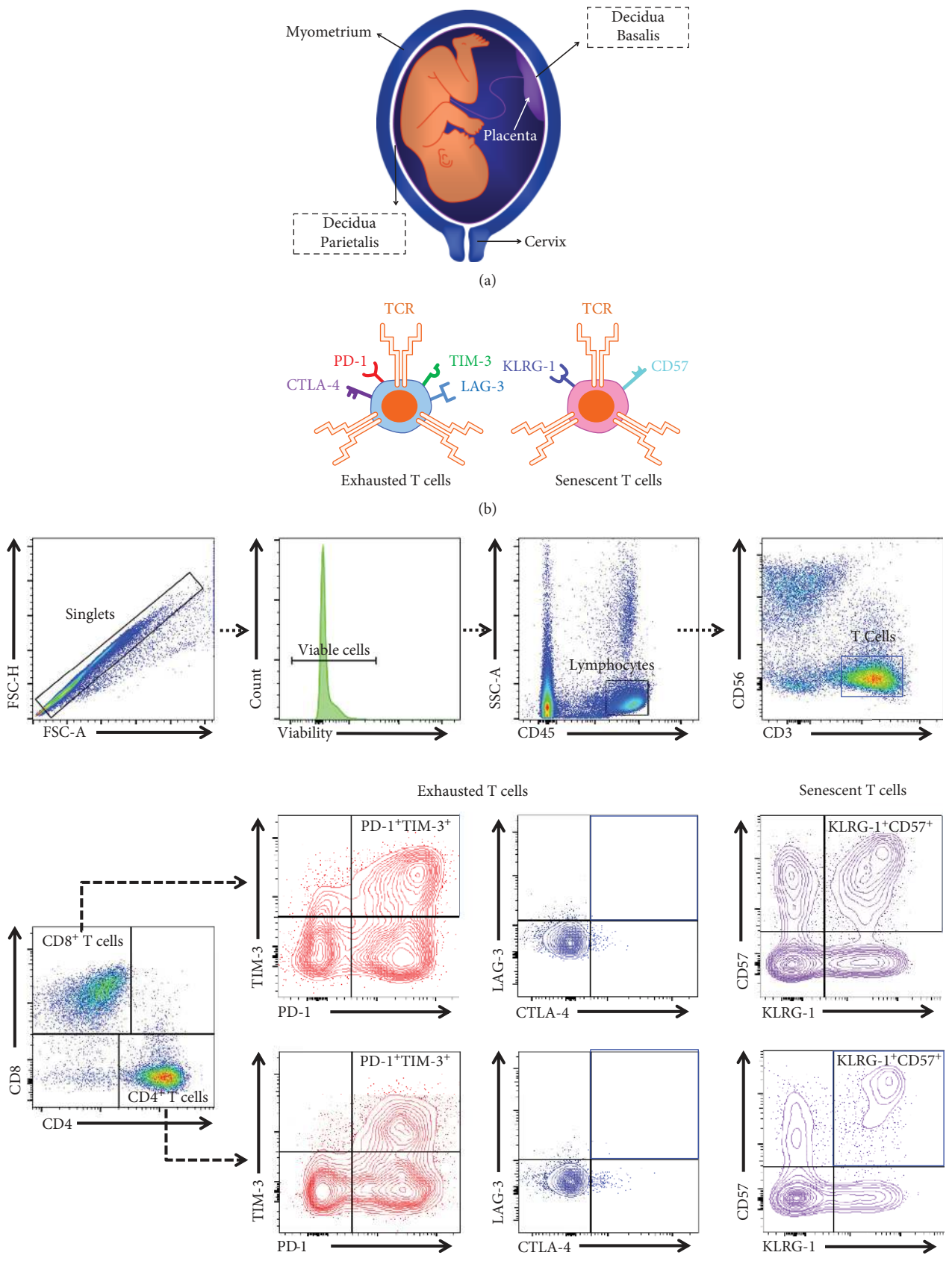
In the current study, we performed immunophenotyping of the maternal-fetal interface (i.e., the decidua basalis and decidua parietalis; Figure 1(a)) to determine whether exhausted and senescent T cells are present in preterm and term gestations. In addition, we investigated whether the presence of pathological (i.e., preterm) or physiological (i.e., term) labor and/or placental inflammation alter exhausted and senescent T cells at the maternal-fetal interface. Lastly, decidual exhausted T cells were sorted and their functionality was tested *in vitro*.

2. Materials and Methods

2.1. Human Subjects, Clinical Specimens, and Definitions. Human placental basal plate (decidua basalis) and chorioamniotic membrane (amnion, chorion, and decidua parietalis) samples were collected from patients within 30 min after delivery at Hutzel Women's Hospital in the Detroit Medical Center, Detroit, MI, USA, in partnership with Wayne State University School of Medicine and the Perinatology Research Branch, an intramural program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, US Department of Health and Human Services (NICHD/-NIH/DHHS), Detroit, MI, USA. The collection and utilization of biological materials for research purposes were approved by the Institutional Review Boards of Wayne State University and NICHD. All participating women provided written informed consent prior to sample collection. The study groups included women who delivered at term with (TIL) or without (TNL) labor and women who delivered preterm with (PTL) or without (PTNL) labor. Preterm birth was defined as delivery before 37 weeks of gestation, and term birth was defined as delivery after 37 weeks of gestation. Labor was defined by the presence of regular uterine contractions at a frequency of at least 2 contractions every 10 min with cervical changes resulting in delivery. The TIL and PTL study groups were subdivided based on the presence of placental inflammation (PI) in the chorioamniotic membranes (see Placental Histopathological Examination for diagnostic criteria). The clinical and demographic characteristics of the study population are shown in Tables 1 and 2.

2.2. Placental Histopathological Examination. Placentas were examined histologically by a perinatal pathologist blinded to clinical diagnoses and obstetrical outcomes according to standardized Perinatology Research Branch protocols [81]. Briefly, three to nine sections of the placenta were examined, and at least one full-thickness section was taken from the center of the placenta; others were taken randomly from the placental disc. Inflammatory lesions of the placenta were diagnosed according to established criteria [82–84]. Placental inflammation was defined by the infiltration of neutrophils into the chorion and amnion [83].

2.3. Isolation of Decidual Leukocytes. Decidual leukocytes were isolated from the decidua basalis and decidua parietalis as previously described [85]. Briefly, the decidua basalis was collected from the basal plate of the placenta and the decidua parietalis was separated from the chorioamniotic membranes (Figure 1(a)). The decidual tissues were homogenized using a gentleMACS Dissociator (Miltenyi Biotec, San Diego, CA, USA) in StemPro Accutase Cell Dissociation Reagent



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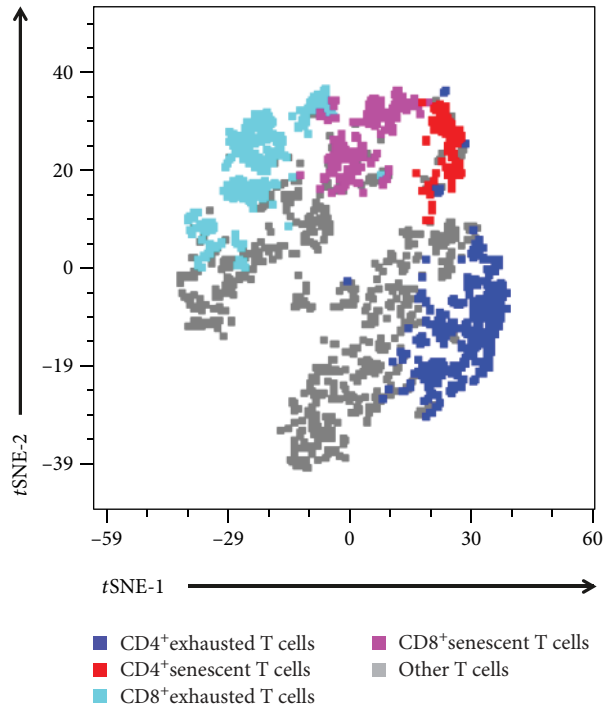

(d)

Figure 1: Immunophenotyping of exhausted and senescent T cells in the decidua basalis and decidua parietalis. (a) Representation of the spatial localization of the decidua basalis and decidua parietalis. (b) Schematic representation of select markers expressed by exhausted and senescent T cells. (c) Flow cytometry gating strategy used to identify exhausted and senescent T cells in the decidual tissues. T cells were gated as $CD3^+CD56^-$ cells within the viability and lymphocytic gates, followed by gating for the $CD4^+$ and $CD8^+$ subsets. Exhausted T cells were gated for expression of PD-1, TIM-3, CTLA-4, and LAG-3. Since expression of CTLA-4 and LAG-3 was low, exhausted T cells were defined as $PD\text{-}1^+TIM\text{-}3^+$ cells within the $CD4^+$ or $CD8^+$ gates. Senescent T cells were gated as $KLRG\text{-}1^+CD57^+$ cells within the $CD4^+$ or $CD8^+$ gates. (d) A representative *t*-distributed stochastic neighbor embedding (*t*-SNE) dot plot visualizing exhausted and senescent $CD4^+$ and $CD8^+$ T cells among decidual T cells. Blue—$CD4^+$ exhausted T cells, red—$CD4^+$ senescent T cells, turquoise—$CD8^+$ exhausted T cells, pink—$CD8^+$ senescent T cells, and grey—other T cells.

Table 1: Clinical and demographic characteristics of the patient population used to perform immunophenotyping of exhausted and senescent T cells in the decidua basalis.

	Term without labor ($n = 17$)	Term with labor ($n = 20$)	Preterm without labor ($n = 8$)	Preterm with labor ($n = 10$)	p value
Maternal age (years; median (IQR))[a]	26 (25-32)	23.5 (21-26.3)	28 (25.3-30.8)	22.5 (21-31.8)	0.04
Body mass index (kg/m^2; median (IQR))[a]	30.1 (26-36.1)[c]	24.7 (23.1-33.5)	32.9 (22.7-42.9)	25.7 (20.5-27.4)[c]	0.3
Primiparity[b]	11.8% (2/17)	35% (7/20)	12.5% (1/8)	20% (2/10)	0.3
Race[b]					0.1
African-American	68.8% (11/16)[c]	90% (18/20)	75% (6/8)	90% (9/10)	
Caucasian	18.8% (3/16)[c]	0% (0/20)	12.5%(1/8)	0% (0/10)	
Asian	12.5% (2/16)[c]	0% (0/20)	0% (0/8)	0% (0/10)	
Other	0% (0/16)[c]	10% (2/20)	12.5% (1/8)	10% (1/10)	
Gestational age at delivery (weeks; median (IQR))[a]	39.1 (39-39.3)	39.2 (38.5-40)	27.6 (26.1-34.5)	35.5 (32.1-36.2)	<0.001
Birthweight (g)[a]	2960 (2775-3285)	3195 (2925-3693.8)	728.5 (595-2078.8)	2305 (1656.3-2446.3)	<0.001
Cesarean section[b]	100% (17/17)	35% (7/20)	100% (8/8)	40% (4/10)	<0.001

Data are given as the median (interquartile range) and percentage (n/N). [a]Kruskal-Wallis test. [b]Fisher's exact test. [c]One missing data.

(Life Technologies, Grand Island, NY, USA). Homogenized tissues were incubated for 45 min at 37°C with gentle agitation. After incubation, tissues were washed in sterile 1X phosphate-buffered saline (PBS) (Life Technologies) and filtered through a 100 μm cell strainer (Falcon, Corning Life Sciences Inc., Durham, NC, USA). The resulting cell

Table 2: Clinical and demographic characteristics of the patient population used to perform immunophenotyping of exhausted and senescent T cells in the decidua parietalis.

	Term without labor ($n = 16$)	Term with labor ($n = 21$)	Preterm without labor ($n = 8$)	Preterm with labor ($n = 10$)	p value
Maternal age (years; median (IQR))[a]	27 (25-32.3)	24 (21-26)	28 (25.3-30.8)	22.5 (21-31.8)	0.05
Body mass index (kg/m^2; median (IQR))[a]	30.1 (27-36.9)[c]	23.5 (23-32.8)	32.9 (22.7-42.9)	25.7 (20.5-27.4)[c]	0.2
Primiparity[b]	12.5% (2/16)	38.1% (8/21)	12.5% (1/8)	20% (2/10)	0.3
Race[b]					0.09
African-American	66.7% (10/15)[c]	90.5% (19/21)	75% (6/8)	90% (9/10)	
Caucasian	20% (3/15)[c]	0% (0/21)	12.5% (1/8)	0% (0/10)	
Asian	13.3% (2/15)[c]	0% (0/21)	0% (0/8)	0% (0/10)	
Other	0% (0/15)[c]	9.5% (2/21)	12.5% (1/8)	10% (1/10)	
Gestational age at delivery (weeks; median (IQR))[a]	39.1 (39-39.3)	39.3 (38.6-40)	27.6 (26.1-34.5)	35.5 (32.1-36.2)	<0.001
Birthweight (g)[a]	2972.5 (2763.8-3290)	3295 (2935-3675)	728.5 (595-2078.8)	2305 (1656.3-2446.3)	<0.001
Cesarean section[b]	100% (16/16)	33.3% (7/21)	100% (8/8)	40% (4/10)	<0.001

Data are given as the median (interquartile range) and percentage (n/N). [a]Kruskal-Wallis test. [b]Fisher's exact test. [c]One missing data.

suspension was centrifuged at 300 x g for 10 min at 4°C. Decidual leukocytes were then separated using a density gradient (Ficoll-Paque Plus; GE Healthcare Biosciences, Uppsala, Sweden), following the manufacturer's instructions. The cells collected from the mononuclear layer of the density gradient were washed with 1X PBS and immediately used for immunophenotyping.

2.4. Immunophenotyping of Decidual T Cells. Isolated decidual mononuclear cells were incubated with BD Fixable Viability Stain 575V (Cat#565694; BD Biosciences, San Jose, CA, USA) for 30 min at 4°C, then washed with 1X PBS. Next, the cells were resuspended in 50 μL of stain buffer (BD Biosciences) and incubated with fluorochrome-conjugated anti-human monoclonal antibodies (Supplementary Table 1) for 30 min at 4°C in the dark. After extracellular staining, the cells were washed with 1X PBS to remove excess antibody, resuspended in 0.5 mL of stain buffer, and acquired using the BD LSRFortessa Flow Cytometer (BD Biosciences) and BD FACSDiva 6.0 software (BD Biosciences). The analysis and figures were performed using FlowJo software version 10 (FlowJo, LLC, Ashland, OR, USA). The cell surface markers used to identify exhausted and senescent T cells were selected based on a literature review (Supplementary Table 2). The effector memory status of exhausted and senescent T cells was determined by the expression of CD45RA and CCR7.

2.5. Cytokine Production by Decidual Exhausted T Cells. Decidual mononuclear cells were isolated as described above and incubated with BD Fixable Viability Stain 510 (Cat#564406; BD Biosciences) for 30 min at 4°C, then washed with 1X PBS. The cells were then resuspended in 50 μL of stain buffer and incubated with fluorochrome-conjugatedanti-human monoclonal antibodies (Supplementary Table 1) for 30 min at 4°C in the dark. After extracellular staining, the cells were washed with 1X PBS to remove excess antibody, resuspended in 0.5 mL of presort buffer (Cat#563503; BD Biosciences), and exhausted $CD4^+$ ($CD45^+CD3^+CD4^+$ $Tim\text{-}3^+PD\text{-}1^+$ cells) and $CD8^+$ ($CD45^+CD3^+CD8^+Tim\text{-}3^+$ $PD\text{-}1^+$ cells) T cells were sorted using the BD FACSMelody cell sorter (BD Biosciences) and BD FACSChorus version 1.3 software (BD Biosciences). For the determination of T cell function, sorted exhausted T cells were stimulated for 4 h with 2 μL/mL of Cell Stimulation Cocktail [phorbol 12-myristate 13-acetate (PMA), ionomycin, brefeldin A, and monensin (Cat#00-4975; Life Technologies)]. Stimulated exhausted T cells were then collected, fixed, and permeabilized using the BD Cytofix/Cytoperm Fixation and Permeabilization Solution (BD Biosciences) and incubated with specific monoclonal antibodies against IFNγ and TNFα (Supplementary Table 1). Nonstimulated sorted exhausted T cells were used as controls. Stained exhausted T cells were acquired using the BD LSRFortessa Flow Cytometer and BD FACSDiva 6.0 software. The analysis and figures were performed using FlowJo version 10 software (FlowJo).

2.6. Statistical Analysis. Data were analyzed using IBM SPSS version 19.0 (IBM Corporation; Armonk, NY, USA). For patient demographics, the Fisher's exact test was used to compare proportions among groups and the Kruskal-Wallis test was used to compare continuous variables among groups. Experimental data were compared between study groups using the Mann-Whitney U-test. Two-tailed (p values without an asterisk) and one-tailed (p values with an asterisk) p values were reported. The t-distributed stochastic neighbor embedding (t-SNE) plot was generated using FlowJo version 10 software. The association between exhausted and senescent T cells and gestational age was assessed using a Spearman's correlation test. p values were adjusted across the T cell subsets using the false discovery rate method [86]. Nonparametric local weighted regression (LOESS) [87] was used to estimate the average percentage of each T cell subset as a function of

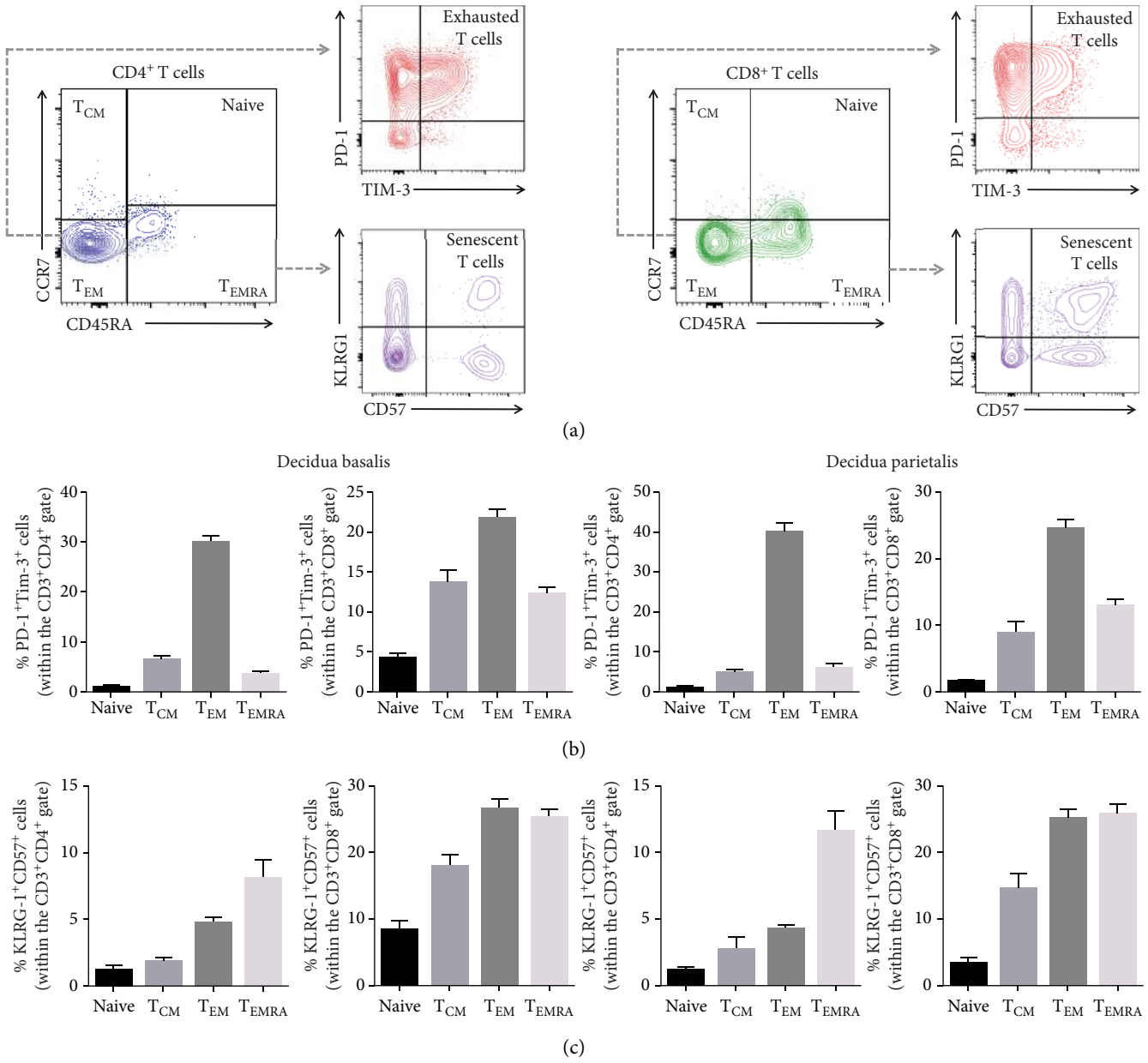

Figure 2: Proportions of exhausted and senescent $CD4^+$ and $CD8^+$ decidual T cells within the effector memory subsets. (a) Flow cytometry gating strategy used to identify exhausted and senescent decidual $CD4^+$ and $CD8^+$ T cells within the naïve, central memory (T_{CM}), effector memory (T_{EM}), and terminally differentiated effector memory (T_{EMRA}) subsets. (b) Proportions of exhausted $CD4^+$ and $CD8^+$ T cells within the naïve, T_{CM}, T_{EM}, and T_{EMRA} subsets in the decidua basalis and decidua parietalis. (c) Proportions of senescent $CD4^+$ and $CD8^+$ T cells within the naïve, T_{CM}, T_{EM}, and T_{EMRA} subsets in the decidua basalis and decidua parietalis. $N = 55$. Data are shown as the means with a standard error of the mean.

gestational age. The R statistical package was used for analysis [88]. A p value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Exhausted and Senescent T Cells Are Present at the Maternal-Fetal Interface. Figure 1(a) shows the spatial localization of the decidua basalis and decidua parietalis. The markers for the identification of exhausted and senescent T cells are shown in Figure 1(b). The gating strategy used to identify exhausted and senescent $CD4^+$ and $CD8^+$ T cells in the decidua basalis and decidua parietalis is shown in Figure 1(c). In the decidual tissues, exhausted $CD4^+$ and $CD8^+$ T cells expressed PD-1 and TIM-3, but lacked expression of LAG-3 and CTLA-4. We considered exhausted T cells as those expressing both PD-1 and TIM-3 (Figure 1(c)). In the decidual tissues, we considered senescent $CD4^+$ and $CD8^+$ T cells as those expressing both KLRG-1 and CD57 (Figure 1(c)). A t-SNE plot representing the abundance of exhausted and senescent $CD4^+$ and $CD8^+$ T cells among decidual T cells is shown in Figure 1(d).

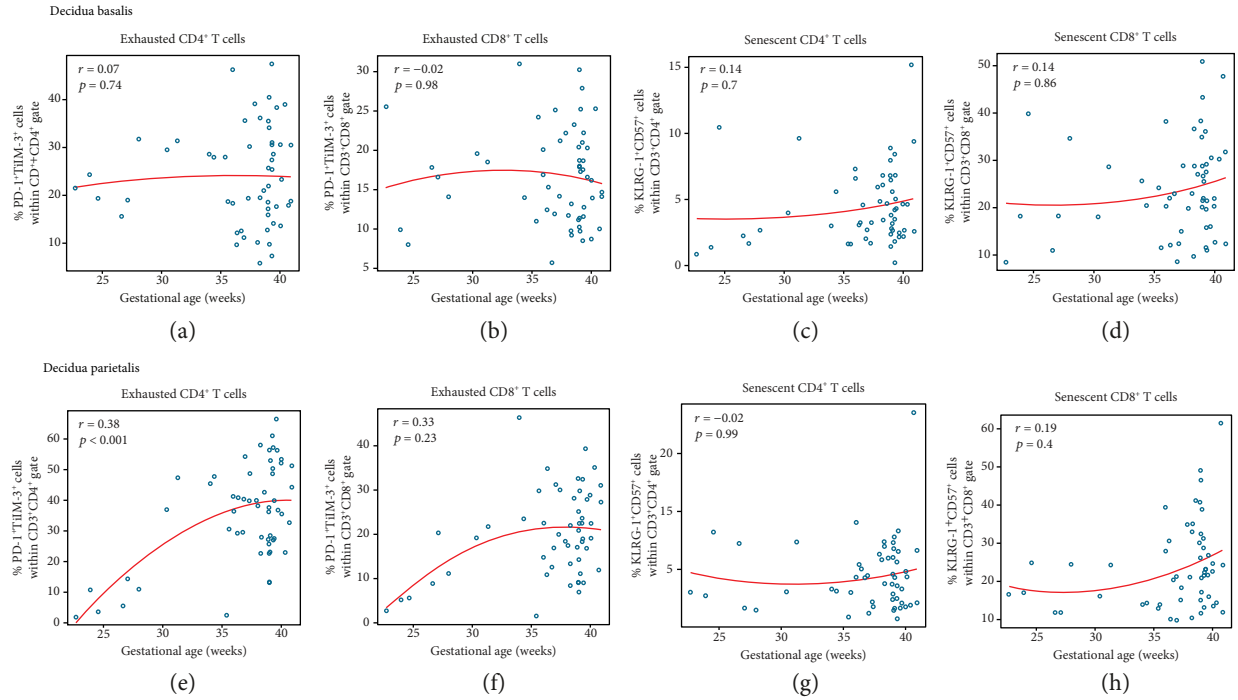

FIGURE 3: Correlations between exhausted or senescent $CD4^+$ and $CD8^+$ decidual T cells and gestational age. The correlations between gestational age and the proportions of exhausted or senescent $CD4^+$ and $CD8^+$ T cells in the decidua basalis (a–d) and decidua parietalis (e–h). The red line represents locally weighted scatter plot smoothing (LOESS) estimating the average cell percentages as a function of gestational age (weeks). The correlations were assessed using a Spearman's correlation test. Correlation coefficients and p values are shown for each plot.

The majority of exhausted $CD4^+$ and $CD8^+$ T cells belong to the effector memory T cell subset (T_{EM}) in the decidua basalis and decidua parietalis (Figures 2(a) and 2(b)). Yet, some of the exhausted $CD8^+$ T cells were also found in the central memory (T_{CM}) and terminally differentiated effector memory (T_{EMRA}) subsets (Figure 2(b)). Most of the senescent $CD4^+$ T cells belonged to the T_{EMRA} subset, whereas senescent $CD8^+$ T cells were found in both the T_{EM} and T_{EMRA} subsets in the decidua basalis and decidua parietalis (Figures 2(a) and 2(c)).

Together, these findings indicate that exhausted and senescent T cells are found at the maternal-fetal interface, where most of them express an effector memory phenotype.

3.2. Exhausted $CD4^+$ and $CD8^+$ T Cells Increase in the Decidua Parietalis as Gestational Age Progresses. Next, we determined whether the abundance of exhausted or senescent T cells changes as gestational age advances, given that the T cell repertoire undergoes alterations throughout gestation [12]. The Spearman correlations between the proportions of exhausted or senescent $CD4^+$ and $CD8^+$ T cells and gestational age are shown in Figure 3. In the decidua basalis, no significant correlations were observed between exhausted or senescent $CD4^+$ and $CD8^+$ T cells and gestational age (Figures 3(a)–3(d)). In the decidua parietalis, exhausted $CD4^+$ T cells significantly increased from preterm to term gestation ($p < 0.001$; Figure 3(e)). The same positive correlation was observed for exhausted $CD8^+$ T cells, yet this did not reach a statistical significance (Figure 3(f)). In the decidua parietalis, senescent $CD4^+$ and $CD8^+$ T cells did not vary as gestational age progressed (Figures 3(g) and 3(h)). These data show that the abundance of exhausted $CD4^+$ and $CD8^+$ T cells in the decidua parietalis increases as gestational age progresses.

3.3. Exhausted $CD4^+$ and $CD8^+$ T Cells Decrease in the Decidua Basalis of Women with Labor at Term. Our previous studies have suggested that T cells participate in the physiological [45, 46, 89, 90] and pathological [56, 60, 61, 91, 92] processes of labor (i.e., labor at term and preterm labor). Therefore, we investigated whether exhausted and senescent T cells were altered with the presence of labor at term or preterm labor. In the decidua basalis, exhausted $CD4^+$ and $CD8^+$ T cells were reduced in women who underwent labor at term compared to those who delivered at term without labor (Figures 4(a) and 4(b)). However, this reduction was not observed when comparing the preterm labor and preterm without labor groups (Figures 4(a) and 4(b)). In the decidua basalis, senescent $CD4^+$ and $CD8^+$ T cells did not vary between the labor and nonlabor groups (Figures 4(c) and 4(d)). In the decidua parietalis, exhausted and senescent $CD4^+$ and $CD8^+$ T cells did not vary between the labor and nonlabor groups at term and preterm gestations (Figures 4(e)–4(h)). Consistent with our previous results, in the absence of labor, exhausted $CD4^+$ T cells were more abundant in the term than in the preterm groups

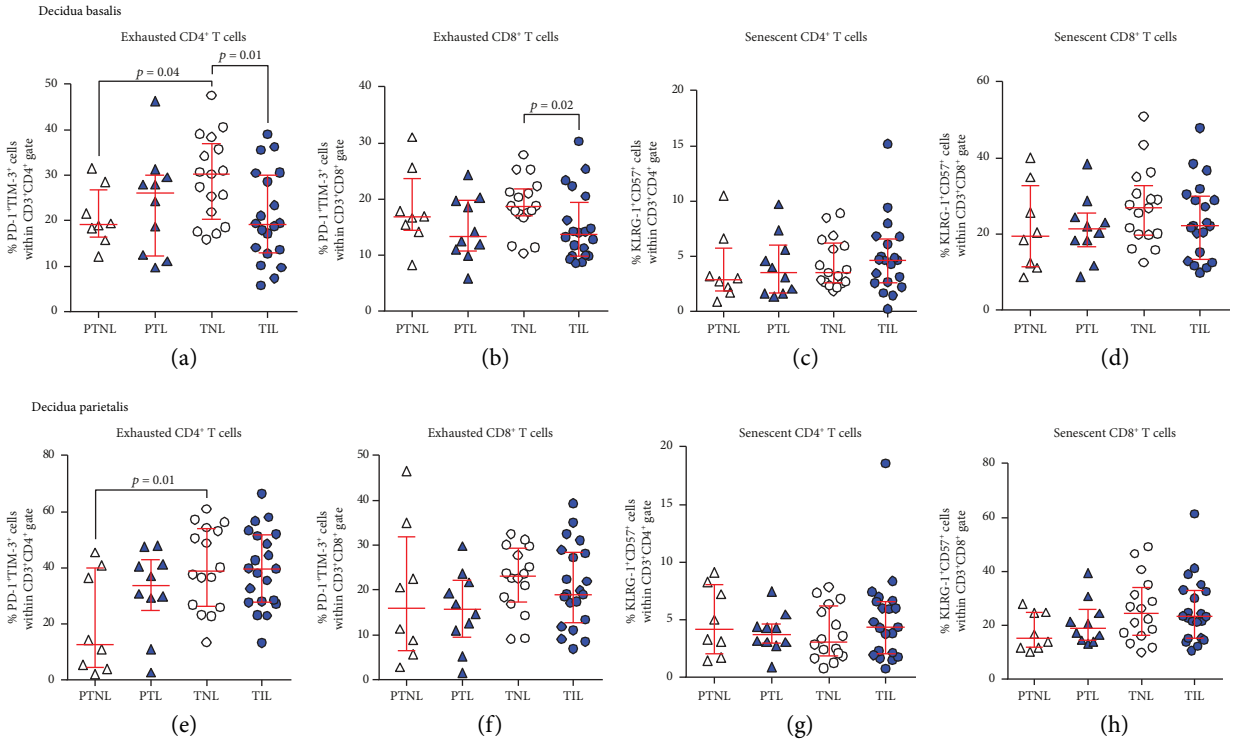

FIGURE 4: Proportions of exhausted and senescent CD4$^+$ and CD8$^+$ T cells in the decidua basalis and decidua parietalis. The proportions of exhausted and senescent CD4$^+$ and CD8$^+$ T cells in the decidua basalis (a–d) and decidua parietalis (e–h) from women who delivered preterm with labor (PTL) or without labor (PTNL) and women who delivered at term with labor (TIL) or without labor (TNL). $N = 8 - 21$ per group. Red midlines and whiskers indicate medians and interquartile ranges, respectively.

(Figures 4(a) and 4(e)). Similar differences in exhausted and senescent T cells between the study groups were observed when such cells were gated within the effector memory subsets (Supplementary Figure 1A–1L). Taken together, these data indicate that the physiological process of labor at term, but not the pathological process of preterm labor, is accompanied by a decline in exhausted CD4$^+$ and CD8$^+$ T cells at the maternal-fetal interface.

3.4. The Impact of Placental Inflammation on Exhausted and Senescent CD4$^+$ T Cells in the Decidual Tissues. Pathological inflammation is associated with an imbalance between immune cells at the maternal-fetal interface [56]. Thus, we next evaluated whether inflammation in the placenta of women who underwent preterm labor or labor at term impacted the abundance of exhausted or senescent T cells in the decidual tissues.

Exhausted CD4$^+$ T cells, but not exhausted CD8$^+$ T cells, were reduced in the decidua basalis of women who underwent preterm labor with placental inflammation compared to those without this condition (Figures 5(a) and 5(c)). In contrast, exhausted CD8$^+$ T cells, but not exhausted CD4$^+$ T cells, were decreased in the decidua basalis of women who underwent labor at term with placental inflammation compared to those without inflammation (Figures 5(b) and 5(d)). Both senescent CD4$^+$ and CD8$^+$ T cells were reduced in the decidua basalis of women who underwent preterm labor with placental inflammation compared to those without this condition (Figures 5(e) and 5(g)). However, senescent CD4$^+$ and CD8$^+$ T cells in the decidua basalis did not vary between term labor women with and without placental inflammation (Figures 5(f) and 5(h)). Placental inflammation did not alter the abundance of exhausted or senescent CD4$^+$ and CD8$^+$ T cells in the decidua parietalis (Figures 5(i)–5(p)). These findings show that placental inflammation can selectively impact the abundance of exhausted and senescent T cells in the decidua basalis of women who underwent preterm labor or labor at term.

3.5. Decidual Exhausted T Cells Are Functional upon In Vitro Stimulation. Exhausted T cells lose their effector functions, whereas senescent T cells do not [78]. Therefore, we sorted exhausted T cells from the decidual tissues and tested their functionality upon *in vitro* stimulation. The purity of sorted exhausted T cells is shown in Figure 6(a). Functionality was tested by the production of IFNγ and TNFα (Figure 6(a)). Consistent with our *in vivo* data (e.g., reduction of exhausted T cells in placental inflammation), exhausted T cells produced inflammatory cytokines upon *in vitro* stimulation, suggesting the restoration of an effector phenotype (Figure 6(b)). These data imply that exhausted T cells restore their functional-effector phenotype during inflammatory conditions at the maternal-fetal interface.

4. Discussion

4.1. Principal Findings. The principal findings of this study are as follows: (1) exhausted and senescent T cells were

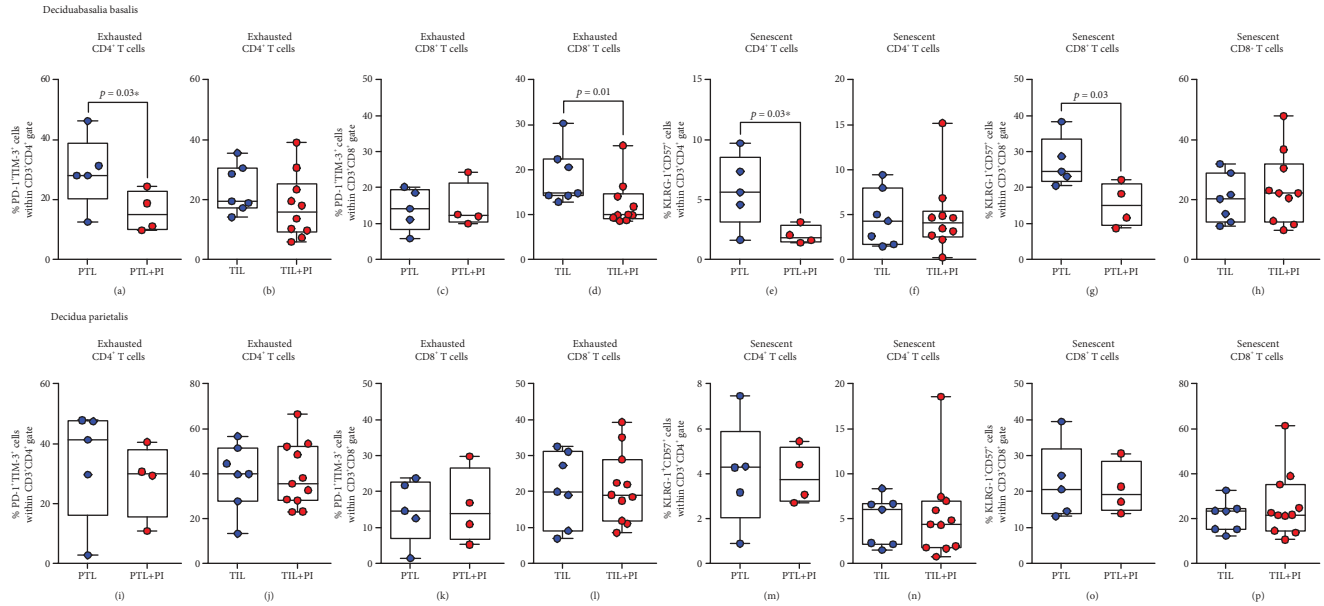

FIGURE 5: Proportions of exhausted and senescent $CD4^+$ and $CD8^+$ T cells in the decidua basalis and decidua parietalis with placental inflammation. The proportions of exhausted and senescent $CD4^+$ and $CD8^+$ T cells in the decidua basalis (a–h) and decidua parietalis (i–p) of women who underwent preterm labor with (PTL+PI) or without (PTL) placental inflammation or labor at term with (TIL+PI) or without (TIL) placental inflammation. $N = 4 - 11$. Midlines—medians, boxes—interquartile ranges, and whiskers—minimum and maximum ranges. PI: placental inflammation.

present at the human maternal-fetal interface and predominantly expressed an effector memory phenotype; (2) exhausted $CD4^+$ T cells increased in the decidua parietalis as gestational age progressed; (3) exhausted $CD4^+$ and $CD8^+$ T cells decreased in the decidua basalis of women who underwent labor at term compared to those without labor; (4) exhausted $CD4^+$ T cells declined with the presence of placental inflammation in the decidua basalis of women with preterm labor; (5) exhausted $CD8^+$ T cells decreased with the presence of placental inflammation in the decidua basalis of women who underwent labor at term; (6) both senescent $CD4^+$ and $CD8^+$ T cells declined with the presence of placental inflammation in the decidua basalis of women who underwent preterm labor; and (7) decidual exhausted T cells produced IFNγ and TNFα upon *in vitro* stimulation. Together, these findings indicate that exhausted and senescent T cells are present at the maternal-fetal interface and undergo alterations in a subset of women either with labor at term or preterm labor and placental inflammation, yet can restore their functionality upon stimulation.

4.2. Exhausted T Cells at the Maternal-Fetal Interface in Term and Preterm Labor. Herein, for the first time, we identified exhausted $CD4^+$ and $CD8^+$ T cells at the human maternal-fetal interface. Such cells display an effector memory phenotype, consistent with that of other tissue-resident exhausted T cells [93, 94]. Recent studies have identified decidual T cells expressing PD-1 and TIM-3 during the first trimester [95, 96] and in term pregnancy [50, 97]. However, the abovementioned studies did not identify such cells as exhausted T cells. It is thought that T cells expressing PD-1 and TIM-3 participate in the mechanisms leading to immune tolerance [76, 77, 98–100]; therefore, such molecules have been implicated in the pathophysiology of pregnancy loss [101–105]. The fact that decidual exhausted T cells expressing PD-1 and TIM-3 are more abundant in term than in preterm gestations suggests that T cell dysfunction represents a regulatory mechanism to prevent exacerbated cellular responses toward the end of pregnancy.

We and others have found that the lack of functionality by decidual T cells can be restored *in vitro* [50], suggesting that the inflammatory milieu that accompanies the physiological process of labor at term [106–113] reinvigorates T cell responses (i.e., reversal of T cell exhaustion [114]) at the maternal-fetal interface. This concept could explain why women who underwent labor at term had reduced proportions of exhausted T cells compared to those who delivered at term without labor.

In the current study, no differences in exhausted T cells were found in the decidual tissues of women who underwent preterm labor compared to those who delivered preterm without labor. This finding supports the hypothesis that the pathological process of preterm labor is distinct from the physiological process of labor at term [115–119] and that, in most cases, occurs in the absence of a reduction in T cell exhaustion. However, acute placental inflammation (the only causal link to spontaneous preterm labor [120–127] and present in a subset of women who deliver preterm [128–131]) decreased the abundance of exhausted T cells at the maternal-fetal interface, suggesting that T cell exhaustion is reduced solely in some cases of preterm labor associated with exacerbated placental inflammation. The mechanisms whereby placental inflammation can reduce T cell exhaustion at the maternal-fetal interface may involve cytokines, given that such inflammatory mediators can reverse T cell dysfunction [73, 132–135].

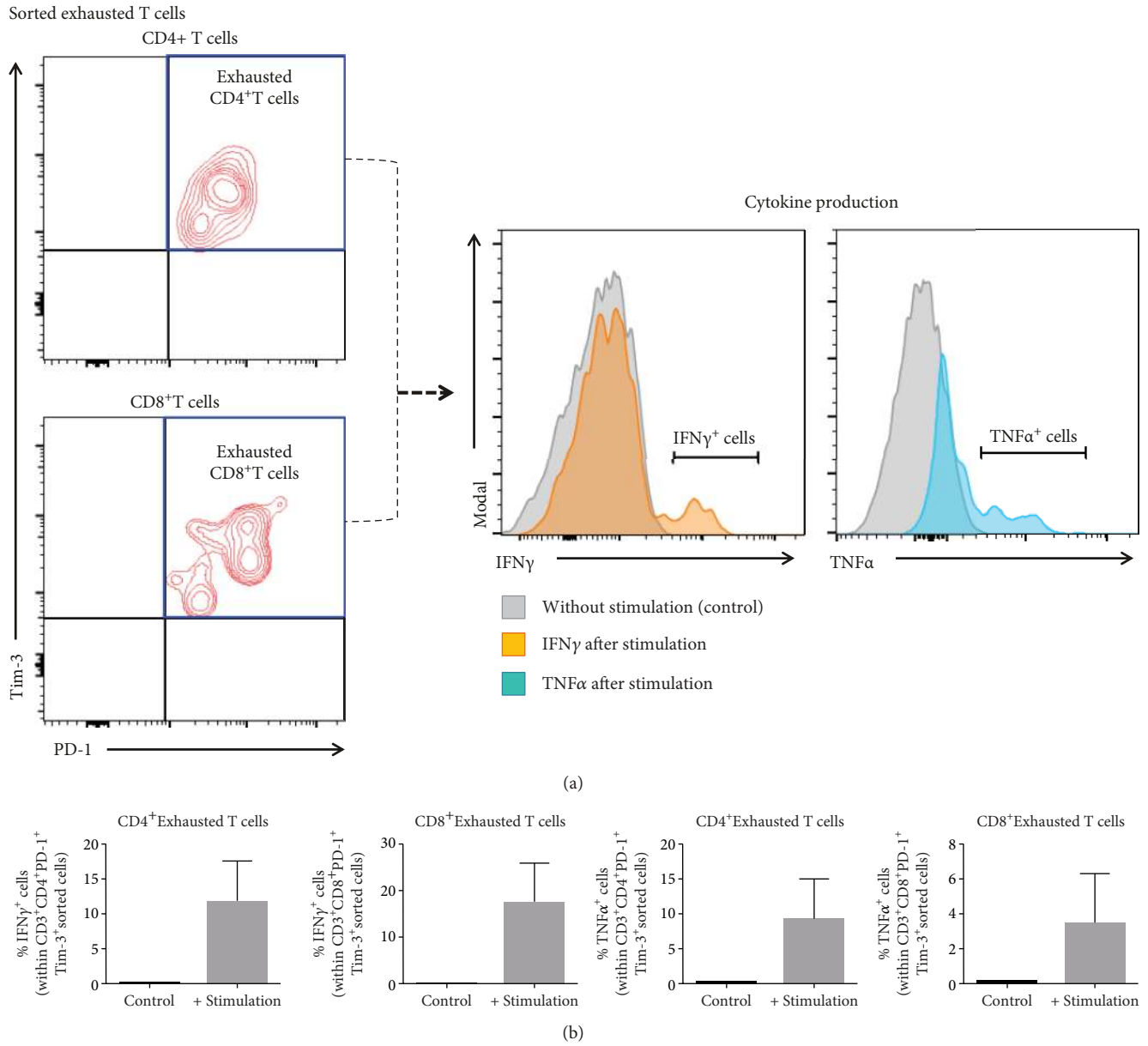

Figure 6: Determination of exhausted T cell functionality upon *in vitro* stimulation. (a) Gating strategy used to determine the purity of sorted $CD4^+$ and $CD8^+$ exhausted decidual T cells. Histograms show the production of IFNγ (orange histogram) and TNFα (blue histogram) by sorted exhausted T cells after *in vitro* stimulation. Grey histograms indicate nonstimulated controls. (b) Proportions of sorted exhausted $CD4^+$ and $CD8^+$ decidual T cells expressing IFNγ or TNFα after *in vitro* stimulation compared to nonstimulated controls. $N = 3$. Data are shown as the means with a standard error of the mean.

Therefore, we surmise that placental inflammation boosts effector T cell function by dampening T cell exhaustion at the maternal-fetal interface in a subset of women who undergo preterm labor.

A central question that arises from this study is whether T cell exhaustion at the maternal-fetal interface can be augmented in order to ameliorate effector T cell responses that lead to pathological inflammation and preterm labor and birth. T cell exhaustion has been manipulated by targeting the TCR and inhibitory receptors (e.g., PD-1, TIM-3, CTLA-4, and LAG-3) as well as by treatment with soluble mediators (e.g., anti-inflammatory cytokines such as IL-10 and TGFβ) and suppressive cells [67, 68, 135–141]. Further research is required to investigate which of the abovementioned strategies could be safely utilized during pregnancy.

4.3. Senescent T Cells at the Maternal-Fetal Interface in Preterm Labor. To our knowledge, we are the first to identify senescent T cells at the human maternal-fetal interface. Decidual senescent T cells express an effector memory phenotype consistent with that displayed by these cells in other tissues [80]. Unlike exhausted T cells, senescent T cells can release proinflammatory mediators such as IFNγ, TNFα, granzyme B, and perforin [80, 142, 143]. We and others have shown that T cells can release such inflammatory mediators at the maternal-fetal interface [50, 61],

suggesting that senescent T cells may contribute to the inflammatory milieu in this microenvironment.

We also found that senescent T cells were reduced in women who underwent preterm labor associated with placental inflammation. This finding is in line with the hypothesis that cellular senescence is implicated in the mechanisms of disease for preterm labor and birth [55, 144, 145]. The mechanisms whereby placental inflammation reduces senescent T cells at the maternal-fetal interface of women with preterm labor may involve the p53 pathway, mitogen-activated protein kinase p38 (MAPKp38), and the cyclin-dependent kinase inhibitors p16 and p21 [78], all of which are implicated in the process of parturition [144–150]. Given that T cells can undergo reversible senescence [71, 78, 143, 151–153], additional research is required to investigate the mechanisms implicated in such a process at the maternal-fetal interface.

It is worth mentioning that the effect of gestational age was observed in the decidua parietalis, whereas the impact of the process of labor and placental inflammation was mainly observed in the decidua basalis. This finding exemplifies the complexity of the maternal-fetal interface and highlights the importance of considering both the maternal (i.e., decidua parietalis is in contact with the endometrium) and fetal (i.e., decidua basalis is attached to the placenta) sides when studying maternal-fetal interactions.

5. Conclusion

In the current study, exhausted and senescent effector memory T cells were identified at the human maternal-fetal interface, where they are more abundant as term approaches. To our knowledge, this is the first time that exhausted T cells have been identified at the human maternal-fetal interface. While the physiological process of labor at term was associated with a decline in exhausted T cells, the pathological process of preterm labor with placental inflammation was linked to a reduction in both exhausted and senescent T cells. Moreover, we show that exhausted T cells restore their functionality upon *in vitro* stimulation. Collectively, these data suggest that exhausted and senescent T cells are physiological components of the maternal-fetal interface and that such cells play a role in homeostasis and disease during pregnancy.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no potential conflicts of interest.

Acknowledgments

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Supplementary Materials

Supplementary Table 1: antibodies used for immunophenotyping and cell sorting. Supplementary Table 2: literature review performed to select markers for identifying exhausted and senescent T cells. Supplementary Figure 1: exhausted and senescent $CD4^+$ and $CD8^+$ T cells display an effector memory phenotype in the decidua basalis and decidua parietalis. The proportions of exhausted and senescent $CD4^+$ and $CD8^+$ T cells within the effector memory (TEM) (A-H) or terminally differentiated effector memory (TEMRA) (I-L) T cell subsets in the decidua basalis and decidua parietalis from women who delivered preterm with labor (PTL) or without labor (PTNL) and women who delivered at term with labor (TIL) or without labor (TNL). $N = 8 – 21$ per group. Red midlines and whiskers indicate medians and interquartile ranges, respectively. *(Supplementary Materials)*

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