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ORIGINAL ARTICLE

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Innate lymphoid cells at the human maternal-fetal interface in spontaneous preterm labor

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Problem: Pathological inflammation is causally linked to preterm labor and birth, the leading cause of neonatal morbidity and mortality worldwide. Our aims were to investigate whether (i) the newly described family of innate lymphoid cells (ILCs) was present at the human maternal-fetal interface and (ii) ILC inflammatory subsets were associated with the pathological process of preterm labor.

Methods of study: Decidual leukocytes were isolated from women with preterm or term labor as well as from gestational age-matched non-labor controls. ILCs ($CD15^-CD14^-CD3^-CD19^-CD56^-CD11b^-CD127^+$ cells) and their subsets (ILC1, T-bet+ ILCs; ILC2, GATA3+ ILCs; and ILC3, RORγt+ ILCs) and cytokine expression were identified in the decidual tissues using immunophenotyping.

Results: (i) The proportion of total ILCs was increased in the decidua parietalis of women with preterm labor; (ii) ILC1s were a minor subset of decidual ILCs during preterm and term gestations; (iii) ILC2s were the most abundant ILC subset in the decidua during preterm and term gestations; (iv) the proportion of ILC2s was increased in the decidua basalis of women with preterm labor; (v) the proportion of ILC3s was increased in the decidua parietalis of women with preterm labor; and (vi) during preterm labor, ILC3s had higher expression of IL-22, IL-17A, IL-13, and IFN-γ compared to ILC2s in the decidua.

Conclusion: ILC2s were the most abundant ILC subset at the human maternal-fetal interface during preterm and term gestations. Yet, during preterm labor, an increase in ILC2s and ILC3s was observed in the decidua basalis and decidua parietalis,

respectively. These findings provide evidence demonstrating a role for ILCs at the maternal-fetal interface during the pathological process of preterm labor.

KEYWORDS
cytokine, decidua, inflammation, innate immunity, interleukin, mucosal immunity, parturition, pregnancy, tolerance

1 | INTRODUCTION

Preterm birth, defined as birth prior to 37 weeks of gestation, is one of the most common obstetrical syndromes[1-3] and the leading cause of perinatal morbidity and mortality worldwide.[4-8] In 2013, 11.39% of all births in the USA were diagnosed as preterm.[9] Premature neonates are at an increased risk of short- and long-term morbidities that represent a substantial burden for society and the healthcare system.[10-13] Approximately 70% of all preterm births are preceded by spontaneous preterm labor[1,14] with multiple pathological processes involved.[15] Therefore, it is essential to determine the mechanisms implicated in spontaneous preterm labor and to develop novel therapies and strategies to prevent this syndrome.

Inflammation is implicated in the pathological process of spontaneous preterm labor.[15-42] Pathological inflammation can result from the activation of innate immunity[43-55] by microorganisms[29,56-59] or endogenous signals derived from necrosis or cellular stress,[48,51,52,60-65] termed damage-associated molecular patterns[66] or alarmins.[67] Moreover, it has been demonstrated that activation of the adaptive immune system can also lead to pathological inflammation.[68] Hence, characterization of innate and adaptive immune cells and their mediators may provide an understanding into the mechanisms that lead to spontaneous preterm labor.

Recently, a new family of immune cells that belong to the lymphoid lineage without expressing antigen-specific receptors was described and termed innate lymphoid cells (ILCs).[69,70] Such cells are defined by 3 main features: (i) the absence of recombination activating gene (RAG)-dependent rearranged antigen receptors; (ii) a lack of myeloid cell and dendritic cell phenotypical markers; and (iii) their lymphoid morphology.[69,70] Despite lacking antigen recognition capabilities, ILCs exhibit a functional diversity, which resembles that of T cells.[71] Two prototypical members of the ILC family have been previously described: natural killer (NK) cells[72] and lymphoid tissue-inducer (LTi) cells.[73] These 2 cell types, while distinct, are related through a shared requirement of the common cytokine receptor γ-chain (IL-2Rγ) and the transcriptional repressor inhibitor of DNA binding 2 (ID2) for development.[70,74]

Distinct ILC subsets have since been described, which rely on signaling through the IL-7 receptor-α (IL-7Rα or CD127) in addition to the abovementioned markers.[70] These new members of the ILC family were classified based on their functional similarities to T-cell subsets.[70] Group 1 ILCs (ILC1) are based on expression of the transcription factor T-bet and include NK cells as well as other IFN-γ-producing Th1-like ILCs.[75] Group 2 ILCs (ILC2) are characterized by Th2-like expression of the cytokines IL-5 and IL-13 and are dependent on the transcription factors GATA-binding protein 3 (GATA3) and retinoic acid receptor–related orphan receptor-α (RORα) for development.[76,77] Finally, group 3 ILCs (ILC3) include cells that produce Th17-like cytokines, eg, IL-17A and IL-22, and depend on expression of RORγt.[78] Yet, there is plasticity among ILC subsets, which makes their characterization and identification challenging.[79,80]

Innate lymphoid cell subsets have been identified in the human decidua during early pregnancy;[81-86] however, whether such cells are present at the human maternal-fetal interface (decidua basalis and decidua parietalis[87]) during preterm and term gestations and are implicated in the pathological process of preterm labor is unknown.

The aims of this study were (i) to determine whether ILCs are present in the decidua of women with preterm and term gestations; (ii) to investigate whether the proportions of ILC subsets in the decidua are altered in women who underwent spontaneous preterm labor; and (iii) to characterize the cytokine signature of decidual ILCs in the pathological process of preterm labor.

2 | MATERIALS AND METHODS

2.1 | Human subjects, clinical specimens, and definitions

Human placental basal plate and chorioamniotic membrane samples were collected from patients within 30 minutes 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, U S 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. 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. Two separate sets of samples were utilized in this study: exploratory and confirmatory. The demographic and clinical characteristics of the study populations are shown in Tables 1 and 2. Preterm birth was defined as delivery before 37 weeks of gestation. Labor was defined by the presence of regular uterine contractions at a frequency of at least 2 contractions every 10 minutes with cervical changes resulting in delivery. For each case, several tissue sections of the

TABLE 1 Demographic and clinical characteristics of the study population (exploratory set of samples)

	TNL (n = 8)	TIL (n = 25)	PTNL (n = 8)	PTL (n = 17)	*P* value
Age (y; median [IQR])[a]	26 (23-32)	24 (22-27)	31.5 (24.3-34.5)	22 (21-25)	NS
Body mass index (kg/m^2; median [IQR])[a]	28.5 (22.5-28.5)	29.2 (24.4-34)	22.5 (22.1-33.2)	27.3 (20.2-32.1)	NS
Gestational age at delivery (wk; median [IQR])[a]	39 (38.6-39.3)	39.3 (38.3-40)	34.4 (31.3-36.5)	33.9 (31.3-34.9)	<.001
Race (n [%])[b]					NS
African American	8 (100%)	25 (100%)	7 (87.5%)	15 (88.2%)	
Caucasian	0 (0%)	0 (0%)	1 (12.5%)	1 (5.9%)	
Other	0 (0%)	0 (0%)	0 (0%)	1 (5.9%)	
Primiparity (n [%])[b]	0 (0%)	5 (20%)	2 (25%)	1 (5.9%)	NS
Cesarean section (n [%])[b]	8 (100%)	2 (8%)	8 (100%)	4 (23.5%)	<.001
Acute chorioamnionitis (n [%])[b]					
Acute subchorionitis/ chorionitis	0/8 (0%)	4/25 (16%)	0/8 (0%)	3/17 (17.6%)	NS
Acute chorioamnionitis	0/8 (0%)	7/25 (28%)	1/8 (12.5%)	2/17 (11.8%)	NS
Necrotizing chorioamnionitis	0/8 (0%)	0/25 (0%)	0/8 (0%)	3/17 (17.6%)	NS
Umbilical cord pathology (n [%])[b]					
Umbilical phlebitis	0/8 (0%)	9/25 (36%)	0/8 (0%)	4/17 (23.5%)	NS
Umbilical arteritis	0/8 (0%)	1/25 (4%)	1/8 (12.5%)	1/17 (5.9%)	NS
Necrotizing funisitis	0/8 (0%)	0/25 (%)	0/8 (0%)	0/17 (0%)	NS

IQR, interquartile range.
[a]Kruskal-Wallis test.
[b]Fisher's exact test.

chorioamniotic membranes, umbilical cord, and placental disk were evaluated by pathologists blinded to the clinical outcome, according to the published criteria.[88] Patients with neonates having congenital or chromosomal abnormalities were excluded from this study.

2.2 | Decidual leukocyte isolation

Decidual leukocytes were isolated from the decidual tissue of patients from each study group as previously described.[87] Briefly, the decidua basalis was collected from the basal plate of the placenta, and the decidua parietalis was separated from the chorioamniotic membranes. The decidual tissues were homogenized in StemPro Accutase Cell Dissociation Reagent (Life Technologies, Grand Island, NY, USA) using a gentleMACS Dissociator (Miltenyi Biotec, San Diego, CA, USA). Homogenized tissues were incubated in Accutase for 45 minutes at 37°C with gentle agitation. After incubation, tissues were washed in 1× phosphate-buffered saline (PBS; Life Technologies) and filtered through a 100-μm cell strainer (Fisher Scientific, Durham, NC, USA). The resulting cell suspensions were centrifuged at 300 *g* for 10 minutes at 4°C. The decidual mononuclear cells were then separated using a density gradient (Ficoll-Paque Plus; GE Healthcare Biosciences, Piscataway, NJ, USA) by following the manufacturer's instructions. The cells collected from the mononuclear layer of the density gradient were washed with 1× PBS and immediately used for immunophenotyping.

2.3 | Immunophenotyping of decidual innate lymphoid cells

Mononuclear cell suspensions from decidual tissues were stained with BD Horizon Fixable Viability Stain 510 dye (BD Biosciences, San Jose, CA, USA) prior to immunophenotyping. Mononuclear cell suspensions were then washed with FACS staining buffer (CAT#554656; BD Biosciences) and incubated with 20 μL of human FcR Blocking Reagent (CAT#130-059-901; Miltenyi Biotec) in 80 μL of FACS staining buffer (BD Biosciences) for 10 minutes at 4°C. The cells were incubated with extracellular fluorochrome-conjugated anti-human monoclonal antibodies for 30 minutes at 4°C in the dark (Table S1). After extracellular staining, the cells were fixed and permeabilized using the Foxp3/Transcription Factor Staining Buffer Set (eBioscience, San Diego, CA, USA) prior to staining with intracellular and intranuclear antibodies (Table S1). Stained cells were washed and resuspended in 0.5 mL of FACS staining buffer and acquired using an LSRFortessa flow cytometer and FACSDiva 6.0 software (BD Biosciences). Data were analyzed using FlowJo software version 10 (TreeStar, Ashland, OR, USA).

2.4 | Statistics

Statistical analyses were conducted using SPSS software version 19.0 (IBM Corporation, Armonk, NY, USA). The Mann-Whitney *U*-test was

TABLE 2 Demographic and clinical characteristics of the study population (confirmatory set of samples)

	TNL (n = 11)	TIL (n = 39)	PTNL (n = 12)	PTL (n = 28)	*P* value
Age (y; median [IQR])[a]	26 (23-31)	24 (20-26)	31 (27.8-32.5)	27 (22.5-30)	.002
Body mass index (kg/m^2; median [IQR])[a]	35.1 (29.2-39)	26.1 (23.1-30.7)	35.1 (29.4-36.3)	31.1 (25.4-39.6)	.009
Gestational age at delivery (wk; median [IQR])[a]	39.1 (39-39.3)	39.3 (38.6-40.2)	34.1 (31.5-36.5)	34.6 (33.6-35.8)	<.001
Birth weight (g; median [IQR])[a]	3370 (3125-3705)	3190 (2960-3352.5)	2017.5 (1393.8-2760)	2223 (1760-2420)	<.001
Race (n [%])[b]					NS
African American	9 (81.8%)	35 (89.7%)	11 (91.7%)	20 (71.4%)	
Caucasian	2 (18.2%)	2 (5.1%)	1 (8.3%)	5 (17.9%)	
Other	0 (0%)	2 (5.1%)	0 (0%)	3 (10.7%)	
Primiparity (n [%])[b]	0 (0%)	4 (10.3%)	1 (8.3%)	6 (21.4%)	NS
Cesarean section (n [%])[b]	11 (100%)	2 (5.1%)	12 (100%)	12 (42.9%)	<.001
Acute chorioamnionitis (n [%])[b]					
Acute subchorionitis/ chorionitis	1/11 (9.1%)	13/38 (34.2%)[c]	1/11 (9.1%)[c]	4/28 (14.3%)	NS
Acute chorioamnionitis	0/11 (0%)	9/38 (23.7%)[c]	0/11 (0%)[c]	4/28 (14.3%)	NS
Necrotizing chorioamnionitis	0/11 (0%)	0/38 (0%)[c]	0/11 (0%)[c]	1/28 (3.6%)	NS
Umbilical cord pathology (n [%])[b]					
Umbilical phlebitis	0/11 (0%)	10/38 (26.3%)[c]	0/11 (0%)[c]	1/28 (3.6%)	.01
Umbilical arteritis	0/11 (0%)	2/38 (5.3%)[c]	0/11 (0%)[c]	4/28 (14.3%)	NS
Necrotizing funisitis	0/11 (0%)	0/38 (0%)[c]	0/11 (0%)[c]	2/28 (7.1%)	NS

IQR, interquartile range.
[a]Kruskal-Wallis test.
[b]Fisher's exact test.
[c]Calculated based on the available placental pathology information.

used for comparisons between study groups or different samples, and the Wilcoxon's signed-rank paired test was used for comparisons of different subpopulations from the same samples. For patient demographics, the Kruskall-Wallis test was performed for continuous variables and the Fisher's exact test for nominal variables. A *P*-value <.05 was considered statistically significant.

3 | RESULTS

3.1 | The proportion of ILCs is increased in the decidua parietalis of women with spontaneous preterm labor

We first conducted an exploratory study to determine the proportions and phenotypes of decidual ILCs in women with spontaneous term or preterm labor and non-labor gestational age-matched controls (Table 1). The gating strategy used to identify ILCs in the decidua parietalis and decidua basalis is shown in Figure 1A. ILCs were identified as CD15$^-$CD14$^-$CD3$^-$CD19$^-$CD56$^-$CD11b$^-$CD127$^+$ cells within the viability gate (Figure 1A). A higher proportion of total ILCs was observed in the decidua parietalis from women who underwent spontaneous preterm labor when compared to patients who delivered preterm without labor (Figure 1B). However, no significant differences were observed in ILCs in the decidua parietalis between women who underwent spontaneous labor at term compared to those who delivered at term in the absence of labor (Figure 1B). No significant differences were observed in the proportion of total ILCs in the decidua parietalis between women who underwent spontaneous preterm labor and those with labor at term (Figure 1B). There were no differences in the proportion of total ILCs in the decidua basalis among the study groups; yet, ILCs tended to be more abundant in the decidua basalis of women with preterm labor than in those who delivered preterm in the absence of labor (Figure 1C). These results show that ILCs are present at the human maternal-fetal interface during preterm and term gestations, and an increase in these cells is associated with spontaneous preterm labor.

3.2 | ILC1s are a minor subset of decidual ILCs during preterm and term gestations

We continued our exploratory study by characterizing the populations of ILC1s, ILC2s, and ILC3s in the decidua parietalis and decidua

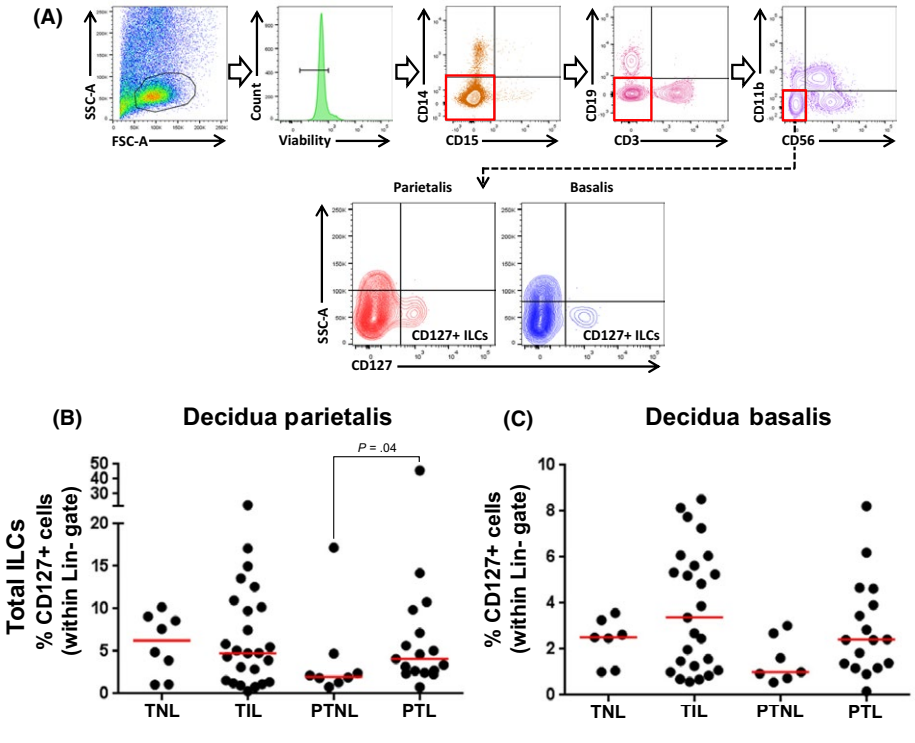

FIGURE 1 Innate lymphoid cells (ILCs) are present at the human maternal-fetal interface. (A) Mononuclear cells were isolated from the decidua parietalis and decidua basalis. Flow cytometry gating strategy used for immunophenotyping of ILCs. ILCs ($CD127^+$) were initially gated within the viability gate and lineage-negative (Lin−; $CD15^-CD14^-CD3^-CD19^-CD56^-CD11b^-$) gate. Red boxes represent the lineage-negative populations. Representative flow cytometry contour plots show the expression of CD127 by ILCs from the decidua parietalis and decidua basalis. The proportion of total ILCs in the decidua parietalis (B) and decidua basalis (C) of women who underwent spontaneous preterm (PTL) or term (TIL) labor and those who delivered preterm (PTNL) or term (TNL) without labor. n = 8-25 per group

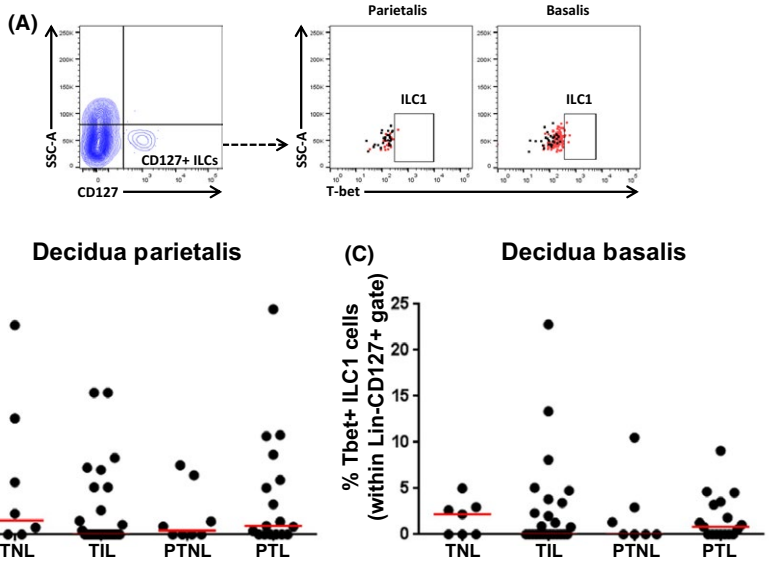

FIGURE 2 ILC1s are a minor population in the decidua. (A) Mononuclear cells were isolated from the decidua parietalis and decidua basalis. Flow cytometry gating strategy used for immunophenotyping of ILC1s. ILCs ($CD127^+$) were initially gated within the viability gate and lineage-negative (Lin−; $CD15^-CD14^-CD3^-CD19^-CD56^-CD11b^-$) gate. Representative flow cytometry contour plots show the expression of T-bet by ILC1s from the decidua parietalis and decidua basalis (red dots). Isotype controls are shown as black dots. The proportion of ILC1s in the decidua parietalis (B) and decidua basalis (C) of women who underwent spontaneous preterm (PTL) or term (TIL) labor and those who delivered preterm (PTNL) or term (TNL) without labor. n = 8-25 per group

basalis (Table 1). ILC1s were distinguished by the expression of the ILC1-associated transcription factor T-bet (Figure 2A). A very small proportion of ILC1s was identified in both the decidua parietalis (median < 2%) and decidua basalis (median < 3%) (Figure 2B,C). No significant differences in the proportion of decidual ILC1s were found among the study groups (Figure 2B,C). These data indicate that ILC1s may not have a significant role in the decidua during preterm and term gestations. Due to the small proportion of ILC1s detected in the decidua, we did not pursue further examination of this population.

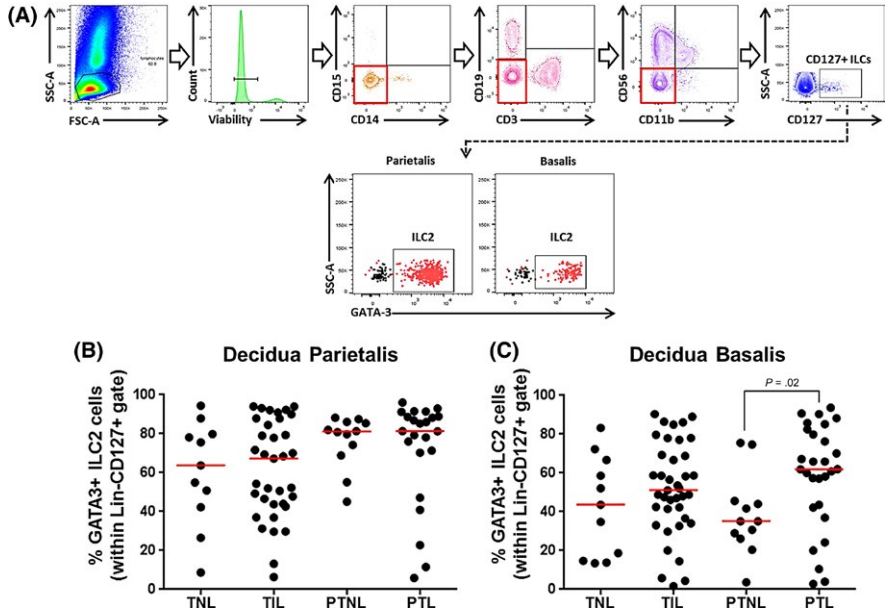

FIGURE 3 ILC2s are the most abundant ILC subset in the decidua. (A) Mononuclear cells were isolated from the decidua parietalis and decidua basalis. Flow cytometry gating strategy used for immunophenotyping of ILC2s. ILCs ($CD127^+$) were initially gated within the viability gate and the lineage-negative (Lin−; $CD15^-CD14^-CD3^-CD19^-CD56^-CD11b^-$) gate. Red boxes represent the lineage-negative populations. Representative flow cytometry contour plots show the expression of GATA3 by ILC2s from the decidua parietalis and decidua basalis (red dots). Isotype controls are shown as black dots. The proportion of ILC2s in the decidua parietalis (B) and decidua basalis (C) of women who underwent spontaneous preterm (PTL) or term (TIL) labor and those who delivered preterm (PTNL) or term (TNL) without labor. n = 11-39 per group

3.3 | ILC2s are the most abundant ILC subset in the decidua during preterm and term gestations

Our exploratory study revealed that ILC2s were the most abundant ILC subset in the human decidua (data not shown); therefore, we conducted a subsequent confirmatory study using a different and larger set of samples (Table 2). Decidual ILC2s were determined by the expression of GATA3 on $CD127^+$ ILCs (Figure 3A). In this second cohort, the ILC2 subset was also the most abundant population of ILCs in both the decidua parietalis and decidua basalis (Figure 3B,C). The proportion of ILC2s was higher in the decidua parietalis (median 60%-80%) than in the decidua basalis (median 35%-60%; Figure 3B,C). No differences in the proportion of ILC2s were observed in the decidua parietalis among the study groups; yet, ILC2s tended to be more abundant in preterm than in term gestations (Figure 3B). However, a higher proportion of ILC2s was found in the decidua basalis of women who underwent spontaneous preterm labor compared to non-labor controls (Figure 3C). There were no differences in the proportion of ILC2s in the decidua basalis of women who underwent spontaneous labor at term compared to those who delivered at term in the absence of labor or those who underwent spontaneous preterm labor (Figure 3C). These data show that ILC2s are the dominant ILC population in the decidua parietalis and decidua basalis and that an increase in these cells in the decidua basalis is associated with spontaneous preterm labor.

3.4 | The proportion of ILC3s is increased in the decidua parietalis of women who underwent spontaneous preterm labor

Next, we evaluated the presence of ILC3s in the decidual tissues (Table 2). The gating strategy used to determine the proportion of decidual ILC3s by the expression of RORγt is shown in Figure 4A. The proportion of ILC3s was significantly increased in the decidua parietalis of women who underwent spontaneous preterm labor compared to that of women who delivered preterm in the absence of labor or those who delivered at term (Figure 4B). The proportion of ILC3s in the decidua basalis did not vary among the study groups (Figure 4C). These findings indicate that an increase of ILC3s in the decidua parietalis is associated with spontaneous preterm labor.

3.5 | Decidual ILC3s express high levels of IL-22, IL-17A, IL-13, and IFN-γ in women with spontaneous preterm labor

To further characterize decidual ILC2s and ILC3s from women with spontaneous preterm labor, we evaluated the expression of cytokines associated with the 3 ILC subsets (Figure 5A). The mean fluorescence intensity (MFI) of IL-22 (Figure 5B,C), IL-13 (Figure 5F,G), and IFN-γ (Figure 5H,I) was higher for ILC3s from the decidua parietalis and decidua basalis compared to that of ILC2s. The MFI of IL-17A was

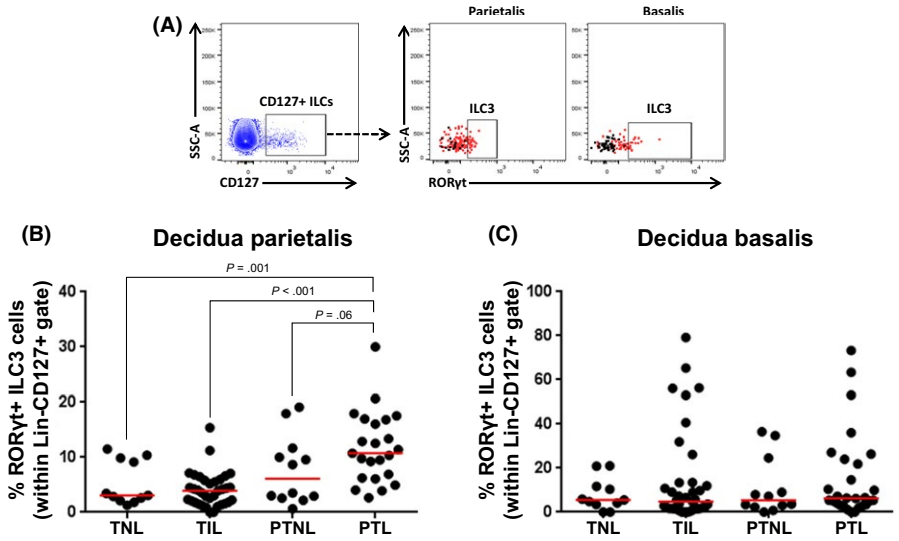

FIGURE 4 ILC3s are increased in the decidua parietalis of women who underwent spontaneous preterm labor. (A) Mononuclear cells were isolated from the decidua parietalis and decidua basalis. Flow cytometry gating strategy used for immunophenotyping of ILC3s. ILCs ($CD127^+$) were initially gated within the viability gate and lineage-negative (Lin−; $CD15^-CD14^-CD3^-CD19^-CD56^-CD11b^-$) gate. Representative flow cytometry contour plots show the expression of RORγt by ILC3s from the decidua parietalis and decidua basalis (red dots). Isotype controls are shown as black dots. The proportion of ILC3s in the decidua parietalis (B) and decidua basalis (C) of women who underwent spontaneous preterm (PTL) or term (TIL) labor and those who delivered preterm (PTNL) or term (TNL) without labor. n = 11-39 per group

increased solely for ILC3s in the decidua parietalis compared to that of ILC2s (Figure 5D). No differences were observed in the expression of IL-5 between decidual ILC2s and ILC3s (data not shown). Together, these data show that ILC3s expressed higher levels of IL-22, IL-17A, IL-13, and IFN-γ than ILC2s in the decidua during the pathological process of preterm labor.

4 | DISCUSSION

4.1 | Principal findings of the study

(i) The proportion of total $CD127^+$ ILCs was increased in the decidua parietalis of women who underwent spontaneous preterm labor; (ii) ILC1s were a minor subset of decidual ILCs during preterm and term gestations; (iii) ILC2s were the most abundant ILC subset in the decidua during preterm and term gestations; (iv) the proportion of ILC2s was increased in the decidua basalis of women who underwent spontaneous preterm labor; (v) the proportion of ILC3s was increased in the decidua parietalis of women who underwent spontaneous preterm labor; (vi) ILC3s had higher expression of IL-22, IL-17A, IL-13, and IFN-γ compared to ILC2s in the decidua of women who underwent spontaneous preterm labor; and (vii) decidual ILC2s and ILC3s had similar expression of IL-5 in the decidua of women who underwent spontaneous preterm labor. Collectively, these findings show that although ILC2s are the most abundant ILC subset at the human maternal-fetal interface during preterm and term gestations, an increase in ILC2s and ILC3s in the decidua basalis and decidua parietalis is observed during the pathological process of preterm labor.

4.2 | ILCs at the human maternal-fetal interface during preterm and term gestations

Innate lymphoid cells have been described in mucosal tissues, such as the lung, where they contribute to asthma and allergy-related processes,[89-91] and the gastrointestinal tract, where they provide defense against parasitic and microbial infections.[92-95] The discovery of enriched ILCs in mucosal tissues suggests that these cells are implicated in chronic intestinal inflammatory disorders, (e.g. Crohn's disease).[96] The association between ILCs and inflammatory diseases has led to the search for these cells at other sites of mucosal immunity, such as the reproductive tissues.[81] The 3 conventional ILC subsets have been described in the murine uterus during early and mid-gestation, although there is controversy as to which ILC subset is dominant during this period.[81,82] Indeed, ILC subsets have also been identified in the non-pregnant state in mice[97] and humans.[82] During early pregnancy, ILC1s and ILC3s are present at the human maternal-fetal interface, where such lymphoid cells crosstalk with neutrophils to modulate their migration and function.[81,85] In the study herein, we extended these observations by demonstrating that ILCs are present at the human maternal-fetal interface during term and preterm gestations (ie, third trimester). Together, these results indicate that ILCs are present at the human maternal-fetal interface; yet, their subsets are dynamically changing throughout gestation and with the onset of preterm labor.

4.3 | A role for ILC2s at the human maternal-fetal interface during the third trimester and in preterm labor

ILC2s are a distinct subset of ILCs that bear a functional resemblance to Th2 cells and were first described in a mouse model of helminth

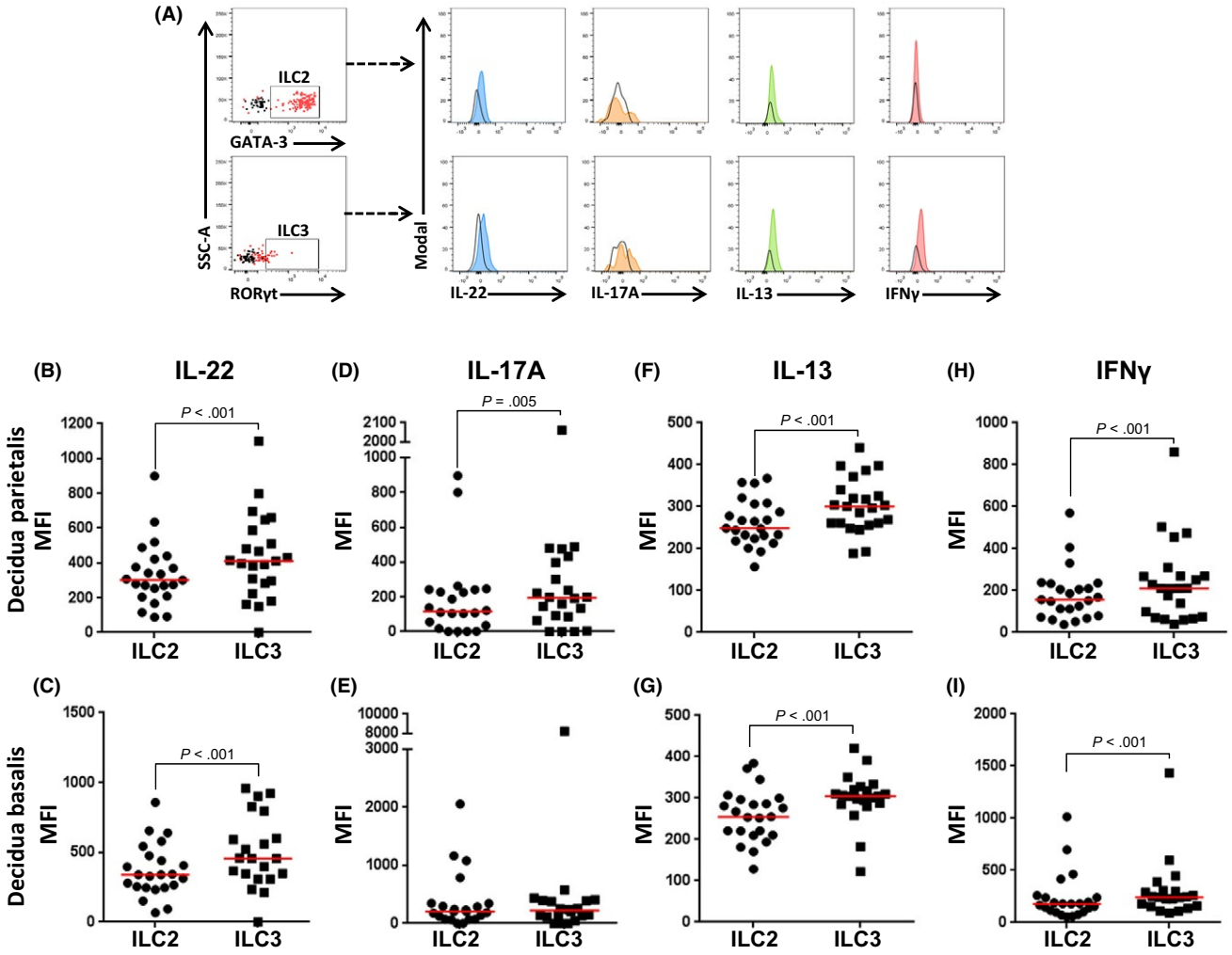

FIGURE 5 Cytokines expressed by decidual ILC2s and ILC3s in women who underwent spontaneous preterm labor. (A) Mononuclear cells were isolated from the decidua parietalis and decidua basalis. Representative flow cytometry histograms show the mean fluorescence intensity (MFI) expression of IL-22 (blue histograms), IL-17A (orange histograms), IL-13 (green histograms), and IFN-γ (red histograms) by decidual ILC2s and ILC3s (red dots). Isotype controls are shown as black outline histograms or as black dots. The MFI of IL-22 (B and C), IL-17A (D and E), IL-13 (F and G), and IFN-γ (H and I) expression by ILC2s and ILC3s in the decidua parietalis (upper row) and the decidua basalis (bottom row) of women who underwent spontaneous preterm labor. n = 23

parasitic infection as novel producers of the Th2-like cytokines IL-4, IL-5, and IL-13.[92] ILC2s have been identified in the mesenteric lymph nodes, spleen, liver, intestines, and airways.[98] More recently, ILC2s were abundantly found in the murine uterus during early pregnancy,[97] where their presence may be regulated by female sex hormones (eg, estrogen).[99] Herein, we show that ILC2s are the most abundant ILC subset at the human maternal-fetal interface (decidua basalis and decidua parietalis). Tissue ILC2s display homeostatic functions through the secretion of tissue repair factors, such as amphiregulin and IL-13,[100-102] whose properties resemble those exhibited by M2 decidual macrophages in term and preterm gestations.[50] Therefore, we suggest that ILC2s, as well as M2 macrophages,[103] display homeostatic roles at the human maternal-fetal interface during the third trimester.

In addition to displaying homeostatic functions, ILC2s also exhibit proinflammatory functions. For example, ILC2s can contribute to the pathogenesis of ulcerative colitis, a chronic disease characterized by elevated concentrations of Th2 cytokines, such as IL-4, IL-5, and IL-13.[104,105] Herein, we found that the proportion of ILC2s was increased in the decidua basalis of women who underwent spontaneous preterm labor. Interestingly, preterm labor is associated with chronic inflammatory lesions of the placenta[106] (eg, chronic deciduitis, infiltration of lymphocytes or plasma cells into the basal plate of the placenta[107]), which provides evidence that preterm labor can also be a chronic inflammatory disease. These data indicate that ILC2s may participate in the chronic inflammatory microenvironment that accompanies the pathological process of preterm labor in the decidua basalis.

4.4 | A role for ILC3s at the human maternal-fetal interface during preterm labor

ILC3s were first described in the small intestine as unique innate cells that express the transcription factor RORγt and the cytokine

IL-22[108,109] and were later shown to produce IL-17A.[110,111] ILC3s have been studied primarily in the context of inflammatory bowel disorders and other gastrointestinal diseases due to their presence in the gut mucosa and interactions with commensal bacteria.[93,112] Moreover, RORγt+ ILC3s can express MHC class II molecules and process and present microbial antigens to gut T cells.[113] This presentation of microbial peptides in the gut results in diminished commensal bacteria-specific T-cell responses.[113] In addition, IL-23-responsive ILC3s producing IL-17A and IL-22 have been implicated in the development of colitis in mouse models and in human studies.[96] In the current study, ILC3s were enriched in the decidua parietalis (decidua attached to the chorioamniotic membranes[87]) of women who underwent spontaneous preterm labor. Such ILCs expressed high levels of IL-22 and IL-17A, suggesting that this subset is implicated in the localized inflammatory milieu that accompanies the pathological process of preterm labor.

Herein, we found that decidual ILC3s expressed a high level of IL-13 (a cytokine mainly produced by ILC2s[91,92]) during the process of preterm labor. In line with this observation, previous studies have demonstrated that IL-13 is expressed by the placental[114] and decidual tissues.[115,116] IL-13 promotes the activation and migration of dendritic cells to the draining lymph nodes, leading to the differentiation of Th2 cells.[117] This cytokine is also important for tissue repair responses, reduction of ILC3-mediated inflammation, and defense against parasitic infections.[101,118] Together, these results allow us to propose that decidual ILC3s express a high level of the anti-inflammatory cytokine IL-13 to regulate the inflammatory responses exhibited by such cells.

Interestingly, ILC3s are also capable of expressing IFN-γ when exposed to IL-12, IL-18, and/or IL-1β by upregulating T-bet, suggesting that a small proportion of ILC1s are derived from ILC3s.[79] In the current study, we found that decidual ILC3s expressed a high level of IFN-γ, besides expressing ILC3 cytokines. Together, these results indicate that decidual ILC3s express ILC1 and ILC2 cytokines, supporting the concept that immune cells at the maternal-fetal interface display unique phenotypical characteristics.[119]

5 | SUMMARY

In the current study, we provide evidence that ILCs are present at the human maternal-fetal interface; yet, their subsets are dynamically changing throughout late gestation and with the onset of preterm labor. First, we found that ILC2s are the most abundant ILC subset at the human maternal-fetal interface and that their proportion in the decidua basalis (decidua attached to the placenta) increased in women who underwent spontaneous preterm labor. Next, we showed that ILC3s are enriched in the decidua parietalis (decidua attached to the chorioamniotic membranes) in women who underwent spontaneous preterm labor. Lastly, we demonstrated that ILC3s expressed high levels of IL-22, IL-17A, IL-13, and IFN-γ at the human maternal-fetal interface during preterm labor. Collectively, these data provide the first evidence demonstrating a role for ILCs at the human maternal-fetal interface during the pathological process of preterm labor.

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CONFLICT OF INTEREST

The authors declare no financial interests in any of the work submitted here.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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