ORIGINAL RESEARCH

Linked Th17 and Calgranulin Responses in Maternal-cord Blood Dyads of Preterm Gestations with Histologic Chorioamnionitis

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Received on: 14 May 2023; *Accepted on*: 07 June 2023; *Published on*: 30 June 2023

Abstract

Introduction: Maternal–fetal immune crosstalk mechanisms are increasingly identified in the pathogenesis of gestational disorders, including histologic chorioamnionitis (HCA). Although an inflammatory Th17 immune phenotype has been described in preterm neonates with HCA, the associated maternal Th17 response is relatively unknown. To refine our understanding of Th17 biology in this context, we examined Th17 responses in maternal-cord blood dyads of preterm gestations.

Materials and methods: Paired maternal and cord blood (CB) samples were prospectively collected from preterm gestations (23–34 weeks) with HCA or controls. Th17-linked cell frequencies and plasma calgranulin (S100A8, S100A12) levels were determined by flow cytometry and enzyme-linked immunoassay, respectively.

Results: Analyses of 47 maternal-cord blood pairs showed striking parallel increases in Th17 cell frequencies as well as plasma calgranulin levels in the presence of fetal inflammation. Cord blood S100A12 levels were directly correlated with Th17 cell frequencies. In CB cultures, rh-S100A12 promoted *in vitro* propagation of Th17-type $CD4^+$ cells.

Conclusions: Maternal and CB Th17-linked responses are dually amplified in gestations with HCA, supporting a biological role for maternal–fetal interactions in this disorder. In addition to advancing current knowledge of neonatal Th17 mechanisms, these data shed new light on their association with maternal inflammation.

Keywords: Fetal inflammation, Gamma–delta T cells, Maternal inflammation, S100, S100A8, S100A12, Treg cells.

Newborn (2023): 10.5005/jp-journals-11002-0064

Highlights

- The key message of our study is that certain parallel T-helper (Th) 17 cells and calgranulin responses can be found in mothers and cord blood (CB) of preterm gestations with histologic chorioamnionitis (HCA), particularly in the presence of fetal inflammation and despite the absence of maternal clinical symptoms.
- The effects of fetal inflammation on maternal and CB Th17 responses support mounting evidence of maternal–fetal inflammatory and immune crosstalk mechanisms.
- Calgranulins may be key mediators of perinatal inflammation modulated by the Th17 pathway.
- Our findings advance still limited understanding of the contributions of Th17 and calgranulin biology to placental, maternal, and CB inflammatory processes.
- This knowledge could be important to the targeted development of strategies to mitigate the pathogenesis of perinatal and neonatal inflammation.

Introduction

Preterm birth is a significant and increasing global health concern. The Centers for Disease Control reported that over 1 in 10 deliveries were preterm in the United States and that this number is increasing.[1,2] Of these, 70% were spontaneous, the result of preterm

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How to cite this article: Buchanan CQ, Lawlor ML, Okafor C, *et al*. Linked Th17 and Calgranulin Responses in Maternal-cord Blood Dyads of Preterm Gestations with Histologic Chorioamnionitis. Newborn 2023;2(2):133–141.

Source of support: This work was supported in part by funding from the Gerber Foundation, the National Institutes of Health, a private institutional foundation, and the institution's Department of Pediatrics. No funding source had involvement in study design, data analysis, or publication decisions. NIH R21 AI094478 (to JMK).

Conflict of interest: None

labor or preterm premature rupture of membranes (PPROM). Histologic chorioamnionitis (HCA) is a placental inflammation closely linked to spontaneous preterm birth.[3] Fetal exposure to HCA can result in adverse outcomes in preterm neonates, including brain injury, sepsis, necrotizing enterocolitis, and chronic lung disease, as detailed in a recent elegant review.[4] A postnatal diagnosis of HCA based on placental pathology is common in extremely preterm gestations, even in the absence of maternal or fetal symptoms associated with intra-amniotic infection (IAI).[5,6]

T-helper (Th) 17 cells represent a unique lymphocyte subset that can bridge adaptive and innate immune responses to protect the host against microbial pathogens.[7,8] Conversely, dysregulated Th17 cells may mediate pathologic processes leading to chronic inflammation of various organs, particularly affecting the brain in neonates.[9] Mounting evidence points to a role for Th17 cells in modulating immune function during normal pregnancy and in healthy neonates; however, much remains unknown, representing a significant knowledge gap.[10,11] In contrast, recent observations suggest potential contributions of Th17 cells to pathologic gestational processes including those leading to preterm delivery.[12–15] Also, Th17 cells may be functionally linked to members of the S100 family of proteins, including the calgranulins (S100A8, S100A9, S100A12).[16,17] While elevated calgranulin expression levels in association with HCA have been reported in placentas, amniotic fluid, and in preterm cord blood (CB) and neonatal blood,[18–21] a link between calgranulins and Th17 cells in the setting of HCA has not been established.

A growing body of literature suggests the importance of maternal–fetal immune crosstalk mechanisms to the pathogenesis of certain placental disorders, including chorioamnionitis.[22] However, whether systemic maternal Th17-type responses mimic the expression patterns found in their neonates, and whether such responses contribute to the pathogenesis of HCA, is presently unknown. Such observations could also be relevant to evidence that women who deliver a preterm infant are at increased risk of future heart disease,[23,24] particularly given the connection between enhanced Th17 responses and cardiovascular disorders.[25] We designed the present study to test the hypothesis that HCA is associated with enhanced circulating Th17 cell frequencies and Th17-linked calgranulin levels in affected pregnant women that parallel responses in the CB of their preterm neonates.

Materials and Methods

Human Subjects

From December 2016 to March 2019, eligible pregnant women admitted to the labor and delivery service at a large perinatal center in St. Louis, Missouri, USA were approached for their own enrollment and that of their delivered preterm neonates. Eligibility criteria included singleton or uncomplicated twin gestations, preterm labor and/or PPROM, and delivery between 23^0 and 34^6 gestational weeks. Potential subjects were excluded from study if mothers or pregnancies were affected by inflammatory conditions or infection other than suspected clinical chorioamnionitis, or if a potential for altered immunity related to congenital or genetic conditions in the fetus or newborn existed. Demographic and clinical details were obtained from the electronic medical record. This prospective observational study was performed with the approval of a protocol and according to the policies of the Institutional Review Board for Human Studies of Saint Louis University, SSM Health Cardinal Glennon Children's Hospital (CGCH), and SSM Health St. Mary's Health Center (SMHC). Informed, written consent was obtained for all study participants.

Diagnosis of Histologic Chorioamnionitis

All placentas were examined by a clinical academic pathologist as part of routine clinical care (Redline criteria[3]). Diagnosis and staging of HCA were based on the involved compartment (maternal and fetal) and the extent of neutrophil invasion.[3,26] A diagnosis of maternal HCA (MHCA) was based on neutrophil infiltration at or below the chorionic plate; fetal HCA (FHCA) was identified by neutrophil invasion of veins or arteries in the chorionic plate and/or of the umbilical CB vessels.[3] Chronic inflammation was diagnosed in placentas with lymphocytic infiltration of the chorionic villi (*chronic villitis*), chorioamniotic membranes/plate (*chronic chorioamnionitis*), or basal plate (*chronic deciduitis*).[27] Gestations were defined as "controls" in placentas without evidence of HCA or chronic chorioamnionitis, or other significant pathology. Medical records were reviewed for maternal or fetal evidence of IAI or clinical chorioamnionitis.[28,29]

Blood Sample Collection

Anticoagulated (citrate phosphate) maternal blood samples were obtained from pregnant women by peripheral venipuncture within 24 hours prior to delivery. For cord blood (CB) samples, anticoagulated blood was aspirated from the placental umbilical vein (cleansed of maternal blood) immediately following delivery. Whole blood was processed for flow cytometric analysis and for the collection of plasma aliquots as described.[13] Plasma samples were stored at −80°C until batch analysis. For *in vitro* studies, anonymous CB samples (collected less than 12 hours postdelivery) were obtained from the SSM Health St. Louis cord Blood Bank.

Flow Cytometric Analyses of Patient Samples

Multiparameter flow cytometric analyses of antibody-stained whole blood samples were used to identify specific immune cell subsets. Briefly, samples stained with fluorochrome-labeled mAb or type-specific immunoglobulin G (IgG) controls were acquired within 24 hours of staining using a BD LSR-II Flow Cytometer, and were analyzed with the FlowJo 7.2.2 software (Tree Star, Ashland, Oregon, USA), as previously described.[13] Within the viable gated $CD3^+$ lymphocyte population, Th17 cells were identified in $CD4^+$ cells with surface expression of $CD161^+$ (progenitor Th17 cells [pTh17])[30] or both $CD161^+$ and $CCR6^+$ (mature Th17 cells [mTh17]) (7). The T regulatory (Treg) cells were identified in $CD4^+$ cells expressing the $CD25^{hi}CD127^{lo}$ phenotype.[31] The $TCR\gamma\delta^+$ T cells were identified within the gated $CD3^+$ cell population.[32] Furthermore, the Th17:Treg ratios were determined by calculating the ratios of pTh17 or mTh17 cell frequencies, respectively, to those of Treg cells.

Determination of Plasma Calgranulins

Calgranulin levels were determined in batched duplicate plasma samples using commercial ELISAs (CircuLex S100A8/MRP8, catalog No. CY-8061; CircuLex S100A12/EN-RAGE, catalog No. CY-8058; Circulex; MBL International Corporation, Woburn, Massachusetts, USA). Readings (405 nm) were compared against an internal standard curve, and the concentrations of S100A8 or S100A12 in each sample were calculated by plotting against a four-parameter logistic equation. Assay limits for detection were: S100A8,

43.4 pg/mL; S100A12, 8.2 pg/mL. Due to variability in plasma sample volumes, S100A8 and/or S100A12 levels were not determined in all subjects.

Th17 Cell Propagation in CB Cultures

For these studies, CB $CD4^+$ cells were purified from mononuclear cells, as we described.[33] Briefly, $CD4^+$ T cells were isolated by negative selection (EasySep™ Human Naïve $CD4^+$ T Cell Isolation Kit (Catalog 19555), STEMCELL Technologies, Vancouver, Canada) according to the manufacturer's instructions. Purified $CD4^+$ cells (2×10^6 cells) were suspended in CTCM (2 mM glutamine, 50-μM β-mercaptoethanol, 10% heat-inactivated human AB type serum, 100-U penicillin/100 μg streptomycin/mL) and cultured either in CTCM alone, or in CTCM containing a Th17-propagating cocktail (10 ng/mL: Interleukin-1β (IL-1β), IL-6, IL-23; 3 ng/mL: TGFβ) or varying concentrations of rh-S100A12. Cell suspensions were added to 24 well plates coated with anti-CD3 Ab (2 μg/mL) and in the presence of IL-2 (50 U/mL) at 37°C, 5% CO_2. Following a 72-hour culture, cells were harvested and stimulated for intracellular staining, including viability, as described.[33] Samples were acquired within 24 hours of staining using a 16-color BD LSRII flow cytometer. Acquired samples were analyzed using the FlowJo 7.2.2 software (Tree Star, Ashland, OR). Within the gated $CD4^+$ T cell population, Th1 cells were identified by the intracellular expression of the nuclear factor, Tbet, and Th17 cells were identified by expression of the nuclear factor, RORγt, or IL-17A. Tregs were identified in $CD4^+$ cells with intracellular expression of the nuclear factor, Foxp3.

Antibodies and Reagents

Fluorochrome-labeled mAb (all, Becton-Dickinson, Franklin Lakes, New Jersey, USA) were used for surface staining: CD3-FITC (clone SK7), CD4-Alexa Fluor 700 (RPA-T4), CD25-PE (2A3), CD45-V450 (HI30), CD127-BV650 (HIL-7R-M21), CD161-APC (DX12), CD196-PerCPCy5.5 (IIA9), and TCRγδ-BV605 (B1). The vital stain, Live/Dead Aqua, was purchased from Invitrogen/Thermo Fisher Scientific (Waltham, MA; catalog No. L34957). Recombinant human (rh) cytokines were purchased from BD Biosciences, San Jose, CA (rhIL-2), Peprotech, Inc., Rocky Hills, New Jersey, USA (rhIL-1β, rhIL-6), and R&D Systems, Minneapolis, Minnasota, USA (rhIL-23, rhTGFβ, rhS100A12).

Statistical Analyses

Experimental data were analyzed using the non-parametric Kruskal–Wallis test for intragroup comparisons across conditions; the non-parametric Mann–Whitney *U* test or independent Student's *t*-test for comparisons of unpaired data; and the non-parametric Wilcoxon rank test for paired data analyses (Prism v7; GraphPad Software, La Jolla, California, USA). Demographic data were compared using the Mann–Whitney *U* test or independent Student's *t*-test for continuous data, or Fisher's exact test for categorical data using Statistical Package for the Social Sciences (SPSS) (v23; IBM, Armonk, New York, USA) or Prism. Correlations between variables were calculated using the Pearson correlation coefficient; $p < 0.05$ was considered significant.

Results

Subject Characteristics and Demographics

We studied 47 women in preterm labor who were enrolled at the time of their admission to labor and delivery, and their delivered neonates. Placental analyses identified 37 gestations with HCA (12 MCHA; 25 FHCA). Seven placentas were unaffected by HCA or other identified placental pathology, and served as controls. Placentas of three gestations were diagnosed with chronic inflammation only (villitis or deciduitis); these were analyzed separately from the HCA or control groups. Key baseline maternal and perinatal characteristics, including clinically suspected IAI, were not different between groups (Table 1). Neonates with HCA were delivered at earlier gestational ages relative to controls (Table 2), especially in the presence of FHCA (29.4 ± 3.1 weeks, $p = 0.02$); however, no age differences were observed between MHCA and FHCA gestations. Birth weights were lower in HCA relative to controls, particularly in the presence of FHCA (1471 ± 542 gm, $p = 0.01$). In contrast, the three neonates with chronic inflammation had higher birth weights (2166 ± 465 gm) relative to CB with any HCA ($p = 0.02$) and FHCA ($p = 0.01$). No differences were observed between groups for the remainder of neonatal outcomes, including early- or late-onset sepsis.

Table 1: Maternal characteristics

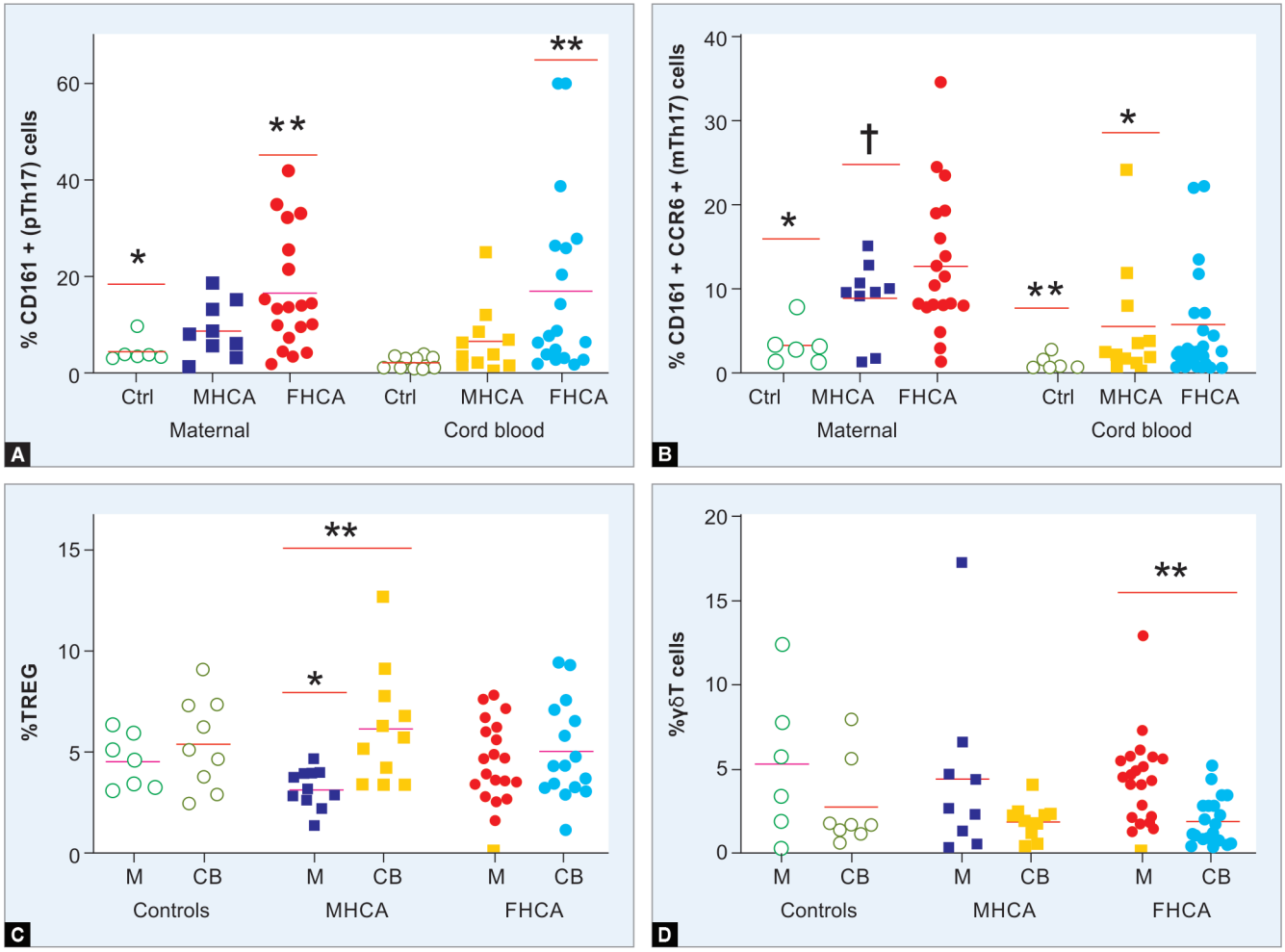
Parameter	*HCA (n = 37)*	*Controls (n = 7)*	*p-value*
Age (year)	26.8 ± 6.5	26.1 ± 7.0	0.80
BMI (kg/m^2)	30.2 ± 6.8	30.6 ± 5.9	0.86
African–American	19 (51%)	3 (43%)	>0.99
Caucasian	18 (49%)	4 (57%)	>0.99
Multiparous	22 (59%)	6 (86%)	0.39
Prior preterm delivery	9 (24%)	4 (57%)	0.17
History of smoking	8 (22%)	3 (43%)	0.34
PPROM	31 (84%)	6 (86%)	>0.99
Suspected IAI	6 (16%)	1 (14%)	>0.99
Antenatal antibiotics	37 (100%)	7 (100%)	>0.99
Antenatal steroids	37 (100%)	7 (100%)	>0.99

Data are shown either as mean ± standard deviation (SD) or as *n* (%). BMI, body mass index; IAI, intraamniotic infection; PPROM, prolonged preterm rupture of membranes

Table 2: Neonatal outcomes

Parameter	*HCA (n = 37)*	*Controls (n = 9)*	*p-value*
Gestational age at delivery (weeks)	29.9 ± 3.0	31.8 ± 2.6	0.04
Birth weight (gm)	1546 ± 612	1898 ± 533	0.03
SGA	2 (5%)	0 (0%)	>0.99
EOS	8 (22%)	1 (11%)	0.66
LOS	3 (8%)	0 (0%)	>0.99
IVH	3 (8%)	0 (0%)	>0.99
NEC	2 (4%)	0 (0%)	>0.99
BPD	5 (14%)	1 (11%)	>0.99
Death	4 (11%)	0 (0%)	0.57

Data are shown as either mean ± SD or as *n* (%). Death at delivery or at any time prior to hospital discharge. BPD, bronchopulmonary dysplasia; EOS, early-onset sepsis; IVH, intraventricular hemorrhage; LOS, late-onset sepsis; NEC, necrotizing enterocolitis; SGA, small for gestational age

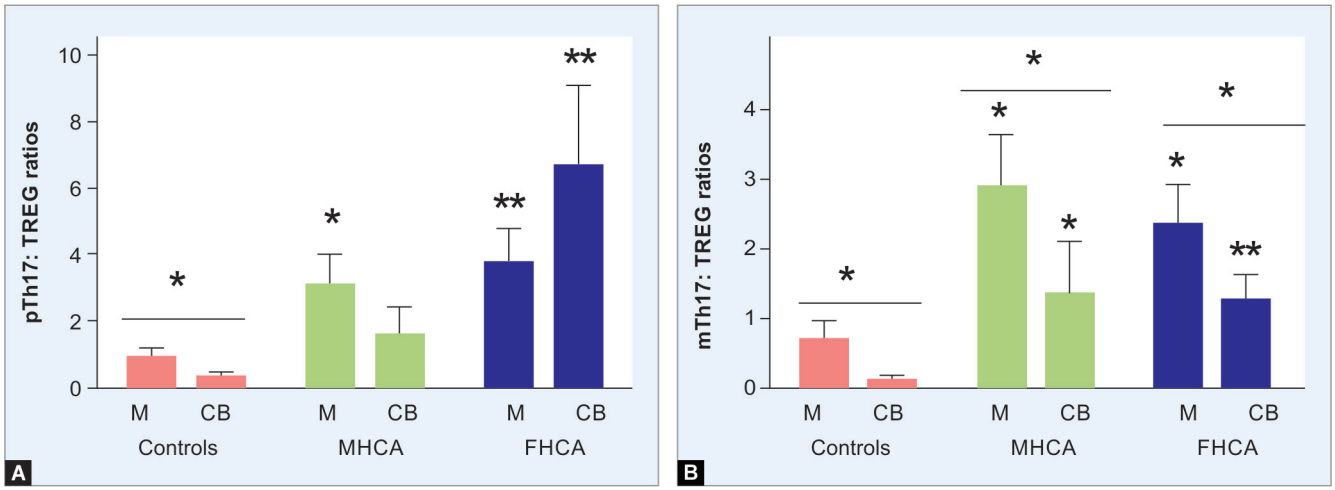


Figs 1A to D: Immune cell frequencies. Gated $CD4^+$ cells in maternal peripheral blood and CB (cord blood) samples from preterm gestations with HCA or from unaffected preterm controls were analyzed for circulating frequencies of Th17 and Treg cell populations by multi-parameter flow cytometric analysis. The analysis of γδ T cell populations was performed within gated $CD3^+$ cells. Each symbol represents a single subject. Horizontal bars represent mean ± SEM. (A) Progenitor (p)Th17 cells. Mean frequencies of $CD4^+CD161^+$ populations. Maternal (M)-CB pairs: Controls (Ctrl), $n = 6$; MHCA, $n = 11$; FHCA, $n = 19$. *$p < 0.05$ vs Ctrl; **$p < 0.01$ vs Ctrl; (B) Mature (m) Th17 cells. Mean frequencies of $CD4^+CD161^+CCR6^+$ populations. M-CB pairs: Ctrl, $n = 7$; MHCA, $n = 11$; FHCA, $n = 19$. *$p < 0.05$ vs Ctrl; **$p < 0.01$ vs Ctrl; $^{\dagger}p < 0.001$ vs Ctrl; (C) Treg cells. Frequencies of $CD4^+CD25^{hi}CD127^{lo}$ Treg populations. M-CB pairs: Ctrl, $n = 7$; MHCA, $n = 11$; FHCA, $n = 16$. *$p < 0.05$ vs Controls; **$p < 0.01$, M vs CB; (D) γδ T cells. Mean frequencies of $CD3^+TCR^+\gamma\delta^+$ cell populations. M-CB pairs: Ctrl, $n = 7$; MHCA, $n = 11$; FHCA, $n = 18$. **$p < 0.01$, M vs CB

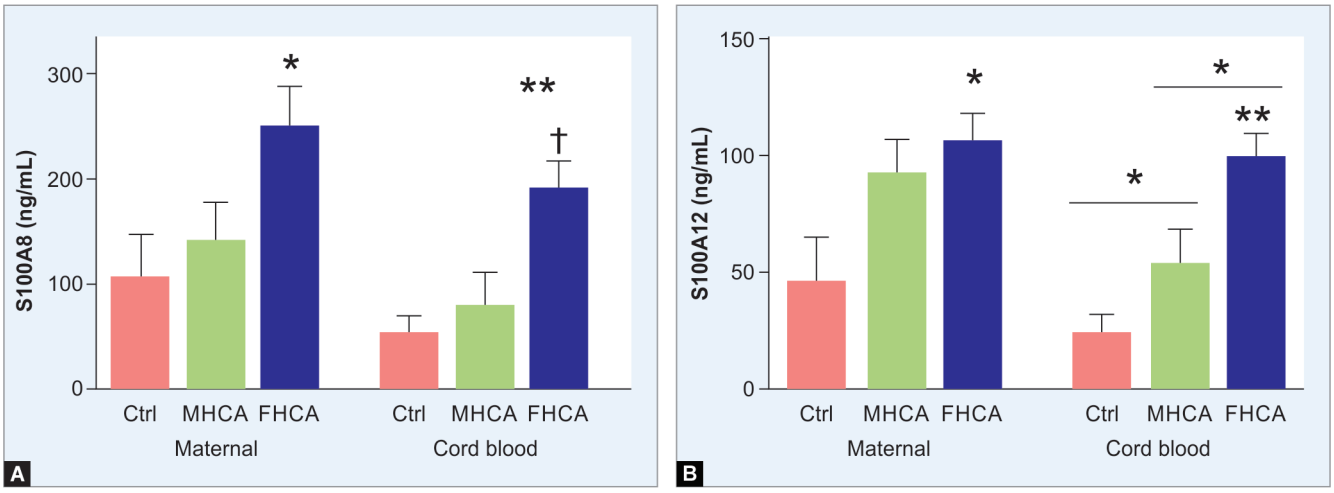
Immune Cell Responses

In flow cytometric studies of maternal peripheral blood and preterm CB, we examined the proportions of Th17 cells subsets with progenitor or mature phenotypes, as these have been variably linked to the pathogenicity of chronic inflammatory disorders.[7,34–36] We determined the highest frequencies of progenitor (p)Th17 cells in FHCA gestations for both mothers and in CB relative to controls, while these were significantly elevated only for mothers in MHCA (Fig. 1A). In paired comparisons, pTh17 cell frequencies were higher in mothers in control gestations ($p = 0.02$ vs CB); frequencies were similar between mothers and in CB with MHCA ($p = 0.42$) or FHCA ($p = 0.61$). In studies of mature (m)Th17 cells, we also observed the highest maternal and CB frequencies in FHCA gestations, similar to our observations for pTh17 cell subsets (Fig. 1B). Maternal and CB mTh17 cell frequencies were also both elevated in MHCA relative to control gestations. In paired studies, maternal mTh17 cell frequencies were higher than in the CB of their neonates in FHCA gestations; however, frequencies were not significantly different between pairs in MHCA ($p = 0.28$) or control ($p = 0.06$) gestations.

Quantitative alterations in Treg cells, which have the capacity to suppress Th17 responses,[37] have been reported in pregnancy-related inflammatory disorders, including in women with preeclampsia.[15] We examined frequencies of Treg cells in paired maternal-CB blood samples in gestations with or without HCA (Fig. 1C). We observed lower circulating Treg cell frequencies in mothers with MHCA ($p < 0.05$ vs controls), while no differences in CB Treg frequencies were determined between HCA and control gestations. In paired comparison studies, maternal Treg cell frequencies were also lower compared with CB in MHCA gestations but were similar to CB Treg cell frequencies in gestations with FHCA ($p = 0.89$) or in controls ($p = 0.05$).



Figs 2A and B: The Th17:Treg ratios. The Th17:Treg ratio was calculated by dividing individual frequencies of pTh17 or mTh17 cells by Treg cell frequencies. Maternal (M)-CB pairs: Ctrl, $n = 7$; MHCA, $n = 11$; FHCA, $n = 13$. (A) pTh17:Treg ratio. *$p < 0.05$, M vs CB; $p < 0.05$ vs Controls; **$p < 0.01$ vs Ctrl; $p < 0.01$, M vs CB; (B) mTh17:Treg ratio. *$p < 0.05$, M vs CB; $p < 0.05$ vs Ctrl; **$p < 0.001$ vs Ctrl



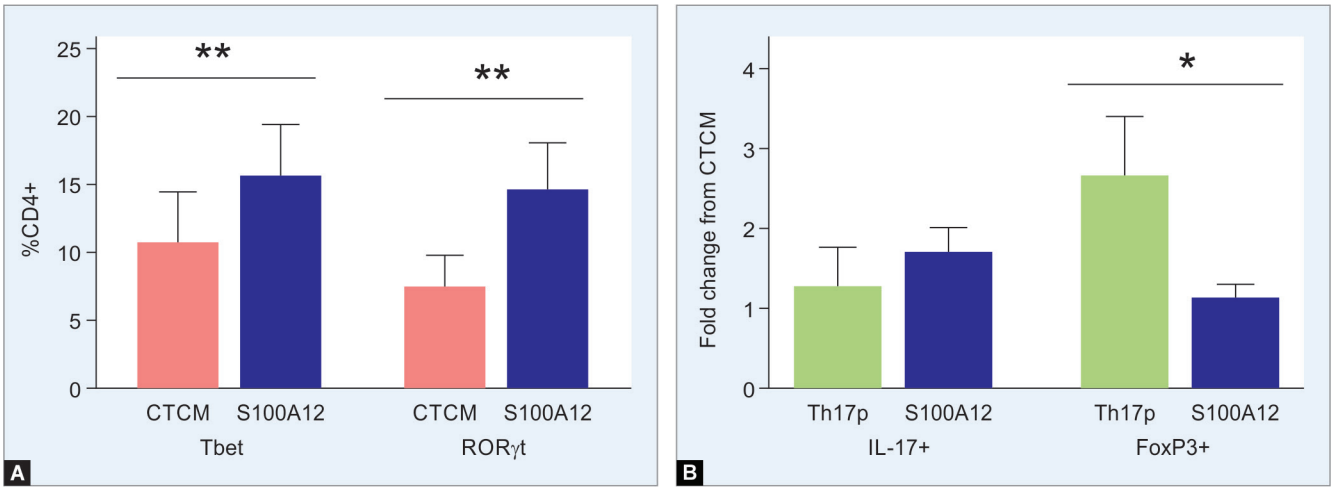
Figs 3A and B: Maternal and CB S100A8 and S100 A12 plasma levels. Plasma levels of S100A8 or S100A12 were determined in maternal (M)-CB pairs from HCA or control gestations. (A) S100A8 levels. Ctrl, $n = 9$; MHCA, $n = 10$; FHCA, $n = 18$. *$p < 0.05$ vs Ctrl; **$p < 0.01$, MHCA vs FHCA; †$p < 0.001$ vs Ctrl; (B) S100A12 levels. Ctrl, $n = 9$; MHCA, $n = 10$; FHCA, $n = 20$. *$p < 0.05$ vs Ctrl; MHCA vs FHCA; **$p < 0.001$ vs Ctrl

Gamma-delta T cells represent a lymphocyte subset that may contribute to inflammatory pathology in part through the release of IL-17.[38] To more fully define Th17-type responses in HCA, we compared maternal and CB γδ T cell frequencies in affected and control gestations (Fig. 1D). No differences in γδ T cell frequencies were determined for either mothers or in CB for any HCA condition relative to controls. However, in pairwise assessments, higher γδ T cell frequencies were determined in mothers vs CB in FHCA, while frequencies were similar between pairs in both MHCA ($p = 0.17$) and control gestations ($p = 0.19$).

The Th17:Treg ratio has been correlated with disease severity in a variety of inflammatory disorders.[39] We observed elevated pTh17:Treg ratios for both mothers and in CB in FHCA vs control gestations, while only maternal pTh17:Treg ratios were elevated in MHCA gestations (Fig. 2A). We also determined elevation of mTh17:Treg ratios for mothers and in CB in gestations with either HCA condition vs relative controls (Fig. 2B). In paired analyses, pTh17:Treg ratios were not different between mothers and CB for a specific HCA condition (MHCA, $p = 0.07$; FHCA, $p = 0.55$), while maternal ratios were higher than CB ratios in controls (Fig. 2A). In contrast, mTh17:Treg ratios in mothers were higher than in the CB of their neonates for any gestational condition (Fig. 2B). In analyses that compared controls with combined MHCA and FHCA ratios (any HCA), maternal and CB ratios were higher in gestations with any HCA (MHCA + FHCA) for both Th17 subsets: pTh17 (maternal, $p = 0.01$, CB, $p = 0.02$); mTh17 (maternal, $p = 0.04$, CB, $p = 0.03$).

S100A8 and S100A12 Levels

As calgranulins and other S100 proteins have been linked to Th17-mediated inflammatory disorders (16, 17), we performed comparison studies of maternal and CB calgranulin levels in HCA or control gestations (Fig. 3). In gestations with FHCA, S100A8 levels in CB were increased three-fold relative to controls while maternal levels were doubled (Fig. 3A). In contrast, in MHCA gestations, S100A8 levels were not elevated for either mothers or CB. In studies of S100A12 expression, in FHCA gestations, CB levels were quadrupled relative



Figs 4A and B: The rh-S100A12 and Th17 propagation in term CB cultures. $CD4^+$ T cells isolated from banked CB were incubated for 72 hours in the presence of complete medium (CTCM) containing either S100A12 (1000 ng/mL) or Th17-propagating cocktail (Th17p), then analyzed by flow cytometry. Data represent the results of about four to six individual studies. *$p < 0.05$; **$p < 0.01$. (A) rh-S100A12 vs CTCM. rh-S100A12 induced greater propagation of $Tbet^+$ and $ROR\gamma t^+$ CD4 cells relative to culture with CTCM alone; (B) S100A12 vs Th17 propagating cocktail (Th17p). The induction of $CD4^+FoxP3^+$ cells by rh-S100A12 alone was only half of those determined in the presence of Th17p

to controls, while maternal S100A12 levels were nearly twice those of controls (Fig. 3B). In MHCA gestations, elevation of S100A12 levels were significantly elevated only for CB. Paired analyses showed similar expression levels of S100A12 between mothers and preterm CB for all gestational conditions (controls, $p = 0.21$; MHCA, $p = 0.16$; FHCA, $p = 0.81$). Paired CB comparisons of S100A12 levels and Th17 cell frequencies showed a correlation for pTh17 frequencies ($r = 0.62$, $p < 0.02$), with a relationship also observed for mTh17 cell frequencies ($r = 0.44$ $p < 0.02$).

rhS100A12 Promotes Th17 Cell Propagation in CB Cultures

The prominent CB S100A12 levels observed in association with fetal inflammation in this study led us to hypothesize that S100A12 might serve as a "transducer" between fetal neutrophil and Th17 responses. In preliminary *in vitro* studies, coculture of term CB $CD4^+$ cells with rhS100A12 resulted in a higher proportion of cell populations expressing the nuclear factor, $ROR\gamma t$ (a Th17 cell marker), as well as the Th1 nuclear factor, Tbet (Fig. 4A), compared to $CD4^+$ cells cultured in media alone. Cultures containing either rh-S100A12 alone or only a potent Th17-propagating cytokine cocktail induced $IL\text{-}17^+$ $CD4^+$ cells to a similar degree ($p = 0.31$) (Fig. 4B). However, the S100A12 effect appeared to be specific for Th17 cell induction, as increased Treg ($CD4^+$ $FoxP3^+$) cell frequencies were observed in cultures containing a Th17-propagating cocktail but not in the presence of S100A12 alone (Fig. 4B).

Discussion

The primary goal of the present study was to determine maternal Th17-type responses relative to those in the CB of their neonates in preterm gestations with HCA. We determined concurrently elevated circulating expression levels of Th17 cells in whole blood and the plasma Th17-associated calgranulins, S100A8 and S100A12, in both mothers and in preterm CB, particularly in the presence of fetal inflammation. To our knowledge, this is the first report describing these combined Th17-related responses in both mothers and in preterm CB in HCA gestations.

Our findings provide added evidence supporting the role of maternal–fetal crosstalk mechanisms and extend existing information regarding Th17 responses in pregnant women and in neonates with HCA gestations.[13,14,40] We observed elevations in circulating Th17 cell subset frequencies in both pregnant women in preterm labor and in the CB of their preterm neonates especially in gestations with fetal inflammation (FHCA). Notably, while elevated in both groups, mTh17 cell frequencies were higher in mothers relative to those in the CB of their neonates in gestations with fetal inflammation. This latter observation may reflect a specific maternal inflammatory response to fetal inflammation, as a strong association between elevated mTh17 cell responses and inflammatory status has been reported in other disorders.[41] Our results differ from a recent report showing higher maternal Th17 frequencies in term vs preterm gestations.[40] However, our results may reflect the focus of our study on the comparison of preterm gestations with a diagnosis of HCA vs those with absent placental or infectious pathology. That report also described elevated circulating maternal IL-6 levels in preterm gestations as a group, although whether maternal IL-6 levels correlated with placental inflammation was not clear (and fetal inflammation was not specifically identified). However, this finding is supportive of increased Th17-linked responses, as IL-6 is critical to the preferential propagation of Th17 cells over anti-inflammatory Treg cells.[42] In our studies of γδ T cells, an immune cell that is an important source of IL-17,[34] we found higher frequencies in mothers than in the CB of their neonates specifically in gestations with fetal inflammation. In light of an established association between Th17-type responses and inflammatory disorders,[25] these maternal Th17-type responses are consistent with the possibility that maternal exposure to fetal inflammation could set the stage for later maternal metabolic or cardiovascular disease.[43]

Enhanced Th17 responses have been ascribed to neuroinflammation in neonates,[9] and have been associated with the severity of chronic inflammation in adults.[39] Prominent circulating Th17 cell populations have also been observed in women with recurrent pregnancy loss and pre-eclampsia, gestational disorders also associated with inflammation.[44,45] Our present findings extend

previous observations of elevated Th17 responses in preterm neonates with HCA[13,14] and provide new evidence of a potential involvement of maternal Th17 cells in its pathogenesis.[46,47] In addition, the observed imbalances between Th17 cells and Tregs could enhance tissue Th17 responses that amplify the inflammatory cascade[8,48] in mothers, in neonates, or in both. Some data suggest that pathologic Th17 cells contribute to preterm labor through processes involving fetal immune activation against maternal antigens.[12,49] In addition, imbalances in Th17 and Treg cell expression levels (such as in this study and in other[13,14] studies) as well as Th17-calgranulin interactions have been observed in conjunction with immune rejection processes.[15,17] Notably, rejection has been identified as a potential mechanism associated with preterm birth[22,49] In addition to its possible role in preterm delivery, intrauterine Th17-mediated inflammation could "imprint" the immune system of the developing fetus, leading to altered postnatal responses.[50,51] Taken together, our present observations highlight a need for improved understanding of the roles of pathogenic Th17 processes and Th17 cell heterogeneity to the development of placental inflammation.[8] Such information could guide the development of novel prenatal therapeutic approaches,[52] for example, by targeting elements of the Th17 pathway.[53]

Our studies included analyses of circulating maternal and CB levels of the calgranulin proteins, S100A8 and S100A12. We found marked elevations of plasma calgranulin levels in mothers and especially in the CB of their preterm neonates in the context of fetal inflammation. Our findings are consistent with the increased S100A12 blood levels previously reported in a small subset of neonates born after HCA,[21] as well as recently described neonatal monocyte and blood expression levels of S100 proteins in chorioamnionitis.[20] While the role of calgranulins in the pathogenesis of fetal inflammation has not been discerned, evidence suggests a link between inflammatory neutrophils, a driving force in this disease process[54,55] and their contribution to circulating S100 proteins.[56] Pertinently, neutrophils can promote Th17 cell propagation and function,[33,57] a process that may involve neutrophil-derived calgranulin mediators (in this study and in other[58,59] studies). Conversely, calgranulins modulate inflammatory responses by promoting neutrophil production and activation.[60,61] Additionally, crosstalk between neutrophils and Th17 cells can amplify the inflammatory cascade.[62] Our finding that rhS100A12 also promoted the expression of Tbet, a canonical nuclear transcription factor for Th1 cells,[63] is consistent with the Th1 polarization bias observed in exometabolomic studies of HCA-exposed preterm neonates.[64] Our preliminary observations in the context of existing data hint at feed-forward mechanisms involving interactions between neutrophils, calgranulins, and Th17 cells in the pathophysiology of fetal inflammation. However, our findings are based on *in vitro* studies that targeted healthy CB $CD4^+$ cells of term gestations. Future studies involving preterm CB cells and *in vivo* models may provide important clues to understanding whether these "pieces fit the puzzle" of mechanisms that drive fetal inflammation and preterm birth and as well as postpartum maternal inflammatory disease.[65]

Our observations suggest an attributable risk of HCA in association with elevated Th17 cell frequencies and calgranulin levels, despite the limited size of our study sample. Our findings also support the potential utility of calgranulin levels in identifying clinically "silent" fetal inflammation, an important task given its association with preterm delivery and adverse neonatal outcomes.[20,66] Such information could also facilitate anticipatory clinical management of HCA and guide effective postpartum and postnatal interventions.[52,67,68] However, studies in much larger, diverse populations are clearly warranted to define the use of calgranulins in this context.

A major strength of this study lies in its prospective design with stringent inclusion and exclusion criteria that minimize the presence of confounding factors associated with other perinatal inflammatory disorders.[69] Thus, despite our limited sample size, the significant intergroup differences in Th17 responses that we now report in maternal-CB dyads with fetal inflammation support the biological relevance of our findings. However, our study was neither powered to identify infectious etiologies nor to specifically correlate our findings with adverse maternal or neonatal outcomes.

In summary, the enhanced Th17-linked response patterns observed in pregnant women parallel those in their preterm neonates especially in the context of fetal inflammation. These findings provide compelling supportive evidence of maternal–fetal crosstalk mechanisms that may influence gestational inflammatory or immune processes.[27] While our understanding of perinatal Th17 responses in HCA continues to evolve, their contributions to maternal and neonatal health and disease remain critical knowledge gaps that will benefit from continued investigations in this area.

Acknowledgments

This work was presented in part at annual meetings of the Pediatric Academic Societies (5–8 May 2018, Toronto, Canada; 24 July 2020; Summer Webinar Series), and the Society for Maternal–Fetal Medicine (6–8 February 2020, Grapevine, Texas, USA). We thank the Labor and Delivery and Neonatal Intensive Care Unit medical staff for their helpful assistance with the success of this project. We thank Dr. Gilad Gross for useful discussions during the course of these studies. We very much appreciate Dr. David Carlton's insightful and thoughtful review of the manuscript.

Author Contributions

Authors CQB and MLL contributed equally to this work.

References

1. Martin JA, Hamilton BE, Osterman MJ. Births in the United States, 2021. NCHS Data Brief 2022;(442):1–8. PMID: 36043891
2. Harrison MS, Goldenberg RL. Global burden of prematurity. Semin Fetal Neonatal Med 2016;21(2):74–79. DOI: 10.1016/j.siny.2015.12.007.
3. Redline RW. Inflammatory response in acute chorioamnionitis. Semin Fetal Neonatal Med 2012;17(1):20–25. DOI: 10.1016/j.siny.2011.08.003.
4. Jung E, Romero R, Yeo L, et al. The fetal inflammatory response syndrome: The origins of a concept, pathophysiology, diagnosis, and obstetrical implications. Semin Fetal Neonatal Med 2020;25(4):101146. DOI: 10.1016/j.siny.2020.101146.
5. Guzick DS, Winn K. The association of chorioamnionitis with preterm delivery. Obstet Gynecol 1985;65(1):11–16. PMID: 3966012.
6. Queiros dMV, Prodhom G, Yan P, et al. Correlation between placental bacterial culture results and histological chorioamnionitis: A prospective study on 376 placentas. J Clin Pathol 2013;66(3):243–248. DOI: 10.1136/jclinpath-2012-201124.
7. Crome SQ, Clive B, Wang AY, et al. Inflammatory effects of *ex vivo* human Th17 cells are suppressed by regulatory T cells. J Immunol 2010;185(6):3199–3208. DOI: 10.4049/jimmunol.1000557.

8. Schnell A, Littman DR, Kuchroo VK. T(H)17 cell heterogeneity and its role in tissue inflammation. Nat Immunol 2023;24(1):19–29. DOI: 10.1038/s41590-022-01387-9.
9. Lawrence SM, Wynn JL. Chorioamnionitis, IL-17A, and fetal origins of neurologic disease. Am J Reprod Immunol 2018;79(5):e12803.
10. Nakashima A, Ito M, Yoneda S, Shiozaki A, Hidaka T, Saito S. Circulating and decidual Th17 cell levels in healthy pregnancy. Am J Reprod Immunol. 2010;63(2):104-9.
11. Lawrence SM, Ruoss JL, Wynn JL. IL-17 in neonatal health and disease. Am J Reprod Immunol. 2018;79(5):e12800. DOI: 10.1111/aji.12803.
12. Ito M, Nakashima A, Hidaka T, S, et al. A role for IL-17 in induction of an inflammation at the fetomaternal interface in preterm labour. J Reprod Immunol 2010;84(1):75–85. DOI: 10.1016/j.jri.2009.09.005.
13. Rito DC, Viehl LT, Buchanan PM, et al. Augmented Th17-type immune responses in preterm neonates exposed to histologic chorioamnionitis. Pediatr Res 2017;81(4):639–645. DOI: 10.1038/pr.2016.254.
14. Jackson CM, Wells CB, Tabangin ME, et al. Pro-inflammatory immune responses in leukocytes of premature infants exposed to maternal chorioamnionitis or funisitis. Pediatr Res 2017;81(2):384–390. DOI: 10.1038/pr.2016.232.
15. Eghbal–Fard S, Yousefi M, Heydarlou H, et al. The imbalance of Th17/Treg axis involved in the pathogenesis of preeclampsia. J Cell Physiol 2019;234(4):5106–5116. DOI: 10.1002/jcp.27315.
16. Lee DG, Woo JW, Kwok SK, et al. MRP8 promotes Th17 differentiation *via* upregulation of IL-6 production by fibroblast-like synoviocytes in rheumatoid arthritis. Exp Mol Med 2013;45(4):e20. DOI: 10.1038/emm.2013.39.
17. Reinhardt K, Foell D, Vogl T, et al. Monocyte-induced development of Th17 cells and the release of S100 proteins are involved in the pathogenesis of graft-versus-host disease. J Immunol 2014;193(7):3355–3365. DOI: 10.4049/jimmunol.1400983.
18. Buhimschi CS, Bhandari V, Han YW, et al. Using proteomics in perinatal and neonatal sepsis: Hopes and challenges for the future. Curr Opin Infect Dis 2009;22(3):235–443. DOI: 10.1097/QCO.0b013e32832a5963.
19. Phillips RJ, Fortier MA, Lopez BA. Prostaglandin pathway gene expression in human placenta, amnion and choriodecidua is differentially affected by preterm and term labour and by uterine inflammation. BMC Pregnancy Childbirth 2014;14:241. DOI: 10.1186/1471-2393-14-241.
20. Golubinskaya V, Puttonen H, Fyhr IM, et al. Expression of S100A alarmins in cord blood monocytes is highly associated with chorioamnionitis and fetal inflammation in preterm infants. Front Immunol 2020;11:1194. DOI: 10.3389/fimmu.2020.01194.
21. Bersani I, De CS, Foell D, et al. Impact of chorioamnionitis on maternal and fetal levels of proinflammatory S100A12. Eur J Pediatr 2021;180(1):39–45. DOI: 10.1007/s00431-020-03695-4.
22. Green ES, Arck PC. Pathogenesis of preterm birth: Bidirectional inflammation in mother and fetus. Semin Immunopathol 2020;42(4):413–429. DOI: 10.1007/s00281-020-00807-y.
23. Kessous R, Shoham–Vardi I, Pariente G, et al. An association between preterm delivery and long-term maternal cardiovascular morbidity. Am J Obstet Gynecol 2013;209(4):368.e1-8. DOI: 10.1016/j.ajog.2013.05.041.
24. Wu P, Gulati M, Kwok CS, S, et al. Preterm delivery and future risk of maternal cardiovascular disease: A systematic review and meta-analysis. J Am Heart Assoc 2018;7(2):e007809. DOI: 10.1161/JAHA.117.007809.
25. von Stebut E, Boehncke WH, Ghoreschi K, et al. IL-17A in psoriasis and beyond: Cardiovascular and metabolic implications. Front Immunol 2019;10:3096. DOI: 10.3389/fimmu.2019.03096.
26. Khong TY, Mooney EE, Ariel I, et al. Sampling and definitions of placental lesions: Amsterdam Placental Workshop Group Consensus Statement. Arch Pathol Lab Med 2016;140(7):698–713. DOI: 10.5858/arpa.2015-0225-CC.
27. Kim CJ, Romero R, Chaemsaithong P, et al. Chronic inflammation of the placenta: definition, classification, pathogenesis, and clinical significance. Am J Obstet Gynecol 2015;213(Suppl. 4):S53–S69. DOI: 10.1016/j.ajog.2015.08.041.
28. Higgins RD, Saade G, Polin RA, et al. Evaluation and management of women and newborns with a maternal diagnosis of chorioamnionitis: Summary of a workshop. Obstet Gynecol 2016;127(3):426–436. DOI: 10.1097/AOG.0000000000001246.
29. Committee on Obstetric P. ACOG Committee Opinion No. 712: Intrapartum management of intraamniotic infection. Obstet Gynecol 2017;130(2):e95-e101. DOI: 10.1097/AOG.0000000000002236.
30. Cosmi L, De PR, Santarlasci V, et al. Human interleukin 17-producing cells originate from a $CD161^{+}CD4^{+}$ T cell precursor. J Exp Med 2008;205(8):1903–916. DOI: 10.1084/jem.20080397.
31. Liu W, Putnam AL, Xu–Yu Z, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human $CD4^{+}$ T reg cells. J Exp Med 2006;203(7):1701–1711. DOI: 10.1084/jem.20060772.
32. Borst J, van Dongen JJ, Bolhuis RL, et al. Distinct molecular forms of human T cell receptor gamma/delta detected on viable T cells by a monoclonal antibody. J Exp Med 1988;167(5):1625–1644. DOI: 10.1084/jem.167.5.1625.
33. Lin J, Haridas S, Barenkamp SJ, et al. Neonatal neutrophils stimulated by group B Streptococcus induce a proinflammatory T-helper cell bias. Pediatr Res 2018;83(3):739–746. DOI: 10.1038/pr.2017.272.
34. Maggi L, Santarlasci V, Capone M, et al. CD161 is a marker of all human IL-17-producing T-cell subsets and is induced by RORC. Eur J Immunol 2010;40(8):2174–2181. DOI: 10.1002/eji.200940257.
35. Basdeo SA, Moran B, Cluxton D, et al. Polyfunctional, pathogenic $CD161^{+}$ Th17 lineage cells are resistant to regulatory T cell-mediated suppression in the context of autoimmunity. J Immunol 2015;195(2):528–540. DOI: 10.4049/jimmunol.1402990.
36. Ramesh R, Kozhaya L, McKevitt K, et al. Pro-inflammatory human Th17 cells selectively express P-glycoprotein and are refractory to glucocorticoids. J Exp Med 2014;211(1):89–104. DOI: 10.1084/jem.20130301.
37. Mai J, Wang H, Yang XF. Th 17 cells interplay with $Foxp3^{+}$ Tregs in regulation of inflammation and autoimmunity. Front Biosci 2010;15:986–1006. DOI: 10.2741/3657.
38. Hassane M, Demon D, Soulard D, et al. Neutrophilic NLRP3 inflammasome-dependent IL-1 beta secretion regulates the gamma-delta T17 cell response in respiratory bacterial infections. Mucosal Immunol 2017;10(4):1056–1068. DOI: 10.1038/mi.2016.113.
39. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. Autoimmun Rev 2014;13(6):668–677. DOI: 10.1016/j.autrev.2013.12.004.
40. Couture C, Brien ME, Boufaied I, et al. Proinflammatory changes in the maternal circulation, maternal–fetal interface, and placental transcriptome in preterm birth. Am J Obstet Gynecol 2023;228(3):332.e1–332.e17. DOI: 10.1016/j.ajog.2022.08.035.
41. Kagami S, Rizzo HL, Lee JJ, et al. Circulating Th17, Th22, and Th1 cells are increased in psoriasis. JInvest Dermatol 2010;130(5):1373–1383. DOI: 10.1038/jid.2009.399.
42. Kimura A, Kishimoto T. IL-6: regulator of Treg/Th17 balance. Eur J Immunol 2010;40(7):1830–1835. DOI: 10.1002/eji.201040391.
43. Lane–Cordova AD, Khan SS, Grobman WA, et al. Long-term cardiovascular risks associated with adverse pregnancy outcomes: JACC Review Topic of the Week. J Am Coll Cardiol 2019;73(16):2106–2116. DOI: 10.1016/j.jacc.2018.12.092.
44. Lee SK, Kim JY, Lee M, et al. Th17 and regulatory T cells in women with recurrent pregnancy loss. Am J Reprod Immunol 2012;67(4):311–318. DOI: 10.1111/j.1600-0897.2012.01116.x.
45. Zolfaghari MA, Arefnezhad R, Parhizkar F, et al. T lymphocytes and preeclampsia: The potential role of T-cell subsets and related MicroRNAs in the pathogenesis of preeclampsia. Am J Reprod Immunol 2021;86(5):e13475. DOI: 10.1111/aji.13475.
46. Saito S, Nakashima A, Ito M, et al. Clinical implication of recent advances in our understanding of IL-17 and reproductive immunology. Expert Rev Clin Immunol 2011;7(5):649–657. DOI: 10.1586/eci.11.49.

47. Fedorka CE, El-Sheikh Ali H, Walker OF, et al. The imbalance of the Th17/Treg axis following equine ascending placental infection. J Reprod Immunol 2021;144:103268. DOI: 10.1016/j.jri.2020.103268.
48. Yang WY, Shao Y, Lopez–Pastrana J, et al. Pathological conditions re-shape physiological Tregs into pathological Tregs. Burns Trauma 2015;3(1):1. DOI: 10.1186/s41038-015-0001-0.
49. Frascoli M, Coniglio L, Witt R, et al. Alloreactive fetal T cells promote uterine contractility in preterm labor *via* IFN-gamma and TNF-alpha. Sci Transl Med 2018;10(438):eaan2263. DOI: 10.1126/scitranslmed.aan2263.
50. Lim AI, McFadden T, Link VM, et al. Prenatal maternal infection promotes tissue-specific immunity and inflammation in offspring. Science 2021;373(6558):eabf3002. DOI: 10.1126/science.abf3002.
51. Sabic D, Koenig JM. A perfect storm: Fetal inflammation and the developing immune system. Pediatr Res 2020;87(2):319–326. DOI: 10.1038/s41390-019-0582-6.
52. Xiong Y, Wintermark P. Therapeutic interventions for fetal inflammatory response syndrome (FIRS). Semin Fetal Neonatal Med 2020;25(4):101112. DOI: 10.1016/j.siny.2020.101112.
53. Cosmi L, Santarlasci V, Maggi L, et al. Th17 plasticity: Pathophysiology and treatment of chronic inflammatory disorders. Curr Opin Pharmacol 2014;17:12–6. DOI: 10.1016/j.coph.2014.06.004.
54. Singareddy A, Lee ASE, Sweeney PL, et al. Elevated neutrophil–lymphocyte ratios in extremely preterm neonates with histologic chorioamnionitis. J Perinatol 2021;41(6):1269–1277. DOI: 10.1038/s41372-021-00964-4.
55. Chaiworapongsa T, Romero R, Berry SM, et al. The role of granulocyte colony-stimulating factor in the neutrophilia observed in the fetal inflammatory response syndrome. J Perinat Med 2011;39(6):653–666. DOI: 10.1515/jpm.2011.072.
56. Foell D, Wittkowski H, Vogl T, et al. S100 proteins expressed in phagocytes: A novel group of damage-associated molecular pattern molecules. J Leukoc Biol 2007;81(1):28–37. DOI: 10.1189/jlb.0306170.
57. Parackova Z, Bloomfield M, Klocperk A, et al. Neutrophils mediate Th17 promotion in COVID-19 patients. J Leukoc Biol 2021;109(1):73–76. DOI: 10.1002/JLB.4COVCRA0820-481RRR.
58. Chellan B, Yan L, Sontag TJ, et al. IL-22 is induced by S100/calgranulin and impairs cholesterol efflux in macrophages by downregulating ABCG1. J Lipid Res 2014;55(3):443–454. DOI: 10.1194/jlr.M044305.
59. Wu DM, Wang S, Shen M, et al. S100A9 gene silencing inhibits the release of pro-inflammatory cytokines by blocking the IL-17 signalling pathway in mice with acute pancreatitis. J Cell Mol Med 2018;22(4):2378–2389. DOI: 10.1111/jcmm.13532.
60. Sreejit G, Abdel–Latif A, Athmanathan B, et al. Neutrophil-derived S100A8/A9 amplify granulopoiesis after myocardial infarction. Circulation 2020;141(13):1080–1094. DOI: 10.1161/CIRCULATIONAHA.119.043833.
61. Li SC, Tsai KW, Huang LH, et al. Serum proteins may facilitate the identification of Kawasaki disease and promote *in vitro* neutrophil infiltration. Sci Rep 2020;10(1):15645. DOI: 10.1038/s41598-020-72695-z.
62. Pelletier M, Maggi L, Micheletti A, et al. Evidence for a cross-talk between human neutrophils and Th17 cells. Blood 2010;115(2):335–343. DOI: 10.1182/blood-2009-04-216085.
63. Szabo SJ, Kim ST, Costa GL, et al. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 2000;100(6):655–669. DOI: 10.1016/s0092-8674(00)80702-3.
64. Matta P, Sherrod SD, Marasco CC, et al. *In utero* exposure to histological chorioamnionitis primes the exometabolomic profiles of preterm $CD4^+$ T lymphocytes. J Immunol 2017;199(9):3074–3085. DOI: 10.4049/jimmunol.1601880.
65. Tyden H, Lood C, Gullstrand B, et al. Increased serum levels of S100A8/A9 and S100A12 are associated with cardiovascular disease in patients with inactive systemic lupus erythematosus. Rheumatology(Oxford) 2013;52(11):2048–2055. DOI: 10.1093/rheumatology/ket263.
66. Fanaroff JM, Donn SM. Medico–legal implications of the fetal inflammatory response syndrome. Semin Fetal Neonatal Med 2020;25(4):101127. DOI: 10.1016/j.siny.2020.101127.
67. Oesterle A, Bowman MA. S100A12 and the S100/calgranulins: Emerging biomarkers for atherosclerosis and possibly therapeutic targets. Arterioscler Thromb Vasc Biol 2015;35(12):2496–2507. DOI: 10.1161/ATVBAHA.115.302072
68. Yang D, Sun YY, Bhaumik SK, et al. Blocking lymphocyte trafficking with FTY720 prevents inflammation-sensitized hypoxic–ischemic brain injury in newborns. J Neurosci 2014;34(49):16467–16481. DOI: 10.1523/JNEUROSCI.2582-14.2014.
69. Faupel–Badger JM, Fichorova RN, Allred EN, et al. Cluster analysis of placental inflammatory proteins can distinguish preeclampsia from preterm labor and premature membrane rupture in singleton deliveries less than 28 weeks of gestation. Am J Reprod Immunol 2011;66(6):488–494. DOI: 10.1111/j.1600-0897.2011.01023.x.