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ORIGINAL RESEARCH ARTICLE

Pregnancy tailors endotoxin-induced monocyte and neutrophil responses in the maternal circulation

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Abstract

Objective To comprehensively characterize monocyte and neutrophil responses to *E. coli* and its product [lipopolysaccharide (LPS) or endotoxin] in vitro during pregnancy.

Material or subjects Peripheral blood was collected from pregnant women during the third trimester ($n = 20$) and from non-pregnant women ($n = 20$).

Methods The number, phagocytic activity, and reactive oxygen species (ROS) production of peripheral monocytes and neutrophils were investigated using flow cytometry. The phenotypes of peripheral monocytes and neutrophils after acute or chronic LPS stimulation were also determined using flow cytometry. Cytokine profiles were quantified for LPS-stimulated peripheral blood mononuclear cells (PBMCs) and a whole blood TruCulture® system using a multiplex immunoassay.

Results Increased number, phagocytic activity, and ROS production capacity of monocytes and neutrophils were found in pregnant compared to non-pregnant women. Additionally, specific subsets of pro-inflammatory monocytes (IL-6$^+$CD14$^+$ or MIP-1α$^+$CD14$^+$ cells) and neutrophils (IL-1β$^+$CD15$^+$ or MIP-1β$^+$CD15$^+$ cells) were increased in pregnant women in response to acute LPS stimulation. Moreover, distinct subsets of intermediate-activated monocytes expressing CD142, IL-6, and IL-1RA were increased in pregnant women upon chronic LPS stimulation. Last, pregnant women displayed a different cytokine profile than non-pregnant women in LPS-stimulated PBMCs and in whole blood.

Conclusions Pregnancy tailors the immune responses of circulating monocytes and neutrophils to endotoxin, a Gram-negative bacterial product.

Keywords Chorioamnionitis · Cytokine · Funisitis · Infection · Inflammation · Phagocytosis

Introduction

Infection during pregnancy is one of the leading causes of maternal mortality and morbidity, accounting for approximately 11–13% of all deaths [1]. The main sources of life-threatening infections in pregnancy are the genital, urinary, and respiratory tracts [2], with the Gram-negative bacterium *Escherichia coli* (*E. coli*) being the most commonly identified microorganism in severe obstetrical infections [2]. The increased susceptibility to some bacterial infections and risk of complications in pregnancy are attributed to maternal physiological and immunological adaptations [3]. If such changes are maladaptive in the third trimester [3], they can manifest as one or more of the "great obstetrical syndromes" (i.e., preeclampsia, fetal growth restriction, and preterm birth) [4–8]. Importantly, a potential consequence of maternal infection in late gestation is spontaneous or indicated (i.e., iatrogenic) preterm birth [9], the leading cause of perinatal morbidity and mortality worldwide [7, 8]. Thus, infections during pregnancy have devastating effects for the mother and the fetus, highlighting the need for a comprehensive understanding of the host immune response during this vulnerable period.

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Pregnancy was traditionally classified as a state of immunosuppression [10]. Yet, in recent decades, this concept has been demonstrated to be erroneous [11, 12], given that maternal immunological adaptations are tightly regulated to allow a critical balance between tolerance of the fetal semi-allograft and defense against infection [11, 13–18]. The former is largely governed by specialized subsets of T cells [19–26] and macrophages [27–30], whereas the latter is orchestrated by the infantry of the immune system (e.g., neutrophils and pro-inflammatory monocytes/macrophages) [29, 31–36]. Neutrophils possess an arsenal of weapons tailored for the killing of invading pathogens, namely degranulation, phagocytosis, reactive oxygen species (ROS) production, cytokine release, and the formation of neutrophil extracellular traps (NETs) [37]. On the other hand, monocytes serve as a major source of pro-inflammatory cytokines and nitric oxide (NO), and represent the primary type of mononuclear phagocyte in the blood [38]. During pregnancy, such innate immune cells increase in number [11, 39] and display an activated phenotype [40–43]. Indeed, the enhanced functionality of neutrophils and monocytes during pregnancy has been compared to that observed in women with sepsis [11]. Recent omics studies have revealed that neutrophil functionality is enhanced in pregnancy [44], which contributes to the immune responsiveness observed in preparation for the onset of labor [45]. However, a deep characterization of the phenotypes and function of circulating monocytes and neutrophils of women in late gestation, compared to non-pregnant women, is still lacking.

In the current study, we performed a comprehensive ex vivo characterization of monocyte and neutrophil responses to a Gram-negative bacterium (*E. coli*) and its product (lipopolysaccharide, LPS) in pregnant and non-pregnant women. We first investigated differences in the baseline phagocytic activity and ROS production by monocytes and neutrophils. Subsequently, conventional monocyte subsets (classical, intermediate, non-classical, and $CD14^{lo}CD16^{-}$) and neutrophils were characterized using flow cytometry. Last, cytokine expression profiles were generated for LPS-stimulated peripheral blood mononuclear cells (PBMCs) and whole-blood samples from pregnant and non-pregnant women. Collectively, this study provides an extensive analysis of pregnancy-induced changes in the response of monocytes and neutrophils to endotoxin, a Gram-negative bacterial product.

Methods

Human subjects, clinical specimens, and definitions

Peripheral blood samples were obtained from eligible healthy pregnant and non-pregnant women recruited by the Perinatology Research Branch, an intramural program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), U.S. Department of Health and Human Services, Wayne State University (Detroit, MI, USA), and the Detroit Medical Center (Detroit, MI, USA). All women provided written informed consent prior to blood collection. The collection and use of biological specimens for research purposes were approved by the Institutional Review Boards of Wayne State University and the Detroit Medical Center. Peripheral blood was collected from pregnant women ($n = 20$) during the third trimester, prior to the administration of any medication. The non-pregnant control group ($n = 20$) was comprised of healthy women of reproductive age.

Phagocytic activity of monocytes and neutrophils

Peripheral blood was obtained by venipuncture, collected into tubes containing heparin (BD, Franklin Lakes, NJ, USA; cat# 367878), and placed on ice for 10 min prior to use. The pHrodo™ Green *Escherichia coli* (*E. coli*) BioParticles™ Phagocytosis Kit (Thermo Fisher Scientific, Life Technologies Limited, Paisley, UK; cat# P35381) was used to assay the phagocytic activity of monocytes and neutrophils. The pH-sensitive pHrodo™ Green *E. coli* BioParticles™ Conjugate was prepared according to manufacturer instructions. Two tubes containing 100 µL of whole blood and 20 µL of pHrodo™ BioParticles™ conjugate were prepared for each experimental sample. One tube was placed on ice, while the other was incubated in a 37 °C water bath for 15 min. Following incubation, the heated tubes were immediately placed on ice. Next, the samples were incubated with Lysis Buffer A at room temperature (RT) for 5 min, then this step was repeated using Lysis Buffer B, per manufacturer instructions. The samples were centrifuged at 350×*g* for 5 min and the resulting cell pellets were washed twice using wash buffer, after which the cells were resuspended in 100 µL of FACS Stain Buffer (BD Biosciences, San Jose, CA, USA; cat# 554656). Fluorochrome-conjugated anti-human monoclonal antibodies CD14-BUV395 and CD15-BV605 (BD Biosciences; Supplementary Table 1) were added to the cells and incubated in the dark at 4 °C for 30 min. Following incubation, the cells were washed in FACS Stain Buffer, then centrifuged at 350×*g* for 5 min and resuspended in 0.5 mL FACS Stain Buffer for analysis on a BD LSRFortessa flow cytometer (BD Biosciences) using FACSDiva software version 7.0 (BD Biosciences). FlowJo software version 10 (FlowJo, Ashland, OR, USA) was used to perform data analysis and to create figures. Phagocytic activity was defined as the percentage of phagocytic cells at 37 °C minus the percentage of phagocytic cells at 4 °C. The absolute number of neutrophils and monocytes were determined using CountBright absolute counting beads (Thermo Fisher Scientific/Molecular Probes, Eugene, OR, USA; cat# C36950).

Production of reactive oxygen species by monocytes and neutrophils

Peripheral blood samples were obtained by venipuncture and collected into EDTA tubes (BD; cat# 366450). A 50 µL aliquot of whole blood was stimulated with an equal volume of ROS Assay Mix containing a 1:250 dilution of ROS Assay Stain into ROS Assay Buffer (Total ROS Assay Kit 520 nm; Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA; cat# 88-5930-74). Incubation proceeded at 37 °C with 5% CO_2 for 60 min before erythrocytes were lysed by the addition of Ammonium–Chloride–Potassium (ACK) lysing buffer (Fisher Scientific; cat# A1049201). Following erythrocyte lysis, the remaining leukocytes were centrifuged at 300×*g* for 5 min and resuspended in 0.5 mL of 1X PBS. Finally, ROS production by monocytes and neutrophils was detected using a BD LSRFortessa flow cytometer and FACSDiva software version 7.0. FlowJo software version 10 was used to perform data analysis and to create figures.

Stimulation of polymorphonuclear leukocytes and peripheral blood mononuclear cells with LPS

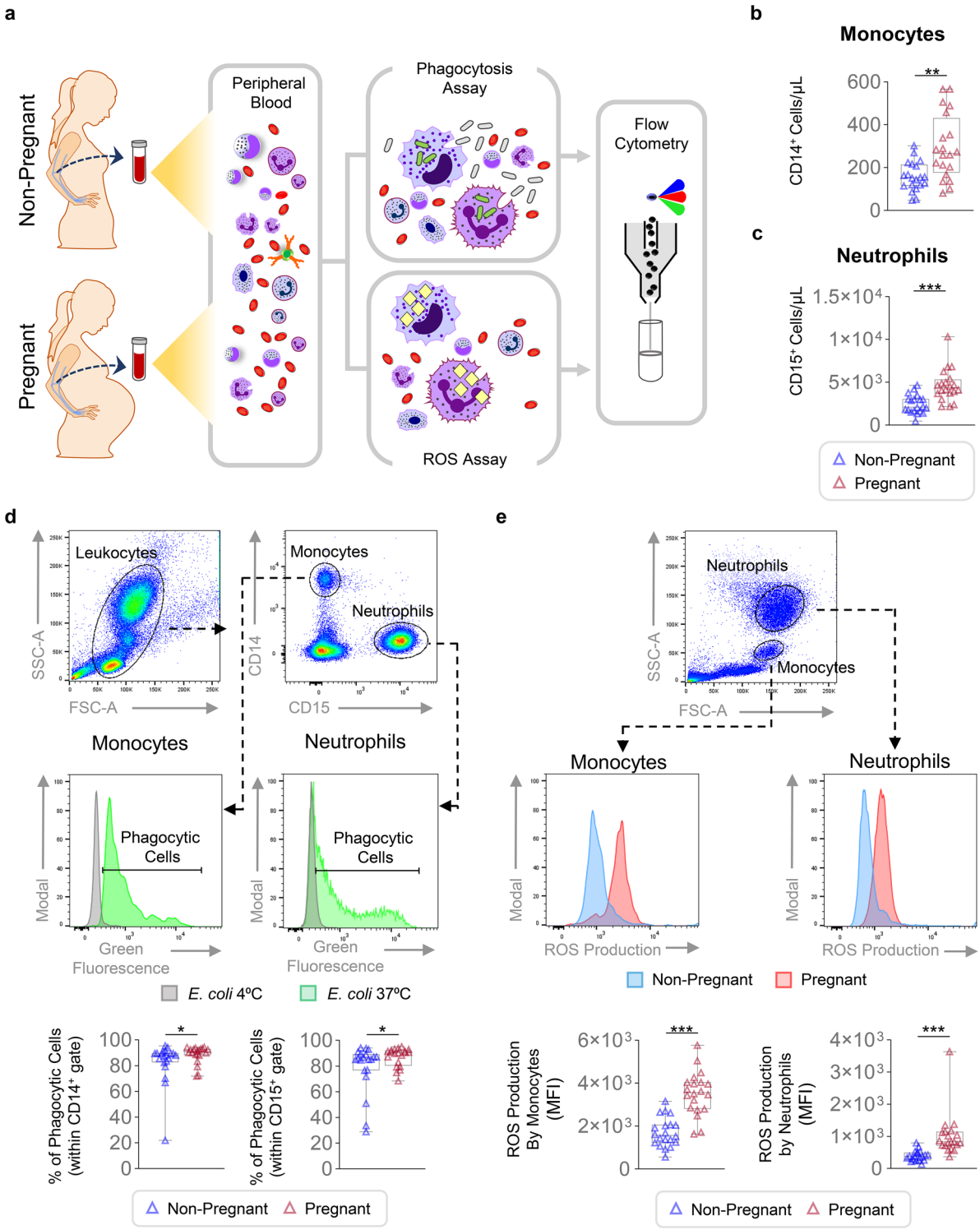
Peripheral blood samples were obtained by venipuncture and collected into EDTA tubes. Polymorphonuclear leukocytes (PMNs) were isolated using the Polymorphprep density gradient (Cosmo Bio USA, Inc., Carlsbad, CA, USA; cat# AXS1114683), per the manufacturer's instructions. The leukocytes isolated using this gradient, while primarily PMNs, also included a portion of monocytes ($CD14^+$ cells). PBMCs were isolated using the Lymphoprep density gradient (Stemcell Technologies Inc., Vancouver, Canada; cat# 07801), per manufacturer instructions. All isolated leukocytes (PMNs or PBMCs) were cultivated in complete RPMI 1640 Medium (Thermo Fisher Scientific; cat# 11-875-093) enriched with 5% human serum (Sigma-Aldrich, St Louis, MO, USA; cat# H3667) and 1% penicillin–streptomycin (Thermo Fisher Scientific; cat# 15-140-122). The cells were plated into cell culture plates at a density of 1×10^6 cells/mL prior to treatment. For acute LPS stimulation, PMNs were incubated at 37 °C with 5% CO_2 for 4 h with ultrapure lipopolysaccharide from *E. coli* K12 (LPS; 100 ng/mL; InvivoGen, San Diego, CA, USA; cat# tlrl-peklps) or an equivalent volume of 1X PBS for stimulated and control samples, respectively, in the presence of a protein transport inhibitor cocktail (Thermo Fisher Scientific; cat# 00-4980-93). For chronic LPS stimulation, PBMCs were incubated with 100 ng/mL LPS at 37 °C with 5% CO_2 for 24 h with the addition of the protein transport inhibitor cocktail for the last 4 h of incubation. Following incubation, the isolated cells (PMNs or PBMCs) were gently collected using a cell scraper and centrifuged at 300×*g* and 4 °C for 5 min. The resulting PMN pellets were immediately processed for immunophenotyping. Finally, for PBMCs, the resulting cell supernatants were stored at −80 °C prior to cytokine profiling, while the cell pellets were immediately processed for immunophenotyping.

Immunophenotyping of polymorphonuclear leukocytes and peripheral blood mononuclear cells

The collected PMN or PBMC pellets were washed and resuspended in 1X PBS, then incubated with 1 µL/mL of Fixable Viability Stain 510 (BD Biosciences; cat# 564406) in the dark at RT for 15 min. Next, cells were washed and resuspended in FACS Stain Buffer. Extracellular anti-human monoclonal antibodies (Supplementary Table 1) were added to the cell suspensions, which were incubated in the dark at 4 °C for 30 min. Cells were then fixed and permeabilized using the BD Cytofix/Cytoperm Fixation/Permeabilization Solution Kit (BD Biosciences; cat# 554714), according to the manufacturer's instructions. Following permeabilization, intracellular anti-human monoclonal antibodies (Supplementary Table 1) were added to cell suspensions, which were incubated in the dark at 4 °C for 30 min. Finally, the cells were washed and resuspended in 0.5 mL of FACS Stain Buffer and acquired using the BD LSRFortessa flow cytometer with FACSDiva 6.0 software. FlowJo software version 10 was used to perform data analysis and to create figures. Monocytes and neutrophils were identified as $CD14^+$ and $CD15^+$ cells, respectively. Monocyte subsets were classified as follows: classical monocytes ($CD14^{hi}CD16^-$ cells), intermediate monocytes ($CD14^{hi}CD16^+$ cells), non-classical monocytes ($CD14^{lo}CD16^+$ cells), and $CD14^{lo}CD16^-$ monocytes. Additional markers (Supplementary Table 1) were used to further phenotype cells within the identified subsets.

Whole blood stimulation with LPS using a TruCulture® system

Peripheral blood samples were obtained by venipuncture and collected into tubes containing heparin. A 1.0 mL aliquot of whole blood was added directly to a TruCulture® tube (Myriad RBM, Inc., Austin, TX, USA) containing LPS (part# 782-001-087) or control (part# 782-001-086) [46]. The tubes were inverted several times for mixing and then incubated in a heating block at 37 °C for 48 h. Following incubation, cell supernatants were collected and stored at −80 °C until cytokine profiling.



Cytokine profiling of LPS-stimulated peripheral blood mononuclear cell and TruCulture® supernatants

PBMCs were isolated and cultured, and the resulting cell supernatants were collected as previously described. Separately, supernatants were obtained from LPS-stimulated whole blood in TruCulture® tubes, as detailed above. To determine the concentration of various cytokines and chemokines, the V-PLEX Proinflammatory Panel 1 (human) was used (Meso Scale Discovery, Rockville, MD, USA; cat# K15049D-1), per the manufacturer's instructions. The

◂**Fig. 1** Phagocytic activity and ROS production of monocytes and neutrophils. **a** Peripheral blood was obtained from pregnant ($n=20$) and non-pregnant ($n=20$) women to determine the phagocytic activity and ROS production of monocytes and neutrophils by flow cytometry. **b** Number of monocytes ($CD14^+$ cells) per µL in the peripheral blood of pregnant (red triangles) and non-pregnant (blue triangles) women. **c** Number of neutrophils ($CD15^+$ cells) per µL in the peripheral blood of pregnant (red triangles) and non-pregnant (blue triangles) women. **d** (Top) Flow cytometry gating strategy used to identify monocytes and neutrophils in peripheral blood samples. (Middle) Representative histograms depicting the increase in phagocytic monocytes and neutrophils (green histograms) upon incubation with fluorescently labeled *E. coli*. (Bottom) The frequencies of phagocytic monocytes and neutrophils in pregnant and non-pregnant peripheral blood samples. **e** (Top) Flow cytometry gating strategy used to identify monocytes and neutrophils in peripheral blood samples. (Middle) Representative histograms depicting basal ROS production by monocytes and neutrophils from pregnant (red histograms) and non-pregnant (blue histograms) women. (Bottom) Comparison of ROS production, represented as mean fluorescence intensity (MFI), by monocytes and neutrophils between pregnant and non-pregnant women. ROS: reactive oxygen species. $^*p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$

following immune mediators were assayed: IFNγ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF. A MESO QuickPlex SQ 120 was used to read the plates, and cytokine concentrations were calculated using Discovery Workbench software version 4.0 (Meso Scale Discovery). The range of the lower limit of detection for each assay is as follows: 0.21–0.62 pg/mL (IFNγ), 0.01–0.17 pg/mL (IL-1β), 0.01–0.29 pg/mL (IL-2), 0.01–0.03 pg/mL (IL-4), 0.05–0.09 pg/mL (IL-6), 0.03–0.14 pg/mL (IL-8), 0.02–0.08 pg/mL (IL-10), 0.02–0.89 pg/mL (IL-12p70), 0.03–0.73 pg/mL (IL-13), and 0.01–0.13 pg/mL (TNF).

Statistical analyses

The R statistical programming language was used to perform all statistical analyses. Linear mixed effects models were fit to compare flow cytometry data and cytokine concentrations between groups to account for repeated measurements. In addition, for comparison of cytokine mean fluorescence intensities, an offset was added to ensure all values were positive, and the data were $\log_2$-transformed to improve normality. The data obtained by flow cytometry were modeled as frequencies. A false discovery rate adjusted p value (q value) <0.05 was considered statistically significant. Selected significant comparisons are displayed as box plots; the remaining immunophenotypic data were transformed into Z scores and are represented as heatmaps. GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com) was used to conduct statistical analysis for the absolute number of monocytes and neutrophils, as well as to evaluate differences in cytokine concentrations, using the Mann–Whitney U test with a p value <0.05 considered statistically significant. To assess for significant correlations between cytokine levels in PBMCs and whole blood from the same subject, Spearman's correlation tests were performed. Correlations were generated for the paired data of each cytokine and significance was determined based on a p value <0.05.

Results

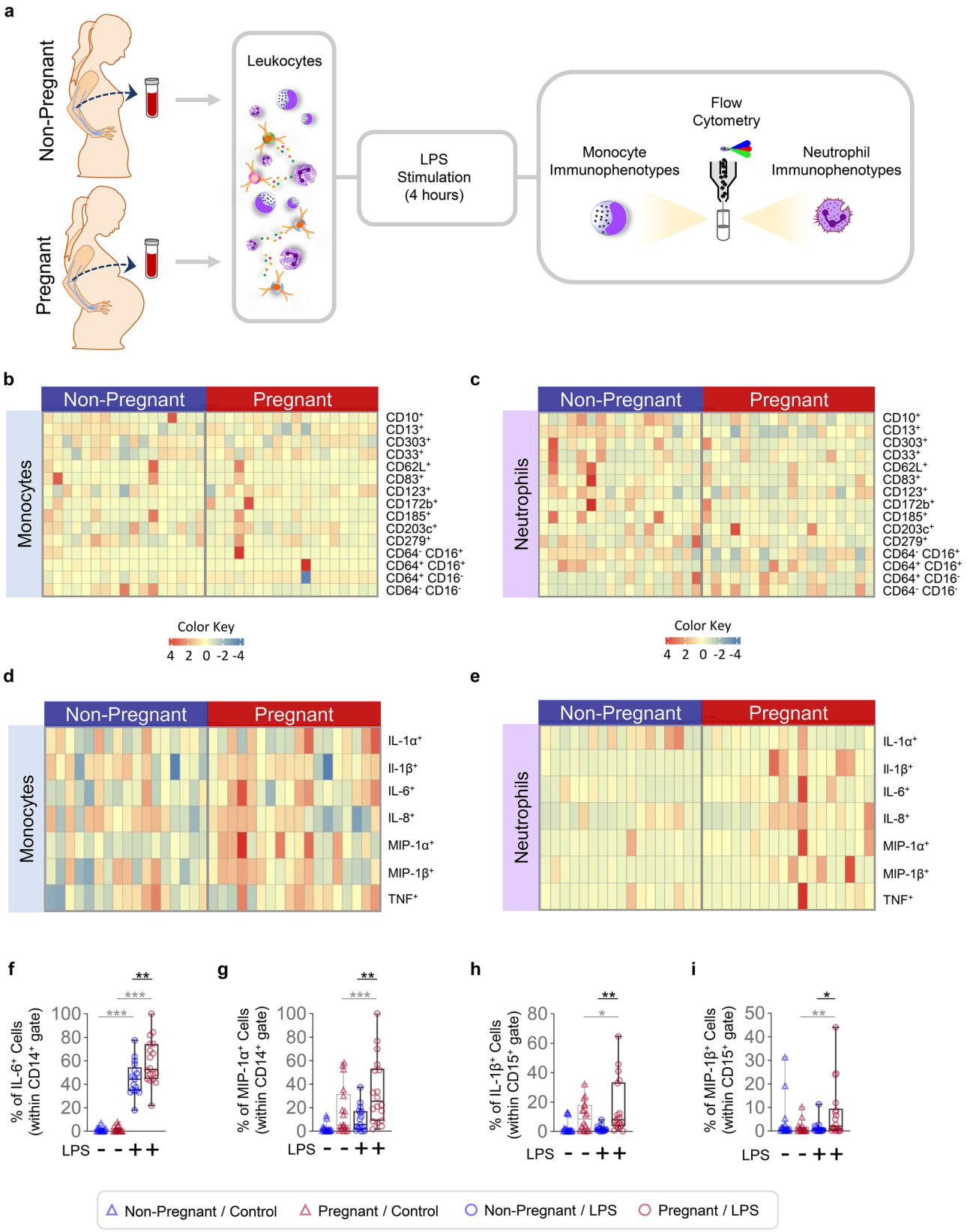
The present study included peripheral blood samples collected from a cohort comprised largely of African–American women in the third trimester, a population at high risk for pregnancy complications [47], as well as from healthy non-pregnant women of reproductive age. Peripheral whole blood or PBMCs were exposed to *E. coli* or LPS to explore the cellular immune responses triggered by Gram-negative bacteria in pregnant and non-pregnant women. Immunophenotyping revealed that pregnant and non-pregnant women strongly responded to such stimuli (Extended Dataset 1). Yet, here we only report the differences in cellular phenotypes between pregnant and non-pregnant women (shown with black asterisks throughout the figures) to document pregnancy-tailored immune responses.

Pregnancy increases the phagocytic activity and ROS production of maternal monocytes and neutrophils

The first line of defense against bacteria includes the active participation of monocytes and neutrophils, which are able to kill pathogens through different mechanisms, such as phagocytosis, degranulation, and ROS production [37, 38]. Pregnancy has been associated with increased numbers and activation of these innate immune cells [11, 40]. Therefore, we quantified the numbers of monocytes and neutrophils as well as their in vitro phagocytic activity and ROS production in pregnant and non-pregnant women (Fig. 1a). As expected, the numbers of monocytes and neutrophils were greater in pregnant women compared to non-pregnant women (Fig. 1b, c). Increased phagocytic activity was observed in monocytes and neutrophils of pregnant women compared to non-pregnant women (Fig. 1d). Moreover, ROS production by monocytes and neutrophils isolated from pregnant women was increased compared to that of cells isolated from non-pregnant women (Fig. 1e). These data show that pregnancy enhances the numbers and effector functions of monocytes and neutrophils in the maternal circulation.

Pregnancy tailors cytokine responses by monocytes and neutrophils

In addition to their primary role of phagocytosis, monocytes and neutrophils undergo phenotypic changes to enhance the


a
Non-Pregnant
Pregnant
Leukocytes
LPS Stimulation (4 hours)
Flow Cytometry
Monocyte Immunophenotypes
Neutrophil Immunophenotypes
b
Non-Pregnant
Pregnant
Monocytes
CD10+
CD13+
CD303+
CD33+
CD62L+
CD83+
CD123+
CD172b+
CD185+
CD203c+
CD279+
CD64- CD16+
CD64+ CD16+
CD64+ CD16-
CD64- CD16-
Color Key
4 2 0 -2 -4
c
Non-Pregnant
Pregnant
Neutrophils
CD10+
CD13+
CD303+
CD33+
CD62L+
CD83+
CD123+
CD172b+
CD185+
CD203c+
CD279+
CD64- CD16+
CD64+ CD16+
CD64+ CD16-
CD64- CD16-
Color Key
4 2 0 -2 -4
d
Non-Pregnant
Pregnant
Monocytes
IL-1α+
Il-1β+
IL-6+
IL-8+
MIP-1α+
MIP-1β+
TNF+
e
Non-Pregnant
Pregnant
Neutrophils
IL-1α+
Il-1β+
IL-6+
IL-8+
MIP-1α+
MIP-1β+
TNF+
f
% of IL-6+ Cells (within CD14+ gate)
LPS - - + +
g
% of MIP-1α+ Cells (within CD14+ gate)
LPS - - + +
h
% of IL-1β+ Cells (within CD15+ gate)
LPS - - + +
i
% of MIP-1β+ Cells (within CD15+ gate)
LPS - - + +
Non-Pregnant / Control
Pregnant / Control
Non-Pregnant / LPS
Pregnant / LPS

◂**Fig. 2** Response of monocytes and neutrophils to acute LPS stimulation. **a** Peripheral blood samples were obtained from pregnant ($n=18$) and non-pregnant ($n=17$) women to isolate leukocytes for acute (4 h) in vitro stimulation with lipopolysaccharide (LPS). Following stimulation, surface marker and cytokine/chemokine expression by monocytes ($CD14^+$ cells) and neutrophils ($CD15^+$ cells) was determined using flow cytometry. **b** Heatmap representation of the relative expression of immune markers by monocytes from pregnant and non-pregnant women following 4 h LPS stimulation. **c** Heatmap representation of the relative expression of immune markers by neutrophils from pregnant and non-pregnant women following 4 h LPS stimulation. **d** Heatmap representation of the relative expression of cytokines and chemokines by monocytes from pregnant and non-pregnant women following 4 h LPS stimulation. **e** Heatmap representation of the relative expression of cytokines and chemokines by neutrophils from pregnant and non-pregnant women following 4 h LPS stimulation. **f** Frequencies of $CD14^+$ cells expressing IL-6 in pregnant (red symbols) and non-pregnant (blue symbols) women following 4 h LPS stimulation (circles) or control (triangles). **g** Frequencies of $CD14^+$ cells expressing MIP-1α in pregnant and non-pregnant women following 4 h LPS stimulation (circles) or control (triangles). **h** Frequencies of $CD15^+$ cells expressing IL-1β in pregnant and non-pregnant women following 4 h LPS stimulation (circles) or control (triangles). **i** Frequencies of $CD15^+$ cells expressing MIP-1β in pregnant and non-pregnant women following 4 h LPS stimulation (circles) or control (triangles). $*p<0.05$; $**p<0.01$; $***p<0.001$. (+) Stimulated; (−) control

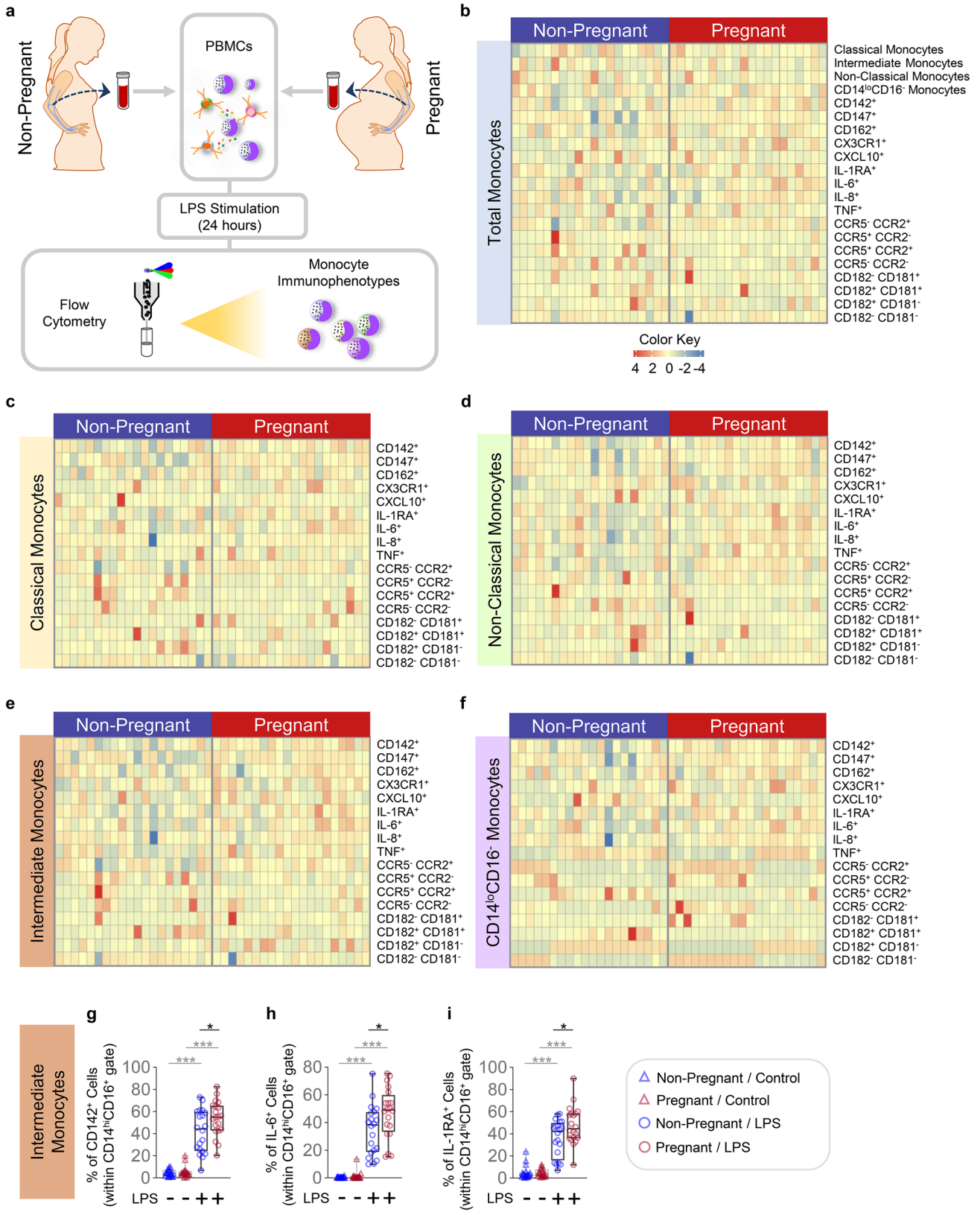
coordinated immune response to bacteria [37, 38]. The timeline of such an inflammatory response is broadly divided into acute and chronic stages: the former occurs within hours of insult and involves both monocytes and neutrophils, whereas the latter extends for days or even weeks and involves only monocytes [48, 49]. Therefore, we first characterized the influence of pregnancy on the phenotypes of monocytes and neutrophils after 4 h stimulation with LPS (Fig. 2a). Stimulation with LPS induced a significant increase in the frequency of specific phenotypes of monocytes (Fig. 2b, Supplementary Fig. 1 and Extended Dataset 1) and neutrophils (Fig. 2c, Supplementary Fig. 1 and Extended Dataset 1) in pregnant and non-pregnant women; yet, pregnancy itself did not have any further effect. Thus, we evaluated the frequency of monocytes and neutrophils expressing relevant pro-inflammatory cytokines and chemokines in response to LPS (Fig. 2d, e, Supplementary Fig. 2, and Extended Dataset 1). We observed a difference in the cytokine profiles displayed by monocytes and neutrophils in pregnant women. Specifically, the frequencies of IL-6^+CD14^+ (Fig. 2f) and MIP-$1\alpha^+CD14^+$ (Fig. 2g) cells were greater in pregnant compared to non-pregnant women in response to LPS. By contrast, the frequencies of IL-$1\beta^+CD15^+$ (Fig. 2h) and MIP-$1\beta^+CD15^+$ (Fig. 2i) cells were increased in pregnant compared to non-pregnant women in response to LPS stimulation. These findings indicate that pregnancy tailors the cytokine response by monocytes and neutrophils upon acute exposure to a Gram-negative bacterial product.

Pregnancy drives specific chronic monocyte responses

Monocytes, as mentioned above, are central players in the chronic inflammatory response triggered by bacteria [38, 48]. In this context, such immune cells are conventionally divided into four subsets based on the expression of the surface markers CD14 and CD16: classical ($CD14^{hi}CD16^-$), intermediate ($CD14^{hi}CD16^+$), non-classical ($CD14^{lo}CD16^+$), and $CD14^{lo}CD16^-$ monocytes [50]. Therefore, we evaluated the phenotypes of monocyte subsets from pregnant and non-pregnant women after 24 h of LPS exposure (Fig. 3a). Flow cytometry was utilized to determine the frequency of each monocyte subset as well as the expression of specific surface markers, cytokines, and chemokines within each subset in response to LPS (Fig. 3b–f, Supplementary Fig. 3). As expected, stimulation with this bacterial product increased the frequencies of monocytes expressing surface markers of activation in pregnant and non-pregnant women (Extended Dataset 1). However, pregnancy status did not confer any additional effect among classical, non-classical, and $CD14^{lo}CD16^-$ monocytes. By contrast, stimulation with LPS was associated with a significant increase in the frequencies of $CD142^+$ (i.e., tissue factor) (Fig. 3g), IL-6^+ (Fig. 3h), and IL-$1RA^+$ (interleukin 1 receptor antagonist) (Fig. 3i) intermediate monocytes in pregnant compared to non-pregnant women. Notably, the increased frequency of IL-6^+CD14^+ cells was consistently observed in response to acute (Fig. 2f) and chronic LPS exposure (Fig. 3h), suggesting that these monocytes could have an early and maintained activation response to this bacterial product. Collectively, these results show that pregnancy drives specific chronic functions of monocytes in response to Gram-negative bacteria.

Pregnancy confers a tailored cytokine and chemokine response

Activated monocytes and neutrophils are characterized by their release of cytokines and chemokines [37, 51]. Therefore, we next investigated whether pregnancy impacts the release of such immune mediators. We evaluated cytokine and chemokine release by PBMCs from pregnant and non-pregnant women in response to LPS stimulation (Fig. 4a). Baseline cytokine production did not differ between pregnant and non-pregnant women (Fig. 4b–k). As expected, stimulation with LPS increased the production of all evaluated cytokines, including IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF, and IFNγ by PBMCs from pregnant and non-pregnant women (Fig. 4b–k). Interestingly, PBMCs from pregnant women showed an enhanced release of IL-2 (Fig. 4c) and IL-13 (Fig. 4i) compared to non-pregnant women in response to LPS. Nevertheless, the


a
Non-Pregnant
PBMCs
Pregnant
LPS Stimulation
(24 hours)
Flow
Cytometry
Monocyte
Immunophenotypes
b
Non-Pregnant
Pregnant
Total Monocytes
Classical Monocytes
Intermediate Monocytes
Non-Classical Monocytes
CD14loCD16- Monocytes
CD142+
CD147+
CD162+
CX3CR1+
CXCL10+
IL-1RA+
IL-6+
IL-8+
TNF+
CCR5- CCR2+
CCR5+ CCR2-
CCR5+ CCR2+
CCR5- CCR2-
CD182- CD181+
CD182+ CD181+
CD182+ CD181-
CD182- CD181-
Color Key
4 2 0 -2 -4
c
Non-Pregnant
Pregnant
Classical Monocytes
d
Non-Pregnant
Pregnant
Non-Classical Monocytes
e
Non-Pregnant
Pregnant
Intermediate Monocytes
f
Non-Pregnant
Pregnant
CD14loCD16- Monocytes
Intermediate Monocytes
g
% of CD142+ Cells (within CD14hiCD16+ gate)
h
% of IL-6+ Cells (within CD14hiCD16+ gate)
i
% of IL-1RA+ Cells (within CD14hiCD16+ gate)
LPS - - + +
*
***
***
Non-Pregnant / Control
Pregnant / Control
Non-Pregnant / LPS
Pregnant / LPS

◂**Fig. 3** Response of total monocytes and monocyte subsets to chronic LPS stimulation. **a** Peripheral blood mononuclear cells (PBMCs) were isolated from the peripheral blood of pregnant ($n=20$) and non-pregnant ($n=20$) women and stimulated with LPS for 24 h. Flow cytometry was performed to evaluate the expression of surface markers and cytokines/chemokines by total monocytes and monocyte subsets. **b** Heatmap representation of the relative expression of immune markers and cytokines/chemokines by total monocytes from pregnant and non-pregnant following 24 h LPS stimulation. **c** Heatmap representation of the relative expression of immune markers and cytokines/chemokines by classical monocytes ($CD14^{hi}CD16^{-}$ cells) from pregnant and non-pregnant women following 24 h LPS stimulation. **d** Heatmap representation of the relative expression of immune markers and cytokines/chemokines by non-classical monocytes ($CD14^{lo}CD16^{+}$ cells) from pregnant and non-pregnant women following 24 h LPS stimulation. **e** Heatmap representation of the relative expression of immune markers and cytokines/chemokines by intermediate monocytes ($CD14^{hi}CD16^{+}$ cells) from pregnant and non-pregnant women following 24 h LPS stimulation. **f** Heatmap representation of the relative expression of immune markers and cytokines/chemokines by $CD14^{lo}CD16^{-}$ monocytes from pregnant and non-pregnant women following 24 h LPS stimulation. Frequencies of intermediate monocytes expressing **g** CD142 (tissue factor), **h** IL-6, and **i** IL-1RA in pregnant (red symbols) and non-pregnant (blue symbols) women following 24 h LPS stimulation (circles) or control (triangles). $*p<0.05$; $***p<0.001$. (+) Stimulated; (−) control

LPS-dependent release of IL-8 was reduced in pregnant women (Fig. 4f).

To assess the contribution of cells omitted in PBMCs, such as neutrophils, which comprise the largest population in peripheral blood [37], we evaluated the release of immune mediators from whole blood using the LPS-containing TruCulture® system [46] (Fig. 5a). Consistent with the results in PBMCs, whole-blood samples from pregnant and non-pregnant women displayed increased concentrations of all evaluated immune mediators upon LPS stimulation (Fig. 5b–k). Yet, analysis of whole blood samples from pregnant women revealed a greater number of differences in the evaluated cytokines and chemokines compared to PBMCs. Indeed, IL-6 (Fig. 5e), IL-8 (Fig. 5f), and IL-10 (Fig. 5g) were increased in pregnancy-derived whole blood samples, but not PBMCs. By contrast, both IL-1β (Fig. 5b) and IFNγ (Fig. 5k) were decreased in whole blood samples from pregnant women, yet this was not observed in PBMCs. The only consistent change between both pregnancy-derived PBMCs and whole blood samples was an increase in IL-2 release (Fig. 4c and Fig. 5c).

Last, the Spearman's correlations between cytokine profiles in PBMCs and whole blood TruCulture® supernatants in response to LPS were determined. Distinct cytokine correlations were observed in pregnant and non-pregnant women. Positive correlations between PBMCs and whole blood for IL-1β and IFNγ concentrations, but a negative correlation for IL-8, were observed in pregnant women (Fig. 6a). By contrast, positive correlations between PBMCs and whole blood for IL-4, IL-6, and IL-10 concentrations were observed in non-pregnant women (Fig. 6b).

Taken together, these results indicate that pregnancy confers a tailored cytokine and chemokine response to endotoxin, a Gram-negative bacterial product.

Discussion

In the current study, we observed an increase in monocyte and neutrophil phagocytosis of live *E. coli* during pregnancy, which is in line with a previous publication reporting an increase in phagocytic activity specific to live microorganisms over inert particles (latex beads) [52]. Furthermore, a significantly higher number of phagocytosed microorganisms per neutrophil was demonstrated in pregnant women, a characteristic that abated following delivery [53]. By contrast, another report found a decrease in the phagocytic function of monocytes and neutrophils during gestation [54]. This observed decrease in phagocytic function during pregnancy may result from a decrease in circulating classical monocytes, which are the main subset of monocytes associated with phagocytic functions [39]. Additionally, we showed increased ROS production in monocytes and neutrophils isolated from the peripheral blood of pregnant women. These results are consistent with prior reports showing elevated ROS production by monocytes and neutrophils from pregnant women [40, 55], a characteristic that seems to be further increased in the context of obstetrical diseases, such as maternal infection or preeclampsia [11, 40]. Indeed, basal and stimulated ROS production was higher in monocytes and granulocytes (i.e., neutrophils) from pregnant women with a positive blood bacterial culture, pyelonephritis, or preeclampsia compared to normal pregnant and non-pregnant women [11, 40, 56]. However, a reduction in superoxide production by granulocytes in healthy pregnant women has also been reported [57], which could partially explain such differences. Regardless, our findings support an increase in the ROS production and phagocytic activity of monocytes and neutrophils during late pregnancy.

Monocytes and neutrophils sense bacterial products through pattern recognition receptors (PRRs) such as Toll-like receptor 4 (TLR4), which upon activation will initiate a series of transduction signals leading to the release of immune mediators such as cytokines [58]. Interestingly, we found that the endotoxin-induced cytokine profiles of monocytes were distinct from those of neutrophils in pregnant women, highlighting potential differences in the cellular signaling pathways initiated by these immune cells in response to microbes. Indeed, we observed an increase in the frequency of $IL\text{-}6^{+}$ and $MIP\text{-}1\alpha^{+}$ monocytes, as opposed to an increase in the frequency of $IL\text{-}1\beta^{+}$ and $MIP\text{-}1\beta^{+}$ neutrophils in response to endotoxin. Hence, while the specific

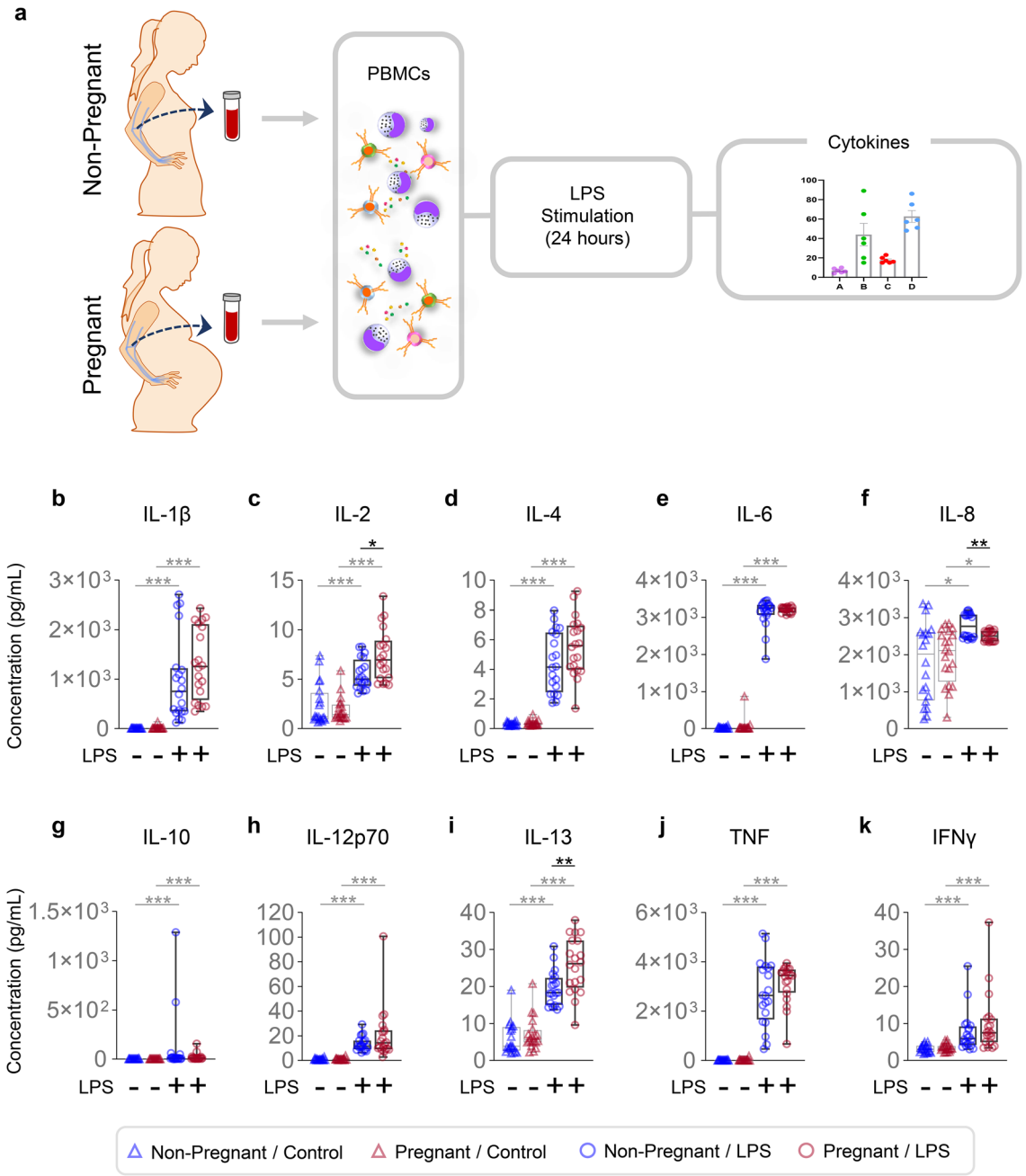


Fig. 4 Cytokine release by PBMCs in response to LPS. **a** Peripheral blood mononuclear cells (PBMCs) were isolated from pregnant ($n=20$) and non-pregnant ($n=20$) women and stimulated with LPS for 24 h. Cytokine concentrations were then determined in the cell culture supernatants. Concentrations (pg/mL) of **b** IL-1β, **c** IL-2, **d** IL-4, **e** IL-6, **f** IL-8, **g** IL-10, **h** IL-12p70, **i** IL-13, **j** TNF, and **k** IFNγ in PBMC culture supernatants from pregnant (red symbols) and non-pregnant (blue symbols) women following 24 h LPS stimulation (circles) or control (triangles). *$p<0.05$; **$p<0.01$; ***$p<0.001$. (+) Stimulated; (−) control

immune markers themselves differed, pregnancy was associated with increased pro-inflammatory subsets of both monocytes and neutrophils, which supports the concept of an enhanced inflammatory response to endotoxin during pregnancy shown in an animal model [59]. Future investigations may focus on elucidating the precise pathways implicated in monocyte and neutrophil activation during late gestation.

Pregnancy has been associated with increased expression of activation markers in peripheral innate immune cells [11, 40, 59, 60], which is postulated to result from gestational factors such as the presence of placental microparticles and

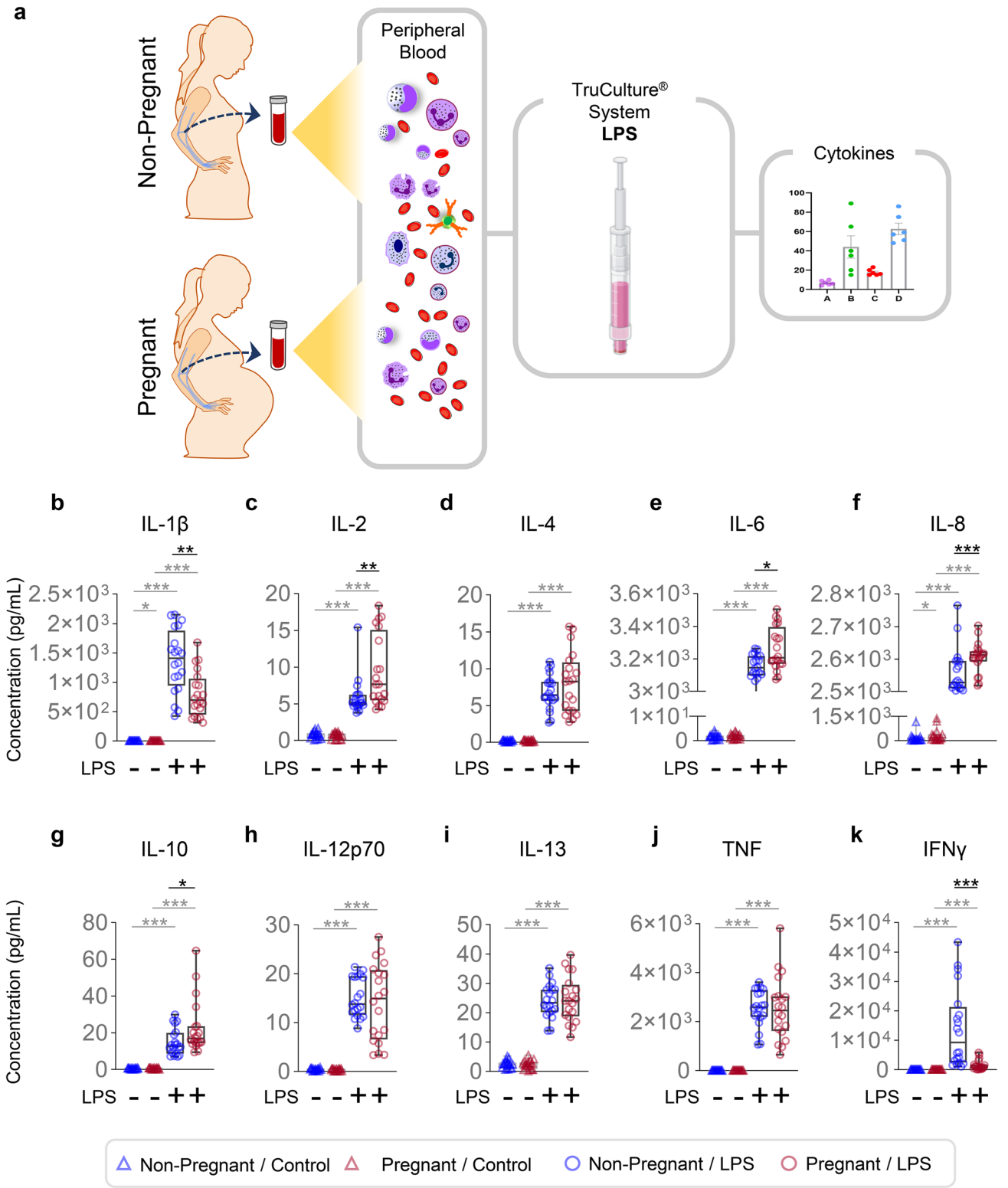


Fig. 5 Cytokine release by whole-blood samples in response to LPS (TruCulture®). **a** Peripheral whole blood was obtained from pregnant ($n=20$) and non-pregnant ($n=20$) women and stimulated using a whole blood culture system (TruCulture®) containing LPS for 48 h. Cytokine concentrations were then determined in the culture supernatants. Concentrations (pg/mL) of **b** IL-1β, **c** IL-2, **d** IL-4, **e** IL-6, **f** IL-8, **g** IL-10, **h** IL-12p70, **i** IL-13, **j** TNF, and **k** IFNγ in whole blood TruCulture® supernatants from pregnant (red symbols) and non-pregnant (blue symbols) women following 48 h LPS stimulation (circles) or control (triangles). *$p<0.05$; **$p<0.01$; ***$p<0.001$. (+) Stimulated; (−) control

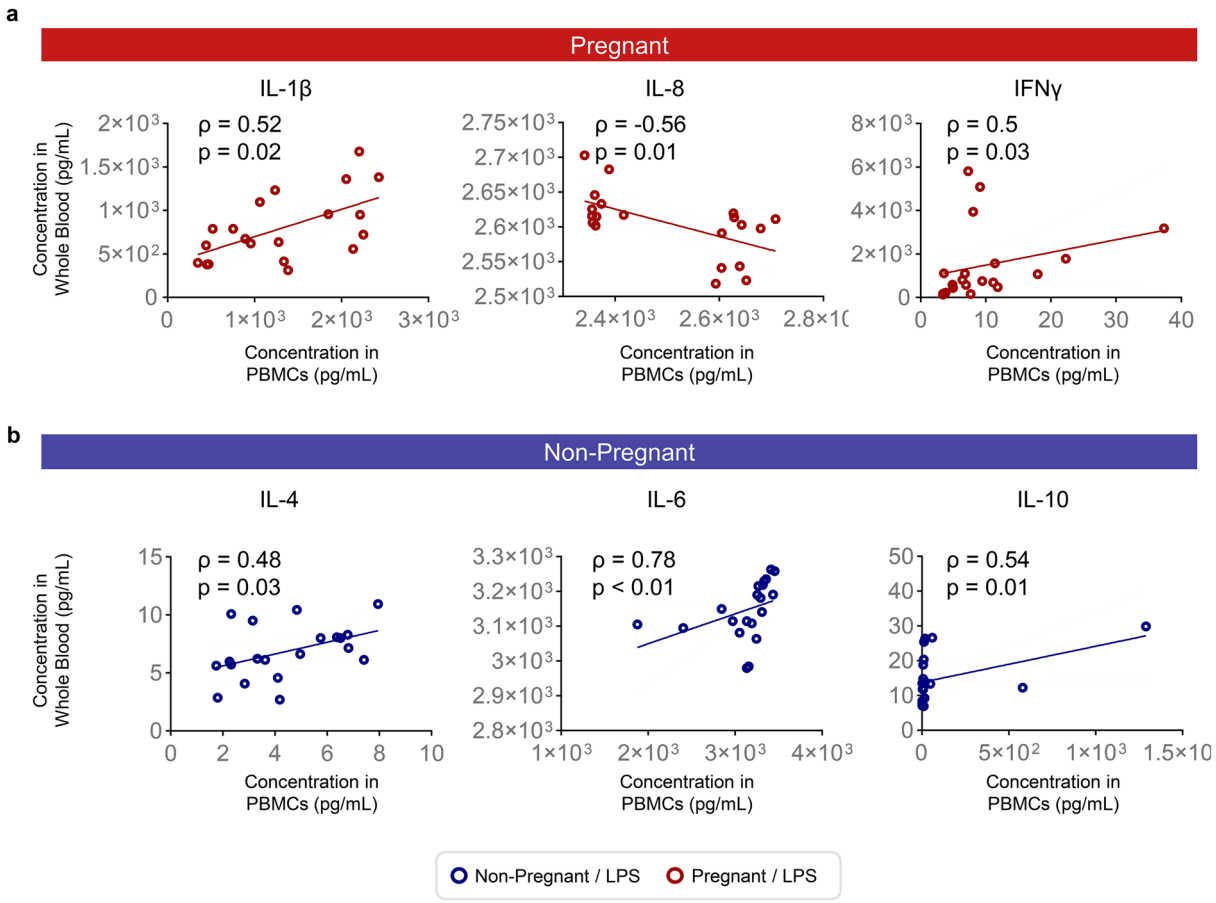


Fig. 6 Correlations between cytokine profiles in whole blood and PBMC samples exposed to LPS. **a** Correlations of IL-1β, IL-8, and IFNγ concentrations between whole blood and PBMC samples of pregnant women exposed to LPS. **b** Correlations of IL-4, IL-6, and IL-10 concentrations between whole blood and PBMC samples of non-pregnant women exposed to LPS. The Spearman's correlation coefficient (ρ) and p value are displayed for each graph. A p value < 0.05 was considered significant

fetal cells in the maternal circulation [41, 61–63]. Accordingly, a significant shift has been reported in the frequency of monocyte subsets from classical to intermediate, the latter displaying the surface signals of activation and differentiation in human pregnancy [64] and in an animal model [65]. Notably, our results demonstrate an additional effect of pregnancy in intermediate monocytes in response to chronic endotoxin exposure, which showed enhanced frequencies of cells expressing CD142 (i.e., tissue factor), IL-6, and IL-1RA. Tissue factor is necessary for thrombin formation in the coagulation cascade [66], and the increased frequency of intermediate monocytes expressing tissue factor in the current study is consistent with a previous report showing elevated tissue factor activity in women with preterm labor and intra-amniotic infection [67]. Similarly, increased expression of multiple immune mediators, including IL-6 and IL-1RA, has been found in response to experimental endotoxemia in human volunteers [68]. In the context of pregnancy, amniotic fluid IL-6 is a clinically established biomarker of intra-amniotic inflammation [69] and was predicted as one of the activated upstream regulators of cytokine signaling in monocytes from the amniotic cavity of women with this condition [36]. In the current study, we observed elevated frequencies of IL-6$^+$ monocytes in response to both acute and chronic endotoxin exposure, suggesting that this cytokine is a key component of monocyte-mediated host defense mechanisms during late pregnancy. Interestingly, we also observed increased frequencies of monocytes expressing IL-1RA, a soluble antagonist for the IL-1β receptor, in response to chronic endotoxin exposure during gestation. This phenomenon may reflect the need to attenuate the cytokine storm that could damage the mother and her fetus. Together, the distinct monocyte phenotype characterized by the expression of pro-inflammatory mediators and the natural antagonist IL-1RA may indicate a tightly-regulated response to bacterial products that is necessary for maternal host defense.

Herein, we reported that exposure to endotoxin increased the concentrations of all evaluated cytokines and chemokines in the PBMCs and whole blood from pregnant and non-pregnant women, which is consistent with observations

in pregnant women with infection [70, 71]. In line with this concept, we found a positive correlation of IL-1β and IFNγ concentrations between the PBMCs and whole blood of pregnant women. We recently performed an extensive microarray analysis of pregnant and non-pregnant mice that received systemic endotoxin, showing an organ-specific response during pregnancy; however, the effect in peripheral blood was not evaluated [72]. In this study, we showed an increased release of immune mediators such as IL-6, IL-8, and IL-10 by pregnant women in whole blood samples but not PBMCs. Given that PBMCs primarily lack neutrophils, it is plausible that the enhanced cytokine release observed in whole blood samples is driven at least partially by the absence of these cells [73].

Consistent with the above hypothesis, the concentrations of IL-8 (i.e., CXCL8), a chemokine that is implicated in infection-associated obstetrical disease such as acute histologic chorioamnionitis [74], were negatively correlated between the PBMCs and whole blood of pregnant women upon endotoxin stimulation. Given that IL-8 is a pro-inflammatory chemokine associated with neutrophil chemotaxis and activation [37, 75], the opposing results from PBMCs and whole blood could also be explained by the low frequency of neutrophils in PBMCs. We also showed that IL-8 was decreased in PBMCs and increased in whole blood derived from pregnant women compared to non-pregnant women. Consistent with this observation, we recently demonstrated the differential release of this chemokine from peripheral leukocytes of pregnant women in response to SARS-CoV-2 [76]. Based on the alterations in IL-8 expression observed upon bacterial and viral challenges in pregnancy, it is tempting to suggest a critical role for this chemokine in maternal host defense mechanisms.

Interleukin-2 is the only cytokine that was increased in PBMCs and whole blood from pregnant women. IL-2 is involved in local immune responses taking place in the amniotic cavity of women with preterm labor [77]. Conversely, the systemic administration of IL-2 improved neonatal parameters in a pregnant ovine model of chorioamnionitis induced by intra-amniotic injection of endotoxin [78], which highlights the pleiotropic role of this cytokine in gestation. Here, we focused on the maternal monocyte and neutrophil responses to endotoxin, given that these are the primary cellular components of the peripheral blood. Yet, given the proposed involvement of systemic adaptive cellular immunity throughout pregnancy [79–82], future studies should evaluate how peripheral T-cell and B-cell responses differ between pregnant and non-pregnant women.

In summary, herein we have characterized the response of monocytes and neutrophils in the maternal circulation to endotoxin, a Gram-negative bacterial product. Overall, our findings show greater phagocytic activity and ROS production in the monocytes and neutrophils of pregnant women, as well as an increased frequency of pro-inflammatory subsets of these innate immune cells and an altered release of immune mediators in response to bacterial challenge. These results provide insight into the pregnancy-tailored immune responses of circulating monocytes and neutrophils to endotoxin, a Gram-negative bacterial product, and shed light on the immune mechanisms that contribute to host response during late gestation.

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Author contributions NG-L and RR: conceived and designed the study. MF-J, JG, YX, LT, and MA-H: performed and analyzed the experiments. MF-J, JG, CD-P, GB, ZL, NK, ZS, and KRT: analyzed the data, contributed to data visualization, and provided intellectual input. TK and TC: performed enrollment of patients and collection of samples and provided intellectual input. NG-L, RR, MF-J, JG, and YX: interpreted the data. All authors revised and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

Ethical approval All women provided written informed consent prior to blood collection. The collection and use of biological specimens for research purposes were approved by the Institutional Review Boards of Wayne State University and the Detroit Medical Center.

References

1. Brizuela V, Cuesta C, Bartolelli G, Abdosh AA, Abou Malham S, Assarag B, et al. Availability of facility resources and services and

infection-related maternal outcomes in the WHO global maternal sepsis study: a cross-sectional study. Lancet Glob Health. 2021;9:e1252–61.
2. Acosta CD, Kurinczuk JJ, Lucas DN, Tuffnell DJ, Sellers S, Knight M, et al. Severe maternal sepsis in the UK, 2011–2012: a national case-control study. PLoS Med. 2014;11:e1001672.
3. Cunningham FG, Leveno KJ, Bloom SL, Dashe JS, Hoffman BL, Casey BM, et al. Maternal Physiology Williams Obstetrics. New York: McGraw-Hill Education; 2018. p. 25e.
4. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. Nat Rev Nephrol. 2014;10:466–80.
5. Chappell LC, Cluver CA, Kingdom J, Tong S. Pre-eclampsia. Lancet. 2021;398:341–54.
6. Figueras F, Caradeux J, Crispi F, Eixarch E, Peguero A, Gratacos E. Diagnosis and surveillance of late-onset fetal growth restriction. Am J Obstet Gynecol. 2018;218:S790-S802 e1.
7. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371:75–84.
8. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Health. 2019;7:e37–46.
9. Kourtis AP, Read JS, Jamieson DJ. Pregnancy and infection. N Engl J Med. 2014;370:2211–8.
10. Medawar PB. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp Soc Exp Biol. 1953;7:320–8.
11. Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. Am J Obstet Gynecol. 1998;179:80–6.
12. Robertson SA. Immune regulation of conception and embryo implantation-all about quality control? J Reprod Immunol. 2010;85:51–7.
13. Chaouat G, Voisin GA, Daeron M, Kanellopoulos J. Enhancing antibodies and supressive cells in maternal anti-fetal immune reaction. Ann Immunol (Paris). 1977;128:21–4.
14. Hunt JS, Petroff MG, McIntire RH, Ober C. HLA-G and immune tolerance in pregnancy. FASEB J. 2005;19:681–93.
15. Moffett A, Loke C. Immunology of placentation in eutherian mammals. Nat Rev Immunol. 2006;6:584–94.
16. Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, et al. A pivotal role for galectin-1 in fetomaternal tolerance. Nat Med. 2007;13:1450–7.
17. Thaxton JE, Sharma S. Interleukin-10: a multi-faceted agent of pregnancy. Am J Reprod Immunol. 2010;63:482–91.
18. Mold JE, Venkatasubrahmanyam S, Burt TD, Michaelsson J, Rivera JM, Galkina SA, et al. Fetal and adult hematopoietic stem cells give rise to distinct T cell lineages in humans. Science. 2010;330:1695–9.
19. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol. 2004;5:266–71.
20. Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S. Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol Hum Reprod. 2004;10:347–53.
21. Zenclussen AC, Gerlof K, Zenclussen ML, Sollwedel A, Bertoja AZ, Ritter T, et al. Abnormal T-cell reactivity against paternal antigens in spontaneous abortion: adoptive transfer of pregnancy-induced CD4+CD25+ T regulatory cells prevents fetal rejection in a murine abortion model. Am J Pathol. 2005;166:811–22.
22. Bizargity P, Del Rio R, Phillippe M, Teuscher C, Bonney EA. Resistance to lipopolysaccharide-induced preterm delivery mediated by regulatory T cell function in mice. Biol Reprod. 2009;80:874–81.
23. Rowe JH, Ertelt JM, Xin L, Way SS. Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. Nature. 2012;490:102–6.
24. Samstein RM, Josefowicz SZ, Arvey A, Treuting PM, Rudensky AY. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. Cell. 2012;150:29–38.
25. Moldenhauer LM, Schjenken JE, Hope CM, Green ES, Zhang B, Eldi P, et al. Thymus-derived regulatory t cells exhibit Foxp3 epigenetic modification and phenotype attenuation after mating in mice. J Immunol. 2019;203:647–57.
26. Gomez-Lopez N, Arenas-Hernandez M, Romero R, Miller D, Garcia-Flores V, Leng Y, et al. Regulatory T cells play a role in a subset of idiopathic preterm labor/birth and adverse neonatal outcomes. Cell Rep. 2020;32:107874.
27. Hunt JS, Manning LS, Wood GW. Macrophages in murine uterus are immunosuppressive. Cell Immunol. 1984;85:499–510.
28. Svensson-Arvelund J, Mehta RB, Lindau R, Mirrasekhian E, Rodriguez-Martinez H, Berg G, et al. The human fetal placenta promotes tolerance against the semiallogeneic fetus by inducing regulatory T cells and homeostatic M2 macrophages. J Immunol. 2015;194:1534–44.
29. Xu Y, Romero R, Miller D, Kadam L, Mial TN, Plazyo O, et al. An M1-like macrophage polarization in decidual tissue during spontaneous preterm labor that is attenuated by rosiglitazone treatment. J Immunol. 2016;196:2476–91.
30. Gomez-Lopez N, Garcia-Flores V, Chin PY, Groome HM, Bijland MT, Diener KR, et al. Macrophages exert homeostatic actions in pregnancy to protect against preterm birth and fetal inflammatory injury. JCI Insight. 2021;6:e146089. https://doi.org/10.1172/jci.insight.146089.
31. Faas MM, Spaans F, De Vos P. Monocytes and macrophages in pregnancy and pre-eclampsia. Front Immunol. 2014;5:298.
32. Gomez-Lopez N, Romero R, Xu Y, Miller D, Unkel R, Shaman M, et al. Neutrophil extracellular traps in the amniotic cavity of women with intra-amniotic infection: a new mechanism of host defense. Reprod Sci. 2017;24:1139–53.
33. Gomez-Lopez N, Romero R, Leng Y, Garcia-Flores V, Xu Y, Miller D, et al. Neutrophil extracellular traps in acute chorioamnionitis: a mechanism of host defense. Am J Reprod Immunol. 2017;77:e12617.
34. Gomez-Lopez N, Romero R, Garcia-Flores V, Xu Y, Leng Y, Alhousseini A, et al. Amniotic fluid neutrophils can phagocytize bacteria: a mechanism for microbial killing in the amniotic cavity. Am J Reprod Immunol. 2017;78:e12723.
35. Galaz J, Romero R, Xu Y, Miller D, Slutsky R, Levenson D, et al. Cellular immune responses in amniotic fluid of women with preterm clinical chorioamnionitis. Inflamm Res. 2020;69:203–16.
36. Gomez-Lopez N, Romero R, Varrey A, Leng Y, Miller D, Done B, et al. RNA sequencing reveals diverse functions of amniotic fluid neutrophils and monocytes/macrophages in intra-amniotic infection. J Innate Immun. 2021;13:63–82.
37. Burn GL, Foti A, Marsman G, Patel DF, Zychlinsky A. The Neutrophil. Immunity. 2021;54:1377–91.
38. Jakubzick CV, Randolph GJ, Henson PM. Monocyte differentiation and antigen-presenting functions. Nat Rev Immunol. 2017;17:349–62.
39. Abu-Raya B, Michalski C, Sadarangani M, Lavoie PM. Maternal immunological adaptation during normal pregnancy. Front Immunol. 2020;11:575197.
40. Naccasha N, Gervasi MT, Chaiworapongsa T, Berman S, Yoon BH, Maymon E, et al. Phenotypic and metabolic characteristics of monocytes and granulocytes in normal pregnancy and maternal infection. Am J Obstet Gynecol. 2001;185:1118–23.

41. Germain SJ, Sacks GP, Sooranna SR, Sargent IL, Redman CW. Systemic inflammatory priming in normal pregnancy and preeclampsia: the role of circulating syncytiotrophoblast microparticles. J Immunol. 2007;178:5949–56.
42. Faas MM, de Vos P. Maternal monocytes in pregnancy and preeclampsia in humans and in rats. J Reprod Immunol. 2017;119:91–7.
43. Zhang J, Shynlova O, Sabra S, Bang A, Briollais L, Lye SJ. Immunophenotyping and activation status of maternal peripheral blood leukocytes during pregnancy and labour, both term and preterm. J Cell Mol Med. 2017;21:2386–402.
44. Aghaeepour N, Ganio EA, McIlwain D, Tsai AS, Tingle M, Van Gassen S, et al. An immune clock of human pregnancy. Sci Immunol. 2017;2:eaan2946. https://doi.org/10.1126/sciimmunol.aan2946.
45. Stelzer IA, Ghaemi MS, Han X, Ando K, Hedou JJ, Feyaerts D, et al. Integrated trajectories of the maternal metabolome, proteome, and immunome predict labor onset. Sci Transl Med. 2021;13:eabd9898. https://doi.org/10.1126/scitranslmed.abd9898.
46. Duffy D, Rouilly V, Libri V, Hasan M, Beitz B, David M, et al. Functional analysis via standardized whole-blood stimulation systems defines the boundaries of a healthy immune response to complex stimuli. Immunity. 2014;40:436–50.
47. Egbe TI, Montoya-Williams D, Wallis K, Passarella M, Lorch SA. Risk of extreme, moderate, and late preterm birth by maternal race, ethnicity, and nativity. J Pediatr. 2021;240:24-30.e2.
48. Stramer BM, Mori R, Martin P. The inflammation-fibrosis link? A jekyll and hyde role for blood cells during wound repair. J Invest Dermatol. 2007;127:1009–17.
49. Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. J Clin Invest. 2018;128:2657–69.
50. Kapellos TS, Bonaguro L, Gemünd I, Reusch N, Saglam A, Hinkley ER, et al. Human monocyte subsets and phenotypes in major chronic inflammatory diseases. Front Immunol. 2019;10:2035.
51. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. Nat Rev Immunol. 2005;5:953–64.
52. Barriga C, Rodriguez AB, Ortega E. Increased phagocytic activity of polymorphonuclear leukocytes during pregnancy. Eur J Obstet Gynecol Reprod Biol. 1994;57:43–6.
53. Saikia TC, Pramanik T, Thapa M. Phagocytic activities of neutrophilic leukocytes in women in various phases of menstrual cycle, and in pregnancy. Southeast Asian J Trop Med Public Health. 2003;34:877–80.
54. Lampe R, Kover A, Szucs S, Pal L, Arnyas E, Adany R, et al. Phagocytic index of neutrophil granulocytes and monocytes in healthy and preeclamptic pregnancy. J Reprod Immunol. 2015;107:26–30.
55. Kindzelskii AL, Huang JB, Chaiworapongsa T, Fahmy RM, Kim YM, Romero R, et al. Pregnancy alters glucose-6-phosphate dehydrogenase trafficking, cell metabolism, and oxidant release of maternal neutrophils. J Clin Invest. 2002;110:1801–11.
56. Gervasi MT, Chaiworapongsa T, Pacora P, Naccasha N, Yoon BH, Maymon E, et al. Phenotypic and metabolic characteristics of monocytes and granulocytes in preeclampsia. Am J Obstet Gynecol. 2001;185:792–7.
57. Lampe R. Superoxide-anion production by neutrophil granulocytes in healthy and preeclamptic pregnant women. Orv Hetil. 2012;153:425–34.
58. Lu YC, Yeh WC, Ohashi PS. LPS/TLR4 signal transduction pathway. Cytokine. 2008;42:145–51.
59. Faas MM, Schuiling GA, Linton EA, Sargent IL, Redman CW. Activation of peripheral leukocytes in rat pregnancy and experimental preeclampsia. Am J Obstet Gynecol. 2000;182:351–7.
60. Luppi P, Haluszczak C, Trucco M, Deloia JA. Normal pregnancy is associated with peripheral leukocyte activation. Am J Reprod Immunol. 2002;47:72–81.
61. Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proc Natl Acad Sci U S A. 1996;93:705–8.
62. Redman CW, Sargent IL. Microparticles and immunomodulation in pregnancy and pre-eclampsia. J Reprod Immunol. 2007;76:61–7.
63. Arenas-Hernandez M, Romero R, Gershater M, Tao L, Xu Y, Garcia-Flores V, et al. Specific innate immune cells uptake fetal antigen and display homeostatic phenotypes in the maternal circulation. J Leukoc Biol. 2021;111:519–38.
64. Pflitsch C, Feldmann CN, Richert L, Hagen S, Diemert A, Goletzke J, et al. In-depth characterization of monocyte subsets during the course of healthy pregnancy. J Reprod Immunol. 2020;141:103151.
65. Melgert BN, Spaans F, Borghuis T, Klok PA, Groen B, Bolt A, et al. Pregnancy and preeclampsia affect monocyte subsets in humans and rats. PLoS One. 2012;7:e45229.
66. Mackman N. Role of tissue factor in hemostasis, thrombosis, and vascular development. Arterioscler Thromb Vasc Biol. 2004;24:1015–22.
67. Erez O, Romero R, Vaisbuch E, Kusanovic JP, Mazaki-Tovi S, Chaiworapongsa T, et al. High tissue factor activity and low tissue factor pathway inhibitor concentrations in patients with preterm labor. J Matern Fetal Neonatal Med. 2010;23:23–33.
68. Beunders R, Schutz MJ, van Groenendael R, Leijte GP, Kox M, van Eijk LT, et al. Endotoxemia-induced release of pro-inflammatory mediators are associated with increased glomerular filtration rate in humans in vivo. Front Med (Lausanne). 2020;7:559671.
69. Yoon BH, Romero R, Moon JB, Shim SS, Kim M, Kim G, et al. Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. Am J Obstet Gynecol. 2001;185:1130–6.
70. Chaiworapongsa T, Romero R, Gotsch F, Kusanovic JP, Mittal P, Kim SK, et al. Acute pyelonephritis during pregnancy changes the balance of angiogenic and anti-angiogenic factors in maternal plasma. J Matern Fetal Neonatal Med. 2010;23:167–78.
71. Gulati S, Agrawal S, Raghunandan C, Bhattacharya J, Saili A, Agarwal S, et al. Maternal serum interleukin-6 and its association with clinicopathological infectious morbidity in preterm premature rupture of membranes: a prospective cohort study. J Matern Fetal Neonatal Med. 2012;25:1428–32.
72. Motomura K, Romero R, Tarca AL, Galaz J, Bhatti G, Done B, et al. Pregnancy-specific transcriptional changes upon endotoxin exposure in mice. J Perinat Med. 2020;48:700–22.
73. Scapini P, Cassatella MA. Social networking of human neutrophils within the immune system. Blood. 2014;124:710–9.
74. Dollner H, Vatten L, Halgunset J, Rahimipoor S, Austgulen R. Histologic chorioamnionitis and umbilical serum levels of pro-inflammatory cytokines and cytokine inhibitors. BJOG. 2002;109:534–9.
75. Yoshimura T, Matsushima K, Tanaka S, Robinson EA, Appella E, Oppenheim JJ, et al. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. Proc Natl Acad Sci U S A. 1987;84:9233–7.
76. Garcia-Flores V, Romero R, Xu Y, Theis KR, Arenas-Hernandez M, Miller D, et al. Maternal-fetal immune responses in pregnant women infected with SARS-CoV-2. Nat Commun. 2022;13:320.
77. Revello R, Alcaide MJ, Dudzik D, Abehsera D, Bartha JL. Differential amniotic fluid cytokine profile in women with chorioamnionitis with and without funisitis. J Matern Fetal Neonatal Med. 2016;29:2161–5.

78. Nikiforou M, Vanderlocht J, Chougnet CA, Jellema RK, Ophelders DR, Joosten M, et al. Prophylactic interleukin-2 treatment prevents fetal gut inflammation and injury in an ovine model of chorioamnionitis. Inflamm Bowel Dis. 2015;21:2026–38.
79. Gomez-Lopez N, Romero R, Hassan SS, Bhatti G, Berry SM, Kusanovic JP, et al. The cellular transcriptome in the maternal circulation during normal pregnancy: a longitudinal study. Front Immunol. 2019;10:2863.
80. Pique-Regi R, Romero R, Tarca AL, Sendler ED, Xu Y, Garcia-Flores V, et al. Single cell transcriptional signatures of the human placenta in term and preterm parturition. eLife. 2019;8:e52004. https://doi.org/10.7554/eLife.52004.
81. Tarca AL, Romero R, Xu Z, Gomez-Lopez N, Erez O, Hsu CD, et al. Targeted expression profiling by RNA-Seq improves detection of cellular dynamics during pregnancy and identifies a role for T cells in term parturition. Sci Rep. 2019;9:848.
82. Ando K, Hédou JJ, Feyaerts D, Han X, Ganio EA, Tsai ES, et al. A peripheral immune signature of labor induction. Front Immunol. 2021;12:725989.

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