The Journal of Immunology

RESEARCH ARTICLE | APRIL 01 2022

IL-22 Plays a Dual Role in the Amniotic Cavity: Tissue Injury and Host Defense against Microbes in Preterm Labor FREE

Meyer Gershater; ... et. al

J Immunol (2022) 208 (7): 1595–1615.
https://doi.org/10.4049/jimmunol.2100439

IL-22 Plays a Dual Role in the Amniotic Cavity: Tissue Injury and Host Defense against Microbes in Preterm Labor

Meyer Gershater,[*,†] Roberto Romero,[*,‡,§,¶,‖] Marcia Arenas-Hernandez,[*,†] Jose Galaz,[*,†] Kenichiro Motomura,[*,†] Li Tao,[*,†] Yi Xu,[*,†] Derek Miller,[*,†] Roger Pique-Regi,[*,†,¶] Gregorio Martinez, III,[*,†] Yesong Liu,[*,†] Eunjung Jung,[*,†] Robert Para,[*,†] and Nardhy Gomez-Lopez[*,†,#]

IL-22 is a multifaceted cytokine with both pro- and anti-inflammatory functions that is implicated in multiple pathologies. However, the role of IL-22 in maternal-fetal immunity in late gestation is poorly understood. In this study, we first showed that IL-22$^+$ T cells coexpressing retinoic acid–related orphan receptor γt (ROR-γt) are enriched at the human maternal-fetal interface of women with preterm labor and birth, which was confirmed by in silico analysis of single-cell RNA sequencing data. T cell activation leading to preterm birth in mice was preceded by a surge in IL-22 in the maternal circulation and amniotic cavity; however, systemic administration of IL-22 in mice did not induce adverse perinatal outcomes. Next, using an ex vivo human system, we showed that IL-22 can cross from the choriodecidua to the intra-amniotic space, where its receptors (*Il22ra1*, *Il10rb*, and *Il22ra2*) are highly expressed by murine gestational and fetal tissues in late pregnancy. Importantly, amniotic fluid concentrations of IL-22 were elevated in women with sterile or microbial intra-amniotic inflammation, suggesting a dual role for this cytokine. The intra-amniotic administration of IL-22 alone shortened gestation and caused neonatal death in mice, with the latter outcome involving lung maturation and inflammation. IL-22 plays a role in host response by participating in the intra-amniotic inflammatory milieu preceding *Ureaplasma parvum*–induced preterm birth in mice, which was rescued by the deficiency of IL-22. Collectively, these data show that IL-22 alone is capable of causing fetal injury leading to neonatal death and can participate in host defense against microbial invasion of the amniotic cavity leading to preterm labor and birth. ***The Journal of Immunology*****, 2022, 208: 1595–1615.**

Interleukin-22 is a pleiotropic cytokine with both pro- and anti-inflammatory functions (1, 2), whose role varies according to location and microenvironment (3). Indeed, IL-22 has been recognized as a cytokine acting as a double-edged sword in the pathogenesis of many diseases, with respect to either inducing or ameliorating inflammation (4). The proinflammatory functions of IL-22 are well documented in skin and autoimmune disorders such as psoriasis (5, 6) and rheumatoid arthritis (7, 8). However, IL-22 also has a protective role in specific compartments such as mucosal tissues or epithelial barriers (9–11). In addition to its bidimensional nature, IL-22 is a unique cytokine because it is primarily produced by lymphoid cells but acts on nonimmune cells such as epithelial or stromal cells (4). Despite the abundance of literature documenting the role of IL-22 in autoimmune diseases, cancers, and intestinal complications, there is a paucity of information regarding the role of IL-22 in maternal-fetal immunity during late gestation, a vulnerable period in which maternal-fetal immune dysregulation can lead to premature birth and adverse neonatal outcomes (12–14).

Most cases of premature birth are preceded by spontaneous preterm labor (12, 15, 16), a syndrome of multiple pathological processes (17, 18). Of these etiologies, only acute pathological inflammation of the amniotic cavity (i.e., intra-amniotic inflammation) has been causally linked to preterm labor leading to premature birth (19–23). Such a local inflammatory process can be induced by microorganisms invading the amniotic cavity (i.e., microbial intra-amniotic inflammation or intra-amniotic infection) or by alarmins released upon cellular stress (i.e., sterile intra-amniotic inflammation) (24–27). In the former, the inflammatory process represents the host response against microbes invading the amniotic cavity (28–33), which leads to preterm labor and birth as an unintended

*Perinatology Research Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD, and Detroit, MI; †Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI; ‡Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI; §Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI; ¶Center for Molecular Obstetrics and Genetics, Wayne State University, Detroit, MI; ‖Detroit Medical Center, Detroit, MI; and #Department of Biochemistry, Microbiology, and Immunology, Wayne State University School of Medicine, Detroit, MI

ORCIDs: 0000-0002-4448-5121 (R.R.); 0000-0002-1178-6112 (M.A.-H.); 0000-0003-1519-5501 (K.M.); 0000-0003-1531-358X (L.T.); 0000-0002-1262-2275 (R.P.-R.); 0000-0003-0099-1505 (G.M.); 0000-0003-0951-4692 (Y.L.); 0000-0003-0897-0039 (E.J.); 0000-0002-3406-5262 (N.G.-L.).

Received for publication May 7, 2021. Accepted for publication January 18, 2022.

This work was supported in part by the Perinatology Research Branch, Division of Obstetrics and Maternal-Fetal Medicine, Division of Intramural Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services (NICHD/NIH/DHHS), and in part with federal funds from NICHD/NIH/DHHS under Contract HHSN275201300006C. This research was also supported by the Wayne State University Perinatal Initiative in Maternal, Perinatal and Child Health. R.R. has contributed to this work as part of his official duties as an employee of the U.S. Federal Government.

Address correspondence and reprint requests to Prof. Nardhy Gomez-Lopez, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Perinatology Research Branch, NICHD/NIH/DHHS, 275 E. Hancock Street, Detroit, MI 48201. E-mail address: nardhy.gomez-lopez@wayne.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: B6, C57BL/6; dpc, day postcoitum; ESI-MS, electrospray ionization mass spectrometry; ILC, innate lymphoid cell; PBST, PBS with 0.1% Tween 20; PTL, preterm with labor; RDS, respiratory distress syndrome; rh, recombinant human; rm, recombinant mouse; ROR-γt, retinoic acid–related orphan receptor γt; scRNA-seq, single-cell RNA sequencing; SP-A, surfactant protein A; TIL, term with labor; TNL, term without labor; t-SNE, *t*-distributed stochastic neighbor embedding.

consequence (34, 35). Both microbial products and alarmins are sensed by pattern recognition receptors, which are mainly present in innate immune cells (36); therefore, most maternal-fetal immunology research has focused on the role of innate immunity (37–54). However, acute inflammation can also be mediated by maternal T cells, whose infiltration is well documented at the maternal-fetal interface (i.e., decidua) (55–62). Indeed, our group has shown that the activation of decidual maternal T cells is associated with the physiological process of labor at term (61–65). Furthermore, we reported that the premature activation of maternal effector T cells can trigger acute inflammatory mechanisms that lead to preterm labor and birth (66, 67) and that such activation can be noninvasively monitored in the maternal circulation (65, 68). However, the soluble mediators produced by effector and activated T cells at the maternal-fetal interface of women with preterm labor, which may represent a potential therapeutic target for premature birth and its adverse neonatal outcomes, are poorly understood.

In this study, we first aimed to identify the cellular sources of IL-22 at the maternal-fetal interface of women who underwent preterm labor and birth. Using available single-cell RNA sequencing (scRNA-seq) human data, we also explored the expression of IL-22 and its receptors by decidual immune cell types. Next, we investigated whether systemic levels of IL-22 were increased upon in vivo maternal T cell activation, and whether the i.v. administration of this cytokine alone could induce adverse pregnancy outcomes in mice. In addition, we explored the expression of *Il22* and its receptors by gestational (fetal membranes and placenta) and fetal (lung and intestine) tissues in late murine pregnancy as a possible explanation of the elevated concentrations of IL-22 in the amniotic cavity (i.e., fetal compartment) of dams upon in vivo maternal T cell activation. We also evaluated whether IL-22 can cross from the maternal to the fetal side using an ex vivo model of the human maternal-fetal interface, and measured the concentrations of this cytokine in amniotic fluid from well-characterized subsets of women who underwent spontaneous preterm labor and birth. Such clinical data led us to propose a dual role for IL-22 in maternal-fetal immunity: 1) IL-22 alone is capable of causing fetal tissue injury leading to neonatal death, and 2) IL-22 participates in host defense in microbial invasion of the amniotic cavity leading to preterm birth. To test the former, IL-22 was intra-amniotically injected in mice under ultrasound guidance to evaluate adverse perinatal outcomes, and its effects on tissue injury were determined in the neonatal lung and intestine. To test the latter, the role of IL-22 in host defense against *Ureaplasma parvum*–induced preterm birth was thoroughly evaluated in wild-type and knockout mice.

Materials and Methods

Human subjects and clinical specimens

Human placental basal plate (decidua basalis) and chorioamniotic membrane (decidua parietalis) samples were collected within 30 min after delivery at the Detroit Medical Center, Wayne State University, Perinatology Research Branch, an intramural program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services, Detroit, MI. Amniotic fluid samples were retrieved from the Biorepository of the Perinatology Research Branch. The collection and utilization of biological materials for research purposes were approved by the Institutional Review Boards of these institutions. All participating women provided written informed consent prior to the collection of samples. Two separate cohorts of women were used in this study.

The first cohort comprised women who delivered at term with labor (TIL), at term without labor (TNL), preterm with labor (PTL), or preterm without labor whose placental basal plate and chorioamniotic membranes were collected and used for immunophenotyping (please see clinical definitions below). The demographic and clinical characteristics of the study populations are shown in Table I.

The second cohort comprised the following: 1) women with spontaneous preterm labor and intact membranes who delivered at term without intra-amniotic inflammation, and women with preterm labor and intact membranes who delivered preterm, who were divided into the following groups: 2) women without microbial or sterile intra-amniotic inflammation, 3) women with sterile intra-amniotic inflammation, and 4) women with microbial intra-amniotic inflammation. Please see the clinical definitions below. In this cohort, amniotic fluid samples (the remnant that was not used for clinical determinations) were used to determine IL-22. The demographic and clinical characteristics of the study population are shown in Table II.

Clinical definitions

Gestational age was determined by the date of the last menstrual period and confirmed by the first ultrasound examination or by ultrasound examination alone when the sonographic determination of gestational age was inconsistent

Table I. Demographic and clinical characteristics of the study population for immunophenotyping of cellular sources of IL-22

	Term Not in Labor (n = 11)	Term in Labor (n = 30)	Preterm Not in Labor (n = 11)	Preterm in Labor (n = 26)	p value
Maternal age (y; median [IQR])[a]	26 (23–31)	24 (20.3–25.8)	31 (27.5–33)	26 (22.3–28.8)	0.004
Body mass index (kg/m^2; median [IQR])[a]	35.1 (29.2–39)	25.6 (23–31.2)	34.7 (29.1–35.8)	31.1 (25.9–40.4)[b]	0.02
Primiparity[c]	0% (0/11)	6.7% (2/30)	0% (0/11)	23.1% (6/26)	0.09
Race/ethnicity[c]					0.4
African American	81.8% (9/11)	90% (27/30)	90.9% (10/11)	76.9% (20/26)	
White	18.2% (2/11)	3.3% (1/30)	9.1% (1/11)	19.2% (5/26)	
Other	0% (0/11)	6.7% (2/30)	0% (0/11)	3.8% (1/26)	
Gestational age at delivery (wk; median [IQR])[a]	39.1 (39–39.3)	39.2 (38.6–40.3)	33.4 (31–36.4)	34.6 (33.7–35.9)	<0.001
Birthweight (g; median [IQR])[a]	3370 (3125–3705)	3188 (2839–3319)	1730 (1383–2508)	2233 (1809–2440)	<0.001
Acute maternal inflammatory response[c]					
Stage 1 (early acute subchorionitis or chorionitis)	9.1% (1/11)	33.3% (10/30)	0% (0/11)	15.4% (4/26)	0.07
Stage 2 (acute chorioamnionitis)	0% (0/11)	23.3% (7/30)	0% (0/11)	15.4% (4/26)	0.14
Stage 3 (necrotizing chorioamnionitis)	0% (0/11)	0% (0/30)	0% (0/11)	0% (0/26)	1
Acute fetal inflammatory response[c]					
Stage 1 (chorionic vasculitis or umbilical phlebitis)	0% (0/11)	23.3% (7/30)	0% (0/11)	3.8% (1/26)	0.06
Stage 2 (umbilical arteritis)	0% (0/11)	6.7% (2/30)	0% (0/11)	15.4% (4/26)	0.4
Stage 3 (necrotizing funisitis)	0% (0/11)	0% (0/30)	0% (0/11)	0% (0/26)	1

Data are given as median (interquartile range [IQR]) and percentage (n/N).
[a]Kruskal–Wallis test.
[b]Three missing data points.
[c]Fisher exact test.

Table II. Demographic and clinical characteristics of the study population for measurements of IL-22 in amniotic fluid

	Term Delivery	Preterm Delivery			
	Preterm Labor Who Delivered at Term (n = 20)	Preterm Labor without Sterile or Microbial Intra-Amniotic Inflammation (n = 27)	Preterm Labor with Sterile Intra-Amniotic Inflammation (n = 27)	Preterm Labor with Microbial Intra-Amniotic Inflammation (n = 16)	p Value
Maternal age (y; median [IQR])[a]	23 (20.8–25.5)	22 (19–25.5)	24 (20.5–27)	23.5 (20–27)	0.7
Body mass index (kg/m^2; median [IQR])[a]	22.7 (20.9–29.4)	25.3 (21.5–28.9)[b]	28.2 (23.2–33.4)[b]	23.7 (21.6–31.7)[c]	0.2
Primiparity[d]	10% (2/20)	29.6% (8/27)	56.3% (9/16)	18.8 (3/16)	0.2
Race/ethnicity[d]					0.6
African American	100% (20/20)	81.5% (22/27)	88.9% (24/27)	93.8% (15/16)	
White	0% (0/20)	11.1% (3/27)	7.4% (2/27)	6.2% (1/16)	
Other	0% (0/20)	7.4% (2/27)	3.7% (1/27)	0% (0/16)	
Gestational age at amniocentesis (wk; median [IQR])[a]	31.3 (30.7–32.3)	30.6 (28.5–32.4)	26.4 (23.8–30.2)	26.4 (22.5–30.2)	0.002
IL-6 (ng/ml; median [IQR])[a]	0.4 (0.3–0.7)	0.5 (0.4–1)	11.2 (5.4–22.9)	98.9 (26.6–130.1)	<0.001
Amniotic fluid glucose (mg/dl; median [IQR])[a]	28.5 (22.8–32)	27 (20–32.5)	21 (19–26)[c]	10 (1–18)[e]	<0.001
Amniotic fluid WBC (cells/mm^3; median [IQR])[a]	0.5 (0–4.3)	1 (0–3)	2.5 (0.8–13.3)[b]	285 (23–458)	<0.001
Gestational age at delivery (wk; median [IQR])[a]	38.7 (37.4–39)	34 (31.8–35.8)	26.7 (24.5–31.3)	26.4 (22.5–30.3)	<0.001
Cesarean section[d]	0% (0/20)	3.7% (1/27)	22.2% (6/27)	12.5% (2/16)	0.047
Birthweight (g; median [IQR])[a]	3049 (2900–3294)	2190 (1588–2364)	917 (593–1545)	985 (458–1261)	<0.001
Acute maternal inflammatory response					
Stage 1 (early acute subchorionitis or chorionitis)	10.5% (2/19)[e]	13% (3/23)[f]	29.2% (7/24)[b]	13.3% (2/15)[e]	0.5
Stage 2 (acute chorioamnionitis)	15.8% (3/19)[e]	21.7% (5/23)[f]	12.5% (3/24)[b]	20% (3/15)[e]	0.9
Stage 3 (necrotizing chorioamnionitis)	0% (0/19)[e]	0% (0/23)[f]	16.6% (4/24)[b]	66.6% (10/15)[e]	<0.001
Acute fetal inflammatory response					
Stage 1 (chorionic vasculitis or umbilical phlebitis)	15.8% (3/19)[e]	8.7% (2/23)[f]	20.8% (5/24)[b]	33.3% (5/15)[e]	0.1
Stage 2 (umbilical arteritis)	5.3% (1/19)[e]	4.3% (1/23)[f]	4.2% (1/24)[b]	46.7% (7/15)[e]	<0.001
Stage 3 (necrotizing funisitis)	0% (0/19)[e]	0% (0/23)[f]	0% (0/24)[b]	0% (0/15)[e]	1.0

Data are given as median (interquartile range, IQR) and percentage (n/N).
[a]Kruskal–Wallis test with multiple comparison.
[b]Three missing data points.
[c]Two missing data points.
[d]Fisher exact test.
[e]One missing data point.
[f]Four missing data points.

with menstrual dating (69). Spontaneous preterm labor was defined as the presence of regular uterine contractions with a frequency of at least two every 10 min and cervical ripening between 20 and 36 (6/7) wk of gestation. Preterm delivery was defined as birth before 37 wk of gestation. Term labor was defined by the presence of regular uterine contractions at a frequency of at least two contractions every 10 min with cervical changes resulting in delivery at term (≥37 wk of gestation). Standard clinical laboratory determinations in amniotic fluid included the evaluation of the WBC count (70), glucose concentration (71), Gram stain (72), and microbiological culture of aerobic/anaerobic bacteria and genital mycoplasmas (73). The study group classifications used in this study (second cohort) were determined by combining the presence or absence of microbes together with the evaluation of amniotic fluid IL-6 concentrations as an indicator of intra-amniotic inflammation, as previously established (74). Thus, a positive microbial signal (either by culture/Gram stain or PCR/electrospray ionization mass spectrometry [ESI-MS]), together with elevated IL-6 (≥2.6 ng/ml), indicates microbial intra-amniotic inflammation (24–26, 75); a negative microbial signal indicated by both culture/Gram stain and PCR/ESI-MS, together with elevated IL-6, indicates sterile intra-amniotic inflammation (24–26, 75); and the absence of microbial signals (indicated by both culture/Gram stain and PCR/ESI-MS), together with low IL-6 levels, indicates no intra-amniotic inflammation. Women with preterm prelabor rupture of membranes, a multiple gestation, or who presented a fetus with chromosomal and/or sonographic abnormalities were excluded from this study. Maternal and neonatal data were obtained by retrospective clinical chart review.

Placental histopathological examination

Placentas were examined histologically by perinatal pathologists blinded to clinical diagnoses and obstetrical outcomes according to standardized Perinatology Research Branch protocols (76). Briefly, three to nine sections of the placenta were examined, and at least one full-thickness section was taken from the center of the placenta; others were taken randomly from the placental disc. Acute inflammatory lesions of the placenta were diagnosed according to established criteria (77–79).

Human decidual leukocyte isolation

Decidual leukocytes were isolated from the decidual tissue of the subjects in each study group (Table I), as previously described (80). Briefly, the decidua basalis was collected from the basal plate of the placenta and the decidua parietalis was separated from the chorioamniotic membranes. The decidual tissues were homogenized by using a gentleMACS dissociator (Miltenyi Biotec, San Diego, CA) in StemPro Accutase cell dissociation reagent (Life Technologies, Grand Island, NY). Homogenized tissues were incubated in Accutase for 45 min at 37°C with gentle agitation. Following incubation, tissues were washed in PBS (Life Technologies) and filtered through a 100-μm cell strainer (Fisher Scientific, Durham, NC). Cell suspensions were collected and centrifuged at 300 × g for 10 min at 4°C. The decidual mononuclear cells were purified with Ficoll-Paque Plus (GE Healthcare Biosciences, Uppsala, Sweden), following the manufacturer's instructions. The mononuclear cells were then collected and washed with PBS and immediately used for immunophenotyping.

Immunophenotyping of human decidual IL-22–expressing cells

Mononuclear cell suspensions from decidual tissues were stained with BD Horizon Fixable Viability Stain 510 dye (BD Biosciences) prior to incubation with extracellular and intracellular mAbs (Supplemental Table I). Mononuclear cell suspensions were then washed with FACS staining buffer

(catalog no. 554656; BD Biosciences) and incubated with 20 μl of human FcR blocking reagent (catalog no. 130-059-901; Miltenyi Biotec) in 80 μl of FACS staining buffer (BD Biosciences) for 10 min at 4°C. The cells were incubated with extracellular fluorochrome-conjugated anti-human mAbs (Supplemental Table I) for 30 min at 4°C in the dark. After extracellular staining, the cells were fixed and permeabilized using the Foxp3/transcription factor staining buffer set (catalog no. 00-5523-00; eBioscience, San Diego, CA), prior to staining with intracellular and intranuclear Abs (Supplemental Table I). Finally, stained cells were washed and resuspended in 0.5 ml of FACS staining buffer and acquired using an LSRFortessa flow cytometer and FACSDiva 6.0 software (BD Biosciences). Leukocyte subsets were gated within the viability gate. Immunophenotyping included the identification of IL-22–expressing cells in the following subsets: neutrophils ($CD14^-CD15^+$); monocytes/macrophages ($CD15^-CD14^+$), T cells ($CD14^-CD15^-CD19^-CD3^+$), B cells ($CD14^-CD15^-CD3^-CD19^+$), NK cells ($CD14^-CD15^-CD3^-CD19^-CD56^+$), and innate lymphoid cells (ILCs; $CD14^-CD15^-CD3^-CD19^-CD11b^-CD56^-CD127^+$). The expression of IL-22 by retinoic acid–related orphan receptor γt ($ROR\text{-}\gamma t)^+$ and by $CD69^+ROR\text{-}\gamma t^+$ T cells was also reported. Flow cytometry analysis was performed with FlowJo software version 10 (FlowJo, Ashland, OR).

In silico scRNA-seq analysis of the human placental tissues

Publically available scRNA-seq data from the decidual tissues (the basal plate and chorioamniotic membranes) of TNL, TIL, and PTL women ($n = 3$ per group) (68) were used to explore the expression of *IL22*, *IL22RA1*, *IL10RB*, *IL22RA2*, and *RORC*. Briefly, raw fastq files were downloaded from previously established resources in National Center for Biotechnology Information dbGaP phs001886.v1.p1 (68). The fastq files were then aligned using kallisto (81), and bustools (82) summarized the cell/gene transcript counts in a matrix for each sample, using the "lamanno" workflow for scRNA-seq. Each sample was then processed using DIEM (83) to eliminate debris and empty droplets. All count data matrices were then normalized and combined using the "NormalizeData," "FindVariableFeatures," and "ScaleData" methods implemented in the Seurat package in R (Seurat version 3.1, R version 3.6.1) (84, 85). Afterward, the Seurat "RunPCA" function was applied to obtain the first 50 principal components, and the different batches and locations were integrated and harmonized using the Harmony package in R (86). To label the cells, the Seurat "FindTransferAnchors" and "TransferData" functions were used for each group of locations separately to assign a cell type identity based on our previously labeled data as reference panel (as performed in Ref. 68). Visualization of gene expression for each cell type was performed using the ggplot2 (87) package in R with gene expression values scaled to transcripts per million and to the proportion of cells expressing the transcript within a given cell type (88).

Maternal-fetal IL-22 transfer assay

Human chorioamniotic membrane samples were collected from women at TNL who delivered via cesarean section. All experiments were performed under sterile conditions. Chorioamniotic membranes were rinsed carefully in sterile PBS and cut into 5 × 5-cm squares. The polyethylene terephthalate membranes of six-well Falcon cell culture inserts (Corning Life Sciences, Glendale, AZ) were removed and chorioamniotic membrane sections were held in place, using orthodontic rubber bands, with the choriodecidual side facing upward. The culture inserts with membrane sections were placed into the six-well culture plates. Then, 1 ml of DMEM supplemented with 10% FBS (Thermo Fisher Scientific, Waltham, MA) and 1% penicillin/streptomycin (Thermo Fisher Scientific) containing either 6 ng ($n = 3$), 60 ng ($n = 4$), or 600 ng ($n = 3$) of the carrier-free recombinant human (rh)IL-22 (catalog no. 782-IL/CF; R&D Systems, Minneapolis, MN) was gently added on top of the culture insert, and 1 ml of the same medium without rhIL-22 was added to the bottom wells of the culture plate. After incubation for 8 h at 37°C with 5% CO_2, the supernatants (media) from upper wells (choriodecidua-maternal side) and lower wells (amnion-fetal side) were collected into separate cryovials and stored at −80°C. The IL-22 concentrations from the upper and lower supernatants were determined by using the human IL-22 Quantikine ELISA kit (R&D Systems), according to the manufacturer's instructions. Assays were read using the SpectraMax iD5 (Molecular Devices, San Jose, CA), and analyte concentrations were calculated with SoftMax Pro 7 (Molecular Devices). The sensitivity of the assay was 2.7 pg/ml. The lower (fetal side) supernatant concentrations were divided by the upper (maternal side) supernatant concentrations to obtain the proportion of IL-22 that was transferred across the chorioamniotic membranes.

Determination of IL-22 concentrations in human amniotic fluid

Human amniotic fluid samples from women with spontaneous preterm labor were retrieved from the Biorepository of the Perinatology Research Branch (Table II). A U-PLEX immunoassay (Meso Scale Discovery, Rockville, MD) was used to measure the concentrations of IL-22 in the human amniotic fluid samples, according to the manufacturer's instructions. The plate was read using the MESO QuickPlex SQ 120 (Meso Scale Discovery), and analyte concentrations were calculated with Discovery Workbench 4.0 (Meso Scale Discovery). The sensitivity of the IL-22 assay was 0.13 pg/ml.

Mice

C57BL/6 (stock no. 000664; hereafter referred to as B6 or $Il22^{+/+}$) and C57BL/6-$Il22^{tm1.1(icre)Stck}$ (stock no. 027524; hereafter referred to as $Il22^{-/-}$) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred in the animal care facility at the C.S. Mott Center for Human Growth and Development at Wayne State University (Detroit, MI). All mice were kept under a circadian cycle (12-h light/12-h dark). Females 8–12 wk of age were bred with males of the same genotype and proven fertility. Female mice were checked daily between 8:00 AM and 9:00 AM for the appearance of a vaginal plug, indicating 0.5 d postcoitum (dpc). Females were then housed separately from the males, their weights were monitored daily, and pregnancy was confirmed by 12.5 dpc by a gain of at least 2 g. Animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Wayne State University (Protocol Nos. A-09-08-12, A-07-03-15, 18-03-0584, and 21-04-3506).

Murine model of maternal T cell activation-induced preterm birth

B6 dams were injected i.p. with 10 μg/200 μl of monoclonal anti-CD3ε Ab (clone 145-2C11; BD Biosciences) in sterile PBS (Fisher Scientific Chemicals, Fair Lawn, NJ) using a 26G needle on 16.5 dpc, as previously described (66, 89). Controls were injected i.p. with 10 μg/200 μl of IgG1κ isotype control (clone A19-3; BD Biosciences) in sterile PBS. Mice were euthanized at 12–16 h postinjection and maternal peripheral blood was collected by cardiac puncture ($n = 18$ [isoytope control] and 19 [anti-CD3ε]). Amniotic fluid was collected from each amniotic sac with a 26G needle ($n = 5$ per group). Serum was separated from the maternal blood samples by centrifugation (1300 × *g* for 10 min at room temperature) and stored at −20°C. Amniotic fluid samples were centrifuged at 1300 × *g* for 5 min at 4°C, and the supernatants were separated and stored at −20°C. The mouse/rat IL-22 Quantikine ELISA kit (R&D Systems) was used to measure the concentrations of IL-22 in the amniotic fluid and serum samples, according to the manufacturer's instructions. The plates were read using the SpectraMax iD5 (Molecular Devices), and analyte concentrations were calculated with SoftMax Pro 7 (Molecular Devices). The sensitivity of the IL-22 assay was 3.2 pg/ml.

Analysis of the basal expression of Il22 and its receptors by the murine fetal tissues

B6 dams were euthanized at 16.5 ($n = 8$) or 17.5 ($n = 8$) dpc to harvest the placenta, fetal membranes, fetal lung, and fetal intestine. The lung of dams i.p. injected with 10 μg/200 μl of LPS (*Escherichia coli*; 055:B5, Sigma-Aldrich, St. Louis, MO) on 16.5 dpc ($n = 3$) (90) were used as a positive control for *Il22* expression. Tissues were collected into RNAlater solution (Thermo Fisher Scientific, Vilnius, Lithuania) and stored at −80°C until RNA isolation.

Ultrasound-guided intra-amniotic injection of IL-22 in mice

B6 dams were anesthetized on 16.5 dpc by inhalation of 2% isoflurane (Aerrane; Baxter Healthcare, Deerfield, IL; Fluriso, isoflurane USP; VetOne, Boise, ID) and 1–2 l/min of oxygen in an induction chamber. Anesthesia was maintained with a mixture of 1.5–2% isoflurane and 1.5–2 l/min of oxygen. Mice were positioned on a heating pad and stabilized with adhesive tape. Fur was removed from the abdomen of the dams by applying Nair cream (Church & Dwight, Ewing, NJ). Body temperature was maintained in the range of 37 ± 1°C and detected with a rectal probe (VisualSonics, Toronto, ON, Canada), and respiratory and heart rates were monitored by electrodes embedded in the heating pad. An ultrasound probe was fixed and mobilized with a mechanical holder, and the transducer was slowly moved toward the abdomen. Syringes were stabilized by a mechanical holder (VisualSonics). Injections were performed in each amniotic sac using a 30G needle (BD PrecisionGlide needle; Becton Dickinson, Franklin Lakes, NJ). Dams were intra-amniotically injected with 1.2 pg/25 μl ($n = 6$), 70 pg/25 μl ($n = 8$), or 612 pg/25 μl ($n = 8$) of carrier-free recombinant mouse (rm) IL-22 (catalog no. 576202; BioLegend, San Diego, CA) into each amniotic sac on 16.5 dpc. Controls were injected with 25 μl of PBS per sac ($n = 8$). After ultrasound examination, mice were placed under heat lamps for recovery (defined as when the mouse resumed normal activities such as walking and responding), which typically occurred within 10 min after removal from anesthesia.

i.v. administration of IL-22 in mice

B6 dams were i.v. injected with 689 pg of rmIL-22 in 100 μl of PBS using a 26G needle on 16.5 dpc ($n = 8$). Controls were injected with 100 μl of PBS alone ($n = 5$). The used dose of rmIL-22 was selected based on the plasma concentrations of IL-22 in women with spontaneous preterm labor (91).

Video monitoring of pregnancy and neonatal outcomes induced by IL-22 in mice

Immediately after intra-amniotic or i.v. injection, B6 dams were monitored until delivery by an infrared camera (Sony, Tokyo, Japan). Gestational length was defined as the time elapsed from the detection of the vaginal plug (0.5 dpc) to the delivery of the first pup. The rate of neonatal mortality at birth was determined for each litter (pups delivered from the same dam) and defined as the proportion of delivered pups found dead among the total litter size. The average of the rates of neonatal mortality per litter was then calculated and plotted for each experimental group. Representative images of neonates (day 0) born to dams injected with rmIL-22 or PBS (controls) are shown. Neonatal weights were recorded at 1, 2, and 3 wk of age.

Tissue analysis of neonates born to mice intra-amniotically injected with IL-22

B6 dams were intra-amniotically injected with 612 pg/25 μl per sac of rmIL-22 ($n = 10$) or 25 μl of PBS per sac (controls, $n = 10$) on 16.5 dpc, and were monitored until delivery. Neonates born to dams were euthanized on day 0 to harvest the neonatal lung and intestine. Tissues were collected into RNAlater solution and stored at −80°C until RNA isolation. Furthermore, the neonatal lung was snap-frozen for preparation of tissue extracts, in which the concentration of surfactant protein A (SP-A) was determined by using a mouse pulmonary SP-A ELISA kit (Cusabio, Wuhan, China), following the manufacturer's instructions. Plates were read using the SpectraMax iD5 (Molecular Devices, San Jose, CA), and analyte concentrations were calculated with SoftMax Pro 7 (Molecular Devices). The sensitivity of the assay was 0.78 pg/ml.

Murine model of U. parvum–induced preterm birth

U. parvum was prepared for intra-amniotic injection, as previously described (92). Briefly, *U. parvum* was isolated from women with intra-amniotic infection, aliquoted, and stored at −80°C with 50% glycerol (Teknova, Hollister, CA; with a final concentration of glycerol 25%). The stocks were inoculated in SP4 broth (Hardy Diagnostics, Santa Maria, CA) and cultured at 37°C for 4–12 h to reach the exponential phase based on growth rate. After incubation, the bacterial cells were counted by flow cytometry, using the Live/Dead BacLight bacterial viability and counting kit (Invitrogen by Thermo Fisher Scientific, Carlsbad, CA). The cells were diluted to a target concentration ($1–5 \times 10^4$ cells/25 μl) with SP4 broth.

B6 dams were intra-amniotically injected with $1–5 \times 10^4$ cells/25 μl of *U. parvum* per sac ($n = 10$) or 25 μl of SP4 broth per sac (controls, $n = 10$) on 16.5 dpc. The rate of preterm birth induced by *U. parvum* in B6 mice is ∼40–50% (92), which is consistently reported herein in *Il22*$^{+/+}$ mice. B6 dams were euthanized at 16 h postinjection, 4–5 h prior to previously observed times of preterm delivery, for collection of peripheral blood by cardiac puncture. Amniotic fluid was also collected from each amniotic sac with a 26G needle. Serum was separated from the maternal blood samples by centrifugation ($1300 \times g$ for 10 min at room temperature) and stored at −20°C. Amniotic fluid samples were centrifuged at $1300 \times g$ for 5 min at 4°C, and the supernatants were separated and stored at −20°C. A ProcartaPlex mouse cytokine and chemokine panel 1A 36-plex (Thermo Fisher Scientific, Vienna, Austria) was used to measure the concentrations of IL-22 in serum and amniotic fluid samples, according to the manufacturer's instructions. The plates were read using the Luminex FLEXMAP 3D (Luminex, Austin, TX), and analyte concentrations were calculated with xPONENT version 4.2 (Luminex). The sensitivity of the IL-22 assay was 0.24 pg/ml.

Collection of amniotic fluid and fetal tissues from mice intra-amniotically injected with U. parvum

B6 dams were intra-amniotically injected with $1–5 \times 10^4$ cells/25 μl of *U. parvum* per sac ($n = 8$) or 25 μl of SP4 broth per sac (controls, $n = 8$) on 16.5 dpc. Dams were euthanized at 16 h postinjection, and amniotic fluid was collected from each amniotic sac with a 26G needle. The placenta, fetal membranes, fetal lung, and fetal intestine were collected into RNAlater solution (Thermo Fisher Scientific) and stored at −80°C until RNA isolation.

Analysis of amniotic fluid from mice intra-amniotically injected with U. parvum

The murine amniotic fluid concentrations of several IL-22 pathway–related cytokines were determined using the U-PLEX Biomarker Group 1 (ms) assay, SECTOR (Meso Scale Discovery), following the manufacturer's instructions. This assay allowed for the determination of IFN-γ, IL-1β, IL-6, IL-13, IL-17A, IL-17C, IL-17E/IL-25, IL-21, IL-22, and IL-23 concentrations. The plate was read using the MESO QuickPlex SQ 120 (Meso Scale Discovery), and analyte concentrations were calculated with the Discovery Workbench 4.0 (Meso Scale Discovery). The sensitivities of the assays were as follows: 0.16 pg/ml (IFN-γ), 3.1 pg/ml (IL-1β), 4.8 pg/ml (IL-6), 2.7 pg/ml (IL-13), 0.30 pg/ml (IL-17A), 2.3 pg/ml (IL-17C), 1.6 pg/ml (IL-17E/IL-25), 6.5 pg/ml (IL-21), 1.2 pg/ml (IL-22), and 4.9 pg/ml (IL-23).

Cytospin slides of amniotic fluid samples ($n = 3$) were prepared using Fisherbrand Superfrost microscope slides (Thermo Fisher Scientific) and a Shandon Cytospin 3 cytocentrifuge (Thermo Fisher Scientific) at 800 rpm for 5 min. After fixation with 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA), the cytospin slides were rinsed with PBS at room temperature for 2 min. Slides were then permeabilized using 0.25% Triton X-100 (Electron Microscopy Sciences) diluted in PBS for 5 min, then rinsed with PBS for 5 min. Nonspecific Ab interaction was blocked by using the BlockAid blocking solution (catalog no. B10710, Thermo Fisher Scientific) for 1 h at room temperature. Afterwards, the rat anti-mouse F4/80 (catalog no. 123140, BioLegend) and rabbit polyclonal IL-22 (catalog no. bs-2623R, Bioss, Woburn, MA) Abs were added and the slides incubated for 60 min. Then, the slides were washed with PBS with 0.1% Tween 20 (PBST) three times for 10 min each time. Next, the slides were stained with the secondary Abs goat anti-rat F4/80-Alexa Fluor 594 (catalog no. A11007, Thermo Fisher Scientific) and goat anti-rabbit IgG-Alexa Fluor 647 (catalog no. A31634, Thermo Fisher Scientific) for 30 min in the dark and washed again three times with PBST for 10 min each time. Then, the slides were stained with rat anti-mouse Ly-6G-Alexa Fluor 488 Ab (catalog no. 127626, BioLegend) and incubated for 60 min. The slides were again washed three times with PBST for 10 min each time, treated with Spectral DAPI (catalog no. 2451506, PerkinElmer, Waltham, MA) for 5 min, then washed in PBST for 10 min. Lastly, ProLong diamond anti-fade mountant with DAPI (Thermo Fisher Scientific) was applied to the slide and sealed with a coverslip. The immunofluorescence images were obtained using an All-in-One fluorescence microscope BZ-X810 (Keyence, Itasca, IL) and prepared with BZ-X800 Analyzer software v1.1.1.8 (Keyence).

RNA isolation and quantitative real-time PCR

RNA was isolated from RNAlater-collected tissues by using the Qiagen RNeasy mini kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The purity, concentration, and integrity of the RNA samples were assessed using the NanoDrop 1000 or 8000 spectrophotometer (Thermo Scientific, Wilmington, DE) and the Agilent Bioanalyzer 2100 (Agilent Technologies, Wilmington, DE). cDNA was synthesized using SuperScript IV VILO master mix (Invitrogen, Thermo Fisher Scientific Baltics, Vilnius, Lithuania). The gene expression of *Il22*, *Il22ra1*, *Il10rb*, and *Il22ra2* was determined by quantitative PCR and the ABI 7500 Fast real-time PCR system (Applied Biosystems, Alameda, CA) with commercial TaqMan gene expression assays. cDNA from the maternal lung was used to generate a standard curve to calculate the limit of detection of *Il22*, *Il22ra1*, *Il10rb*, and *Il22ra2*. Briefly, a standard curve based on duplicate 2-fold serial dilutions ranging from 100 ng of cDNA to 2.4×10^{-4} ng was generated, and the performance of the quantitative PCR assay by estimation of its efficiency based on the slope of regression lines was reported as the limit of detection.

Gene expression profiling was performed on the BioMark system for high-throughput quantitative real-time PCR (Fluidigm, San Francisco, CA) using commercial TaqMan gene expression assays (Applied Biosystems, Life Technologies, Pleasanton, CA). The Δ threshold cycle (ΔCt) values were determined using multiple reference genes (*Actb*, *Gusb*, *Gapdh*, and *Hsp90ab1*) averaged within each sample to determine gene expression levels. The −ΔCt values were normalized by calculating the *Z* score of each gene, and heatmaps were created using the Subio platform (Subio, Kagoshima, Japan; https://www.subioplatform.com/).

IL-22 detection in human amniotic fluid leukocytes by immunofluorescence

Cytospin slides of human amniotic fluid samples from women with microbial intra-amniotic inflammation ($n = 3$; see clinical definitions above) were prepared using Fisherbrand Superfrost microscope slides and a Shandon Cytospin 3 cytocentrifuge at 800 rpm for 5 min. After fixation with 4% paraformaldehyde, the cytospin slides were rinsed with PBS at room temperature for 2 min. The slides were then permeabilized using 0.25% Triton X-100

diluted in PBS for 5 min and rinsed with PBS for 5 min. Nonspecific Ab interaction was blocked by incubating the slides with BlockAid blocking solution for 1 h at room temperature. The mouse anti-human CD14 (catalog no. 347490, BD Biosciences) and the rabbit IL-22 polyclonal Abs were added and the slides were incubated for 60 min at room temperature. The slides were then washed three times for 10 min each time, after which the secondary Abs goat anti-mouse IgG Alexa Fluor 594 (catalog no. A32742, Thermo Fisher Scientific) and goat anti-rabbit IgG Alexa Fluor 488 (catalog no. A11034, Thermo Fisher Scientific) were added and the slides were incubated for 30 min. The slides were again washed three times with PBST for 10 min each time. A third primary Ab, mouse anti-human CD15-Alexa Fluor 647 (catalog no. 562369, BD Pharmingen), was added and the slides were incubated for 60 min. The slides were washed in PBST three times for 10 min each time. The slides were then incubated with Spectral DAPI for 5 min and washed with PBST for 10 min. Lastly, the ProLong diamond anti-fade mountant was applied to the slide and sealed with a coverslip. The immunofluorescence images were obtained using an All-in-One fluorescence microscope BZ-X810 and prepared with BZ-X800 Analyzer software v1.1.1.8.

Intra-amniotic injection of U. parvum into Il22$^{+/+}$ and Il22$^{-/-}$ mice

Il22$^{+/+}$ and *Il22*$^{-/-}$ dams were intra-amniotically injected with 5×10^4 cells/25 μl of *U. parvum* per sac ($n = 10$ each genotype) or 25 μl of SP4 broth per sac (controls, $n = 10$ each genotype) on 16.5 dpc. Dams were video monitored to determine the rate of preterm birth (preterm birth was defined as delivery occurring before 18.5 dpc, and its rate was represented by the percentage of females delivering preterm among the total number of mice injected) as well as to calculate the gestational length and rate of neonatal mortality, as described above.

Statistical analysis

Statistical analyses were performed with SPSS v19.0 (IBM, Armonk, NY) or GraphPad Prism version 8.0.1 for Windows (GraphPad Software, San Diego, CA). For human demographic data, the group comparisons were performed using Kruskal–Wallis tests for nonnormally distributed continuous variables or the Fisher exact test for nominal variables. The Mann–Whitney *U* test was used to compare differences between term and preterm (human) and control and treatment (mouse) groups. For comparisons of more than two groups, Kruskal–Wallis tests followed by a Dunn post hoc test for multiple comparisons were performed. The Mantel–Cox test was used when Kaplan–Meier survival curves were plotted and compared. Linear regression tests were performed to determine correlations. A p value ≤ 0.05 was considered statistically significant.

Results

IL-22 is predominantly expressed by T cells at the maternal-fetal interface of women with preterm labor

The main cellular source of IL-22 belongs to the lymphoid lineage, including innate and adaptive immune cells such as αβ T cells, γδ T cells, NKT cells, and ILCs (4). Therefore, we first investigated the expression of IL-22 by total T cells, B cells, NK cells, and ILCs at the human maternal-fetal interface. Herein, we use the term human maternal-fetal interface to refer to the decidua basalis and parietalis, which are the sites of contact between the uterine endometrium and the placental tissues (Fig. 1A). Fig. 1B represents the gating strategy used to identify major cell types in the decidual tissues, which are depicted in a *t*-distributed stochastic neighbor embedding (t-SNE) plot in Fig. 1C. Notably, IL-22$^+$ T cells were more abundant in the decidual tissues of women with preterm labor compared to term labor controls (Fig. 1D). Such T cells coexpressed ROR-γt; therefore, IL-22$^+$ROR-γt$^+$ T cells were enriched in the decidual tissues of women with preterm labor (Fig. 1E). Intriguingly, IL-22$^+$ T cells lacked expression of IL-17A, IFN-γ, IL-5, and IL-13, while simultaneously expressing moderate levels of CD127 (a marker of T cell memory [93, 94]) and high levels of CD69 (a marker of early T cell activation [95, 96]) (Fig. 1F). Indeed, CD69$^+$ROR-γt$^+$IL-22$^+$ T cells were present in high proportions in the human decidua, suggesting that most of such T cells are activated (Supplemental Fig. 1A). By contrast, IL-22$^+$ B cells, IL-22$^+$ NK cells (cells previously reported in the uterine mucosa and decidua [97, 98]), and IL-22$^+$ ILCs (cells previously reported in the uterine tissues [99–101]) were not significantly altered in the decidual tissues of women with preterm labor compared to term labor controls (Supplemental Fig. 1B–D). IL-22 can also be produced by myeloid and nonhematopoietic cells such as macrophages, neutrophils, and fibroblasts (4). Macrophages and neutrophils play central roles in host defense in the maternal (49, 68, 102) and fetal (28–33, 103, 104) compartments of women with spontaneous preterm labor who were diagnosed with intra-amniotic infection. Therefore, we also determined the expression of IL-22 by neutrophils and macrophages in the decidual tissues. Consistent with the concept that IL-22 is primarily produced by T cells, the expression of this cytokine by decidual macrophages and neutrophils did not differ between women with preterm labor and those with a term pregnancy (Supplemental Fig. 1E, 1F). Collectively, these results indicate that the main cellular source of IL-22 at the maternal-fetal interface is T cells and that such cells are enriched in women with preterm labor. It is noteworthy that the increase in IL-22$^+$ T cells was not due to differences in gestational age, as no differences in the cell types expressing IL-22 were found between women who delivered preterm and those who delivered at term in the absence of labor (Supplemental Fig. 2).

In silico scRNA-seq analysis of IL22 expression by immune cells at the human maternal-fetal interface

Next, we explored the unbiased expression of *IL22* by decidual immune cells at single-cell resolution. For this, we used the previously reported cellular landscape of human placental tissues (basal plate and chorioamniotic membranes) during normal and pathological parturition (68) (Fig. 1G). In silico analysis of these scRNA-seq data confirmed our immunophenotyping findings, showing that *IL22* is mainly expressed by activated T cells in the decidua basalis that also express *RORC* in the basal plate (Fig. 1H). NK cells in the decidua parietalis also displayed minimal expression of *IL22* and *RORC* in the chorioamniotic membranes (Fig. 1I). Other immune cells displayed negligible coexpression of *IL22* and *RORC* (Fig. 1H, 1I). As expected, the transcripts of the activating receptor for IL-22 (IL-22RA1) as well as its binding protein (IL-22RA2) were minimally expressed by decidual immune cells (Fig. 1H, 1I). By contrast, *Il10rb* was highly expressed by all decidual immune cells, consistent with its pleiotropic functions (105–107) (Fig. 1H, 1I). These results support the concept that IL-22 is mainly expressed by activated T cells at the human maternal-fetal interface.

IL-22 is increased in the maternal circulation upon in vivo T cell activation in mice

We previously showed that the in vivo activation of T cells induces preterm birth and neonatal mortality in mice in the context of a systemic cytokine storm (66). Given that IL-22 has been implicated in pathologies associated with systemic inflammation (108–110), we next investigated whether this mediator contributes to the cytokine storm resulting from in vivo maternal T cell activation prior to preterm birth. Dams were i.p. injected with an mAb against CD3ε (Fig. 2A), which consistently induces preterm birth in 80–90% of cases, as previously reported (66, 89). Circulating levels of IL-22 were increased in dams injected with anti-CD3ε compared to those injected with the isotype (Fig. 2B). Therefore, we next investigated whether the systemic administration of IL-22 alone, at pathophysiological concentrations (91), was sufficient to induce preterm birth and/or adverse neonatal outcomes in mice. The exogenous administration of IL-22 neither shortened gestational length nor induced neonatal mortality in mice (Supplemental Fig. 3A, 3B), suggesting that activation of the IL-22 axis in the mother alone is insufficient to induce adverse perinatal outcomes, and that the fetal compartment could be involved. To further explore this concept, we examined the

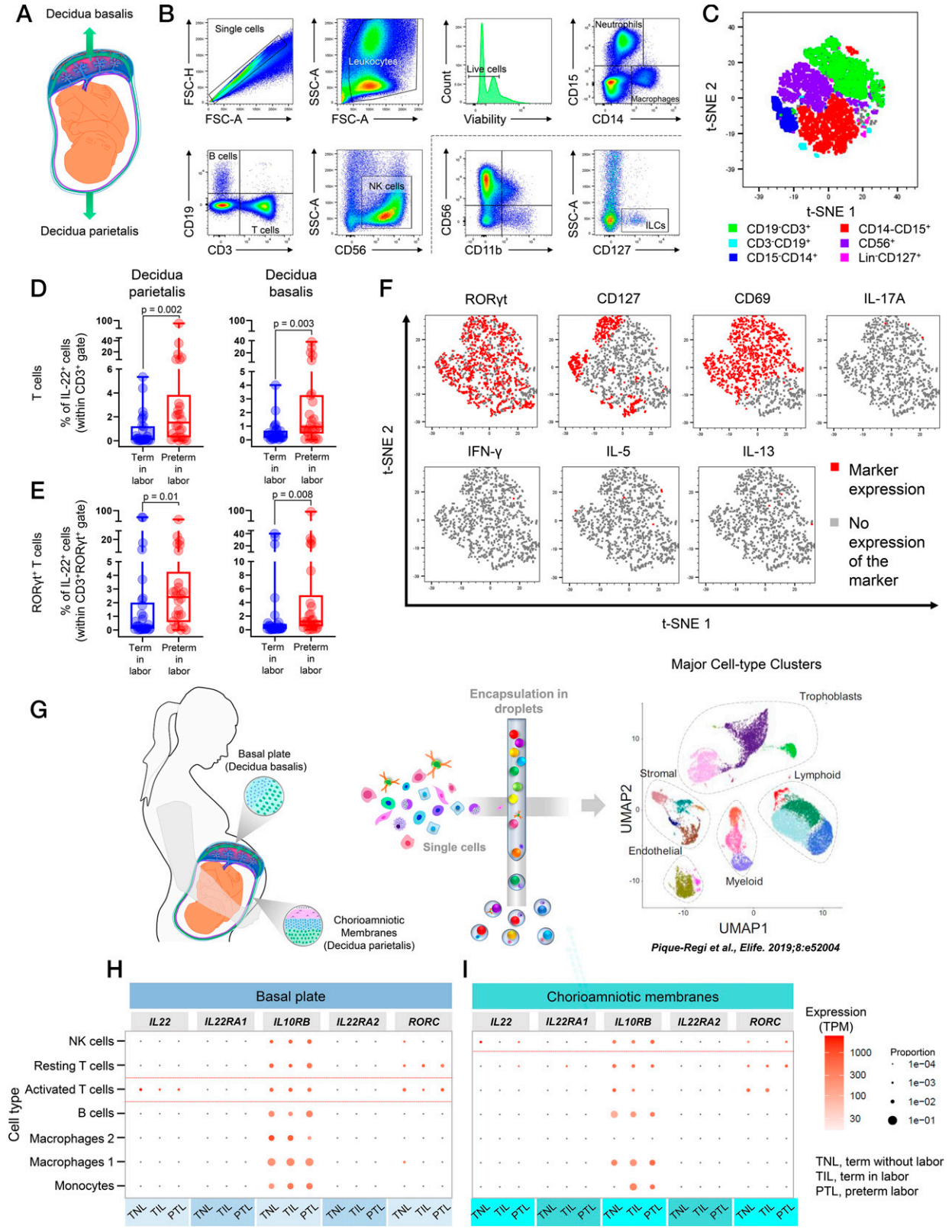

FIGURE 1. Immunophenotyping of cellular sources of IL-22 in human decidual tissues. (**A**) Spatial localization of the human decidua basalis and parietalis. (**B**) Gating strategy used to identify neutrophils ($CD14^{-}CD15^{+}$), macrophages ($CD15^{-}CD14^{+}$), T cells ($CD14^{-}CD15^{-}CD19^{-}CD3^{+}$), B cells ($CD14^{-}CD15^{-}CD3^{-}CD19^{+}$), NK cells ($CD14^{-}CD15^{-}CD3^{-}CD19^{-}CD56^{+}$), and innate lymphoid cells [ILCs; $CD14^{-}CD15^{-}CD3^{-}CD19^{-}CD11b^{-}CD56^{-}(Lin^{-})CD127^{+}$] in the human decidua parietalis and basalis. (**C**) Representative t-SNE plot showing the relative distribution of immune cell populations in the human decidual tissues. (**D**) Proportions of $IL\text{-}22^{+}$ T cells in the decidua parietalis of women with term labor (TIL, $n = 29$) or preterm labor (PTL, $n = 26$) and decidua basalis of women with TIL ($n = 30$) or PTL ($n = 26$). (**E**) Proportions of $IL\text{-}22^{+}ROR\text{-}\gamma t^{+}$ T cells in the decidua parietalis of women with TIL ($n = 29$) or PTL ($n = 26$) and decidua basalis of women with TIL ($n = 30$) or PTL ($n = 26$). The p values were determined using Mann–Whitney U tests. Data are shown as scatterplots with medians, interquartile *(Figure legend continues)*

concentrations of IL-22 in the amniotic cavity upon maternal T cell activation in mice. Amniotic fluid concentrations of IL-22 tended to increase in dams injected with anti-CD3ε compared to those injected with isotype control (3.5-fold increase) (Fig. 2C). These data suggest that maternal T cell activation causes a surge in systemic IL-22 that may be transferred to the fetal compartment, given that this cytokine is not normally expressed in the fetal tissues (e.g., intestine) in late gestation (111).

Il22 receptors, but not Il22, are highly expressed in the murine fetal tissues in late gestation

To complement a prior report showing that IL-22 is not expressed by the murine fetal intestine (111), we next determined whether this cytokine was transcribed by gestational tissues (e.g., the placenta and fetal membranes) and the fetal lung in mice. Consistently, *Il22* was minimally expressed in the fetal intestine, fetal lung, placenta, or fetal membranes in late murine gestation, which contrasted with the high expression of this cytokine by the lung of pregnant mice undergoing systemic inflammation (Fig. 2D). However, the transcripts encoding the primary receptors for IL-22, *Il22ra1* and *Il10rb*, were highly expressed in the fetal and gestational murine tissues (Fig. 2E, 2F). Similarly, *Il22ra2*, the transcript encoding the IL-22 binding protein, was also highly expressed by the fetal and gestational murine tissues, with the placenta demonstrating the highest expression (Fig. 2G). Taken together, these results show that the fetus possesses the machinery for responding to IL-22 signaling in late murine gestation, but it does not express this cytokine under physiological conditions.

IL-22 can cross from the maternal compartment to the fetal compartment, where it is associated with intra-amniotic inflammation and preterm labor and birth in humans

The above data suggested to us that the pathological effects of IL-22 may be localized to the fetal compartment rather than the maternal circulation, and that this cytokine may be capable of crossing the human maternal-fetal interface and reaching the amniotic cavity, inducing inflammation and disease. Therefore, we generated a novel ex vivo human system to test this hypothesis (Fig. 3A). IL-22 was capable of crossing from the maternal decidua through the chorioamnion at different doses, suggesting that this maternally derived cytokine can indeed reach the fetus (Fig. 3B). Next, we evaluated the clinical relevance of increased levels of IL-22 in the human amniotic cavity by investigating the concentrations of this cytokine in different subsets of women with preterm labor. Patients who presented with an episode of preterm labor and delivered at term were considered as controls (Fig. 3C). Notably, amniotic fluid IL-22 concentrations were greater in women with sterile or microbial intra-amniotic inflammation than in those without these clinical conditions who delivered preterm or in those who delivered at term (Fig. 3D). In addition, amniotic fluid concentrations of IL-22 tended to increase in women with microbial intra-amniotic inflammation compared to those with sterile intra-amniotic inflammation (Fig. 3D). Taken together, these data led us to propose that, within the amniotic cavity, IL-22 plays a dual role in maternal-fetal immunity: 1) IL-22 alone is capable of causing fetal tissue injury leading to neonatal death, and 2) IL-22 participates in host defense against microbial invasion leading to preterm birth.

The intra-amniotic administration of IL-22 alone shortens gestational length and induces neonatal death in mice

To establish the role of intra-amniotic IL-22 in fetal injury leading to neonatal death (Fig. 4A), we performed ultrasound-guided intra-amniotic injection of this cytokine in mice (Fig. 4B) at pathophysiological concentrations found in women with intra-amniotic inflammation leading to preterm birth (Fig. 3D). The intra-amniotic administration of IL-22 shortened gestational length (Fig. 4C); however, this reduction did not reach statistical significance. Sample size calculations revealed that 46 animals per group would be required to reach statistical significance with a confidence level of 0.95 and a power of 0.9. Regardless, neonates born to mice intra-amniotically injected with 612 pg of IL-22 showed a 50% increase in mortality compared to those born to controls, which was statistically significant (Fig. 4D). Neonates born to control mice appeared healthy compared to those born to IL-22–injected dams, of which one-half were hypoxic and ultimately died (Fig. 4E). Neonatal weights at weeks 1, 2, and 3 were not significantly different among the groups (Fig. 4F–H). Taken together, these results provide mechanistic evidence that elevated concentrations of IL-22 alone in the amniotic cavity shorten gestational length and, more importantly, induce neonatal death in mice.

Intra-amniotic injection of IL-22 alone induces lung injury in the murine offspring

IL-22 has been previously shown to induce lung inflammation (112, 113). Therefore, to investigate the mechanisms whereby intra-amniotic IL-22 causes neonatal death, we first examined whether this cytokine induces a respiratory distress syndrome (RDS)-like phenomenon, which is a common clinical complication of premature neonates (114, 115). As a readout of RDS, we measured SP-A, a molecule produced in the lung by type II alveolar cells that is essential for maintaining a patent airway (116, 117), in the murine neonatal lung (Fig. 5A). The intra-amniotic injection of IL-22 in mice significantly reduced the concentrations of SP-A (Fig. 5B), suggesting that this cytokine impairs lung maturation in the offspring. Next, we examined the expression of key markers of inflammation (e.g., transcripts related to RDS, necrotizing enterocolitis, the IL-22 pathway) in the neonatal lung (Fig. 5C) and intestine (Fig. 5D) to evaluate the effects of IL-22 in these murine tissues. IL-22 significantly upregulated the expression of *Il33*, a proinflammatory cytokine implicated in the pathogenesis of preterm birth and neonatal mortality induced by alarmins (27, 118, 119), in the murine neonatal lung (Fig. 5E) and intestine (Fig. 5F). Furthermore, IL-22 upregulated the transcription of *Defb1*, a defensin increased in the amniotic cavity of women with intra-amniotic inflammation (120, 121), in the murine neonatal lung (Fig. 5E). Moreover, IL-22 upregulated the expression of the gene encoding for IL-22RA1 in the murine neonatal lung (Fig. 5E) and tended to decrease the expression of the gene encoding for the binding protein (Fig. 5C), suggesting that this cytokine orchestrates its engagement in neonatal life. Consistently, the

ranges, and minimum/maximum ranges. Demographic and clinical characteristics of the study population are shown in Table I. (**F**) Representative t-SNE plots showing the relative distribution of ROR-γt, CD69, CD127, IL-17A, IFN-γ, IL-5, and IL-13 expression by IL-22$^+$ T cells in the human decidual tissues. (**G**) Overview of the single-cell RNA-sequencing (scRNA-seq) of the human decidual tissues showing the major cell types identified in a uniform manifold approximation and projection (UMAP) as reported in Pique-Regi et al. (68). (**H** and **I**) Gene expression (scaled to transcripts per million) of *IL22*, *Il22RA1*, *IL10RB*, *IL22RA2*, and *RORC* by immune cells in the (H) basal plate and (I) chorioamniotic membranes of women who delivered at TNL ($n = 3$), TIL ($n = 3$), or PTL ($n = 3$). Dot size corresponds to the proportion of cells expressing each transcript within a given cell type.

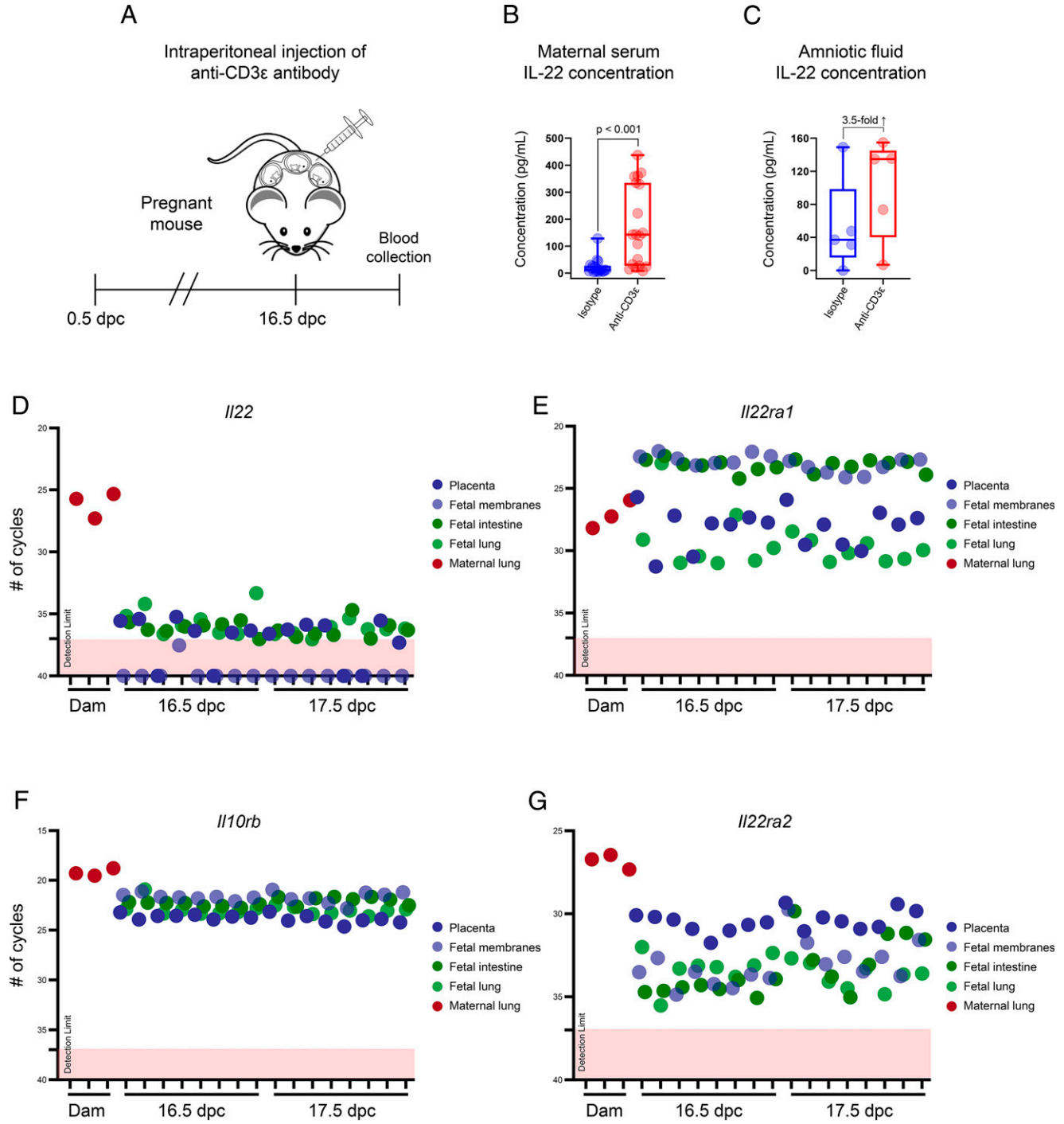

FIGURE 2. Maternal T cell activation induces elevated IL-22 in the maternal circulation and amniotic cavity and the expression of IL-22 and its receptors by gestational and fetal tissues in late gestation in mice. (**A**) Study design illustrating the i.p. injection of an anti-CD3ε Ab on 16.5 dpc to induce T cell activation-induced preterm birth in mice. Maternal serum and amniotic fluid were collected 12–16 h after injection for IL-22 determination. (**B**) Concentrations of IL-22 (pg/ml) in the maternal serum of mice injected with anti-CD3ε ($n = 19$) or isotype control ($n = 18$). (**C**) Concentrations of IL-22 (pg/ml) in the amniotic fluid of mice injected with anti-CD3ε ($n = 5$) or isotype control ($n = 5$). The p values were determined using Mann–Whitney U tests. Data are shown as scatterplots with medians, interquartile ranges, and minimum/maximum ranges. (**D**–**G**) Dot plots representing the expression of (D) *Il22*, (E) *Il22ra1*, (F) *Il10rb*, and (G) *Il22ra2* in the murine placenta, fetal membranes, fetal intestine, and fetal lung at 16.5 and 17.5 dpc, as well as the lung of pregnant mice i.p. injected with LPS (positive control) at 17.5 dpc. Limits of detection for each transcript are denoted by pink boxes.

expression of *Il22ra2* is significantly reduced in the murine neonatal intestine upon IL-22 administration (Fig. 5F). In addition, IL-22 downregulated the transcription of several inflammatory mediators in the murine neonatal lung such as *Ifng*, *Ccl5*, *Tslp*, *Il27*, and *Ahr* (Fig. 5E).

Collectively, these data suggest that the mechanisms whereby IL-22 induces neonatal death in mice primarily involve impaired maturation and dysregulation of inflammatory cascades in the neonatal lung.

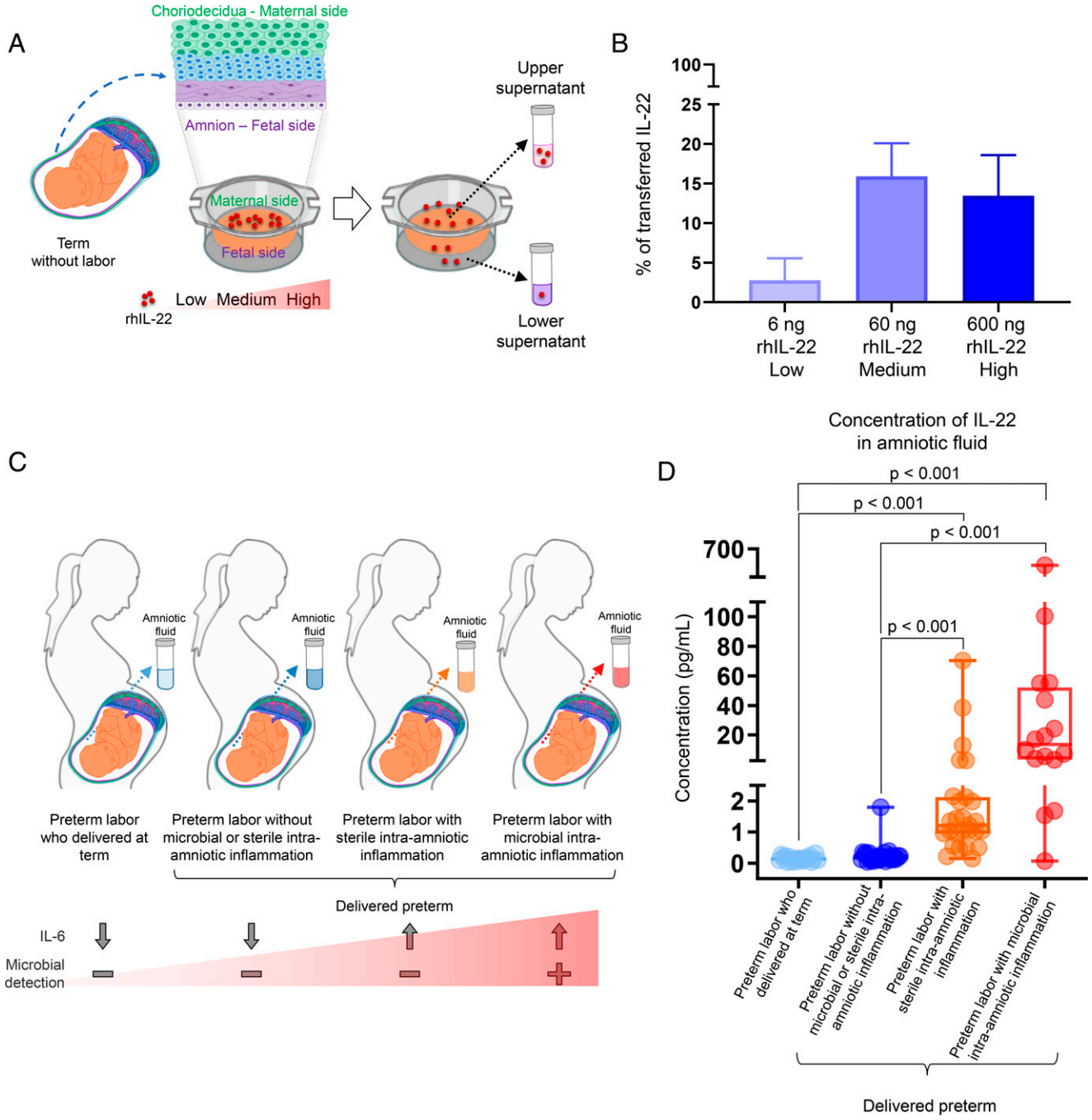

FIGURE 3. IL-22 can cross from the maternal to the fetal compartment and is implicated in intra-amniotic inflammation in women with preterm labor and birth. (**A**) Study design illustrating the ex vivo human model of rhIL-22 transfer across an explant of chorioamniotic membranes from women without labor at term (TNL). (**B**) Percent of transferred rhIL-22 at low (6 ng/ml; $n = 3$), medium (60 ng/ml; $n = 4$), and high (600 ng/ml; $n = 3$) concentrations. Data are shown as bar plots (mean ± SEM). BioRender was used to create part of the study design (transwells). (**C**) Study design illustrating the study groups of women who underwent spontaneous preterm labor and characterization of their intra-amniotic inflammatory and microbiological status and outcomes. (**D**) Concentrations of IL-22 in amniotic fluid of women with preterm labor who delivered at term ($n = 20$), preterm labor without microbial or sterile intra-amniotic inflammation who delivered preterm ($n = 27$), preterm labor with sterile intra-amniotic inflammation who delivered preterm ($n = 27$), or preterm labor with microbial intra-amniotic inflammation who delivered preterm ($n = 16$). The p values were determined using a Kruskal–Wallis test followed by the Dunn post hoc test. Data are shown as scatterplots with medians, interquartile ranges, and minimum/maximum ranges. Demographic and clinical characteristics of the study population are shown in Table II.

IL-22 participates in host defense induced by microbial invasion of the amniotic cavity in mice and humans

During intra-amniotic infection, the inflammatory process represents the host response against microbes invading the amniotic cavity (28–33), which leads to preterm labor and birth as an unintended consequence (34, 35). To establish the role of IL-22 in host defense against microbial invasion of the amniotic cavity leading to preterm birth (Fig. 6A), we used a previously established murine model of preterm birth induced by *U. parvum* (92), the most common microorganism found in women with microbial intra-amniotic inflammation (73, 122–125). Amniotic fluid concentrations of IL-22 were significantly increased in dams injected with

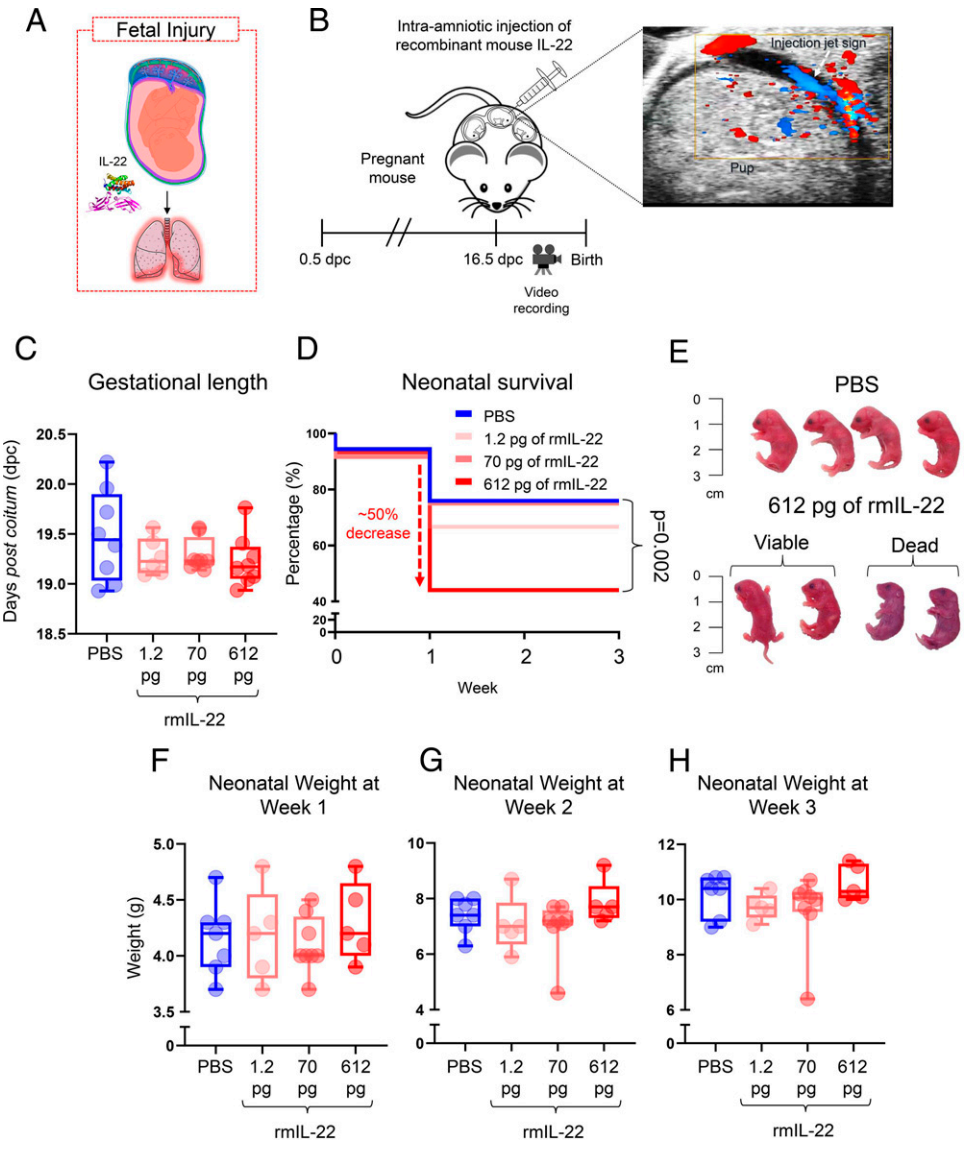

FIGURE 4. Intra-amniotic administration of IL-22 shortens gestational length and causes neonatal death in mice. (**A**) In the absence of microbial invasion of the amniotic cavity, increased IL-22 could lead to fetal injury. (**B**) Mice received an ultrasound-guided intra-amniotic injection of rmIL-22 on 16.5 dpc and were monitored until delivery. Color Doppler was used to identify the injection jet sign to confirm successful intra-amniotic injection. (**C**) Gestational length (dpc) of mice that received an intra-amniotic injection of rmIL-22 at 1.2 (pink; $n = 6$), 70 (dark pink; $n = 8$), or 612 (red; $n = 8$) pg/amniotic sac or PBS (blue; $n = 8$) as vehicle control. (**D**) Kaplan–Meier survival curves representing survival at weeks 1, 2, and 3 of neonates born to mice that received intra-amniotic injection of rmIL-22 at 1.2 (pink; $n = 42$), 70 (dark pink; $n = 60$), or 612 (red; $n = 59$) pg/amniotic sac or PBS (blue; $n = 54$). p values were determined using the Mantel–Cox test. (**E**) Representative images of neonates born to mice that received intra-amniotic injection of PBS (top row) or rmIL-22 (612 pg/amniotic sac) (bottom row). (**F**–**H**) Weights at weeks 1, 2, and 3 of neonates born to mice that received intra-amniotic injection of rmIL-22 at 1.2 (pink; $n = 5$ litters), 70 (dark pink; $n = 8$ litters), or 612 (red; $n = 5$ litters) pg/amniotic sac or PBS (blue; $n = 7$ litters). Each dot corresponds to the mean weight of a litter of pups. Data are shown as scatterplots with medians.

U. parvum compared to those injected with control medium (Fig. 6B). However, the intra-amniotic injection of *U. parvum* in mice did not increase the systemic concentrations of IL-22 (Supplemental Fig. 3C). To appreciate the role of our cytokine of interest in the local host response to *U. parvum*, we determined the correlation between IL-22 concentrations and the concentrations of proinflammatory cytokines as well as the expression of IL-22 by infiltrating leukocytes in the murine amniotic cavity, both of which are clinical manifestations of intra-amniotic infection (27, 30–32, 70, 104, 119, 126–128). Consistent with a role for IL-22 in the host response, the concentrations of this cytokine were positively correlated with IL-6 (the clinical marker of intra-amniotic inflammation in humans [74, 129]) as well as other proinflammatory cytokines such as IL-21 and IL-17A (Fig. 6C). Moreover, amniotic fluid neutrophils and monocytes/macrophages found in mice injected with *U. parvum* expressed IL-22 (Fig. 6D). The translational value of the latter finding was confirmed in human amniotic fluid samples from women with intra-amniotic infection associated with genital mycoplasmas, where neutrophils and monocytes/macrophages expressed IL-22 (Fig. 6E, 6F). To further examine the sources and sensing of IL-22 in the amniotic cavity upon microbial invasion, the expression of this cytokine and its receptors was explored in the fetal membranes and placentas of *U. parvum*–injected mice. The expression of *Il22* was significantly upregulated in the murine fetal membranes (Fig. 7A) and the placenta (Fig. 7B) compared to controls, suggesting that these gestational tissues express this cytokine as part of the host response triggered by genital mycoplasmas invading the amniotic cavity. Interestingly, the murine placenta upregulated the expression of *Il22ra1* and *Il10rb* (Fig. 7B), suggesting that intra-amniotic infection induced by genital mycoplasmas promotes the sensing of IL-22 by this fetal organ. Lastly, we evaluated the inflammatory status of the murine fetal lung and intestine as a consequence of the host intra-amniotic immune response triggered by microbes. Consistent with the clinical scenario of fetal inflammatory response syndrome induced by intra-amniotic infection (130–132), fetuses of mice injected with *U. parvum*

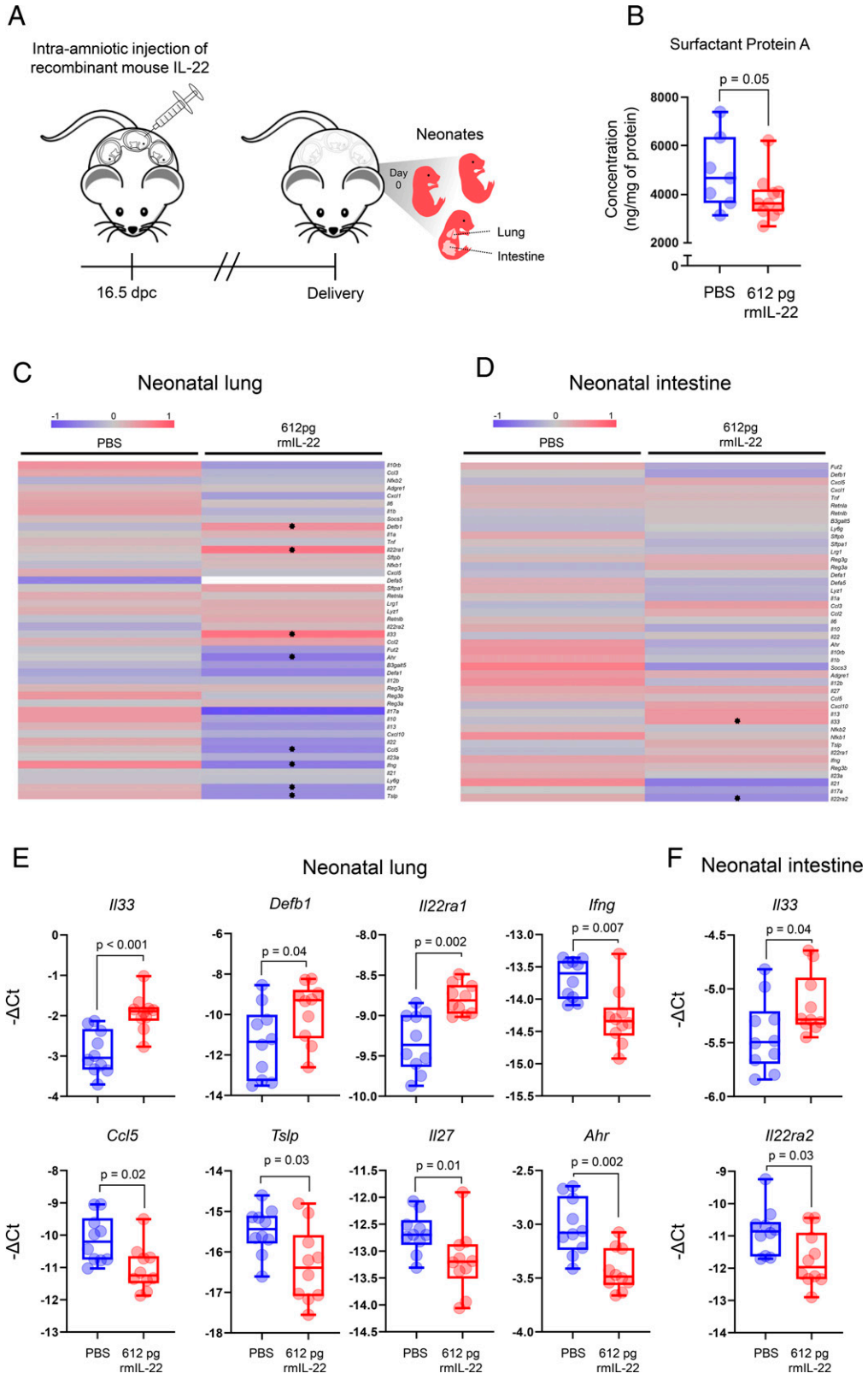

FIGURE 5. Intra-amniotic administration of IL-22 in mice induces lung injury and inflammation in the offspring. (**A**) Mice received an ultrasound-guided intra-amniotic injection of rmIL-22 (612 pg/amniotic sac) (n = 10) or PBS (vehicle control; n = 7–10) on 16.5 dpc and were monitored until delivery, after which the neonatal lung and intestine were collected. (**B**) Surfactant protein A concentrations (ng/mg of protein) in the lung of neonates born to mice intra-amniotically injected with PBS (n = 7) or rmIL-22 (n = 10). p value was determined using a Mann–Whitney U test. (**C** and **D**) Heatmap representations showing inflammatory gene expression in the (C) lung and (D) intestine of neonates born to mice intra-amniotically injected with PBS (n = 10) or rmIL-22 (n = 10). Red indicates upregulated expression and blue indicates downregulated expression. Stars indicate significant differentially expressed genes. (**E**) Expression of *Il33*, *Defb1*, *Il22ra1*, *Ifng*, *Ccl5*, *Tslp*, *Il27*, and *Ahr* in the murine neonatal lung. (**F**) Expression of *Il33* and *Il22ra2* in the murine neonatal intestine. p values were determined using Mann–Whitney U tests. Data are shown as scatterplots with medians, interquartile ranges, and minimum/maximum ranges.

displayed a massive upregulation of proinflammatory mediators in the fetal lung and intestine (Fig. 7C–F). Taken together, these data provide a solid role for IL-22 in the host response to *U. parvum*–induced intra-amniotic inflammation preceding preterm birth in both mice and humans.

Il22 deficiency protects against U. parvum–induced preterm birth and neonatal mortality

Lastly, to demonstrate causality between IL-22 and adverse perinatal outcomes due to microbial intra-amniotic inflammation, we performed intra-amniotic injection of *U. parvum* in *Il22*-sufficient and

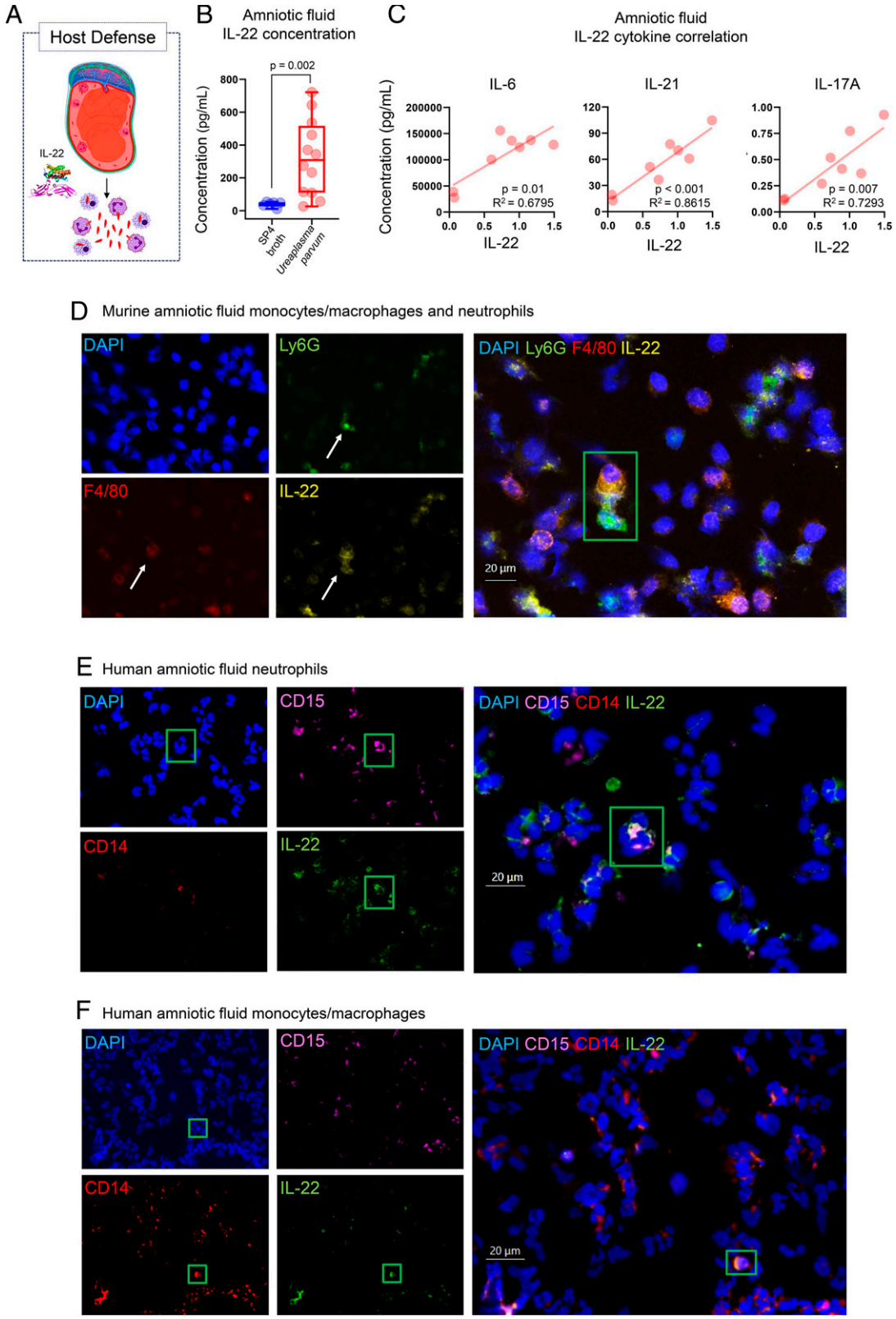

FIGURE 6. IL-22 is expressed by neutrophils and monocytes/macrophages in the human and murine amniotic cavity upon microbial infection. (**A**) IL-22 participates in the local host immune response to microbial invasion of the amniotic cavity. (**B**) Concentrations of IL-22 in the amniotic fluid of mice intra-amniotically injected with *U. parvum* ($n = 12$) or SP4 broth ($n = 6$). Data are shown as scatterplots with medians, interquartile ranges, and minimum/maximum ranges. *p* value was determined using a Mann–Whitney *U* test. (**C**) Linear regressions showing the correlations between amniotic fluid concentrations of IL-22 and those of IL-6, IL-21, and IL-17A in mice intra-amniotically injected with *U. parvum* ($n = 8$). (**D**) Representative immunofluorescence imaging of amniotic fluid neutrophils and monocytes/macrophages from mice intra-amniotically injected with *U. parvum* showing the single and merged expression of DAPI (nuclei, blue), Ly6G (neutrophils, green), F4/80 (monocytes/macrophages, red), and IL-22 (yellow). (**E** and **F**) Representative immunofluorescence imaging of human amniotic fluid (E) neutrophils and (F) monocytes/macrophages obtained from women with intra-amniotic infection showing the single and merged expression of DAPI (blue), CD15 (neutrophils, violet), CD14 (monocytes/macrophages, red), and IL-22 (green). Green boxes correspond to cells of interest. All images were taken at original magnification ×40. Scale bars, 20 µm.

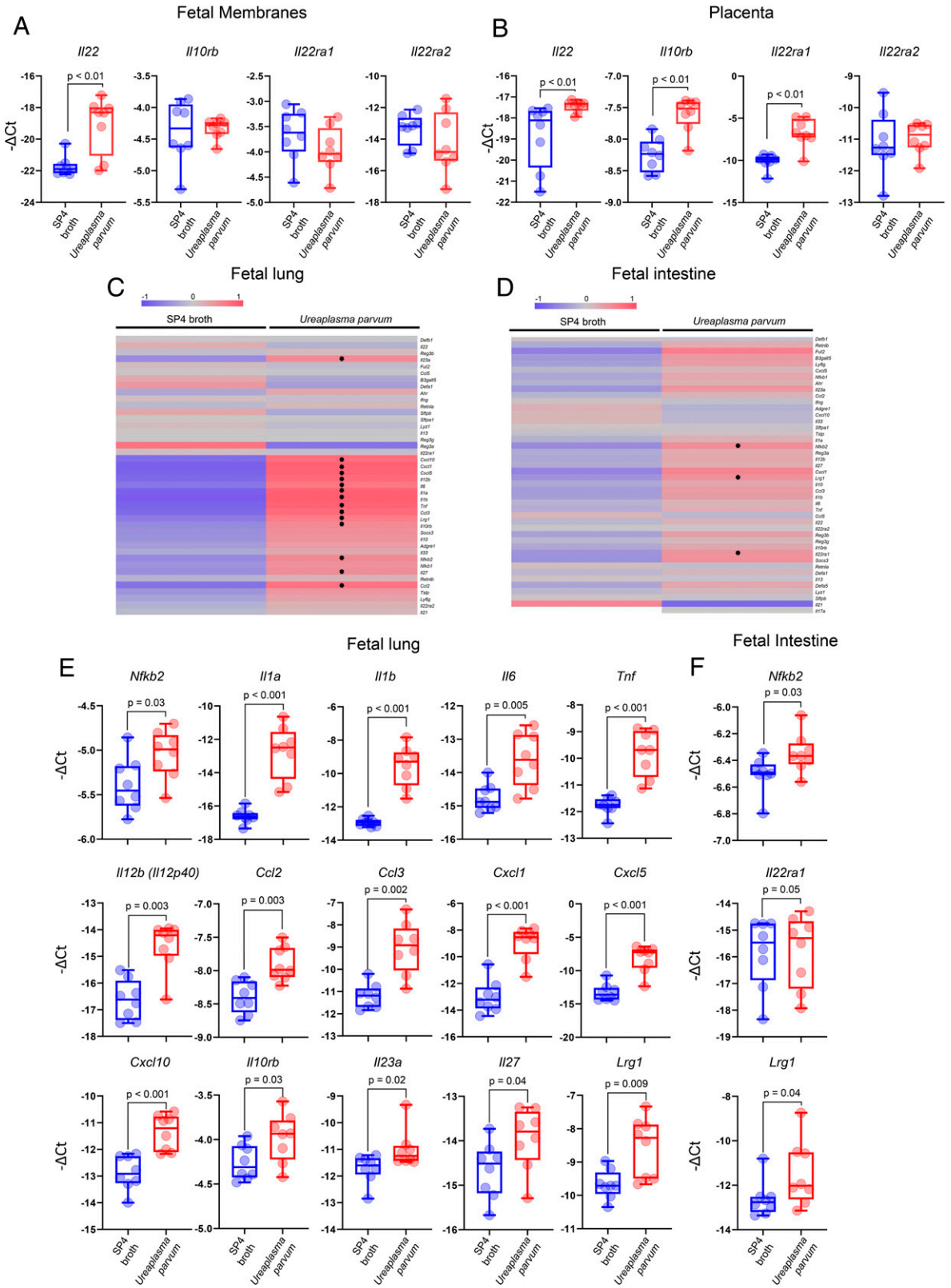

FIGURE 7. IL-22 participates in host response to *U. parvum* in the placenta, fetal membranes, fetal lung, and fetal intestine in mice. (**A**) Expression of *Il22*, *Il10rb*, *Il22ra1*, and *Il22ra2* in the fetal membranes of mice intra-amniotically injected with *U. parvum* ($n = 8$) or SP4 broth ($n = 8$) on 16.5 dpc. (**B**) Expression of *Il22*, *Il10rb*, *Il22ra1*, and *Il22ra2* in the placentas of mice intra-amniotically injected with *U. parvum* ($n = 8$) or SP4 broth ($n = 8$). (**C** and **D**) Heatmap representations showing inflammatory gene expression in the (C) lung and (D) intestine of fetuses of mice intra-amniotically injected with *U. parvum* ($n = 8$) or SP4 broth ($n = 8$). Stars indicate significant differentially expressed genes. (**E**) Expression of *Nfkb2*, *Il1a*, *Il1b*, *Il6*, *Tnf*, *Il12b* (*Il12p40*), *Ccl2*, *Ccl3*, *Cxcl1*, *Cxcl5*, *Cxcl10*, *Il10rb*, *Il23a*, *Il27*, and *Lrg1* in the lung of fetuses of mice intra-amniotically injected with *U. parvum* ($n = 8$) or SP4 broth ($n = 8$). (**F**) Expression of *Nfkb2*, *Il22ra1*, and *Lrg1* in the intestine of fetuses of mice intra-amniotically injected with *U. parvum* ($n = 8$) or SP4 broth ($n = 8$). *p* values were determined using Mann–Whitney *U* tests. Data are shown as scatterplots with medians, interquartile ranges, and minimum/maximum ranges.

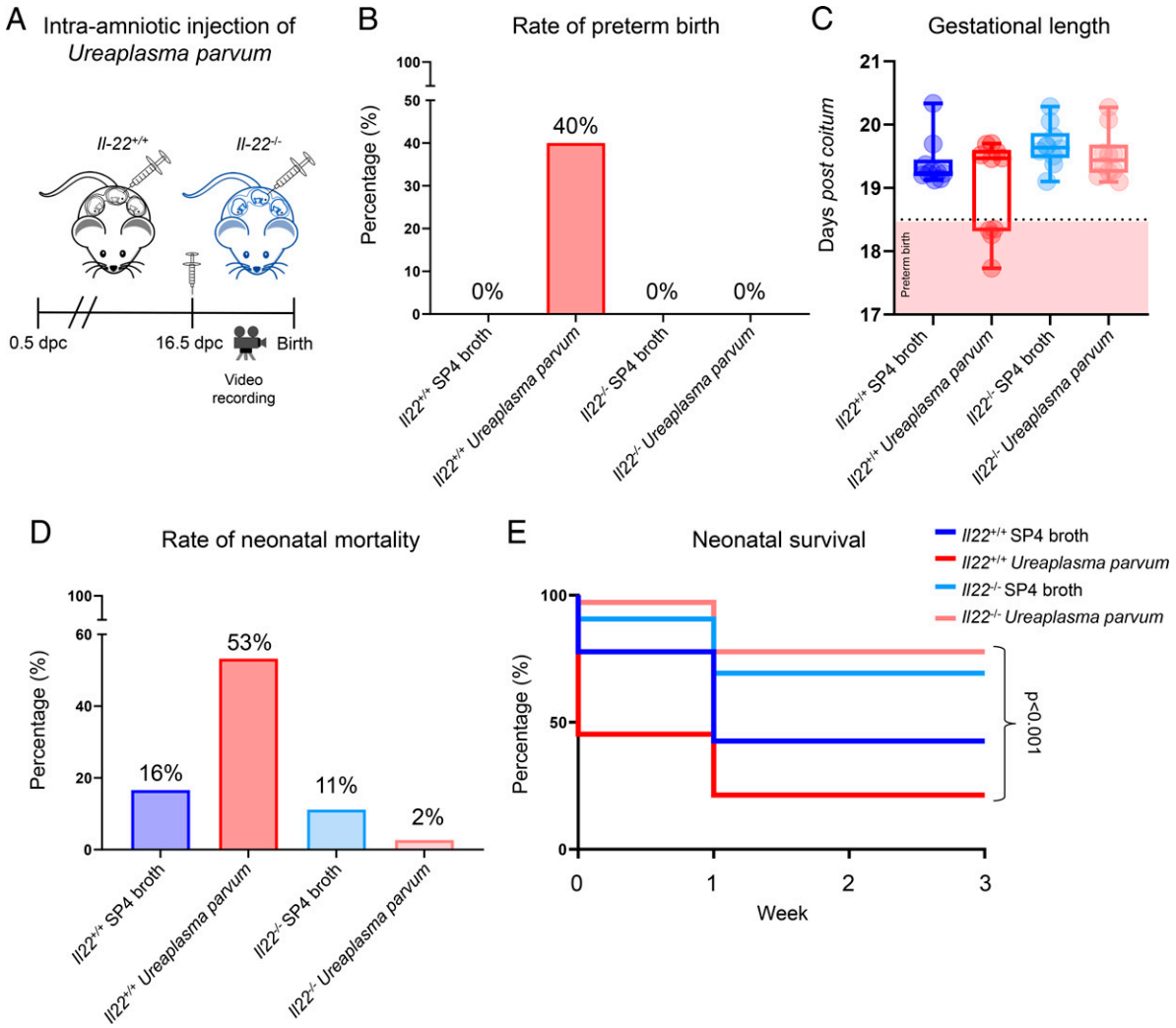

FIGURE 8. *Il22* deficiency protects against intra-amniotic *U. parvum*–induced preterm birth and neonatal mortality. (**A**) $Il22^{+/+}$ and $Il22^{-/-}$ mice were intra-amniotically injected with *U. parvum* or SP4 broth (control) on 16.5 dpc and monitored until delivery. (**B**) Preterm birth rates of $Il22^{+/+}$ and $Il22^{-/-}$ mice intra-amniotically injected with *U. parvum* ($n = 10$ per genotype) or control ($n = 10$ per genotype). (**C**) Gestational lengths of $Il22^{+/+}$ and $Il22^{-/-}$ mice intra-amniotically injected with *U. parvum* or control. Dotted line and pink box represent the cutoff for preterm birth (<18.5 dpc). (**D**) Mortality rates of neonates born to $Il22^{+/+}$ and $Il22^{-/-}$ mice intra-amniotically injected with *U. parvum* or control. (**E**) Kaplan–Meier survival curves representing survival at weeks 1, 2, and 3 of neonates born to $Il22^{+/+}$ and $Il22^{-/-}$ mice intra-amniotically injected with *U. parvum* or SP4 broth. *p* values were determined using the Mantel–Cox test.

-deficient mice (Fig. 8A). *U. parvum* induced high rates (40%) of preterm birth in $Il22^{+/+}$ mice, confirming our previously established model of microbial intra-amniotic inflammation-induced preterm birth (92). Notably, $Il22^{-/-}$ mice were protected against preterm birth and delivered later than $Il22^{+/+}$ dams (Fig. 8B, 8C). Importantly, although *U. parvum* induced high rates of neonatal mortality in $Il22^{+/+}$ mice, neonates born to $Il22^{-/-}$ dams experienced minimal mortality rates (Fig. 8D) and thrived up to 3 wk of age. This last set of results provides a mechanistic demonstration of a role for IL-22 in the inflammatory response induced by genital mycoplasmas as part of the host defense against infection of the amniotic cavity, which leads to preterm birth and adverse neonatal outcomes as unintended consequences.

Discussion

In this study, we present evidence that IL-22 plays a dual role in maternal-fetal immunity. Specifically, we demonstrated that IL-22^{+} T cells coexpressing ROR-γt are enriched at the human maternal-fetal interface of women with preterm labor and birth, as confirmed by in silico analysis of available scRNA-seq data. Next, we showed that systemic concentrations of IL-22 were elevated upon maternal T cell activation leading to preterm birth in mice. However, the i.v. administration of IL-22 alone in mice did not induce adverse perinatal outcomes, suggesting that this cytokine must reach the fetal compartment to cause damage. In line with our hypothesis, concentrations of IL-22 were increased in the amniotic cavity upon in vivo maternal T cell activation where it may be sensed by its receptors (*Il22ra1*, *Il10rb*, and *Il22ra2*), which were highly expressed by gestational and fetal tissues in late murine pregnancy. Next, we confirmed that IL-22 can cross from the maternal (choriodecidua) to the fetal (amniotic cavity) side using an ex vivo human system. Importantly, amniotic fluid concentrations of IL-22 were elevated in women with sterile or microbial intra-amniotic inflammation, leading us to investigate the role of this cytokine in the presence and absence of microbes. A role for IL-22 in fetal tissue injury was evidenced by the intra-amniotic administration of this cytokine alone in mice, which led to shortened gestation and neonatal death, with the latter involving impaired lung maturation and

tissue inflammation. A role for IL-22 in the host response against microbes invading the amniotic cavity was demonstrated by the participation of this cytokine in the intra-amniotic inflammatory milieu preceding *U. parvum*–induced preterm birth in mice, which was rescued by the deficiency of IL-22. Collectively, these data show that IL-22 alone is capable of causing fetal tissue injury leading to neonatal death and that this cytokine participates in host defense against microbial invasion of the amniotic cavity leading to preterm labor and birth.

T cells of maternal origin are present at the human (56–60, 66, 133–138) and murine (48, 50, 51, 64, 66, 139–141) maternal-fetal interface (67, 142–146). In early pregnancy, Ag-specific and nonspecific events are thought to modulate the highly regulated, but limited, recruitment of effector T cells (147), which contribute to the proinflammatory milieu at the maternal-fetal interface that is required for successful implantation (148, 149). Such an inflammatory response may be counteracted by the concomitant increase in regulatory T cells that occurs both locally and systemically (150–154) and persists throughout gestation (152). The importance of regulatory T cells in late gestation has been underscored by mechanistic studies showing that the depletion of such cells in early (152, 153, 155–159) and late (160) pregnancy results in pregnancy loss and preterm birth, respectively. Moreover, we reported that a reduction in regulatory T cells is accompanied by an increased influx of effector T cells at the maternal-fetal interface of women who underwent spontaneous preterm labor (160). These T cells express effector molecules such as granzyme B and perforin that may exert their lytic functions at the maternal-fetal interface (66), thereby inducing tissue damage and chronic decidual inflammation, a hallmark of spontaneous preterm labor (161, 162). In this study, we expand on this concept by demonstrating that a subset of decidual T cells coexpress IL-22 and ROR-γt, and such cells are enriched in women with preterm labor and birth. The expression of such molecules, together with the absence of IL-17A, led us to propose that such decidual T cells belong to the recently described Th22 subset (163–169). Our finding that Th22-like cells are present at the maternal-fetal interface of women with preterm labor is in tandem with a prior study showing that decidual IL-22–expressing T cells are implicated in pregnancy loss (170). Therefore, T cells expressing IL-22 are present at the maternal-fetal interface in early and late pregnancy, where they seem to participate in the mechanisms involved in obstetrical disease.

Consistent with the above concept, we demonstrated that the in vivo injection of an anti-CD3ε Ab can activate systemic and decidual T cells in mice, resulting in preterm birth and neonatal mortality (66). In the current study, we show that such activation of T cells is accompanied by elevated concentrations of IL-22 in the murine maternal circulation. In line with this finding, elevated concentrations of IL-22 or numbers of IL-22$^+$ T cells have been reported in systemic inflammatory disorders as well as in host response to pathogens (171–175). Furthermore, IL-22$^+$ T cells are elevated in the circulation of women with preeclampsia (176), a population at increased risk of medically indicated preterm birth (12, 177). However, the concentrations at which IL-22 displays deleterious effects likely vary according to systemic pathophysiological processes. For example, we report in the present study that the systemic administration of IL-22, at concentrations reported in women with spontaneous preterm labor (91), is not sufficient to induce preterm birth in mice. However, it is plausible that the cytokine storm induced by maternal T cell activation (66) facilitates the crossing of IL-22 from the maternal to the fetal compartment (i.e., amniotic cavity), as evidenced by the elevated amniotic fluid concentrations of this cytokine in this murine model. Such a concept was confirmed in the current study by demonstrating that maternally derived IL-22 crosses the human maternal-fetal interface, reaching the fetal compartment. Transfer of cytokines through the placental tissues is not unique to IL-22, as it has been previously demonstrated for IL-8 (178), IL-2 (179), and IL-6 (180, 181).

IL-22 is minimally expressed by murine fetal tissues, as demonstrated in the present study and previously reported (111). However, its canonical receptors are highly expressed by the murine fetal and placental tissues, as shown in the present study, or by the human placenta (182), suggesting that the fetus can sense IL-22 under pathological conditions. Indeed, we demonstrate a pathological role for IL-22 in the fetal compartment by showing that the intra-amniotic administration of this cytokine alone shortens gestational length and, more importantly, causes neonatal death in mice. This finding is in tandem with our previous investigation showing that activated fetal T cells, which are capable of producing IL-22 as well as other Th cytokines, are implicated in a subset of preterm labor cases without microbial intra-amniotic inflammation (68). Consistently, the intra-amniotic administration of activated neonatal T cells in mice induced a proportion of preterm births (68), suggesting that the release of multiple T cell cytokines is necessary to activate the premature cascade of parturition, and it provides an explanation as to why IL-22 alone shortened only the gestational length. Nevertheless, intra-amniotic IL-22 was sufficient to induce neonatal death in mice, which could result from the multiorgan pathological effects attributed to this cytokine (4, 183), as evidenced in the present study by tissue inflammation and impaired lung maturation in neonates.

The indispensable role of IL-22 in host defense mechanisms against infection with bacteria, yeast, and protozoa is well documented (4, 173, 184–187). Specifically, during bacterial infection, this cytokine enhances antibacterial defense by epithelial cells and actively participates in the recruitment and activation of immune cells, thus limiting colonization (188–190). Consistently, in this study, we report that IL-22 is part of the intra-amniotic inflammatory response driven by the host response against microbes, such as *U. parvum*, in humans and mice. Although we did not investigate the direct actions of IL-22 on *U. parvum*, a previous study reported that IL-22 alone inhibits the growth of *Mycobacterium tuberculosis* in macrophages (191). The ability of IL-22 to perform such a function with respect to *U. parvum* in the amniotic cavity warrants further investigation. Moreover, we show that fetal innate immune cells infiltrating the amniotic cavity in response to infection (30, 31, 33) are sources of IL-22 in humans and mice. The mechanisms whereby IL-22 participates in the inflammatory milieu induced by *U. parvum* involve the upregulation of its transcription as well as that of its receptors in the placental tissues, as shown in the present study. Furthermore, we provide evidence that IL-22 is implicated in the inflammatory pathways leading to preterm birth and fetal injury, given that *Il22*-deficient mice are resistant to the deleterious effects of *U. parvum* in the amniotic cavity. The mechanisms whereby *Il22* deficiency protects against neonatal mortality may involve resolution of the acute fetal inflammatory response syndrome, which is a common complication that can result from microbial invasion of the amniotic cavity and involves massive activation of the innate limb of fetal immunity as part of the host response (130, 132, 192).

In summary, this study provides a previously unrecognized dual role for IL-22 in maternal-fetal immunity. IL-22 is expressed by maternal T cells in the uterine decidua of women with preterm labor and birth. Under pathological circumstances associated with maternal T cell activation, IL-22 can cross the maternal-fetal interface and reach the amniotic cavity, where it can be sensed by the fetal and gestational tissues, causing fetal injury leading to neonatal death. By contrast, IL-22 contributes to the host response against microbes invading the amniotic cavity in the context of preterm labor and birth, which involves a

fetal inflammatory response leading to neonatal demise. Collectively, these findings shed light on the biology of IL-22 during late gestation and of host response mechanisms implicated in intra-amniotic infection resulting in preterm birth, the leading cause of neonatal morbidity and mortality worldwide.

Acknowledgments

We thank Dr. Valeria Garcia-Flores for intellectual input and helpful discussions. We also thank the physicians, nurses, and research assistants from the Center for Advanced Obstetrical Care and Research, Intrapartum Unit, Perinatology Research Branch Clinical Laboratory, and Perinatology Research Branch Perinatal Translational Science Laboratory (Rona Wang) for help with collecting and processing samples. Lastly, we thank Dr. Marcelo Farias-Jofre, Chengrui Zou, and David Kracht for help with the execution of some assays.

Disclosures

The authors have no financial conflicts of interest.

References

1. Zenewicz, L. A., and R. A. Flavell. 2011. Recent advances in IL-22 biology. *Int. Immunol.* 23: 159–163.
2. Saxton, R. A., L. T. Henneberg, M. Calafiore, L. Su, K. M. Jude, A. M. Hanash, and K. C. Garcia. 2021. The tissue protective functions of interleukin-22 can be decoupled from pro-inflammatory actions through structure-based design. *Immunity* 54: 660–672.e9.
3. Rutz, S., X. Wang, and W. Ouyang. 2014. The IL-20 subfamily of cytokines—from host defence to tissue homeostasis. *Nat. Rev. Immunol.* 14: 783–795.
4. Dudakov, J. A., A. M. Hanash, and M. R. van den Brink. 2015. Interleukin-22: immunobiology and pathology. *Annu. Rev. Immunol.* 33: 747–785.
5. Ma, H. L., S. Liang, J. Li, L. Napierata, T. Brown, S. Benoit, M. Senices, D. Gill, K. Dunussi-Joannopoulos, M. Collins, et al. 2008. IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation. *J. Clin. Invest.* 118: 597–607.
6. Lowes, M. A., M. Suárez-Fariñas, and J. G. Krueger. 2014. Immunology of psoriasis. *Annu. Rev. Immunol.* 32: 227–255.
7. Ikeuchi, H., T. Kuroiwa, N. Hiramatsu, Y. Kaneko, K. Hiromura, K. Ueki, and Y. Nojima. 2005. Expression of interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine. *Arthritis Rheum.* 52: 1037–1046.
8. Xie, Q., C. Huang, and J. Li. 2015. Interleukin-22 and rheumatoid arthritis: emerging role in pathogenesis and therapy. *Autoimmunity* 48: 69–72.
9. Sonnenberg, G. F., L. A. Fouser, and D. Artis. 2011. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat. Immunol.* 12: 383–390.
10. Lo, B. C., S. B. Shin, D. Canals Hernaez, I. Refaeli, H. B. Yu, V. Goebeler, A. Cait, W. W. Mohn, B. A. Vallance, and K. M. McNagny. 2019. IL-22 preserves gut epithelial integrity and promotes disease remission during chronic *Salmonella* infection. *J. Immunol.* 202: 956–965.
11. Keir, M., Y. Yi, T. Lu, and N. Ghilardi. 2020. The role of IL-22 in intestinal health and disease. *J. Exp. Med.* 217: e20192195.
12. Goldenberg, R. L., J. F. Culhane, J. D. Iams, and R. Romero. 2008. Epidemiology and causes of preterm birth. *Lancet* 371: 75–84.
13. Blencowe, H., S. Cousens, D. Chou, M. Oestergaard, L. Say, A. B. Moller, M. Kinney, and J. Lawn; Born Too Soon Preterm Birth Action Group. 2013. Born too soon: the global epidemiology of 15 million preterm births. *Reprod. Health* 10(Suppl 1): S2.
14. Chawanpaiboon, S., J. P. Vogel, A. B. Moller, P. Lumbiganon, M. Petzold, D. Hogan, S. Landoulsi, N. Jampathong, K. Kongwattanakul, M. Laopaiboon, et al. 2019. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob. Health* 7: e37–e46.
15. Romero, R., and C. J. Lockwood. 2009. Pathogenesis of spontaneous preterm labor. In *Creasy and Resnik's Maternal-Fetal Medicine: Principles and Practice*, 6th Ed., R. K. Creasy, R. Resnik, and J. Iams, eds. Elsevier, Philadelphia, p. 521–543.
16. Muglia, L. J., and M. Katz. 2010. The enigma of spontaneous preterm birth. *N. Engl. J. Med.* 362: 529–535.
17. Romero, R., S. K. Dey, and S. J. Fisher. 2014. Preterm labor: one syndrome, many causes. *Science* 345: 760–765.
18. Barros, F. C., A. T. Papageorghiou, C. G. Victora, J. A. Noble, R. Pang, J. Iams, L. Cheikh Ismail, R. L. Goldenberg, A. Lambert, M. S. Kramer, et al.; International Fetal and Newborn Growth Consortium for the 21st Century. 2015. The distribution of clinical phenotypes of preterm birth syndrome: implications for prevention. *JAMA Pediatr.* 169: 220–229.
19. Romero, R., M. Mazor, Y. K. Wu, M. Sirtori, E. Oyarzun, M. D. Mitchell, and J. C. Hobbins. 1988. Infection in the pathogenesis of preterm labor. *Semin. Perinatol.* 12: 262–279.
20. Gravett, M. G., S. S. Witkin, G. J. Haluska, J. L. Edwards, M. J. Cook, and M. J. Novy. 1994. An experimental model for intraamniotic infection and preterm labor in rhesus monkeys. *Am. J. Obstet. Gynecol.* 171: 1660–1667.
21. Whidbey, C., M. I. Harrell, K. Burnside, L. Ngo, A. K. Becraft, L. M. Iyer, L. Aravind, J. Hitti, K. M. Adams Waldorf, and L. Rajagopal. 2013. A hemolytic pigment of group B *Streptococcus* allows bacterial penetration of human placenta. *J. Exp. Med.* 210: 1265–1281.
22. Combs, C. A., M. Gravett, T. J. Garite, D. E. Hickok, J. Lapidus, R. Porreco, J. Rael, T. Grove, T. K. Morgan, W. Clewell, et al.; ProteoGenix/Obstetrix Collaborative Research Network. 2014. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. *Am. J. Obstet. Gynecol.* 210: 125.e1–125.e15.
23. McCartney, S. A., R. Kapur, H. D. Liggitt, A. Baldessari, M. Coleman, A. Orvis, J. Ogle, R. Katz, L. Rajagopal, and K. M. Adams Waldorf. 2021. Amniotic fluid interleukin 6 and interleukin 8 are superior predictors of fetal lung injury compared with maternal or fetal plasma cytokines or placental histopathology in a nonhuman primate model. *Am. J. Obstet. Gynecol.* 225: 89.e1–89.e16.
24. Romero, R., J. Miranda, T. Chaiworapongsa, P. Chaemsaithong, F. Gotsch, Z. Dong, A. I. Ahmed, B. H. Yoon, S. S. Hassan, C. J. Kim, et al. 2014. A novel molecular microbiologic technique for the rapid diagnosis of microbial invasion of the amniotic cavity and intra-amniotic infection in preterm labor with intact membranes. *Am. J. Reprod. Immunol.* 71: 330–358.
25. Romero, R., J. Miranda, T. Chaiworapongsa, S. J. Korzeniewski, P. Chaemsaithong, F. Gotsch, Z. Dong, A. I. Ahmed, B. H. Yoon, S. S. Hassan, et al. 2014. Prevalence and clinical significance of sterile intra-amniotic inflammation in patients with preterm labor and intact membranes. *Am. J. Reprod. Immunol.* 72: 458–474.
26. Romero, R., J. Miranda, P. Chaemsaithong, T. Chaiworapongsa, J. P. Kusanovic, Z. Dong, A. I. Ahmed, M. Shaman, K. Lannaman, B. H. Yoon, et al. 2015. Sterile and microbial-associated intra-amniotic inflammation in preterm prelabor rupture of membranes. *J. Matern. Fetal Neonatal Med.* 28: 1394–1409.
27. Romero, R., J. C. Grivel, A. L. Tarca, P. Chaemsaithong, Z. Xu, W. Fitzgerald, S. S. Hassan, T. Chaiworapongsa, and L. Margolis. 2015. Evidence of perturbations of the cytokine network in preterm labor. *Am. J. Obstet. Gynecol.* 213: 836.e1–836.e18.
28. Gomez-Lopez, N., R. Romero, Y. Xu, D. Miller, R. Unkel, M. Shaman, S. M. Jacques, B. Panaitescu, V. Garcia-Flores, and S. S. Hassan. 2017. Neutrophil extracellular traps in the amniotic cavity of women with intra-amniotic infection: a new mechanism of host defense. *Reprod. Sci.* 24: 1139–1153.
29. Gomez-Lopez, N., R. Romero, V. Garcia-Flores, Y. Xu, Y. Leng, A. Alhousseini, S. S. Hassan, and B. Panaitescu. 2017. Amniotic fluid neutrophils can phagocytize bacteria: a mechanism for microbial killing in the amniotic cavity. *Am. J. Reprod. Immunol.* 78: e12723.
30. Gomez-Lopez, N., R. Romero, Y. Xu, Y. Leng, V. Garcia-Flores, D. Miller, S. M. Jacques, S. S. Hassan, J. Faro, A. Alsamsam, et al. 2017. Are amniotic fluid neutrophils in women with intraamniotic infection and/or inflammation of fetal or maternal origin? *Am. J. Obstet. Gynecol.* 217: 693.e1–693.e16.
31. Gomez-Lopez, N., R. Romero, Y. Leng, Y. Xu, R. Slutsky, D. Levenson, P. Pacora, E. Jung, B. Panaitescu, and C. D. Hsu. 2019. The origin of amniotic fluid monocytes/macrophages in women with intra-amniotic inflammation or infection. *J. Perinat. Med.* 47: 822–840.
32. Galaz, J., R. Romero, Y. Xu, D. Miller, R. Slutsky, D. Levenson, C. D. Hsu, and N. Gomez-Lopez. 2020. Cellular immune responses in amniotic fluid of women with preterm clinical chorioamnionitis. *Inflamm. Res.* 69: 203–216.
33. Gomez-Lopez, N., R. Romero, A. Varrey, Y. Leng, D. Miller, B. Done, Y. Xu, G. Bhatti, K. Motomura, M. Gershater, et al. 2021. RNA sequencing reveals diverse functions of amniotic fluid neutrophils and monocytes/macrophages in intra-amniotic infection. *J. Innate Immun.* 13: 63–82.
34. Romero, R., J. Espinoza, L. F. Gonçalves, J. P. Kusanovic, L. Friel, and S. Hassan. 2007. The role of inflammation and infection in preterm birth. *Semin. Reprod. Med.* 25: 21–39.
35. Lee, J., H. S. Seong, B. J. Kim, J. K. Jun, R. Romero, and B. H. Yoon. 2009. Evidence to support that spontaneous preterm labor is adaptive in nature: neonatal RDS is more common in "indicated" than in "spontaneous" preterm birth. *J. Perinat. Med.* 37: 53–58.
36. Takeuchi, O., and S. Akira. 2010. Pattern recognition receptors and inflammation. *Cell* 140: 805–820.
37. Kim, Y. M., R. Romero, T. Chaiworapongsa, G. J. Kim, M. R. Kim, H. Kuivaniemi, G. Tromp, J. Espinoza, E. Bujold, V. M. Abrahams, and G. Mor. 2004. Toll-like receptor-2 and -4 in the chorioamniotic membranes in spontaneous labor at term and in preterm parturition that are associated with chorioamnionitis. *Am. J. Obstet. Gynecol.* 191: 1346–1355.
38. Mittal, P., R. Romero, J. P. Kusanovic, S. S. Edwin, F. Gotsch, S. Mazaki-Tovi, J. Espinoza, O. Erez, C. L. Nhan-Chang, N. G. Than, et al. 2008. CXCL6 (granulocyte chemotactic protein-2): a novel chemokine involved in the innate immune response of the amniotic cavity. *Am. J. Reprod. Immunol.* 60: 246–257.
39. Gotsch, F., R. Romero, T. Chaiworapongsa, O. Erez, E. Vaisbuch, J. Espinoza, J. P. Kusanovic, P. Mittal, S. Mazaki-Tovi, C. J. Kim, et al. 2008. Evidence of the involvement of caspase-1 under physiologic and pathologic cellular stress during human pregnancy: a link between the inflammasome and parturition. *J. Matern. Fetal Neonatal Med.* 21: 605–616.
40. Cardenas, I., R. E. Means, P. Aldo, K. Koga, S. M. Lang, C. J. Booth, A. Manzur, E. Oyarzun, R. Romero, and G. Mor. 2010. Viral infection of the placenta leads to fetal inflammation and sensitization to bacterial products predisposing to preterm labor. [Published erratum appears in 2011 *J. Immunol.* 187: 2835.] *J. Immunol.* 185: 1248–1257.

41. Ilievski, V., and E. Hirsch. 2010. Synergy between viral and bacterial Toll-like receptors leads to amplification of inflammatory responses and preterm labor in the mouse. *Biol. Reprod.* 83: 767–773.
42. Abrahams, V. M. 2011. The role of the Nod-like receptor family in trophoblast innate immune responses. *J. Reprod. Immunol.* 88: 112–117.
43. Romero, R., T. Chaiworapongsa, Z. Alpay Savasan, Y. Xu, Y. Hussein, Z. Dong, J. P. Kusanovic, C. J. Kim, and S. S. Hassan. 2011. Damage-associated molecular patterns (DAMPs) in preterm labor with intact membranes and preterm PROM: a study of the alarmin HMGB1. *J. Matern. Fetal Neonatal Med.* 24: 1444–1455.
44. Lappas, M. 2013. NOD1 and NOD2 regulate proinflammatory and prolabor mediators in human fetal membranes and myometrium via nuclear factor-kappa B. *Biol. Reprod.* 89: 14.
45. Jaiswal, M. K., V. Agrawal, T. Mallers, A. Gilman-Sachs, E. Hirsch, and K. D. Beaman. 2013. Regulation of apoptosis and innate immune stimuli in inflammation-induced preterm labor. *J. Immunol.* 191: 5702–5713.
46. Koga, K., G. Izumi, G. Mor, T. Fujii, and Y. Osuga. 2014. Toll-like receptors at the maternal-fetal interface in normal pregnancy and pregnancy complications. *Am. J. Reprod. Immunol.* 72: 192–205.
47. Agrawal, V., M. K. Jaiswal, V. Ilievski, K. D. Beaman, T. Jilling, and E. Hirsch. 2014. Platelet-activating factor: a role in preterm delivery and an essential interaction with Toll-like receptor signaling in mice. *Biol. Reprod.* 91: 119.
48. St Louis, D., R. Romero, O. Plazyo, M. Arenas-Hernandez, B. Panaitescu, Y. Xu, T. Milovic, Z. Xu, G. Bhatti, Q. S. Mi, et al. 2016. Invariant NKT cell activation induces late preterm birth that is attenuated by rosiglitazone. *J. Immunol.* 196: 1044–1059.
49. Xu, Y., R. Romero, D. Miller, L. Kadam, T. N. Mial, O. Plazyo, V. Garcia-Flores, S. S. Hassan, Z. Xu, A. L. Tarca, et al. 2016. An M1-like macrophage polarization in decidual tissue during spontaneous preterm labor that is attenuated by rosiglitazone treatment. *J. Immunol.* 196: 2476–2491.
50. Arenas-Hernandez, M., R. Romero, D. St Louis, S. S. Hassan, E. B. Kaye, and N. Gomez-Lopez. 2016. An imbalance between innate and adaptive immune cells at the maternal-fetal interface occurs prior to endotoxin-induced preterm birth. *Cell. Mol. Immunol.* 13: 462–473.
51. Gomez-Lopez, N., R. Romero, M. Arenas-Hernandez, G. Schwenkel, D. St Louis, S. S. Hassan, and T. N. Mial. 2017. In vivo activation of invariant natural killer T cells induces systemic and local alterations in T-cell subsets prior to preterm birth. *Clin. Exp. Immunol.* 189: 211–225.
52. Musilova, I., C. Andrys, J. Krejsek, M. Drahosova, B. Zednikova, L. Pliskova, H. Zemlickova, B. Jacobsson, and M. Kacerovsky. 2018. Amniotic fluid pentraxins: potential early markers for identifying intra-amniotic inflammatory complications in preterm pre-labor rupture of membranes. *Am. J. Reprod. Immunol.* 79: e12789.
53. Negishi, Y., Y. Shima, T. Takeshita, and H. Takahashi. 2017. Distribution of invariant natural killer T cells and dendritic cells in late pre-term birth without acute chorioamnionitis. *Am. J. Reprod. Immunol.* 77: e12658.
54. Xu, Y., R. Romero, D. Miller, P. Silva, B. Panaitescu, K. R. Theis, A. Arif, S. S. Hassan, and N. Gomez-Lopez. 2018. Innate lymphoid cells at the human maternal-fetal interface in spontaneous preterm labor. *Am. J. Reprod. Immunol.* 79: e12820.
55. Vince, G. S., P. M. Starkey, M. C. Jackson, I. L. Sargent, and C. W. Redman. 1990. Flow cytometric characterisation of cell populations in human pregnancy decidua and isolation of decidual macrophages. *J. Immunol. Methods* 132: 181–189.
56. Vargas, M. L., J. L. Sántos, C. Ruiz, M. J. Montes, P. Alemán, C. García-Tortosa, and E. García-Olivares. 1993. Comparison of the proportions of leukocytes in early and term human decidua. *Am. J. Reprod. Immunol.* 29: 135–140.
57. Sindram-Trujillo, A., S. Scherjon, H. Kanhai, D. Roelen, and F. Claas. 2003. Increased T-cell activation in decidua parietalis compared to decidua basalis in uncomplicated human term pregnancy. *Am. J. Reprod. Immunol.* 49: 261–268.
58. Sindram-Trujillo, A. P., S. A. Scherjon, P. P. van Hulst-van Miert, H. H. Kanhai, D. L. Roelen, and F. H. Claas. 2004. Comparison of decidual leukocytes following spontaneous vaginal delivery and elective cesarean section in uncomplicated human term pregnancy. *J. Reprod. Immunol.* 62: 125–137.
59. Tilburgs, T., D. L. Roelen, B. J. van der Mast, J. J. van Schip, C. Kleijburg, G. M. de Groot-Swings, H. H. Kanhai, F. H. Claas, and S. A. Scherjon. 2006. Differential distribution of $CD4^+CD25^{bright}$ and $CD8^+CD28^-$ T-cells in decidua and maternal blood during human pregnancy. *Placenta* 27(Suppl A): S47–S53.
60. Taglauer, E. S., A. S. Trikhacheva, J. G. Slusser, and M. G. Petroff. 2008. Expression and function of PDCD1 at the human maternal-fetal interface. *Biol. Reprod.* 79: 562–569.
61. Gomez-Lopez, N., L. Vadillo-Perez, A. Hernandez-Carbajal, M. Godines-Enriquez, D. M. Olson, and F. Vadillo-Ortega. 2011. Specific inflammatory microenvironments in the zones of the fetal membranes at term delivery. *Am. J. Obstet. Gynecol.* 205: 235.e15-24.
62. Gomez-Lopez, N., R. Vega-Sanchez, M. Castillo-Castrejon, R. Romero, K. Cubeiro-Arreola, and F. Vadillo-Ortega. 2013. Evidence for a role for the adaptive immune response in human term parturition. *Am. J. Reprod. Immunol.* 69: 212–230.
63. Gomez-Lopez, N., G. Estrada-Gutierrez, L. Jimenez-Zamudio, R. Vega-Sanchez, and F. Vadillo-Ortega. 2009. Fetal membranes exhibit selective leukocyte chemotaxic activity during human labor. *J. Reprod. Immunol.* 80: 122–131.
64. Gomez-Lopez, N., D. M. Olson, and S. A. Robertson. 2016. Interleukin-6 controls uterine Th9 cells and $CD8^+$ T regulatory cells to accelerate parturition in mice. *Immunol. Cell Biol.* 94: 79–89.
65. Tarca, A. L., R. Romero, Z. Xu, N. Gomez-Lopez, O. Erez, C. D. Hsu, S. S. Hassan, and V. J. Carey. 2019. 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.* 9: 848.
66. Arenas-Hernandez, M., R. Romero, Y. Xu, B. Panaitescu, V. Garcia-Flores, D. Miller, H. Ahn, B. Done, S. S. Hassan, C. D. Hsu, et al. 2019. Effector and activated T cells induce preterm labor and birth that is prevented by treatment with progesterone. *J. Immunol.* 202: 2585–2608.
67. Miller, D., M. Gershater, R. Slutsky, R. Romero, and N. Gomez-Lopez. 2020. Maternal and fetal T cells in term pregnancy and preterm labor. *Cell. Mol. Immunol.* 17: 693–704.
68. Pique-Regi, R., R. Romero, A. L. Tarca, E. D. Sendler, Y. Xu, V. Garcia-Flores, Y. Leng, F. Luca, S. S. Hassan, and N. Gomez-Lopez. 2019. Single cell transcriptional signatures of the human placenta in term and preterm parturition. *eLife* 8: e52004.
69. Romero, R., R. Quintero, and C. Brekus. 1991. Assessment of gestational age. In *Abnormal Fetal Growth*. M. Y. Divon, ed. Elsevier Science Publishing, New York, p. 47–65.
70. Romero, R., R. Quintero, J. Nores, C. Avila, M. Mazor, S. Hanaoka, Z. Hagay, L. Merchant, and J. C. Hobbins. 1991. Amniotic fluid white blood cell count: a rapid and simple test to diagnose microbial invasion of the amniotic cavity and predict preterm delivery. *Am. J. Obstet. Gynecol.* 165: 821–830.
71. Romero, R., C. Jimenez, A. K. Lohda, J. Nores, S. Hanaoka, C. Avila, R. Callahan, M. Mazor, J. C. Hobbins, and M. P. Diamond. 1990. Amniotic fluid glucose concentration: a rapid and simple method for the detection of intraamniotic infection in preterm labor. *Am. J. Obstet. Gynecol.* 163: 968–974.
72. Romero, R., M. Emamian, R. Quintero, M. Wan, J. C. Hobbins, M. Mazor, and S. Edberg. 1988. The value and limitations of the Gram stain examination in the diagnosis of intraamniotic infection. *Am. J. Obstet. Gynecol.* 159: 114–119.
73. Romero, R., M. Sirtori, E. Oyarzun, C. Avila, M. Mazor, R. Callahan, V. Sabo, A. P. Athanassiadis, and J. C. Hobbins. 1989. Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. *Am. J. Obstet. Gynecol.* 161: 817–824.
74. Yoon, B. H., R. Romero, J. B. Moon, S. S. Shim, M. Kim, G. Kim, and J. K. Jun. 2001. Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. *Am. J. Obstet. Gynecol.* 185: 1130–1136.
75. Romero, R., J. Miranda, T. Chaiworapongsa, P. Chaemsaithong, F. Gotsch, Z. Dong, A. I. Ahmed, B. H. Yoon, S. S. Hassan, C. J. Kim, et al. 2015. Sterile intra-amniotic inflammation in asymptomatic patients with a sonographic short cervix: prevalence and clinical significance. [Published erratum appears in 2020 *J. Matern. Fetal Neonatal Med.* 33: 2506.] *J. Matern. Fetal Neonatal Med.* 28: 1343–1359.
76. Romero, R., Y. M. Kim, P. Pacora, C. J. Kim, N. Benshalom-Tirosh, S. Jaiman, G. Bhatti, J. S. Kim, F. Qureshi, S. M. Jacques, et al. 2018. The frequency and type of placental histologic lesions in term pregnancies with normal outcome. *J. Perinat. Med.* 46: 613–630.
77. Redline, R. W. 2006. Inflammatory responses in the placenta and umbilical cord. *Semin. Fetal Neonatal Med.* 11: 296–301.
78. Kim, C. J., R. Romero, P. Chaemsaithong, N. Chaiyasit, B. H. Yoon, and Y. M. Kim. 2015. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. *Am. J. Obstet. Gynecol.* 213(4 Suppl): S29–S52.
79. Redline, R. W. 2015. Classification of placental lesions. *Am. J. Obstet. Gynecol.* 213(4, Suppl)S21–S28.
80. Xu, Y., O. Plazyo, R. Romero, S. S. Hassan, and N. Gomez-Lopez. 2015. Isolation of Leukocytes from the Human Maternal-fetal Interface. *J. Vis. Exp.* 99: e52863.
81. Bray, N. L., H. Pimentel, P. Melsted, and L. Pachter. 2016. Near-optimal probabilistic RNA-seq quantification. [Published erratum appears in 2016 *Nat. Biotechnol.* 34: 888.] *Nat. Biotechnol.* 34: 525–527.
82. Melsted, P., A. S. Booeshaghi, L. Liu, F. Gao, L. Lu, K. H. J. Min, E. da Veiga Beltrame, K. E. Hjörleifsson, J. Gehring, and L. Pachter. 2021. Modular, efficient and constant-memory single-cell RNA-seq preprocessing. *Nat. Biotechnol.* 39: 813–818.
83. Alvarez, M., E. Rahmani, B. Jew, K. M. Garske, Z. Miao, J. N. Benhammou, C. J. Ye, J. R. Pisegna, K. H. Pietiläinen, E. Halperin, and P. Pajukanta. 2020. Enhancing droplet-based single-nucleus RNA-seq resolution using the semi-supervised machine learning classifier DIEM. *Sci Rep.* 10: 11019.
84. Hafemeister, C., and R. Satija. 2019. Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression. *Genome Biol.* 20: 296.
85. Stuart, T., A. Butler, P. Hoffman, C. Hafemeister, E. Papalexi, W. M. Mauck, 3rd, Y. Hao, M. Stoeckius, P. Smibert, and R. Satija. 2019. Comprehensive integration of single-cell data. *Cell* 177: 1888–1902.e21.
86. Korsunsky, I., N. Millard, J. Fan, K. Slowikowski, F. Zhang, K. Wei, Y. Baglaenko, M. Brenner, P. R. Loh, and S. Raychaudhuri. 2019. Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat. Methods* 16: 1289–1296.
87. Wickham, H. 2011. ggplot2. *Wiley Interdiscip. Rev. Comput. Stat.* 3: 180–185.
88. Booeshaghi, A. S., and L. Pachter. 2021. Normalization of single-cell RNA-seq counts by $\log(x + 1)$ or $\log(1 + x)$. *Bioinformatics* 37: 2223–2224.
89. Gomez-Lopez, N., R. Romero, M. Arenas-Hernandez, H. Ahn, B. Panaitescu, F. Vadillo-Ortega, C. Sanchez-Torres, K. S. Salisbury, and S. S. Hassan. 2016. In vivo T-cell activation by a monoclonal αCD3ε antibody induces preterm labor and birth. *Am. J. Reprod. Immunol.* 76: 386–390.
90. Garcia-Flores, V., R. Romero, D. Miller, Y. Xu, B. Done, C. Veerapaneni, Y. Leng, M. Arenas-Hernandez, N. Khan, B. Panaitescu, et al. 2018. Inflammation-

induced adverse pregnancy and neonatal outcomes can be improved by the immunomodulatory peptide exendin-4. *Front. Immunol.* 9: 1291.
91. Bersani, I., M. P. De Carolis, D. Foell, T. Weinhage, E. D. Rossi, S. De Carolis, S. A. Rubortone, C. Romagnoli, and C. P. Speer. 2015. Interleukin-22: biomarker of maternal and fetal inflammation? *Immunol. Res.* 61: 4–10.
92. Motomura, K., R. Romero, Y. Xu, K. R. Theis, J. Galaz, A. D. Winters, R. Slutsky, V. Garcia-Flores, C. Zou, D. Levenson, et al. 2020. Intra-amniotic infection with *Ureaplasma parvum* causes preterm birth and neonatal mortality that are prevented by treatment with clarithromycin. *MBio* 11: e00797-20.
93. Kaech, S. M., J. T. Tan, E. J. Wherry, B. T. Konieczny, C. D. Surh, and R. Ahmed. 2003. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nat. Immunol.* 4: 1191–1198.
94. Huster, K. M., V. Busch, M. Schiemann, K. Linkemann, K. M. Kerksiek, H. Wagner, and D. H. Busch. 2004. Selective expression of IL-7 receptor on memory T cells identifies early CD40L-dependent generation of distinct $CD8^+$ memory T cell subsets. *Proc. Natl. Acad. Sci. USA* 101: 5610–5615.
95. Hara, T., L. K. Jung, J. M. Bjorndahl, and S. M. Fu. 1986. Human T cell activation. III. Rapid induction of a phosphorylated 28 kD/32 kD disulfide-linked early activation antigen (EA 1) by 12-o-tetradecanoyl phorbol-13-acetate, mitogens, and antigens. *J. Exp. Med.* 164: 1988–2005.
96. Testi, R., J. H. Phillips, and L. L. Lanier. 1989. T cell activation via Leu-23 (CD69). *J. Immunol.* 143: 1123–1128.
97. Male, V., T. Hughes, S. McClory, F. Colucci, M. A. Caligiuri, and A. Moffett. 2010. Immature NK cells, capable of producing IL-22, are present in human uterine mucosa. *J. Immunol.* 185: 3913–3918.
98. Vacca, P., C. Vitale, E. Montaldo, R. Conte, C. Cantoni, E. Fulcheri, V. Darretta, L. Moretta, and M. C. Mingari. 2011. $CD34^+$ hematopoietic precursors are present in human decidua and differentiate into natural killer cells upon interaction with stromal cells. *Proc. Natl. Acad. Sci. USA* 108: 2402–2407.
99. Vacca, P., E. Montaldo, D. Croxatto, F. Loiacono, F. Canegallo, P. L. Venturini, L. Moretta, and M. C. Mingari. 2015. Identification of diverse innate lymphoid cells in human decidua. *Mucosal Immunol.* 8: 254–264.
100. Doisne, J. M., E. Balmas, S. Boulenouar, L. M. Gaynor, J. Kieckbusch, L. Gardner, D. A. Hawkes, C. F. Barbara, A. M. Sharkey, H. J. Brady, et al. 2015. Composition, development, and function of uterine innate lymphoid cells. *J. Immunol.* 195: 3937–3945.
101. Filipovic, I., L. Chiossone, P. Vacca, R. S. Hamilton, T. Ingegnere, J. M. Doisne, D. A. Hawkes, M. C. Mingari, A. M. Sharkey, L. Moretta, and F. Colucci. 2018. Molecular definition of group 1 innate lymphoid cells in the mouse uterus. *Nat. Commun.* 9: 4492.
102. Gomez-Lopez, N., R. Romero, Y. Leng, V. Garcia-Flores, Y. Xu, D. Miller, and S. S. Hassan. 2017. Neutrophil extracellular traps in acute chorioamnionitis: a mechanism of host defense. *Am. J. Reprod. Immunol.* 77: e12617.
103. Gomez-Lopez, N., R. Romero, Y. Xu, D. Miller, Y. Leng, B. Panaitescu, P. Silva, J. Faro, A. Alhousseini, N. Gill, et al. 2018. The immunophenotype of amniotic fluid leukocytes in normal and complicated pregnancies. *Am. J. Reprod. Immunol.* 79: e12827.
104. Gomez-Lopez, N., R. Romero, J. Galaz, Y. Xu, B. Panaitescu, R. Slutsky, K. Motomura, N. Gill, R. Para, P. Pacora, et al. 2019. Cellular immune responses in amniotic fluid of women with preterm labor and intra-amniotic infection or intra-amniotic inflammation. *Am. J. Reprod. Immunol.* 82: e13171.
105. Cai, G., X. Nie, W. Zhang, B. Wu, J. Lin, H. Wang, C. Jiang, and Q. Shen. 2012. A regulatory role for IL-10 receptor signaling in development and B cell help of T follicular helper cells in mice. *J. Immunol.* 189: 1294–1302.
106. Shouval, D. S., J. Ouahed, A. Biswas, J. A. Goettel, B. H. Horwitz, C. Klein, A. M. Muise, and S. B. Snapper. 2014. Interleukin 10 receptor signaling: master regulator of intestinal mucosal homeostasis in mice and humans. *Adv. Immunol.* 122: 177–210.
107. Shouval, D. S., A. Biswas, J. A. Goettel, K. McCann, E. Conaway, N. S. Redhu, I. D. Mascanfroni, Z. Al Adham, S. Lavoie, M. Ibourk, et al. 2014. Interleukin-10 receptor signaling in innate immune cells regulates mucosal immune tolerance and anti-inflammatory macrophage function. *Immunity* 40: 706–719.
108. Weber, G. F., S. Schlautkötter, S. Kaiser-Moore, F. Altmayr, B. Holzmann, and H. Weighardt. 2007. Inhibition of interleukin-22 attenuates bacterial load and organ failure during acute polymicrobial sepsis. *Infect. Immun.* 75: 1690–1697.
109. Bingold, T. M., E. Ziesché, B. Scheller, C. D. Sadik, K. Franck, L. Just, S. Sartorius, M. Wahrmann, H. Wissing, B. Zwissler, et al. 2010. Interleukin-22 detected in patients with abdominal sepsis. *Shock* 34: 337–340.
110. Aziz, M., A. Jacob, W. L. Yang, A. Matsuda, and P. Wang. 2013. Current trends in inflammatory and immunomodulatory mediators in sepsis. *J. Leukoc. Biol.* 93: 329–342.
111. Mihi, B., Q. Gong, L. S. Nolan, S. E. Gale, M. Goree, E. Hu, W. E. Lanik, J. M. Rimer, V. Liu, O. B. Parks, et al. 2021. Interleukin-22 signaling attenuates necrotizing enterocolitis by promoting epithelial cell regeneration. *Cell Rep. Med.* 2: 100320.
112. Leyva-Castillo, J. M., J. Yoon, and R. S. Geha. 2019. IL-22 promotes allergic airway inflammation in epicutaneously sensitized mice. [Published erratum appears in 2019 *J. Allergy Clin. Immunol.* 144: 1142.] *J. Allergy Clin. Immunol.* 143: 619–630.e7.
113. Starkey, M. R., M. W. Plank, P. Casolari, A. Papi, S. Pavlidis, Y. Guo, G. J. M. Cameron, T. J. Haw, A. Tam, M. Obiedat, et al. 2019. IL-22 and its receptors are increased in human and experimental COPD and contribute to pathogenesis. *Eur. Respir. J.* 54: 1800174.
114. Colin, A. A., C. McEvoy, and R. G. Castile. 2010. Respiratory morbidity and lung function in preterm infants of 32 to 36 weeks' gestational age. *Pediatrics* 126: 115–128.
115. Stoll, B. J., N. I. Hansen, E. F. Bell, S. Shankaran, A. R. Laptook, M. C. Walsh, E. C. Hale, N. S. Newman, K. Schibler, W. A. Carlo, et al.; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. 2010. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. *Pediatrics* 126: 443–456.
116. Whitsett, J. A., S. E. Wert, and T. E. Weaver. 2015. Diseases of pulmonary surfactant homeostasis. *Annu. Rev. Pathol.* 10: 371–393.
117. Matthay, M. A., R. L. Zemans, G. A. Zimmerman, Y. M. Arabi, J. R. Beitler, A. Mercat, M. Herridge, A. G. Randolph, and C. S. Calfee. 2019. Acute respiratory distress syndrome. *Nat. Rev. Dis. Primers* 5: 18.
118. Çekmez, Y., F. Cekmez, E. Ozkaya, O. Pirgon, Z. Yõlmaz, E. A. Yõlmaz, G. Kaya, N. Süer, and T. Küçüközkan. 2013. uPAR, IL-33, and ST2 values as a predictor of subclinical chorioamnionitis in preterm premature rupture of membranes. *J. Interferon Cytokine Res.* 33: 778–782.
119. Bhatti, G., R. Romero, G. E. Rice, W. Fitzgerald, P. Pacora, N. Gomez-Lopez, M. Kavdia, A. L. Tarca, and L. Margolis. 2020. Compartmentalized profiling of amniotic fluid cytokines in women with preterm labor. *PLoS One* 15: e0227881.
120. Erez, O., R. Romero, A. L. Tarca, T. Chaiworapongsa, Y. M. Kim, N. G. Than, E. Vaisbuch, S. Draghici, and G. Tromp. 2009. Differential expression pattern of genes encoding for anti-microbial peptides in the fetal membranes of patients with spontaneous preterm labor and intact membranes and those with preterm prelabor rupture of the membranes. *J. Matern. Fetal Neonatal Med.* 22: 1103–1115.
121. Varrey, A., R. Romero, B. Panaitescu, D. Miller, T. Chaiworapongsa, M. Patwardhan, J. Faro, P. Pacora, S. S. Hassan, C. D. Hsu, and N. Gomez-Lopez. 2018. Human β-defensin-1: a natural antimicrobial peptide present in amniotic fluid that is increased in spontaneous preterm labor with intra-amniotic infection. *Am. J. Reprod. Immunol.* 80: e13031.
122. Yoon, B. H., J. W. Chang, and R. Romero. 1998. Isolation of *Ureaplasma urealyticum* from the amniotic cavity and adverse outcome in preterm labor. *Obstet. Gynecol.* 92: 77–82.
123. DiGiulio, D. B., R. Romero, J. P. Kusanovic, R. Gómez, C. J. Kim, K. S. Seok, F. Gotsch, S. Mazaki-Tovi, E. Vaisbuch, K. Sanders, et al. 2010. Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes. *Am. J. Reprod. Immunol.* 64: 38–57.
124. Romero, R., J. Miranda, J. P. Kusanovic, T. Chaiworapongsa, P. Chaemsaithong, A. Martinez, F. Gotsch, Z. Dong, A. I. Ahmed, M. Shaman, et al. 2015. Clinical chorioamnionitis at term I: microbiology of the amniotic cavity using cultivation and molecular techniques. *J. Perinat. Med.* 43: 19–36.
125. Rittenschober-Böhm, J., T. Waldhoer, S. M. Schulz, B. Pimpel, K. Goeral, D. C. Kasper, A. Witt, and A. Berger. 2019. Vaginal Ureaplasma parvum serovars and spontaneous preterm birth. *Am. J. Obstet. Gynecol.* 220: 594.e1–594.e9.
126. Romero, R., C. Avila, U. Santhanam, and P. B. Sehgal. 1990. Amniotic fluid interleukin 6 in preterm labor. Association with infection. *J. Clin. Invest.* 85: 1392–1400.
127. Martinez-Varea, A., R. Romero, Y. Xu, D. Miller, A. I. Ahmed, P. Chaemsaithong, N. Chaiyasit, L. Yeo, M. Shaman, K. Lannaman, et al. 2017. Clinical chorioamnionitis at term VII: the amniotic fluid cellular immune response. *J. Perinat. Med.* 45: 523–538.
128. Galaz, J., R. Romero, R. Slutsky, Y. Xu, K. Motomura, R. Para, P. Pacora, B. Panaitescu, C. D. Hsu, M. Kacerovsky, and N. Gomez-Lopez. 2020. Cellular immune responses in amniotic fluid of women with preterm prelabor rupture of membranes. *J. Perinat. Med.* 48: 222–233.
129. Chaemsaithong, P., R. Romero, S. J. Korzeniewski, A. Martinez-Varea, Z. Dong, B. H. Yoon, S. S. Hassan, T. Chaiworapongsa, and L. Yeo. 2016. A rapid interleukin-6 bedside test for the identification of intra-amniotic inflammation in preterm labor with intact membranes. *J. Matern. Fetal Neonatal Med.* 29: 349–359.
130. Gomez, R., R. Romero, F. Ghezzi, B. H. Yoon, M. Mazor, and S. M. Berry. 1998. The fetal inflammatory response syndrome. *Am. J. Obstet. Gynecol.* 179: 194–202.
131. Jung, E., R. Romero, L. Yeo, R. Diaz-Primera, J. Marin-Concha, R. Para, A. M. Lopez, P. Pacora, N. Gomez-Lopez, B. H. Yoon, et al. 2020. The fetal inflammatory response syndrome: the origins of a concept, pathophysiology, diagnosis, and obstetrical implications. *Semin. Fetal Neonatal Med.* 25: 101146.
132. Para, R., R. Romero, D. Miller, J. Galaz, B. Done, A. Peyvandipour, M. Gershater, L. Tao, K. Motomura, D. M. Ruden, et al. 2021. The distinct immune nature of the fetal inflammatory response syndrome type I and type II. *Immunohorizons* 5: 735–751.
133. Wang, S. C., Y. H. Li, H. L. Piao, X. W. Hong, D. Zhang, Y. Y. Xu, Y. Tao, Y. Wang, M. M. Yuan, D. J. Li, and M. R. Du. 2015. PD-1 and Tim-3 pathways are associated with regulatory $CD8^+$ T-cell function in decidua and maintenance of normal pregnancy. *Cell Death Dis.* 6: e1738.
134. Lissauer, D., M. D. Kilby, and P. Moss. 2017. Maternal effector T cells within decidua: the adaptive immune response to pregnancy? *Placenta* 60: 140–144.
135. Powell, R. M., D. Lissauer, J. Tamblyn, A. Beggs, P. Cox, P. Moss, and M. D. Kilby. 2017. Decidual T cells exhibit a highly differentiated phenotype and demonstrate potential fetal specificity and a strong transcriptional response to IFN. *J. Immunol.* 199: 3406–3417.
136. van der Zwan, A., K. Bi, E. R. Norwitz, Â. C. Crespo, F. H. J. Claas, J. L. Strominger, and T. Tilburgs. 2018. Mixed signature of activation and dysfunction allows human decidual $CD8^+$ T cells to provide both tolerance and immunity. *Proc. Natl. Acad. Sci. USA* 115: 385–390.
137. Slutsky, R., R. Romero, Y. Xu, J. Galaz, D. Miller, B. Done, A. L. Tarca, S. Gregor, S. S. Hassan, Y. Leng, and N. Gomez-Lopez. 2019. Exhausted and

senescent T cells at the maternal-fetal interface in preterm and term labor. *J. Immunol. Res.* 2019: 3128010.
138. Terzieva, A., V. Dimitrova, L. Djerov, P. Dimitrova, S. Zapryanova, I. Hristova, I. Vangelov, and T. Dimova. 2019. Early pregnancy human decidua is enriched with activated, fully differentiated and pro-inflammatory gamma/delta T cells with diverse TCR repertoires. *Int. J. Mol. Sci.* 20: 687.
139. Pinget, G. V., T. M. Corpuz, J. Stolp, E. L. Lousberg, K. R. Diener, S. A. Robertson, J. Sprent, and K. E. Webster. 2016. The majority of murine γδ T cells at the maternal-fetal interface in pregnancy produce IL-17. *Immunol. Cell Biol.* 94: 623–630.
140. Furcron, A. E., R. Romero, T. N. Mial, A. Balancio, B. Panaitescu, S. S. Hassan, A. Sahi, C. Nord, and N. Gomez-Lopez. 2016. Human chorionic gonadotropin has anti-inflammatory effects at the maternal-fetal interface and prevents endotoxin-induced preterm birth, but causes dystocia and fetal compromise in mice. *Biol. Reprod.* 94: 136.
141. Levenson, D., R. Romero, V. Garcia-Flores, D. Miller, Y. Xu, A. Sahi, S. S. Hassan, and N. Gomez-Lopez. 2020. The effects of advanced maternal age on T-cell subsets at the maternal-fetal interface prior to term labor and in the offspring: a mouse study. *Clin. Exp. Immunol.* 201: 58–75.
142. Gomez-Lopez, N., L. J. Guilbert, and D. M. Olson. 2010. Invasion of the leukocytes into the fetal-maternal interface during pregnancy. *J. Leukoc. Biol.* 88: 625–633.
143. Erlebacher, A. 2013. Immunology of the maternal-fetal interface. *Annu. Rev. Immunol.* 31: 387–411.
144. Gomez-Lopez, N., D. StLouis, M. A. Lehr, E. N. Sanchez-Rodriguez, and M. Arenas-Hernandez. 2014. Immune cells in term and preterm labor. *Cell. Mol. Immunol.* 11: 571–581.
145. Bonney, E. A. 2016. Immune regulation in pregnancy: a matter of perspective? *Obstet. Gynecol. Clin. North Am.* 43: 679–698.
146. Bonney, E. A., and M. R. Johnson. 2019. The role of maternal T cell and macrophage activation in preterm birth: cause or consequence? *Placenta* 79: 53–61.
147. Nancy, P., E. Tagliani, C. S. Tay, P. Asp, D. E. Levy, and A. Erlebacher. 2012. Chemokine gene silencing in decidual stromal cells limits T cell access to the maternal-fetal interface. *Science* 336: 1317–1321.
148. Griffith, O. W., A. R. Chavan, S. Protopapas, J. Maziarz, R. Romero, and G. P. Wagner. 2017. Embryo implantation evolved from an ancestral inflammatory attachment reaction. *Proc. Natl. Acad. Sci. USA* 114: E6566–E6575.
149. Chavan, A. R., O. W. Griffith, D. J. Stadtmauer, J. Maziarz, M. Pavlicev, R. Fishman, L. Koren, R. Romero, and G. P. Wagner. 2021. Evolution of embryo implantation was enabled by the origin of decidual stromal cells in eutherian mammals. *Mol. Biol. Evol.* 38: 1060–1074.
150. Aluvihare, V. R., M. Kallikourdis, and A. G. Betz. 2004. Regulatory T cells mediate maternal tolerance to the fetus. *Nat. Immunol.* 5: 266–271.
151. Sasaki, Y., M. Sakai, S. Miyazaki, S. Higuma, A. Shiozaki, and S. Saito. 2004. Decidual and peripheral blood $CD4^+CD25^+$ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. *Mol. Hum. Reprod.* 10: 347–353.
152. Rowe, J. H., J. M. Ertelt, L. Xin, and S. S. Way. 2012. Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. *Nature* 490: 102–106.
153. Samstein, R. M., S. Z. Josefowicz, A. Arvey, P. M. Treuting, and A. Y. Rudensky. 2012. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. *Cell* 150: 29–38.
154. Robertson, S. A., A. S. Care, and L. M. Moldenhauer. 2018. Regulatory T cells in embryo implantation and the immune response to pregnancy. *J. Clin. Invest.* 128: 4224–4235.
155. Zenclussen, A. C., K. Gerlof, M. L. Zenclussen, A. Sollwedel, A. Z. Bertoja, T. Ritter, K. Kotsch, J. Leber, and H. D. Volk. 2005. 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.* 166: 811–822.
156. Darrasse-Jèze, G., D. Klatzmann, F. Charlotte, B. L. Salomon, and J. L. Cohen. 2006. $CD4^+CD25^+$ regulatory/suppressor T cells prevent allogeneic fetus rejection in mice. [Published erratum appears in 2006 *Immunol. Lett.* 102: 241.] *Immunol. Lett.* 102: 106–109.
157. Kahn, D. A., and D. Baltimore. 2010. Pregnancy induces a fetal antigen-specific maternal T regulatory cell response that contributes to tolerance. *Proc. Natl. Acad. Sci. USA* 107: 9299–9304.
158. Rowe, J. H., J. M. Ertelt, M. N. Aguilera, M. A. Farrar, and S. S. Way. 2011. $Foxp3^+$ regulatory T cell expansion required for sustaining pregnancy compromises host defense against prenatal bacterial pathogens. *Cell Host Microbe* 10: 54–64.
159. Chen, T., G. Darrasse-Jèze, A. S. Bergot, T. Courau, G. Churlaud, K. Valdivia, J. L. Strominger, M. G. Ruocco, G. Chaouat, and D. Klatzmann. 2013. Self-specific memory regulatory T cells protect embryos at implantation in mice. *J. Immunol.* 191: 2273–2281.
160. Gomez-Lopez, N., M. Arenas-Hernandez, R. Romero, D. Miller, V. Garcia-Flores, Y. Leng, Y. Xu, J. Galaz, S. S. Hassan, C. D. Hsu, et al. 2020. Regulatory T cells play a role in a subset of idiopathic preterm labor/birth and adverse neonatal outcomes. *Cell Rep.* 32: 107874.
161. Kim, C. J., R. Romero, J. P. Kusanovic, W. Yoo, Z. Dong, V. Topping, F. Gotsch, B. H. Yoon, J. G. Chi, and J. S. Kim. 2010. The frequency, clinical significance, and pathological features of chronic chorioamnionitis: a lesion associated with spontaneous preterm birth. *Mod. Pathol.* 23: 1000–1011.
162. Kim, C. J., R. Romero, P. Chaemsaithong, and J. S. Kim. 2015. Chronic inflammation of the placenta: definition, classification, pathogenesis, and clinical significance. *Am. J. Obstet. Gynecol.* 213(4, Suppl): S53–S69.
163. Nograles, K. E., L. C. Zaba, A. Shemer, J. Fuentes-Duculan, I. Cardinale, T. Kikuchi, M. Ramon, R. Bergman, J. G. Krueger, and E. Guttman-Yassky. 2009. IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing T_H17 T cells. *J. Allergy Clin. Immunol.* 123: 1244–52.e2.
164. Liu, Y., B. Yang, M. Zhou, L. Li, H. Zhou, J. Zhang, H. Chen, and C. Wu. 2009. Memory IL-22-producing $CD4^+$ T cells specific for *Candida albicans* are present in humans. *Eur. J. Immunol.* 39: 1472–1479.
165. Duhen, T., R. Geiger, D. Jarrossay, A. Lanzavecchia, and F. Sallusto. 2009. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat. Immunol.* 10: 857–863.
166. Trifari, S., C. D. Kaplan, E. H. Tran, N. K. Crellin, and H. Spits. 2009. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T_H-17, T_H1 and T_H2 cells. *Nat. Immunol.* 10: 864–871.
167. Eyerich, S., K. Eyerich, D. Pennino, T. Carbone, F. Nasorri, S. Pallotta, F. Cianfarani, T. Odorisio, C. Traidl-Hoffmann, H. Behrendt, et al. 2009. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J. Clin. Invest.* 119: 3573–3585.
168. Plank, M. W., G. E. Kaiko, S. Maltby, J. Weaver, H. L. Tay, W. Shen, M. S. Wilson, S. K. Durum, and P. S. Foster. 2017. Th22 cells form a distinct Th lineage from Th17 cells in vitro with unique transcriptional properties and Tbet-dependent Th1 plasticity. *J. Immunol.* 198: 2182–2190.
169. Lin, X., S. Tawch, H. T. Wong, S. Roy, S. Gaudino, P. Castillo, W. Elsegeiny, N. Wakabayashi, T. D. Oury, D. Pociask, et al. 2021. Nrf2 through aryl hydrocarbon receptor regulates IL-22 response in $CD4^+$ T cells. *J. Immunol.* 206: 1540–1548.
170. Logiodice, F., L. Lombardelli, O. Kullolli, H. Haller, E. Maggi, D. Rukavina, and M. P. Piccinni. 2019. Decidual interleukin-22-producing $CD4^+$ T cells ($Th17/Th0/IL\text{-}22^+$ and $Th17/Th2/IL\text{-}22^+$, $Th2/IL\text{-}22^+$, $Th0/IL\text{-}22^+$), which also produce IL-4, are involved in the success of pregnancy. *Int. J. Mol. Sci.* 20: 428.
171. Wolk, K., E. Witte, U. Hoffmann, W. D. Doecke, S. Endesfelder, K. Asadullah, W. Sterry, H. D. Volk, B. M. Wittig, and R. Sabat. 2007. IL-22 induces lipopolysaccharide-binding protein in hepatocytes: a potential systemic role of IL-22 in Crohn's disease. *J. Immunol.* 178: 5973–5981.
172. Kagami, S., H. L. Rizzo, J. J. Lee, Y. Koguchi, and A. Blauvelt. 2010. Circulating Th17, Th22, and Th1 cells are increased in psoriasis. *J. Invest. Dermatol.* 130: 1373–1383.
173. Basu, R., D. B. O'Quinn, D. J. Silberger, T. R. Schoeb, L. Fouser, W. Ouyang, R. D. Hatton, and C. T. Weaver. 2012. Th22 cells are an important source of IL-22 for host protection against enteropathogenic bacteria. *Immunity* 37: 1061–1075.
174. Sakamoto, K., Y. G. Kim, H. Hara, N. Kamada, G. Caballero-Flores, E. Tolosano, M. P. Soares, J. L. Puente, N. Inohara, and G. Núñez. 2017. IL-22 controls iron-dependent nutritional immunity against systemic bacterial infections. *Sci. Immunol.* 2: eaai8371.
175. Huggins, M. A., F. V. Sjaastad, M. Pierson, T. A. Kucaba, W. Swanson, C. Staley, A. R. Weingarden, I. J. Jensen, D. B. Danahy, V. P. Badovinac, et al. 2019. Microbial exposure enhances immunity to pathogens recognized by TLR2 but increases susceptibility to cytokine storm through TLR4 sensitization. *Cell Rep.* 28: 1729–1743.e5.
176. Zhang, Z., H. Liu, Y. Shi, N. Xu, Y. Wang, A. Li, and W. Song. 2017. Increased circulating Th22 cells correlated with Th17 cells in patients with severe preeclampsia. *Hypertens. Pregnancy* 36: 100–107.
177. Chappell, L. C., C. A. Cluver, J. Kingdom, and S. Tong. 2021. Pre-eclampsia. *Lancet* 398: 341–354.
178. Reisenberger, K., C. Egarter, S. Vogl, B. Sternberger, H. Kiss, and P. Husslein. 1996. The transfer of interleukin-8 across the human placenta perfused in vitro. *Obstet. Gynecol.* 87: 613–616.
179. Ponzio, N. M., R. Servatius, K. Beck, A. Marzouk, and T. Kreider. 2007. Cytokine levels during pregnancy influence immunological profiles and neurobehavioral patterns of the offspring. *Ann. N. Y. Acad. Sci.* 1107: 118–128.
180. Zaretsky, M. V., J. M. Alexander, W. Byrd, and R. E. Bawdon. 2004. Transfer of inflammatory cytokines across the placenta. *Obstet. Gynecol.* 103: 546–550.
181. Dahlgren, J., A. M. Samuelsson, T. Jansson, and A. Holmäng. 2006. Interleukin-6 in the maternal circulation reaches the rat fetus in mid-gestation. *Pediatr. Res.* 60: 147–151.
182. Xu, W., S. R. Presnell, J. Parrish-Novak, W. Kindsvogel, S. Jaspers, Z. Chen, S. R. Dillon, Z. Gao, T. Gilbert, K. Madden, et al. 2001. A soluble class II cytokine receptor, IL-22RA2, is a naturally occurring IL-22 antagonist. *Proc. Natl. Acad. Sci. USA* 98: 9511–9516.
183. Sabat, R., W. Ouyang, and K. Wolk. 2014. Therapeutic opportunities of the IL-22-IL-22R1 system. *Nat. Rev. Drug Discov.* 13: 21–38.
184. De Luca, A., T. Zelante, C. D'Angelo, S. Zagarella, F. Fallarino, A. Spreca, R. G. Iannitti, P. Bonifazi, J. C. Renauld, F. Bistoni, et al. 2010. IL-22 defines a novel immune pathway of antifungal resistance. *Mucosal Immunol.* 3: 361–373.
185. Kisand, K., A. S. Bøe Wolff, K. T. Podkrajsek, L. Tserel, M. Link, K. V. Kisand, E. Ersvaer, J. Perheentupa, M. M. Erichsen, N. Bratanic, et al. 2010. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. *J. Exp. Med.* 207: 299–308.
186. Tripathi, D., R. K. Radhakrishnan, R. S. Thandi, P. Paidipally, K. P. Devalraju, V. S. K. Neela, A. R. Tvinnereim, V. L. Valluri, and R. Vankayalapati. 2019. IL-22 reduces the mortality of type 2 diabetes mellitus (T2DM) mice infected with Mycobacterium tuberculosis. *J. Immunol.* 202(1 Suppl): 181.19.
187. Nadeem, A., H. Deshmukh, J. Gray, and T. Wang. 2020. Intestinal commensal bacteria promote AT2 self-renewal and AT1 differentiation in an IL-22 dependent fashion and prepare the newborn to fight potentially fatal respiratory pathogens. *J. Immunol.* 204(1 Suppl): 225.30.
188. Aujla, S. J., Y. R. Chan, M. Zheng, M. Fei, D. J. Askew, D. A. Pociask, T. A. Reinhart, F. McAllister, J. Edeal, K. Gaus, et al. 2008. IL-22 mediates mucosal

host defense against Gram-negative bacterial pneumonia. *Nat. Med.* 14: 275–281.
189. Zheng, Y., P. A. Valdez, D. M. Danilenko, Y. Hu, S. M. Sa, Q. Gong, A. R. Abbas, Z. Modrusan, N. Ghilardi, F. J. de Sauvage, and W. Ouyang. 2008. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat. Med.* 14: 282–289.
190. Laurence, A., J. J. O'Shea, and W. T. Watford. 2008. Interleukin-22: a sheep in wolf's clothing. *Nat. Med.* 14: 247–249.
191. Dhiman, R., M. Indramohan, P. F. Barnes, R. C. Nayak, P. Paidipally, L. V. Rao, and R. Vankayalapati. 2009. IL-22 produced by human NK cells inhibits growth of *Mycobacterium tuberculosis* by enhancing phagolysosomal fusion. *J. Immunol.* 183: 6639–6645.
192. Madsen-Bouterse, S. A., R. Romero, A. L. Tarca, J. P. Kusanovic, J. Espinoza, C. J. Kim, J. S. Kim, S. S. Edwin, R. Gomez, and S. Draghici. 2010. The transcriptome of the fetal inflammatory response syndrome. *Am. J. Reprod. Immunol.* 63: 73–92.