# NLRP3 mediates trophoblastic inflammasome activation and protects against *Listeria monocytogenes* infection during pregnancy

**Yu Gao[1#], Min Zhou[2,3#], Wen Zhang[4#], Jinxing Jiang[5], Zhibin Ouyang[5], Yuanfang Zhu[6,7], Ning Li[8,9]**

[1]Obstetrics and Gynecology, The Second Clinical Medical College (Shenzhen People's Hospital), Jinan University, Shenzhen, China; [2]Key Laboratory of Zoonosis, Ministry of Education, College of Veterinary Medicine, Jilin University, Changchun, China; [3]School of Life Sciences, Tsinghua University, Beijing, China; [4]Emergency Department, The Second Clinical Medical College (Shenzhen People's Hospital), Jinan University, Shenzhen, China; [5]Cytotherapy Laboratory, The Second Clinical Medical College (Shenzhen People's Hospital), Jinan University, Shenzhen, China; [6]Obstetrics and Gynecology, Shenzhen Bao'an Maternal and Child Health Hospital Affiliated to Jinan University, Jinan University, Shenzhen, China; [7]Obstetrics and Gynecology, The First Affiliated Hospital of Jinan University, Jinan University, Guangzhou, China; [8]Biotherapy Research Center, The Second Affiliated Hospital, School of Medicine, Southern University of Science and Technology, Shenzhen, China; [9]Biotherapy Research Center, National Clinical Research Center for Infectious Disease, Shenzhen Third People's Hospital, Shenzhen, China

*Contributions:* (I) Conception and design: N Li; (II) Administrative support: N Li, Y Zhu; (III) Provision of study materials or patients: Y Gao, M Zhou, W Zhang; (IV) Collection and assembly of data: M Zhou, J Jiang, Z Ouyang, N Li; (V) Data analysis and interpretation: N Li, Y Zhu, Y Gao; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

*Correspondence to:* Ning Li. Biotherapy Research Center, The Second Affiliated Hospital, School of Medicine, Southern University of Science and Technology, Shenzhen, China; Biotherapy Research Center, National Clinical Research Center for Infectious disease, Shenzhen Third People's Hospital, Shenzhen, China. Email: lining.yatu@hotmail.com; Yuanfang Zhu. Obstetrics and Gynecology, Shenzhen Bao'an Maternal and Child Health Hospital Affiliated to Jinan University, Shenzhen, China; Obstetrics and Gynecology, The First Affiliated Hospital of Jinan University, Jinan University, Guangzhou, China. Email: zhuyf1027@163.com.

**Background:** Intrauterine *Listeria monocytogenes* (*L. monocytogenes*) infections pose a major threat during pregnancy via affecting placental immune responses. However, the underlying mechanisms of placental defense against this pathogen remain ill-defined. Therefore, this study aims to investigate the function and the mechanism of inflammasomes on against *L. monocytogenes* infection during pregnancy.

**Methods:** A listeriosis murine model and cell culture system was used to investigate the role of trophoblastic nucleotide-binding oligomerization domain-like receptor pyrin domain-containing 3 (NLRP3) in orchestrating innate immune responses to *L. monocytogenes* infection. Caspase-1 activity was determined using a caspase-1 activity colorimetric kit. NLRP3 and apoptosis-associated speck-like protein containing a CARD (ASC) in placental tissue was detected by immunohistochemistry. NLRP3 in HTR-8/SVneo cells was also detected by immunofluorescence. The expression of interleukin 1β (IL-1β), NLRP3, ASC, and caspase-1 was detected by Western blot. We characterized the NLRP3 inflammasome in trophoblast cells according to whether *L. monocytogenes* infection increased the activation of caspase-1 and the release of IL-1β. For human or mouse IL-1β in the culture supernatants and mouse tissue lysates were analyzed using ELISA Kits.

**Results:** Trophoblast cells constitutively expressed the components of the NLRP3 inflammasome. *In vitro*, *L. monocytogenes* triggers NLRP3 inflammasome activation in trophoblast cells by inducing caspase-1 activation, increasing the NLRP3 protein levels, IL-1β maturation and secretion in HTR-8/SVneo cells. *In vivo*, *L. monocytogenes* induces fetal resorption and IL-1β processing in pregnant mice. In addition, NLRP3-deficient mice were more prone to fetal loss than their wild-type counterparts following infection with *L. monocytogenes* at a lower infective dose.

**Conclusions:** We conclude that trophoblast cells respond to *L. monocytogenes* infection through the NLRP3 receptor, resulting in inflammasome activation and IL-1β production, which prevents listeriosis during pregnancy.

**Keywords:** Pregnancy; trophoblast; *Listeria monocytogenes* (*L. monocytogenes*); inflammasome; nucleotide-binding oligomerization domain-like receptor pyrin domain-containing 3 (NLRP3)

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## Introduction

Preterm birth (PTB), defined as delivery before 37 weeks of gestation, accounting for 70% of perinatal morbidity and mortality. It has become a significant public health problem (1), and microbes are increasingly implicated in triggering pregnancy complications and infection-induced disruptions in fetal tolerance because of the unique susceptibility to infection for pregnancy (2). The infecting organisms usually invade the pregnant uterus via originate from the lower genital tract and the oral cavity which will reach the uterus through hematogenous transmission (3,4). There is accumulating epidemiological and experimental evidence that maternal infection is a significant risk factor for adverse pregnancy outcomes (5). Therefore, it is important to explore the effective anti-intrauterine infection mechanisms in maintaining a viable pregnancy. However, the molecular mechanisms of the innate immune responses at the maternofetal interface remain ill-defined.

Recent studies have suggested that the placenta functions as an active immunologic barrier by trophoblasts recognizing and responding to microbes through innate immune pattern recognition receptors (PRRs) (6-9). PRRs are essential for innate immune system responses to infectious microorganisms and recognize conserved sequences known as pathogen-associated molecular patterns (PAMPs). The cytoplasmic-based Nod-like receptors (NLRs), a family of PRRs, have been identified in both the first-trimester and term placenta (10-13), suggesting that the placenta can control microorganisms that may injure the embryo/fetus, thereby protecting the pregnancy.

Nucleotide-binding oligomerization domain-like receptor pyrin domain-containing 3 (NLRP3), a NLR family member, can drive the assembly of a caspase-1-activating inflammasome. The NLRP3 inflammasome is currently the most fully characterized inflammasome and consists of the NLRP3 scaffold, the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) including a caspase recruitment domain, and caspase-1 (14). NLRP3 is activated upon exposure to bacteria (15), viruses (16), fungi (17), components of dying cells (18), and crystal particles (19,20), resulting in the activation of caspase-1 and production of the proinflammatory cytokines interleukin (IL)-1β and IL-18. Furthermore, this multi-protein complex plays a critical role in pathogen clearance and the induction of an adaptive immune response.

In the early phase of infection, NLRP3 inflammasome activation is critical for the host's defense against many pathogens, including *Listeria monocytogenes* (*L. monocytogenes*) (21). During human pregnancy, *L. monocytogenes* is a clinically relevant microbe which can replicate at the maternofetal interface in the decidua basalis and the placenta (22). It can also be transmitted from mother to fetus, substantially increasing the risk of abortion, stillbirth, and neonatal morbidity and mortality (23). Pregnant women are highly susceptible to infection by *L. monocytogenes*, leading to miscarriage, PTB, and neonatal infection. Fetal complications caused by *L. monocytogenes* largely occur in the absence of overt illness in the mother, delaying medical intervention (24,25). Previous studies have established the role of the trophoblast as a component of the innate immune system during pregnancy to organize the placental defense against *L. monocytogenes* (21,26). Therefore, we sought to determine whether the trophoblastic NLRP3 inflammasome plays a role in the host defense against *L. monocytogenes* infection during pregnancy.

In this study, we investigated the involvement of the NLRP3 inflammasome in placental immunity and established a mouse model for fetal loss induced by *L. monocytogenes* infection. The trophoblast expressed the components of the NLRP3 inflammasome, and activation of this inflammasome by *L. monocytogenes* resulted in IL-1β processing and secretion. Furthermore, NLRP3 was required for the trophoblast to synthesize IL-1β, and NLRP3-deficient ($NLRP3^{-/-}$) mice were more prone to fetal loss. Thus, during pregnancy, the trophoblastic NLRP3 inflammasome response to *L. monocytogenes* acts to organize the innate immune response to bacterial infection at the

 

maternofetal interface. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4120/rc).

## Methods

### *Reagents and antibodies*

Lipopolysaccharide (LPS) was purchased from Invivogen (San Diego, CA, USA). Rabbit polyclonal antibodies to ASC (sc-22514-R), IL-1β (sc-7884), caspase-1 (sc-514), and the goat anti-NLRP3 polyclonal antibody (sc-34410) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit anti-human cleaved IL-1β polyclonal antibody (#2021) was obtained from Cell Signaling Technology (CST, Shanghai, China). β-Tubulin monoclonal antibody (Tianjin Sungene Biotech, China) was used as an internal control.

### *Bacterial staining*

*L. monocytogenes* 10403*S* was grown to the log phase in a brain-heart infusion broth (BHI) and collected by centrifugation. The pellet was washed twice, diluted to the required concentration in sterile phosphate-buffered saline (PBS), and prepared for *in vivo* and *in vitro* infection.

### *Animal studies*

Animal experiments were performed under a project license (approval No. LL-KY-2019083) granted by Animal Welfare and Research Ethics Committee at Shenzhen People's Hospital, in compliance with national guidelines for the care and use of animals. A protocol was prepared before the study without registration. NLRP3-deficient mice were obtained from The Jackson Laboratory (ME, USA), and C57BL/6J mice were provided by the Model Animal Research Center of Nanjing University. All mice were housed in a specific pathogen-free facility at the Laboratory Animal Center of Shenzhen People's Hospital (The Second Clinical Medical College of Jinan University).

To establish the mouse model, adult mice (8–12 weeks of age) were mated and the appearance of a vaginal plug was designated embryonic day (ED) 0.5. Pregnant wide-type mice (n=6) were injected intravenously (i.v.) on ED8.5 with either PBS (vehicle) or $7\times10^4$ colony forming unit (CFU) of *L. monocytogenes* (in a volume of 100 µL). On ED12.5–14.5, the animals were sacrificed and the placentas were harvested.

To investigate the role of NLRP3, pregnant wide-type and NLRP3$^{-/-}$ mice (n=10) were injected i.v. on ED8.5 with either PBS (vehicle) or $1\times10^4$ CFU of *L. monocytogenes* (in a volume of 100 µL). On ED13.5, the animals were sacrificed and the placentas were harvested.

Mouse tissue lysates were prepared by homogenization in 4 mL of lysis buffer containing 1% Triton X-100, 1 mM PMSF (Phenylmethanesulfonyl Fluoride), 5 µg/mL aprotinin, 5 µg/mL pepstatin A, and 5 µg/mL leupeptin in 50 mM Tris-HCl (Hydrochloric acid) (pH 7.4) per gram of tissue.

### *Cell culture*

The human trophoblast cell lines HTR-8/Svneo, JAR, and JEG3 were gifts from Prof. Jian Zhang (Laboratory for Reproductive Health, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China). The trophoblast cells were cultured in DMEM/F12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) supplemented with 10% fetal bovine serum (FBS), 1 mM L-glutamine, 2 mM sodium pyruvate, and penicillin/streptomycin.

### *Isolation of mouse trophoblast cells*

Mouse trophoblast cells were isolated according to the previously described methods (27,28). Briefly, the placentas (ED13.5) were separated from the endometrium and cut into small pieces. The fragments were then incubated in a dissociation medium (DMEM/F12, 1 mg/mL collagenase, 40 µg/mL DNase (Deoxyribonuclease), and 0.125% trypsin) for 1 h at 37 ℃, with periodic pipetting to separate the cells. Subsequently, the dissociation medium in the cells was removed by washing with PBS, then collected and filtered to remove the undigested tissue. The cells were separated using Percoll gradient (GE Healthcare, CA, USA). The trophoblast cell layer was collected and cultured in NCTC-135 medium (Thermo Fisher Scientific, MA, USA) containing 10% FBS and penicillin/streptomycin, of which 80–90% were cytokeratin positive.

### *Caspase-1 activity analysis*

Caspase-1 activity was determined using a caspase-1 activity colorimetric kit (Beyotime Biotechnology, China) according to the manufacturer's instructions. In brief, the cells were

 

lysed in lysis buffer, then clarified at 16,000 g for 20 min at 4 ℃, and the supernatant protein concentrations were determined using Bradford Protein Assay (Thermo Fisher Scientific, MA, USA). Caspase-1 activity was determined using the caspase-1 substrate Ac-YVAD-pNA (Ac-Tyr-Val-Ala-Asp-pNA, pNA=p-nitroaniline) and incubated at 37 ℃ for 12 h. Caspase-1 activity was calculated as [Δ(p – NA)/Δtime]/total protein.

### *RNA extraction and polymerase chain reaction (PCR)*

Total RNA was extracted using TRIzol reagent (Sigma-Aldrich, USA) according to the manufacturer's instructions. Semi-quantitative reverse transcription (RT)-PCR reactions were performed using a RT-PCR Kit (TaKaRa, Japan). The following primers were used: GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase), 5'-ACATCATCCCTGCCTCTACTG-3' (sense) and 5'-ACCACCTGGTGCT CAGTGTA-3' (anti-sense); ASC, 5'-AGCCAAGCCAGGCCTGCACTTTA-3' (sense) and 5'-TCCGCATCTTGCTTGGGTTGGTG-3' (anti-sense); NLRP3, 5'-ATTCGGAG ATTGTGGTTGGGG CG-3' (sense) and 5'-GAAGTCACCGAGGGCGTTGTC AC-3' (anti-sense); caspase-1, 5'-CTTTCCAGCTCCTCA GGCAGTGC-3' (sense) and 5'-AGACGTGTGCGGCT TGACTTGTC-3' (anti-sense).

### *Immunohistochemistry and immunofluorescence*

Placental tissue sections (4 μm) were deparaffinized, rehydrated. And then the sections were subjected to an antigen retrieval step, followed by blocking for 1 h at room temperature. To analyze the expressions of NLRP3 and ASC, the samples were incubated overnight at 4 ℃ with NLRP3 or ASC antibodies at 1:100 or 1:200. After washing three times with PBS, specific staining was detected using the UltraSensitive™ S-P Kit and DAB (3,3'-diaminobenzidine) Detection Kit (Maixin-Bio, China) according to the manufacturer's directions. The tissue sections were subsequently counterstained with hematoxylin (Sigma-Aldrich, USA) before dehydration with ethanol and Histosolve. The slides were mounted with Permount (Sigma-Aldrich, USA) and visualized by light microscopy.

To investigate the protein expression of NLRP3, HTR-8/SVneo cells were cultured on glass coverslips and fixed with 4% paraformaldehyde for 15 min at room temperature. The cells were permeabilized with 0.1% Triton X-100 in PBS for 10 min and blocked with 5% donkey serum for 1 h at room temperature, and then incubated overnight at 4 ℃ with goat anti-human NLRP3 (1:100). Alexa 488-conjugated anti-goat IgG (Immunoglobulin G, 1:1,000, Invitrogen) was used as the secondary antibody. The nuclei were stained with DAPI (4',6-diamidino-2-phenylindole, 1 μg/mL). After sequential excitation, images of the same cell were captured using cellSens Dimension software (Olympus, Japan) and analyzed using ImageJ2x software (National Institutes of Health, USA).

### *Western blot analysis*

Protein concentration was measured using the BCA (bicinchoninic acid) method (Thermo Fisher Scientific, USA). Samples containing equal amounts of proteins were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes, which were blocked with 5% non-fat dry milk in a Tris-buffer containing 0.05% Tween-20 (TBST) for 1 h and probed with specific antibodies. The membranes were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies. Chemiluminescence was performed using an enhanced chemiluminescence (ECL) Kit (Millipore, Germany).

### *Cytokine studies*

The culture supernatants and mouse tissue lysates were analyzed using ELISA Kits (R&D Systems, USA) for human or mouse IL-1β.

### *Statistical analysis*

Results were presented as the mean ± standard error (SE). The experiments were performed with at least three independent experiments. Statistical significance was determined using analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test. $P<0.05$ was considered statistically significant.

## Results

### *L. monocytogenes induces fetal resorption and IL-1β processing in pregnant mice*

To investigate the role of the trophoblastic inflammasome

 

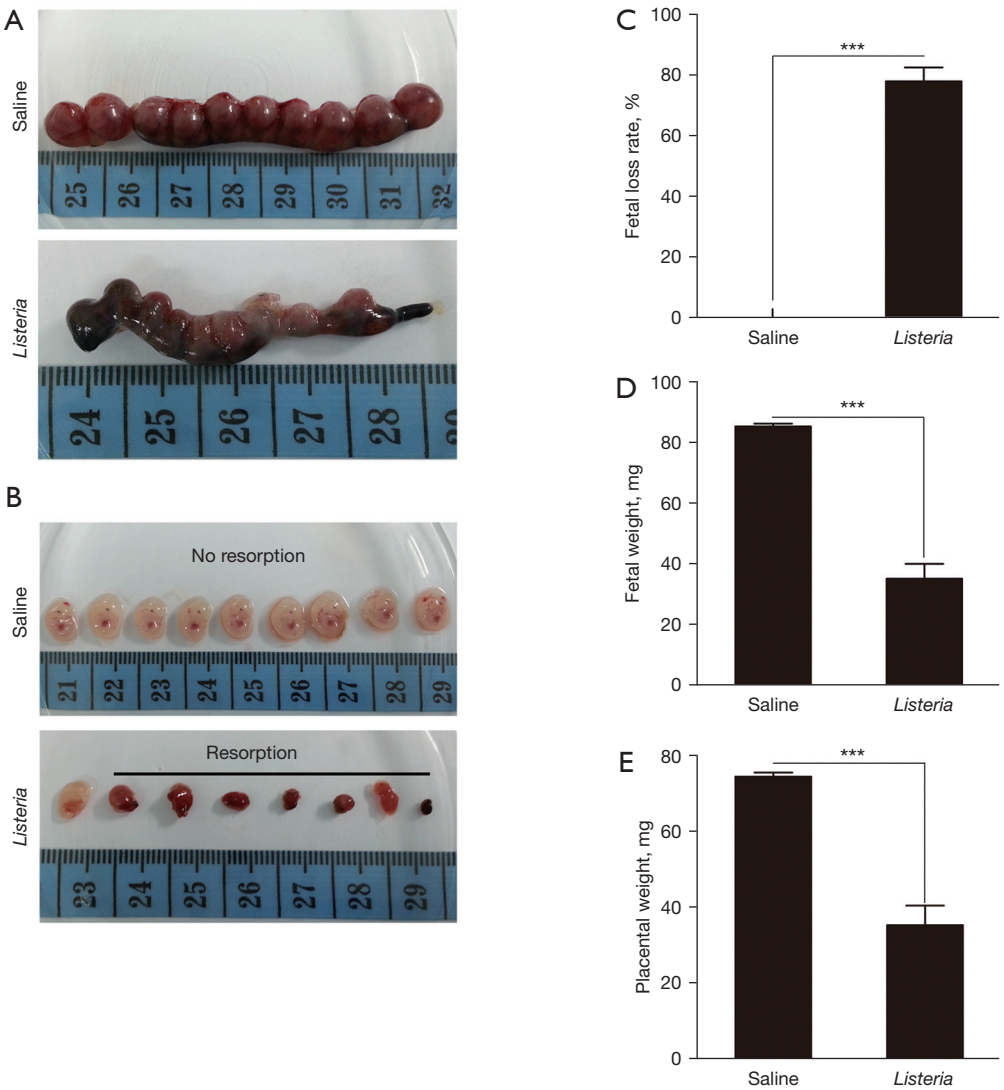


**Figure 1** *Listeria monocytogenes* infection results fetal loss during pregnancy. (A) Representative uterine horns of control and $7\times10^4$ CFU of *Listeria monocytogenes*-infected mice (n=6). (B) Images of fetuses isolated from the implantation sites of representative mice on ED12.5. The underlined fetuses were resorbed. (C) The fetal loss rate was calculated as the ratio of resorption sites to the total number of implantation sites in the pregnant mice. Fetal weights (D) and placental weights (E) were evaluated. The data are expressed as the means ± SE. ***, P<0.001, *Listeria monocytogenes* infection versus the value for control mice. CFU, colony forming unit; ED, embryonic day; SE, standard error.

in *L. monocytogenes*-induced adverse pregnancy outcomes, $7\times10^4$ CFU of *L. monocytogenes* or saline was injected into the pregnant mice through the tail vein on ED8.5. Compared with control mice, infected mice displayed a significantly higher fetal resorption rate (*Figure 1A-1C*). Moreover, fetal resorption appeared to be correlated with marked reductions in fetal and placental weight in infected mice (*Figure 1D,1E*).

To further assess the effect of *L. monocytogenes* on trophoblastic inflammasome activation, IL-1β processing was measured. As shown in *Figure 2*, *L. monocytogenes* infection increased the NLRP3 protein levels, induced the proteolytic cleavage of caspase-1, and the production of mature IL-1β in mouse placentas. These data indicate that *L. monocytogenes* induces trophoblastic inflammasome-dependent caspase-1 activation and IL-1β secretion.

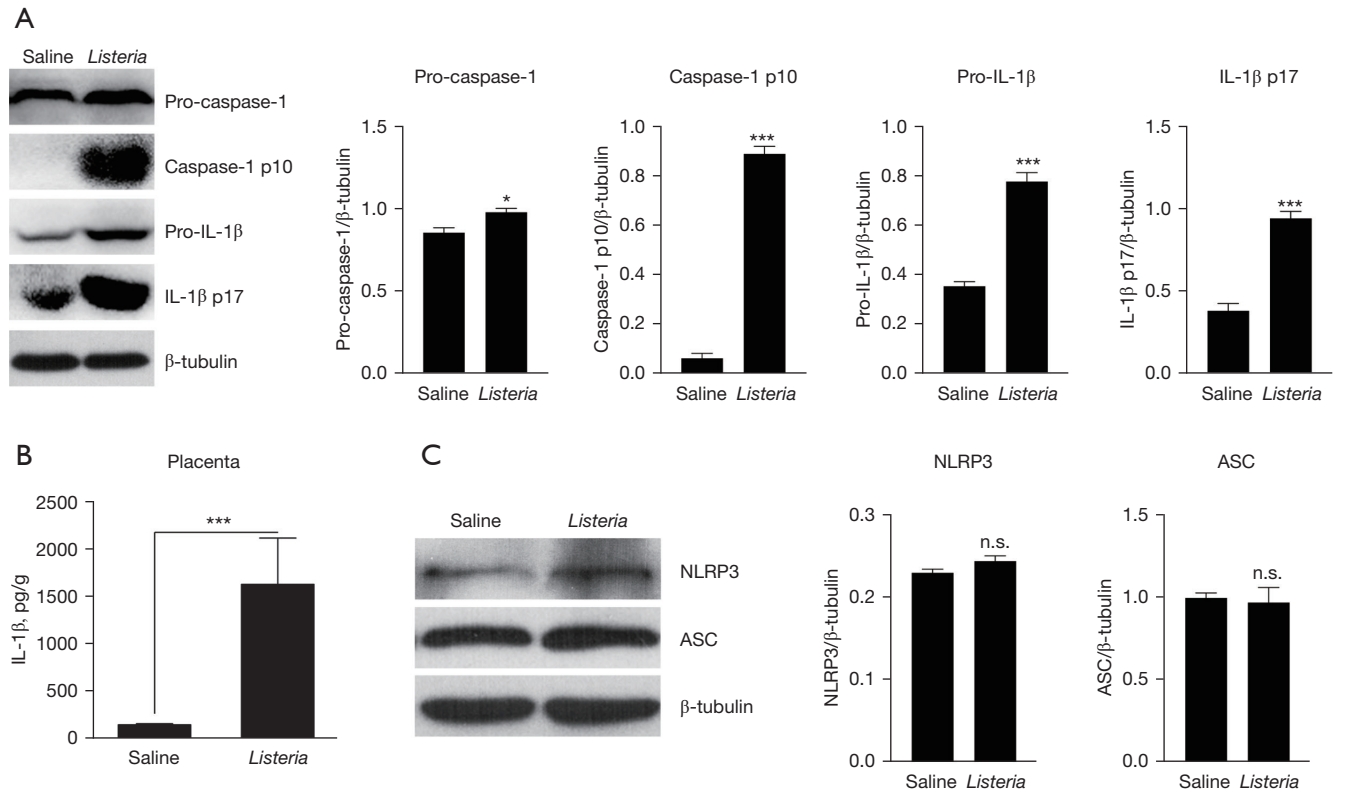


**Figure 2** *Listeria monocytogenes* triggers caspase-1 activation and IL-1β secretion in mouse placentas. (A,C) Western blot analysis of caspase-1, IL-1β, NLRP3, and ASC in mouse placentas. β-tubulin served as a loading control. (B) IL-1β ELISA in the mouse placentas (n=18 placentas). The data are expressed as the means ± SE. *, P<0.05; ***, P<0.001. IL, interleukin; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; SE, standard error. n.s., not statistically significant.

### *NLRP3 inflammasome components are expressed by trophoblast cells*

After demonstrating that *L. monocytogenes* activated NLRP3 inflammasome during infection, we next addressed the expression profiles of NLRP3 inflammasome components in trophoblast cells. All three human trophoblast cell lines (HTR-8/SVneo, JAR, and JEG-3 cells) constitutively expressed NLRP3, ASC, and caspase-1 mRNA (messenger RNA), and THP-1 cells served as the positive control (*Figure 3A*). Immunoblot analysis revealed that the ASC protein was expressed in HTR-8/SVneo cells and the placental villi of normal pregnant women (*Figure 3B*). The expression pattern of NLRP3 and ASC in human placental villous was detected by immunohistochemical staining. ASC protein was observed both in the syncytiotrophoblast (STB) and the cytotrophoblast (CTB) layers, and the NLRP3 protein was mainly stained at the CTB layer (*Figure 3C*). The isotype control antibodies failed to stain in these tissue sections. Moreover, these NLRP3 inflammasome components were also expressed in the mouse placentas (*Figure 2A,2C*). Taken together, these observations suggest that the trophoblastic NLRP3 inflammasome may play an important role in the host defense against *L. monocytogenes* infection during pregnancy.

### *L. monocytogenes triggers NLRP3 inflammasome activation in trophoblast cells*

To investigate the effect of *L. monocytogenes* on trophoblastic NLRP3 inflammasome activation, LPS-primed HTR-8/SVneo cells were treated with *L. monocytogenes* for 4 h. We found that *L. monocytogenes* infection dramatically induced caspase-1 activation and IL-1β maturation and secretion in HTR-8/SVneo cells (*Figure 4A-4C*).

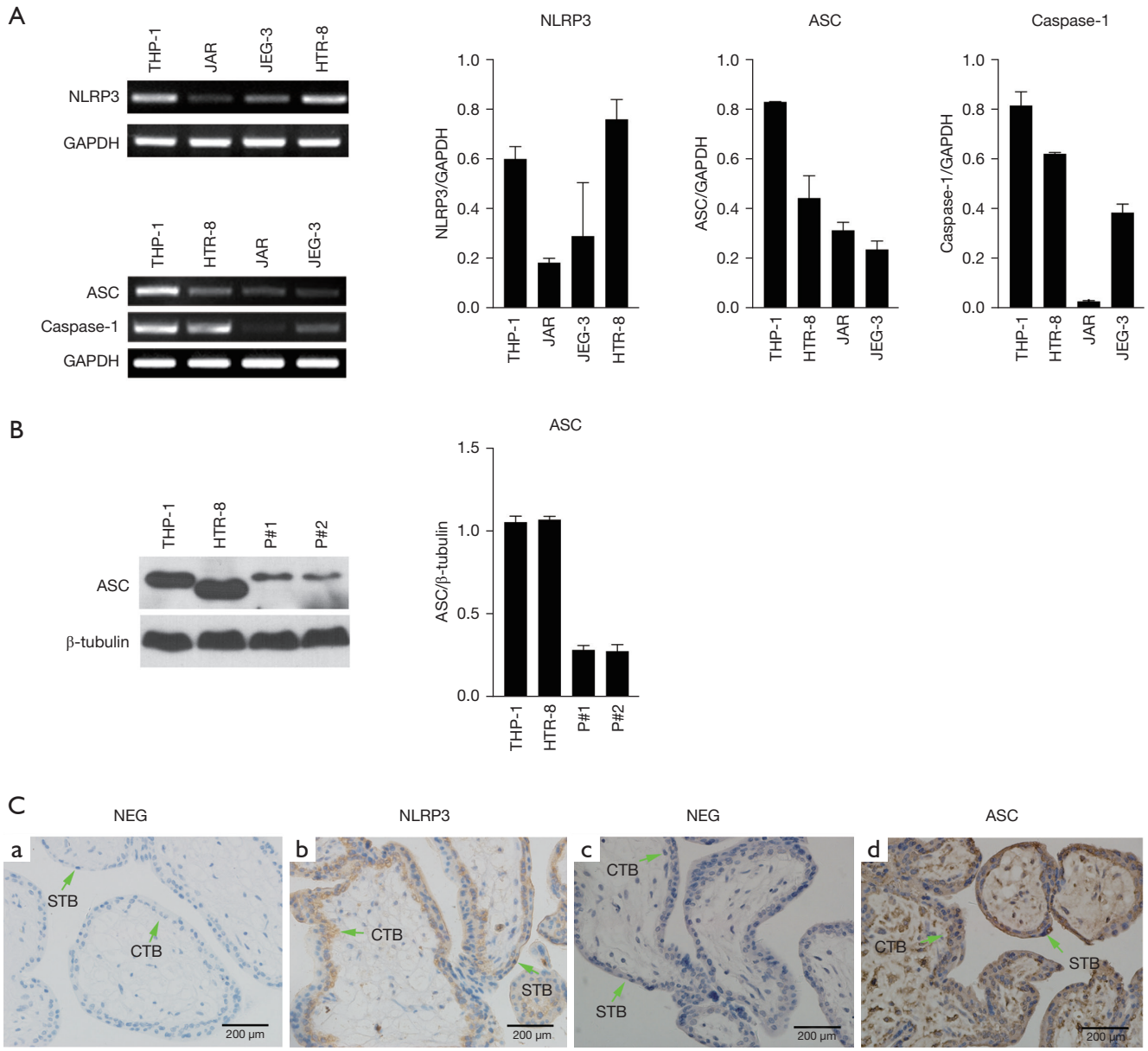


**Figure 3** Human first-trimester trophoblast cells express the components of the NLRP3 inflammasome. (A) Expression of NLRP3, ASC, and caspase-1 mRNA in three human first-trimester trophoblast cell lines and THP-1 (positive control) cells were determined by semi-quantitative RT-PCR. GAPDH served as a loading control. (B) Expression of the ASC protein in HTR-8/SVneo cells and first-trimester placental villous tissue was determined by Western blot analysis. P, normal pregnant woman. (C) Immunohistochemical staining of NLRP3 and ASC was performed in first-trimester placental villous. Green arrows indicate the STB and CTB layers. NEG: rabbit or goat IgG isotype control (original magnification, ×400). ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; CTB, cytotrophoblast; STB, syncytiotrophoblast; NLRP3, nucleotide-binding oligomerization domain-like receptor pyrin domain-containing 3.

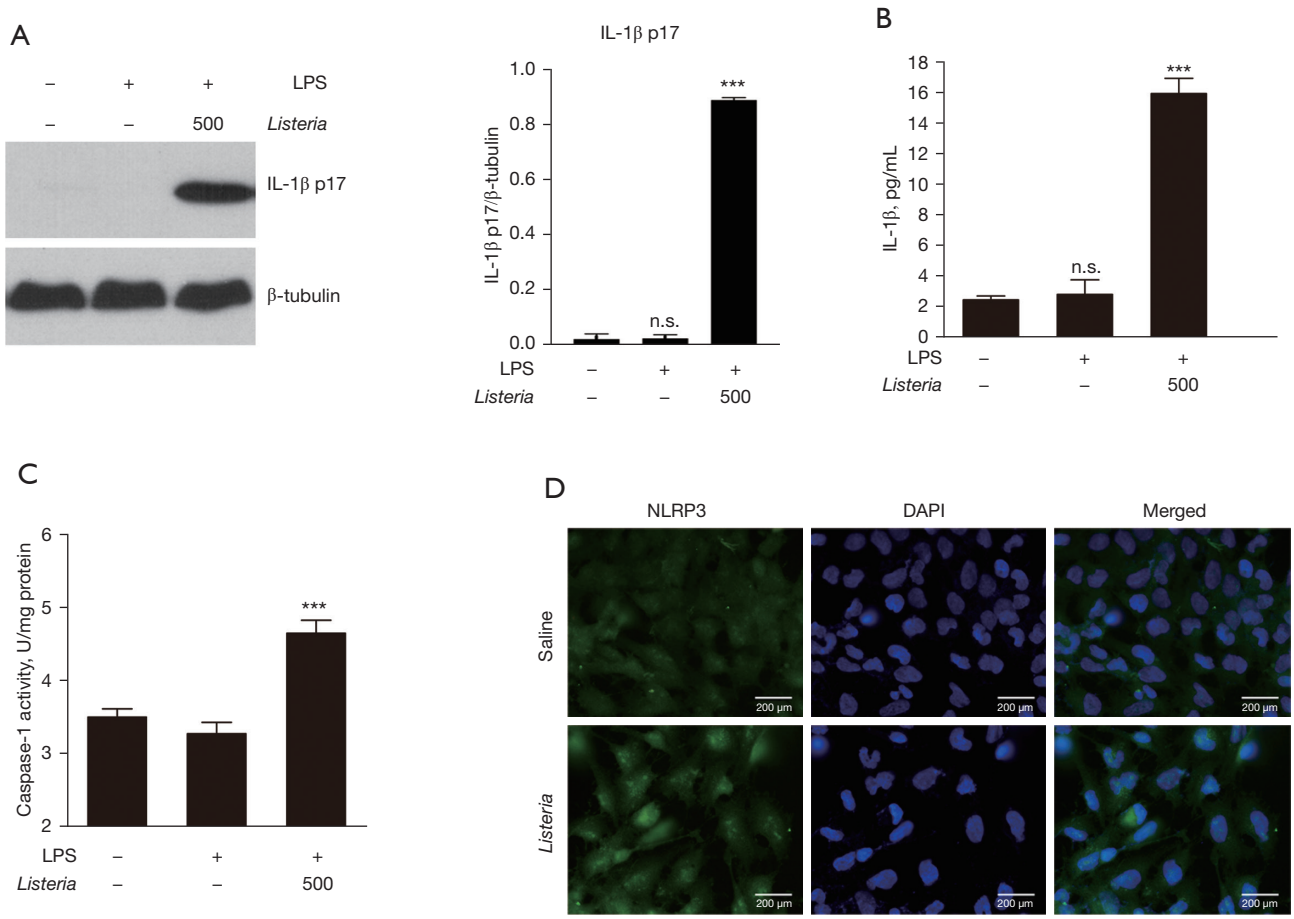


**Figure 4** *Listeria monocytogenes* induces inflammasome-mediated caspase-1 activation and IL-1β secretion in trophoblast cells. LPS-primed HTR-8/SVneo cells were stimulated with *Listeria monocytogenes* (MOI =500, 4 h). (A) Cell extracts were immunoblotted for IL-1β maturation. β-tubulin served as a loading control. (B) The culture supernatants were analyzed for IL-1β secretion. (C) Caspase-1 activity was determined as described in the "Methods" section. (D) HTR-8/SVneo cells were stimulated with *Listeria monocytogenes* (MOI =5, 4 h). Localization of NLRP3 (green) and nuclei (blue) using fluorescence microscopy (original magnification, × 400). The data are expressed as the means ± SE. ***, P<0.001, *Listeria monocytogenes* infection vs. the value for control group. IL, interleukin; LPS, lipopolysaccharide; MOI, multiplicity of infection; SE, standard deviation. n.s., not statistically significant; NLRP3, nucleotide-binding oligomerization domain-like receptor pyrin domain-containing 3.

Moreover, *L. monocytogenes* infection significantly increased the NLRP3 protein levels in HTR-8/SVneo cells (*Figure 4D*). To evaluate whether IL-1β secretion is specific to the NLRP3 inflammasome, mouse primary trophoblast cells from wild-type (WT) and NLRP3-deficient mice were isolated. As shown in *Figure 5*, NLRP3 deficiency blocked *L. monocytogenes*-induced IL-1β secretion in mouse primary trophoblast cells. Taken together, these results demonstrate that trophoblastic NLRP3 inflammasome plays a critical role in regulating the caspase-1-dependent maturation of IL-1β in response to *L. monocytogenes*.

### *NLRP3-deficient mice are more prone to fetal loss after L. monocytogenes infection during pregnancy*

To assess the role of NLRP3 in *L. monocytogenes*-induced adverse pregnancy outcomes, $1\times10^4$ CFU of *L. monocytogenes* or saline was injected into pregnant mice on ED 8.5. Macroscopic analysis of the mothers and the uterine horns harvested on ED13.5 revealed that NLRP3-deficient mice developed severe vaginal bleeding, uterine size reduction, and fetal resorption compared to their WT counterparts (*Figure 6A*). As shown in *Figure 6B*, a significant increase in

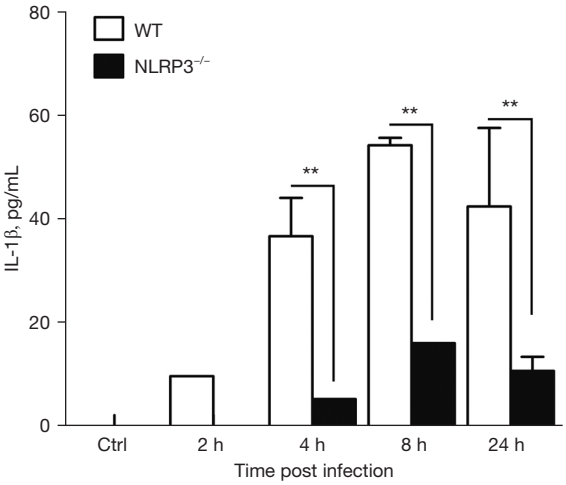


**Figure 5** NLRP3 deficiency prevents *Listeria monocytogenes*-mediated IL-1β secretion in trophoblast cells. Primary mouse trophoblast cells from WT and NLRP3$^{-/-}$ mice were isolated as described in the "Methods" section. IL-1β secretion in trophoblast cells infected with *Listeria monocytogenes* for indicated times. The data are expressed as the mean ± SE of three independent experiments. **, P<0.01. WT, wild-type; IL, interleukin; SE, standard deviation; NLRP3, nucleotide-binding oligomerization domain-like receptor pyrin domain-containing 3.

the fetal resorption rate was observed in NLRP3-deficient mice compared to WT mice (90.90% *vs.* 19.34%, P<0.001). These results indicate that in the absence of NLRP3, mice are more susceptible to pregnancy loss when infected with *L. monocytogenes* during pregnancy.

## Discussion

Placental infection can lead to adverse pregnancy outcomes, including abortion, stillbirth, PTB, as well as perinatal and neonatal morbidity and mortality. Listeriosis during pregnancy usually manifests as an unremarkable febrile illness in the mother but can be fatal for fetuses and newborns. NLRP3 assembles a canonical caspase-1-dependent inflammasome in the cytoplasm that responds to *L. monocytogenes*. In this study, we proposed the hypothesis that the trophoblastic NLRP3 inflammasome plays a role in antibacterial resistance in listeriosis during pregnancy. Consistent with a previous study (21), we demonstrated that placental trophoblasts constitutively expressed the components of the NLRP3 inflammasome. *L. monocytogenes* infection induced NLRP3-dependent IL-1β secretion and NLRP3-deficient mice were more prone to fetal loss. Our

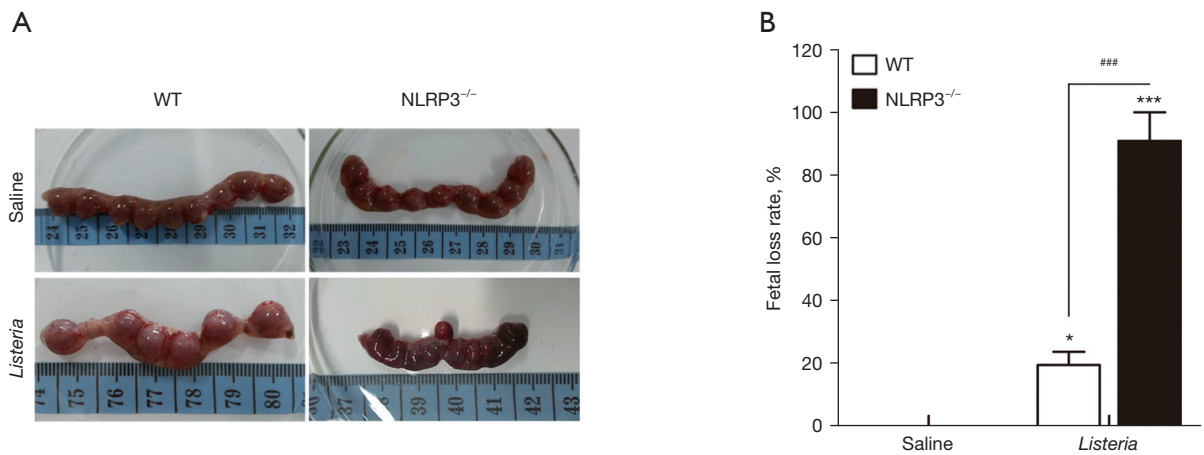


**Figure 6** NLRP3-deficient mice are more prone to fetal loss after *Listeria monocytogenes* infection during pregnancy. (A) Representative uterine horns of control and *Listeria monocytogenes*-infected WT and NLRP3$^{-/-}$ mice (n=10) harvested on ED13.5. (B) The fetal loss rate was calculated as the ratio of resorption sites to the total number of implantation sites in pregnant mice. The data are expressed as the means ± SE. *, P<0.05; ***, P<0.001, *Listeria monocytogenes* infection *vs.* the value for control mice; ###, P<0.001, comparing the WT and NLRP3$^{-/-}$ groups. WT, wild-type; ED, embryonic day; NLRP3, nucleotide-binding oligomerization domain-like receptor pyrin domain-containing 3.

study identifies a pathway through which the placenta regulates local innate immunity during pregnancy.

Although previous work on PRRs in the placenta has demonstrated an association with pathogenic conditions such as preeclampsia (29-33) and preterm delivery (34,35), the role of these pathways in pregnancy infection has not yet been fully investigated. Recent studies have demonstrated the production of antimicrobial factors in the placenta via TLR (Toll-like receptor) and NLR signaling, suggesting that trophoblasts might play a role in the placental defense and the local innate response proceeds independently (11,17,21,36,37). Within this context, our data implied that placenta-derived NLRP3 inflammasome activation was associated with *L. monocytogenes* infection. Consistent with this, bacterial LPS stimulation induced trophoblastic NLRP3 inflammasome activation and fetal absorption, and TANK-binding kinase 1 (TBK1) inhibition attenuated NLRP3 inflammasome activation by targeting the mammalian target of rapamycin complex 1 (mTORC1) (38,39). Moreover, LPS-induced pregnancy abnormalities were improved in NLRP3-deficient mice (40). These findings suggest that this innate immune complex could play a key role in placental immune defense.

Inflammasomes mediate microbial clearance via a suite of cytokine-dependent and/or cytokine-independent mechanisms, and inflammasome pathway-deficient mice exhibit heightened susceptibility to infections (41). Caspase-1-deficient mice that are infected with *L. monocytogenes* have an impaired ability to clear the bacterial burden (42). Inflammasomes are multi-protein complexes that assemble in response to cellular infection and in turn trigger caspase-1 activation as well as the maturation and secretion of IL-1β and IL-18. IL-1β is a potent antibacterial factor, which has been demonstrated to play a critical role in host resistance to pathogens such as *Mycobacterium tuberculosis* and *L. monocytogenes* (21,43). Previous studies have suggested that endogenously produced IL-1 plays an important role in anti-*Listeria* resistance (44,45). Accordingly, Hirsch *et al.* showed that mice lacking the IL-1R antagonist have improved survival upon infection with *L. monocytogenes* (46). Furthermore, IL-1β is a potent inflammatory cytokine that exerts direct and indirect effects to mobilize and activate immune cells into the site of infection. The control of invading pathogens by innate host defense relies strongly on phagocytosis and the killing of microorganisms by neutrophils, monocytes, and macrophages (47). In murids, macrophages are excluded from the placenta, and instead, neutrophils are the major infiltrative cell (26). The immediate action of an immune cell reaching a site of infection is to seek cellular reinforcement, and inflammasome-derived IL-1β is a major driver of neutrophils. Mature IL-1β amplifies the phagocytic capability of neutrophils, enhances the production of reactive oxygen species (ROS), and triggers neutrophil degranulation and the release of antimicrobial proteins, ultimately inhibiting bacterial replication (41). In the present study, we demonstrated that NLRP3-deficient mice were more prone to fetal loss after *L. monocytogenes* infection, suggesting that NLRP3 protects against *L. monocytogenes* infection during pregnancy. Furthermore, we demonstrated that NLRP3 deficiency blocked *L. monocytogenes*-induced IL-1β secretion in mouse primary trophoblast cells. It has been proved that increased maternal levels of IL-1β to be associated with PTB protection by regulating intense inflammatory response (48,49). Thus, we demonstrated that NLRP3 inflammasome protects against *L. monocytogenes* infection during pregnancy by regulating the secretion of trophoblastic IL-1β to organize the innate immune response at the maternofetal interface.

Although neonatal outcomes have been improved with symptomatic treatment of pregnancies presenting in preterm labor with corticosteroids and antibiotics. However, the incidence of PTB has not been reduced (50,51). It is of great significance to understand the mechanism of PTB induced by *L. monocytogenes* which will prevent negative outcome in a timely manner. This study provided a potential strategy to treat PTB induced by *L. monocytogenes* by activating NLRP3 inflammasome. However, the treatment effect needs more *in vitro*/*in vivo* experiment or clinical verification.

In summary, this study provides insight into the role of the trophoblastic NLRP3 inflammasome in response to *L. monocytogenes* infection during pregnancy. Trophoblast cells constitutively expressed the components of the NLRP3 inflammasome. Upon *L. monocytogenes* infection, trophoblastic NLRP3 signals an inflammasome-dependent pathway, resulting in the production of IL-1β and the promotion of a potent immune response against *L. monocytogenes* infection. In contrast, NLRP3-deficient mice were more prone to pregnancy loss. Overall, these findings demonstrate that the trophoblastic NLRP3 inflammasome plays an important role in mounting protective innate immune responses against *L. monocytogenes* infection at the maternofetal interface.

 

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## Footnote

*Reporting Checklist:* The authors have completed the ARRIVE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4120/rc

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*Ethical Statement:* The authors are accountable for all aspects of the work, including ensuring that any questions related to the accuracy or integrity of any part of the work have been appropriately investigated and resolved. Animal experiments were performed under a project license (approval No. LL-KY-2019083) granted by Animal Welfare and Research Ethics Committee at Shenzhen People's Hospital, in compliance with national guidelines for the care and use of animals.



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