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Role of NLRC3 in modulating inflammatory responses in neonates

Meng Zhang[1] and Mingming Zhang[2*]

Abstract

Objective This study sought to investigate the role and molecular mechanisms of nucleotide-binding oligomerization domain (NOD)-like receptor family caspase activation and recruitment domain (CARD)-containing 3 (NLRC3) in the inflammatory responses of neonates, thereby developing new clinical insights into the occurrence and prevention of neonatal infections.

Methods Peripheral blood samples were collected from full-term infants ($n=49$) and preterm infants ($n=41$) without any signs of intrauterine infection, as well as from healthy non-pregnant adults ($n=45$). A real-time polymerase chain reaction was used to assess the expression levels of NLRC3 and NOD-containing protein 1 (NOD1) in the isolated mononuclear cells. Whole blood from the adults, full-term infants, and preterm infants was stimulated for four hours with a mixture of herpes simplex virus type 60 DNA (HSV-60 DNA) and lipopolysaccharides (LPS) or LPS alone or blank medium. An enzyme-linked immunosorbent assay was employed to measure the tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and interleukin 1 beta (IL-1β) levels in the supernatant.

Results The gene expression levels of NLRC3 were significantly lower in the full-term and preterm infants than in the adults, with the preterm infants showing notably lower levels when compared with the full-term infants. A positive correlation was found between the NLRC3 and NOD1 expression levels in the neonates (both full-term and preterm), indicating lower NLRC3 expression to be associated with lower NOD1 expression. After LPS stimulation, the production of TNF-α, IL-6, and IL-1β in the whole blood of the preterm and full-term infants was significantly lower than in that of the adults. Moreover, stimulation with a combination of LPS and HSV-60 DNA resulted in similar TNF-α, IL-6, and IL-1β production across the blood samples from preterm infants, full-term infants, and adults. When compared with LPS stimulation alone, the LPS and HSV-60 DNA mixture significantly reduced the release of TNF-α, IL-6, and IL-1β in the adults. In the neonates, however, only the release of TNF-α was significantly reduced, as no notable difference was observed in the IL-6 and IL-1β levels.

Conclusion The reduced expression and functional impairment of NOD-like receptors, such as NLRC3 and NOD1, in neonates, may contribute to their heightened susceptibility to severe infections. This finding indicates new avenues for the prevention and treatment of neonatal infections.

Keywords NLRC3, Neonate, Immune response, Inflammatory response

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Introduction

Microbial infections represent a significant risk factor for severe illness during infancy, while severe infections are a leading cause of neonatal mortality. Indeed, reports indicate that millions of newborns succumb to infectious diseases annually [1, 2], a phenomenon that is primarily attributed to the immature state of both innate and acquired immunity in neonates [3].

Previous studies have focused on the innate immune components in neonates (e.g., nucleotide-binding oligomerization domain-containing protein 1 [NOD1]) in the context of responses to microbial infections. Both NOD1 and NOD-like receptor family caspase activation and recruitment domain (CARD)-containing 3 (NLRC3) are members of the pattern recognition receptor (PRR) family that play essential roles in the detection of pathogens and tissue damage [4]. They also fulfill critical functions in terms of the immune response and inflammatory signal transduction. For instance, through its CARD domain, NOD1 recruits and binds to receptor-interacting protein 2 (RIP2) from the receptor-interacting protein (RIP) family, thereby activating downstream signaling via the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, which subsequently induces the production of inflammatory cytokines [5, 6]. Moreover, NLRC3, when activated by herpes simplex virus type 60 DNA (HSV-60 DNA), inhibits the degradation of NF-κB by suppressing the activation of the inhibitor of nuclear factor kappa-B kinase (IKK), leading to the dissociation and inhibition of the activation of the NF-κB heterodimer p50. As a consequence, p50 is unable to enter the nucleus to initiate specific gene transcription, which suppresses T-cell activation and reduces cytokine release. Upon activation, NOD1 and NLRC3 act on the same downstream pathways and can competitively bind with the apoptosis-associated speck-like protein via CARD–CARD interactions, thereby bridging pro-caspase-1 and pro-caspase-5. This interaction regulates the cleavage of pro-caspase and both the maturation and secretion of pro-interleukin 1 beta (pro-IL-1β) and pro-interleukin 6 (pro-IL-6) [7]. Additionally, both receptors have tumor necrosis factor receptor-associated factor (TRAF)-binding sequences on their nucleotide-binding domains (NBDs), enabling them to interact with the adaptor molecule TRAF6, altering its ubiquitination pattern and modulating the TNF-α release levels [8]. In terms of the immune responses of neonates to microbial infections, NOD1 not only functions as a pro-inflammatory component but may also be involved in mechanisms that modulate and potentially inhibit the inflammatory response, a role that has received less research attention and so remains poorly understood.

This study sought to investigate the expression and functional mechanisms of NLRC3 in neonates, particularly its role during infection-induced immune responses. By elucidating the involvement of NLRC3, this study aimed to provide new clinical insights concerning the treatment and prevention of neonatal infections.

Table 1 Clinical information concerning premature newborns, full-term newborns, and adults ($\overline{x} \pm SD$)

Group	Adult Control	Full-Term Neonates	Preterm Neonates
Number of cases (n)	45	49	41
Gender (male/female)	22/23	21/28	20/21
Weight (kg) ($\overline{x} \pm SD$)	58.93 ± 10.65	3.4 ± 0.41	2.03 ± 0.39
Age (years/weeks) ($\overline{x} \pm SD$)	26.1 ± 2.58	38.96 ± 1.68	31.54 ± 3.23

Subjects and methods

Study population

The research protocol for this study has been approved by the Ethics Committee of Xuzhou Central Hospital, with the approval number XZXY-LK-20200103-016. Informed consent has been obtained from the parents or legal guardians of the participating neonates. The neonatal peripheral blood specimens were obtained from newborns admitted to the Obstetrics and Neonatology Departments of Xuzhou Central Hospital in China between January 2020 and December 2022. The mothers of the participating neonates were under 35 years of age and without malignancies, genetic disorders, or infectious diseases such as hepatitis B, hepatitis C, or AIDS. Moreover, they did not experience severe complications during pregnancy. Newborns showing any signs of intrauterine infections, such as chorioamnionitis, umbilical cord abnormalities, chorionic plate abnormalities, or placental infections, were excluded from the specimen collection. The adult peripheral blood specimens were obtained from volunteers at Xuzhou Central Hospital (with written consent for their use in scientific research). Adults exhibiting clinical symptoms of infection or elevated clinical infection markers (including C-reactive protein [CRP], white blood count [WBC], etc.) were not included in the specimen collection (see Table 1).

Specimen collection

Peripheral blood samples were collected from the neonates within two hours of birth. More specifically, using a 5 mL sterile disposable syringe (Shanghai Double-Dove Industry CO., LTD., Shanghai, China), 2 mL of peripheral venous blood was drawn via the elbow vein and placed in heparinized anticoagulant tubes (Becton, Dickinson and Company, NJ, USA). Prior to the collection, routine disinfection of the collection site and surrounding skin was performed to minimize the risks of infection and contamination. This study utilized residual blood samples collected during the routine examinations performed upon the admission of pediatric patients after the blood

needed for essential tests had been extracted. No additional blood was drawn specifically for the purposes of this study. Samples of less than 2 mL were excluded from the analysis.

Peripheral blood specimens were collected from the healthy non-pregnant adult volunteers using a similar method, with 2 mL drawn via the elbow vein and transferred to heparinized anticoagulant tubes. Routine disinfection of the collection site and surrounding skin was also conducted prior to the collection.

Isolation of peripheral blood mononuclear cells (PBMCs)

Each peripheral blood sample was transferred to a 15 ml centrifuge tube (Corning, USA) and diluted with phosphate-buffered saline (PBS) at a ratio of 1:1 (PBS: blood). Another 15 ml centrifuge tube (Corning, USA) was prepared and 4 ml of lymphocyte separation medium (RPMI 1640 medium; HyClone, USA) was added to it. The diluted blood mixture was slowly layered on top of the lymphocyte separation medium, with care taken not to disturb the interface between the blood and the lymphocyte separation medium. The mixture was then centrifuged at 1600 rpm for 30 min. After centrifugation, the liquid separated into five layers, with the PBMC layer (i.e., a narrow, white, cloudy band in the middle) primarily containing lymphocytes and monocytes. The PBMC layer was transferred to a new 15 ml centrifuge tube and PBS was added at twice its volume. Next, the sample was thoroughly mixed and centrifuged at 1600 rpm for 10 min. The supernatant was carefully discarded, 1 ml of TRIzol was added, and the sample was mixed thoroughly and transferred to a 1.5 ml Eppendorf (EP) tube. The sample was then stored at -80 °C.

Real-Time quantitative polymerase chain reaction (qPCR) analysis

PBMCs were isolated from the blood samples of the neonates (preterm and full-term) and adults (Tianjin Haoyang Biotechnology Co., Ltd., China). Next, mRNA was extracted from the cells, reverse-transcribed into cDNA under specific conditions using reverse transcriptase (Promega, China), and assessed for the NLRC3 mRNA expression levels via real-time PCR technology using the 2-ΔΔCt method. The following real-time PCR primers were used:

NLRC3

Forward: 5′-GTGCCGACCGACTCATCTG-3′.
Reverse: 5′-GTCCTGCACTCATCCAAGC-3′.

NOD1

Forward: 5′-CAGGTCTCCGAGAGGGTACTG-3′.
Reverse: 5′-TGTGTCCATATAGGTCTCCTCCA-3′.
β-actin (internal control):
Forward: 5′-CACGAAACTACCTTCAACTCC-3′.
Reverse: 5′-CATACTCCTGCTTGCTGATC-3′.

Treatment

In a 48-well culture plate, 50 μl of anticoagulated whole blood was added to each well. Then, 450 μl of RPMI 1640 medium containing either lipopolysaccharide (LPS; 100 ng/ml; G-Clone (Beijing) Biotechnology Co., Ltd., Beijing, China), a mixture of LPS (100 ng/ml) and HSV-60 DNA (100 ng/ml; InvivoGen, Toulouse, France), or blank medium as a control, with 5% fetal calf serum (FCS), was added. The plate was then incubated in a 37 °C incubator for four hours. After incubation, the medium containing the whole blood was transferred to a 1.5 ml centrifuge tube and centrifuged at 3000 rpm for 10 min. The supernatant was carefully collected and stored at -80 °C until required for future analysis.

Cytokine detection

After culturing the peripheral blood for four hours, the sample was centrifuged, and the supernatant was collected. The release levels of tumor necrosis factor-alpha (TNF-α), IL-6, and IL-1β were measured using an enzyme-linked immunosorbent assay (ELISA). The ELISA kits were provided by Hangzhou Lianke Biotechnology Co., Ltd., and all the procedures were carried out according to the manufacturer's instructions. The lower detection limits for TNF-α, IL-6, and IL-1β are 1.0 pg/mL, 3.9 pg/mL, and 1.17 pg/mL, respectively. When the measured value falls below the lower detection limit, missing results may occur. In such cases, half of the lower detection limit is used as the recorded result. All collected blood supernatants were stored at -80 °C until all samples were collected, after which they were analyzed together using ELISA.

Statistical analysis

Statistical analysis of the experimental data was conducted using Statistical Package for the Social Sciences (SPSS) version 20.0 software. The results are presented as the mean ± standard deviation ($\bar{x} \pm$ SD). The comparison of gene expression and cytokine levels was performed using a one-way analysis of variance (ANOVA), followed by Tukey's Honest Significant Difference (HSD) test. Statistical significance was set at $P < 0.05$.

Results

Participant demographics

Based on the inclusion and exclusion criteria, a total of 45 adults were included in the adult group, comprising 22 males and 23 females, with an average weight of 58.93 ± 10.65 kg and an average age of 26.1 ± 2.58 years. In the full-term group, 49 neonates were included, comprising 21 males and 28 females, with an average weight of

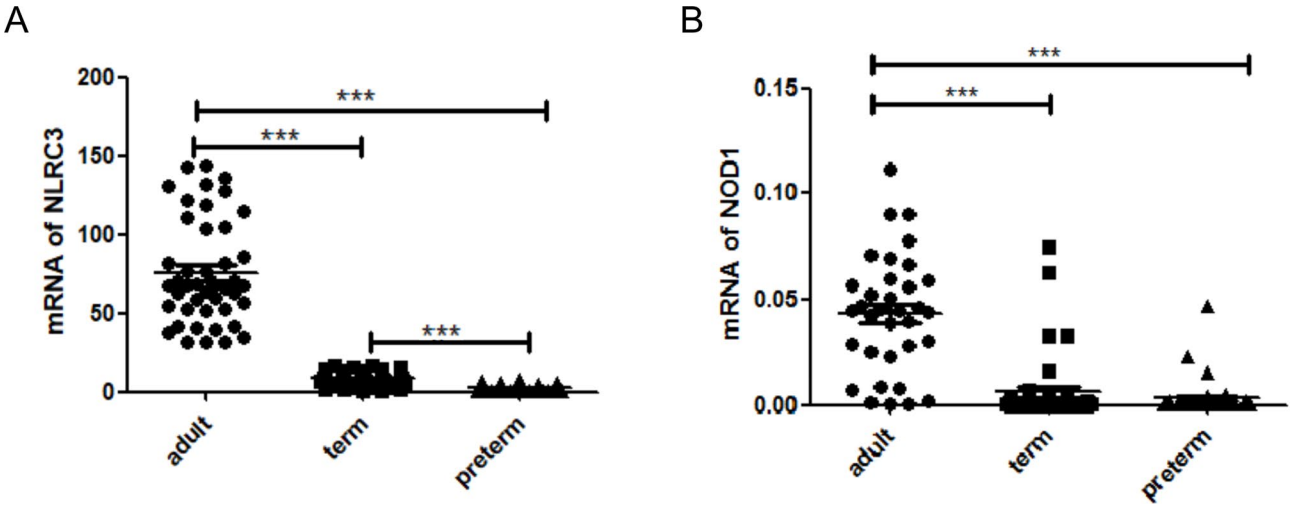

Fig. 1 Comparison of the NLRC3 **(A)** and NOD1 **(B)** mRNA levels among the preterm newborns, full-term newborns, and adults. Gene expression levels are normalized to the level of housekeeping gene (*GAPDH*). Note: ***, $P < 0.001$

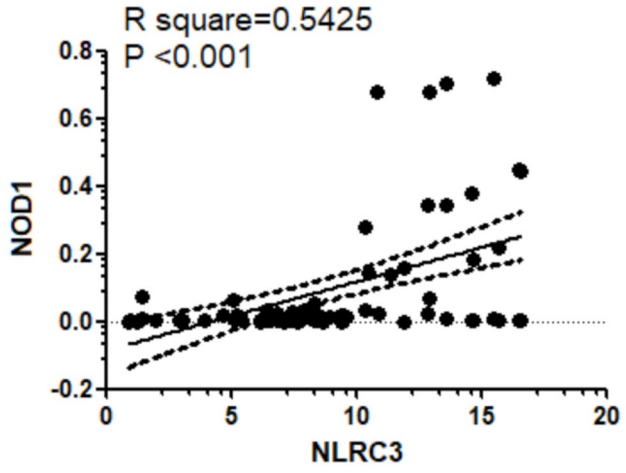

Fig. 2 Correlation analysis of the NLRC3 and NOD1 expression in the neonates

3.4 ± 0.41 kg and an average gestational age of 38.48 ± 5.27 weeks. In the preterm group, 41 neonates were included, comprising 20 males and 21 females, with an average weight of 2.03 ± 0.39 kg and an average gestational age of 33.54 ± 1.6 weeks (Table 1).

Moreover, among the 49 full-term neonates, 2 developed sepsis, and 7 developed neonatal pneumonia in the later stages. Among the 41 preterm infants, there was 1 case of pyogenic meningitis, 3 cases of sepsis, and 11 cases of neonatal pneumonia in the later stages.

Comparison of the NLRC3 and NOD1 expression levels

As shown in Fig. 1, real-time PCR analysis of the NLRC3 and NOD1 mRNA expression levels in the adults, full-term infants, and preterm infants revealed that both were expressed at measurable levels in the full-term and preterm infants. Here, the gene expression level of NLRC3 in the full-term and preterm infants was significantly lower than that in the adults ($P < 0.01$). Furthermore, the NLRC3 gene level in the preterm infants was markedly lower than that in the full-term infants ($P < 0.001$, Fig. 1A). As shown in Fig. 1B, when compared with the adults, both the preterm and full-term infants had significantly lower NOD1 expression levels ($P < 0.01$). While the NOD1 expression level in the full-term infants was higher than that in the preterm infants, this difference was not statistically significant.

Correlation of NLRC3 and NOD1 expression in neonates

Spearman correlation analysis was conducted on the NLRC3 and NOD1 expression in the PBMCs from the neonates (both full-term and preterm). The results indicated a positive correlation between the NLRC3 and NOD1 expression levels (Fig. 2). More specifically, lower NLRC3 expression levels corresponded to lower NOD1 expression levels.

Differences in inflammatory responses mediated by the NLRC3 agonist HSV-60 DNA

Following LPS stimulation of the whole blood samples obtained from the neonates and adults, the levels of TNF-α, IL-6, and IL-1β produced by the full-term and preterm infants were found to be significantly lower than those produced by the adults ($P < 0.01$). However, after stimulation with a mixture of LPS and HSV-60 DNA, no significant differences were observed in the levels of TNF-α, IL-6, and IL-1β among the full-term infants, preterm infants, and adults. When compared with the LPS-alone stimulation group, the levels of TNF-α, IL-6, and IL-1β produced by the adults in the LPS and HSV-60 DNA mixture stimulation group were significantly

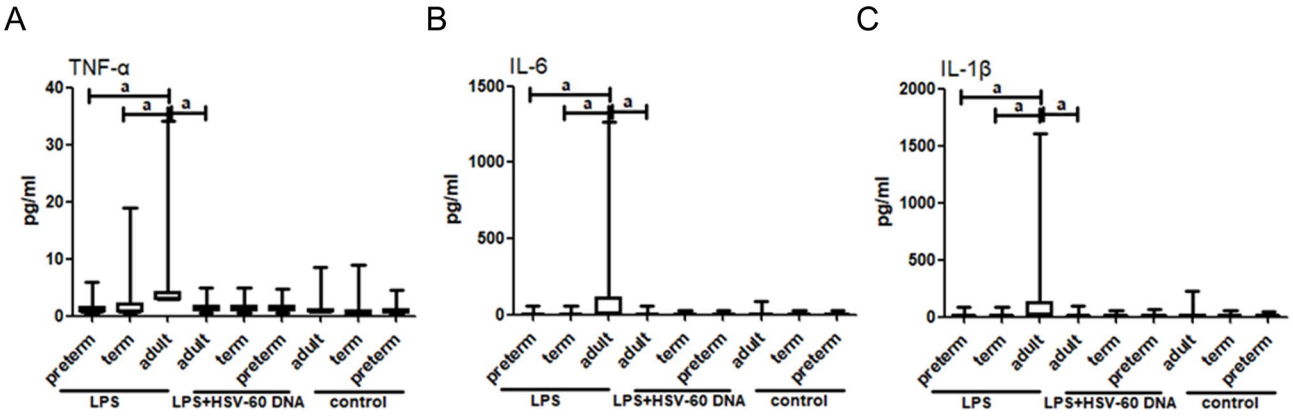

Fig. 3 Comparison of the pro-inflammatory factor (TNF-α **[A]**, IL-6 **[B]**, and IL-1β**[C]**) release after LPS-alone and LPS plus HSV-60 DNA stimulation. Note: a, $P < 0.05$

reduced ($P < 0.01$), whereas the levels of TNF-α, IL-6, and IL-1β produced by the full-term and preterm infants showed no significant changes (Fig. 3).

Discussion

When compared with adults, neonates exhibit higher susceptibility to microbial infections and systemic inflammatory response syndrome (SIRS). This susceptibility primarily stems from the immature state of both the innate and adaptive immune systems in neonates [3]. Notably, PRRs such as NOD1, which are representative of pro-inflammatory receptors, are expressed at lower levels and functionally impaired in neonates. This deficiency significantly contributes to PRRs' delayed recognition and sluggish initiation of inflammatory responses against microbial infections, which has been reported in prior studies [9, 10]. However, a comprehensive immune response to infection requires not only the involvement of pro-inflammatory receptors but also the regulation of immune checkpoints to terminate inflammatory responses in a timely fashion and prevent the occurrence of cytokine storms, although this is currently an under-research area. Previous studies have demonstrated that NLRC3 negatively influences NF-κB activation, thereby both inhibiting the function of NLRs and terminating immune responses [11, 12]. In the present study, we investigated the expression of NLRC3 in neonates and the inflammatory response following stimulation with NLRC3 agonists such as HSV-60 DNA, LPS, or a combination of the two. Our aim in so doing was to explore the functional characteristics and mechanisms of anti-inflammatory receptors involved in neonatal immune responses.

Using real-time PCR technology, we determined the expression levels of NLRC3 in preterm infants, full-term infants, and adults. We observed significantly lower levels of NLRC3 expression in both the preterm and full-term infants when compared with the adults, although the preterm infants exhibited even lower expression levels when directly compared with the full-term infants. NLRC3, which is known as an inhibitory NLR, can prevent overactivation of the immune system. Moreover, activation of NLRC3 competitively inhibits downstream pathways, including the TLR/STING/NF-kB and PIK3/mTOR pathways, thereby negatively regulating immune–inflammatory responses [13–15]. Consequently, the defective expression of NLRC3 in neonates can lead to inaccurate and delayed regulation of immune responses, resulting in the increased release of inflammatory mediators and higher susceptibility to severe infections such as SIRS, sepsis, and septic shock. Schneider et al. [16] found that LPS increases the serum concentrations of IL-6 and TNF-α in *NLRC3*-/- mice, leading to more severe symptoms of endotoxin shock due to the production of cytokine storms. Additionally, prior reports indicated that overexpression of NLRC3 in macrophages reduces the mRNA expression of TNF-α and IL-6, which further confirms the anti-inflammatory role of NLRC3 [17].

Furthermore, we performed a Spearman correlation analysis between the NLRC3 and NOD1 expression levels in the neonates (both preterm and full-term), revealing a significant positive correlation between the expression within mononuclear cells. This suggests that reduced NLRC3 expression correlates with decreased NOD1 expression, thereby contributing to the broad reduction in PRR expression in innate immune cells and potentially exacerbating severe infections. Both NOD1 and NLRC3 are recognized as critical innate immune receptors [18], which are essential for rapid immune responses to pathogen invasion or tissue damage. Abnormal expression of these receptors exacerbates the severity of infections, as evidenced by the delayed bacterial clearance seen in *NOD1*-/- mice [19] and the impaired cytokine responses of mononuclear cells in preterm infants, primarily due to defective NOD1 responses [20]. Moreover, activation of NLRC3 in dendritic cells (DCs) reduces Th1 and Th17

differentiation, whereas *NLRC3*-/- mice show enhanced Th1 and Th17 differentiation in DCs [21].

To elucidate the differences in inflammatory responses between the neonates and the adults, we compared the production of inflammatory cytokines in neonatal and adult peripheral blood stimulated with LPS alone or LPS plus HSV-60 DNA in vitro. HSV-60 DNA is a 60 bp oligonucleotide that contains a viral DNA motif derived from the genome of HSV type 1 (HSV-1), which exhibits the biological functions of HSV-1. Previous reports have indicated that NLRC3 can recognize the double-stranded DNA of HSV-1 with high affinity. Furthermore, upon binding to HSV-1 DNA, NLRC3 is specifically activated, and once activated, NLRC3 can inhibit the release of downstream inflammatory factors via suppressing proteasome components such as IKK, ASC, and TRAF [7, 14, 22]. Kollmann et al. observed that neonatal blood produces significantly lower levels of both TNF-α and IL-6 than adult blood when stimulated with LPS, which is suggestive of impaired inflammatory responses in neonates [23]. Thus, we utilized whole blood stimulation to simulate the inflammatory response following bacterial infection and observed that following LPS stimulation, the preterm and full-term infants produced significantly lower levels of TNF-α, IL-6, and IL-1β when compared with the adults. Moreover, following stimulation with a mixture of LPS and HSV-60 DNA, there was no significant difference in the levels of TNF-α, IL-6, and IL-1β among preterm infants, full-term infants, and adults. Notably, post-stimulation with the LPS and HSV-60 DNA mixture, the adults exhibited markedly reduced levels of TNF-α, IL-6, and IL-1β release when compared with stimulation using LPS alone, whereas the neonates showed a significant reduction in only the TNF-α release, with no significant differences observed in the IL-6 and IL-1β release levels. This underscores that while NLRC3's function in neonates is intact, it does not exert the same inhibitory regulation of inflammatory responses as in adults, possibly contributing to neonates' increased susceptibility to severe infections. Additionally, Xu et al. [24] discovered that NLRC3 negatively regulates the K63-linked ubiquitination of TRAF6, potentially reducing the downstream signaling. After LPS stimulation, *Nlrc3*-/- macrophages exhibited significantly higher nuclear p65 levels when compared with wild-type cells. Moreover, the phosphorylation of p65 in macrophages lacking NLRC3 occurred earlier and persisted for longer when compared with wild-type cells, indicating a minimal impact on signaling transduction via this pathway in the absence of NLRC3. Conversely, enhanced NLRC3 expression suppressed the functionality of receptors such as NOD1, thereby attenuating the inflammatory responses.

During the research phase, the data collection proved challenging due to the impact of the COVID-19 epidemic. As a result, this study had a small sample size, which may have influenced the final results. Furthermore, investigation of the molecular signaling pathway of NLRC3 is still ongoing, but upon completion, the results may provide a more comprehensive understanding of the role of NLRC3 in neonatal infections.

Conclusion

Our findings indicated that when compared with adults, both full-term and preterm infants exhibited significantly lower expression levels of NLRC3 and NOD. Moreover, in neonatal mononuclear cells, there was a notable positive correlation between the NLRC3 and NOD1 expression levels. Following stimulation with an LPS and HSV-60 DNA mixture, the levels of TNF-α, IL-6, and IL-1β were markedly reduced in the adult group. By contrast, in the neonatal group, only the release level of TNF-α was significantly reduced, whereas the IL-6 and IL-1β release levels showed no significant difference. These findings reveal widespread deficiencies in the PRRs within neonates. On the one hand, abnormalities in pro-inflammatory receptors, as exemplified by NOD1, contribute to neonatal difficulties in terms of pathogen recognition and the delayed initiation of inflammatory responses. On the other hand, defects in anti-inflammatory receptors such as NLRC3 impair the timely regulation of inflammation, resulting in cytokine storms and thus exacerbating the occurrence of severe infections. Such insights deepen our understanding of the characteristics of the neonatal innate immune system and suggest new immunological bases for the development of strategies to prevent and treat neonatal infections.

Abbreviations

NOD1	Domain-containing protein 1
CARD	Caspase activation and recruitment domain;
NLRC3	NOD-like receptor family CARD domain-containing 3;
PRR	Pattern recognition receptor;
RIP	Receptor-interacting protein;
NF-κB	Nuclear factor kappa B;
MAPK	Mitogen-activated protein kinase;
HSV-60	Herpes simplex virus type 60;
IKK	Kappa-B kinase;
pro-IL-1β	pro-interleukin 1 beta;
pro-IL-6	pro-interleukin 6;
TRAF	Tumor necrosis factor receptor-associated factor;
NBDs	Nucleotide-binding domains;
CRP	C-reactive protein;
WBC	White blood count;
PBMCs	Peripheral blood mononuclear cells;
PBS	Phosphate-buffered saline;
EP	Eppendorf;
qPCR	quantitative polymerase chain reaction;
LPS	Lipopolysaccharide;
FCS	Fetal calf serum;
TNF-α	Tumor necrosis factor-alpha;
ELISA	Enzyme-linked immunosorbent assay;
SPSS	Statistical Package for the Social Sciences;
$\overline{x} \pm SD$	mean ± standard deviation;
ANOVA	Analysis of variance;
HSD	Honest Significant Difference;

SIRS Systemic inflammatory response syndrome;
DCs Dendritic cells

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None.

Author contributions
Mingming Zhang designed the study, performed the data analysis, and wrote the manuscript. Meng Zhang collected the data, reviewed the manuscript, and provided critical revisions. Both authors approved the final manuscript.

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Data availability
The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The research protocol for this study has been approved by the Ethics Committee of Xuzhou Central Hospital, with the approval number XZXY-LK-20200103-016. This research was conducted in accordance with the principles outlined in the Declaration of Helsinki (https://www.wma.net/policies-post/wma-declaration-of-helsinki/). Informed consent has been obtained from the parents or legal guardians of the participating neonates.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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References

1. Tzialla C, Borghesi A, Perotti GF, Garofoli F, Manzoni P, Stronati M. Use and misuse of antibiotics in the neonatal intensive care unit. J Matern Fetal Neonatal Med. 2012;25(Suppl 4):35–7.
2. Chacko B, Sohi I. Early onset neonatal sepsis. Indian J Pediatr. 2005;72(1):23–6.
3. PrabhuDas M, Adkins B, Gans H, King C, Levy O, Ramilo O, et al. Challenges in infant immunity: implications for responses to infection and vaccines. Nat Immunol. 2011;12(3):189–94.
4. Elinav E, Strowig T, Henao-Mejia J, Flavell RA. Regulation of the antimicrobial response by NLR proteins. Immunity. 2011;34(5):665–79.
5. Ghorpade DS, Kaveri SV, Bayry J, Balaji KN, Withdrawal. Cooperative regulation of NOTCH1 protein-phosphatidylinositol 3-kinase (PI3K) signaling by NOD1, NOD2, and TLR2 receptors renders enhanced refractoriness to transforming growth factor-β (TGF-β)- or cytotoxic T-lymphocyte antigen 4 (CTLA-4)-mediated impairment of human dendritic cell maturation. J Biol Chem. 2019;294(50):19449.
6. Pham AT, Ghilardi AF, Sun L. Recent advances in the development of RIPK2 modulators for the treatment of inflammatory diseases. Front Pharmacol. 2023;14:1127722.
7. Zha LH, Zhou J, Li TZ, Luo H, Zhang MQ, Li S, et al. NLRC3 inhibits MCT-induced pulmonary hypertension in rats via attenuating PI3K activation. J Cell Physiol. 2019;234(9):15963–76.
8. Holley CL, Coll RC, Schroder K. NLRC3 restrains responses to a T. Immunity. 2018;49(6):989–91.
9. Chuphal B, Rai U, Roy B. Teleost NOD-like receptors and their downstream signaling pathways: A brief review. Fish Shellfish Immunol Rep. 2022;3:100056.
10. Chen Y, Yu SL, Li YP, Zhang MM. Nucleotide-binding oligomerization domain (NOD) plays an important role in neonatal infection. Int J Biol Macromol. 2019;121:686–90.
11. Zhou JT, Ren KD, Hou J, Chen J, Yang G. α–rhamnrtin–3–α–rhamnoside exerts anti–inflammatory effects on lipopolysaccharide–stimulated RAW264.7 cells by abrogating NF–κB and activating the Nrf2 signaling pathway. Mol Med Rep. 2021;24(5):799.
12. Li ZT, Liu H, Zhang WQ. NLRC3 alleviates hypoxia/reoxygenation induced inflammation in RAW264.7 cells by inhibiting K63-linked ubiquitination of TRAF6. Hepatobiliary Pancreat Dis Int. 2020;19(5):455–60.
13. Li W, Zhang Y, Hu Z, Xu Y. Overexpression of NLRC3 enhanced Inhibition effect of Sevoflurane on inflammation in an ischaemia reperfusion cell model. Folia Neuropathol. 2020;58(3):213–22.
14. Li X, Deng M, Petrucelli AS, Zhu C, Mo J, Zhang L, et al. Viral DNA binding to NLRC3, an inhibitory nucleic acid sensor, unleashes STING, a Cyclic dinucleotide receptor that activates type I interferon. Immunity. 2019;50(3):591–e96.
15. Zha L, Yu Z, Fang J, Zhou L, Guo W, Zhou J. NLRC3 delays the progression of AD in APP/PS1 mice via inhibiting PI3K activation. Oxid Med Cell Longev. 2020;2020:5328031.
16. Schneider M, Zimmermann AG, Roberts RA, Zhang L, Swanson KV, Wen H, et al. The innate immune sensor NLRC3 attenuates Toll-like receptor signaling via modification of the signaling adaptor TRAF6 and transcription factor NF-κB. Nat Immunol. 2012;13(9):823–31.
17. Fu Y, Zhan X, Wang Y, Jiang X, Liu M, Yang Y, et al. NLRC3 expression in dendritic cells attenuates CD4(+) T cell response and autoimmunity. EMBO J. 2019;38(16):e101397.
18. Li D, Wu M. Pattern recognition receptors in health and diseases. Signal Transduct Target Ther. 2021;6(1):291.
19. Shimada K, Chen S, Dempsey PW, Sorrentino R, Alsabeh R, Slepenkin AV, et al. The NOD/RIP2 pathway is essential for host defenses against Chlamydophila pneumoniae lung infection. PLoS Pathog. 2009;5(4):e1000379.
20. Strunk T, Richmond P, Prosser A, Simmer K, Levy O, Burgner D, et al. Method of bacterial killing differentially affects the human innate immune response to Staphylococcus epidermidis. Innate Immun. 2011;17(6):508–16.
21. Hu S, Du X, Huang Y, Fu Y, Yang Y, Zhan X, et al. NLRC3 negatively regulates CD4 + T cells and impacts protective immunity during Mycobacterium tuberculosis infection. PLoS Pathog. 2018;14(8):e1007266.
22. Zahid A, Ismail H, Li B, Jin T. Molecular and structural basis of DNA sensors in antiviral innate immunity. Front Immunol. 2020;11:613039.
23. Kollmann TR, Crabtree J, Rein-Weston A, Blimkie D, Thommai F, Wang XY, et al. Neonatal innate TLR-mediated responses are distinct from those of adults. J Immunol. 2009;183(11):7150–60.
24. Xu J, Gao C, He Y, Fang X, Sun D, Peng Z, et al. NLRC3 expression in macrophage impairs Glycolysis and host immune defense by modulating the NF-κB-NFAT5 complex during septic immunosuppression. Mol Ther. 2023;31(1):154–73.

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