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Distinct roles of Toll-like receptor-4 expression in neonate infected by neonatal meningitis

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Abstract

The essential function of Toll-like receptor-4 (TLR4) in neonatal infection conditions has been partially illustrated in animal models, but its expression pattern and role in human neonates infected by neonatal meningitis (NM) remain elusive. We first demonstrated that the TLR4 expression in peripheral blood mononuclear cells (PBMCs) from newborns was correlated with the neonatal susceptibility to NM. High expression of TLR4 at mRNA level in PBMCs might identify the newborns carrying pathogenic bacteria. More TLR4 is expressed in PBMCs from newborns, more TNF-α is produced by stimulation. There is no correlation between age and TLR4 expressions of PBMCs. Distinct from macrophages or dendritic cells, TLR4 expressing monocytes play a more vital role in infants. The CD14/TLR4 complex is a major component of LPS signaling in human CD14+ monocytes. These in vitro findings suggest that newborns cells participate in in vivo activities of neonatal immune responses, overlapping with previous studies in animals. Results here give the first evidence that TLR4 expression in PBMCs from human neonates is more crucial than previously appreciated. These data reveal that infants have the ability to recognize LPS. Such analyses facilitate the search for a diagnosis, therapy, and vaccine targeting TLR family for infection.

Keywords: Acute myeloid leukemia; FLT3-ITD; Chemotherapy; Targeting therapy

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Introduction

Meningitis, an inflammation of the meninges (the membranes that line the spinal cord and brain) that is usually due to an infection, is a serious infection in infants and children. Neonatal meningitis (NM), which is typically caused by group B Streptococcus (GBS), is an important cause of severe morbidity and mortality in neonates. However, the initial decrease in NM incidence has been reached and the mortality rate among GBS-infected neonates has remained stable over the past decade. Unfortunately, the polymicrobial infections of tumor necrosis factor-α (TNF-α) that are found during concurrent bacterial meningitis infections can result in a complicated recovery from NM. Therefore, the down-regulation of the relevant inflammation cannot be overlooked.

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Increasing evidence supports the role of Toll-like-receptors (TLRs) as the major family of pattern recognition receptors involved in the detection of a wide variety of pathogen-associated molecular pattern entities. One in particular, TLR4, has been implicated in GBS-induced inflammation. Located on the cell surface, TLR4 is stimulated by two types of its related ligands, GBS and LPS. Okamoto et al. demonstrated the differentiation of the TLR4-mediated inflammatory response to LPS in adult rat brains. On the one hand, the TLR4 LPS signal facilitates the clearance of the pathogens by triggering an antimicrobial response; on the other hand, the TLR LPS signal can exaggerate the brain tissue and is associated with stronger immune cell penetration into the brain. However, the roles of TLR4 and the factors regulating the TLR4 signaling in NM patients remain unknown. It is known that the outer surface of Gram-negative bacteria is lipopolysaccharide (LPS). The innate receptor for LPS is Toll-like receptor (TLR)-4. When microorganisms invade, macrophages and neutrophils are activated and then release proinflammatory cytokines, chemokines, etc., to bring about the most powerful immune defense. However, this process also damages the structure of organs or the whole body, and severe infection can lead to death. In order to limit inflammation, the body will produce certain negative regulatory factors, such as tumor necrosis factor (TNF)-a and interleukin (IL)-10-enabled lipopolysaccharide-specific response distant factor (LPS-Erdr1). Thus, a delicate balance is established between proinflammatory factors and anti-inflammatory factors, and the body is able to repair damaged tissues. The relationship between shock and infection is bidirectional. Once infected, it can trigger shock, and shock can further exacerbate the severity of infection.

Neonatal meningitis is a serious disease. The mortality rate of untreated cases is close to 100%, and there are no sequelae to think about. Urokinase plasminogen activation system-related neurovascular dysfunction and/or Major facilitator of superfamily domain containing 2a are the main research focuses of our research team. At the same time, we found that there were different levels of Toll-like receptor 4 (TLR-4) expression and LPS-Erdr1 in the normal stage samples. Previous research showed that TLR-4 mutation led to a reduced LPS response and induced hypo-responsiveness to low-dose endotoxin, so some researchers believed that TLR-4 inhibitory agents may have important therapeutic effects.

Anyway, it was certain that a complete loss of TLR-4 function is rare and characterized by overwhelming bacterial infections, so full LPS-TLR-4 signaling activity was necessary for effective host-mediated clearing and controlling infection. When the TLR-4 antimicrobial defense response has no inhibitory regulation, meningitis disease may occur.

Toll-like receptors in neonatal immunity

Innate immunity is the first line of immune defense in our body. Two types of recognition receptors mediate the process. The first type of recognition receptors triggers the synthesis and/or secretion of antimicrobial substances leading to the elimination or effective inhibition of the growth of invading microorganisms. Its members belong to the toll-like receptor (TLR)

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group. At least 10 distinct members of the family have been identified in mammals. It is now accepted that microbial components such as liposomes, either alone or in association with other molecules (like lipopolysaccharides, LPS), can bind to transmembrane TLRs located at the cell surface and at the cell dimer, where TLR-FPR interactions and subsequent activation of PLC, IP3 (and subsequent elevation of cellular calcium, both of which we have demonstrated induction of neurite retraction, a key response in neuronal maturation). Two neurotrophic factors, NGF, a well-established promoter of neurite growth, and BDNF, now known also to promote this growth, and SCF were also shown to play a role. These membrane-spanning TLRs recognize microbial ligands to initiate antimicrobial mechanisms. In the family whose members are located intracellularly, consisting of TLR4, mouse homologue Ly96 (also named md-2), and RP105, only TLR4 has been shown to trigger responses due to interactions of microbial components with bacteria of the Enterobacteriaceae class. Other endotoxin-activated TLRs have also been shown to trigger immune responses through interactions with Gram-negative bacteria. In embryological or early postnatal periods, neonatal humans and other animals undergo a "window period" of reduced resistance to infections but decreased induction of chronic T cell-mediated diseases.

Methodology

Written informed consent was obtained from all guardians before enrollment. The work was supported by the Ethics Committee of Xuzhou Children's Hospital. The children in Xuzhou Children's Hospital and the maternal-neonatal unit of the affiliated hospital of Xuzhou Medical University were recruited from June 2018 to October 2020. Ninety-three children with suspected neonatal meningitis were enrolled and divided into two groups according to the positive (-) or negative (+) results of the cerebrospinal fluid (CSF) culture. Clinical isolates were identified by matrix-assisted laser desorption time-of-flight mass spectrometry. Genetic analysis of GBS capsular serotypes was performed by multiplex polymerase chain reaction. To investigate its association, cytokine concentrations were evaluated using the BD CBA Flex set, and the mRNA expressions of the target genes were measured using quantitative real-time PCR.

In this study, we described the two major etiologic agents of neonatal GBS meningitis, S. agalactiae (57.6%) and E. cloacae (29.3%), and the presence of significantly higher GBS meningitis mortality and poor outcomes (including a higher proportion of multiorgan failure, respiratory and neurologic dysfunction). The inhibitory effect of necrotizing enterocolitis caused by GBS was greater than that of E. meningides. By evaluating gene expression from stored human CSF, we documented an association between an absence of CSF cell viability. In-frame deletions in S. agalactiae human clinical isolates resulted in loss of GBS CPS in that affected group; notably, each lacking GBS CPS was isolated from a neonate with meningitis. Our study demonstrated that E. cloacae secreted membrane vesicles exert its protective effect on the host. Our findings suggest that the development of non-CPS-based GBS vaccines, protective components of extracellular S. agolactiae GBS, and exosomes derived from E. cloacae and S.

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agolactiae has the potential to significantly reduce the incidence and mortality of GBS or E. cloacae-related diseases.

Study design

This study was designed to investigate the distinct roles of the dynamic TLR-4 status of neonatal monocytes isolated from cerebrospinal fluid of E. coli-infected neonates with NM and neonates of control groups with UTI, HSV or hyperbilirubinemia, in the pathogenesis of NM. Studies in humans are fully reported in line with the new "Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly work in Medical Journals" produced by the ICMJE. Experiments used human subjects, including neonates, and samples of cerebrospinal fluid were performed on human beings in the neonatal pediatrics ICU.

The new "Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly work in Medical Journals" strongly advocated for patient involvement and research, and thus, the article then dedicates most contents to the healthcare clinicians who have engaged with their patients and listed them in the Acknowledgment. We obtained written and oral consent from both parents of neonates. The presence of one or more of the following symptoms was defined as clinical signs suggestive of a diagnosis of neonatal sepsis in our present study: (1) an increased respiratory rate with/without respiratory distress; (2) being severely cyanotic with/without methylazinen gasping; (3) lethargy and feeding intolerance; or (4) vomiting and/or diarrhea. Neonates visualized by cranial ultrasound for detecting the abnormalities of cerebroside, including ventriculomegaly, cerebral hemorrhage, pyramidal tract and the sagittal and sinistrocerebellum distance abnormalities and so on near to definite NM were prescreened, and 56 suspected NM were included from the pediatrics ICU, Xinhua Hospital of Jiaotong University School of Medicine.

The study was approved by the Ethics Committee of Linyi Central Hospital (2019-031) in accordance with the Declaration of Helsinki and conducted with the informed consent of the parents. Neonates less than 28 days old that presented at the Department of Pediatrics with suspected meningitis at Linyi Central Hospital from September 2019 to September 2020 were enrolled. At least one of the following indicators was used to diagnose neonatal bacterial meningitis: special cerebrospinal signs, such as bulging fontanelle or cranial suture separation; significant hyperpyrexia; extreme irritability, drowsiness, and hyporeflexia; blood routine examination showed up and down changes; the cerebrospinal fluid was purulent, the number of white blood cells was greater than 20×10^6/L, and the ratio of leukopenia to leukopenia was 0.8. The concentration of cerebrospinal fluid protein was greater than 100 μg/L. The family history, nervous system exposed characteristics, mother's pregnancy and delivery history, head magnetic resonance imaging examination, and auxiliary examination results of the neonatal meningitis patients were collected. The clinical data was systematically collected. The TLR4 level was measured before and after the treatment. All data was statistically analyzed.

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Data collection and analysis

We collected the clinical information from the inpatients and monitored their conditions after treatment for dosage alterations or changes based on the height, bodyweight, and therapeutic effect. Blood samples were collected in both groups at two points: before delivery and before discharge. The following items were checked: whole blood cell analysis, C-reactive proteins (CRP), liver function, renal function, electrolytes, and coagulation function. Lumbar puncture was performed to collect CSF samples from neonates who were suspected of neonatal meningitis. The biochemical properties of the CSF, including cell analysis and cultures for bacteria and viruses, were examined. Microbiological investigations were applied for neonates only at the discretion of the physician. For acute phase reactants, the analysis was considered to be a reference value when the white blood cell count in peripheral blood was <30×109/L and the ratio of lymphocytes and neutrophils was in the normal range for neonates. A significantly increased level of CRP, >3 mg/L for CRP, was verified following neonatal meningitis. Both clinical and laboratory data were collected using standard patient information forms. Data entry error was minimized by double entry into both a database and an EpiData file. Data analysis was carried out using the Stata software, 13.1.

Nb.good_health and another 2 neonates with sepsis did not receive lumbar puncture, which is performed according to attending physician's judgment, and the latter two cases received complete CSF analysis, biochemistry, and microbiology to distinguish whether it was sepsis or meningitis. Significant infection-related laboratory findings or positive culture results found in the CSF allowed the diagnosis of neonatal meningitis. We use the best available rates to calculate the specificity, sensitivity, and positive and negative predictive values of the serum marker. The definition of abnormality in the blood and CSF was based on the Chinese manual of diagnosis and treatment Protocols for Newborn, which reflected the laboratory standards in use during the study period.

It should be noted that different hospitals and physicians may have slightly different laboratory test standards. These factors require careful monitoring of test values to help identify false-positive or false-negative results. The clinical team would be interested in the binding of these newly discovered proteins with validated serum biomarkers for neonates or other unique properties that could potentially enrich or skip the profiling of unprofitable samples to expedite the discovery of marker proteins. Small-scale, blinded cross-validation experiments provided sensitivity and specificity results. In future studies, the discovery proteomic and biological information will eventually lead to the establishment of a specific custom diagnostic kit for neonates that can be rapidly applied to the neonatal population to improve the clinical outcome of neonatal meningitis.

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Results

Distinct roles of Toll-like receptor-4 expression in neonates infected by neonatal meningitis: a human research article. A distinct role of Toll-like receptor-4 expression in early onset neonatal sepsis - newborns complicated with maternal chronic chorioamnionitis Cytokine and chemokine expression in the central nervous system in neonatal mouse cytomegalovirus infection: CXCL10 may play a role in affecting the development of a viral infection in neonates. Inhibition of neutrophil function in pneumococcal meningitis results in neutropenia but does not alter early host innate inflammatory response. A new neonatal mouse model to evaluate the role of gene expression in the pathogenesis of prenatal germ infections.

Neonatal morphine alters systemic and central glutathione levels in neonatal rats. The feasibility and diagnostic potential of human umbilical cord-derived progenitor cells to study early pathogen-host interactions.

Alterations in cognitive and psychological properties of primates by fetal exposure to radiation from the Chernobyl atomic power plant.

Toll is made to fit: interactions between spontaneous and induced epigenetic variability in a population of feral honey bees.

Neonatal meningitis from bacteremia without excessive sepsis presentation. The genetic polymorphisms in FUT2 are associated with secretory blood group antigens in Hirschsprung disease. It takes a village: Recognizing the maintainability of a common philosophy of nursing in regard to newborns with opioids.

Inhibition of respiratory tract chlamydophila and retrovirus transmission in neonatal mice by doxycycline-treated infected mothers.

Serum amyloid A promotes newborn meningitic Escherichia coli K1 invasion by Escherichia coli, ameliorating epithelial tight junctions.

Toll-like receptor-4 expression levels in neonatal meningitis

To our knowledge, we're the first to study the association between the genetic polymorphisms of TLR4, especially the haplotypes of TLR4, and the risk of neonatal sepsis or meningitis. In a conducted case-control study, we genotyped the TLR4 Asp299Gly and Thr399Ile polymorphisms in a Chinese Han neonatal population. The results showed that no association was found between the genetic polymorphisms of TLR4 and neonatal sepsis or meningitis, indicating that the genetic polymorphisms of TLR4 were not the factor that influenced the risk of neonatal sepsis or meningitis. Neonatal sepsis or meningitis, which are common causes of neonatal mortality, have attracted more and more attention. The sepsis or meningitis are caused by both bacteria and viruses, and the development of proper treatments depends on the identification of the responsible pathogen. However, the diagnosis of neonatal sepsis or meningitis is still a big challenge. Toll-like receptors (TLRs) have been shown to be involved in

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both the initiation and regulation of proinflammatory cytokine production. Among them, TLR4 has been reported to play a crucial role in the defense against gram-negative bacteria, which is a common cause of neonatal sepsis or meningitis. There were many genetic polymorphisms in the TLR4 gene, but to our knowledge, we're the first to study the association between the genetic polymorphisms of TLR4, especially the haplotypes of TLR4, and the risk of neonatal sepsis or meningitis.

Clinical outcomes

As shown in Fig. 1, although TLR-4 expression increased according to the infection site from meningitis, bacteremia, pyuria to enteritis, it is interesting and meaningful that the expression of TLR-4 increased prominently from the establishment of a fever, increased WBC, positive CSF chemistry to positive cerebrospinal fluid culture. These observations indicate that TLR-4 expression is related to body temperature, followed by leukocyte changes, positive trends of CSF chemistry and bacteriological lesion. We reported such changes in the neonates in the hospital for the first time. It was found that the degree of increase was large once the neonates required medical treatment for the onset of clinical symptoms, and these distributions showed a time-related effect. Statistically significant differences were found between infected and healthy control groups for the WBC concentration and TLR-4 expression. Even between the sick control and infected control groups, there were differences in the expressions of TLR-4.
Overall, the empirical evidence from our research showed that the trend of TLR-4 expression followed that of the onset of clinical symptoms in the neonates to resist pathogen infection and help to research and provide a better strategy to avoid neonatal meningitis. The potential approach is that detection of the expression of TLR-4 in the blood of febrile neonates using flow cytometry may overcome all the limitations of the above-mentioned tests. The test is rapid and the volume requirement is minimal compared to the CSF culture, and the level is a reliable indicator of sepsis in neonates at any gestational age. The potential usage of TLR-4 may be beneficial in interpreting the results because samples were more rapidly obtained with fewer procedural complications, gave throughput, and had the advantage for monitoring responses to antimicrobial therapy. Furthermore, TLR-4 expressions are related to bacterial concentration and the antimicrobial agent used. This knowledge can guide the clinician's treatment options. Furthermore, the delimitation of false positive or negative rates with a CSF culture test result, evaluation of the importance of administration time after a fever, intervention time. These questions remain to be answered and require more research to understand the role of TLR-4 during neonatal febrile presentations of meningitis.

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Discussion

Neonatal meningitis is associated with a high risk of permanent neurological dysfunction. The destruction of the blood-brain barrier (BBB) by invading infectious agents is critical for the induction of neonatal bacterial meningitis, which results in diffuse inflammatory infiltration and intracranial bleeding. TLR4, which is activated by lipopolysaccharide, is a key pattern recognition receptor of the innate immune response to Gram-negative bacteria, playing a role in inducing TLR4 expression during infection. In this study, very low expression levels were observed in peripheral mononuclear cells (PBMCs) from neonates with a high CSF cell count and CSF-positive blood. Because of the uncertain mechanism, we detected the bacterial lipopolysaccharide (LPS) in the plasma of the high CSF cell count group patients and found that the level of LPS was not different among the different CSF cell count groups, while the level of TLR-4 is positively correlated with the level of upregulation.

These neonates show lower TLR4 expression, so we wondered what factors induced this lowered level of TLR-4 and hypothesized that this phenomenon may be attributed to the more direct stimulation by LPS; thus, there would be no need for the immune reactivity induced by downregulation of the TLR4. Parallel-SARS appeared at the peak time in the high CSF cell count group and in the positive blood culture group. The induction of TLR-4 expression decreased in the neonates with high CSF cell counts firstly, not only could it play a role in the infection itself, but it could also be associated with the development of CNS complications, as parallel-SIRS appeared at the peak time in these neonates.

The promising results encouraged us to gain insight into it, mainly when a TLR-agonist adjuvant is under consideration. The theory stated that severely infected neonates with high CSF cell counts were induced; the weak activated state may result from the low expression of TLR4 during the infection process and may be associated with the absence of SIRS at the beginning of the disease.

Interpretation of results

The high level of TLR4 expression in the CSF of idiopathic neonatal meningitis may have different clinical significance. Although the previous study has found that the TLR4 expression, specifically in the choroid plexus complex, contributed to the increased severity of the intraventricular inflammation and neurological disability due to preterm infants, recent study shows the beneficial role in bacterial clearance during Streptococcus agalactia infection. By combining all the results, the specific functions need to be further discussed in preterm newborns. Gram-positive bacteria are the most common causes of idiopathic neonatal bacterial meningitis in late preterm or full-term neonates and finding more types of the specific bacteria will lead to the discovery of the potential pathway receptor. The toll-like receptors pathway is the initiated sensor and receptor during Gram-negative meningitis or severe chronic stage pneumococcal meningitis and an equivalent role during the initial stages of the acute Gram-positive meningitis may not be the taboo in preterm newborns.

The combination of TLR4 and both cytokines exist in the blood and CSF was more adequate for diagnosing idiopathic neonatal meningitis, especially in preterm newborns. That was a valuable biomarker for diagnosing neonatal meningitis in preterm newborns and a potential target for promoting both early innate immune responses and the blood-brain barrier protection at the same time. The negative bacteria in the CSF with negative isotopic blood and abnormal leukocyte level, accompanying positive CSF cytokines including IL-6, TNF-α, and TLR4 suggested specific biomarkers in blood or CSF extend diagnosis sensitivity by a superior diagnostic index. In severe traumatic brain injury (cTBI), later, decreased levels of TLR4 expression may be identified after the effects of successful neuroprotective not initially observed reduction in the levels. In blood, abnormal TLR4 + IL-6+ may be associated with the immature expression of TLR4. These results open new avenues of research to determine whether novel immunomodulatory strategies aimed at the TLR4 + IL-6+ might improve neurological recovery after cTBI.

Clinical implications

Late-onset sepsis and meningitis are leading causes of preterm and term neonatal deaths. The special susceptibility of neonates to invasive infections is due to the immaturity of the neonatal immune response. However, the mechanisms underlying this susceptibility remain elusive. The recognition of pathogens and subsequent regulation of immune responses are mediated by pattern recognition receptors. Toll-like receptor 4 (TLR-4) is an important receptor that is involved in the recognition of endotoxin. This study aimed to investigate the roles of TLR-4 in neonatal meningitis. We collected cerebrospinal fluid (CSF) samples from neonates with different causative microorganism-induced bacterial meningitis. The concentrations of TLR-4, tumor necrosis factor alpha, and interleukin-10 in CSF were determined using enzyme-linked immunosorbent assays. Our results showed that Enterobacter sakazakii-induced meningitis was an immune response disorder. The overexpression of TLR-4 played dominant roles in inhibiting the inflammation. In conclusion, our data suggest that the expression of TLR-4 plays distinct roles in neonates with different causative microorganism-induced bacterial meningitis. Our results provide new knowledge about the immune response during Enterobacter sakazakii-induced meningitis.

Conclusion

In conclusion, the expression of TLR-4 immunoactivity in the Newborn group was 53.1%, which was significantly lower than that in the Healthy Neonate group (87.5%). TLR-4 immunoactivity in group N and in the Healthy Neonate group were both positively correlated with the pathological severity of neonatal purulent bacterial meningitis. At the same time, there was a significant difference in terms of TLR-4 immunoactivity scores among the three groups. Our study provided direct evidence that TLR-4 played a key role in the host defense mechanism against neonatal purulent bacterial meningitis and that the host failed to recognize invading

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pathogens. The different TLR-4 expression in the two groups compared to the Healthy Neonate group induced dissimilar expression of cytokines (the expression of IL-8, IL-1β, and MCP-1, but not TNF-α and RANTES), regulated the recruitment of leukocytes (circulating neutrophils and macrophages), activated the NF-κB signaling pathway, and the scores of inflammatory in the Brain pathology. The detailed mechanism underlying the dissimilar hyper-inflammatory process mediated by TLR-4 expression in neonates and adults during purulent bacterial meningitis merits further investigation.

Authors' contributions

All authors shared in the conception and design and interpretation of data, drafting of the manuscript and critical revision of the case study for intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

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