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Plasma Receptor Interacting Protein3 in Neonatal Sepsis

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Abstract

The gold standard for diagnosing baby sepsis continues to be blood culture isolation of the causing bacterium, despite the fact that technique has substantial practical limitations due to its high cost and low sensitivity. White blood cell count (WBC), platelet count (PLT), C-reactive protein (CRP), and pro calcitonin (PCT) are examples of traditional non-specific markers that may not have the sensitivity or specificity needed for early diagnosis. Recent research has focused on a variety of biomarkers, including cell surface antigens, bacterial surface antigens, and genetic biomarkers. To identify newborn sepsis, researchers are closely observing protein biomarkers, cytokines, and chemokines. The optimal biomarker with high sensitivity and specificity does not yet exist, though. Receptor Interacting Protein (RIP) 3 belongs to the RIP kinase family. According to recent research, several diseases cause RIP3-dependent necroptosis. When activated by biological or physicochemical factors, tumor necrosis factor receptor binds to ligand and recruits Fas-associated protein with death domain (FADD), RIP1, and caspase-8 to form complex I, which promotes apoptosis. When the activity of caspase-8 is decreased, RIP1 binds to RIP3 and recruits FADD and caspase-8 to form complex II. Through the activation of the programmed necrosis pathway and the suppression of apoptosis, this causes cell expansion and rupture.

Keywords: RIP3, Neonatal Sepsis, Receptor Interacting Protein 3 (RIP3), Biomarkers, Apoptosis

Mini review article **Corresponding Author*, e-mail: *amanyelsayed84@gmail.com*

1. Introduction

Neonatal sepsis poses a significant health burden and is associated with high morbidity and mortality. Low birth weight infants are especially vulnerable to sepsis and appropriate interventions are needed as early as possible in improving survival outcome [1]. The pathogenesis of neonatal sepsis is characterized by an exaggerated inflammatory response leading to single or multi-organ dysfunctions. Increase in pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 are frequently observed in both animal models and in infants suffering from neonatal sepsis. While there is much improvement in diagnosis of neonatal sepsis, little is known about the molecular factors responsible for the immune response leading to end organ damage and cell death during neonatal sepsis [2]. Receptor interacting protein kinase 3 (RIPK3) is a member of the RIP kinase family and is expressed in multiple tissues including the tissues of the hemopoietic cell lineages. Receptor interacting protein belongs to a family of serine/threonine kinases that are sensors of cellular stress [3]. Two members of the RIPK family, RIPK1 and RIPK3, interact together and mediate programmed necrosis or necroptosis. Necroptosis is a type of cell death that is controlled by the kinase activity of RIPK1.

The crucial role of RIPK1 in cellular models of necrosis in tumor necrosis factor (TNF) receptor 1, TNF-related apoptosis inducing ligand receptor (TRAIL), Fas, Toll-Like Receptor (TLR) 3 and 4 has been shown in a number of studies [4]. Allosteric inhibition of the kinase activity of RIPK1 by necrostatin blocks necrosis without affecting the activation of NF-kB or MAPK pathways. Interestingly, in presence of caspase inhibitor, zVAD-fmk, cells were protected from TNF-induced apoptosis whereas in other cells, zVAD treatment caused TNF-induced necrosis. At first, RIPK3 was regarded as a protein associated with RIPK1. However, several studies implicated RIPK3 in the regulation of apoptosis and NF-kB signaling [5]. Mutagenesis studies showed that phosphorylation of RIPK1 by RIPK3 is crucial in inhibiting either RIPK1 or TNF-induced NF-kB activation. Therefore, although RIPK3 first identified as a protein associated with RIPK1, more recent work identified RIPK3 as a mediator of necroptosis which is caspase-independent [6]. A combination of genome wide siRNA analysis and biochemical studies revealed that RIPK3 is key determinant of necrotic cell death downstream to RIPK1.

Later it was confirmed that RIPK3 expression determines the cells to undergo necroptosis implicating RIPK3 as the molecular switch which determines whether cells undergo apoptosis or necroptosis [7]. RIPK3 mediated necrotic cell death accelerates systemic inflammation and mortality. In addition, in animal models of cerulean-induced pancreatitis, in adult severe sepsis models and in high dose endotoxemia, the expression of RIPK3 was dramatically increased [8]. To delineate importance of RIPK3 signaling, mice deficient in RIPK3 generated by gene targeting. Mice lacking RIPK3 were healthy and fertile and showed no signs of impairment in either TNF receptor or Toll like receptor-

induced NF-kB activation. When embryonic fibroblasts from wild type mice experienced cell death, those cells from RIPK3 knockout mice were resistant to necrosis following treatment with various necrosis inducing agents [9]. Neonatal immune response is considered immature and the cytokine responses are polarized to Thelper (Th) 2 and Th-17 with impairment in Th-1 cytokines.

As such, the immunological profiles of the neonates are distinct from adults [10]. Neonatal sepsis is a systemic inflammatory response occurring in neonates during the first 28 days of their birth. It is the third leading cause of neonatal death and low birth weight infants are more prone to sepsis than term infants. This increased susceptibility has been attributed to their immature or underdeveloped immune system [11]. The pathogenesis of neonatal sepsis is characterized as the exaggerated inflammatory response due to infection with the presence of high levels of pro inflammatory cytokines, i.e., TNF-α, IL-1β and IL-6 [12]. While there is much improvement in diagnosis, the molecular factors mediating such inflammatory conditions were not completely elucidated. In a previous study using an established CS model in newborn mice, serum IL-6 and IL-1β were significantly elevated in the WT mice whereas in the KO mice these levels were markedly attenuated. While lung injury score, lung neutrophil infiltration and lung and gut apoptosis were significantly increased in the WT mice, the KO mice showed a more attenuated phenotype in all these parameters. These data suggest that RIPK3 could be involved in uncontrolled inflammatory response leading to end organ damage and cell death observed during neonatal sepsis [13].

Cells may die through multiple means and the two predominant ways of death are apoptosis and necrosis. Apoptosis involves activation of caspases which manifests as condensed chromatin with the plasma membrane being intact. For decades unlike apoptosis, necrosis has been considered as merely fortuitous and unregulated form of cell death caused by permeabilization of the plasma membrane and subsequent cytoplasmic swelling with no signs of nuclear shrinkage [14]. However, recent evidence suggests that as in the case of apoptosis, necrosis can also be regulated. At the forefront of such regulation is the receptor interacting protein kinase (RIPK). Although RIPK3 was first identified as a protein associated with RIPK1, more recent work established RIPK3 as molecular switch that determines cell to undergo apoptosis or necrosis [15]. In an animal model of cerulein-induced pancreatitis, expression of RIPK3 was dramatically increased in pancreas and 67% of the mice showed pancreas acinar cell loss and necrosis whereas these effects were prevented in the KO mice [16]. In an adult severe sepsis model and in high dose endotoxemia, RIPK3 protein increased significantly and RIPK3 deficiency attenuated sepsis-induced increases in cytokines and neutrophil infiltration to lung tissues.

A previous study in newborn mice, sepsis-induced increases in cytokines, neutrophil infiltration to the lungs, and lung and gut apoptosis were attenuated in the KO mice. It can be speculated that the effect could be due to attenuation of neonatal sepsis associated necrosis in the lungs and the gut [17]. How RIPK3 deficiency attenuates necrosis in the lungs and gut in neonatal sepsis has not been determined. Alarmins or damage associated molecular patterns (DAMPs) released from the stressed cells undergoing necrosis act as endogenous danger signals to exacerbate inflammatory response [18]. Increased levels of these DAMPs have been associated with inflammatory conditions such as adult sepsis, arthritis, lupus, Crohn's disease and cancer. In previous CS model of the newborn mice, IL-1β and IL-6 were significantly elevated at 10 h after sepsis induction. These cytokines are pro inflammatory cytokines which are crucial and sensitive biomarkers shown to be elevated in neonatal sepsis [19]. In addition, RIPK3 has shown to be a stimulator of IL-1 activation. However, studies in TNF-α induced mouse adult sepsis model, RIPK3 is not essential for cytokine transcription but it sustains cytokine secretion presumably by DAMP release induced by cell necrosis [20].

Lung integrity was severely compromised in the WT mice 10 h after cecal slurry injection whereas no substantial tissue damage was observed in the gut. The circulating levels of IL6 and IL-1β could come from other organs such as the liver or the circulating immune cells. Neutrophils in the alveolar space, expression of the lung MIP-2 mRNA and lung MPO activity attenuated in the CS injected RIPK3 deficient mice as compared to the WT counterpart. Presence of neutrophils in the alveolar space, MIP-2 mRNA expression and MPO activity are measures of neutrophil infiltration to the tissues [21]. Increased neutrophil infiltration to tissues, particularly lungs, has implicated in systemic inflammatory response. Several studies also support that necroptosis caused by DAMPs released from cells promote neutrophil infiltration. Therefore, it is possible that deficiency in RIPK3 attenuates cellular necrosis thereby prevents release of these DAMPs and decreases inflammation in neonatal sepsis [22]. In pathological state, homeostasis is maintained by apoptosis, which is generally considered to be a non-inflammatory biological process.

Once this process is inhibited, necrosis can be activated as an alternative pathway to cell death and participates in a series of inflammatory reactions [23]. At present, the mechanisms of necrosis include mainly programmed necrosis, ferroptosis, inflammatory necrosis and other pathways. Previous studies have shown the critical role of programmed necrosis in the regulation of systemic inflammatory response syndrome (SIRS). Linde Duprez et al. showed that the deletion of RIP3 reduced the amounts of circulating DAMPs in mice and conferred complete protection against lethal SIRS [24]. Apostolos Polykratis et al. had similar results in mice pre-treated with the RIP1 inhibitor necrostatin-1 [25]. The latest studies showed that RIP3 protein deficiency attenuated the inflammatory response and organ damage in new-born mice with sepsis, suggesting that RIP3 may participate in the pathogenesis of neonatal sepsis. To date, the role of RIP3 in neonatal sepsis has not been reported in the clinic [26]. A previous study evaluated the clinical value of plasma RIP3 for LOS. They demonstrated that the levels of RIP3 in the sepsis group before treatment were significantly higher than those in the control group, revealing that plasma RIP3 may be an important biomarker of LOS.

Their finding is consistent with reports about the expression of plasma RIP3 in adult patients in the intensive care unit. RIP3 was detectable in the plasma of adult patients with severe sepsis, and the expression of RIP3 in the plasma of patients with sepsis-related death was significantly higher than that in the plasma of survivors, suggesting that the abnormal production or clearance of RIP3 may predict the poor prognosis of sepsis [27]. In a prospective study of 953 patients in five intensive care units, elevated levels of plasma

were associated with in-hospital mortality and organ failure, which indirectly confirmed that high expression of RIP3 could indicate the adverse consequences of critical illness. They further observed that plasma RIP3 levels in the sepsis group significantly decreased after effective treatment. It is speculated that RIP3 would be helpful to judge the clinical efficacy and prognosis of LOS [28]. At present, the early diagnosis of neonatal sepsis in practice depends mainly on nonspecific infection indicators such as WBC, PLT, CRP and PCT. In this study, hs-CRP and PLT were also investigated and compared with RIP3 to evaluate the value of different indicators in the early diagnosis of LOS.

The results showed that sensitivity, negative predictive values and Youden index of hs-CRP and PLT were lower than those of RIP3, indicating that RIP3 was a biomarker with high diagnostic efficiency [29]. Previous studies have shown that diagnostic value can be improved by combining different biomarkers. According to this point, further comparison of diagnostic value of multiple combined indicators for LOS was conducted, showing that sensitivity, specificity, positive predictive value and negative predictive value of RIP3 + hs-CRP + PLT in the diagnosis of LOS were higher than those of RIP3 + PLT and hs-CRP + PLT [30]. Additionally, the AUC of RIP3 + hs-CRP + PLT in the diagnosis of LOS was the highest. It is suggested that the combination of RIP3, hs-CRP and PLT has more robust diagnostic performance for LOS [31]. Furthermore, it is reported that the scoring systems based on physical examination coupled with laboratory findings feature great value in neonatal sepsis predicting. Therefore, encompassing physical examination, laboratory findings plus RIP3 level might be more helpful for LOS early diagnosis [31].

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