Emerging Role of the NLRP3 Inflammasome and Interleukin-1 β in Neonates

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Abstract

Infection and persistent inflammation have a prominent role in the pathogenesis of brain injury and cerebral palsy, as well as other conditions associated with prematurity such as bronchopulmonary dysplasia. The NLRP3 inflammasome-interleukin (IL)-1 β pathway has been extensively studied in adults and pre-clinical models, improving our understanding of innate immunity and offering an attractive therapeutic target that is already contributing to clinical management in many auto-inflammatory disorders. IL-1 blockade has transformed the course and outcome of conditions such as chronic infantile neurological, cutaneous, articular (CINCA/NOMID) syndrome. Inflammasome activation and upregulation has recently been implicated in neonatal brain and lung inflammatory disease and may be a novel therapeutic target.

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Introduction

Neonates and preterm infants in particular are susceptible to infection and inflammatory conditions [1]. The incidence of neonatal sepsis varies from 23 per 1,000 live births in Africa to 9 per 1,000 in the USA [2], causing a disproportionately high mortality and morbidity in preterm and very-low-birth-weight neonates [1, 3]. Infection and persistent inflammation have been implicated in the pathogenesis of brain injury and cerebral palsy [2], as well as other comorbidities associated with prematurity such as bronchopulmonary dysplasia (BPD) [4], retinopathy of prematurity [5], and necrotising enterocolitis [6]. The foetus and neonate primarily depend on their innate immunity as a barrier against infection [7]. Pro-inflammatory cytokines induce leucocyte migration to inflamed tissues and boost their proliferation. They stimulate the release of lytic enzymes, the production of prostaglandins, and the synthesis and secretion of secondary cytokines.

Fig. 1. Activation of interleukin-1 β (IL-1 β) via NLRP3 inflammasome: IL-1 β activation depends on 2 signals, NF κ B and the inflammasome. After the recognition of DAMPs by Toll-like receptors (TLRs) on the cell membrane, the NF κ B pathway is activated, inducing the synthesis of pro-IL-1 β . The second signal triggers the assembly of the inflammasome; this occurs when the cell senses ATP or nigericin and crystals. The inflammasome complex consists of 3 parts: NLRP3, apoptosis-related speck-like protein containing a caspase recruitment domain (ASC), and pro-caspases. Once activated, pro-caspase will cleave caspase 1 into the active form and caspase 1 cleaves pro-IL-1 β and pro-IL-18 into the mature forms IL-1 β and IL-18.

Pro-inflammatory cytokines have been implicated in neonatal disorders especially in preterm infants [8]. Interleukin-1 β (IL-1 β), a key initiator of inflammation by the innate immune system, has been implicated in hypoxia-ischaemia and LPS-induced neonatal brain injury, especially involving the hippocampus [9, 10]. IL-1 β is a pro-inflammatory cytokine that alters cell proliferation, differentiation, and apoptosis, and its role in the initiation, regulation, and maintenance of inflammation is well established [11]. IL-1 β production is tightly regulated, requiring a dual signal. First, pro-IL-1 β is synthesised upon stimulation of cell membrane pattern-recognition receptors. The second signal triggers the assembly of the NLRP3 inflammasome, which is essential for active caspase 1 production that in turn cleaves pro-IL-1 β to create the biologically active IL-1 β [12] (Fig. 1).

Many animal studies demonstrate the possibility of reducing brain injury by the use of an IL-1 receptor antagonist [13–15]. Yoon et al. [16] found a correlation between higher amniotic fluid IL-1 β concentrations in women with complicated preterm gestation and white matter injury sustained by their newborns. Higher IL-1 β can be detected in neonates with culture-proven sepsis compared to controls at diagnosis, and these elevated concentrations persisted even after a week of antibiotic therapy [17].

The Inflammasome

The innate immune response relies on cell membrane Toll-like receptors (TLR) and intracellular Nod-like receptors (NLR) to detect invading microbes. After sensing

danger signals, some of the NLRs trigger the activation of inflammatory caspases by forming a large multiprotein complex called the inflammasomes [18]. The term inflammasome describes the caspase-activating complex that plays a major role in innate immune responses [19]. Five inflammasomes have been described to date, and each respond to a different set of stimuli. The NLRP3 inflammasome is the most extensively studied, and its components are NLRP3 (NLR family, pyrin domain containing 3), apoptosis-related speck-like protein containing a caspase recruitment domain (ASC), and procaspase-1 [20, 21] (Fig. 1).

The NLRP3 inflammasome can be detected upon induction of monocytes, macrophages, neutrophils, keratinocytes, and B and T lymphocytes [22–24]. In a mouse model, Mankan et al. [25] established the important role of NLRP3 inflammasome in the production of active IL-1 β by neutrophils. This has been confirmed by a murine model of bacterial corneal infection, where the NLRP3 inflammasome activated IL-1 β in neutrophils after infection [26]. Inflammasome-dependent caspase 1 is crucial for pro-IL-1 β activation, but other inflammasome-independent mechanisms utilise proteases, such as neutrophil-derived serine proteases, to also activate pro-IL-1 β [27].

Inflammasome and Pregnancy

The inflammasome is associated with human development, and several studies have shown that IL-1ra concentration in amniotic fluid is associated with foetal gender.

Pregnant women with a female foetus have higher levels of IL-1ra, which are not altered with gestation or the presence or absence of labour [28, 29]. However, this is controversial since more recent studies showed no link between IL-1ra levels and foetal gender [30, 31].

The inflammasome also plays a role in pregnancy and labour. Caspase-1 and IL-1 β are expressed in the placenta and chorioamniotic membranes during normal pregnancy. Activation of the NLRP3 inflammasome in the chorioamniotic membranes is associated with term labour, and NLRP3 inflammasome activation in the placenta is involved in preeclampsia and preterm labour [32]. A study of 20 women with full-term normal pregnancies and 20 women with pre-eclampsia showed a significant increase in NLRP3, caspase-1, IL-1 β , TNF- α , and HMGB1 at mRNA level in placenta and in placental homogenate. On the contrary, IL-18 was lower in placentae from women with preeclampsia [33].

IL-1 β is known to increase expression of tryptophan 2,3-dioxygenase and to stimulate tryptophan catabolism in endometrioma stromal cells. Tryptophan 2,3-dioxygenase along with indoleamine 2,3-dioxygenase (IDO) suppresses T cell and NK cell proliferation and induces Tregs and myeloid-derived suppressor cells. It has been shown that these enzymes play a role in pregnancy by the suppression of anti-foetal immunity, whereas abnormal expression and function of indoleamine 2,3-dioxygenase are associated with spontaneous abortion, preeclampsia, preterm labour, and foetal growth restrictions [34, 35]. This may suggest that the regulation of the inflammasome and its products could be a novel therapy for the prevention and treatment of complications in pregnancy.

Inflammasome-Mediated Diseases

The NLRP3 inflammasome is associated with a variety of conditions in adults such as Alzheimer's disease, atherosclerosis, multiple sclerosis, type 2 diabetes, age-related macular degeneration and gout, epilepsy, stroke, and neurodevelopmental disorders such as schizophrenia and autism [36, 37]. Beta-amyloid plaques, drusen deposits, cholesterol crystals, and monosodium urate crystals induce the production of IL-1 β and IL-18. Investigating the abnormal activation of the NLRP3 inflammasome may be important to understand the molecular mechanisms underlying these disease processes [38–41]. Ruscitti et al. [42] showed a significant increase in NLRP3 expression and IL-1 β production by monocytes from patients with both rheumatoid arthritis and type 2 diabetes when

monocytes were cultured with different concentrations of glucose. In peripheral blood samples from adults with acute respiratory distress syndrome, NLRP3-related mRNA transcripts (caspase 1, IL-1 β , and IL-18) were elevated, suggesting a crucial role of NLRP3 activation in this disease [43]. Siddeek et al. [44] reported the correlation of obesity in children with cardiac failure in adulthood. In this study, increased expression of the cardiac proteins NLRP3 and ETS-1 was found in postnatally overfed mice. Three pathways have been described to explain the link between IL-1 β and neurodevelopmental disorders. The first pathway is the alteration of dendritic morphology, possibly through the c-Jun N-terminal kinase (JNK) pathway activating mitogen-activated protein kinase pathway. The second related to increased expression of Mecp2 leading to alteration of synaptic proteins by chromatin remodelling mechanisms. The final pathway suggests the modulation of GABA by the reduction of the chloride transporter KCC2, incrementing the susceptibility to seizures [37].

Cryopyrin-associated periodic syndromes (CAPS) are a spectrum of autoinflammatory disorders resulting from mutations in the *NLRP3* gene, leading to the overproduction of IL-1 β [45]. Milder forms of CAPS include familial cold autoinflammatory syndrome (FCAS) and Muckle-Wells syndrome (MWS), while the most severe form is represented by chronic infantile neurological, cutaneous, articular (CINCA) syndrome, collectively known as neonatal-onset multisystem inflammatory disease (NOMID) [46]. FCAS is characteristically self-limiting with onset in infancy persisting into adulthood; it presents with recurrent pyrexia, fatigue, headaches, and/or nausea starting within hours of exposure to cold and last for 24 h.

MWS also presents in infancy with various features including continuous pyrexia, joint pains, and skin rashes with exacerbations triggered by cold, heat, stress, and exercise, and flares can last up to 48 h; some patients can develop progressive sensorineural hearing loss and painful red eyes, as well as systemic amyloidosis leading to renal failure later in life [47–49].

CINCA/NOMID is a very rare disorder with approximately 100 affected individuals with different ethnic backgrounds have been reported and is characterised by persistent inflammation and tissue damage primarily affecting the nervous system, eyes, skin, and joints [50]. Infants present within the first few weeks of life with urticarial rash, followed by recurrent episodes of fever, arthropathy, and neurological manifestations such as chronic aseptic meningitis, raised intracranial pressure, mental retardation, and seizures. Some patients exhibit

MRI brain changes such as abnormal small vessel enhancement in the basal ganglia, periventricular white matter lesions on FLAIR sequences, ventriculomegaly, and cerebral atrophy. Other clinical presentations include progressive hearing impairment secondary to cochlear inflammation, hepatosplenomegaly, eye inflammation, bony overgrowth, and short stature [46, 51]. The presence of *NLRP3* germline missense mutations can be confirmed in approximately 60% of patients, while somatic mosaicism accounts for up to two-thirds of the remainder [52–54]. Patients with CINCA/NOMID have high risk mortality due to the overproduction of IL-1 β , which leads to inflammation-mediated organ damage. Importantly, disease symptoms and some end-organ damage can be prevented by blocking IL-1 [55].

Neonatal Inflammation, Infection, and the Inflammasome

Sharma et al. [56] demonstrated significantly lower caspase-1 activity in cord blood monocytes from preterm infants in the presence of histological chorioamnionitis compared to preterm infants without chorioamnionitis, suggesting a role of foetal infection in controlling NLRP3 inflammasome responses. Non-classical monocytes (CD14low/CD16pos) in human preterm cord blood samples did not express pro-IL-1 β on LPS stimulation, while the other monocyte subsets (CD14high/CD16neg and CD14high/CD16pos) had preserved pro-IL-1 β expression but significantly reduced mature IL-1 β secretion due to impaired NLRP3 inflammasome assembly, and profound reduction in caspase 1 activity especially in preterm infants below 29 gestational weeks. They observed increase in IL-1 β secretion capability in neonates born between 24 and 29 gestational weeks, beyond the first week of neonatal life [56]. Higher concentrations of IL-18 have also been observed in preterm neonates <28 gestational weeks compared to preterm neonates of 28–32 gestational weeks when the neonates presented with late-onset sepsis [57–59].

The inflammasome is also involved in severe brain damage associated with Zika virus microcephaly. Zika virus is a disease transmitted mainly by mosquitos, and it is associated with microcephaly and congenital malformations. de Sousa et al. [60] showed significantly higher atrophy of the brain with higher expression of NLRP1, NLRP3, IL-1 β , and caspase 1 in brain tissue samples from Zika virus-positive neonates that died after birth compared to controls, suggesting that the inflammasome increases brain damage in Zika virus-positive neonates. Lie

et al. [61] showed that an IL-1 blockage lessens microglia activation, increases foetal viability, and reduces neurobehavioral deficits in offspring of ZIKV-infected pregnant mice.

Inflammasome gene expression can be altered by malnutrition. Gomes de Moraes et al. [62] measured inflammasome gene expression in mononuclear cells from nourished and malnourished Wistar rats after treatment with either LPS or *Staphylococcus aureus*. Malnutrition reduced LPS-stimulated expression of TLR-2, TLR-4, NLRP3 receptors, as well as caspase 1 and IL-1 β in macrophages. On the contrary, direct treatment with *S. aureus* promoted gene expression of receptors and enzymes in macrophages in the malnourished rats [62]. These studies show that the inflammasome plays a significant role in neonatal responses to inflammation and infection, and alterations in the inflammasome pathway are associated with worse prognosis to infection, especially in neonates under 29 gestational weeks [62].

The Inflammasome, IL-1 β , and Neonatal Brain Injury

Inflammasomes mediate neuroinflammation, which is an innate immune response in the central nervous system against noxious stimuli such as chronic stress, microbes, and toxic by-products of metabolism. Neuroinflammation has an established role in the pathogenesis of neurodegenerative diseases and psychiatric illnesses such as Parkinson's disease, Huntington's disease, multiple sclerosis, Alzheimer's disease, depression, and hypoxia-ischaemia [63]. Hypoxia-ischaemia is associated with the activation of stress sensor signalling pathways like inositol-requiring enzyme-1 alpha, which activates the NLRP3 inflammasome via miR-17/thioredoxin-interacting protein, aggravating brain damage [64].

Many cytokines including IL-1 β are expressed at low concentrations in the healthy brain and may be important for many physiological processes such as synaptic plasticity and pruning as well as memory formation. However, IL-1 β has a key role in various types of brain injuries such as traumatic brain injury, cerebral ischaemia, subarachnoid and intracerebral haemorrhage, as well as many pathological conditions including sepsis, lung injury, arthritis, and autoimmune dysfunction [65].

Brain-derived neurotrophic factor (BDNF) is a growth factor that plays an important role in neuronal survival, growth, and plasticity, and it is essential for learning and memory. Lower levels of the growth factor BDNF are associated with autism disorders, whereas higher levels of

BDNF in preterm infants are associated with a more normal development [66, 67]. A murine study showed up-regulation of BDNF with a single injection of IL-1 β , whereas daily administration of IL-1 β decreased BDNF levels [68], suggesting the use of IL-1 β as treatment for these conditions.

In newborn hypoxic-ischaemic lambs resuscitated with 100% oxygen, the expression of IL-1 β was increased in cortex/subcortex after resuscitation compared with those resuscitated with 21% O₂. Increased cellular expression of IL-1 β was localized to sub-meningeal cortical layers and to sub-cortical white matter. Hyperoxic resuscitation at birth following foetal asphyxia therefore seems to induce a cerebral pro-inflammatory condition, which may be leading to increased tissue damage after exposure to hyperoxaemia at birth [69].

Swaroop et al. [70] showed that in IL-1 β -induced inflammation in microglia, heat shock protein 60 induces NLRP3 expression, leading to the activation of caspase 1 and increase in IL-1 β secreted by the microglia both in vitro and in vivo. Further, knockdown of heat shock protein 60 reduced IL-1 β production and decreased inflammation. IL-1 β may induce neuronal injury through various mechanisms including induction of nitric oxide production, activation of necroptotic and apoptotic pathways, modulating synaptic plasticity, and influencing elements of the mitogen-activated protein kinase pathway, which has a role in the control of neurodegeneration [71, 72].

Several animal studies have defined the deleterious effects of IL-1 β on the brain with worsening of ischaemic damage following exogenous administration of recombinant IL-1 β in experimental models of stroke [73, 74]. Jedrzejowska-Szypulka et al. [75] showed that IL-1 β has a significant role in blood-brain barrier disruption and leucocyte migration following subarachnoid haemorrhage in adult rats. In a mouse model of intrauterine inflammation, Leitner et al. [76] observed elevated levels of IL-1 β in foetal brains and placenta, and an improvement in foetal brain injury with the systemic administration of IL-1 receptor antagonist to pregnant mice. In addition, intracerebral haemorrhage impairs endogenous hydrogen sulphide production, and this damage can be restored using sodium hydrosulphide, a classical exogenous hydrogen sulphide donor, which decreases the expression of P2X7 and NLRP3. These data suggest that enhancing endogenous hydrogen sulphide production is a potential therapeutic option for managing post-intracerebral haemorrhage [77].

Stolp et al. [78] found that mice with IL-1 β -induced white matter damage expressed a significant reduction in

the number of parvalbumin-positive interneurons at 10 days. Girard et al. [79] demonstrated that the IL-1 β pro-inflammatory system is activated and matrix metalloproteinase 9 is induced in perinatal human brain injury. The expression of IL-1 receptors (IL-1R1 and IL-1R2) and IL-1RA as well as the induction of downstream effectors from 9 human brains with white matter damage were examined. IL-1 β overexpression was associated with IL-1R1 and IL-1R2 immunoreactivity in areas with white matter damage, and IL-1 receptor antagonist was significantly less upregulated than that of IL-1 β . This imbalance in the expression of IL-1 β versus IL-1 receptor antagonist was especially pronounced in the damaged brains of very pre-term compared to near term infants; they also noted the overexpression of matrix metalloproteinase 9 co-localised with IL-1 β in affected cerebral areas [79].

Ystgaard et al. [80] showed in a newborn mice model that hypoxic-ischaemic insult initiated an inflammatory response including an approximately 2-fold upregulation of NLRP3 in astrocytes in the hippocampus, thalamus, and striatum at 24 h. An upregulation could be detected in astrocytes within 3 h after hypoxia-ischaemia. By contrast, expression in microglia developed slower and was prominent 72 h after the insult. There was no reduced infarct size in NLRP3 knockout mice at 24 h. However, after 7 days, NLRP3-deficient mice had increased infarction volumes and more activated microglia compared to wild type, while ASC-deficient mice had smaller infarct size and less activated microglia compared to wild type. RNA sequencing in hippocampus showed an altered inflammatory transcriptional response and increased expression of genes involved in protein translation and genes that are positive regulators of so-called epithelial mesenchymal transition. NLRP3 deficiency therefore increases hypoxic-ischaemic brain injury in newborn mice, while ASC deficiency seems to be neuroprotective [80, 81]. Activation of NLRP3 therefore occurs following hypoxia-ischaemia in neonatal mice brain and its deficiency aggravates the injury not early but later in the course. By contrast, ASC deficiency is neuroprotective.

Respiratory Disease in Neonates and the Inflammasome

A number of studies have shown an association between increased IL-1 β and the development of BPD [82–86]. Indeed, Bry et al. [86] using a mouse with conditional expression of mature IL-1 β in a lung-specific manner showed that activation of the transgene in both perinatal

and postnatal periods resulted in downstream cytokine and growth factor elaboration, inflammation, and decreased alveolarization even in the absence of lung injury.

Several groups have used recombinant IL-1RA as a potential therapeutic agent in neonatal hyperoxia models of BPD [87, 88]. Studies by Liao et al. [89], in a neonatal mouse hyperoxia model of BPD, demonstrated NLRP3 inflammasome activation, with increased IL-1 β and abnormal lung alveolarization by postnatal day 15 in wild-type neonatal mice exposed to 85% oxygen from postnatal day 3 to postnatal day 14. In contrast NLRP3-deficient mice (*Nlrp3*^{-/-}) exposed to the same conditions did not exhibit the IL-1 β surge and had normal lung architecture. They also evaluated the response to recombinant IL-1RA, which blocks IL-1 interaction with its receptor, and glyburide, a compound that prevents the assembly of NLRP3 inflammasome, in hyperoxia-exposed WT mice, showing blockade of the inflammasome and preservation of lung architecture in comparison to placebo-treated mice [89].

In a preterm (equivalent to 28-week gestation in humans) baboon model of BPD, preterm baboons ventilated and exposed to oxygen had a significantly increased IL-1 β :IL-1 receptor antagonist ratio when compared with gestational age controls. Moreover, they showed that IL-1 β , IL-1 receptor antagonist, and the IL-1 β :IL-1 receptor antagonist ratio in tracheal aspirates obtained in the first 3 days after birth from human preterm infants with respiratory failure were independently predictive of adverse outcome at 36 weeks postmenstrual age [89]. Therefore, activation of the NLRP3 inflammasome is an important mechanism linked to the development of BPD and may be a potential therapeutic target.

Therapeutic Interventions

The neuroprotective role of IL-1 receptor antagonist has been explored in many animal models. Pang et al. [90] demonstrated a significant reduction in LPS-induced brain injury by administering IL-1 receptor antagonist to neonatal rats. This has also been confirmed by Savard et al. [14, 72]. Pups (at a developmental stage equivalent to full term human neonate) were pre-treated with LPS and then exposed to a hypoxic-ischaemic event, an early surge of neuronal IL-1 β production was evident, and the brain insult in this population was attenuated by IL-1 receptor antagonist. Girard et al. [91] also showed that the postnatal administration of IL-1 receptor antagonist can be protective against brain injury in animal model of prenatal inflammation and/or postnatal hypoxia and ischaemia.

However, in human infants, neonatal encephalopathy is heterogeneous with multiple aetiologies [92] including hypoxia-ischaemia. Most human studies are not divided by aetiology, making it difficult to stratify treatment based on aetiology, and this may affect immunomodulatory therapies [93, 94].

A number of other blockers of this pathway have been described. For instance, Chen et al. [95] reported that ginkgolide B, a component of *Ginkgo biloba* extract, prevents NLRP3 inflammasome activation in neonatal rats with hypoxic-ischaemic brain injury by a reduction in NLRP3 and caspase 1 expression in microglia, leading to reduced IL-1 β and IL-18 production in the neocortex. Pentoxifylline, dexamethasone, and azithromycin have also been suggested as treatment to prevent neonatal inflammation. All 3 inhibit inflammatory mediator cytokine production in whole blood from neonates in vitro regardless of the time of treatment [96, 97].

In mouse models of MWS and FCAS, Youm et al. [98] demonstrated inhibition of the NLRP3 inflammasome by β -hydroxybutyrate or ketogenic diet by interrupting potassium ion efflux as well as interfering with the assembly of the NLRP3 inflammasome components. Coll et al. [99] described the potential of MCC950 as a selective inhibitor of NLRP3 in a mouse model of multiple sclerosis, where MCC950 reduced IL-1 β production in vivo and delayed the onset and severity of experimental autoimmune encephalomyelitis. They also demonstrated in a mouse model of CAPS, the increased survival of NLRP3 mutant mice treated with MCC950 up to day 45.

The introduction of specific agents targeting the NLRP3 inflammasome and IL-1 β has a proven record in debilitating and previously lethal conditions such as NOMID/CINCA [55]. Anakinra commercially known as Kineret (inhibits IL-1 activity by binding to IL-1 receptor), rilonacept (dimeric fusion protein formed by portions of IL-1R and the IL-1R accessory protein linked to the Fc portion of immunoglobulin G1 that binds to IL-1), and canakinumab (neutralizing monoclonal anti-IL-1 β antibody) (Fig. 2) are FDA-approved IL-1 blockers that effectively reduce IL-1 activation in rheumatoid arthritis, osteoarthritis, and hereditary auto-inflammatory diseases such as hyperimmunoglobulinaemia D with periodic fever syndrome, familial Mediterranean fever, systemic juvenile idiopathic arthritis and adult-onset Still's disease, Schnitzler syndrome, and CAPS/NOMID with few side effect profiles [55, 100–102]. Bui et al. [103] showed the long-term protection after anakinra treatment in a BPD mouse model induced by antenatal LPS and postnatal hyperoxia of 65% oxygen. Mice were treated with dai-

Fig. 2. Interleukin-1 β (IL-1 β) antagonists: 3 molecules have been designed as IL-1 β antagonists. Anakinra, is a drug that competitively binds to IL-1 type I receptor blocking IL-1; canakinumab is monoclonal antibody that binds specifically to IL-1 β ; and rilonacept is a protein formed by portions of IL-1R and the IL-1R accessory protein linked to the Fc portion of immunoglobulin G1 that binds to IL-1, blocking its biologic activity.

ly injections of anakinra for 28 days and at 60 days of age, which represents ~ 20 years in human age, a well-developed distal vascular region was observed [103]. Showing the long effect of anakinra even after the treatment has been suspended. A follow-up of 10 patients with CINCA/NOMID for 26–42 months demonstrated a rapid control of systemic inflammation with variable efficacy with regard to central nervous system manifestations and growth parameters [51]. However, treatment must be initiated before lesion and bone deformation begins, anakinra does not reverse the damage [51, 104]. IL-1 antagonists are only used to treat signs and symptoms of inflammation associated with diseases such as rheumatoid arthritis and Still's disease, and they are not good enough on their own [105]. Side effects include nausea, vomiting, diarrhoea, stomach pain, headache, joint pain, and cold symptoms [106].

Conclusion

The NLRP3-IL-1 β pathway is an attractive target in the management of neonatal conditions, with potential diagnostic therapeutic and prognostic value. Modulating this intricate system can be pivotal in symptom resolution as well as alleviating end-organ damage. Further, the safety and efficacy of several agents are established in preclinical models, adult disorders, as well as some neonatal onset conditions such as CINCA/NOMID. Because of their in-

herent susceptibility to infection, broader utilization of these immunomodulatory interventions in a neonatal context requires careful consideration and further characterisation of the ontogeny of NLRP3-IL-1 β pathway in preterm and term neonates.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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