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Toll-like Receptors, crucial role in the progress of Necrotizing Enterocolitis

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Abstract: Necrotizing enterocolitis(NEC), the most common gastrointestinal disease and one of the major causes of high mortality and morbidity in premature infants, especially in very low and extra low birth weight premature infants, is a common difficulty seen in neonatal intensive care unit (NICU). As the immune system of neonates is immature, microbes infection becomes an important risk factor of NEC, but the detailed pathogenesis of NEC still remains ambigious. Toll-like receptors(TLRs), a group of pattern recognition receptors of innate immune system, play a crucial role in recognizing pathogen-associated molecular patterns (such as lipopolysaccharide, nucleic acid of microbes, etc) and danger-associated molecular patterns (such as self DNA released from damaged cells). In addition, the signaling pathways of TLRs could activate immune inflammation cascade reaction to break the balance between pro-inflammation and anti-inflammation in gastrointestinal tract. Moreover, they also regulate the activities of enterocytes including apoptosis, migration, autophagy and proliferation, which are partici-pated in the pathogenesis of necrotizing enterocolitis (NEC). In particularly, TLR4 as the important adaptor of intestine innate immune system is essential to the pathogenesis of this disease through the regulation of not only the production of inflammation cytokines but also various activities of intestine epithelia cells. In this review, we summarize recent studies on the role of TLRs, especially TLR4, in the intestine immunity and pathogenesis of NEC, in order to explore the novel approaches in preventing or delaying NEC progression.

Key words: Toll-like receptor, TLR4, Intestinal inflammation, intestine innate immune system, Necrotizing enterocolitis.

Introduction

With the development of the respiratory support level and intravenous nutrition technology, necrotizing entero-colitis (NEC) is regarded as one of the major severe di-seases causing high mortality and morbidity rates in pre-mature infants (1). NEC is histopathologically characte-rized by leukocyte infiltration, mucosal edema, ulceration, hemorrhages, mucosa and transmural necrosis, however, its pathogenesis, effective way of precaution and treat-ment are still unclear (2). Although the level of treatment is improved in NICU, nearly 40 percent of the patients re-quire intestinal resection with the almost 50 percent mor-tality and high morbidity of sequelae (1, 3). Currently, one of the important risk factors of NEC is microbes infection
(4). Microbes disruption could damage the intestine bar-rier and lead to clinical consequences (5). Therefore, as the components of innate immunity system, TLRs display pathogen-associated molecular patterns and danger-asso-ciated molecular patterns so that they play a crucial role in the NEC progress (6, 7). TLRs are expressed on both immunocytes (such as dendritic cells, T cells and B cells) and tissue cells (such as intestinal epithelial cells and intestinal endothelial cells) (8-11). TLRs, particularly TLR4, could not only activate downstream signal to pro-duce inflammation cytokines but also interfere several cell activities by binding to the ligands of TLRs. TLR4 signal is activated by binding with LPS, followed by the eleva-tion of several inflammatory cytokines expression (12). Moreover, TLR4 activation could reduce enterocytes pro-liferation, migration, and induce enterocytes apoptosis, leading to mucosal injury and mucosal repairment delay (13, 14). Here we explore the role of TLRs in the pathoge-nesis of NEC, and summarize recent developments of the activities of TLRs in NEC. In particular, we discuss the role of TLR4, in order to throw more light of the unders-tanding of the pathogenesis and etiology of this disease, to establish potential novel approaches to treatment.

Toll-like Receptors

Receptor-mediated signaling pathways are extensively studied in cell biology, and one of the main receptors in the immune system is the TLR (15-19). TLRs are evolutiona-rily conserved molecules which can sense pathogen-as-sociated molecular patterns (PAMPs) and danger-asso-ciated molecular patterns (DAMPs), so they serve a cru-cial role on innate immune system. Till now, 10 types of

Table 1. The PAMPs ligands of each TLRs in human.

TLRs type(s)	Distribution	Ligand(s)
TLR1, 2, 6, 10	Cell membrane (TLR2 also is expressed on the endosome)	Pam3Cys , MALP-2, LTA, PGN, and Zymosan
TLR3	Subcellular structure(e.g.endosomes and endoplasmic reticulum)	RNA of microbes
TLR4	Both the plasma membrane and endosome	lipopolysaccharide
TLR5	Cell membrane	flagellin
TLR7	Subcellular structure(mainly endoplasmic reticulum)	RNA of microbes
TLR8	Subcellular structure(mainly endoplasmic reticulum)	RNA of microbes
TLR9	endosome	unmethylated CpG ODN

TLRs: Toll-like receptors; PAMPs: pathogen-associated molecular patterns.

TLRs have been identified in human. Among them, TLR1, TLR5, TLR6, and TLR10 are membrane receptors that could sense the extracellular ligands. TLR3, TLR7, TLR8, TLR9 function on the subcellular structures, i.e.TLR9 locates on endosomes, and recognizes the nucleic acids derived from microbes and self-damaged cells (20, 21). TLR2 and TLR4 are expressed on both cell membrane and subcellular structures (22-25). TLR2 is regarded as the he-terdimer of TLR1 or TLR6, and TLR10 is identified as the precursor of TLR1/TLR6 (26). PAMPs that recognized by TLR1, TLR2, TLR6, TLR10 are the components of microbes cell walls and membranes, including bacterial lipoproteins such as Pam3Cys and MALP-2, lipoteichoic acid (LTA), Peptidoglycan (PGN), and Zymosan (20, 27). TLR3, TLR7, TLR8 could sense the RNA of microbes (28). TLR9 could recognize the unmethylated CpG ODN of bacteria genome while the ligand for TLR5 is flagel-lin (27). TLR4 is the receptor of lipopolysaccharide, the component of gram-negative bacteria cell wall (29). The detailed PAMPs ligands for different types of TLRs are listed in Table 1. Besides, TLRs recognize the DAMPs in-cluding proteins and peptides (TLR1, TLR2, TLR4, TLR7 and TLR8), fatty acids and lipoproteins, proteoglycans and glycosaminoglycans (TLR2 and TLR4) ,and nucleic acids and protein-nucleic acids complexes (TLR3, TLR7, TLR8and TLR9) (6, 28).

TLRs are composed of nearly 200 amino acids in their transmembrane domain called Toll/IL-1R (TIR) domain, which is significant for the downstream signaling (30-33). Once TLRs combine with their ligands, they could trigger a wide range of increase in immune cytokines and chemokines expression level (26, 34). Myeloid diffe-rentiation factor 88 (MyD88), as the molecular that TIR domain recruits, is important to induce cytokines release such as TNF-a and IL-12, and it could mediate most TLRs signaling (30, 35). MyD88 could recruit IRAK-4 then facilitate phosphorylation of IRAK-1. Association of IRAK-1 with TRAF6 activates transcription factors protein (AP)-1, nuclear factor-κB (NF-κB) and interferon response factor (IRF)-1, IRF-5, IRF-7 to induce several cytokines expression via activation of mitogen-activate protein kinases (MAPK) (20, 36, 37). On the other hand, though MyD88-dependent signal is important to TLRs in mediating several cytokines production, NF-κB could be activated in MyD88-deficient macrophages by TRIF/MyD88-independent pathway, suggesting that MyD88-in-dependent pathway is another pathway of TLRs (38). For example, TLR3 and TLR4 could mediate related inflam-mation cytokines release via TIR domain-containing adap-tor-inducing interferon-β (TRIF) and TRIF-related adap-tor molecule (TRAM) (36). TRIF is essential adaptor in MyD88-deficient pathway by recruitment of TRF6, RIP, IKK, TBK1 and IRF-3 to activate NF-κB, then leads to IFN-β and type I interferon expression(39). This pathway is associated with various cell activities, such as apoptosis and autophagy (40-43). (Figure 1)

TLRs, as the important adaptors of sensing pathogens, are highly expressed in hematopoietic cells such as neutrophils, macrophages, dendritic cells and lympho-cytes (44). TLRs are also expressed in various non-hema-topoietic cells including epithelial cells, endothelial cells and fibroblasts (45). Normally, the environment in uterus is sterile. The normal bacteria balance is not built in gas-trointestinal tract in neonates contributing to prone to in-testinal flora disturbance. Moreover, there are a variety of conditions to prematurity, such as hypoxia, infection, and prematurity with impaired host defense (20). Given these conditions, the intestinal epithelial barrier is vulnerable to

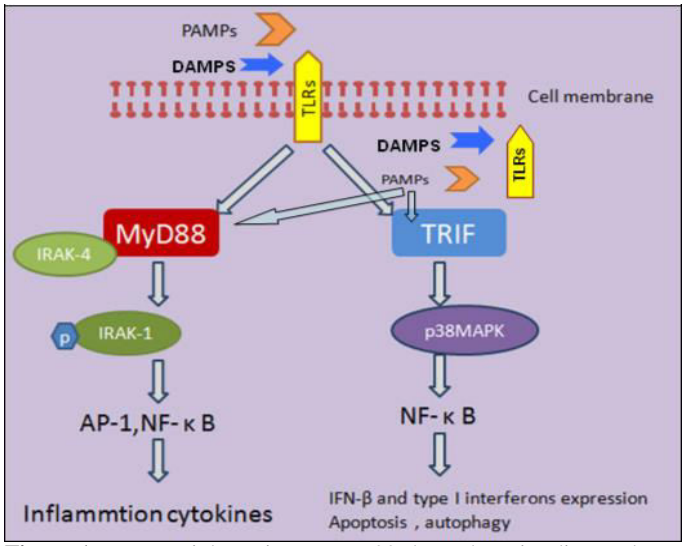

Figure 1. TLRs mainly activate MyD88-dependent signaling pathway and TRIF/MyD88-independent signaling pathway via binding with their ligands. MyD88 could recruit IRAK-4, then facilitate IRAK-1 phosphorylation and activate NF-κB and AP-1 to increase the expres-sion level of inflammation cytokines. TRIF pathway could activate p38MAPK and NF-κB to increase IFN-β, type I interferon expression, meanwhile, this pathway could participate in the cell apoptosis and au-tophagy.

damage, and the neonates, especially premature infants, are more susceptible to necrotizing enterocolitis. So TLRs as the important components of immune system that lo-cate on the intestinal epithelium cells are particularly im-port to the NEC development (46).

TLRs signaling in the pathogenesis of NEC

TLRs signaling pathway mediates the production of im-mune cytokines in NEC

It has been demonstrated that intestinal inflammation is associated with the host-microbial communication, inclu-ding sensing the production of microbes and the response of innate immunity to the microbes (47). Currently, it is widely accept that TLRs, especially TLR4, are heavily in-volved in the process of NEC in intestine. Haruki and his colleagues performed the experiment on the pre-suckling newborn swine and found that TLR2, TLR9 of the imma-ture gut-associated lymphoid tissues, including ileal Peyer patches and mesenteric lymph nodes, could promote the production of a variety of cytokines (48). Zymosan pro-motes IFN-γ, IL-12p35, IL-6, IL-10 and TGF-β gene trans-cripts in ileal Peyer patches, and induces IL-6 and TGF-β expression in mesenteric lymph nodes. CpG2006, the li-gands of TLR9, could increase the expression level of IL-6 and TGF-β in mesenteric lymph nodes, while it increases all tested cytokines production in ileal Peyer patches. Be-sides, it has also be demonstrated by Doyle that the li-gands of TLR2 could improve the ability of phagocytosis in human macrophage through the IL1 receptor-associated kinase 4 and MAPK (49). Different from the adults, TLR2 and TLR4 are overexpressed in the intestinal epithelium of neonates (50, 51). In neonatal rat model of NEC, the overexpression of TLR2 and phosphorylated NF-κB are associated with the increase of intestinal epithelial cells apoptosis and impaired cell proliferation (52). However, studies on C57BL/6 TLR2-deficient mice of intestinal ischemia model showed that the mucosal innate immune response is in disorder with the level of immune cytokines reduced and the intestine injury score increased. Moreo-ver, *Lactobacillus lactis* activated TLR2 pathway in the animal model of inflammatory colitis could be a protec-tive signaling pathway (53-55). All these results suggest the controversial and complicate role of TLRs in NEC.

It has been demonstrated by numerous studies that TLR4 exhibits a pathogenic role in the progress of NEC (55-57). TLR4 is expressed on the intestinal endothelial cells, intestinal epithelia and intestinal fibroblasts (58-61). TLR4 mutant and knockout mice could protect them-selves from the process of NEC (47, 62, 63). TLR4 me-diates phagocytosis of enterocytes and the translocation of Gram-negative bacteria (22). In TLR4 signaling, bin-ding of lipopolysaccharide (LPS) with CD14 molecular is sensed by the complex of TLR4 and MD2, then a lot of cytokines expression is enhanced, including TNF-a, IL-8 and IL-6 via the MyD88 (1, 58, 64, 65). In the pathogene-sis of NEC, platelet activating factor (PAF) can also acti-vate TLR4. *In vitro*, PAF could stimulate the TLR4 mRNA and IL-8 secretion in a dose-dependent manner, leading to intestinal injury of NEC. This phenomenon was blocked by the inhibitors of STAT3 and NF-κB (66). TLR4 activa-tion also leads to the downregulated expression of endo-thelial nitric oxide synthase(eNOS), which facilitates the nitric oxide production and affects the microcirculatory perfusion in intestine, resulting in the increased incidence of NEC (67). This inflammation injury caused by TLR4 plays a key role in the pathophysiology of the NEC.

TLRs signaling pathway regulates the Enterocyte apop-tosis and intestinal injury

Apoptosis, a programmed cell death pathway, is the initial injury of intestine enterocytes which leads to the loss of epithelial villi, break of epithelial barrier and the translocation of microbes or other antigens. This process could facilitate the development of NEC. TLR4 recently is regarded as a key role in promoting the apoptosis leading to the intestine injury in neonates (7, 47). Probably as the result of the ontogeny of TLR4 , the expression of TLR4 is higher in the gut in neonates compared with the adults. Once TLR4 signaling pathway is activated, the effects of the signaling are exaggerated. While TLR4 expression le-vels are quite low, the effects of the signaling are minimal. Given the difference in the expression level of TLR4 com-pared to adults, TLR4 may play the pathological role in the intestine of neonates (68, 69).

TLR4 and its downstream cytokine tumor necrosis fac-tor-a (TNF-a) could recruit Myeloid Differentiation Pri-mary Response gene (MyD88) or the Fas-associated death domain protein (FADD) to the death domain of the recep-tor, then promote the formation of the Death-Inducing Signaling Complex (DISC) (70-72). Then the complexes activate the caspase-3 and finally lead to the enterocyte apoptosis (73). TLR4 could also mediate the intestinal epithelial cells apoptosis by activating NF-κB (74). TLR4 is demonstrated both *in vivo* and in intestinal stem cells organoids to reduce the proliferation and promote apop-tosis of intestinal stem cells through the p53-up-regulated modulator of apoptosis (PUMA). However, in order to implement this function, the recruitment of TRIF, but not MyD88 and TNF-a, is required (75). Afrazi indentified that TLR4 promoted the apoptosis of Lgr5 (leucine-rich repeat-containing G-protein-coupled receptor 5)-positive ISCs by induction of endoplasmic reticulum stress, which leads to the NEC severity (76). It requires to activate protein kinase related PKR-like ER kinase (PERK), C/ EBP homologous protein (CHOP), and myeloid differen-tiation primary response gene 88 (MyD88). Inhibition of PERK, CHOP, and MyD88 could reduce the ISC apopto-sis and intestine injury.

TLR9 as the homologue of TLR4 plays an important role in the intestine inflammation. It could be activated by CpG-DNA and exists the crosstalk with TLR4 (29). Bacterial genome with the cytosin-guanosin dinucleotide (CpG) motifs could be sensed by intercellular TLR9, and leads to the activation of TLR9 signaling pathway, resul-ting in the production of several cytokines, such as TNF-α, IFN-c, IL-6, IL-10 and IL-12 (77, 78). Besides the activa-tion of inflammation cytokines, TLR9 is also indentified to reduce the degree of the intestinal enterocytes apoptosis and the intestinal inflammation (79). In the mice model of NEC, TLR9 knockout mice showed the more intense NEC process (69). A variety of studies *in vitro* and *in vivo* have demonstrated that the administration of CpG ODN could inhibit the inflammation and apoptosis promoted by TLR4 signal pathway, resulting in the decrease of intestinal in-jury and development of NEC. One of the mechanisms that TLR9 inhibits TLR4 signaling pathway is dependent

on the increased expression level of IL-1R-associated kinases-M (IRAK-M), which is a negative molecular of TLR4 signal. Another possible mechanism is related to the ontogeny specificity of TLR9. Different from TLR4, the level of TLR9 expression is decreased in the fetal and neonatal period (6, 69, 80). As the result of the crosstalk between TLR9 and TLR4, TLR9 plays a protective role in NEC. As the description of CpG ODN, the administration of CpG ODN could be a promising treatment strategy of NEC (81, 82).

Autophagy is a conserved pathway of cell self-degra-dation and metabolic energy recycling induced by several stress such as starvation. The marker of autophagy is the formation of the autophagosome which could be self-di-gested by reaction with lysosome hydrolases (82-84). The process of NEC requires the increase of autophagy induced of TLR4 signaling pathway. In the mice model of NEC, NEC did not developed automatically in the mice lacking the autophagy-related protein 7 gene, which is necessary to autophagy. Different from the prevailing recognition that autophagy acts a protective role in cells, autophagy is the cause of NEC through the negative re-gulation of enterocyte migration (85). Inhibition of auto-phagy could reduce the degree of experimental NEC. The adverse effect of autophagy in NEC may depend on the exaggerated activation of autophagy in the result of TLR4 overexpression in the neonates (86). Helicobacter hepati-cus, a gram negative bacteria, could increase the incidence of NEC from 39% to 71% and promote the expression of TLR4. Helicobacter hepaticus infection activates the TLR4 signaling pathway and increases the level of auto-phagy in intestinal epithelial cells with the overexpression of cytokines including CXCL1, IL-1, IL-12, and IL-23 in-duced by TLR4 (87). Given the causative role of TLR4-in-duced-autophagy in NEC, the insights into the molecular mechanism of autophagy in the disease may provide a no-vel approach for the future clinic therapy.

Role of TLR4 in the intestinal repair of NEC

The state of intestine injury and repairment should be balanced to maintain the normal homeostasis of gas-trointestinal environment. The generation of enterocytes from the intestinal stem cells and the consequent migra-tion of the healthy enterocytes could repair the intestinal barrier where the enterocytes apoptosis and necrosis can limit bacterial translocation (3, 44). It is demonstrated that TLR4 signaling reduces the enterocyte proliferation and migration in premature gastrointestinal tract leading to the injury of mucosal repairment (47, 75, 88). TLR4 adversely affects the ability of enterocyte proliferation via the phosphorylation of glycogen synthase kinase 3β, and plays a negative role on wnt-β-catenin pathway which re-gulates the cell division in intestine (63).

TLR4 plays an important role on the migration of en-terocytes through regulation of cell-matrix interactions and enterocyte adhesion (6). Lipopolysaccharide depends on the phosphatidylinositol 3-kinase pathway to increase the Ras homolog gene family member A(RhoA) and fo-cal adhesions expression, as the result, the ability of en-terocytes migration is decreased (61). HMGB1, released from the damaged enterocytes by TLR4 activation, could also active RhoA to inhibit the migration and affect the cell-matrix adhesiveness of enterocytes (89). Moreover, intestinal epithelium autophagy activated by TLR4 signa-ling could impair the enterocytes migration by activation of Rho-GTPase in enterocytes (6). As a consequence, the injury of the intestine repairment could break the balance of the intestine barrier and lead to the development of the NEC.

Negative Regulation Signal of TLR4 in intestine Considering that TLR4 signaling pathway promotes the process of NEC, host has evolved various effector mo-lecules to dampen the TLR4 signal response (44). As the crucial downstream effector of TLR4 pathway, NF-κB is the target of many negative regulation molecules. Toll-in-teracting protein (TOLLIP) could inhibit TLR2 and TLR4 by decrease the MyD88-mediated NF-κB activation(90). IL-1 receptor-associated kinase 1 single immunoglobulin IL-1R-related molecule (SIGIRR), MAPK phosphatase 1, peroxisome proliferator activated receptor-γ (PPARγ) secretory leukocyte peptidase inhibitor (SLPI) and A20 could also antagonize TLR4 effect by inhibiting NF-κB activation to reduce the inflammation cytokines release and reduce the degree of the inflammation injury in intes-tine (6, 89, 91-99). Besides, it is demonstrated that TLR9, the receptor of CpG ODN and nucleotide oligomerization domain 2 (NOD2), and receptor of bacterial component muramyl-di-peptide (MDP) could also negatively regu-late the exaggerated TLR4 signaling in NEC. As descri-bed above, the activation of TLR9 could inhibit TLR4 signaling and play a protective role in the pathogenesis of NEC. NOD2 could down-regulate the mitochondrial-de-rived proapoptosis protein, second mitochondria-derived activator of caspase (SMAC-diablo) (74). These nega-tive pathway of TLR4 may provide the effective therapy strategies to cure NEC, which would be described in de-tails in the following part.

Genetic polymorphisms of the key molecules in TLRs signaling pathway associated with NEC

Although NEC is the severe gastrointestinal disease in premature infants, it only happens in a minority of patients. This phenomenon suggests that there are some other factors participating in the individual susceptibility to NEC. Genetic polymorphisms have been regarded as a role associated with the susceptibility of this disease (100). As the studies on the TLRs signaling pathway ef-fect in NEC increase, more and more researchers explore the functional single nucleotide polymorphisms (SNP) of the key molecules in TLRs signaling pathway. This may provide a novel understanding of the susceptibility to the disease. Venkatesh explored the SNPs of TLR2, TLR4, TLR5, TLR9, IRAK1, TIRAP, NFκB1 and NFκBIA in a cohort of 271 very low birth weight infants, in which 15 infants suffered from NEC (101). The results showed that NFκB1(rs28362491) and NFκBIA(rs3138053) is si-gnificantly different between non-NEC and NEC infants. Szebeni and colleagues analyzed CD14 -260T, TLR4 +896G, +1196T, and caspase-recruitment domain (CARD)15+2722C, +2104T in 118 very low birth weight infants with 41 of those developed NEC (102). However, they did not find any association between any of the SNP site and NEC. It is accept that the functional gene polymorphisms of cytokines may affect the level of the cytokines secretion. Recently, the function of TNF-α , IL-1b , IL-4, IL-6, IL-

10, IL-18 polymorphisms have been analyzed (103-109). For example, IL-18 607AA genotype was higher in stage III of NEC, compared to stage II and I, and IL-4 recep-tor AA1902 was associated with the risk of NEC. Though other SNP sites of cytokines in these studies did not show the relation with the susceptibility and severity of NEC, it still needs multi-center clinical trial and meta-analysis to explore the key gene polymorphisms in the pathogenesis of NEC in order to predict the susceptibility and response to the individual treatment of NEC (110).

Treatment of NEC by targeting TLRs

Given the important role of TLRs in the development of NEC, a variety of studies have explored the preventive and therapeutic approaches on regulating the TLRs signa-ling pathway. In both infants and animal models, it exhi-bits that human milk could protect against NEC while the formula-feeding leads to the susceptibility of NEC (111, 112). In human milk, significant quantities of TLR2 and soluble CD14 (sCD14) participating in TLR4 signaling are found (113). It is suggested that TLR2 and sCD14 in the human milk may bind to the bacteria in the gastrointes-tinal tract in order to prevent the bacteria being sensed by their receptors in the intestine, leading to the protection against the microbes translocation (114).

Many stressors could cause the high expression of HSP70 , a member of heat shock protein family (115). However, HSP70 leads to the ubiquitination of TLR4 which results in the degradation of TLR4 via the ubiqui-tin dependent pathway. As the result of this process, NEC severity is down-regulated (116). Furthermore, recent stu-dy showed that glutamine can protect against the intestine injury through induction of HSP70 and decrease the level of TLR4 (117).

Polyunsaturated fatty acid (PUFA) is demonstrated to induce the development of NEC through the suppression of TLR 4 and platelet-activating factor receptor (PAFR) gene expression in epithelial cells, which play an impor-tant role in the pathogenesis of NEC. Administration of both AA and DHA could block PAF-induced TLR4 activa-tion and reduce PAFR mRNA expression in enterocytes. It is suggested that PUFA could regulate the key molecules expression to decrease the process of NEC (33).

Probiotics treatment is a focus on prevention of NEC. The mechanism of probiotics treatment is still uncovered. A probable mechanism may be that the administration of probiotics could increase CpG ODN and MDP, which res-pectively activate TLR9 and NOD2 to limit the function of TLR4 signaling pathway (118). Moreover, probiotic conditioned media exposure can significantly attenuate LPS and IL-1β-induced IL-8 and IL-6 expression, level of TLR2 mRNA and TLR4 mRNA, and increase mRNA levels of SIGIRR and TOLLIP (119). Khailova and the colleagues have demonstrated that administration of Bi-fidobacterium bifidum significantly reduced apoptosis via facilitating the expression of COX-2 and production of PGE(2) in the ileum to protect against mucosal injury and preserves intestinal integrity (120). Moreover, Bifido-bacterium bifidum can reduce apoptosis in the intestinal epithelium in NEC. Strains of Lactobacillus reuteri can si-gnificantly increase the survival rate and decrease the inci-dence and severity of NEC by down-regulation of mRNA expression of IL-6, TNF-α, TLR4, and NF-κB with the up-regulation of anti-inflammatory cytokine IL-10. These results support the idea that administration of probiotics may provide a valuable treatment to prevent NEC (121).

IL-10 is an anti-inflammatory cytokine, which can reduce the degree of the inflammatory response. Bovine IL-10 could inhibit TLRs activation in monocytes, and LPS-induced activation of monocyte-derived DCs. The-refore, Bovine IL-10-containing dairy products such as infant foods and immunomodulatory diets may be poten-tially used to prevent NEC (122) .

Summary and Future Directions

NEC is a severe gastrointestinal disease that cause the high morbidity and mortality in neonates, especially in preterm infants. Although a variety of NEC therapies have been assessed in experiment and human trails, there are no effective prevention or cure strategies of this disease since the pathogenesis of NEC is not well understood. Thus, it is essential to explore the detailed mechanism for novel targets on the therapy of NEC. In the considera-tion of the prematurity of intestinal barrier defense to the microbes, the immune system plays an important role on the process of the disease. TLRs, as the key components of the innate immune system, are pioneer to activate the immune response through sensing the pathogen antigen and facilitating the downstream signaling pathway. It is demonstrated that TLR4 signaling pathway is necessary in the development of NEC. It not only promotes the expres-sion of inflammation cytokines but also participates in the activity of the enterocytes. Different from the intestine of adults, TLR4 expression is highly expressed and the role of TLR4 pathway is also specific important in the neo-nates gastrointestinal tract. TLR4 promotes the expression of cytokines via the MyD88-dependent pathway through the activation of transcription factor NF-κB. Another downstream pathway of TLR4 is MyD88-indepent/TRIF signaling pathway, which could recruit TRF6, RIP, IKK, TBK1 and IRF-3 to activate NF-κB, then leads to IFN-β and type I interferon expression. TRIF signaling pathway directly regulates apoptosis and autophagy in enterocytes, as a result, the migration and proliferation of enterocytes is negatively affected, leading to increased mucosa injury and decreased intestine repairment.

In summary, the role of TLRs in the progress of NEC is extensively studied and there is bright prospect for TLRs-based NEC therapies. Further improvement for the speci-fic TLR targeting in NEC will not only benefit the basic scientific research to deeply uncover the pathogenesis of the disease, but also direct the individual therapy. Howe-ver, it is still needed to explore the effective approaches of predicting the therapeutic effect of different patients and early discovering the susceptible factors, in order to re-duce the mortality and morbidity of NEC.

Conflict of interest

The authors declare that they have no conflict of interests.

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