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Inflammatory signaling in necrotizing enterocolitis

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Introduction

The pathogenesis of necrotizing enterocolitis (NEC) remains poorly understood. Many factors potentially predispose the premature intestine to injury: 1) A lack of adequate substrate and O₂ delivery to the intestinal epithelial cells, due to an incomplete microvasculature development or to an immature regulation of the intestinal vascular tone; 2) an inadequate intestinal barrier; 3) an inflammatory response triggered by abnormal bacterial colonization; and 4) an immature immune response, leading to inefficient killing of microbes which then translocate through the epithelium (Fig. 1). At the same time, an excessive production of inflammatory mediators leads to the recruitment of neutrophils and subsequent tissue injury and necrosis.

The pathogenesis of NEC is complex and its speed of progression is quite variable. In an attempt to gain understanding of the disease, researchers have examined tissues resected from patients with NEC. However, as these are obtained at late stages of the disease, they do not yield clues about the early pathogenic events leading to NEC. Therefore animal models have been used and have helped to identify a role for several mediators of the inflammatory network in NEC. In this chapter, we discuss the evidence for the role of these inflammatory mediators and conclude with a current unifying hypothesis regarding NEC pathogenesis.

Bacterial – Lipopolysaccharide

During the birth process, the newborn intestine is exposed to the many microbes from the environment and colonization occurs. In term infants, the intestine is colonized with microbes derived from the maternal birth canal and the environment. Exposure to breast milk induces the development of a rich balanced microflora and the growth of *bifidobacteria*, which have many protective properties for the neonatal gut. Compared with full term infants, the colonization process in preterm infants is altered as the growth of fewer

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but more aggressive bacteria¹ is facilitated by the lack of breast milk, the use of antibiotics and anti-acids² and the NICU environment. This flora contains more “pro-inflammatory” bacteria and less commensals, which are known to induce genes that promote the epithelial barrier, digesting enzymes and angiogenesis³. Bacterial overgrowth is further facilitated by the immaturities of intestinal motility and digestion, the intestinal barrier and innate immunity. There is evidence that bacteria play a role in acute bowel injury in animal models. For example, germ-free rats were protected against injury in a model of acute bowel injury induced by PAF⁴. In humans, an abnormal intestinal microflora skewed toward gram-negative bacteria has been found to be associated with the early stage of NEC⁵. Gram-negative bacteria contain lipopolysaccharides (LPS or endotoxin) on their outer membranes. LPS is a potent activator of the host immune response and may play a central role in NEC. LPS, when injected to rats and mice, produces intestinal injury and shock⁶. Increased serum LPS has been found in patients with NEC⁷. LPS is a potent activator of the transcription factor nuclear factor- κ B (NF- κ B)⁸ and stimulates the production of many cytokines, including IL1, IL6, chemokines, TNF⁹ and PAF¹⁰, which all amplify the inflammatory response.

Cytokines – Inflammation

The intestinal epithelial cells (IECs) are in contact with bacteria and their products. The bacterial-IEC interaction may be facilitated in the premature intestine by bacterial overgrowth and by an immature mucous layer. Components of the bacteria (called microbial-associated molecular patterns (MAMPs)) interact with Toll-like receptors (TLRs) on the IEC and on the submucosal inflammatory cells. Once activated, TLRs elicit the activation of several signal transduction pathways including the inhibitor of NF- κ B (I κ B) kinase (IKK)¹¹, a critical upstream kinase that activates NF- κ B, a major regulator of inflammation. This leads to the production of many inflammatory mediators, including cytokines and chemokines, which lead to immune cell recruitment, especially neutrophils, and to intestinal inflammation.

There are several pieces of evidence which suggest a role for excessive inflammation in NEC: 1) In patients with the disease, several cytokines have been found to be increased in the plasma and intestinal tissue^{12, 13}; 2) The incidence of NEC is prevented by prenatal use of glucocorticoids¹⁴, which are known potent inhibitors of the inflammatory response; 3) The production of IL-8 by enterocytes is developmentally regulated¹⁵; and 4) Blocking the activation of NF- κ B in a neonatal rat model protects against NEC¹⁶.

NF- κ B

NF- κ B is a transcription factor that regulates the expression of many pro-inflammatory cytokines, chemokines and leukocyte adhesion molecules^{17, 18}. It consists of five subunits (p50, p65, p52, cRel and RelB) that homo- or heterodimerize to form active NF- κ B^{17, 18}. The dimers of NF- κ B mostly found in intestinal tissues are p50-p50 and p50-p65^{19, 20}. NF- κ B is constitutively present in the cytoplasm of most cells, bound to inhibitory proteins I κ Bs (Fig. 2). Following stimulation, I κ B is phosphorylated by the upstream IKK complex¹⁷. This complex consists of two catalytic subunits, IKK α and IKK β and a regulatory component, NEMO (NF- κ B essential modulator)²¹. Phosphorylation of I κ B by the IKK complex leads to its ubiquitination and subsequent degradation by the 26S proteasome, leaving NF- κ B free to translocate to the nucleus and regulate the gene expression of many inflammatory mediators^{17, 21}. Our laboratory has shown that NF- κ B is constitutively present at low levels in adult rat intestine, and is activated in PAF-induced acute bowel injury¹⁹. We also found that, in fetal rat intestines, the constitutive NF- κ B activation appears at 20 days of gestation (length of gestation: 21 days)¹⁶.

NF- κ B has multiple functions in the intestine, some protective, other potentially detrimental. Several interventions known to decrease NEC such as breastfeeding and use of probiotics attenuate IEC NF- κ B activation^{22, 23}. Breast milk induces the production of I κ B α in IECs²² and probiotics inhibit NF- κ B activation through the proteasome²³. Mice with deletion of IKK β in IECs are protected against systemic inflammation and multiple organ dysfunction syndromes following intestinal ischemia-reperfusion²⁴. However, in several *in vivo* models, the activation of IKK β and NF- κ B in IECs has been shown to be protective and to limit intestinal mucosal damage^{24–26}. Therefore, NF- κ B in IECs might have both protective and detrimental properties in the intestine.

Previous work done in animal models by our laboratory and others suggests a role for NF- κ B in NEC. In a neonatal rat NEC model, increased NEC severity (by histology) correlated with increased IEC staining for TLR-2 and activated NF- κ B as well as increased IEC apoptosis and impaired IEC proliferation²⁷. We have shown that NF- κ B is persistently activated in the intestine in a neonatal rat model of NEC¹⁶ and neonatal rats treated with a specific NF- κ B inhibitory peptide but not a control peptide had decreased mortality and bowel injury¹⁶, suggesting a central role for NF- κ B in NEC. This same NF- κ B inhibitory peptide inhibits LPS-induced chemokine CXCL2 (or MIP-2) gene expression in IECs²⁸ and inhibits LPS-induced interleukin-1 β (IL-1 β), IL-6 and TNF-alpha gene expression in macrophages *in vitro* (J774.1)²⁹.

There is evidence that NF- κ B is developmentally regulated with higher activation and cytokine production in immature IECs¹⁵ and inflammatory cells^{30, 31}. Intestinal NF- κ B is strongly activated at birth, and is downregulated within a day in dam fed newborn rats¹⁶. In contrast, NF- κ B remains strongly activated at both day 1 (D1) and D2 in stressed animals and this is accompanied by a significant decrease in the levels of the endogenous NF- κ B inhibitory proteins I κ B α and I κ B β at D2¹⁶. Immature enterocytes expressed lower levels of specific I κ B genes compared with mature enterocytes¹⁵ and had a higher IL-8 response to bacterial infection¹⁵.

While activation of NF- κ B is an essential component of host immunity against pathogens³², in premature infants, a marked and prolonged NF- κ B activation may contribute to intestinal tissue injury¹⁶.

TLR

TLRs are receptors for bacterial products (also called microbial-associated molecular patterns (MAMPs)), and are found on many cells, including IECs and inflammatory cells. 10 different TLRs have been identified to date in humans. In the intestine, when MAMPs, generally from commensal bacteria, interact with TLRs located on the intestinal epithelium, epithelial cell proliferation, IgA production, integrity of tight junctions and antimicrobial peptide production are promoted, all of which help maintaining a healthy intestinal barrier³³. However, the interaction of MAMPs with TLRs on underlying lamina propria immune cells³³ can trigger a pro-inflammatory response. The location of these interactions may influence the response: Apical exposure of IECs with CpG-DNA results in inhibition of NF- κ B activation, while basolateral exposure leads to activation of NF- κ B³⁴. This suggests that invasive bacteria that can penetrate the epithelial barrier elicit a pro-inflammatory response at the basolateral site, while bacteria that cannot cross the barrier, generally non-pathogenic bacteria, remaining on the apical site, elicit a homeostatic, anti-inflammatory response³³.

Murine and human NEC have been associated with increased intestinal TLR2 and TLR4³⁵ and decreased TLR9 expression³⁶.

TLR4 is the receptor for endotoxin. While a study found that TLR4 was protective in ischemia/reperfusion injury in neonatal mice³⁷, there are many pieces of evidence that suggest that TLR4 has an injurious role in NEC 1) Intestinal TLR4 gene expression is increased in animals exposed to formula feeding and cold asphyxia stress during experimental NEC, while it is normally downregulated in dam fed animals during the first 72 hours of life³⁸; and 2) TLR4-deficient mice are protected against NEC^{38,39}. TLR4 activation in IECs has been found to delay mucosal repair by inducing HMGB1 signaling which increases stress fibers and focal adhesions⁴⁰ and reduce enterocyte proliferation by inhibiting beta-catenin signaling⁴¹.

While the activation of TLR4 contributes to NEC, TLR9, a cell receptor for unmethylated CpG dinucleotides originated from bacterial DNA, has been shown to be protective. Indeed, TLR9-deficient mice exhibited increased NEC severity³⁶ and the activation of TLR9 by its ligand CpG-DNA inhibits LPS-mediated TLR4 signaling in enterocytes and reduces NEC severity³⁶.

IFN gamma

Interferon gamma (or type II interferon)(IFN γ) is a cytokine produced mainly by T cells and natural killer cells, but also by B cells, NK-T cells, dendritic cells and macrophages⁴². IFN γ is produced by macrophages early during infection. Its synthesis is induced in response to IL 12 and IL 18 and is inhibited by IL-4, IL-10, TGF β and glucocorticoids⁴². IFN γ activates several signaling pathways, including STAT1, PI-3kinase/Akt and MAPKs which regulates the transcription of over 500 genes in the cell that modulate several cellular functions, such as apoptosis, proliferation, leukocyte migration and epithelial permeability⁴².

A role for IFN γ in NEC has been suggested by the following evidence: A trend toward an increase in IFN γ in ileal tissues has been shown in experimental NEC⁴³. IFN γ knock-out mice are protected against NEC and show increased epithelial cell restitution compared to wild-type controls when exposed to the NEC model⁴⁴. IFN γ has been shown to inhibit enterocyte migration by reversibly displacing connexin43 from lipid rafts⁴⁵.

IL-6

IL-6 is a cytokine produced by macrophages, T cells and endothelial cells under the control of NF- κ B. IL-6 triggers the production of acute phase proteins in the liver, B cell proliferation and antibody production. IL-6 levels have been found to be elevated in the plasma and the stools of patients with NEC⁴⁶ and correlated with the severity of disease¹².

IL-8

IL-8 is a chemokine produced by macrophages, endothelial cells and epithelial cells. IL-8 is a potent chemoattractant for neutrophils and an angiogenic factor. IL-8 has been shown to be elevated in the plasma of infants with NEC⁴⁷ and to correlate with disease severity⁴⁷. Fetal enterocytes have upregulated IL-8 gene expression compared to mature enterocytes¹⁵ which might contribute to the susceptibility of the premature intestine to inflammation.

IL-10

IL-10 is a cytokine secreted by Th2-cells that inhibits cytokine production in macrophages and other antigen presenting cells (APCs)⁴⁸. IL-10 modulates both innate and adaptive immune responses⁴⁸ and is a major anti-inflammatory cytokine in the intestine. In humans, IL-10 has been found to be increased in infants with severe NEC⁴⁷, which could be a compensatory mechanism to attenuate the intestinal inflammatory response. A role for IL10

in NEC is suggested by the following work: IL10-deficient mice are predisposed to inflammatory colitis⁴⁹. The production of IL-10 by stimulated blood mononuclear leukocytes is diminished in the premature infant compared to term infant⁵⁰ and may increase the susceptibility of the premature infant to inflammation. IL-10 levels were more frequently not detectable in the breast milk of premature infants that developed NEC compared to those that did not⁵¹. In a mouse model of NEC, IL-10 has been shown to be protective against NEC by attenuating the degree of intestinal inflammation, epithelial apoptosis, decreased junctional adhesion molecule-1 localization, and increased intestinal inducible nitric oxide synthase expression⁵².

IL-12

IL-12 is a cytokine released by macrophages, neutrophils, B cells and dendritic cells in response to bacteria, viruses and their products. It induces IFN γ and activates Th1 cells and macrophages. While one study found that IL-12 is downregulated in NEC⁴³, another has shown that IL-12 is upregulated in the ileum of neonatal rats with NEC which correlates with the progression of the tissue damage⁵³.

IL-18

IL-18, a pro-inflammatory cytokine released by macrophages, dendritic cells and IECs, induces the production of IL-1 β , IL-8, TNF-alpha and IFN γ by inflammatory cells. Several pieces of evidence suggest an involvement of IL-18 in NEC: 1) A polymorphism of IL18 has been associated with NEC⁵⁴; 2) IL18 is upregulated in the ileum of neonatal rats with NEC^{53, 55}; 3) IL-18 knock-out mice have been shown to have decreased incidence of NEC⁵⁶. They were also found to have higher levels of IkappaB-alpha and IkappaB-beta, suggesting less NF- κ B activation⁵⁶. They were also shown to have fewer ileal macrophages⁵⁶.

TNF-alpha

TNF-alpha has been shown to mediate inflammatory bowel disease in adults⁵⁷. While TNF-alpha levels have not been found to be consistently increased in the plasma of infants with NEC^{12, 46, 58}, TNF-alpha protein has been found to be increased in resected NEC intestinal tissues⁵⁹. When examined by *in situ* hybridization, tissue obtained from patients with NEC had a marked increase in TNF-alpha mRNA in Paneth cells, as well as in infiltrating eosinophils and macrophages⁶⁰. TNF-alpha has been found to be increased in a model of bowel injury in neonatal rats induced by hypoxia/reoxygenation⁶¹ but not in a neonatal rat NEC model⁴³. Our laboratory also did not find any increase in intestinal TNF-alpha in the neonatal rat NEC model (unpublished data). Two independent studies found that anti-TNF-alpha antibodies improved the intestinal injury in a neonatal rat NEC model^{62, 63}. Also, pentoxifylline, a drug with many effects including TNF-alpha inhibition has been shown to decrease the incidence of NEC in neonatal rats⁶⁴. However, our lab did not find any protective effect of anti-TNF in both a model of acute bowel injury induced by PAF and in a neonatal rat NEC model where we compared rats treated with anti-TNF antibodies versus control immunoglobulin (unpublished data). TNF-alpha has been shown to cause a marked loss of mucous-containing goblet cells in immature mice⁶⁵. TNF-alpha causes apoptosis in IECs via production of mitochondrial ROS (reactive oxygen species) and activation of the JNK/p38 signaling pathway⁵⁹.

PAF

Platelet-activating factor (PAF) is an endogenous phospholipid mediator released by many cells, including neutrophils, mast cells, eosinophils, macrophages, platelets, endothelial cells

and bacteria, including *E. coli*^{66–68}. PAF binds to a G protein-coupled receptor, PAF-R, preferentially expressed in the ileum, but also abundant in the jejunum and the spleen⁶⁹. PAF-R is present in many cells, e.g. neutrophils, macrophages and epithelial cells^{69, 70}. Activation of PAF-R causes a prolonged effect *in vivo* by the activation of a downstream cascade (e.g. activation of NF- κ B¹⁹ and of PI3kinase/Akt⁷¹), and the production of endogenous PAF (by phospholipase A2 (PLA2))⁷², oxygen radicals by xanthine oxidase (a major oxidant producing enzyme in the small intestine)⁷³ and TNF- α ⁷⁴. *In vivo*, free PAF is rapidly degraded by its degrading enzyme, PAF-acetylhydrolase (PAF-AH). PAF has been shown to play an important role in NEC: 1) PAF-receptor antagonists prevent experimental NEC in a neonatal rat model⁷⁵ and in a piglet model⁷⁶, and PAF-AH prevents experimental NEC in rats⁷⁷; 2) Infants with NEC have elevated circulating PAF⁷⁸ and decreased amounts of plasma PAF-acetylhydrolase⁷⁹, compared to age-matched controls; 3) PAF-AH is present in human milk⁸⁰; 4) intravenous infusion of recombinant PAF-AH prevents NEC in rats⁸¹, and PAF-AH knock-out mice are more susceptible to NEC, although their survival is higher⁸²; and 5) the ileum, the site of predilection of NEC, has the highest amounts of PAF-R⁶⁹. PAF also mediates the intestinal injury induced by hypoxia/reperfusion⁸³, TNF- α ⁸⁴ and LPS⁸⁵. Exogenous administration of PAF induces systemic hypotension, increased vascular permeability, hemoconcentration, and a dose-dependent isolated bowel necrosis⁸⁶ which predominates in the small intestine (frequently the ileum) as seen in NEC. Therefore, PAF administration has been used as a model of acute bowel necrosis and NEC⁸⁶. LPS administration potentiates PAF-induced bowel injury⁶ and PAF mediates LPS⁸⁷ and hypoxia-induced bowel necrosis⁸⁷. When injected intravenously, even at a dose below that which causes bowel necrosis, PAF activates NF- κ B very rapidly (within 5 minutes, peaking at 30 minutes)¹⁹ and induces the release of leukotrienes C4⁸⁸, the gene expression of PLA2⁷², TNF- α ⁷⁴, NF- κ B p50 precursor p105⁸⁹, PAF-R⁶⁹ and TLR4 in IECs⁹⁰. PAF is an important mediator of the allergic and inflammatory response and causes systemic and mesenteric vasodilation, increased permeability, platelet aggregation and neutrophil aggregation⁹¹.

NO

Nitric oxide (NO) is a free radical that regulates numerous physiological as well as pathological processes in the gastrointestinal tract. Small amounts of endogenous NO are constitutively produced in the intestine, mainly via the 2 constitutive isoforms of the enzyme NO synthase: endothelial NOS (eNOS) and neuronal NOS (nNOS). Several studies have found that eNOS is protective against intestinal inflammation in a DSS colitis model^{92, 93}. However, using the same model, other investigators showed that eNOS and iNOS were detrimental while nNOS was beneficial⁹⁴. In normal rat small intestine, nNOS was found to suppress the gene expression of iNOS through NF- κ B down-regulation⁹⁵. nNOS suppression led to I κ B α degradation, NF- κ B activation, and iNOS expression⁹⁵. In neonatal rats, formula feeding downregulates eNOS and nNOS, while it upregulates iNOS⁹⁶ and in piglets, it downregulates eNOS⁹⁷. iNOS is upregulated in a neonatal rat NEC model⁴³. Intravenous infusion of L-arginine, a NO synthase substrate, attenuates intestinal injury in this neonatal piglet model of NEC while L-NAME, a NO synthase inhibitor, worsened the injury⁹⁸. NO released by activated macrophages has been found to inhibit enterocyte migration⁹⁹. The localized production of nitric oxide by villus enterocytes results in an increase in enterocyte apoptosis¹⁰⁰ and impaired proliferation¹⁰¹. NO mediates dendritic cell apoptosis¹⁰², while protecting against apoptosis in other cell types¹⁰³.

ROS

Reactive oxygen species (ROS) are likely the final effector of PAF and many other cytokines. In the intestine, one of the main producers of ROS is the xanthine oxidase/

dehydrogenase system. This enzyme is first synthesized as xanthine dehydrogenase, which catalyzes the transformation of xanthine into uric acid ($\text{Xanthine} + \text{H}_2\text{O} + \text{NAD} \rightarrow \text{Uric acid} + \text{NADH} + \text{H}^+$). Xanthine dehydrogenase is constitutively and abundantly expressed in the intestinal villous epithelium¹⁰⁴. However, during ischemia, xanthine dehydrogenase is converted into xanthine oxidase, which not only catalyzes the transformation of xanthine into uric acid, but also leads to the production of superoxide ($\text{Xanthine} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{Uric acid} + 2\text{O}_2^- + 2\text{H}^+$). During oxidative stress, ROS leads to activation of the intestinal mitochondrial apoptotic signaling pathway¹⁰⁵ and IEC apoptosis via the activation of intestinal p38 MAPK¹⁰⁶. In experimental NEC, intestinal ischemia has been shown to be associated with a shift from NO^* to O_2^* production in a NOS-dependent manner¹⁰⁷. Xanthine oxidase and superoxide have been shown to play a central role in intestinal reperfusion injury¹⁰⁸. A central role for XO and ROS in causing intestinal injury is further supported by the protective role of allopurinol, a xanthine oxidase inhibitor, on PAF-induced bowel necrosis⁷³.

Neutrophils/Macrophages

Neutrophils are thought to play an important role in NEC. Neutrophils have been shown to mediate PAF-induced bowel injury, hypotension, hemoconcentration¹⁰⁹ and NF- κ B activation¹⁹. Mice deficient in P-selectin¹¹⁰ and mice treated with antibody against beta-2 integrin¹¹¹ (adhesion molecules necessary for neutrophils to roll and adhere to the endothelium) are protected against PAF-induced bowel injury. Our laboratory has found that treatment of mice with antibodies against CXCL2, a major chemokine for neutrophils, attenuated the systemic inflammation, the hypotension and the acute intestinal injury induced by PAF¹¹². However, in studies using live bacteria to induce NEC, e.g., NEC induced by *Cronobacter sakazakii* infection, depletion of PMN and macrophages from the lamina propria impeded bacterial killing, decreased *C. sakazaki* clearance and exacerbated cytokine production and bowel injury¹¹³. In this same model, *C. sakazakii* infection was shown to result in epithelial damage by recruiting dendritic cells (DCs) into the gut¹¹⁴. The interaction of DC with IECs led to increased TGF- β production, iNOS production, apoptosis and epithelial cell damage¹¹⁴. The role of macrophages has been recently explored by other investigators who have found that prematurity is associated with a hyper-inflammatory intestinal macrophage phenotype that leads to increased bowel injury¹¹⁵. They showed that, during pregnancy, intestinal macrophages progressively acquire a non-inflammatory profile. They found that TGF- β (2) isoform, suppresses macrophage inflammatory responses in the developing intestine and protects against inflammatory mucosal injury¹¹⁵. Activated macrophages have been shown to block IEC restitution by inhibiting enterocyte gap junction via the release of nitric oxide⁹⁹.

Systemic inflammation

In NEC, the inflammatory response is not limited to the intestine, and there is evidence that the liver might play an important role in amplifying the inflammation¹¹⁶. Hepatic IL-18 and TNF- α and the number of Kupffer cells (KC) were found to be increased in experimental NEC and to correlate with the progression of the intestinal damage¹¹⁶. TNF- α levels found in the intestinal lumen of rats with NEC were significantly decreased when KC were inhibited with gadolinium chloride¹¹⁶.

SUMMARY - Current Hypothesis (Fig. 3)

The pathogenesis of NEC remains poorly understood. The current but limited understanding of NEC pathogenesis is that it results from a local intestinal inflammation initiated by perinatal stress. Following the introduction of feedings, there is proliferation of intestinal bacteria, favored by the immaturity of the neonatal mucosal immune system. Intestinal

bacteria and their products adhere to the epithelium, breach the immature and fragile intestinal mucosal barrier and activate NF- κ B in lamina propria immunocytes, causing them to secrete pro-inflammatory mediators, chemokines (CXCL2), cytokines (TNF, IL), prostanoids, platelet-activating factor, and nitric oxide. (We hypothesized that the bowel injury in NEC results from inappropriately elevated and prolonged NF- κ B activity in inflammatory cells). These inflammatory agents attract further inflammatory cells, in particular neutrophils, induce the production of reactive oxygen species, and inflict further damage to the intestinal barrier resulting in increased bacterial translocation, intestinal epithelial damage, impaired epithelial cell restitution, apoptosis and mucosal necrosis. Thus, a vicious cycle characteristic of severe NEC is created by bacterial invasion, immune activation, uncontrolled inflammation with production of reactive oxygen and nitrogen species, vasoconstriction followed by ischemia-reperfusion injury, gut barrier failure, intestinal necrosis, sepsis and shock.

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Key points

- The pathogenesis of NEC remains poorly defined and likely due to a complex mechanism.
- The production of many inflammatory mediators is developmentally regulated in the intestine.
- Immaturities of several pathways that regulate inflammation may predispose the premature infants to inflammation.
- Excessive inflammation may play an important role in NEC.

Fig. 1.
Schematic representation of the risk factors of the premature intestine to injury.

Fig. 2.
Signaling events leading to NF-κB activation

Fig. 3.
Pathogenesis of NEC-Current hypothesis.