Necrotising enterocolitis

Patricia W Lin, Barbara J Stoll

Necrotising enterocolitis is one of the most common gastrointestinal emergencies in newborn infants. Here we review the epidemiology, clinical presentation, and pathophysiology of the disease, as well as strategies for diagnosis, management, and prevention. Necrotising enterocolitis is one of the most devastating and unpredictable diseases affecting premature infants. Despite decades of research, its pathogenesis remains unclear; diagnosis can be difficult; and treatment is challenging. We will need to improve our understanding of intestinal defences in premature infants, dietary and bacterial factors, and genetic effects that could predispose infants to necrotising enterocolitis before we can develop new strategies for prevention and treatment.

Lancet 2006; 368: 1271–83

Division of Neonatal-Perinatal Medicine, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322, USA (P W Lin MD, Prof B J Stoll MD)

Correspondence to:
Dr Patricia W Lin
patricia_lin@oz.ped.emory.edu

Introduction

Necrotising enterocolitis is one of the most common gastrointestinal emergencies in newborn infants. First described over a century ago, this disease remains an enigma. Pathogenesis is still unproven; treatment is difficult and often proves inadequate; and no effective prevention strategy has been agreed. The disease is especially poignant because it mainly affects premature infants who have survived the early neonatal period and subsequently face a disease with high morbidity and mortality. We also note that spontaneous intestinal perforation has become an increasingly common gastrointestinal emergency in preterm infants. Although the emergence of this disorder is of concern, it probably represents a separate disease entity that is beyond the scope of this Seminar.

Epidemiology

Although multiple case-control studies have attempted to identify demographic or clinical risk factors (or both) for the development of necrotising enterocolitis, prematurity and low birthweight are the most consistently recorded risk factors. Over 90% of infants who develop the disease are born preterm, and the risk is inversely related to birthweight and gestational age.[1–5] Advances in obstetric and neonatal care have improved survival rates for smaller, more immature infants, and as more very low birthweight (VLBW) preterm infants (<1500 g) survive the early neonatal period, the population at risk for necrotising enterocolitis increases.

The disease occurs postnatally; it is not seen in stillborn infants and is rare in infants who have never been fed. Over 90% of infants with necrotising enterocolitis

Search strategy and selection criteria

We searched the Cochrane Library (1980 to present) and MEDLINE (1980 to present) using the search terms "necrotizing enterocolitis" or "NEC". We selected articles published in English, focusing on publications from the past 5 years, but including older publications that are commonly referenced or highly regarded. We also searched the reference lists of articles identified by this search strategy and selected additional publications that we judged relevant. Several reviews and book chapters were included because they provide comprehensive overviews that are beyond the scope of this Seminar.

Author	Year	Study population	Total number studied	Number NEC cases	% total	Rate per 100 livebirths	% surgically treated	Overall CFR*	CFR for medically treated NEC	CFR for surgically treated NEC	Other findings
Holman[9]	2006	Hospital discharges in the USA: 2000 kids' inpatient database	Sample of all neonatal hospitalisations (4 058 814 livebirths)	4464	..	1·1	27%	15%	10%	29%	Rates higher in VLBW and in black infants
Guillet and others[10]	2006	NICHD neonatal research network	11 936 VLBW	787	7%	..	50%	24%	11%†	36%†	Use of antecedent H2 blocker increased risk
Luig and Lui[11]	2005	New South Wales NICU study	4649 (24–31 weeks' gestation)	178	4%	..	39%	30%	24%	36%	Immaturity and growth restriction increased risk
Sankaran and others[12]	2004	Canadian neonatal network	18 234 (3628 VLBW)	336	7% VLBW	1·8	38%	..	..	..	Rates higher in VLBW infants
Guthrie and others[5]	2003	Pediatrix medical group	15 072 (23–34 weeks' gestation)	390	3%	..	37%	12%	5%	23%	Rates higher in VLBW and in black infants
Llanos and others[4]	2002	New York state	117 892	85	..	0·72	28%	19%	..	..	Rates higher in VLBW and black infants

*CFR=case-fatality rate among patients with NEC. †Based on coded cause of death; Guillet R, Glantz M, NICHD and RTI, personal communication.

Table: **Epidemiology of necrotising enterocolitis (NEC)**

have received enteral feeds.[6] Feeding human milk, however, can be protective, with an estimated three-fold to ten-fold risk reduction in infants fed human milk compared with those fed formula milk.[7,8]

Although many single-centre studies have reported rates of necrotising enterocolitis, few population-based or multicentre studies have been reported (table). In selected series, frequency ranges from fewer than 1% to 5% of neonatal intensive care unit (NICU) admissions, or from 0·5 to five patients per 1000 livebirths.[3,4,13] Both the incidence of necrotising enterocolitis and its fatality rates increase in inverse proportion to birthweight and gestational age.[2,4,10,12,14] Rates have remained stable for VLBW infants born or cared for, or both, at the centres of the National Institute of Child Health and Human Development (NICHD) neonatal research network. In the latest published NICHD neonatal network cohort (1999–2001), about 7% of 11072 VLBW infants developed proven necrotising enterocolitis (≥stage II), with about half undergoing surgery.[10] In this cohort, rates were inversely related to birthweight, with necrotising enterocolitis affecting 11·5% of infants weighing 401–750 g, 9% of infants 751–1000 g, 6% 1001–1250 g, and 4% 1251–1500 g. Several groups from the NICHD neonatal network have reported that rates of the disease varied across different clinical centres and periods, ranging from 1% to 22% of VLBW infants between 1987 and 2000.[15,16] A study from Canada showed variation in crude rates from 0% to 13·3%, but there were no significant differences in risk-adjusted rates across centres (118488 VLBW infants from 362 centres).[12] The Vermont Oxford network reported rates of necrotising enterocolitis of 6·0% to 7·1% between 1991 and 1999.[17] A Canadian population-based study (20488 admissions to 17 NICUs from 1996 to 1997) showed similar rates of 7% in VLBW infants, with almost half needing surgery.[14] The New South Wales intensive care unit study showed that the incidence of necrotising enterocolitis in infants of less than 29 weeks' gestation decreased from 12% in 1992–93 to 6% in 1998–99,[18] and the researchers noted that this decline occurred despite increasing VLBW admissions and survivors at NICUs. Mortality and surgery rates remained stable. Although encouraging, this is the only large study to report a decline in the rate of the disease in VLBW preterm infants.

No consistent association between sex and rates of necrotising enterocolitis has been identified. However, male VLBW infants are at greater risk of death.[19] Several studies have also reported an increased risk of necrotising enterocolitis in black infants; this is most often attributed to the high risk of prematurity in this group.[4,9] Furthermore, mortality associated with necrotising enterocolitis has been shown to be higher for black infants than other groups.[19] This reported racial disparity in deaths from the disease remained significant even after controlling for birthweight and other characteristics, and needs further investigation.[19]

Mortality rates from necrotising enterocolitis range from 15% to 30%. Several studies have reported that higher fatality rates are associated with falling birthweight and gestational age.[19,11] In 1979, the International Classification of Diseases established a unique code for necrotising enterocolitis that allowed investigators to distinguish the disease from other gastrointestinal causes of death. On this basis, Holman and colleagues[19] summarised the relevant trends and risk factors for infant mortality in the USA, reporting that, from 1979 to 1992, there were 6629 infant deaths associated with necrotising enterocolitis, representing an average of 474 deaths per year. They showed that the average yearly infant death rate was 12·4 deaths per 100000 livebirths, and that deaths from necrotising enterocolitis were highest in VLBW infants who were black and male.

Although most cases of necrotising enterocolitis are managed medically, an estimated 20–40% of infants undergo surgery.[5,11,12,20] The case fatality rate with surgical intervention is as high as 50%, and is highest for the smallest, least mature infants.[21,22] Mortality for this group is related to underlying clinical status and surgical treatment.[21–23] Furthermore, infants needing surgery can develop postoperative complications, including wound dehiscences, intra-abdominal abscesses, and intestinal strictures.[21,24] The most serious long-term postoperative complication that has been reported in infants that undergo surgery for necrotising enterocolitis is short bowel syndrome.[25]

Holman and colleagues[9] provided the first estimates of hospitalisation rates and mortality associated with necrotising enterocolitis in the USA. They used the 2000 kids' inpatient database to estimate that the rate of hospitalisation associated with the disease was 1·1 per 1000 livebirths (4464 hospital admissions [SE 262]). 66% of these infants weighed less than 1500 g and 27% weighed 1500–2499 g at birth. The rate of hospitalisation from necrotising enterocolitis was highest in non-Hispanic black infants, but did not differ by sex. The median length of stay was 49 days, and in-hospital mortality was 15%. Neonates who needed surgery (27%) were smaller, had a longer length of stay, and higher mortality than those who did not.[9]

Most cases of necrotising enterocolitis are sporadic, with no clear seasonal distribution, but outbreaks do occur.[26] Observations made during these epidemics suggest that they are infectious outbreaks. No one infectious agent has been linked to epidemic necrotising enterocolitis, but common infectious agents have been isolated from blood, stool, and peritoneal fluid during outbreaks. Outbreaks have been recorded more commonly in crowded nurseries and where there are high rates of gastrointestinal illness among carers.[27]

Necrotising enterocolitis occurs rarely in full-term infants.[28] In full-term infants the disease might differ from that in preterm infants; the clinical and pathological findings are similar, but the initiating events vary.[12,29,30] In

full-term infants, necrotising enterocolitis is usually associated with predisposing or underlying disorders, such as perinatal asphyxia, polycythaemia, respiratory distress, and congenital anomalies, such as myelomeningocele and congenital heart disease.[31–34] Necrotising enterocolitis in full-term infants can, however, result in much the same morbidity and mortality as that in preterm infants, if not recognised and treated early in the course of the disease.

VLBW survivors of necrotising enterocolitis are at increased risk for neurodevelopmental, neurosensory, and functional disabilities.[35,36] Stoll and colleagues[37] reported that between 18 and 22 months of corrected gestational age, infants with sepsis and necrotising enterocolitis were at high risk for adverse outcomes, including poor growth, cerebral palsy, vision and hearing impairment, and low scores on the Bayley scales of infant development. The need for surgical intervention might be used as a surrogate marker for severity of the disease.[5,38] In a cohort study of almost 3000 extremely low birthweight (ELBW) infants (<1000 g), 245 of whom survived necrotising enterocolitis, Hintz and colleagues[39] showed that infants who were surgically treated were more likely to have growth impairment and adverse neurodevelopmental outcomes at 18–22 months of corrected gestational age than infants without the disease, or those that could be treated medically.

Pathophysiology

The pathophysiology of necrotising enterocolitis remains poorly understood. Premature infants are at high risk because of developmental immaturity of key functions, in particular gastrointestinal motility, digestive ability, circulatory regulation, intestinal barrier function, and immune defence. Other potential contributing factors include hypoxic-ischaemic injury, feeding with formula milk, and colonisation by pathological bacteria (figure 1).[1,40]

Immature intestinal motility and digestion

Immature intestinal motility and digestion might predispose preterm infants to necrotising enterocolitis.

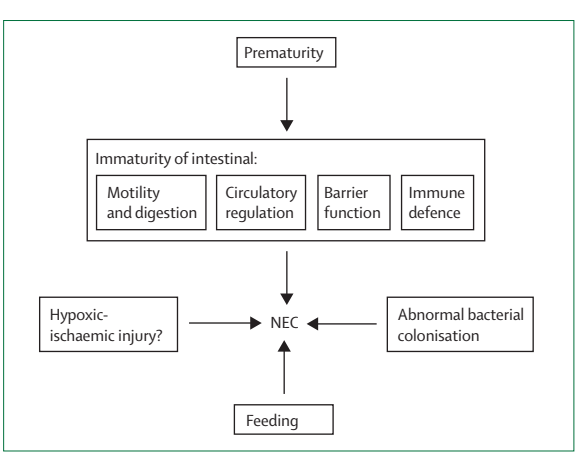

Figure 1: **Pathophysiology of necrotising enterocolitis (NEC)**

Fetal studies in both people and animals suggest that development of gastrointestinal motility begins in the second trimester, but matures in the third trimester.[41–43] Studies of intestinal motility have shown that premature infants can have immature motility patterns when compared with full-term infants, but that enteral feeding can mature these responses.[44–48] Maternal-fetal disease states, including intrauterine growth restriction, might contribute to immature motility since studies in people and animals have shown that fetal hypoxia or perinatal asphyxia can further reduce postnatal intestinal motility.[49,50] In addition to impaired intestinal motility, premature infants have not yet developed the ability to digest and absorb nutrients,[51] and incompletely digested molecules could contribute to intestinal injury.[52,53] Thus, impaired digestion of nutrients, coupled with delayed transit time, could result in injury of intestines with immature host and barrier defences.

Immature intestinal circulatory regulation

Hypoxic-ischaemic injury might play a part in the pathogenesis of necrotising enterocolitis. One plausible mechanism that is often cited is the so-called diving reflex, whereby blood flow is preferentially diverted to the heart and brain in preference to less vital organs. Although the disease occurs mainly in premature infants, full-term infants at increased risk for necrotising enterocolitis often have comorbidities that cause hypoxic or ischaemic states, such as cyanotic congenital heart disease or post-bypass surgery.[31–34] For many years, basic science research in necrotising enterocolitis has relied on animal models that utilise hypoxic or ischaemic injury, or both, to induce pathological changes resembling necrotising enterocolitis in people. Although hypoxic-ischaemic stress can cause intestinal injury, the hypothesis that this stress is the primary inciting factor in the pathogenesis of necrotising enterocolitis has been seriously questioned.[54–56] In fact, studies show a stronger association with prematurity, rapid feeding, abnormal intestinal colonisation, and inflammatory mediators than with asphyxia or ischaemia.[2,3,54,57,58] Hypoxia-ischaemia might contribute to the pathogenesis of necrotising enterocolitis, but it probably has a secondary role. In this respect, another animal model, which induces similar symptoms by injection of certain solutions into the intestine, might prove more useful.[59]

Immature regulation of intestinal circulation might lead to intestinal hypoxia-ischaemia in response to feeding or to the presence of abnormal bacteria. Some studies indicate that immature animals have altered circulatory regulation in response to ischaemia or haemorrhage; others suggest that they do not.[60,61] Reduced endothelial production of nitric oxide might result in an impaired transition of the premature intestinal circulation from the prenatal to the postnatal state, and produce a predisposition to ischaemic injury.[56,62–66] Work in people and new animal models for studying necrotising

enterocolitis should help to establish the role of intestinal circulatory regulation in this disease process.

Immature intestinal barrier function

If either the structural or biochemical component of the intestinal epithelial barrier is not fully developed, bacteria could gain access to deeper tissues and cause inflammation. Intestinal epithelia need to exist symbiotically with commensal bacteria and to protect against potential pathogens.[67–69] Intestinal epithelia are joined by tight junctional complexes, which form by 10 weeks' gestation.[70,71] When fully developed, the intestinal epithelial barrier can allow selective permeability to small ions, absorption of nutrients, and control of bidirectional fluid flow. By selectively controlling the movement of small ions across the epithelial monolayer, enterocytes use Cl^- ions and water secretion (secretory diarrhoea) to flush unwanted pathogens or toxins from the intestinal lumen.[72] These functions tend to be underdeveloped in the preterm infant since fetal intestinal secretion and absorption mature gradually, under the influence of amniotic fluid, from 26 weeks' gestation to full-term.[51] Therefore, pathogens or toxins might not be efficiently washed from the intestinal lumen in preterm infants (figure 2).

Preterm infants might also have immature goblet cells. These specialised enterocytes secrete gram quantities of mucins, forming a thick protective layer over the intestinal mucosa. This mucus layer hampers direct microbial-epithelial binding, aggregates adherent bacteria, and enhances bacterial removal.[72] Developmental expression of mucin genes changes throughout the intestine and seems to mimic adult pattern expression between 23 and 27 weeks' gestation.[73] An immature mucin layer might lead to increased intestinal permeability and enhanced bacterial adherence, potentially resulting in a breach of the intestinal epithelial barrier, and increasing susceptibility to injury by pathogenic or even non-pathogenic stimuli (figure 2).

Another aspect of the intestinal epithelial barrier that may not be functioning correctly in preterm infants consists of biochemical defences. Paneth cells, which are specialised secretory enterocytes located at the base of small intestinal crypts, secrete lysozyme, phospholipase A_2, and small antimicrobial peptides (also secreted by absorptive enterocytes) that can regulate the composition and distribution of bacterial populations.[74,75] The two main families of antimicrobial peptides produced by intestinal cells are the defensins (α and β) and cathelicidins.[74,76] Paneth cells secrete α-defensins in response to microbial stimuli.[76,77] Intestinal epithelial cells mainly secrete β-defensins, and some cells can upregulate expression of defensins in response to pro-inflammatory stimuli.[74,78,79] These antimicrobial peptides have bioactivity against a wide range of microbes, including bacteria, viruses, fungi, protozoa, and spirochaetes, and the immature intestine may be vulnerable to such pathogens (figure 2).[78,80,81]

In-vitro studies have also shown that some antimicrobial peptides have a pro-inflammatory role (in secreting cytokines and recruiting immune cells) and Cl^- secretory activity (presumably in flushing the crypt of unwanted pathogens and toxins).[82–85] A better understanding of the way defensin and cathelicidin modulate host immune defences in vivo should contribute to understanding the pathophysiology of necrotising enterocolitis.

Studies in mice and people have elucidated how developmental regulation alters the expression of α-defensin by Paneth cells.[86,87] Paneth cell number and α-defensin expression are lower in the intestines of premature infants (24 weeks' gestation) than in adults. Furthermore, although pathological specimens from infants undergoing surgery for necrotising enterocolitis have high numbers of Paneth cells and α-defensin transcripts, the amounts of α-defensin are lower in the Paneth cell than in controls.[88] The question is, can developmental disorders in α-defensin translation explain the low peptide levels detected in these infants, or their susceptibility to necrotising enterocolitis?

Prostaglandins have a role in restitution of the intestinal barrier, as measured by increased paracellular resistance through tight junctions. This might partly explain the link between indometacin, which is a prostaglandin inhibitor, and spontaneous perforation of the intestine.[89] Evidence suggests that excessive production of nitric oxide by enterocytes causes intestinal barrier failure, increasing susceptibility to necrotising enterocolitis.[90–92] Studies show that intestinal permeability is highest in premature infants, particularly in those diagnosed with the disease.[93,94] However, the clinical markers used to measure intestinal permeability, such as lactulose or mannitol, are not ideal; we need to find and use better markers. Research on the ontogeny of the intestinal epithelial barrier and its functional and biochemical regulation will probably provide insights into the pathogenesis of this disease.

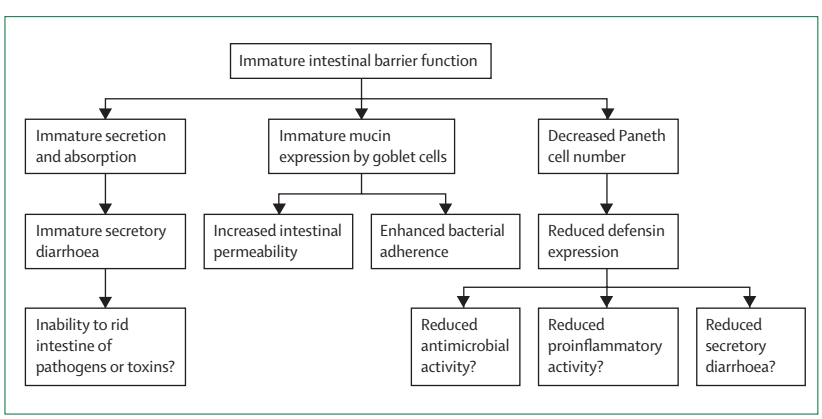

Figure 2: **Immature intestinal barrier function**

Abnormal bacterial colonisation

Commensal bacteria interact symbiotically with the mammalian intestine to regulate the expression of genes important for barrier function, digestion, and

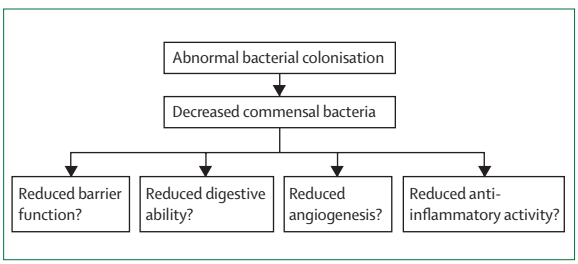

Figure 3: **Abnormal bacterial colonisation**

angiogenesis (figure 3).[95] Pathogenic host-bacterial interactions have been well characterised: a family of pattern recognition receptors (PRRs) transmits signals from microbial-associated molecular patterns (MAMPs) to induce pro-inflammatory and pro-apoptotic and anti-apoptotic responses in host cells, as in the NF-κB signalling pathway (figure 4).[85,96–104] The neonatal intestine does not encounter MAMPs in utero and is instantly challenged at birth with the introduction of normal flora. Since necrotising enterocolitis does not occur in utero, intestinal bacteria might have a role in its pathogenesis, especially if abnormal colonisation occurs. Little is known about the functional status of innate immune signalling pathways during prenatal and postnatal development in vivo, but intestinal colonisation might affect maturation.[105]

Commensal bacteria can inhibit inflammatory pathways and perhaps contribute to the maintenance of homoeostasis.[102] In vitro experiments show that a wide range of commensal bacteria can reduce inflammatory signalling in intestinal epithelia by inhibition of the NF-κB signalling pathway (figure 4).[96,106–109]

Conceivably, hyperactive inflammation in premature infants could be caused by inadequate or altered colonisation by commensal bacteria, and a subsequent lack of bacterially-mediated dampening of inflammatory pathways. In fact, investigators have reported that duodenal colonisation of *Enterobacteriaceae* is abnormal in VLBW infants and that early abnormal colonisation of stools with *Clostridium perfringens* is correlated with later development of necrotising enterocolitis.[110,111] Premature infants are especially susceptible to intestinal colonisation by pathological bacteria because of their daily exposure to nosocomial flora and the likelihood of exposure to antibiotics on admission to NICUs.[112] Furthermore, reports indicate that pathogenic stimuli, including *Salmonella* and *Escherichia coli*, produce exaggerated pro-inflammatory responses in immature intestinal epithelial cells.[113–115] Abnormal expression of certain pattern recognition receptors (which recognise microbial signatures or MAMPs) might also affect the way in which the intestine in premature infants responds to bacterial colonisation.[116] In rats, intestinal epithelial cells upregulate expression of a PRR known as toll-like receptor 4 (TLR4) in response to stress-induced production of platelet activating factor (PAF); upregulation of TLR4 might explain how necrotising enterocolitis develops in this animal model.[116]

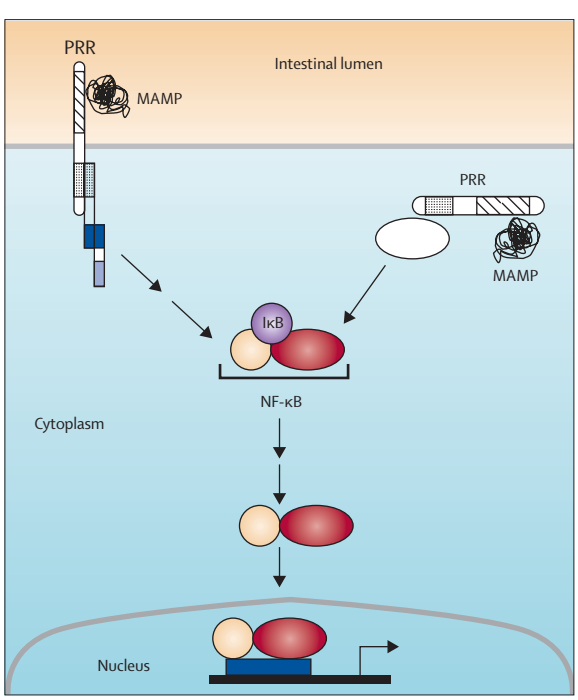

Figure 4: **NF-κB signalling pathway in intestinal epithelial cells**
PRRs transmit signals that activate NF-κB by freeing it from its inhibitor (IκB). NF-κB then enters the nucleus and induces transcription of proinflammatory and antiapoptotic genes.

Immature intestinal innate immunity

A series of events probably induces the inflammatory response that ultimately causes the mucosal oedema, coagulation necrosis, and haemorrhage that characterise necrotising enterocolitis (figure 5).[93,117] Inflammatory mediators implicated in the pathogenesis of necrotising enterocolitis include PAF, tumour necrosis factor (TNFα), and interleukins (IL-1, IL-6, IL-8, IL-10, IL-12, and IL-18).[116,118–121] Inflammation is a tightly regulated and programmed host response that recruits leucocytes to aid in the defence against potential pathogens and in the

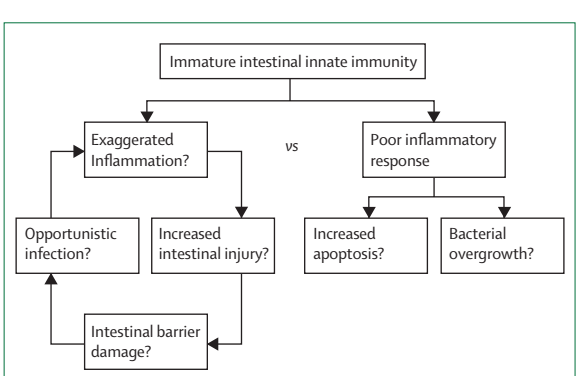

Figure 5: **Immature intestinal innate immunity**

initial response to damaged tissue. The inflammatory process begins when signals of potential danger induce local release of soluble inflammatory mediators and chemotactic agents that increase vascular permeability and attract inflammatory cells.

Long-term survival requires inflammation as a key defence mechanism in the microbe-rich environment of the intestine. However, the inflammatory response results in collateral damage caused by the release of neutrophil-derived oxidants and proteases. These molecules can cause barrier damage and allow opportunistic access for micro-organisms that cannot normally breach the epithelial barrier. These organisms could further stimulate pro-inflammatory activation and tissue damage. Some in-vitro studies suggest that immature intestinal cells seem to have a propensity for exaggerated inflammatory responses to pathogenic stimuli,[113,114] and researchers postulate that developmentally deficient expression of the NF-κB inhibitor IκB might allow greater NF-κB activity. In this model, an exaggerated inflammatory response (which might be caused by immature or abnormal PRR signalling) could cause increased cellular inflammation and potentially uncontrolled tissue damage. For instance, some researchers suggest that abnormal expression of certain PRRs (such as TLR4) may cause exaggerated intestinal pro-inflammatory responses.[116] They hypothesise that abnormal upregulation of intestinal epithelial TLR4 in response to stress (hypoxia and formula feeding) causes increased inflammatory signalling in response to normal bacterial colonisation.[116] But this study was conducted in an animal model of necrotising enterocolitis; studies in human infants are needed to elucidate how PRR expression might affect intestinal epithelial responses.

By contrast, another possible mechanism for the pathophysiology of the disease is that reduced inflammatory signalling could allow bacterial overgrowth. Failure to activate inflammatory pathways in premature infants might prejudice induction of anti-apoptotic, cytoprotective factors.[122] In mice with gut enterocytes that are conditionally null for NF-κB activation, epithelial apoptosis ensues in response to transient hypoxia.[123] Thus, developmental immaturity of the inflammatory response could increase susceptibility to apoptosis when cells are challenged by environmental stress. Host health is dependent on the balance between exaggerated pro-inflammatory activation (causing tissue injury and clinical sequelae) and insufficient inflammation (leaving mucosa vulnerable to uncontrolled bacterial growth, poised to self-destruct, or both).[102] In a rat model of necrotising enterocolitis, early apoptosis proved a factor in the pathogenesis of the disease.[124] We need to know whether excessive or hypoactive inflammation in vivo has a greater role in pathogenesis. Both could be important during different clinical scenarios or distinct stages of pathogenesis. Once epithelial damage is initiated and propagated, inflammation could result in the syndrome of lesions recognised as necrotising enterocolitis.

Advances and questions in pathophysiology

Investigation of factors that might cause a genetic predisposition for necrotising enterocolitis might eventually allow specific treatments or preventive strategies for the infants most at risk for this disease. A family of intracytoplasmic PRRs have been shown to sense invading bacteria and activate gene transcription pathways that regulate immune and inflammatory responses.[125] In a recent clinical study, VLBW infants with mutations in a member of this family, NOD2, demonstrated increased susceptibility to bacterial sepsis.[126] NOD2 mutations have been shown to confer a predisposition to Crohn's disease,[127] but a link to necrotising enterocolitis has not yet been established.[128] Another possible genetic factor is TNFα, which is one of many cytokines shown to be released during the development of the disease. In animal models, pretreatment with anti-TNFα reduces the incidence and severity of necrotising enterocolitis.[129,130] However, investigators have not so far reported a genetic link between TNFα gene variants and the disease.[131] On the other hand, IL-4 receptor alpha-chain genetic variants might confer a protective effect.[132]

Clinical presentation

Necrotising enterocolitis presents with both gastrointestinal and systemic signs.[13,133] Age at presentation is inversely related to gestational age at birth,[2,134] with full-term infants often presenting in the first few days of life.[29,135] Neonates most commonly present with feeding intolerance, delayed gastric emptying, abdominal distention or tenderness (or both), occult or gross blood in the stool, lethargy, apnoea, respiratory distress, or poor perfusion. Because early signs of this disease are non-specific, sepsis may be suspected before necrotising enterocolitis. Infants might either have a benign disease with mainly gastrointestinal symptoms or a catastrophic illness characterised by sudden fulminant onset with circulatory compromise, respiratory and metabolic acidosis, disseminated intravascular coagulopathy, grossly bloody stools, and multiorgan system failure.[133] In severe cases of the disease, there is intestinal perforation, peritonitis, and profound shock. In 1978, Bell and colleagues[136] proposed a system for the uniform clinical staging of infants with necrotising enterocolitis. They classified infants as having stage I (suspect), stage II (definite), or stage III (advanced) disease (panel 1).[136] Guidelines for management of necrotising enterocolitis are based on diagnosis according to these criteria.

Diagnosis and management

When necrotising enterocolitis is clinically suspected, radiological and laboratory studies should be obtained to confirm the diagnosis and to aid in management (figure 6). Clinicians examine serial abdominal films (supine anterioposterior view) for signs of the disease. A horizontal view can reveal free air (supine cross-table lateral or a left-side down decubitus). Early non-specific signs include

diffuse distention and asymmetric bowel gas pattern. Definite signs include pneumatosis intestinalis (intramural air) and portal venous gas. Pneumatosis can have a linear appearance when intramural air is subserosal or a bubbly appearance when the intramural air is submucosal. Submucosal intramural gas can be confused with stool, but the radiographic appearance of colonic stool is rare in premature infants younger than 2 weeks. Subsequent films (demonstrating either movement of stool or a fixed pattern of bubbly pneumatosis) and prone films can help in distinguishing between intramural gas and stool.[94,137] Portal venous gas appears as linear, branching lucencies overlying the liver, and can be detected on cross-table lateral films and on ultrasound.[138–140] Clinicians can also use serial films to follow disease progression and guide clinical management, especially if the infant fails to respond to treatment or deteriorates.

No one laboratory examination is diagnostic of necrotising enterocolitis, but haematological studies and blood chemistry tests can lend support to the diagnosis (figure 6). Severe or persistent thrombocytopenia, neutropenia, coagulopathy, or acidosis might indicate severe disease.[93,141] Serial C-reactive protein could be useful in the management of the disease, and Pourcyrous and colleagues[142] have successfully used serial C-reactive protein to distinguish Bell's stage I necrotising enterocolitis from ileus or benign pneumatosis. They showed that persistently high C-reactive protein indicated developing complications, such as stricture or abscess, or the need for surgical intervention.[142]

When necrotising enterocolitis is suspected, infants are given bowel rest (ie, nil by mouth), bowel decompression (low-intermittent orogastric suction), and broad-spectrum antibiotics (after cultures are obtained). Anaerobic coverage should be considered, especially if pneumoperitoneum is suspected or confirmed. The patient should be closely monitored during confirmation of the diagnosis. Adjunctive therapy includes cardiovascular support (pressors, volume), pulmonary support (oxygen, ventilation), and haematological support (blood product transfusion) as clinically indicated. If the clinical course and results from radiological and laboratory tests remain consistent with suspected necrotising enterocolitis or Bell's stage I disease, the length of medical treatment will usually be dictated by clinical judgment. If definite necrotising enterocolitis or Bell's stage II disease is confirmed, medical treatment should be continued for 7 to 14 days while monitoring for severe disease. If Bell's stage III disease is suspected or confirmed, intensive cardiovascular and respiratory support might be required and surgical intervention should be considered. The decision to operate is difficult, since pneumoperitoneum, an indication of bowel perforation, is the only clear indication for surgery. However, some radiological signs (persistent fixed loop, portal venous gas, ascites, or generalised intestinal distention progressing to asymmetrical intestinal distention[94]) and laboratory features (severe thrombocytopenia, neutropenia, or acidosis) also might indicate sufficiently severe disease to warrant surgical intervention. Portal venous gas alone, however, might not indicate more severe disease, as previously believed.[20,94,143] Ultimately, neonatal paediatricians must use radiological tests in conjunction with clinical course, laboratory studies, and consultation with radiologists and surgeons when making decisions on the diagnosis and management of necrotising enterocolitis.

***Panel 1:* Bell's stages of necrotising enterocolitis**

I. Suspected disease
Mild systemic signs (apnoea, bradycardia, temperature instability)
Mild intestinal signs (abdominal distention, gastric residuals, bloody stools)
Non-specific or normal radiological signs

II. Definite disease
Mild to moderate systemic signs
Additional intestinal signs (absent bowel sounds, abdominal tenderness)
Specific radiologic signs (pneumatosis intestinalis or portal venous air)
Laboratory changes (metabolic acidosis, thrombocytopaenia)

III. Advanced disease
Severe systemic illness (hypotension)
Additional intestinal signs (striking abdominal distention, peritonitis)
Severe radiological signs (pneumoperitoneum)
Additional laboratory changes (metabolic and respiratory acidosis, disseminated intravascular coagulopathy)

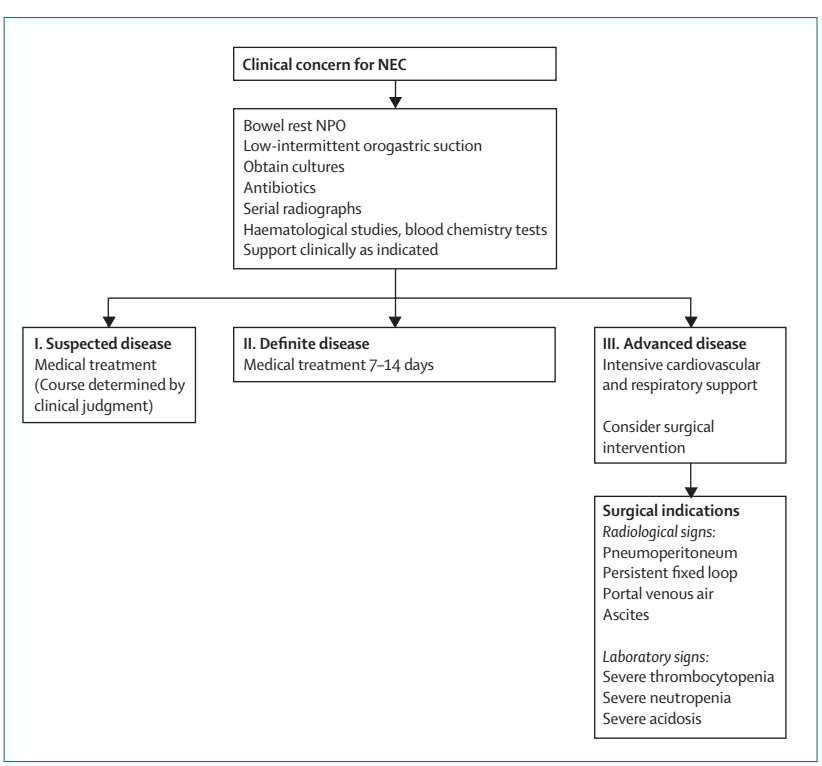

Figure 6: **Suggested management of necrotising enterocolitis**

Advances and questions in diagnosis and management

Some infants present so acutely and severely that morbidity or mortality cannot be avoided despite best treatment. Identification of a biological marker for early disease should allow more timely diagnosis and treatment, but no ideal marker has yet been identified. The serum of symptomatic infants tends to contain high concentrations of certain cytokines, such as IL-8, IL-10, and IL-1 receptor antagonist.[118] Some studies suggest that serum concentrations of fatty acid binding protein in the intestine and liver (I-FABP and L-FABP, respectively) could also be used as markers for necrotising enterocolitis.[144,145] L-FABP concentrations at the onset of clinical signs are highest in infants later diagnosed with stage I necrotising enterocolitis, and I-FABP concentrations are highest in infants who later develop stage III necrotising enterocolitis.[144,145] The identification of other markers, which can be used before the onset of symptoms should allow earlier intervention, and improved outcomes.

More sensitive and accurate imaging studies, such as ultrasonography, could become helpful adjuncts to abdominal films in the diagnosis of necrotising enterocolitis. Ultrasound can be used to detect portal venous gas, necrotic bowel, and other signs that are not easily diagnosed by abdominal films (which can be negative for pneumatosis). Ultrasound with Doppler might be more sensitive than abdominal films for detecting necrotic bowel requiring surgical intervention, and routine abdominal ultrasound might also be used to diagnose early necrotising enterocolitis from the presence of echogenic dots or dense granular echogenicities.[146,147]

There is insufficient work on new approaches for the medical management of necrotising enterocolitis that might prevent the progression of disease. By contrast, surgical management is being actively investigated. The decision to do peritoneal drainage as an alternative to laparotomy is controversial and under investigation.[21,22,148] Some advocate resection and primary anastomosis as an alternative intervention.[149] We need further research to determine how to improve surgical outcome and reduce complications such as short bowel syndrome.

Prevention

Strategies to prevent necrotising enterocolitis should improve both short-term and long-term outcomes for VLBW preterm infants (panel 2). Feeding of human milk and conservative feeding practices, especially in infants suspected to be at higher risk, can reduce the incidence of the disease.[6,7,150] However, researchers do not agree on whether human milk from a donor is as protective as mother's milk.[8,151] Antenatal steroids,[110] IgA supplementation,[152,153] arginine supplementation,[154] erythropoietin,[155] and oral antiobiotics[156,157] might also reduce the incidence or severity of necrotising enterocolitis, but no authoritative recommendations can yet be made about any of these preventive strategies.

When making decisions about introducing feeds, it should be remembered that diet plays an important role in intestinal development and defence, and that non-nutritive dietary substances, such as epidermal growth factor and polyamines, stimulate intestinal epithelial growth.[158,159] Furthermore, some nutrients (such as glutamine, arginine, and omega-3 fatty acids) counteract pro-inflammatory activation.[93] Therefore, many advocate the initiation of trophic feeds rather than extended bowel rest, which can lead to gut atrophy and worsened inflammatory responses. Trophic feeds improve the activity of digestive enzymes, enhance the release of digestive hormones, and increase intestinal blood flow and digestive motility in premature infants.[160,161] In addition, infants given early trophic feeds seem to have better feeding tolerance, improved growth, reduced period of hospitalisation, and decreased likelihood of sepsis compared with infants who are not. Furthermore, early trophic feeds do not increase susceptibility to developing necrotising enterocolitis.[160,161] However, studies have not yet clearly delineated the best feeding strategies for premature infants.

Since bacterial colonisation can affect the course of many intestinal diseases, probiotics are emerging as a promising therapy. Probiotics are "living micro-organisms, which upon digestion in sufficient numbers, exert health benefits beyond basic nutrition".[162] Micro-organisms that are commonly used in therapies include *Lactobacilli*, *Bifidobacterium*, and *Saccharomyces*. Clinical trials show that probiotic supplements can reduce the incidence and severity of necrotising enterocolitis.[163,164] Larger follow-up studies are needed to confirm this beneficial effect before wider use can be recommended. Some patient populations have reportedly suffered invasive disease as a result of probiotic administration, but this has not yet been reported in VLBW infants.[165,166]

Another potential preventative strategy is to administer prebiotics—non-digestible dietary supplements, such as long-chain carbohydrates or mucins, which promote proliferation of beneficial commensal bacteria.[167] Preliminary studies show increased *Bifidobacterium* stool colonisation and decreased pathogenic bacterial

Panel 2: **Potential preventive strategies**

Common practice
Human milk feedings
Conservative feeding
Trophic feeding

Research needed
Antenatal steroids
IgA supplemtation
Arginine supplementation
Erythropoietin
Oral antibiotics
Probiotics

colonisation in preterm infants fed with formula containing prebiotics (90% short-chain galacto-oligosaccharide, 10% long-chain fructo-oligosaccharide) compared with infants fed control formula.[168] Furthermore, prebiotic treatment may have a positive effect on host immune function.[169] Because prebiotic supplements do not contain live micro-organisms, they carry less risk of infection than probiotic therapies. However, prebiotic administration has also been associated with unwanted (but reversible) side-effects such as flatulence, bloating, and diarrhoea.[167]

Another potential therapy involves bacterial metabolites or postbiotics, such as butyric acid, a short-chain fatty acid produced by commensal bacteria in the colon through anaerobic catabolism of complex carbohydrates. Butyrate is a major energy source for colonic enterocytes and has a widely recognised but poorly understood role in intestinal growth and differentiation,[170,171] inflammatory suppression,[172–174] and apoptosis.[175,176] Butyrate and other small molecule products might generate some of the beneficial effects of the normal flora (and exogenous probiotics and prebiotics), and could be a safe alternative therapeutic strategy. Butyrate has been administered with limited success in human inflammatory bowel disease,[177] but there are as yet no studies in neonates.

Other products of commensal bacteria can induce protective responses (such as those mediated by toll-like receptors) that promote intestinal health.[178–180] The beneficial effects of probiotic bacteria can be replicated by treatment with isolated MAMPs—eg, in mice, unmethylated probiotic CpG DNA ameliorates colitis induced by dextran sodium sulphate.[181] Oral administration of inactivated probiotics (heat-killed commensals) or bio-available toll-like receptor ligands could potentially induce beneficial TLR-mediated protective effects without carrying the infectious risk of probiotic therapies. But the neonatal epithelia may not respond to MAMPs from the normal flora in the same manner and intensity as the mature epithelium. We need to improve our understanding of the disease to find out whether probiotics (live or inactivated), prebiotics, or some alternative medical or surgical treatment will be beneficial and safe to use in the prevention and treatment of necrotising enterocolitis in preterm infants. Prematurity and low birthweight are the most important risk factors for the disease. Strategies to address these problems will have the greatest effect in preventing necrotising enterocolitis in newborn infants.

Conflict of interest statement

P Lin has received research funding from NIH K08 DK062851-01A1 for Paneth cell cryptdins in innate intestinal host defense (9/03-6/08), and from Emory Egleston Children's Research Center Seed Grant for Bacterial Colonization and Development of Innate Intestinal Host Defense (1/06-12/06). B Stoll has received funding from the National Institute of Child Health and Human Development for the multicenter network of neonatal intensive care units 2006–2011, and from NIH/NICHHD (9/26/2003–7/31/2008) for research on the scope and causes of stillbirth in the USA.

References

1 Hsueh W, Caplan MS, Qu XW, Tan XD, De Plaen IG, Gonzalez-Crussi F. Neonatal necrotizing enterocolitis: clinical considerations and pathogenetic concepts. *Pediatr Dev Pathol* 2003; **6:** 6–23.

2 Stoll BJ, Kanto WP Jr, Glass RI, Nahmias AJ, Brann AW Jr. Epidemiology of necrotizing enterocolitis: a case control study. *J Pediatr* 1980; **96:** 447–51.

3 Stoll BJ. Epidemiology of necrotizing enterocolitis. *Clin Perinatol* 1994; **21:** 205–18.

4 Llanos AR, Moss ME, Pinzon MC, Dye T, Sinkin RA, Kendig JW. Epidemiology of neonatal necrotising enterocolitis: a population-based study. *Paediatr Perinat Epidemiol* 2002; **16:** 342–49.

5 Guthrie SO, Gordon PV, Thomas V, Thorp JA, Peabody J, Clark RH. Necrotizing enterocolitis among neonates in the United States. *J Perinatol* 2003; **23:** 278–85.

6 Berseth CL. Feeding strategies and necrotizing enterocolitis. *Curr Opin Pediatr* 2005; **17:** 170–73.

7 Lucas A, Cole TJ. Breast milk and neonatal necrotising enterocolitis. *Lancet* 1990; **336:** 1519–23.

8 McGuire W, Anthony MY. Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. *Arch Dis Child Fetal Neonatal Ed* 2003; **88:** F11–14.

9 Holman RC, Stoll BJ, Curns AT, Yorita KL, Steiner CA, Schonberger LB. Necrotizing enterocolitis hospitalizations among neonates in the United States. *Paediatr Perinat Epidemiol* (in press).

10 Guillet R, Stoll BJ, Cotten CM, et al. Association of H2-blocker therapy and higher incidence of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 2006; **117:** e137–42.

11 Luig M, Lui K. Epidemiology of necrotizing enterocolitis—Part II: Risks and susceptibility of premature infants during the surfactant era: a regional study. *J Paediatr Child Health* 2005; **41:** 174–79.

12 Sankaran K, Puckett B, Lee DSC, et al. Variations in incidence of necrotizing enterocolitis in Canadian Neonatal Intensive Care Units. *J Pediatr Gastroenterol Nutr* 2004; **39:** 366–72.

13 Piazza AJ, Stoll BJ. Digestive system disorders. In: Behrnman RE, Kliegman RM, Jensen HB, eds. Nelson's Textbook of Pediatrics. 18th edn. Philadelphia: WB Saunders (in press).

14 Lee SK, McMillan DD, Ohlsson A, et al. Variations in practice and outcomes in the Canadian NICU network: 1996–1997. *Pediatrics* 2000; **106:** 1070–79.

15 Lemons JA, Bauer CR, Oh W, et al. Very low birth weight outcomes of the National Institute of Child Health and Human Development neonatal research network, January 1995 through December 1996. NICHD Neonatal Research Network. *Pediatrics* 2001; **107:** e1.

16 Fanaroff AA, Hack M, Walsh MC. The NICHD neonatal research network: changes in practice and outcomes during the first 15 years. *Semin Perinatol* 2003; **27:** 281–87.

17 Horbar JD, Badger GJ, Carpenter JH, et al. Trends in mortality and morbidity for very low birth weight infants, 1991–1999. *Pediatrics* 2002; **110:** 143–51.

18 Luig M, Lui K. Epidemiology of necrotizing enterocolitis—Part I: Changing regional trends in extremely preterm infants over 14 years. *J Paediatr Child Health* 2005; **41:** 169–73.

19 Holman RC, Stoll BJ, Clarke MJ, Glass RI. The epidemiology of necrotizing enterocolitis infant mortality in the United States. *Am J Public Health* 1997; **87:** 2026–31.

20 Sharma R, Tepas JJ 3rd, Hudak ML, et al. Portal venous gas and surgical outcome of neonatal necrotizing enterocolitis. *J Pediatr Surg* 2005; **40:** 371–76.

21 Blakely ML, Lally KP, McDonald S, et al. Postoperative outcomes of extremely low birth-weight infants with necrotizing enterocolitis or isolated intestinal perforation: a prospective cohort study by the NICHD Neonatal Research Network. *Ann Surg* 2005; **241:** 984–94.

22 Henry MC, Lawrence Moss R. Surgical therapy for necrotizing enterocolitis: bringing evidence to the bedside. *Semin Pediatr Surg* 2005; **14:** 181–90.

23 Ehrlich PF, Sato TT, Short BL, Hartman GE. Outcome of perforated necrotizing enterocolitis in the very low-birth weight neonate may be independent of the type of surgical treatment. *Am Surg* 2001; **67:** 752–56.

24 Horwitz JR, Lally KP, Cheu HW, Vazquez WD, Grosfeld JL, Ziegler MM. Complications after surgical intervention for necrotizing enterocolitis: a multicenter review. *J Pediatr Surg* 1995; **30:** 994–99.

25 Petty JK, Ziegler MM. Operative strategies for necrotizing enterocolitis: The prevention and treatment of short-bowel syndrome. *Semin Pediatr Surg* 2005; **14:** 191–98.

26 Boccia D, Stolfi I, Lana S, Moro ML. Nosocomial necrotising enterocolitis outbreaks: epidemiology and control measures. *Eur J Pediatr* 2001; **160:** 385–91.

27 Gerber AR, Hopkins RS, Lauer BA, Curry-Kane AG, Rotbart HA. Increased risk of illness among nursery staff caring for neonates with necrotizing enterocolitis. *Pediatr Infect Dis* 1985; **4:** 246–49.

28 Ng S. Necrotizing enterocolitis in the full-term neonate. *J Paediatr Child Health* 2001; **37:** 1–4.

29 Wiswell TE, Robertson CF, Jones TA, Tuttle DJ. Necrotizing enterocolitis in full-term infants. A case-control study. *Am J Dis Child* 1988; **142:** 532–35.

30 Maayan-Metzger A, Itzchak A, Mazkereth R, Kuint J. Necrotizing enterocolitis in full-term infants: case-control study and review of the literature. *J Perinatol* 2004; **24:** 494–99.

31 Bolisetty S, Lui K, Oei J, Wojtulewicz J. A regional study of underlying congenital diseases in term neonates with necrotizing enterocolitis. *Acta Paediatr* 2000; **89:** 1226–30.

32 Wilson R, del Portillo M, Schmidt E, Feldman RA, Kanto WP Jr. Risk factors for necrotizing enterocolitis in infants weighing more than 2,000 grams at birth: a case-control study. *Pediatrics* 1983; **71:** 19–22.

33 McElhinney DB, Hedrick HL, Bush DM, et al. Necrotizing enterocolitis in neonates with congenital heart disease: risk factors and outcomes. *Pediatrics* 2000; **106:** 1080–87.

34 Ostlie DJ, Spilde TL, St Peter SD, et al. Necrotizing enterocolitis in full-term infants. *J Pediatr Surg* 2003; **38:** 1039–42.

35 Vohr BR, Wright LL, Dusick AM, et al. Neurodevelopmental and functional outcomes of extremely low birth weight infants in the National Institute of Child Health and Human Development Neonatal Research Network, 1993–1994. *Pediatrics* 2000; **105:** 1216–26.

36 Salhab WA, Perlman JM, Silver L, Sue Broyles R. Necrotizing enterocolitis and neurodevelopmental outcome in extremely low birth weight infants <1000 g. *J Perinatol* 2004; **24:** 534–40.

37 Stoll BJ, Hansen NI, Adams-Chapman I, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. *JAMA* 2004; **292:** 2357–65.

38 Adesanya OA, O'Shea TM, Turner CS, Amoroso RM, Morgan TM, Aschner JL. Intestinal perforation in very low birth weight infants: growth and neurodevelopment at 1 year of age. *J Perinatol* 2005; **25:** 583–89.

39 Hintz SR, Kendrick DE, Stoll BJ, et al. Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotizing enterocolitis. *Pediatrics* 2005; **115:** 696–703.

40 Claud EC, Walker WA. Hypothesis: inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. *Faseb J* 2001; **15:** 1398–403.

41 Sase M, Lee JJ, Park JY, Thakur A, Ross MG, Buchmiller-Crair TL. Ontogeny of fetal rabbit upper gastrointestinal motility. *J Surg Res* 2001; **101:** 68–72.

42 Sase M, Miwa I, Sumie M, et al. Gastric emptying cycles in the human fetus. *Am J Obstet Gynecol* 2005; **193:** 1000–04.

43 Sase M, Miwa I, Sumie M, Nakata M, Sugino N, Ross MG. Ontogeny of gastric emptying patterns in the human fetus. *J Matern Fetal Neonatal Med* 2005; **17:** 213–17.

44 Berseth CL. Gestational evolution of small intestine motility in preterm and term infants. *J Pediatr* 1989; **115:** 646–51.

45 Berseth CL. Neonatal small intestinal motility: motor responses to feeding in term and preterm infants. *J Pediatr* 1990; **117:** 777–82.

46 Berseth CL, Ittmann PI. Antral and duodenal motor responses to duodenal feeding in preterm and term infants. *J Pediatr Gastroenterol Nutr* 1992; **14:** 182–86.

47 al Tawil Y, Berseth CL. Gestational and postnatal maturation of duodenal motor responses to intragastric feeding. *J Pediatr* 1996; **129:** 374–81.

48 Ittmann PI, Amarnath R, Berseth CL. Maturation of antroduodenal motor activity in preterm and term infants. *Dig Dis Sci* 1992; **37:** 14–19.

49 Sase M, Lee JJ, Ross MG, Buchmiller-Crair TL. Effect of hypoxia on fetal rabbit gastrointestinal motility. *J Surg Res* 2001; **99:** 347–51.

50 Berseth CL, McCoy HH. Birth asphyxia alters neonatal intestinal motility in term neonates. *Pediatrics* 1992; **90:** 669–73.

51 Lebenthal A, Lebenthal E. The ontogeny of the small intestinal epithelium. *JPEN J Parenter Enteral Nutr* 1999; **23** (suppl 5)**:** S3–6.

52 Di Lorenzo M, Bass J, Krantis A. An intraluminal model of necrotizing enterocolitis in the developing neonatal piglet. *J Pediatr Surg* 1995; **30:** 1138–42.

53 Lin J. Too much short chain fatty acids cause neonatal necrotizing enterocolitis. *Med Hypotheses* 2004; **62:** 291–93.

54 Neu J. The 'myth' of asphyxia and hypoxia-ischemia as primary causes of necrotizing enterocolitis. *Biol Neonate* 2005; **87:** 97–98.

55 Nowicki PT, Nankervis CA. The role of the circulation in the pathogenesis of necrotizing enterocolitis. *Clin Perinatol* 1994; **21:** 219–34.

56 Reber KM, Nankervis CA, Nowicki PT. Newborn intestinal circulation. Physiology and pathophysiology. *Clin Perinatol* 2002; **29:** 23–39.

57 Covert RF, Neu J, Elliott MJ, Rea JL, Gimotty PA. Factors associated with age of onset of necrotizing enterocolitis. *Am J Perinatol* 1989; **6:** 455–60.

58 De Curtis M, Paone C, Vetrano G, Romano G, Paludetto R, Ciccimarra F. A case control study of necrotizing enterocolitis occurring over 8 years in a neonatal intensive care unit. *Eur J Pediatr* 1987; **146:** 398–400.

59 Miller MJ, Adams J, Gu XA, Zhang XJ, Clark DA. Hemodynamic and permeability characteristics of acute experimental necrotizing enterocolitis. *Dig Dis Sci* 1990; **35:** 1257–64.

60 Gosche JR, Harris PD, Garrison RN. Age-related differences in intestinal microvascular responses to low-flow states in adult and suckling rats. *Am J Physiol* 1993; **264:** G447–53.

61 Chun K, Drugas G, Ferguson D, Biewer J, Clemens MG. Intestinal villus microcirculatory response to hemorrhage in adult and immature rats. *J Pediatr Surg* 1992; **27:** 322–28.

62 Di Lorenzo M, Bass J, Krantis A. Use of L-arginine in the treatment of experimental necrotizing enterocolitis. *J Pediatr Surg* 1995; **30:** 235–41.

63 Zamora SA, Amin HJ, McMillan DD, et al. Plasma L-arginine concentrations in premature infants with necrotizing enterocolitis. *J Pediatr* 1997; **131:** 226–32.

64 Becker RM, Wu G, Galanko JA, et al. Reduced serum amino acid concentrations in infants with necrotizing enterocolitis. *J Pediatr* 2000; **137:** 785–93.

65 Akisu M, Ozmen D, Baka M, et al. Protective effect of dietary supplementation with L-arginine and L-carnitine on hypoxia/reoxygenation-induced necrotizing enterocolitis in young mice. *Biol Neonate* 2002; **81:** 260–65.

66 Amin HJ, Zamora SA, McMillan DD, et al. Arginine supplementation prevents necrotizing enterocolitis in the premature infant. *J Pediatr* 2002; **140:** 425–31.

67 Madara JL. Warner-Lambert/Parke-Davis Award lecture. Pathobiology of the intestinal epithelial barrier. *Am J Pathol* 1990; **137:** 1273–81.

68 Xu J, Gordon JI. Inaugural Article: Honor thy symbionts. *Proc Natl Acad Sci USA* 2003; **100:** 10452–59.

69 Hooper LV, Gordon JI. Commensal host-bacterial relationships in the gut. *Science* 2001; **292:** 1115–18.

70 Nusrat A, Turner JR, Madara JL. Molecular physiology and pathophysiology of tight junctions. IV. Regulation of tight junctions by extracellular stimuli: nutrients, cytokines, and immune cells. *Am J Physiol Gastrointest Liver Physiol* 2000; **279:** G851–57.

71 Polak-Charcon S, Shoham J, Ben-Shaul Y. Tight junctions in epithelial cells of human fetal hindgut, normal colon, and colon adenocarcinoma. *J Natl Cancer Inst* 1980; **65:** 53–62.

72 Hecht G. Innate mechanisms of epithelial host defense: spotlight on intestine. *Am J Physiol* 1999; **277:** C351–58.

73 Buisine MP, Devisme L, Savidge TC, et al. Mucin gene expression in human embryonic and fetal intestine. *Gut* 1998; **43:** 519–24.

74 Otte JM, Kiehne K, Herzig KH. Antimicrobial peptides in innate immunity of the human intestine. *J Gastroenterol* 2003; **38:** 717–26.

75 Scott MG, Hancock RE. Cationic antimicrobial peptides and their multifunctional role in the immune system. *Crit Rev Immunol* 2000; **20:** 407–31.

76 Ganz T. Defensins: antimicrobial peptides of innate immunity. *Nat Rev Immunol* 2003; **3:** 710–20.

77 Ayabe T, Satchell DP, Wilson CL, Parks WC, Selsted ME, Ouellette AJ. Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. *Nat Immunol* 2000; **1:** 113–18.

78 Eckmann L. Innate immunity and mucosal bacterial interactions in the intestine. *Curr Opin Gastroenterol* 2004; **20:** 82–88.

79 O'Neil DA, Porter EM, Elewaut D, et al. Expression and regulation of the human beta-defensins hBD-1 and hBD-2 in intestinal epithelium. *J Immunol* 1999; **163:** 6718–24.

80 Ouellette AJ. Paneth cell alpha-defensins: peptide mediators of innate immunity in the small intestine. *Springer Semin Immunopathol* 2005; **27:** 133–46.

81 Chen H, Xu Z, Peng L, et al. Recent advances in the research and development of human defensins. *Peptides* 2006; **27:** 931–40.

82 Eckmann L. Defence molecules in intestinal innate immunity against bacterial infections. *Curr Opin Gastroenterol* 2005; **21:** 147–51.

83 Lencer WI, Cheung G, Strohmeier GR, et al. Induction of epithelial chloride secretion by channel-forming cryptdins 2 and 3. *Proc Natl Acad Sci USA* 1997; **94:** 8585–89.

84 Merlin D, Yue G, Lencer WI, Selsted ME, Madara JL. Cryptdin-3 induces novel apical conductance(s) in Cl- secretory, including cystic fibrosis, epithelia. *Am J Physiol Cell Physiol* 2001; **280:** C296–302.

85 Lin PW, Simon PO Jr, Gewirtz AT, et al. Paneth cell cryptdins act in vitro as apical paracrine regulators of the innate inflammatory response. *J Biol Chem* 2004; **279:** 19902–07.

86 Mallow EB, Harris A, Salzman N, et al. Human enteric defensins. Gene structure and developmental expression. *J Biol Chem* 1996; **271:** 4038–45.

87 Ouellette AJ, Greco RM, James M, Frederick D, Naftilan J, Fallon JT. Developmental regulation of cryptdin, a corticostatin/defensin precursor mRNA in mouse small intestinal crypt epithelium. *J Cell Biol* 1989; **108:** 1687–95.

88 Salzman NH, Polin RA, Harris MC, et al. Enteric defensin expression in necrotizing enterocolitis. *Pediatr Res* 1998; **44:** 20–26.

89 Liu Z, Li N, Neu J. Tight junctions, leaky intestines, and pediatric diseases. *Acta Paediatr* 2005; **94:** 386–93.

90 Potoka DA, Nadler EP, Upperman JS, Ford HR. Role of nitric oxide and peroxynitrite in gut barrier failure. *World J Surg* 2002; **26:** 806–11.

91 Upperman JS, Potoka D, Grishin A, Hackam D, Zamora R, Ford HR. Mechanisms of nitric oxide-mediated intestinal barrier failure in necrotizing enterocolitis. *Semin Pediatr Surg* 2005; **14:** 159–66.

92 Hackam DJ, Upperman JS, Grishin A, Ford HR. Disordered enterocyte signaling and intestinal barrier dysfunction in the pathogenesis of necrotizing enterocolitis. *Semin Pediatr Surg* 2005; **14:** 49–57.

93 Neu J. Neonatal necrotizing enterocolitis: an update. *Acta Paediatr Suppl* 2005; **94:** 100–05.

94 Buonomo C. The radiology of necrotizing enterocolitis. *Radiol Clin North Am* 1999; **37:** 1187–98, vii.

95 Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. *Science* 2001; **291:** 881–84.

96 Neish AS, Gewirtz AT, Zeng H, et al. Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. *Science* 2000; **289:** 1560–63.

97 Neish AS. The gut microflora and intestinal epithelial cells: a continuing dialogue. *Microbes Infect* 2002; **4:** 309–17.

98 Neish AS. Molecular aspects of intestinal epithelial cell-bacterial interactions that determine the development of intestinal inflammation. *Inflamm Bowel Dis* 2004; **10:** 159–68.

99 Neish AS. Bacterial inhibition of eukaryotic pro-inflammatory pathways. *Immunol Res* 2004; **29:** 175–86.

100 Young AN, de Oliveira Salles PG, Lim SD, et al. Beta defensin-1, parvalbumin, and vimentin: a panel of diagnostic immunohistochemical markers for renal tumors derived from gene expression profiling studies using cDNA microarrays. *Am J Surg Pathol* 2003; **27:** 199–205.

101 Zeng H, Carlson AQ, Guo Y, et al. Flagellin is the major proinflammatory determinant of enteropathogenic Salmonella. *J Immunol* 2003; **171:** 3668–74.

102 Collier-Hyams LS, Neish AS. Innate immune relationship between commensal flora and the mammalian intestinal epithelium. *Cell Mol Life Sci* 2005; **62:** 1339–48.

103 Collier-Hyams LS, Zeng H, Sun J, et al. Cutting edge: Salmonella AvrA effector inhibits the key proinflammatory, anti-apoptotic NF-kappa B pathway. *J Immunol* 2002; **169:** 2846–50.

104 Gewirtz AT, Simon PO Jr, Schmitt CK, et al. Salmonella typhimurium translocates flagellin across intestinal epithelia, inducing a proinflammatory response. *J Clin Invest* 2001; **107:** 99–109.

105 McCracken VJ, Lorenz RG. The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. *Cell Microbiol* 2001; **3:** 1–11.

106 Wallace TD, Bradley S, Buckley ND, Green-Johnson JM. Interactions of lactic acid bacteria with human intestinal epithelial cells: effects on cytokine production. *J Food Prot* 2003; **66:** 466–72.

107 Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 1999; **116:** 1107–14.

108 Kelly D, Campbell JI, King TP, et al. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nat Immunol* 2004; **5:** 104–12.

109 Collier-Hyams LS, Sloane V, Batten BC, Neish AS. Cutting edge: bacterial modulation of epithelial signaling via changes in neddylation of cullin-1. *J Immunol* 2005; **175:** 4194–98.

110 Hoy CM, Wood CM, Hawkey PM, Puntis JW. Duodenal microflora in very-low-birth-weight neonates and relation to necrotizing enterocolitis. *J Clin Microbiol* 2000; **38:** 4539–47.

111 de la Cochetiere MF, Piloquet H, des Robert C, Darmaun D, Galmiche JP, Roze JC. Early intestinal bacterial colonization and necrotizing enterocolitis in premature infants: the putative role of Clostridium. *Pediatr Res* 2004; **56:** 366–70.

112 Stoll BJ, Gordon T, Korones SB, et al. Early-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. *J Pediatr* 1996; **129:** 72–80.

113 Nanthakumar NN, Fusunyan RD, Sanderson I, Walker WA. Inflammation in the developing human intestine: a possible pathophysiologic contribution to necrotizing enterocolitis. *Proc Natl Acad Sci USA* 2000; **97:** 6043–48.

114 Claud EC, Lu L, Anton PM, Savidge T, Walker WA, Cherayil BJ. Developmentally regulated IkappaB expression in intestinal epithelium and susceptibility to flagellin-induced inflammation. *Proc Natl Acad Sci USA* 2004; **101:** 7404–08.

115 Claud EC, Savidge T, Walker WA. Modulation of human intestinal epithelial cell IL-8 secretion by human milk factors. *Pediatr Res* 2003; **53:** 419–25.

116 Caplan MS, Simon D, Jilling T. The role of PAF, TLR, and the inflammatory response in neonatal necrotizing enterocolitis. *Semin Pediatr Surg* 2005; **14:** 145–51.

117 Kliegman RM. Models of the pathogenesis of necrotizing enterocolitis. *J Pediatr* 1990; **117:** S2–5.

118 Edelson MB, Bagwell CE, Rozycki HJ. Circulating pro- and counterinflammatory cytokine levels and severity in necrotizing enterocolitis. *Pediatrics* 1999; **103:** 766–71.

119 Caplan MS, Sun XM, Hseuh W, Hageman JR. Role of platelet activating factor and tumor necrosis factor-alpha in neonatal necrotizing enterocolitis. *J Pediatr* 1990; **116:** 960–64.

120 Harris MC, D'Angio CT, Gallagher PR, Kaufman D, Evans J, Kilpatrick L. Cytokine elaboration in critically ill infants with bacterial sepsis, necrotizing entercolitis, or sepsis syndrome: correlation with clinical parameters of inflammation and mortality. *J Pediatr* 2005; **147:** 462–68.

121 Harris MC, Costarino AT Jr, Sullivan JS, et al. Cytokine elevations in critically ill infants with sepsis and necrotizing enterocolitis. *J Pediatr* 1994; **124:** 105–11.

122 Zeng H, Wu H, Sloane V, et al. Flagellin/TLR5 responses in epithelia reveal intertwined activation of inflammatory and apoptotic pathways. *Am J Physiol Gastrointest Liver Physiol* 2006; **290:** G96–108.

123 Chen Z, Hagler J, Palombella VJ, et al. Signal-induced site-specific phosphorylation targets I kappa B alpha to the ubiquitin-proteasome pathway. *Genes Dev* 1995; **9:** 1586–97.

124 Jilling T, Lu J, Jackson M, Caplan MS. Intestinal epithelial apoptosis initiates gross bowel necrosis in an experimental rat model of neonatal necrotizing enterocolitis. *Pediatr Res* 2004; **55:** 622–29.

125 Girardin SE, Tournebize R, Mavris M, et al. CARD4/Nod1 mediates NF-kappaB and JNK activation by invasive Shigella flexneri. *EMBO Rep* 2001; **2:** 736–42.

126 Ahrens P, Kattner E, Kohler B, et al. Mutations of genes involved in the innate immune system as predictors of sepsis in very low birth weight infants. *Pediatr Res* 2004; **55:** 652–56.

127 Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411:** 599–603.

128 Habib Z, Arnaud B, Pascal de L, et al. CARD15/NOD2 is not a predisposing factor for necrotizing enterocolitis. *Dig Dis Sci* 2005; **50:** 1684–87.

129 Halpern MD, Clark JA, Saunders TA, et al. Reduction of Experimental Necrotizing Enterocolitis with Anti-TNF-alpha. *Am J Physiol Gastrointest Liver Physiol* 2006; **290:** G757–64.

130 Seitz G, Warmann SW, Guglielmetti A, et al. Protective effect of tumor necrosis factor alpha antibody on experimental necrotizing enterocolitis in the rat. *J Pediatr Surg* 2005; **40:** 1440–45.

131 Treszl A, Kocsis I, Szathmari M, Schuler A, Tulassay T, Vasarhelyi B. Genetic variants of the tumour necrosis factor-alpha promoter gene do not influence the development of necrotizing enterocolitis. *Acta Paediatr* 2001; **90:** 1182–85.

132 Treszl A, Heninger E, Kalman A, Schuler A, Tulassay T, Vasarhelyi B. Lower prevalence of IL-4 receptor alpha-chain gene G variant in very-low-birth-weight infants with necrotizing enterocolitis. *J Pediatr Surg* 2003; **38:** 1374–78.

133 Kanto WP Jr, Hunter JE, Stoll BJ. Recognition and medical management of necrotizing enterocolitis. *Clin Perinatol* 1994; **21:** 335–46.

134 Wilson R, Kanto WP Jr, McCarthy BJ, Burton A, Lewin P, Feldman RA. Age at onset of necrotizing enterocolitis: an epidemiologic analysis. *Pediatr Res* 1982; **16:** 82–85.

135 Andrews DA, Sawin RS, Ledbetter DJ, Schaller RT, Hatch EI. Necrotizing enterocolitis in term neonates. *Am J Surg* 1990; **159:** 507–09.

136 Bell MJ. Neonatal necrotizing enterocolitis. *N Engl J Med* 1978; **298:** 281–82.

137 Johnson JF. Pneumatosis in the descending colon: preliminary observations on the value of prone positioning. *Pediatr Radiol* 1988; **19:** 25–27.

138 Merritt CR, Goldsmith JP, Sharp MJ. Sonographic detection of portal venous gas in infants with necrotizing enterocolitis. *AJR Am J Roentgenol* 1984; **143:** 1059–62.

139 Bomelburg T, von Lengerke HJ. Sonographic findings in infants with suspected necrotizing enterocolitis. *Eur J Radiol* 1992; **15:** 149–53.

140 Kirks DR, O'Byrne SA. The value of the lateral abdominal roentgenogram in the diagnosis of neonatal hepatic portal venous gas (HPVG). *Am J Roentgenol Radium Ther Nucl Med* 1974; **122:** 153–58.

141 Hutter JJ Jr, Hathaway WE, Wayne ER. Hematologic abnormalities in severe neonatal necrotizing enterocolitis. *J Pediatr* 1976; **88:** 1026–31.

142 Pourcyrous M, Korones SB, Yang W, Boulden TF, Bada HS. C-reactive protein in the diagnosis, management, and prognosis of neonatal necrotizing enterocolitis. *Pediatrics* 2005; **116:** 1064–69.

143 Kosloske AM. Indications for operation in necrotizing enterocolitis revisited. *J Pediatr Surg* 1994; **29:** 663–66.

144 Edelson MB, Sonnino RE, Bagwell CE, Lieberman JM, Marks WH, Rozycki HJ. Plasma intestinal fatty acid binding protein in neonates with necrotizing enterocolitis: a pilot study. *J Pediatr Surg* 1999; **34:** 1453–57.

145 Guthmann F, Borchers T, Wolfrum C, Wustrack T, Bartholomaus S, Spener F. Plasma concentration of intestinal- and liver-FABP in neonates suffering from necrotizing enterocolitis and in healthy preterm neonates. *Mol Cell Biochem* 2002; **239:** 227–34.

146 Faingold R, Daneman A, Tomlinson G, et al. Necrotizing enterocolitis: assessment of bowel viability with color doppler US. *Radiology* 2005; **235:** 587–94.

147 Kim WY, Kim WS, Kim IO, Kwon TH, Chang W, Lee EK. Sonographic evaluation of neonates with early-stage necrotizing enterocolitis. *Pediatr Radiol* 2005; **35:** 1056–61.

148 Dimmitt RA, Meier AH, Skarsgard ED, Halamek LP, Smith BM, Moss RL. Salvage laparotomy for failure of peritoneal drainage in necrotizing enterocolitis in infants with extremely low birth weight. *J Pediatr Surg* 2000; **35:** 856–59.

149 Hall NJ, Curry J, Drake DP, Spitz L, Kiely EM, Pierro A. Resection and primary anastomosis is a valid surgical option for infants with necrotizing enterocolitis who weigh less than 1000 g. *Arch Surg* 2005; **140:** 1149–51.

150 Patole S. Strategies for prevention of feed intolerance in preterm neonates: a systematic review. *J Matern Fetal Neonatal Med* 2005; **18:** 67–76.

151 Schanler RJ, Lau C, Hurst NM, Smith EO. Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. *Pediatrics* 2005; **116:** 400–6.

152 Eibl MM, Wolf HM, Furnkranz H, Rosenkranz A. Prevention of necrotizing enterocolitis in low-birth-weight infants by IgA-IgG feeding. *N Engl J Med* 1988; **319:** 1–7.

153 Foster J, Cole M. Oral immunoglobulin for preventing necrotizing enterocolitis in preterm and low birth-weight neonates. *Cochrane Database Syst Rev* 2004; **1:** CD001816.

154 Shah P, Shah V. Arginine supplementation for prevention of necrotising enterocolitis in preterm infants. *Cochrane Database Syst Rev* 2004; **4:** CD004339.

155 Ledbetter DJ, Juul SE. Erythropoietin and the incidence of necrotizing enterocolitis in infants with very low birth weight. *J Pediatr Surg* 2000; **35:** 178–82.

156 Egan EA, Nelson RM, Mantilla G, Eitzman DV. Additional experience with routine use of oral kanamycin prophylaxis for necrotizing enterocolitis in infants under 1,500 grams. *J Pediatr* 1977; **90:** 331–32.

157 Siu YK, Ng PC, Fung SC, et al. Double blind, randomised, placebo controlled study of oral vancomycin in prevention of necrotising enterocolitis in preterm, very low birthweight infants. *Arch Dis Child Fetal Neonatal Ed* 1998; **79:** F105–09.

158 Pacha J. Development of intestinal transport function in mammals. *Physiol Rev* 2000; **80:** 1633–67.

159 Rumbo M, Schiffrin EJ. Ontogeny of intestinal epithelium immune functions: developmental and environmental regulation. *Cell Mol Life Sci* 2005; **62:** 1288–96.

160 McClure RJ. Trophic feeding of the preterm infant. *Acta Paediatr Suppl* 2001; **90:** 19–21.

161 Newell SJ. Enteral feeding of the micropremie. *Clin Perinatol* 2000; **27:** 221–34, viii.

162 Bourlioux P, Koletzko B, Guarner F, Braesco V. The intestine and its microflora are partners for the protection of the host: report on the Danone Symposium The Intelligent Intestine, held in Paris, June 14, 2002. *Am J Clin Nutr* 2003; **78:** 675–83.

163 Bin-Nun A, Bromiker R, Wilschanski M, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. *J Pediatr* 2005; **147:** 192–96.

164 Lin HC, Su BH, Chen AC, et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 2005; **115:** 1–4.

165 Kliegman RM, Willoughby RE. Prevention of necrotizing enterocolitis with probiotics. *Pediatrics* 2005; **115:** 171–72.

166 Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Shetty AK. Lactobacillus sepsis associated with probiotic therapy. *Pediatrics* 2005; **115:** 178–81.

167 Ouwehand AC, Derrien M, de Vos W, Tiihonen K, Rautonen N. Prebiotics and other microbial substrates for gut functionality. *Curr Opin Biotechnol* 2005; **16:** 212–17.

168 Knol J, Boehm G, Lidestri M, et al. Increase of faecal bifidobacteria due to dietary oligosaccharides induces a reduction of clinically relevant pathogen germs in the faeces of formula-fed preterm infants. *Acta Paediatr Suppl* 2005; **94:** 31–33.

169 Fanaro S, Boehm G, Garssen J, et al. Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: a review. *Acta Paediatr Suppl* 2005; **94:** 22–26.

170 Tsukahara T, Iwasaki Y, Nakayama K, Ushida K. Stimulation of butyrate production in the large intestine of weaning piglets by dietary fructooligosaccharides and its influence on the histological variables of the large intestinal mucosa. *J Nutr Sci Vitaminol (Tokyo)* 2003; **49:** 414–21.

171 Bartholome AL, Albin DM, Baker DH, Holst JJ, Tappenden KA. Supplementation of total parenteral nutrition with butyrate acutely increases structural aspects of intestinal adaptation after an 80% jejunoileal resection in neonatal piglets. *JPEN J Parenter Enteral Nutr* 2004; **28:** 210–23.

172 Kanauchi O, Andoh A, Iwanaga T, et al. Germinated barley foodstuffs attenuate colonic mucosal damage and mucosal nuclear factor kappa B activity in a spontaneous colitis model. *J Gastroenterol Hepatol* 1999; **14:** 1173–79.

173 Yin L, Laevsky G, Giardina C. Butyrate suppression of colonocyte NF-kappa B activation and cellular proteasome activity. *J Biol Chem* 2001; **276:** 44641–46.

174 Venkatraman A, Ramakrishna BS, Shaji RV, Kumar NS, Pulimood A, Patra S. Amelioration of dextran sulfate colitis by butyrate: role of heat shock protein 70 and NF-kappaB *Am J Physiol Gastrointest Liver Physiol* 2003; **285:** G177–84.

175 Avivi-Green C, Polak-Charcon S, Madar Z, Schwartz B. Apoptosis cascade proteins are regulated in vivo by high intracolonic butyrate concentration: correlation with colon cancer inhibition. *Oncol Res* 2000; **12:** 83–95.

176 Mentschel J, Claus R. Increased butyrate formation in the pig colon by feeding raw potato starch leads to a reduction of colonocyte apoptosis and a shift to the stem cell compartment. *Metabolism* 2003; **52:** 1400–05.

177 Scheppach W, Weiler F. The butyrate story: old wine in new bottles? *Curr Opin Clin Nutr Metab Care* 2004; **7:** 563–67.

178 Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 2004; **118:** 229–41.

179 Strober W. Epithelial cells pay a Toll for protection. *Nat Med* 2004; **10:** 898–900.

180 Abreu MT, Vora P, Faure E, Thomas LS, Arnold ET, Arditi M. Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. *J Immunol* 2001; **167:** 1609–16.

181 Rachmilewitz D, Katakura K, Karmeli F, et al. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology* 2004; **126:** 520–28.