Gastrointestinal development and meeting the nutritional needs of premature infants¹–⁴

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ABSTRACT
The fear of necrotizing enterocolitis and feeding intolerance are major factors inhibiting the use of the enteral route as the primary means of nourishing premature infants. Parenteral nutrition may help to meet many of the nutritional needs of these infants, but has significant detrimental side effects that include intestinal atrophy, sepsis, and increased susceptibility to inflammatory stimuli and systemic inflammatory responses. Being able to minimize the use of the parenteral route and still maintain appropriate nutrition safely would be a major advance in neonatology. At the basis of our inability to use the enteral route is a poorly understood immature gastrointestinal tract. Approaches such as minimal enteral nutrition or trophic feedings may partially alleviate these problems. However, if we are to progress in greater utilization of the gastrointestinal tract, other factors need to be considered. These include the macronutrient composition of minimal enteral nutrition or trophic feedings and the microecology of the intestinal lumen. Some of the developmental aspects of the intestine, which include intestinal growth, motor activity, barrier and other innate immune functions, and the microecology of the developing intestine, are briefly reviewed here. The purpose of this review is to suggest important areas of future research in neonatal and developmental gastroenterology that could affect several conditions that are related to immaturity of the gastrointestinal tract. Am J Clin Nutr 2007;85(suppl):629S–34S.

KEY WORDS Developmental gastroenterology, intestinal growth, microecology, premature infants, necrotizing enterocolitis

INTRODUCTION
Feeding intolerance and necrotizing enterocolitis (NEC) are all too commonly encountered problems in neonatal intensive care that result in significant mortality and morbidity, extended hospital stays, and significant costs of care. At the basis of these problems is an immature gastrointestinal tract that remains poorly understood. Our inability to adequately utilize the intestinal tract for nutrition of premature infants necessitates the use of the parenteral route, which is associated with major complications; these include an increased risk of sepsis, intestinal atrophy, and an increased susceptibility to inflammatory stimuli, liver failure, and thromboses.

The purpose of this review is to briefly discuss intestinal immaturity and to suggest areas of future research in neonatal and developmental gastroenterology that could affect feeding intolerance and NEC as well as several other conditions, such as late-onset sepsis.

NECROTIZING ENTEROCOLITIS
NEC is one of the most feared diseases in the neonatal intensive care unit (NICU) because it can progress rapidly from mild abdominal distension and feeding intolerance to fulminating septic shock, necrosis of the entire intestine, and death. Mortality ranges from 20% to 50% and morbidity includes but is not limited to strictures, adhesions, and short bowel syndrome. The specter of NEC has caused many neonatologists and pediatric surgeons to routinely withhold enteral feedings in neonates for prolonged periods during a highly sensitive phase of development.

The primary risk factor for NEC is prematurity, because the incidence varies inversely with gestational age. About 90% of cases occur in premature infants, and NEC is rarely seen in older infants and children (1–3). Other entities seen in adults and older children, such as “darmbrand,” pigbel, and typhilitis, show some resemblance to NEC, but appear to have different pathogenic origins (4). Furthermore, compared with that in preterm infants, NEC in term and late preterm infants has a greater association with other predisposing factors, such as low APGAR scores, chorioamnionitis, exchange transfusions, prolonged rupture of membranes, congenital heart disease, and neural tube defects (5).

The more premature the infant, the later NEC appears to occur after birth. Another entity, spontaneous intestinal perforations, which is sometimes confused with NEC, frequently is not accompanied by significant intestinal necrosis, occurs earlier than NEC, and is associated with the use of glucocorticoids and indomethacin, but probably not enteral feeding (6, 7).

INTESTINAL IMMATURITY, GROWTH, AND MOTILITY
As the premature infant matures, several aspects of gastrointestinal development become important issues in terms of the capability of the gastrointestinal tract to function as an organ of digestion and absorption. However, the intestine not only serves as a digestive absorptive organ, it also is one of the largest immune organs of the body, plays a major endocrine and exocrine role, and encompasses neural tissue equivalent to that of the...
Entire spinal cord. As the gastrointestinal tract develops, tremendous growth occurs, with a doubling of intestinal length in the last trimester of pregnancy; however, the surface area increase is even more dramatic largely because of the villus and microvillus growth during this period of development.

Various aspects of intestinal motor function appear to be one of the most critical problems resulting in feeding intolerance in low-birth-weight infants. Suck-swallow coordination usually not developed until \( \geq 34 \) wk gestation. Motility and gastric emptying can be delayed, and some infants take considerably longer to feed normally. This is certainly a factor keeping many infants from being able to tolerate enteral feedings. This may extend to later gestational ages (8). Esophageal tone is considerably lower in infants <34 wk gestation, but may extend to later gestational ages (8).

The motility of the small intestine is considerably less organized in premature infants than in term infants (8, 10). This is caused by an intrinsic immaturity of the enteric nervous system that delays transit, causing subsequent bacterial overgrowth and distension from gases that are the byproducts of fermentation. It is likely that this immature motility contributes to the milieu in which the interaction of nutrients, immature host defenses, and other factors initiates the cascade of events including transgression of microbes or their toxic products through a immature intestinal mucosal barrier, which eventually culminates in an inflammatory cascade, leading to NEC (11).

Immature mucosal barrier function and immune response are thought to make premature neonates particularly susceptible to intestinal inflammation and injury (12, 13). The incomplete innervation and poor motility of the premature gastrointestinal tract leads to stasis and bacterial overgrowth. The premature gastrointestinal tract also has increased permeability, low levels of protective mucus and secretory immunoglobulin A, and decreased regenerative capabilities, which results in a greater potential for tissue damage (14).

**INNATE IMMUNITY**

The development of the innate and adaptive immune systems of human infants remains largely unexplored. Several aspects of the innate immune system are beginning to emerge as not only critical in short-term diseases during the immediate neonatal period, but also to play a role during later life. The intestinal barrier is critical in terms of preventing bacterial translocation and initiating the inflammatory response, which might affect the well-being of not only the intestine but distal organs such as the lung and central nervous systems as well (15–17).

One of the first lines of defense against ingested pathogens and toxins is luminal digestion. Immature physiochemical luminal factors include a lower hydrogen ion output in the stomach (18) and low pancreatic proteolytic enzyme activity (19). A relatively low enterokinase activity and subsequent low tryptic activity is likely to suppress the hydrolysis of toxins that can damage the intestine. Thus, immature luminal digestion can predispose entry of pathogens from the environment and allow colonization by pathogens in the distal gastrointestinal tract. In fact, recent studies suggest that further decreasing the already low acid output of the stomach by use of H2 blockers in premature infants is associated with a higher incidence of NEC (20).

What we know of the barrier function and the inflammatory potential of the intestine comes primarily from studies in animals and cell culture, but a few studies in human infants are also beginning to provide some clues. Studies in animals have shown that the intestinal mucin blanket seems to be scant in newborns and has a different composition than in adults (2), which appears to make the immature intestine more permeable to high-molecular-weight molecules. This may also facilitate greater bacterial adherence to the epithelium.

Intestinal permeability also is higher in immature neonates than in older children and adults (21). Preterm infants born at <33 wk of gestation have higher serum concentrations of \( \beta \)-lactoglobulin than do term infants given equivalent milk feedings (22). The permeability of the preterm human intestine to intact carbohydrate markers such as lactulose exhibits a developmental pattern of increased permeability with maturation (21). Little is currently known about the maturation of tight junction proteins such as occludin and claudins, which constitute the major paracellular barrier of the epithelium (23).

**INFLAMMATORY MEDIATORS**

Similar to sepsis and adult respiratory stress syndrome, NEC seems to involve a final common pathway that includes the endogenous production of inflammatory mediators involved in the development of intestinal injury. Endotoxin lipopolysaccharide, platelet-activating factor (PAF), tumor necrosis factor, and other cytokines together with prostaglandins and leukotrienes and nitric oxide are thought to be involved in the final common pathway of NEC pathogenesis (24).

Certain bacteria possess endotoxins that instigate the inflammatory cascade by activating PAF, tumor necrosis factor, and interleukin 1. Interleukin 8, a potent neutrophil attractant chemokine also appears to be involved in this process (25, 26). Other cytokines that appear to play a role, such as interleukin 12 and interleukin 18, have also recently been implicated (27). A better understanding of this cascade is critical because intervention or prevention of the cascade by nutritional or pharmacologic means could be the key to prevention of NEC. For example, PAF injected into the aorta of adult rats has been found to cause necrosis of the bowel that can be prevented by pretreatment with PAF-acetylhydrolase and can be exacerbated by a nitric oxide synthase inhibitor (24). Certain nutrients such as omega-3 fatty acids (28), glutamine (29), arginine (30), and probiotic bacteria (31) may also play a role in the prevention of NEC via the interruption or prevention of the inflammatory cascade.

**MICROBIAL ENVIRONMENT**

Humans and other mammals are colonized by a vast array of microorganisms, the so-called microbiota of the gastrointestinal tract. The function of this large quantity of microorganisms, which comprises mainly commensals and symbionts, is just beginning to be understood. Some of the currently known functions involve luminal digestion of otherwise unabsorbed carbohydrates (32) and secretion of fatty acids such as butyrate that play a role in the maintenance of intestinal barrier function and proliferation (33, 34). A cross-talk exists between microbes and the
intestine whereby stimulation of the secretion of peptides by Paneth cells promotes angiogenesis, growth, and also an environment that prevents the growth of potentially pathogenic microorganisms (32, 35–38).

Several lines of evidence support the thesis that infection is necessary for the development of NEC (39). Bacteria are often isolated from the blood of infants with NEC. However, it can be debated whether these isolated bacteria play the primary role in the pathogenesis of NEC. The large variety of bacteria associated with NEC suggests that they are actually bystanders that amplify another process or set of processes. It is also possible that the microorganisms that have been isolated in association with NEC have a not yet found common pathogenic feature that incites the disease.

Most very premature infants in the NICU are started on broad-spectrum antibiotic therapy shortly after birth during a “rule-out-sepsis” workup. This can alter the normal flora with which the neonate would become colonized (40). Rather than Lactobacillus, Bifidobacter, or other symbiotic microorganisms, resistant species indigenous to the NICU may colonize the infant’s intestines. Whether or how much of a role this has in the pathogenesis of NEC is not known, but certain pathogenic microorganisms have a greater propensity to activate cell surface receptors that transduce signaling molecules such as nuclear factor kappa B to the nucleus, which in turn incites a proinflammatory response via the synthesis of proinflammatory cytokines and chemokines (36).

The commensal microflora may represent a key regulatory checkpoint for the intestinal inflammatory response. The intestinal epithelium partially relies on toll-like receptors (TLRs) to act as an interface between the luminal microflora and cellular signal transduction pathways. TLRs are cell surface receptors that recognize specific microbial ligands, from both pathogens and commensals, which enables the innate immune system to recognize non-self and activates both innate and adaptive immune responses (41). Recent studies suggest that the epithelium and resident immune cells do not simply tolerate commensal microorganisms but are dependent on them (42). This is important for not only disease entities that we see in the NICU such as NEC, which was recently shown to decrease with the use of probiotics (43–45), but also diseases that affect the infant later in life, such as allergy and atopy (46). The mechanisms of the effects of probiotics on later health are fascinating, and studies on the relation between innate and adaptive immunity of the developing gastrointestinal tract are beginning to shed light on their mechanisms of action (47–49).

PROBIOTICS

Probiotics are defined by the World Health Organization as “live microorganisms, which when administered in adequate amount confer a health benefit on the host” (Internet: http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf#search=%20health%20organization%20definition%20of%20probiotics). Recent studies evaluating the use of probiotics in preterm infants for the prevention of NEC (43, 45, 50) have poised neonatologists for routine use in the very near future. In fact, some NICUs in the United States have already started using these agents. However, numerous misadventures from hastily instituted interventions in neonatal intensive care, such as high oxygen therapy causing blindness and prolonged high-dose dexamethasone therapy leading to cerebral palsy (51, 52), mandate that we proceed carefully. Currently, we know little about correct probiotic dosages, their mechanisms of action, which of the numerous probiotics are most efficacious, and whether there might be better, potentially safer alternatives such as inactivated probiotic bacteria or their components. If probiotics are widely used in the near future without additional studies, it is this author’s opinion that we are permitting the use of these agents without the same standards that we would apply to safety testing for pharmacologic or nutraceutical agents. For example, dose-response studies in infant animal models have not been performed, and recent studies that used cell cultures suggest that high doses may be detrimental (53, 54). The case reports of sepsis from probiotics in immunocompromised infants (55, 56) and studies showing significant mortality and pathology in immunocompromised neonatal mice administered probiotics (57) should also be taken seriously. It is possible that inactivated probiotics or their products may prove to be just as efficacious, but safer than live bacteria. Studies that delineate the role of the intestinal microflora in developing mammals under different nutritional situations (eg, breast versus formula feeding) have not been done, despite studies that suggest that microflora endogenously present in human milk (58, 59) may play a protective role, potentially obviating the effect of probiotics. Dose-response studies in animals would be important to evaluate safety before widespread use in human infants. Furthermore, our understanding of basic mechanisms of action of commensal intestinal microbes and probiotics are just beginning to emerge (60). Although common dogma suggests that a biotherapeutic agent must be alive to be useful, recent data suggest that inactivated bacteria or their products may also exert beneficial effects (61–63). If inactivated probiotic bacteria or their products are able to provide similar beneficial effects through similar mechanisms of action, without the potential of overgrowth, sepsis, long-term establishment of a potentially harmful foreign microbial intestinal ecosystem, and other safety concerns, this would certainly warrant additional studies of these agents before routine use in these highly vulnerable premature neonates. These concerns are similar to those we (64) and others (65) addressed in written commentaries on the recently published trials of probiotics in premature infants (43, 45).

Two recent reports showed that preterm human infants randomly assigned to receive a daily feeding supplement of a probiotic mixture (Bifidobacteria infantis, Streptococcus thermophilus, and Bifidobacteria bifidus in one study, and Lactobacillus acidophilus and Bifidobacterium infantis in the other) had a relative risk reduction in NEC and death and also appeared to have decreased late-onset sepsis (43, 45). It also bears recalling that a large multicenter trial conducted in 12 Italian NICUs that included 565 patients did not elicit a statically significant beneficial effect of probiotics (Lactobacillus GG) on NEC (66). Whether the differences in outcomes in these studies are associated with the use of different probiotic preparations, a different baseline incidence of NEC in the various NICUs, or other factors such as breast milk feeding remain speculative. This is important because previous studies suggested that human breast milk may actually contain beneficial microbes that are independent of those derived from the amniotic fluid (58, 59). The role of human milk versus formula feedings in the probiotic studies in premature infants (43, 45, 50, 66) was not critically examined. Commentary on the Lin et al trial of prevention of

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NEC with probiotics favors proceeding with multicenter trials (65, 67). However, exposure of nearly 2000 infants (which would be required for an adequate sample size for such a study) should be undertaken with appropriate precaution. Choice of probiotic, dosage, specific outcome measures to be evaluated, and indicators of safety are all issues that need to be addressed in such a study in human infants, and preliminary evaluation in an infant animal model can at least partially address these issues.

IS IT POSSIBLE TO DECREASE THE USE OF PARENTERAL NUTRITION BY INCREASING THE USE OF THE IMMATURE GASTROINTESTINAL TRACT?

In many NICUs, the critically ill, low-birth-weight infant's gastrointestinal tract is either not exposed to nutrients at all for several weeks after birth or is provided with "minimal enteral nutrition," and most nutrients are provided by the parenteral route. Considerable evidence is accumulating that this approach is far from ideal. Studies in animals and adults have shown that a lack of luminal nutrients results in mucosal atrophy, a lack of stimulation of trophic hormones, sepsis, increased polymorphonuclear attraction, and an increased likelihood of development of the systemic inflammatory response syndrome (68) and translocation of intestinal microbes (69). The intestinal surface epithelium is polarized with an apical microvillus surface that usually derives most of its nutrients from the luminal rather than the basal surface. Because of higher requirements for intestinal growth in the neonatal period, the lack of luminal nutrients may be even more important in infants than in adults. It is reasonable to posit that a lack of luminal nutrients may actually underlie some of the non-intestinal pathology seen in the NICU, such as hospital-acquired sepsis and chronic lung disease.

A continuous flux of amniotic fluid through the gastrointestinal tract during the last trimester of pregnancy is a component of normal physiology. The fetus swallows ≈450 mL of amniotic fluid per day. This is suddenly interrupted at the time of premature birth. However, the extraterine and intrauterine environments are quite different, and the foods currently available to premature infants are very different from amniotic fluid. Even if amniotic fluid were available, the intestinal environment is no longer sterile and the nutritional needs for the gastrointestinal tract would likely be quite different from the in utero needs. An understanding of the anatomical, mechanical, and digestive absorptive aspects of intestinal development should aid in finding ways to improve the capability of premature infants to safely utilize the gastrointestinal tract for nutrition. Two specific areas of interest relate to mechanical problems and increasing the surface area and subsequent digestive absorptive capability.

Mechanical problems include suck-swallow incoordination, which necessitates tube feeding; poor esophageal sphincter tone, which increases reflux; and delayed gastric emptying and disorganized intestinal motility, which lead to feeding intolerance and intestinal stasis. Currently, we have a poor understanding of the development of neurological and endocrine factors that control intestinal motility. For example, it has clearly been shown that migrating motor complexes are disorganized in the immature gastrointestinal tract (8), but the mechanisms are not understood. Is this caused by a lack of development of motilin receptors, dysregulation of hormones such as peptide YY, poor neuronal innervations, or a combination of these factors? Being able to dissect specific immaturities could help direct therapies, such as those that have been accomplished with the immature lung, eg, surfactant therapy.

The surface area of the intestine is arguably the largest interface between the internal and external milieu of the body. Significant growth in length and especially mucosal surface area occurs during the last trimester of pregnancy. Do we inadvertently blunt some of this growth by our NICU feeding practices? Animals and humans that are exclusively fed by the parenteral route demonstrate a marked decrease in mucosal growth (70). Although several physiologic benefits can be provided by minimal enteral feedings (71), one study suggested that at least 40% of nutritional requirements need to be supplied by the enteral route before significant advantages in terms of mucosal growth are elicited (72). It is highly likely that protein provided by the enteral route would serve as a much greater stimulus for growth than would similar quantities of lipid or carbohydrate. Several studies have suggested that there are likely to be specific milkborne factors that are important for intestinal mucosal growth. Examples include glucagon-like peptide 2 (73) and several other factors found in human milk (74). Although these factors hold promise, it is unlikely that they will provide a meaningful trophic effect to the intestine in the face of undernutrition. Relatively simple interventions such as supplying proteins in complete or hydrolyzed form to the gastrointestinal tract without the osmotic load incurred by carbohydrates and other solutes should probably be evaluated first.

Certain nutrients may play an active role in maintenance of the barrier function of the intestine as well as down-regulating inflammation. Butyrate, a short-chain fatty acid produced by bacterial fermentation, has been found to play a major role in intercellular junction integrity (75). Certain drugs that are commonly used in the NICU, such as indomethacin, cause a breakdown of intercellular junctions (76). Certain nutrients, such as n–3 fatty acids and amino acids such as glutamine, have been found to play a potential role in immunity and the down-regulation of intestinal inflammation (28, 77) and offer exciting areas for future investigation.

SUMMARY

This review has described areas relating to the neonatal gastrointestinal tract in which some of our research efforts should be focused. These include improving our understanding of gastrointestinal development to develop means to enhance intestinal morphology (surface area), physiology (motility), and the innate immune system. The importance of the intestinal microflora and its interaction with the developing intestinal mucosa offers exciting new areas for investigation, including live and inactivated probiotics, microbial components, and Toll-like receptor agonists.

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