Adverse Host Responses to Bacterial Toxins in Human Infants

Uzma Shah and W. A. Walker

Harvard Medical School, Boston, MA and Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital and Children’s Hospital, Boston, MA

ABSTRACT Bacterial toxin interaction with the intestinal epithelium is regulated developmentally as well as by nutritional factors. It is the binding of bacterial toxins to the epithelium followed by several events that forms the basis of infantile diarrhea, a leading cause of morbidity and mortality world-wide. There has been increasing interest in bacterial toxin interaction with the enterocyte, postreceptor events that follow and the effect of developmental regulation on necrotizing enterocolitis. Diet and environmental factors can provide a major influence on bacterial-enterocyte interaction. Particularly important is the role of breast milk and its constituents, as well as probiotics, in this regard. The purpose of this review is to provide a brief overview on this complex interaction. J. Nutr. 130: 420S–425S, 2000.

KEY WORDS: • bacterial toxin • enterocyte • developmental regulation

Developmental regulation and nutritional processes influence bacterial toxin interaction with the intestinal epithelium. The binding of bacterial toxins to microvillous membrane receptors, signal transduction and fluid secretion by the developing intestine form the basis of toxigenic diarrhea in the infant population. Several factors may influence this diarrhea in infants. For example, developmental control of receptor expression may involve the regulation of individual glycosyltransferases responsible for the addition of receptor sugar sequences to glycolipids and glycoproteins at the transcriptional level (Chu et al. 1989, Chu and Walker 1991, Cohen et al. 1986, Field et al. 1989). The role of human milk, host defenses and luminal factors in the prevention of diseases such as necrotizing enterocolitis (NEC)1 have been the focus of research in recent years. The purpose of this review will be to provide a brief insight into the significance of intracellular events that follow toxin-receptor interactions in the developing gut.

The mucosa forms the first barrier to antigens presented at the epithelial surface. The mucosal barrier comprises several components, including factors such as gastric pH, gastric and pancreatic enzymes, a glycoprotein-rich mucin layer and an intact microvillous enterocyte surface under growth factor control. Surface immunoglobulin (Ig)A and Ig M also provide surface protection at the epithelial surface (Table 1).

On exposure to antigens and bacteria, the intestinal epithelium mounts an immune response. This immune response depends on the balance between the antigen-handling and antigen-presenting capabilities of the intestinal mucosa. It is also dependent on bacterial adherence and colonization, and the release of inflammatory cytokines from the epithelium. There is a fine balance between the release of proinflammatory and anti-inflammatory cytokines from the mucosa that determines the manifestation of the inflammatory response at the epithelial surface. Proinflammatory cytokines released include interleukin (IL)-1, IL-6, γ-interferon, tumor necrosis factor (TNF) and IL-8, whereas anti-inflammatory cytokines include IL-1Ra, transforming growth factor-β, IL-4 and IL-10. Apart from the actions of cytokines, there are a variety of other events that occur at the epithelial surface on exposure to bacterial colonization or adherence; these include the release of prostaglandins (PG), up-regulation of molecules such as class II antigens, poly A receptor, cytokine receptors and the release of growth factors and growth factor–binding proteins.

Toxin interaction with the intestine

Exotoxin interaction with the enterocyte. Much of our understanding of bacterial epithelial interaction comes from studies on cholera toxin and the mucosa. Cholera toxin (CT) is an 84-kDa protein secreted by Vibrio cholera that colonizes the upper small intestine. The binding component consists of five identical B- (binding) subunits that bind specifically to the upper small intestine. The binding component consists of five identical B- (binding) subunits that bind specifically to apical ganglioside GM1 receptors activating adenylate cyclase...
TABLE 1

Components of the neonatal mucosal defense barrier

<table>
<thead>
<tr>
<th>Nonimmunologic</th>
<th>Immunologic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric acidity</td>
<td>Intraepithelial T cells (CD8)</td>
</tr>
<tr>
<td>Gastric enzymes</td>
<td>Lamina propria T cells (CD4)</td>
</tr>
<tr>
<td>Pancreatic enzymes</td>
<td>B cells</td>
</tr>
<tr>
<td>Peristalsis</td>
<td>Gut-associated lymphoid tissue (Peyer’s patches)</td>
</tr>
<tr>
<td>Mucin layer</td>
<td>Secretory immunoglobulin (IgA) system</td>
</tr>
<tr>
<td>Enterocyte molecules (cytokines, growth factors)</td>
<td>Non-IgA immunoglobulin</td>
</tr>
<tr>
<td></td>
<td>IgE-mucosal mast cell system</td>
</tr>
</tbody>
</table>

1 Source: Insoft et al. (1996).

to induce a massive secretory response. The A- (activation) subunit is comprised of two peptides linked by noncovalent interactions and a disulfide bond. CT activation of adenylate cyclase involves several steps. Binding of the B-subunits to the ganglioside GM1 on the cell surface is followed by translocation of the A-subunit across the membrane. The disulfide bond is then reduced to form the enzymatically active A-subunit that catalyzes GDP-ribosylation of the regulatory GTPase Gs-α and in turn activates adenylate cyclase. GM1 receptors with which CT makes contact lie on the apical surface of polarized cells, whereas the activation of adenylate cyclase occurs at the basolateral membrane. The intracellular route that CT takes to achieve these effects was previously unclear. One of the possibilities was that the A-subunit penetrates the plasma or possibly the endosomal membrane. The hypothesis that endocytosis and vesicular transport are required for the action of CT on polarized intestinal epithelial cells was tested using a T84 human intestinal epithelial cell line that forms confluent monolayers of polarized columnar cells displaying features of crypt cells. When applied to apical or basolateral surfaces, CT induced a cAMP-dependent chloride secretory response. Lencer et al. (1995) showed that the toxin requires endocytosis and processing in intracellular compartments to elicit its effect. They then proceeded to show that cholera toxin in fact entered basolaterally directed transport vesicles and both B- and A-subunits were hence delivered intact to the basolateral membrane, identifying a possible explanation for the surface receptor and basolateral effects seen with cholera toxin. Toxin-induced signal transduction depended on the specificity of the ganglioside receptor and on the coupling of cholera toxin with caveolae or caveolae-related membrane domains (Wolf et al. 1998). CT may also stimulate intestinal secretions by an activation of the enteric nervous system and a secondary activation of arachidonic acid metabolites, causing an increase in the production of PG. Cholera toxin and heat labile Escherichia coli have been considered previously to be identical in their mode of action by increasing intracellular adenosine 3–5 cyclic monophosphate concentration. As recently shown by Turvill et al. (1998), the secretagogues 5-hydroxytryptamine (5-HT) is involved in CT secretion but not in labile toxin (LT)secretions. CT-mediated secretions were reduced by 5-HT depletion and inhibited by 5-HT antagonists.

Endotoxin interaction. Endotoxin or lipopolysaccharide (LPS) is the outer membrane glycolipid of gram-negative bacteria. On release from bacteria, it stimulates mediators from host cells and may lead to septic shock. LPS-responsive cell types include monocytes/macrophages, polymorphonuclear leukocytes (PMN), and endothelial and epithelial cells. Endotoxin may compromise basal colonic water and electrolyte transport as well as increase intestinal permeability (Detch et al. 1987, Odwyer et al. 1988). Increased release of cytokines, TNF, IL-1, platelet-activating factor (PAF) and oxygen-derived free radicals from the gastrointestinal tract has been shown to occur with endotoxins (Ciancio et al. 1992). Recognition of LPS triggers gene induction by myeloid and non-myeloid cells; these genes encode proteins that include cytokines, adhesive proteins and enzymes that produce low-molecular-weight inflammatory mediators. Thus they upregulate host defense mechanisms that work toward elimination of the bacteria (Ulevitch and Tobias 1995). CD 14 is a protein that plays a key role in LPS-induced cell activation. Sheiffele et al. (1987) have shown an association between endotoxemia and thrombocytopenia in NEC.

Other bacterial products that may play an important role in the intestinal epithelium include butyrate, a short-chain fatty acid that is a product of bacterial fermentation of carbohydrates. Butyrate may not only be an energy source for colonocytes (Roediger 1982) but may also stimulate epithelial cell proliferation and cytokine release (Kripke et al. 1989). Sodium butyrate may increase significantly the release of IL-8 from intestinal epithelial cells, and this effect is particularly marked when the cells are also stimulated with IL-1β or LPS. Butyrate also enhanced IL-8 mRNA in Caco2 cells, suggesting that the intestinal epithelial cell may regulate intestinal inflammation in response to changes in the intestinal luminal contents (Fusunyan et al. 1998).

Clinical consequences of toxin interaction

Necrotizing enterocolitis. NEC is a disease of the immature bowel. It is a clinicopathologic syndrome defined as idiopathic coagulation necrosis and inflammation of the intestine in a neonatal patient (Kleinhaus et al. 1992). Its pathogenesis is multifactorial, with several major factors implicated; these include enteral feeding, intestinal ischemia, bacterial adherence, invasion and proliferation (Kleigman et al. 1993), along with host factors such as gut immaturity. Manifestations vary from a benign feeding intolerance to abdominal distension, diarrhea, intestinal perforation, sepsis and shock. Pathologic changes include mucosal edema, hemorrhage, ischemia, necrosis and bacterial overgrowth (Santulli et al. 1975). NEC is a leading cause of morbidity and mortality in newborns; some reports estimate a >10% incidence among infants weighing <1500 g (Lencer et al. 1995) (Fig. 1). Extensive research done to understand the pathophysiology of NEC suggests that newborn infants may have systemic as well as gastrointestinal mucosal impairments that may predispose them to aberrant gut bacterial overgrowth and subsequent mucosal inflammation and injury (Spencer et al. 1990). In addition to reduced numbers of B cells, decreased IgA-producing plasma cells and decreased T cells in the intestinal mucosa, there may also be an increased intestinal permeability to macromolecules and bacteria that would contribute further to inflammation and fulminant NEC (Spencer et al. 1990). Animal studies done have helped in increasing the understanding of NEC. Neonatal rats fed under hypoxic conditions developed features of NEC. Intravenously infused cytokines such as PAF, TNF-α and LPS have led to pathologic changes in the bowel consistent with NEC (Kleigman and Walsh 1987, Sun and Hsieh 1988, Tracey et al. 1986). Intestinal colonization with cytokinin-producing organisms can also induce different degrees of enterocolitis (Schiefelbe 1990).

The role of infectious organisms in NEC has been also
tridium difficile, cells. Organisms associated with these findings included, development, suggesting that initial colonization was with studied extensively (Book et al. 1977, Goman et al. 1979, Book et al. 1978, Han et al. 1983, Schiefele et al. 1987). In vitro and in vivo animal studies have demonstrated a decrease in intestinal permeability to orally fed protein on administration of growth factors. Similarly, steroid and thyroid hormone may induce maturation of surface enzymes (Chu and Walker 1986, Jumawann et al. 1977), membrane fluidity (Israel et al. 1987), surface glycoconjugates and receptors (Pang et al. 1987). Changes in intestinal permeability (Daniels et al. 1973, Israel et al. 1986) and bacterial colonization have also been demonstrated with the use of steroids. Prenatal and postnatal steroid treatment (Bauer et al. 1984, Halac et al. 1990) have also been associated with a decrease in NEC. In a study that reported use of intravenous epidermal growth factor (EGF) was also efficacious in a neonate with NEC (Sullivan et al. 1991). Animal experiments in rat pups with prenatal steroid use showed a decrease in NEC. The number of bacterial colonies was also reduced in steroid-treated pups, suggesting that it may render the pups less susceptible to bacterial organisms.

One of the first immune responses to enteric pathogens is the development of an IgA response with the production of specific IgA antibodies to the antigen. Passively administered antibodies through breast milk concentrates confer a very high level of protection in adults challenged with E. coli or Shigella. Intracellular bacterial infections in the neonate may also elicit a powerful MHC class I restricted cytotoxic lymphocyte response (Brown et al. 1980, Kaufmann 1988). An Fc receptor specific to IgG has been demonstrated in fetal intestine and may play a role in protection against the development of NEC in neonatal intestine.

Enterotoxin induced diarrhea. Toxin intestinal interactions require the following three important steps: 1) toxin binding to the microvillus receptor, 2) signal transduction response, and 3) an enterocyte-effector response. Developmental differences may exist in the intestine at all three levels of this interaction.

Bacterial toxin receptors at the microvillus membrane. Specific oligosaccharides on surface receptors may serve as important factors in bacterial interaction at the enterocyte (Karlsson 1989, Paulson 1989, Rademacher et al. 1988). Binding sites at the surface of the cell may exist as glycolipid or glycoprotein structures. Using a rat glial C6 cell line, Fishman et al. (1980) showed the importance of a glycoprotein in CT binding. This binding also requires the presence of three sugar moieties, galactose, N-acetylgalactosamine and sialic acid. These cell surface carbohydrates are regulated developmentally and are species and tissue specific; hence they may affect age-specific susceptibility to intestinal infections.

Glycolipid receptors. Although the core sequences of glycolipid receptors are structurally similar, it is the terminal oligosaccharide sequence that determines toxin binding specificity. Several such receptors have been identified (Table 2).

Glycoprotein receptors. E. coli produces both an LT and an ST. Although LT binds principally to GM1 on the enterocyte,
one of the receptors for ST is a plasma membrane form of guanylate cyclase (Schulz et al. 1990). Recent evidence also indicates that C. difficile toxin A binds a glycoprotein receptor in hamsters and rabbits (Pothulakis et al. 1988). Tucker and Wilkins (1991) demonstrated that three human carbohydrate antigens with a similar conformation can serve as the binding site to toxin A (Tucker and Wilkins 1991). The three antigens, I, X and Y, can be expressed as either surface glycolipids or glycoproteins.

Developmental changes in membrane receptors and host response

Developmental differences in the number and affinity of surface receptors may play an important role in the development of toxigenic diarrhea. These age-related differences have been studied with the use of binding assays or glycoprotein analysis.

In animal experiments, it was demonstrated earlier that there was a great difference in fluid secretion in the intestine of preweaned rats exposed to CT compared with mature intestine (Chu et al. 1989, Fig. 2). On comparison of receptor numbers and affinity between mature and immature intestine, a similar number of receptors but with a slight increase in binding affinity were noted in the immature intestine. On estimation of coupling efficiency for receptor binding and response, a decrease was seen from 30% in the preweaned to 1% in the weaned rat, suggesting that cellular maturation may account for the decreased receptor effector coupling seen. Other factors that may influence this response include luminal factors such as the presence of enzymes, microflora and mucin at the intestinal cell surface.

ST produces an enhanced response in the immature rat, pig and human gut, a feature that can be explained by an increased number of toxin receptors in the immature intestine (Isberg and Leong 1990). Postreceptor events may also contribute to the developmental variations in host response to bacterial toxins. Although ST acts via guanylate cyclase to increase cGMP, CT acts via activation of cAMP, both producing a secretory response.

Receptor-dependent decrease in host responsiveness. Animal studies have shown a direct correlation between receptor expression and toxin effects with Shiga toxin and C. difficile toxin A. Rabbit intestine shows an age-dependent increase in receptor expression for Shiga toxin with the receptor Gb3 detectable in rat intestine only postweaning (Cohen et al. 1988, Mobasseleh et al. 1988 and 1989). This may explain the relative resistance to Shigella infections in the neonate. With C. difficile, however, receptor underdevelopment in combination with mucosal factors may be important in protection against the toxin. C. difficile receptors in human intestine differ from those present in rabbit intestine, and the organism does not produce disease in the infant even though it is frequently detected in the stool (Eglow et al. 1992, Vanden Waaj 1989).

Developmental control of receptor expression. Because a specific oligosaccharide structure is important in receptor binding by bacteria, regulation of specific sugar expression would control effective bacterial binding to the enterocyte. Although incompletely understood, one of the mechanisms is through the control of glycosyltransferase expression. In the rat small intestine, there is a shift from sialylation to fucosylation of the microvillous membrane with weaning (Torres Pinedo and Mahmood 1984). This increased sialylation is reflected in an increased surface expression of GM3 but not GM1 (Bouhors and Bouhors 1983). An increase in fucosylation of gangliosides has been demonstrated in the neonatal rat gut (Isberg and Leong 1990). The extent to which this increase may contribute to the enhanced response of the immature intestine to CT is uncertain.
transferease activity may also control an increased expression of fucosyl lipids and proteins and therefore enhance C. difficile receptor expression. The activities of galactosyltransferase and N-acetyl galactosaminyltransferase also increase with age but their importance in receptor expression remains to be elucidated.

**External factors.** External factors such as phorbol esters (Ozaki et al. 1989), sodium butyrate (Simmons et al. 1975), nutrients in the diet and various deficiencies such as that of vitamin A (Sato et al. 1984), cortisone and thyroxine (Chu et al. 1989) as well as bacterial products may also influence the expression of surface receptors. Cortisone, known to promote enterocyte maturation when injected into suckling rats, decreased host sensitivity to CT significantly (Chu et al. 1989). These factors may exert control at the transcriptional level by influencing gene expression of glycosyltransferases or by phosphorlation-dephosphorylation.

**Postreceptor events.** In comparison to secretory responses to CT/LT in mature enterocytes, the immature intestine has an exaggerated response (Chu et al. 1989). An increased response in adenylate cyclase activity may be a possible explanation and suggests that postreceptor signal transduction events may be important in the exaggerated secretory response seen in neonates on exposure to toxin (Seo et al. 1989). Gs-α is the target protein for CT. A postweaning decline in this protein has been demonstrated in the rat small intestine. Developmental regulation of Gs-α expression in rat intestine may influence responsiveness to CT (Chu et al. 1991 and 1992).

Additional factors that may influence toxin receptor interaction in the developing gut may include factors such as membrane fluidity in immature vs. mature enterocytes, allowing for greater uptake and migration of the A1 component of CT (Pang et al. 1983). Other factors such as immaturity of the Na+/K+ ATPase Na/Cl cotransporters and the chloride channels may also influence fluid secretion from the enterocyte (Zemelman et al. 1992). With the use of rat microvillous membranes, previous work showed that developmental changes in membrane structure that influence binding affinity but not receptor density may contribute to the increased sensitivity of immature enterocytes to CT.

**Prevention and treatment**

Breast milk contains a variety of factors that have been demonstrated to have anti-inflammatory properties and may protect the neonate from the development of toxin-induced diarrheas. Such cytoprotective factors include PGE₂, PGF₂α, EGF and lactoferrin. Maturational factors such as steroids and thyroid hormones are also protective. Enzymes that degrade inflammatory mediators such as PAF-acetylhydrolase, inflammatory modulators such as lysozymes, secretory IgA, antioxid- dants such as α-tocopherol, ascorbate, β-carotene and uric acid may also play a role in protection.

**Probiotics.** Probiotics such as lactobacilli stimulate mucosal barrier function, by competing with pathogenic bacteria for mucosal colonization and by metabolizing nutrients to promote host defense. These are live microorganisms belonging to normal flora and have low or no pathogenicity. It is becoming increasingly evident that probiotics may be effective tools in controlling bacteria at the mucosal surface. They can control various enteric pathogens such as Salmonella typhimurium, Shigella, Campylobacter and E. coli (Corthier et al. 1985, O’Sullivan et al. 1992, Perdition et al. 1990). Lactobacillus rhamnosus strain GG (ATCC 53103) has proven to be effective in preventing and treating diarrhea in premature infants, newborns, children and travelers (Isolauri et al. 1995, Millar et al. 1993, Sepp et al. 1993). Lactobacillus plantarum prevents the adherence of E. coli to the mucosa and hence interferes with the delivery of endotoxin to the epithelium.

**SUMMARY**

The complex environment that exists within the intestinal lumen is tightly regulated by an interaction between the normal flora and ingested nutrients. Diet and environmental factors can influence this equilibrium. Modified enteric nutrients may serve to down-regulate the inflammatory response induced by bacteria not only by altering bacterial colonization but also by affecting directly enterocyte gene expression of inflammatory cytokines. Specific nutrients such as probiotics may act to enhance intestinal host defense. Further work is required to elucidate the role of nutrients in the mucosal inflammatory response and particularly the role of breast milk in the development of the mucosal immune response.

**LITERATURE CITED**


