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TOPIC HIGHLIGHT

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Recent advances in small bowel diseases: Part I

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Abstract

As is the case in all parts of gastroenterology and hepatology, there have been many advances in our knowledge and understanding of small intestinal diseases. Over 1000 publications were reviewed for 2008 and 2009, and the important advances in basic science as well as clinical applications were considered. In Part I of this Editorial Review, seven topics are considered: intestinal development; proliferation and repair; intestinal permeability; microbiotica, infectious diarrhea and probiotics; diarrhea; salt and water absorption; necrotizing enterocolitis; and immunology/allergy. These topics were chosen because of their importance to the practicing physician.

Key words: Diarrhea; Infectious diarrhea; Intestinal development; Intestinal proliferation and repair; Intestinal permeability; Microbiotica; Necrotizing enterocolitis; Probiotics

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INTESTINAL DEVELOPMENT

The development of the small intestine and its implications for nutritional support has been reviewed previously[1]. The mucosa of the small intestine has many functions other than nutrient absorption and fluid secretion. These functions include host defense and immune responses. All of these small intestinal functions may be influenced by diet as well as by the intestinal microbiotica, and may also be developmentally regulated.

At birth, there is rapid colonization of the intestinal lumen of the neonate, both with bacteria from the mother's milk, such as Gram-positive lactobacilli and bifidobacteria, and colonization with Gram-negative anaerobic bacteria. These bacteria are essential for growth and development of the gastrointestinal (GI) tract. In response to luminal microorganisms, intestinal Paneth cells produce microbicidal peptides, such as α-defensin. Paneth cells differentiate while migrating towards the crypt base, where these cells are eventually cleared by phagocytosis. In addition to the importance of Paneth cells in the innate immune defense against microorganisms, they are also involved in intestinal angiogenesis.

There is a complex interaction between bacteria in the intestinal lumen, the epithelial surface, and the underlying matrix, immune cells and enteric nervous system.

Enterocytes sense pathogenic bacteria, occupy the Toll-like receptors (TLRs) in response to their activation *via* pathogen-associated molecular patterns (PAMPs), leading to nuclear factor (NF)-κB activation. Pattern recognition molecules (PRMs) detect any conserved bacterial products. PRMs, such as TLRs and nucleotide-binding oligomerization domain containing (NOD) receptors, are further important regulators of the intestinal barrier. TLRs are pattern-recognition receptors (PRRs) expressed by immune and nonimmune cells that signal in response to PAMPs expressed by bacteria in the intestinal lumen. Most TLR molecules signal through the adapter molecule MyD88 to interleukin (IL)-1-receptor-associated kinase. MyD88 activates the NF-κB pathway, which is a critical regulator of tumor necrosis factor (TNF)-α and IL-1β. Deficiency in MyD88 increases susceptibility to bowel inflammation, presumably by increasing brush border membrane (BBM) permeability. TLR ligands alter the properties of the intestinal barrier by redistributing zonula occludens (ZO)-1 and β-defensin 2. Cyclooxygenase-2-derived prostaglandin (PG)E_2 is important in TLR mucosal repair[2].

When a child is born prematurely, its intestine is also premature, and is potentially ill-prepared for the extrauterine environment and contact with food bacteria. Microbial manipulation with antibiotic treatment or exposure of the pregnant rat dam to *Escherichia coli* (*E. coli*) results in long-lasting and potentially adverse effects on postnatal microbiotica of the offspring[3]. PAMPs produce an intestinal immune response through interaction with PRRs, including the TLRs. Transmembrane signaling may occur through TLRs[4]. TLRs trigger signaling pathways which lead to NF-κB activation and propagation of inflammatory responses. In addition to exogenous inflammatory mediators, TNF-α as well as IL-1β trigger cytokine production through activation of this NF-κB pathway. The NF-κB proteins activate transcription, resulting in immune and inflammatory responses.

Intestinal alkaline phosphatase (AP) detoxifies lipopolysaccharide (LPS) by hydrolyzing it so that it can no longer activate TLR-dependent inflammatory responses[5,6]. AP is involved in maintenance of homeostasis between the luminal bacterial microbiotica and the body[5,6]. About two-thirds of persons taking short or long courses of nonsteroidal anti-inflammatory drugs (NSAIDs) develop small intestinal damage. The NSAID diclofenac may induce enteropathy by way of the c-Jun-N-terminal kinase (JNK) pathway[7]. This concept of stress-related changes in intestinal barrier and secretory function has therapeutic implications, as does the potential of JNK inhibitors to protect the GI tract from the damage of NSAIDs. For example, the NSAID indomethacin may enter the small intestine through a TLR4/MyD88-dependent pathway[8]. In animals with stress as a result of immobilization, there will be an increase in TNF-α along with an increase in tight junction (TJ) permeability. These permeability changes are associated with alteration in the TJ proteins ZO-1, claudin-2, claudin-4, claudin-5, and β-catenin[9].

IL-8 secretion in response to bacteria, IL-1β and TNF-α are increased more in human fetal small intestinal epithelial cells than in older children or adults. This increased activation of NF-κB DNA binding and transcriptional activity is due to reduced inhibition of signaling, resulting in increased phosphorylation, ubiquitination and degradation of the inhibitor of NF-κB, in conjunction with decreased baseline expression and delayed resynthesis of this inhibitor[10]. This represents a mechanism explaining the enhanced inflammatory response occurring in immature intestinal tissue.

The intestinal epithelial stem cells (ISCs) reside in the crypts of Lieberkuhn, giving rise to five daughter cell lines: Paneth cells, enterocytes, goblet cells, enteroendocrine cells, and M cells. In the upper third of the intestinal crypt, daughter cells differentiate into enterocytes, goblet cells, enterochromaffin cells, and Paneth cells. The Hedgehog platelet-derived growth factor and bone morphogenetic protein (BMP) signaling pathway are key mediators in morphogenesis of the intestinal villi and crypts, and maintain tenancy of ISCs. The ISCs are of two types: the fast cycling "crypt base columnar" (CBC) cells residing among the Paneth cells at the base of the crypt, and the slow cycling ISCs above the Paneth cells (+4ISC). Only one clonogenic cell is needed to regenerate a crypt and to maintain the appropriate number of crypts after injury. It is the +4ISCs that are highly sensitive to modulation and to chemotherapeutic agents such as doxorubicin[11]. The ISCs produce progenitor cells, which form the enterocytes, goblet cells, enteroendocrine cells, and Paneth cells. Paneth cells are a major component of the innate immunity in the intestine, releasing granules containing β-defensins, matrix metalloproteinase-7, synovial phospholipase A2, and lysozyme. Colony stimulating factor (CSF)1 acts in a direct juxtacrine/paracrine fashion, or acts indirectly through macrophages in the lamina propria, to regulate Paneth cell development[12]. Fibroblast growth factor receptor (FGFR)-3 acts on the ISCs in the lower portion of the crypts to signal through both the β-catenin/Tcf-4 dependent and independent pathways, the expansion of the ISCs, expanding ISCs and crypt macrophages, including Paneth cells[13]. The behavior of ISCs is regulated by canonical Wnt signals. Inhibition of Wnt signaling reduces epithelial proliferation in adult mouse intestine. In the developing intestine, the relationships among Wnt signaling, epithelial proliferation, and tissue differentiation are reversed[14].

The intestinal epithelium is renewed through a series of programmed developmental transitions in the form and function of the intestine. For example, lactase-phlorizin hydrolase (LPH), fatty acid binding protein (Fabp1), and sucrase-isomaltase (SI) are intestinal proteins that are important for nutrition during different stages of development. LPH hydrolyzes milk lactose, SI hydrolyzes α-saccharidases, and Fabp1 is a cytoplasmic protein important for intracellular lipid transport. A member of the zinc finger transcription factor family (Gata4) and the hepatocyte nuclear factor α1 (Hnf1α) are essential for

LPH and Fabp1 gene expression but they do not mediate the glucocorticoid-induced precocious maturation of the intestine. Rather, specific intestinal genes have differential requirements for Gata4 and Hnf1α, and these genes are dependent on the developmental time-frame in which each gene is expressed[15].

Protective factors for the intestinal epithelium include secretion of mucins, as well as IgA and defensin peptides that are produced from epithelial cells. Epithelial cells also produce cytoprotective stress proteins, such as heat shock proteins (HSPs). N-formylmethionyl-leucyl-phenylalanine (fMLP) is a tripeptide produced by many enterobacteria as a by-product of protein synthesis. fMLP is a chemotactic agent from neutrophils and may be proinflammatory in nature. fMLP induces expression of HSPs and inhibits activation of the proinflammatory transcription factor NF-κB[16]. This finding needs to be translated to a clinical application.

Amniotic fluid and breast milk contain intestinal trophic factors that stimulate enterocyte development, and enhance various intestinal defense processes. Hydrocortisone is a trophic factor that is present in large amounts in human breast milk. Hydrocortisone reduces the cholera toxin (CT)-induced secretory response observed in fetal human small intestinal epithelial T84 cells, by inducing a pathway change from a clathrin-mediated to a caveolae-mediated endocytic process[17]. Hydrocortisone also increases ganglioside/lipid raft association, which may also reduce intestinal secretion.

Dietary fat in mother's milk is a major modifiable environmental factor that influences intestinal growth and development of the baby. The n-6 fatty acid, linoleic acid, is the metabolic precursor for synthesis of arachidonic acid (ARA). α-linolenic acid is a precursor for synthesis of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Eicosanoids derived from ARA are proinflammatory, whereas the n-3 fatty-acid-derived metabolites are anti-inflammatory. EPA and DHA have direct effects on intestinal cells through gene expression and ion channels, as well as on G-coupled protein receptor activities. As a result of the potential for dietary substances to affect normal growth and development of neonatal intestine, the consumption of early-life non-maternal milk products must be undertaken with the greatest of care.

In adult animals, glucagon-like peptide (GLP)-2 has a trophic effect on the intestine, and enhances nutrient absorption. In contrast, in young animals, there is malabsorption of several lipids with GLP-2 or GLP-2 plus dexamethasone, but not with dexamethasone itself[18]. The loss of body weight and jejunal atrophy induced by dexamethasone is prevented by giving GLP-2. Giving dexamethasone or GLP-2 and dexamethasone to lactating rat dams enhances glucose and lipid uptake in their suckling offspring[19].

The premature human neonate has higher fasting levels of GLP-1 and GLP-2 compared to adults, and feeding increases these levels even further[20]. GLP-2 levels are correlated with residual small intestinal length in the infant with nutrient malabsorption following intestinal surgery[21]. GLP-2 levels are directly correlated with absorption of fat, carbohydrate, and protein.

In suckling rats, GLP-2 plus dexamethasone increased the maximal transport rate (V_{MAX}) for jejunal glucose uptake, and the combination of these may be useful in stimulating glucose uptake in the developing intestine[22]. In suckling animals, dexamethasone reduces jejunal lipid uptake to levels similar to those seen in weanling animals. Giving dams GLP-2 or dexamethasone during pregnancy and lactation reduces lipid uptake in the offspring, and this reduction persists for at least 1 mo[23]. The impact this may have on the nutritional wellbeing of the animal in later life is unknown. This important consideration needs to be explored in terms of its long-term implications if nursing mother or child were to be exposed to steroids or GLP-2.

PROLIFERATION AND REPAIR

Homeostasis of the intestine is balanced between the process of proliferation, migration, differentiation, mitosis and apoptosis. Remodeling of the epithelium results from cell-matrix and cell-cell contacts. The proliferating regions are usually maintained by Wmt/β-catenin signaling, whereas differentiation is regulated by BMP, transforming growth factor (TGF)-β, and Hedgehog signaling. These interact within the stem and progenitor cells in the lower regions of the intestinal crypts, and regulate the expansion and cell fate decision of the interstitial epithelial cells (IECs). Several proteins involved in the contact between cells act as the source (i.e., Notch signaling, which regulates the development of enterocytes)[24] or the downstream effectors of signaling pathways such as E-cadherin/β-catenin. Notch signaling regulates the formation of the Epbrin-B2-positive proliferating crypt cells, as well as the Ephrin-B1-positive differentiated cells[25].

Homeostasis of the intestinal epithelium is a dynamic process created by a balance between proliferation, differentiation and apoptosis. This process is regulated by luminal nutrients, hormones, growth factors and cytokines. When acute damage is done to the GI tract, the usual repair process does not involve proliferation, but rather involves mucosal restitution. Restitution occurs by epithelial cell migration to reseal the superficial wound. Polyamines are involved in stimulation of intestinal epithelial cell migration during restitution. These polyamines (including spermidine, spermine, and precursor, putrescine) regulate calcium homeostasis by inducing alterations in cytosolic free Ca^{2+} concentration. Phospholipase C (PLC)-γ1 modulates calcium store mobilization and calcium influx, thereby promoting intestinal epithelial restitution after wounding[26].

Especially early in life, the BBM of the enterocyte is involved in the process of endocytosis, in which a tiny portion of membrane forms a bud around a luminal constituent, and breaks away from the rest of the BBM. This

hole in the remaining BBM must be quickly repaired. The subapical terminal web cytoskeleton inhibits movement of endosomes, and thereby helps to maintain permeability characteristics of the epithelium by keeping the endocytic activity occurring at the BBM under regulated control[27].

Differential display and microarray analyses have been used to identify genes that may be involved in molecular mechanisms regulating proliferation and differentiation of the intestinal epithelium[28]. Examples of transcription factors include the BMP expressed in the mesenchyme, Wnt which is essential for regulation of crypt stem cell proliferation and maintenance, and Notch signaling within the epithelium influencing differentiation of enterocytes, goblet cells, Paneth cells and enteroendocrine cells.

Glutamine is an amino acid that is the preferred fuel source for enterocytes. Glutamine activates extracellular signal-regulated kinase (ERK) and inhibits phosphorylated Akt, suggesting that ERK has an important role in the glutamine-mediated intestinal homeostasis[29]. Activation of phosphoinositide 3-kinase (PI3K)/Akt during periods of glutamine deprivation limits apoptosis, and thereby may have a protective role. Glutamine-response genes influence the expression of a number of signaling pathways such as NF-κB[30] and peroxisome proliferator-activated receptor (PPAR)α[31]. This potential basis for protection with glutamine, for example, in necrotizing enterocolitis, remains to be tested.

The Notch signaling pathway is essential for the development of enterocytes, and the Wnt cascade is required for the development of Paneth, goblet and enteroendocrine cells (secretory lineage). The commitment of crypt stem cells to differentiate into enterocytes reduces the differentiation of these secretory cells. This interaction by Notch to activate the Hes1 transcription factor, which then blocks the MATH1 transcription factor, influences the Ets-domain transcription factor Spdef, thereby blocking steps in the differentiation of the stem cells into the secretory lineage[32].

Notch, Wnt and Hedgehog signaling pathways are involved in epithelial cell proliferation and differentiation. Indian hedgehog (Ihh) is a signal sent by mature Paneth cells to their stem cell precursors, negatively regulating their differentiation. PPARβ is a nuclear hormone receptor activated by fatty acids in the intestinal lumen. PPARβ acts on Paneth cell homeostasis by downregulating expression of Ihh[33]. Hedgehog proteins are members of a family of secreted signaling factors that orchestrate development of many organs and tissues, including those of the GI tract[34]. In addition to epithelial differentiation being influenced by this variety of signaling cascades, amino acids and carbohydrates regulate gene expression and cellular function. For example, coenzyme A (CoA)-thioester derivatives contribute to cellular renewal along the crypt-villus axis in human small intestine[35].

The Wnt/β-catenin pathway involves molecules secreted by the mesenchyme, and controls proliferation and migration of enterocytes and Paneth cells. Transcription regulators such as the CDX2 and CDX3 homeoproteins, members of the homeobox gene family of transcription factors, activate several intestine-specific genes such as SI and LPH, as well as the adhesion proteins L1-cadherin and claudin-2. Furin is a calcium-dependent serine proteinase which processes many proteins such as BMP-4, the insulin receptor, the Notch1 receptor, and the cell adhesion protein E-cadherin. CDX2 regulates furin expression in intestinal epithelial cells[36]. Hepatocyte growth factor-1α cooperates with CDX2, and regulates transcription of SI, LPH, claudin-2, calbindin 3 and liver Fabp1 gene[37]. The crosstalk between lamina propria lymphocytes and intestinal epithelial cells may also be mediated by keratinocyte growth factor (KGF), such that lymphoepithelial interactions may both promote barrier function as well as regulate mucosal immune responses[38].

Mitogen-activated protein kinases (MAPKs) and PI3K are activated in a cascade-like fashion by various growth factors. Three of the MAPKs that have been identified include ERK, JNK and p38 MAPK. Rac1 also mediates apoptosis irrespective of ERK1/2 and Akt activation[39]. Signaling cascades such as the NF-κB and MAPK pathways provide transcriptional activation of inflammatory mediators such as chemokines, adhesion molecules and antibacterial peptides. In addition, reactive oxygen species (ROS) are important mediators in cellular signal transduction cascades regulating proliferation, apoptosis and migration. TNF-α is an inducer of apoptosis that requires the small GTPase Rac1, which has a role in the control of ROS production[40]. Protein-tyrosine kinase 6 (PTK6) is a stress-reduced kinase that promotes differentiation, and is a target of the serine threonine kinase AKT. After DNA damage, PTK6 is induced to promote apoptosis[11].

The intestinal mucosa in constantly being deformed by peristalsis, movement of the villi, and passage of chyme along the surface of the intestine. This strain by a variety of signals stimulates the migration of epithelial cells across fibronectin [such as Scn, focal adhesion kinase (FAK), and ERKs][41].

INTESTINAL PERMEABILITY

The intestinal BBM of enterocytes constitutes the small intestinal epithelial barrier, which helps to protect the body against invasion by millions of luminal bacteria. This barrier function is a balance between the bacterial microbiotica, and homeostasis provided by the preservation of the integrity of the BBM. TJs, innate immune cell function, and microbial peptides are important in establishing and maintaining this barrier integrity.

Modifications in NOD2 alter intestinal homeostasis, and may be associated in some individuals with chronic intestinal inflammation. PRMs, TLRs and NOD receptors are regulators of the intestinal epithelial barrier, which is essential along with innate immune cells and antimicrobial peptides, to maintain normal intestinal permeability[42].

TJs between the intestinal epithelial cells provide a dynamic structure, altering cellular permeability. TJs are

formed by organization of a number of specific proteins including occludin, ZO-1, ZO-2, ZO-3, claudins, and junctional adhesion molecules. Mucosal hydration occurs through activation of ion channels and transporters, as well as through secretion of mucous and antibacterial protein. These are additional defense pathways against pathogens[43]. Under some circumstances such as intestinal ischemia, there may be increased mucosal penetration and translocation of bacteria, as well as transcytotic movement of bacteria through intestinal epithelium by way of lipid rafts. This movement of bacteria across the disrupted intestinal barrier may precede cytokine-induced disruption of TJs[44].

Adherens junctions (AJs) are a major part of the junctional complexes. The main transmembrane protein of AJ is E-cadherin. E-cadherin mediates adhesion or anchorage of the enterocytes to the matrix by a process that is Ca^{2+}-dependent. Epidermal growth factor receptor (EGFR) is induced in the process by which there is deprivation of the E-cadherin-dependent junctions, and enterocytes lose their interactions with the basal lamina. This leads to anoikis, a form of apoptosis caused by the loss of cell anchorage[45]. Symplekin is also a component of the TJ cytoskeletal plaques. Symplekin co-operates with the transcriptor factor ZONAB to regulate negatively the differentiation of goblet cells in the intestine[46].

Mast cells (MCs) in the lamina propria contain both preformed bioactive mediators such as tryptases and histamine, as well as synthesized mediators like prostanoids and leukotrienes. MCs express corticotrophin-releasing factor receptor 1 (CRFr1) and CRF receptor 2 (CRFr2), G-protein-coupled corticotrophin-releasing factor receptors[47]. In pigs, when CRFr1 is activated during early weaning stress in pigs, the epithelial barrier becomes leaky, whereas CRFr2 may be protective.

When the serotonin reuptake transporter (SERT) is lost, there is enhanced intestinal translocation of bacterial endotoxin in a murine model of chronic increased intake of fructose[48].

The permeability of the intestinal mucosa is determined by the relative balance of uptake from the intestinal lumen, across the BBM and into the cytosol of the enterocyte, and efflux from the cytosol across the BBM and into the lumen, through transporters such as P-glycoprotein (P-gp; ABCB1), multidrug resistance-associated protein 2 (MRP2; ABCC2), and breast cancer resistance protein (BCRP; ABCG2). Changes in the activity of these efflux transporters will modify net drug absorption. For example, sulfasalazine uptake across the BBM is adequate, but sulfasalazine may be transported out of the enterocytes (efflux) by MRP2 and BCRP, contributing to the apparent low permeability of the small intestine to this drug[49].

The transport and barrier properties of the intestinal epithelium are regulated, influenced by food and the microbiotica that colonize mucus overlying the epithelium. Epithelial transport mechanisms are present to regulate the long-term adaptation of intestinal function. Specific molecular components of TJs decrease or increase transepithelial resistance[50]. In enterocytes in the upper portion of intestinal crypts, there are cell cycle promoting and inhibiting genes, as well as transcription/translation related genes. In the enterocytes, in the middle of the villi, there are metabolism-related genes, and vesicle/transport-related genes[51].

Stressful events will increase intestinal permeability to macromolecules, deplete intestinal mucus, and alter interaction of bacteria in the intestinal lumen with the intestinal epithelial cells. Stress may even lead to inflammation, bacterial invasion of the mucosa, as well as T-cell activation. The disturbance of epithelial cell kinetics depends on the duration of the stress. The stress-induced increase of intestinal permeability may be related to an increased number of apoptotic cells in the epithelium[52]. Perfusion of a jejunal segment in persons with low *vs* moderate background stress (called "pain stress"), results in an increase in chloride-related peak secretory response, as well as enhanced permeability to albumin[53].

In addition to glucocorticoid-induced precocious maturation of the intestine, "stress" may also play a role in disease susceptibility through its actions on CRF, and subsequent activation of CRF receptors expressed locally in the intestine. It is predominantly the CRF-r1 activation that alters intestinal function in times of stress. One such type of stress in the developing animal is weaning, when the increased intestinal permeability and secretory activity are mediated by the intestinal CRF receptors. This is possibly through activation of enteric neural and PG synthesis pathways in weaned intestinal tissues[54].

In humans, the increased intestinal permeability due to the acute effects of radiotherapy may be reflected by increased fecal excretion of DHA and calprotectin[55]. From 50% to 70% of NSAID users have damage to the GI tract beyond the stomach. When capsule endoscopy, fecal calprotectin measurement, and urinary recovery of orally administered lactulose and mannitol are used, about half of NSAID users will have defects in the small bowel[56].

The TJ (also known as the zonula occludens) has both a gate function (regulating the passage of molecules through the paracellular pathway), and a fence function. In some intestinal disorders, the permeability of the intestine may increase ("leaky" TJs), and endogenous PPARβ ligands may reduce the degree of TJ permeability, thereby reducing the degree of leakiness[56]. This raises the therapeutic potential of using PPARβ ligands to correct the "leaky gut". Adrenomedullin (AM) may also prove to be useful to reduce intestinal permeability. AM is a 52-amino-acid peptide formed in the mucosa. AM increases cAMP, activates protein kinase (PK)A, and stabilizes the barrier function of the intestine (i.e., prevents increased permeability and decreases leakiness[57]. EGF binds to its receptor, and improves epithelial barrier function (i.e., decreases intestinal permeability). During sepsis there is barrier dysfunction, increased apoptosis, and higher levels of cytokines. Potentially all of these may lead to multiple

organ failure. Systemic EGF prevents peritonitis-induced intestinal barrier dysfunction[58], and may thereby reduce mortality from noninfectious inflammation and intestinal injury. It remains to be established whether this modification of intestinal permeability will have any therapeutic benefits in humans.

MICROBIOTICA, INFECTIOUS DIARRHEA AND PROBIOTICS

Probiotics

The World Health Organization defines probiotics as "live microorganisms which, when consumed in adequate amounts as part of food, confer a health benefit on the host". Probiotic therapy has been used for traveler's diarrhea, to prevent antibiotic-associated diarrhea, and to prevent relapse of pouchitis (after colectomy and ileoanal pouch formation). *Lactobacillus rhamnosus* GG (LGG) is a probiotic bacteria that prevents cytokine-induced apoptosis by way of two LGG-produced viable proteins p75 and p40. These activate Akt and regulate intestinal epithelial cells as well as antiapoptotic responses by a PKC-MAPK-dependent mechanism[59]. The clinically tested VSL#3 probiotic formula and its secreted components may augment the protective mucus layer through MUC2 gene expression[60]. Treating infectious diarrhea in children with *L. rhamnosus* shortens the duration of diarrhea, and also reduces the duration of the need for parental rehydration[61].

A meta-analysis of five randomized controlled trials of *Saccharomyces boulardii* has shown moderate effectiveness in preventing antibiotic-associated diarrhea (AAD) in children and adults. *S. boulardii* reduced the risk of AAD from 17% to 7%, with the number needed to treat to prevent one case of AAD being only 10[62].

The probiotic bacterium *Lactobacillus casei* strain Shirota (LCS) suppresses the LPS/TLR4 signaling pathway and prevents indomethacin-induced intestinal injury, possibly by way of the production of lactic acid[63]. The nonpathogenic yeast *S. baoulardii* inhibits EGFR and thereby also inhibits the downstream ERK1/2 MAPK pathway[64]. This raises the possibility that *S. baoulardii* may have both anti-inflammatory and antineoplastic therapeutic potential.

It will soon be possible to target the human microbiome with antibiotics, probiotics and prebiotics. "Culture-independent strategies such as high-throughput parallel sequencing and comparative genomes, metabolic proliferating and functional genomes, fluorescence *in situ* hybridization, and phylogenetic microarrays will provide new insights into the composition, architecture and functional roles of the human microbiotica"[65].

A therapeutic approach to enteric infections is to express molecular mimics of toxin receptors on the surface of harmless bacteria, so that the toxin binds to the bacterium rather than to its natural target, the intestinal mucosa. This strategy using molecular mimicry has been used to develop recombinant probiotics for treatment and prevention of diarrheal disease caused by enterotoxigenic *E. coli*[66], and for prophylaxis and treatment of cholera[67].

Infections

The protozoon *Giardia lamblia* is a human GI parasite that is common in Western Europe and in North America. Some 15% of infected individuals develop chronic symptomatic disease. *G. lamblia* infection increases intestinal permeability due to downregulation of TJ occludin 1, reduced sodium-dependant glucose absorption, and by increased epithelial apoptosis, as well as increased active electrogenic anion secretion[68]. Exposure to DDT, organochlorine, reduces the efficacy of treatment of mice with experimental giardiasis[69].

Cyclospora responds variably to albendazole[70]. Nitazoxanide is effective in treating diarrhea and enteritis caused by *Cryptosporidium* in immunodeficient as well as non-immunodeficient patients aged ⩾ 12 years[71].

The synthesis of NO is increased in infectious diarrhea, but its physiological role is unknown. Inducible NO synthase (iNOS) is constitutively expressed by the intestinal villous cells. After acute injury of the intestinal epithelium rapidly induces iNOS and synthesizes NO. The induction of iNOS in response to neonatal piglet *Cryptosporidium parvum* is a nonspecific response[72].

Campylobacter spp. are a common cause of diarrhea in developing countries. They causes mild to severe bloody diarrhea, chronic relapsing infection, and invasion of the mucosa. This may be associated with extraintestinal complications such as neuropathy in the peripheral nervous system, (e.g., Guillian Barré syndrome). Norepinephrine (NE) is present in high concentrations in the noradrenergic neurons in the intestine. Exposure to NE increases the virulence-associated properties of *Campylobacter*[73]. This suggests that microorganisms may have evolved a "sense and respond" system. The evidence linking inflammatory bowel disease (IBD) and *Campylobacter jejuni* infection has been reviewed[74].

Diarrhea in the traveler may be termed "traveler's diarrhea," therefore, detection of bacterial enteropathogens is not uniform. When diarrhea occurs in travelers, ciprofloxacin (500 mg *bid* for 3 d), levofloxacin (500 mg *qid* for 3 d), rifaximin (200 mg *qid* for 3 d), or azithromycin (1000 mg, single dose) should be taken in conjunction with an antidiarrheal agent[75]. Indeed, there is an advantage of adding loperamide to fluroquinolone, rifaximin, and azithromycin treatment of traveler's diarrhea. Two new antisecretory drugs that have been shown to be useful for reducing stool frequency of traveler's diarrhea including crofelemer, a chloride channel blocker[76], and zaldaride, a calmodulin inhibitor[77]. Racecadotil, an encephalinase inhibitor, may also be useful for the treatment of acute diarrhea[78-80].

Vibrio cholerae secretes CT, which binds to mucosal enterochromaffin cells, and release large amounts of 5-hydroxytrytamine (5-HT). The sustained hyperexcitability of CT on the submucosal secretomotor neuronal pathway depends on 5-HT3, and nicotinic receptors[81]. The small GTP-binding proteins increase the CT-catalyzed ADP-ribosylation of the heterotrimeric guanine nucleo-

tide-binding (G) protein Gsβ. There is increased susceptibility of neonates to infectious diarrhea. For example, there is increased uptake of CT by a developmentally regulated clathrin-endocytic pathway. Immature human enterocytes, DRF1, play a role in clathrin-mediated CT trafficking through the endoplasmic reticulum and Golgi, and ARF6 enhances Gsβ activation by CT[82].

Shiga toxin (Stx) 1 and 2 are virulence factors for noninvasive enterohemorrhagic *E. coli* (EHEC) infection, such as by *E. coli* 0157:H7 infection. Stx is taken up by microendocytosis in cells that lack the Stx1 receptor, mediated by Gc3 on the BBM[83].

Clostridium difficile accounts for 15%-25% of episodes of AAD. Methicillin-resistant *Staphylococcus aureus* (MRSA), and particularly the enterotoxin-producing strains, may also cause nosocomial AAD[84].

Rotavirus is the major cause of life-threatening diarrheal disease in infants and young children. Rotavirus-induced diarrhea can be prevented by use of a live-attenuated vaccine[85]. Following acute mucosal injury such as from rotavirus infection, there is enterocyte migration that is proliferation-independent. This restitution or migration of cells requires NO, PGs and polyamines. Acute regulation of protein synthesis is achieved through changes in the rates of translation of mRNA, by way of alterations in peptide chain initiation. This chain initiation process is influenced by mammalian target of rapamycin (mTOR). mTOR is an amino acid-sensing mechanism that targets ribosomal p70 S6 kinase ($p70^{S6K}$). $p70^{S6K}$ is activated during an active state of mucosal regeneration produced by a piglet rotavirus enteritis[86].

The etiological agent of Whipple's disease (WD) is *Tropheryma whipplei*, a PAS-positive, rod-shaped actinomycete. Some healthy persons harbor *T. whipplei* in their bowel, and there is no correlation between *T. whipplei* genotypes and clinical manifestations. The cumulative odds ratio (OR) for disease are 2.23 for the HLA-DRB1 X 13 allele, and 2.25 for the DBQ1*06 allele[87]. A randomized controlled trial of 40 persons with previously untreated WD showed that daily infusions of ceftriaxone 1 × 2 g or meropenem 3 × 1 g for 14 d, followed by 12 mg oral trimethoprim-sulfamethoxazole, both gave an OR of 0.95 for remission for at least 3 years[88]. One patient with asymptomatic cerebrospinal fluid infection with WD was resistant to ceftriaxone and to meropenem, but responded to choloroquine and minocycline. This compares favorably to retrospective case series with treatment of *T. whipplei* with tetracycline, penicillin, streptomycin and low trimethoprim-sulfamethoxazole, in which only about three quarters of WB patients had an initial response, and approximately a quarter of those who had an initial response subsequently relapsed.

Both Crohn's disease (CD) and intestinal tuberculosis (TB) (caused mainly by *Mycobacterium tuberculosis* or *Mycobacterium bovis*) have a predilection for the tumor ileum. An algorithm has been developed for the investigation of persons in whom the diagnosis includes CD and TB[89].

Diarrhea is the most common GI symptom in persons with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), affecting 50%-90% of those with a low CD4 lymphocyte count. However, in some 30%-40% of cases of HIV/AIDS, enteric pathogens cannot be identified. Conceptually, HIV enteropathy has been supported by the detection of HIV proteins in the intestinal epithelial cells as well in gut lymphocytes and macrophages. In a case report, a 33-year-old woman with HIV enteropathy was described with structural changes in the duodenal mucosa, regressing after triple antiviral therapy[90].

About half of the HIV-infected persons in the United States have diarrhea. The intestinal immune system is central to the pathogenesis of AIDS (transmission, viral complication, $CD4^+$ T-cell destruction), with the progression of AIDS due to a decrease in the expression of genes that maintain the mucosal barrier, and HIV translocation[91]. Endoscopy and biopsy of the upper and lower GI tract has identified pathogens in about half of those HIV-infected persons who were initially thought to have an idiopathic disorder or AIDS enteropathy. Viral replication occurs early in HIV in the gut-associated lymphoid tissue $CD4^+$ T cells and macrophages. AIDS enteropathy is "an idiopathic, pathogen-negative diarrhea, and treatment includes highly active antiretroviral therapy (HAART), fluid and electrolyte balance, and nutritional support"[92]. The opportunistic infections can still occur in HIV-positive persons taking HAART, especially in those with a high viral load and a low T-cell count. Also note that these opportunistic disorders occur even if the HIV load is low and the $CD4^+$ T-cell count is near normal.

In a young patient with chronic diarrhea, bacterial rDNA (16S rDNA) has been extracted from the duodenal biopsy, and was thought to be due to *Stenotrophomonas maltophilia*[93]. Such genomic techniques may be used for the diagnosis of chronic infections, including those with HIV enteropathy.

Matrix metalloproteinases (MMPs) are a group of enzymes that are capable of degrading all components of the extracellular matrix. Fibroblasts are the major source of MMPs in the human intestine. Fibroblasts are a potential target of IL-21, a T cell-derived cytokine that acts on the IL-21 receptor, and then interacts with the common γ chain receptor IL-21 that controls MMP secretion by fibroblasts[94].

Enteropathogenic *E. coli* (EPEC) is a leading cause of infantile diarrhea in developing countries. The EPEC-induced inflammation is a balance between pro- and anti-inflammatory proteins[95]. Nosocomial infections with *Pseudomonas aeruginosa* are associated with a high fatality rate. A virulence circuitry of *P. aeruginosa* is activated by both soluble and contact-mediated elements of the intestinal epithelium[96]. *P. aeruginosa* metabolizes adenosine to inosine, which enhances virulence of the organism[97].

Malnutrition may be associated with prolonged episodes of EPEC infection. In the human epithelial Caco-2 cell model, EPEC inhibits uptake of thiamine[98]. EPEC also inhibits SERT, the serotonin inhibitor; reduction in

SERT by EPEC may represent an additional and new mechanism of EPEC-mediated diarrhea[99].

Severe acute respiratory syndrome (SARS) is characterized clinically by fever, nonproductive cough, dyspnea, lymphopenia, and rapidly progressing changes on chest X-ray, however, it is not uncommon for SARS patients to have diarrhea, nausea and vomiting, as well as abdominal pain. This may be associated with atrophy of the mucosal lymphoid tissue, staining of intestinal epithelial cells and lymphocytes for corona virus (*via in situ* hybridization), and demonstration by electron microscopy of SARS-coronal virus-like particles in the mucosal epithelial cells[100].

The total microbial population within the GI tract exceeds the total number of mammalian cells in the body by at least an order of magnitude. There is a complex environment of microbes that interfaces with the mucosal lining of the GI tract. This coexistence between eukaryotes and prokaryotes has both beneficial and unfortunately harmful interactions[101]. The intestinal mucosa downregulates proinflammatory signaling pathways, expresses antimicrobial proteins, and initiates epithelial repair after mucosal injury. Indeed, commensal bacteria are actively involved in shaping the very barriers that confine them to the gut lumen[102].

Lipid rafts are cholesterol-sphingolipid-rich microdomains in the enterocyte BBM. These lipid rafts contain digestion and transport enzymes that are important in trafficking and signaling of various proteins. For example, Cx43 is present on enterocyte lipid rafts, and interferon (IFN) inhibits enterocyte migration by displacing Cx43 from these lipid rafts in enterocytes[103]. Some pathogens recognize these lipid-rich specialized areas, and modify function of the nutrient digestive and transport proteins. Anti-glycosyl antibodies are induced by a glycosyl antigen, and are associated with lipid rafts. These anti-glycosyl antibodies act as competitors to pathogens, having a protective role on the lipid rafts by preventing lactin-like pathogens from gaining access to the intestinal BBM[104].

SMALL INTESTINAL BACTERIAL OVERGROWTH

Small intestinal bacterial overgrowth (SIBO) occurs as a result of a reduction in gastric acid secretion, a failure of mucosal and systemic barrier functions, as well as a failure of immune responses. SIBO also occurs in the presence of anatomical abnormalities such as intestinal fistulae or multiple small intestinal (not colonic) diverticulae. Gram-positive and Gram-negative bacteria, including both aerobes and anaerobes, contribute to the diarrhea and malabsorption of nutrients. While the prevalence of SIBO is increased in persons with irritable bowel syndrome (IBS), especially those with diarrhea-predominant IBS, the exact rates depend upon the method used to diagnose SIBO, with low prevalence rates of 4% using the gold standard test of a positive jejunal aspirate and culture, and a prevalence of 54% using the lactulose or glucose hydrogen breath test[105]. The crux of this association of IBS and SIBO is whether treatment of the positive test for SIBO will improve the patient's symptoms.

SIBO may recur after it has been successfully treated with appropriate antibiotics (e.g., metronidazole or ciprofloxacin). The recurrence rates after initially successfully treated SIBO are about 13% at 3 mo, 28% at 6 mo and 44% at 9 mo[106]. SIBO recurrence is more likely in older persons, those treated chronically with proton pump inhibitors, and curiously, those with a previous history of appendectomy. Retreatment with the same or different antibiotics will be necessary, and some persons with recurrent SIBO require maintenance use of antibiotics.

The absorbable antibiotic metronidazole is more effective than the nonabsorbable antibiotic rifaximin for treatment of SIBO in persons with blind loop syndrome[107]. Rifaximin may safely prevent traveler's diarrhea: in a study in travelers to Mexico, rifaximin reduced the risk of diarrhea from 54% in those taking placebo to 15% in those taking rifaximin[108].

In persons with chronic rosacea, 46% had SIBO, *vs* 5% of healthy controls. After eradication of the SIBO, the cutaneous rosacea lesions cleared in 71% and greatly improved in 21%, whereas those treated with placebo remained unchanged in 90% or worsened in 10%[109].

DIARRHEA, SALT AND WATER ABSORPTION

The topic of diagnosis and treatment of acute or persistent diarrhea has been reviewed[110]. Host genetic polymorphisms may influence susceptibility to traveler's diarrhea. The topic of chronic diarrhea has been reviewed and can be accessed for a tutorial (BMJ Learning http://gut.dmj.com/tutorials/collection.dtl)[111].

Na^+/H^+ exchange (NHE) is the principal exchanger isoform responsible for transepithelial Na^+ absorption at the BBM of the intestinal epithelium. Coupled activity includes both Na^+/H^+ and Cl^-/HCO_3^- exchangers. The Cl^-/HCO_3^- exchanger, SLC26A3 [also known as down-regulated in adenoma (DRA)], also exchanges sulfate and formate. Electroneutral NaCl absorption is mediated through NHE_3 as well as DRA as the major Cl^-/HCO_3^- exchanger[112]. NHE is highly regulated by a number of second messenger systems including cAMP-dependent protein kinase (PKA), cGMP dependent kinase, Ca^{2+}, PKC, PI3K, and Akt. The phosphorylation of NHE by PKA alters the clathrin-mediated cAMP-stimulated endocytosis of NHE_3, thereby reducing Na^+ absorption. Synaptotagmin 1 (Syt1) directs NHE_3 to the adaptor protein 2-clathrin complex[113].

There are two Cl^-/OH^- exchangers, DRA (SLC26A3) and PAT-1 (putative anion transporter-1; SLC26A6). DRA is in the colon, whereas PAT-1 is in the human jejunum and ileum. Cytokines such as IL-1β, TNF-α and IFN-γ increase secretion in models of inflammatory diarrhea. IL-1β decreases DRA mRNA, as also does IFN-γ by means of the STAT1/JAK (Janus kinase) pathway[114]. This opens the way for the development of potential antidiarrheal

agents for the treatment of persons with inflammatory bowel disease. For example, *Lactobacillus acidophilus* prevents enteroinvasive *E. coli*-mediated disruption of the BBM barrier in Caco 2 cells, and increases Cl^-/OH^- exchange by enhancing the mRNA and activity of DRA[115].

Lysophosphatidic acid (LPA) is a glycerophospholipid present in abundance in foods such as soybeans and egg yolk. There are seven LPA receptors, and LPA[116] acts through these receptors to inhibit the activity of the cystic fibrosis transmembrane conductance regulator (CFTR)-dependent Cl^- channel. LPA also stimulates BBM Cl^-/OH^- exchange and levels of DRA[117]. This combined mechanistic effect of LPA may explain its antidiarrheal effect. This raises the possibility of using LPA for the treatment of diarrhea.

Serotonin (5-HT) is a neurotransmitter and hormone that influences many physiological functions. Over 97% of the serotonin in the body is in the enterochromaffin cells. SERT is present in the human intestine[118], and the enterochromaffin cells have apical microvilli that protrude into the intestinal lumen. Serotonin is secreted from granules at the basolateral pole of the enterochromaffin cells. Abnormalities in the release, reuptake and catabolism of serotonin may be responsible for altered secretion, motility and pain sensation in patients with IBS, inflammatory bowel disease, or in persons with diarrhea or constipation. Release of serotonin may be mediated through central and enteric nervous system regulation, or by way of the enterochromaffin cells acting as sensors in the gut luminal milieu, responding to tastants such as (caffeine, tyramine and octopamine), olfactants (thymol and eugenol), as well as glutamine, deoxycolate, 2-deoxyglucose, and the artificial sweetener, sucralose. There are 11 secretory-associated genes, including the vesicle docking inhibitor STXBP3, which may be upregulated in response to luminal glutamine or bile acids, whereas antisense knockdown reduces serotonin secretion[119].

NHE_3 is regulated by cAMP, cGMP, intracellular calcium, PI3K, glucocorticoid kinase-1 and PKC. The acute regulation involves changes in both functional activity and membrane trafficking. This acute effect on NHE_3 may be mediated by the glucocorticoid receptor, which stimulates SGK-1. Membrane insertion of NHE_3 may be caused by Na^+-K^+-ATPase-induced changes in A^+ translator Na^+ flux[120].

NHEs help to maintain intracellular pH homeostasis, cell volume regulation, and electroneutral sodium chloride (NaCl) absorption. NHE_2, NHE_3 and NHE_8 are present in the apical membrane of enterocytes and colonocytes. Sp3 is the major activator of NHE2 gene transcription in the intestinal epithelial cells[121]. NHE_3 is also regulated by certain growth factors, protein kinase, and IFN-γ. The Sp transcription factor is involved in cell cycle regulation, hormonal activation, and development patterning. NHE_3 facilitates intestinal neutral NaCl absorption, and thereby increases the net transmucosal absorption of bicarbonate in rat duodenal mucosa. This mechanism is similar to the proximal renal tubular bicarbonate absorption[122].

The epithelial sodium channels (ENaCs) are a rate-limiting component for electrogenic sodium absorption in the distal colon. ENaC function is regulated by mineralocorticoid and glucocorticoid hormones. Steroid hormones act through transcriptional induction of β- and γ-ENaC subunits. In macroscopically noninflamed colon in persons with active CD, there is impaired ENaC-mediated Na^+ absorption, which contributes to the diarrhea arising from the inflamed mucosa[123].

Water movement across the intestinal epithelium is driven osmotically, and is therefore influenced by the balance between sodium absorption and chloride secretion. These processes, absorption and secretion, are regulated. Intestinal motor and sensory function is altered by the action of transmitters and by changes in ion channel function. CFTR is a cAMP-dependent Cl^- channel. CFTR is located in the intestinal epithelium, and is activated by PKA-mediated phosphorylation. A minor component of Cl^- transport occurs by way of intestinal and colonic epithelial cell transport, the type 2 Cl^- channel (ClC-2) volume-regulated channel. CFTR acts as an entry pathway for Cl^- into epithelial cells, in parallel with Na^+ entry through apical ENaCs. ClC-2 promotes anion secretion, with little anion reabsorption. The properties of ClC-2 differ depending upon the conditions in the tissue in which the ClC-2 channels are studied[124].

Diarrhea is common after pelvic radiotherapy, and this may be associated with modification in the diversity of luminal microbiotica, as reflected by a drop in the similarity index of the enteric bacteria. Cluster analysis of the microbial profile 5 wk after radiotherapy displayed a dentogram which was different in patients who presented with or without diarrhea[125]. It is not clear why there is this differential effect of radiation on the intestinal organisms.

Newer and even more effective antidiarrheal agents are needed. In the presence of malabsorption of Na^+ and water in the small intestine, the ENaCs in the distal colon are upregulated in order to absorb more Na^+/water, and hopefully thereby reduce diarrhea. ENaCs are responsive to mineral corticoids as well as to glucocorticoids. Glucocorticoids act through p38 MAPK to enhance the transcription of the B- and J- subunits of ENaCs, thereby rapidly increasing water absorption[126].

DRA is a Cl^-/HCO_3^- exchanger. When DRA is eliminated in mice, there is an approximately 50% reduction of basal and cAMP-stimulated HCO_3^- secretion[127]. Microfluorimetry of villi shows that DRA is the major Cl^-/HCO_3^- exchanger in the lower villous epithelium. In humans, loss-of-function mutations cause congenital Cl^- losing diarrhea[128].

The development and control of the inflammatory reactions, which occur in food allergies, are mediated by T helper (Th)2 or regulatory responses, in which IL-4 is a factor. Allergic responses include IL-4 induction of the differentiation of committed effector Th2 lymphocytes for mRNA for TNFβ, Th1, IFNβ, IL-12p40, regulatory cytokines or Foxp3 (forkhead box P3). These may serve

as "rescue channels" for all Cl^- secretion, but lubiprostone activation of Cl^- secretion requires CFTR. The PPAR-β agonist rosiglitazone reduces cAMP-dependent Cl^- secretion as a result of reduced expression of the CFTR Cl^- channel, KCNQ, K^+ channel, and Na+/K+/2Cl cotransporter proteins[129].

IBS

About half of diarrhea-predominant IBS patients report an exacerbation of their symptoms within 90 min of eating. Normally, the duodenal and ileal breaks slow the passage of chyme along the intestine, and this helps to prevent exceeding the approximately 2-L composition of the colon to absorb water. Indeed, the small bowel water content initially falls, and then rises after a meal of liquids and solids[130]. This could possibly be due to MC products being released from the small bowel mucosa, causing rapid transit of mill as intestinal fluid secretion.

IBS may aggregate in families, and twin studies demonstrate both a genetic as well as an environmental basis for IBS. Human intestinal smooth muscle cells and ICCs express SCN5 (gene encoded Na^+ channel, expressed in interstitial cells). There may be an association between genetic defects in SCN5A and GI symptoms, and SCN5A may be a candidate gene in the pathophysiology of IBS[131].

In a group of 62 patients with presumed idiopathic chronic watery diarrhea, HLA-DQ2/DQ8 genotyping, eHCAT abdominal retention test, small bowel follow-through, and hydrogen breath test were undertaken. In 45% of these patients, bile acid was presumed to be the cause of diarrhea. Sugar malabsorption was seen in 16%, gluten sensitive enteropathy in 16%, and both bile acid and sugar absorption in 3% of patients. It is important to note that not only was the cause of the chronic watery diarrhea not idiopathic in 81% of the patients, but it responded to specific treatment[132].

NECROTIZING ENTEROCOLITIS

Intestinal mucus is one of the important factors protecting the intestinal epithelium. Without this well-formed gel layer, the underlying mucosa is more susceptible to attack by bacteria. Much of the oral intake of threonine is used for mucin production. A threonine-deficient diet will lead to a reduced number of acidic mucin-producing goblet cells in the small intestine. The abnormalities in mucus resulting from the impaired oral intake of threonine can be prevented through parenteral administration of this amino acid[133].

The mucus layer over the intestinal epithelium acts as a barrier, retarding the access of bacteria and bacterial products to the BBM. Goblet cells secrete mucin 2 (MUC2), a glycoprotein with high density and viscoelasticity. MUC2 contains large amounts of threonine and proline. Studies determining the fractional synthetic rate of MUC2 in human neonates have shown rapid incorporation of systemic threonine into MUC2[134].

Premature infants are at increased risk of developing necrotizing enterocolitis (NEC). The initial colonization of the intestine by bacteria, and the use of enteral feeds, contribute to the development of NEC. The maldigestion of carbohydrate may also be a risk factor[135]. This is possibly because maternal diets are high in n-6/n-3 fatty acids. These dietary lipids are epigenetic factors that contribute to the increased incidence of ischemia by altering the composition of amniotic fluid and intestinal membrane structural lipid essential fatty acids[136].

There is a wide range of mortality rates of infants with NEC, depending on the number of risk factors (e.g., prematurity, use of feeding formula, bacterial colonization of the GI tract), as well as the extent of the damage to the bowel (inflammation, necrosis, perforation, peritonitis, sepsis, and systemic inflammatory response). In the healthy child, there is high blood flow and low resting vascular resistance. One mechanism of development of NEC is reduced blood flow in the arteries penetrating the wall of the intestine, as well as the submucosal arterial plexus.

NEC is a major cause of death from GI disease in neonates. The mucosal damage and increased intestinal permeability lead to translocation of enterobacteria, activation of macrophages in the lamina propria, and initiation of a systemic inflammatory response. NO is released in a response to cytokine induction of the enzyme iNOS. Restitution is regulated in part by the Rho family of small-molecular-weight GTPases. NO inhibits enterocyte migration through protein tyrosine phosphatase (SHP-2)[137].

Intestinal barrier function can also be altered as a result of impaired intestinal restitution. This restitution is decreased in, for example, hemorrhagic shock, sepsis, intestinal ischemia and NEC. The increase in intestinal permeability that occurs with stress and the presence of viable nonpathogenic *E. coli* is exaggerated by TNF-α release from macrophages[138]. The proinflammatory mediators IL-18 and TNF-α are involved in the development of NEC. As noted by these authors, this raises the possibility that "…anti-TNF-α could be used as a potential therapy for human NEC"[139]. The use of TNF-α modifiers may also be effective to normalize *E. coli*-associated increased intestinal permeability. It is unknown whether the inhibition of membrane or soluble TNF-α may be used clinically to prevent or to treat NEC.

Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is produced by epithelial cells, where it decreases apoptosis, preserves the integrity of the cytoskeleton, and rebuilds depleted intracellular stores of ATP. In newborn rat pups, HB-EGF maintains the blood flow of the microcirculation that normally would be decreased in NEC[140]. Histological abnormalities correlate with changes in blood flow, and less histological damage occurs with the use of HB-EGF. This may prove to be a useful preventive or therapeutic agent in newborn human infants who are at risk of developing NEC.

The probiotics lactobacilli and bifidobacteria may help to prevent the development of NEC. TLRs on the

intestinal BBM are PRRs that identify components of the usual intestinal microbiotica. TLR4 recognizes the Gram-negative LPS, which is increased in NEC. The TLR changes occur before the histological changes, suggesting that the activator of the inflammatory response through TLRs may be causative in the development of NEC[141].

As a result of the importance of the formation of the microbiotica in the development of NEC, the possibility has been raised for the therapeutic potential of altering the bacterial contents by the use of probiotics[142]. Feeding premature rats the probiotic *Bifidobacterium bifidum* reduced the prevalence of NEC from 57% to 17%. This dramatic protective effect was associated with reduced and indeed normalized levels of proinflammatory IL-6, trefoil factor 3 and mucin-3, as well as TJ and AJ proteins[143].

The release of bacterial LPS from enterobacteria associated with NEC increases the enterocyte expression of integrin. Integrin leads to adhesion of the enterocyte to the underlying matrix and healing of the damaged mucosa by the migration process of restitution. Dystroglycans (DGs) are a component of the dystrophin-glycoprotein complex that are involved in integrin activation in the intestinal epithelium[144]. With restitution, the migrating cells transiently adhere to the underlying matrix, reducing fibrillar adhesions containing a structural core of transmembrane integrins. These integrins provide anchors (adhesion complexes) and also tensors for mechanical events, as well as signal transducers. Removal of neurocytes requires disassembly of the integrin-based adhesions structures. β1-Integrin is endocytosed *via* a dynamin-dependent lipid-raft-mediated pathway that helps to regulate intestinal epithelial cell migration and wound closure[145].

The bacterial ligand activated TLRs signal intracellularly to upregulate both pro- and anti-inflammatory cytokines. There are several genes induced in the inflammatory process such as cytokines, chemokines and adhesion molecules. A factor that is central to the regulation of these various genes is NF-κB. For example, NF-κB is induced in NEC. Platelet activating factor (PAF) is an endogenous proinflammatory phospholipid that rapidly activates NF-κB. PAF also stimulates neutrophils to produce the chemokine that used to be known as macrophage inflammatory protein (MIP)-2, now called $CXCL_2$. The bowel injury produced by PAF is mediated through the processing of the NF-κB p105 into NF-κB p50[146].

PAF induces Cl^- channel activation associated with intracellular acidosis. The Cl^- channel activation occurs through caspase-3 activation and DNA fragmentation, as well as by apoptosis[147]. In NEC, PAF and accelerated apoptosis of enterocytes results in tissue necrosis. Inhibition of caspase activation prevents the progression of disease in a neonatal rat model of NEC. Palmitoylation is a post-translational modification that is necessary for efficient signal transduction by many G protein-coupled receptors (GPCRs). Nutrients in the intestinal lumen may be sensed. For example, free fatty acids lead to release of peptide YY. This affects GPCRs, thereby modifying cell proliferation and differentiation as well as hormonal secretion[148]. Inhibition of the PI3K/Akt signaling pathway is the main mechanism of PAF-induced apoptosis in enterocytes. Polyunsaturated fatty acids block this mechanism early in the signaling cascade, probably by way of an effect on protein palmitoylation[149].

After a period of bowel ischemia, the reperfusion of blood actually increases damage to the tissue. As oxygenation of the reperfused tissue occurs, free radicals are produced, MCs degranulate, leukocytes adhere to the endothelial wall, and thus ischemia/reperfusion (I/R) damages especially the mucosal cell layer and villous structure, as well as mucosal permeability and mitochondrial activity. 3,4-Dihydroxy-phenyl lactic acid reduces the prodirection of peroxide and the expression of adhesion molecules in neutrophils and thereby minimizes the disturbance in the microcirculation[150]. If the initial period of ischemia is short (about 15 min in mice), these adverse effects may reverse, but in longer periods of ischemia, the changes in structure and function are irreversible[151].

During hypoxia-associated inflammation, endogenous adenosine is protective. In hypoxia, the levels of equilibrative nucleoside transporter-2 (ENT2) falls in human epithelial cells[148], whereas hypoxia-inducible-factor-12-dependent repression of ENT2 increases mucosal adenosine signaling, thereby reducing the hypoxia-associated inflammation of the intestine. During hypoxia, the actin cytoskeleton is aggregated in endothelial, epithelial and vascular smooth muscle cells. This aggregation results from the disposition of IgM and complement (C3), causing further tissue injury[152].

IMMUNOLOGY/ALLERGY

Immune-associated disease is of epidemic nature in postindustrial society. For example, adverse reactions to food are common, and true food allergies are believed to affect about 7% of children under the age of 10 years, and 2% of the adult population. These immune reactions are mediated by IgE-dependent or -independent mechanisms involving MCs, eosinophils, and other immune cells[153].

The intestinal immune system includes Peyer's patches, isolated lymphoid follicles, mesentery lymph nodes, lamina propria mononuclear cells, and intraepithelial lymphocytes. The human intestine has unique natural killer (NK) cells derived from hemopoietic stem cells in the bone marrow. These NK cells contribute to maintenance of immune homeostasis in the intestine[154].

Chemokines and their receptors control the influx of T and B cells into the Peyer's patches of the intestine. There is different expression of chemokines along the length of the small intestine[155].

The M (microfold) cells are a clonal population derived from a single stem cell in the small intestinal crypts, within the follicle-associated epithelium of the Peyer's patches. M cells transport antigens and microorganisms

into the underlying lymphoid tissues. Mature enterocytes may convert to the M cell phenotype under the influence of either T and B lymphocytes or microorganisms[156].

Macrophages in the intestinal tract are important to host defense by way of recognizing, phagocytizing and killing microorganisms. Classically, activated Th1 immune response macrophages are major effector cells in the Th1 immune responses. These mediate the production of NO by inducing NOS-2. Alternatively, activated macrophages are induced by T helper (Th)2 cytokines, including IL-4 and IL-13, as well as signal transducer and activator of transcription 6[157].

Under normal circumstances, the gut immune system is poorly responsive to food allergens and to commensal bacteria. This hyporesponsiveness occurs through a process of immune tolerance. The Th2 phenotype is involved in development of allergic disease through release of IL-4, IL-5 and IL-13. These cytokines play a role in antigen-specific IgE production by B cells. IL-4, IL-5 and IL-13 are also important in mucous secretion, muscle contraction and eosinophilia.

IL-4 is the key Th2 cytokine involved in switching antibody responses to IgE. IL-4 induces the expression of IgE receptors on MCs, as well as on other cell types. IL-4 gene transfer to the small bowel serosa leads both to intestinal inflammation and smooth muscle hyperresponsiveness[158]. Sensitization induces expression of CD23 on intestinal epithelial cells. This CD23 enhances IgE-dependant transepithelial antigen uptake by encoding a functional IgE receptor on human intestinal epithelial cells[159].

A localized Th2 milieu has been observed in the intestine of subjects with food allergic disorders. A gut-homing phenotype is induced by mesenteric lymph node, with selective upregulation of the Th2 chemoattractants[160]. TGFβ is an immunoregulatory cytokine that is constitutively expressed in the intestine by epithelial cells, fibroblasts and lamina propria mononuclear cells. As noted by Di Sabatino *et al*[161], "one of the paradoxes of human mucosal T-cell responses in health is the dominant Th1 profile and interferon γ (IFNγ) secretion of normal human gut T cells".

It is of interest that there is a link between intestinal immunology and motility. The Th2 cytokines are implicated in intestinal muscle dysfunction following tissue injury. Products of the innate and adaptive immune responses contribute to the activation and sensitization of primary sensory neurons. T lymphocytes are an important determinant of visceral nociception, and may provide an important opoid-mediated antinociceptive influence in the gut[162].

Cell surface molecules are required for development of intestinal allergy[163]. Disruption of the enteric glial cell (EGC) network is associated with intestinal and mesenteric T-cell infiltration, leading to Th1 cytokine-associated bowel inflammation. EGCs express major histocompatibility complex (MHC) class Ⅰ, and regulate MHC class Ⅱ in persons with bowel inflammation. CD8 and CD4 T cells trigger autoimmunity. Direct presentation of antigen by lymph node stromal cells protects against CD8 T-cell-mediated intestinal autoimmunity[164].

CD23 is a tight Ⅱ intercoil membrane glycoprotein with a carboxy terminal C-type lectin head that binds its ligand, IgE, in a calcium-dependent manner. CD23a is expressed constitutively on mature activated B cells, whereas CD23b is induced by IL-4 or IL-23. CD23a is increased in food-allergic patients, and CD23a is expressed by primary human IECs. The increase in antigen uptake by specific IgE is through the process of diverting antigen from delivery to lysosomes. Delivery of antigen-IgE complexes across the intestinal epithelial barrier may induce deactivation of MCs, and contribute to food-induced pathophysiology of the GI tract[165].

The *in vitro* treatment of smooth muscle tissue with IL-1β decreases expression of CPI-17. Smooth muscle serine-threonine protein phosphorylases are endogenous inhibitory proteins. This TNF-α-mediated process inhibits its muscle contraction in the small intestine[166]. It is not yet known whether intestinal motility may be modulated for therapeutic purposes by immune alterations.

The vagus nerve regulates GI motor and digestive functions through the release of acetylcholine (ACh). This parasympathetic neurotransmitter activates mAChRs, the nicotinic ACh receptors. The afferent vagal nerve, working through the hypothalamic pituitary adrenal axis, acts to inhibit the activation of NF-κB. This reduces proinflammatory cytokine secretion by macrophages, as well as modifying macrophage endocytosis and phagocytosis. Thus, "vagus nerve efferent activity may stimulate surveillance in the intestinal mucosa and peritoneal compartment[167]. This role of the vagus nerve awaits study as a potential therapeutic target in persons with allergies.

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