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Pathogenesis of Malabsorption Syndrome: Issues on Gut Flora and Innate Immunity

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The enormously large gastrointestinal epithelium is dedicated to two seemingly contrasting roles. In its fundamental role in nutrient absorption, the small intestine offers one of the largest epithelial surfaces of the body. The surface is evolutionarily adapted for efficient absorption—manifested by a thin epithelial barrier and cells highly active in membrane transport. This allows the vast diversity of nutrient molecules to be absorbed using many different active and passive transport mechanisms through transcellular and paracellular routes. However, the functional emphasis on extracellular uptake of small molecules and metabolites also present a concurrent risk for entry of toxins and infectious agents in the intestinal lumen. Besides, the nutritionally rich milieu and anatomical features that help transport—i.e., a single epithelial barrier, numerous villi and crypts—also provide an excellent niche for bacterial attachment, colonization, and translocation in the small intestine. While host–pathogen relationships of enteric pathogens in context are better understood, it is the remarkable discretion of this epithelial barrier for either permission or

discrimination of bacterial association at anatomic site(s) of choice that enjoys major interest at present. Fundamental advances in the past two decades have helped understand the unique functional features of the intestinal epithelium: (*i*) *Selectivity of absorption*—enterocytes, the major absorptive cells in the intestine, are polar in nature and offer significant selectivity to paracellular transport through their tight junctions.[1,2] These junctional complexes are much more than epithelial barriers in paracellular transport or diffusion, they also contain proteins involved in stimuli response, signal transduction, and the maintenance of homeostasis.[3] Selectivity is also exerted by phenotypic differences in enterocytes along the longitudinal intestinal axis by the number and type of transporter molecules in their plasma membrane and the structure of the tight junctions they form. Even within a specific intestinal segment, there are major differences in the

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type of transport that occurs, for example, cells in the crypts transport very differently than cells on the tips of villi.[2,4] (*ii*) *Selectivity of association*—the epithelia also present a remarkably complex immune system with highly efficient innate (quick reaction and non-specific) and adaptive (long term and specific) immune system. The intestinal innate immune system is unique in two ways: (*i*) it is closely associated with a diversity of commensal flora, a critical intestinal homeostasis and yet an immunologically "non-self" entity. It has to tolerate this flora and accurately discriminate food- and water-borne pathogens. (*ii*) When stimulated, it can produce an extremely large range of broad-spectrum antibiotics, cytokines and others that can neutralize wide ranging bacteria without resistance selection in the commensals and stimulate the adaptive immune system.

Nutrient absorption is a function of balance between these two roles of the intestinal epithelium. Malabsorption occurs when this

delicate balance between the absorptive capacity and barrier function of the intestine is compromised and result in impaired absorption of nutrients. This may result from congenital defects in the membrane transport mechanisms in the intestinal epithelium (primary malabsorption) or from acquired defects in the epithelial absorptive surface (secondary malabsorption). In clinical practice malabsorption is often construed with maldigestion, although the mechanisms of malabsorption are distinct from the latter. Malabsorption may also be either partial or global. Partial or isolated malabsorption results from diseases that interfere with the absorption of specific nutrients.[5] An example of this is pernicious anemia. Global malabsorption is generally manifested by non-specific impairment of all nutrient classes due to reduced absorptive surface or diffuse mucosal involvement. A typical example is celiac sprue—a diffuse mucosal disease, or tropical sprue,

Two fundamental factors of intestinal homeostasis that play often contrasting roles in SIBO-mediated global malabsorption: (i) intestinal innate immunity that protects and (ii) colonizing bacteria that exacerbate SIBO pathophysiology.

caused by small intestinal bacterial overgrowth (SIBO).[6,7] However, it increasingly seems that SIBO's pathophysiology may extend well beyond just malabsorption and encompass disorders as diverse as irritable bowel syndrome (IBS), celiac sprue, non-alcoholic fatty liver disease, and others, which may not even be associated with the current ambit of gastroenterology. This section will focus on two fundamental factors of intestinal homeostasis that play often contrasting roles in SIBO-mediated global malabsorption: (*i*) intestinal innate immunity that protects and (*ii*) colonizing bacteria that exacerbate SIBO pathophysiology.

INTESTINAL INNATE IMMUNITY

Overall, gastrointestinal host defense system follows the same innate immune paradigm elsewhere: (*i*) non-specific, steady-state epithelial

barrier, (*ii*) a rudimentary, yet effective sensor system to detect "threat", and (*iii*) a set of quick-acting response(s) to this threat. However, the GI innate immunity also deals with a phenomenon not encountered anywhere in the human system—a close association with an enormous bacterial flora that is immunologically "non-self". Not surprisingly therefore, the GI host defense is far more complex and multidimensional than other epithelial defenses, like the airway. Many layers of protection and redundancy exist, including but not limited to tight junctions, a diversity of sensor molecules that can accurately discriminate and signal various kinds of threat, infectious or metabolic and an array of effectors like IgA, antimicrobial peptide, proteins, and lectins. Intestinal motility plays an enormously significant role with its contractions of the phase III of the inter-digestive migrating motor complex (MMC) that "sweeps" the small intestinal contents toward the colon every 90–120 minutes and thereby reduce colonization risks.[8]

The gut flora themselves also contribute to these defenses by producing antimicrobial factors to limit entry of alien bacteria.

An interesting corollary is that the gut flora themselves also contribute to these defenses by producing antimicrobial factors to limit entry of alien bacteria.[9,10] Together, the gastrointestinal innate immunity presents a non-inflammatory, energy-efficient host defense that allows absorption of small (nutrient) molecules to entire (microbial) cells (participated by M cells), switching on adaptive immune functions only when required.

The Innate Immune Sensors

Given their limited repertoire, innate immune sensors in the gut have evolved a simple, yet extremely effective way to discriminate commensal flora from the pathogens. Instead of identifying specific bacteria, the sensors recognize pathogen-associated molecular patterns (PAMPs) that are related to pathogenic/virulent determinants in bacteria.[11] Typically, these include parts of bacterial cell envelope,

such as lipopolysaccharide (LPS), peptidoglycan, and bacterial DNA. Consequently, these innate immune sensors have come to be known as "pattern recognition receptors" (PRRs). Two major groups of PRRs are known, the "toll-like receptors" (TLRs) and "nod-like receptors" (NLRs), which sense extracellular and intracellular (cytoplasmic) PAMPs, respectively.[12] TLRs formerly have attracted substantial attention due to their role in cellular signaling and initiation of the adaptive immune response.[13] There are 13 different TLRs reported in the human genome, each specific for unique class(es) of PAMP(s).[13,14] While TLRs are membrane-bound primary proximal sensors for extracellular PAMPs in general, NLRs are present in the cytosol and operate intracellularly by responding to microbes that have invaded or translocated.[12,15] The mammalian NLR family is composed of more than 20 members.[16] Similar to the TLRs, many members of the NLR family also exhibit specificity toward one or more class of PAMPs. In gastrointestinal epithelial cells,

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the best characterized NLRs are the Nod (nucleotide-binding oligomerization domain 1) proteins.[11] Each Nod protein is characterized by a unique caspase activation and recruitment domain (CARD).[11] Nod1 (*CARD4*) was the first NLR reported as a potent sensor for an enteroinvasive form of *Shigella flexneri*.[16] This was followed by Nod2 (*CARD15*). In terms of specificity toward PAMPs, both Nod1 and Nod2 detect distinct substructures from bacterial peptidoglycan.[17] Nod1 senses peptidoglycan containing *meso*-diaminopimelic acid (*meso*-DAP), more commonly found in Gram-negative bacteria. In contrast, Nod2 detects muramyl dipeptide, the largest molecular motif common to Gram-negative as well as Gram-positive bacteria.[17] Downstream events of engagement of either the Nods or TLRs with their ligand(s) are complex and include, but are not limited to, the induction of NF-κB-mediated inflammatory pathways, upregulation and/or secretion of

antimicrobial peptides, and stimulating the adaptive immune response.[15] Overall, by the innate immune system, the gut practices regulated immune tolerance for non-invasive bacteria in the (intestinal) lumen by low steady-state expression of TLRs (which sense luminal bacteria).[15] For example, the healthy columnar intestinal epithelial cells (IECs) of the gut do not express TLR2, TLR4, and CD14, minimizing the recognition of bacterial PAMPs. Among the others, TLR5 (which recognizes bacterial flagellin) is expressed exclusively on the basolateral surfaces of IECs, and TLR3, TLR7, TLR8, and TLR9 are expressed in endosomes. Together this indicates that the intestinal innate immune system is geared more to respond to invasive bacteria than commensal on the luminal surface.

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The Innate Immune Effectors

Arrays of antimicrobial effectors protect the small intestinal epithelium from bacterial invasion, overgrowth, and consequent malabsorption. In the context of host defense, these may be simple inorganic acid in the stomach, which can very effectively deter food- and water-borne bacteria.[18] In the intestine, the bile acids—owing to their chaotropic property—exert significant antimicrobial activities.[18] However, the most significant antimicrobial effectors in the intestine are wide array of cationic antimicrobial peptides, proteins, and lectins.[11,18] These are products of distinct genes, which may be either constitutive (i.e., alfa-defensins or lysozyme) or inducible (i.e., beta-defensins) by infectious stimuli. The major antimicrobial gene products are defensins, lysozyme, cathelicidins (LL-37), secretory phospholipase A2 (sPLA2), and lectins.[19,20] They all have broad-spectrum antimicrobial activity; interestingly several also exhibit signaling, immunoregulatory and angiogenic functions. Defensins—a group of small (~3 kDa) cationic, membrane active, amphipathic molecules—are the most significant in

this group. Based on differences in their primary structures, they are differentiated into two subgroups—alfa- and beta-defensins. In the human genome, 8 alfa- and 10 beta-defensin genes are known. Of these, 2 alfa- and 4 beta-defensins express in (human) intestine. Interestingly, expression profile of each class of these defensins varies along the intestinal axis; in healthy adults, alfa-defensins are exclusively expressed in the Paneth cells of the small intestine; whereas beta-defensins are predominantly expressed in the colonocytes of the large intestine.

Paneth Cells—Sentinels of Intestinal Host Defense

The two small intestinal alfa–defensins, human defensin 5 and 6 (HD5 and HD6), are expressed in the Paneth cells, which reside in the base of small intestinal crypts of Lieberkühn. The defensins are stored intracellularly as proforms within azurophilic granules in these cells.[21]

The processed defensins along with other Paneth cell antimicrobial determinants exert broad spectrum, multi-molecular-target based antibiotic activity against food- and water-borne pathogens.

The granules also store copious amounts of lysozyme, secretory phospholipase A2, and lectins.[19,20] Interestingly, Paneth cell granules also store trypsinogen-II, a specific anionic isoform of the well known enzyme, along with alfa 1 antitrypsin and pancreatic secretory trypsin inhibitor (PSTI).[22] In response to cholinergic or infectious stimulus like bacteria or their PAMPs (LPS, muramyl dipeptide, and CpG DNA), the granules are secreted out into the crypt lumen.[19,23,24] Both HD5 and HD6 are subsequently processed by trypsin in the crypt lumen. The processed defensins along with other Paneth cell antimicrobial determinants exert broad spectrum, multi-molecular-target based antibiotic activity against food- and water-borne pathogens. We have shown defensins like HD5 can independently deter potent enteric pathogens like Salmonella sp. from infection.[25] In the lumen, at lower concentrations lower than that in the crypts, HD5 can still reduce

total bacterial count of Salmonella to sub-infectious (MIC) levels.[25] Expression of HD5 in a transgenic murine HD5 model developed by us lead to significant alteration of the bacterial flora in the large intestine with a shift in the composition of bacterial microbiota from predominantly small bacilli and cocci (wild-type mice) to a mixed population of bacilli and predominantly fusiform bacteria (transgenic mice).[24,25] This indicated that Paneth-cell defensins can also control the type of gut flora.

Cells that are so pivotal to host defense necessitate sophisticated control of function. Indeed, Paneth cells are regulated at many levels. Secretion is controlled cholinergic stimuli that activate G proteins coupled muscarinic receptors.[26] This indicates an evolutionary conserved link between Paneth cell secretion/innate immunity and vagal activity that may be stimulated by the ingestion of meal. Paneth

Paneth cell secretion may also be induced independently by bacterial PAMPs.

cell secretion may also be induced independently by bacterial PAMPs.[27] Given that the most significant Paneth cell innate sensor is Nod2, it is conceivable that the latter plays a major role in this process.[12] Nod2 activates the NF-κB complex via RICK/RIP2 kinase to induce an inflammatory response.[12] While the cholinergic neuronal inflammatory reflex pathway is well characterized, the exact mechanism of Nod2 activation that may lead to Paneth cell secretion is not known. However, the functional significance of these convergence immunological and neurological pathways in the Paneth cell is easy to understand in context of the demand for immediate host–defense response against food/water borne threat. The major significance of Nod2 mediated control over Paneth cell functions came by the seminal discovery of mutations in Nod2 (*CARD15*) that confers genetic susceptibility to at least one group of Crohn's disease (ileal CD).[28] Significantly, this mutation apparently leads to a sharp decrease in Paneth cell defensins.[28] Recently, it has been reported that TCF-4 (also

named TCF7L2), a Wnt signalling pathway transcription factor which is critical for Paneth cell differentiation, also directly controls (Paneth cell) defensin expression.[29] Interestingly, similar Nod2 mutations have not been detected in Asian CD patients although it is increasingly clear that the incidence of the disease is comparable between Caucasians and Asians (Chinese, Japanese, and Koreans).[30,31] There are reasons to believe such; the Indian demographics may also not agree with the Nod2 polymorphism profile observed in the west and other factors may play more fundamental roles.[32,33] While there is no doubt about the link between Nod2 and/or TCF-4 in contributing to Paneth cell dysregulation, the true extent of this in malabsorption pathophysiology is not clear. Inflammation, all too frequent in IBD (and at least in some

In sharp contrast to alfa-defensins, most beta-defensins are highly inducible owing to a NF-κB inducible site in the gene.[37] Expression of the enteric beta-defensins, HBD-2, 3, and 4 are all induced by various inflammatory and bacterial stimuli.

subsets of IBS), results in extensive damage/loss of intestinal epithelium.[34–36] It is not inconceivable that Paneth cell numbers and functions may also be affected in such cases. The consequent controversy whether Paneth cell deficiencies are the *cause* or rather the *effect* of IBD will require more extensive studies.

In sharp contrast to alfa-defensins, most beta-defensins are highly inducible owing to a NF-κB inducible site in the gene.[37] Expression of the enteric beta-defensins, HBD-2, 3, and 4 are all induced by various inflammatory and bacterial stimuli. The only exception is HBD-1, which is not upregulated by pro-inflammatory stimuli or bacterial infection. In contrast, HBD-2 expression is highly upregulated by inflammatory or bacterial stimulus; also in IBS.[37,38] There is usually little or no expression of HBD-2 in the normal colon, but abundant HBD-2 expression by the epithelium of inflamed colon. Fahlgren et al. investigated HBD-3 and HBD-4 mRNAs in Crohn's and ulcerative colitis (UC) patients, using

real-time quantitative reverse transcription-polymerase chain reaction (QRT-PCR) and by in situ hybridization.[39] They observed significant upregulation of HBD-3 and HBD-4 in UC patients but none in CD. Interestingly, HBD-3 has recently been shown to be the most active defensin against anaerobic bacteria in the gut.[40] Nuding et al. have recently demonstrated that major anaerobic gut bacteria Bacteroides and Parabacteroides, were most effectively controlled by the HBD-3.[40] Other defensins like HD5, HBD-1, and HBD-2 are largely ineffective against these bacteria, but remarkably effective against other bacteria even at strain level.[40,41]

An emerging hypothesis is that the different intestinal antimicrobial peptides and/or their induction mechanisms may possess specificity toward one or more bacteria and help in their selection within the gut microbial ecosystem. This "healthy" flora in-turn helps digestion, nutrient absorption, and production of bacteria derived products like

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short-chain fatty acid beneficial to the host. This hypothesis would predict that deficiency/defects in this innate immune selection (due to either down-regulation or low copy number of the defensins, post-translational processing, innate immune sensor(s) or secretory apparatus) would lead to systemic change in bacterial flora in the large intestine and perhaps their PAMPs.[42] This would result in gradual or chronic colonization of the small intestine by this flora, giving rise to the conditions of SIBO and ultimately permit or facilitate translocation of those bacteria or bacterial components (antigen) across the intestinal barrier leading to loss of tolerance to gut bacteria.[43] Not surprisingly, accurate understanding of the bacterial flora in disease and health is assumed of enormous significance at present.

BACTERIAL FLORA AND SMALL INTESTINAL BACTERIAL OVERGROWTH

The Gut Flora—Implications in Intestinal Homeostasis and Health

Metagenomic profiling studies using ribosomal DNA (r-DNA) typing have already revealed that the human gut flora consists of more than 400 distinct bacterial species adding up to a concentration of 10^{12}–10^{14} bacteria per mL of luminal contents in the adult human large intestine.[9] More than 90% of the bacterial population is obligate anaerobes, predominant species being Bacteroides, Eubacterium, Bifidobacterium, Fusobacterium, Peptostreptococcus, and others. Interestingly, the

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bacterial components of this community are not static and may vary widely between different individuals, dietary changes, or between health and disease in the same individual. Across the ileocecal valve there is a sharp decrease in bacterial numbers in the ileum. The healthy ileum contains between 10^4 and 10^6 bacteria that vary between the flora from the upper GI and large intestine. A major innate immune apparatus that has withstood extremely long selection pressures is evidence for the significance of limiting access to this anatomical area to further colonization from the large intestine. In the jejunum, there are ~10^3/mL bacteria, largely from the upper GI. Therefore, under healthy conditions, there are at least 10^6 times more bacteria in the large intestine than anywhere in the GI tract. In context of sheer cell number, this amounts to ~10 times higher than the combined number of somatic and stem cells in the human body. Recent studies on the human gut "microbiome" (the term given to the microbial community in the gut) have revealed that the gut flora encode approximately 9 million genes (~300 times the number of genes in the human genome).

The emerging concept from this striking information is the remarkable diversity of the translated products of these genes result in human "superorganisms" capable of adapting to selection pressures more diverse than any other living system across evolution.[10] Indeed, accumulating pool of evidences indicate the gut flora play incredibly diverse roles in human physiology that includes, but is not limited to nutrient digestion/absorption, maturation, and proliferation of human intestinal cells, immune homeostasis, as well as diseases like cancer, obesity, IBS, and IBD.[10,44–46] The intraluminal flora also influences the release of bioactive gastrointestinal peptides, organic acids containing phenyl groups, and regulates gastrointestinal endocrine cells.[47,48] The gut flora also produces short-chain fatty acids, which control proliferation and differentiation, thereby reducing colon cancer risk.[46,49]

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In patients with major loss of small intestinal functions, short-chain fatty acid production supports survival by releasing up to 1000 kcal energy/d.[49] The flora also has a direct implication on mammalian blood metabolites. In a seminal investigation Wikoff et al. examined plasma extracts from germ-free mice against conventional animals.[48] The group observed extremely varied changes; both in terms of unique metabolic signals and in the relative signal intensity of shared signals between the two groups. Amino acid metabolites were particularly affected. For example, the bacteria-mediated production of bioactive indole-containing metabolites derived from tryptophan such as indoxyl sulfate were markedly altered.[48] This group as well as others has indicated that gut bacteria are capable of performing a range of biotrans-formations on xenobiotics, in ways that can affect absorption, bioavailability, and overall physiology in much complex and fundamental ways than currently understood.[50–52] Recently, using systems biology Francois-Pierre J et al. compared the metabolomics of germ-free mice colonized by human baby flora (HBF) or a normal flora to conventional

mice.[53] They observed compared to normal, the mice with HBF flora had significantly lower ability to metabolize lipids and showed higher ileal concentrations of tauro-conjugated bile acids, reduced plasma levels of lipoproteins but higher hepatic triglyceride content associated with depletion of glutathione.[53] These data strongly support that the microbiome modulates absorption, storage and the energy harvest from the diet at the systems level.

These and many other researches on animal models and in silico analysis have now set up the base for analysis of human subjects. In a first well-conducted comparative metagenomics study of the microbiome from two unrelated, healthy adults showed significant enrichment for genes involved in many different metabolic pathways including metabolism of xenobiotics, glycans and amino acids and isoprenoids and vitamin biosynthesis through the 2-methyl-D-erythritol 4-phosphate pathway.[10] This remarkable enrichment and diversity in metabolic pathways owing to not the host directly but a transferable microbiome is thought to result in "superorganisms" capable of adapting

The microbiome modulates absorption, storage and the energy harvest from the diet at the systems level.

to extremely wide range of selection pressures across evolution.[10] There are many questions on the diverse aspects of these relationships. For example: (*i*) What are the selection pressures on the microbiome that requires such large diversity and concentration of genetic material? (*ii*) Do all humans have an identifiable "core" microbiome that exists across the lifespan and if so, how is it acquired and transmitted? (*iii*) Does the genetic diversity of the flora follow hereditary or geographical lineage? (*iv*) Can life style, disease, pharmaceutical interventions change this flora and if so, can these independently be a determinant to diseases? These and many other questions on the far reaching roles of the human microbiome in intestinal homeostasis and health are expected to be addressed by the massive Human Microbiome Project (http://nihroadmap.nih.gov/hmp/) launched recently.[54–56] This

prestigious program will (*i*) develop a reference set of microbial genome sequences and (*ii*) perform systems biology characterization of the human microbiome and its relationship between disease and changes in the human microbiome. The ultimate goal is to understand and define the role of individual bacteria or specific bacterial communities in the gut that may define host physiological states in health or disease.

Gut Flora in Disease—The Determinants of SIBO and Malabsorption

Given the wide-ranging roles of normal gut flora on human health and homeostasis, it seems extremely likely that change in the gut bacterial ecosystem may have significant impact on health and disease. Unfortunately, technical challenges in identifying the microbiome in SIBO or IBD and understanding their significance has limited current understanding in context. However, an emerging pool of data indicates the validity of the concept. For example, there are some reports that bacterial flora in IBD differs from healthy subjects.[42,57,58] Differences in

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microbiota were in bacteria isolated from mucosal biopsies of inflamed versus non-inflamed tissue of newly diagnosed and untreated patients of CD and UC patients.[58] The increasingly accepted case of bacteria playing in a role in at least one or more sets of IBS, makes SIBO an exciting area of research at present.[59]

Small intestinal bacterial overgrowth is defined as a condition when the bacterial content of the small intestine exceeds 10^5 CFU/mL.[60] The immediate and easily understood consequence of this colonization is competition for nutrients. Consequently, malabsorption, weight loss, clinical vitamin B_{12} deficiency, and finally IBS are fairly routine in SIBO patients.[59,61] SIBO associated with steatorrhea may result in osteomalacia, osteoporosis, increased renal calculi, and dermatitis (the latter due to micronutrient deficiency); vision related complications

due to vitamin A deficiencies have also been attributed.[7] Following are the consequences of SIBO-related malabsorption that are particularly significant in light of our knowledge of innate host–defense microbe interactions.

Deconjugation of Bile Acids

An increasing pool of evidence indicates that bile salts and their modulation by bacteria in SIBO may play a defining role in the pathophysiology of this disease. A school of thought is that bacteria in SIBO or at least a subset of these are actively involved in deconjugation of bile acids. The primary bile acids, cholic and chenodeoxycholic acid, are synthesized de novo in the liver from cholesterol, and the hydrophobic steroid nucleus is conjugated as an *N*-acyl amidate with either glycine (glycoconjugated) or taurine (tauroconjugated) prior to secretion.[62] Conjugation makes bile acids amphipathic and solubilize lipids to form mixed micelles that are then absorbed in the intestine. However, bile acids function as signaling molecules regulating their own biosynthesis, regulate cholesterol homeostasis, and exhibit potent innate immune functions, thereby affecting roles larger than digestion.[62–64] Certain bacteria in the colon flora that enjoy bile salt hydrolase (BSH) enzymes (EC 3.5.1.24) have capacity to deconjugate these bile salts.[65] The mechanism involves hydrolysis of peptide linkage of conjugated bile acids between bile acid carboxyl and amino group of taurine or glycine, thereby liberating the amino acid moiety (deconjugation). This is also called as the "gateway" reaction, since it affects a much wider pathway of host physiology by the gut flora. Jones et al. showed BSH activity is a conserved microbial adaptation to the human gut environment with a high level of redundancy.[62] Under normal conditions, the deconjugation activities in the large intestine are beneficial for the host. However, excessive deconjugation of bile acids or that in the small intestine by SIBO bacteria impair lipid

Conjugation makes bile acids amphipathic and solubilize lipids to form mixed micelles that are then absorbed in the intestine.

absorption and increase risk of metabolic diseases such as obesity, diabetes, and atherosclerosis.[62,64] Besides, free bile acid also results in malabsorption of carbohydrate and amino acid in the small intestine. Further, at physiologic concentrations deconjugated bile salts can profoundly damage epthelial junctiona integrity, ultimately leading to exfoliation of the epithelium. Using scanning electron microscopy of jejunal mucosa exposed to different conjugated (taurocholate, taurodeoxycholate, taurochenodeoxycholate, tauroursodeoxycholate) and unconjugated (cholate, deoxycholate, chenodeoxycholate, ursodeoxycholate) bile salts, Oumi et al. recently demonstrated that unconjugated bile salts induced morphological changes starting from damaged upper villi to complete denudation of the jejunal villi, depending on the kind and dose of bile salts.[66] Unconjugated deoxycholate induced severe damage even 1.0 mM concentration, whereas conjugated bile salts did not (damage) even at 40 fold higher concentrations.[66] Accumulating pool of evidences argue for a direct relationship of bacterial deconjugation (of bile acids) in the small intestine and pathobiology of malabsorption.[66–68] Using high-resolution 1H-NMR spectroscopy analysis of upper-gut aspirates of patients with malabsorption syndrome (MAS) with and without SIBO Bala et al. showed MAS patients with SIBO had higher quantities of unconjugated bile acids than MAS without SIBO.[68] The presence of unconjugated bile acids correlated with the degree of steatorrhea in these patients and provided a direct relationship of SIBO and MAS in context of biliary deconjugation.

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Modulation of Appetite

The postprandial symptoms commonly seen in SIBO patients discourage appetite. In context of host defense it would seem that reduced food intake in response to bacteria is primarily advantageous. Acute phase

events and their relationship in conditioned taste aversion (CTA) is an accepted paradigm, and there is a large body of evidence in support of anorectic response to LPS.[69] Additionally, interleukins like IL-1 and IL-18 have been shown to reduce food consumption.[70] Earlier, Exton et al. demonstrated that LPS-mediated anorexia can be conditioned and is dependent on prostaglandin. The group also showed that gastric emptying was also conditionable, and was itself prostaglandin dependent. Given the advantages of exerting nutritional stress on colonizing bacteria, does the activation of host intestinal immune system with infectious stimulus result in anorexia? Indeed, several innate immune PRRs like CD14, TLR2, TLR4, and TLR7 have been implicated in bacterial product-induced anorexia.[71] The mechanism for

Activated intestinal mast cells can induce diarrhea through histamine and serotonin release, further exacerbating the malabsorption and nutritional status.

this process has not been elucidated yet, but at least in case of TLR7 the anorexic effects seem to be exerted through mast cells.[72] Interestingly, mast cells themselves are armed with TLRs and Nod1 to detect and respond to bacteria.[73] Activated intestinal mast cells can induce diarrhea through histamine and serotonin release, further exacerbating the malabsorption and nutritional status.[73] Ikeda et al. have recently observed a diverse group of TLRs, including TLR7 are up-regulated in response to total parenteral nutrition (TPN) administration, particularly in the distal small intestine.[74] Given that TPN is often known to increase risk for SIBO and subsequent bacterial translocation, expression of PRRs and lowering local nutritional availability may be a mechanism of innate defense against colonization risk. However, it should be noted that anorexia as a mechanism of defense against SIBO is “double-edged sword”. While lower nutritional availability in the small intestine may discourage bacterial growth, prolonged anorexia may also affect host health and innate immune response. The latter would make the host more vulnerable to intestinal colonization and

infections. However, the possible connection of anorexia—one of the most common symptoms of illness, injury, or inflammation—and innate immune modulators is interesting, given the current interest in the bidirectional communication between the neuroendocrine and immune systems.

Production of Metabolic Byproducts

Accumulating research indicates that specific microbial communities in the intestine can generate metabolic byproducts which, above a critical concentration, pose health challenge. Carbohydrate fermentation generates hydrogen and carbon dioxide and in some cases methane, besides organic acids. When in excess, the immediate result of these gases ranges from bloating, cramping, abdominal pain, and

Hydrogen, one of the most common diagnostic tools for SIBO, can impair NAD regeneration and inhibit cellular metabolism.

borborygmi to nausea. However, the more complex side effects of volatile microbial metabolites are beginning to be elucidated. Hydrogen, one of the most common diagnostic tools for SIBO, can impair NAD regeneration and inhibit cellular metabolism.[6,75] During the anaerobic metabolism of the colonic bacterial flora, short-chain fatty acids and hydrogen and carbon dioxide gases are produced. Methane is a biologically active gas that is capable of slowing intestinal transit by shifting the pattern of motility from peristaltic to nonperistaltic.[76]

Besides this, a competition exists for the common substrate hydrogen, which is potentially regulated by the availability of sulfate in the colonic lumen. When this is available (commonly from high protein diet), the metabolites of sulfate reduction (mercaptides, hydrogen sulfide) may be toxic.[77] Gallego et al. showed that hydrogen sulfide (released from externally administered sodium hydrogen sulfide) caused a concentration-dependent inhibition of spontaneous motor complexes (MCs) in the murine colon and jejunum.[78] In strips from human colon and rat colon, the gas inhibited spontaneous motility in

concentration-dependent manner.[78] This effect was critically dependent on K channels, particularly apamin-sensitive SK channels and glybenclamide-sensitive K (ATP) channels.[78] The interference or dysregulation of motility, otherwise one of the major mechanisms of intestinal host defense, allows the bacteria critical time for planktonic to biofilm conversion and colonization.[8,79] It is likely that H_2S and other volatile sulfur compounds like mercaptans may play even more significant role in pathobiology of malabsorption caused by SIBO than currently known. There is strong possibility that hydrogen sulfide independently may play a direct role in IBS.[80] With the availability of improved analytical techniques for analysis of volatile byproducts of SIBO and/or growth of specific bacterial groups in the large intestine many other processes may be elucidated.

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