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The Intestinal Microenvironment and Disorders of Gut–Brain Interaction

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The past decade has witnessed a tremendous profusion of data on the luminal contents of the gastrointestinal tract and their interactions with the host, many of which have been implicated in the pathophysiology of disorders of gut–brain interaction (DGBI). The role of food in DGBI-related symptoms has attracted much attention and although many alterations in gut microbiome composition have been described, the multitude of factors that confound study design and interpretation in DGBI has precluded the discovery of a specific microbial "signature." The complexities of the gut barrier, its immune and enteroendocrine systems, so critical to the transmission of signals from lumen to host, continue to be revealed. Along the way, concepts such as the microbiome–gut–brain axis have emerged to explain symptom generation in DGBI, forming the basis for novel diagnostic approaches and therapeutic interventions. Taken together, recent research findings have renewed interest in luminal and enteric phenomena in DGBI.

Keywords: Diet; Microbiome; Gut Barrier; Enteroendocrine Cells.

Disorders of gut–brain interaction (DGBI) are driven by variable and complex pathophysiological mechanisms. Peripheral (gut-driven) signaling plays a central role in the onset, clinical presentation, and progression of DGBI. This signaling is mediated in the intestinal microenvironment via dietary, microbial, and host components. These components are in constant crosstalk and are further influenced by extrinsic innervation from the central nervous system, enteroendocrine factors, and circadian rhythms. Progress over the past 2 decades, particularly in microbiome research, has advanced our understanding of the pathophysiology of DGBI. However, challenges remain as many studies are small, cross-sectional, and lack standardized protocols. This is particularly challenging as DGBI are heterogeneous conditions which commonly overlap and feature overlay of environmental and psychosocial factors. In addition, patient experience, treatment, and disease course likely play a significant role in modulating the intestinal microenvironment. This review covers the role of dietary, microbial, epithelial, immune, and neuroendocrine factors in shaping the intestinal microenvironment in DGBI. In addition, the review provides advances in diagnostic strategies and future considerations on how the microenvironment can be tapped for biomarker discovery in, and treatment of, DGBI.

Diet

Considering the frequency of postprandial symptoms and reports of food intolerances among patients with DGBI, diet has become an area of ever-increasing interest. A recent analysis from the Rome Foundation Global Epidemiology Study showed that dietary patterns adjusted by age, country, and religion were associated with the global prevalence and severity of irritable bowel syndrome (IBS).[1] In other studies, dietary habits were linked to higher functional dyspepsia (FD) symptom severity,[2] as well as abdominal bloating and distension.[3] More recently, mechanistic studies have started to reveal the underlying pathophysiology of diet-induced gut dysfunction.

Food Allergies and Intolerances

Classical (ie, systemic immunoglobulin [Ig]E-mediated) food allergies are rare among DGBI sufferers, and their reports of symptoms related to specific foods usually

Abbreviations used in this paper: **5-HT, 5-hydroxytryptamine; CLE, confocal laser endomicroscopy; DGBI, disorders of gut–brain interaction; ECs, enterochromaffin cells; EECs, enteroendocrine cells; FD, functional dyspepsia; FODMAPs, fermentable oligosaccharides, disaccharides, monosaccharides and polyols; GI, gastrointestinal; IBS, irritable bowel syndrome; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; Ig, immunoglobulin; IL, interleukin; NCGS, nonceliac gluten sensitivity; NF-κB, nuclear factor kappa B; PBMCs, peripheral blood mononuclear cells; PDS, postprandial distress syndrome; PI-IBS, postinfection irritable bowel syndrome; PPI, proton pump inhibitor; SCFAs, short-chain fatty acids; SERT, serotonin transporter; SI, sucrase-isomaltase; SIBO, small intestinal bacterial overgrowth; SID, sucrase-isomaltase deficiency; SNP, single nucleotide polymorphism; TLR, toll-like receptor 2; TRPV1, transient receptor potential vanilloid 1.**

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reflect food intolerances rather than allergies. Indeed, over time, it would appear that the prevalence and range of reported intolerances expand, which would not be expected with an allergy. However, some novel mechanisms of food allergic responses have been revealed of late. In the context of postinfection irritable bowel syndrome (PI-IBS), it has been shown that an enteric pathogen can break immune tolerance to certain foods, leading to the induction of an intestinal mucosal IgE immune response on subsequent exposure to the dietary component to which the individual had previously been tolerant.[4] Although largely dismissed in the past, a recent trial has resurrected interest in the potential for IgG-based food responses to be relevant to symptom induction by demonstrating that a diet based on the elimination of foods identified by IgG antibody testing alleviated abdominal pain in patients with IBS.[5] In addition, the alpha-gal syndrome, wherein an individual becomes sensitized to alpha-gal transmitted via a lone-star tick bite, only to then develop an allergic response when mammalian meats are consumed, is increasingly recognized.[6] Although urticaria is common in these patients, many primarily experience gastrointestinal (GI) symptoms, which typically present several hours after consumption of culprit foods and may mimic those of DGBI.

Dietary Constituents

Fat. Patients with FD experience more profound GI symptoms with high-fat meals compared with high-carbohydrate meals. Infusion of lipids into the small bowel has been shown to induce greater pain in both FD and IBS patients compared with healthy volunteers.[7,8] In FD, the pain was more profound than that caused by glucose infusion and associated with cholecystokinin release.[9] In patients with IBS, duodenal transient receptor potential vanilloid 1 (TRPV1) expression correlated with symptom response to intraluminal lipids.[7] Saturated fat intake has been associated with harder stool.[10]

Fermentable oligosaccharides, disaccharides, monosaccharides, and polyols. Fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) induce symptoms in patients with DGBI (particularly IBS) through osmotic effects, gas production, lipopolysaccharide-induced immune activation and changes in microbiota composition. In both adults and children, a low-FODMAP diet has been shown to provide symptom relief.[11] The response rates vary from 40% to 80% and are probably influenced by baseline FODMAP consumption as well as host factors such as epithelial sucrase-isomaltase (SI) expression and microbiota composition.[12,13] Accordingly, those with SI mutations are less likely, and conversely, those whose commensal microbiota enriched with histamine-producing *Klebsiella aerogenes* are more likely to respond.[14,15] A low-FODMAP diet delivered via a mobile app proved superior to an antispasmodic in a primary care–based study.[16] Dietary strategies (low in FODMAPs and low in carbohydrate) were found to be superior to optimized medical therapy in a single-blind randomized controlled trial.[17] In addition, although the recommended approach involves gradual and personalized reintroduction after elimination, this strategy is often poorly implemented, resulting in patients being on chronic elimination, which risks nutrient deficiencies as well as depletion of commensal microbiota. In a pilot study, a simplified low-FODMAP diet, which restricted only fructans and galactooligosaccharides, led to similar improvements in IBS symptoms and was better tolerated than a diet that restricted all 5 FODMAPs.[18]

Fiber. Long-chain polysaccharides are variably soluble and fermentable carbohydrates that are subjected to complex metabolic processing, resulting in downstream effects that will vary according to fiber type. Although the effects of fiber intake in FD have shown mixed results, impacts in IBS vary according to fiber type, with little evidence of benefit for insoluble fibers, whereas some benefit may derive from the use of partially soluble fibers like psyllium.[19] In relation to chronic constipation, a joint American College of Gastroenterology–American Gastroenterological Association clinical practice guideline provided a conditional recommendation for bran, inulin, and psyllium, although with a low certainty of evidence.[20]

Gluten. The role of gluten in nonceliac gluten (or wheat) sensitivity (NCGS) continues to be explored but remains poorly understood, with some studies suggesting that GI effects occurring on ingestion of wheat are mediated by its FODMAP (or fructan) rather than gluten content.[21] In contrast, others have demonstrated a low-grade immune response to gluten as well as amylase-tryptase inhibitors contained in wheat among those classified as NCGS.[22] On the other hand, a study among a small number of subjects with self-reported gluten sensitivity found no differences in symptom exacerbation on exposure to wheat, gluten, or a sham food, with more than 90% experiencing food-related symptoms regardless of exposure.[23]

Milk. Some patients with IBS report symptoms in response to milk and dairy products. Although a few trials have found an improvement in symptoms in a substantial proportion of patients on exclusion of these products, these trials were often unblinded; thus, a placebo effect cannot be discounted.[24,25]

Spicy foods. Symptom induction by spicy foods may be mediated by capsaicin and involve activation of TRPV1 receptors on nociceptive C fibers. An increase in the density of sensory fibers expressing TRPV1 receptors has been reported in DGBI.[7,26,27] In contrast, desensitization of C fibers by repeated capsaicin exposure may underlie the observation that symptoms were reduced in patients with IBS who chronically ingested red pepper in capsules.[28]

Other Mechanisms

Other pathophysiological mechanisms also can be invoked to explain food-related symptoms in DGBI and include an exaggerated colonic motor response (as has been described in IBS), overproduction of intestinal fluids (eg, osmotic effects, gastric acid hypersecretion), or impaired inhibition of proteases resulting in intestinal barrier compromise.

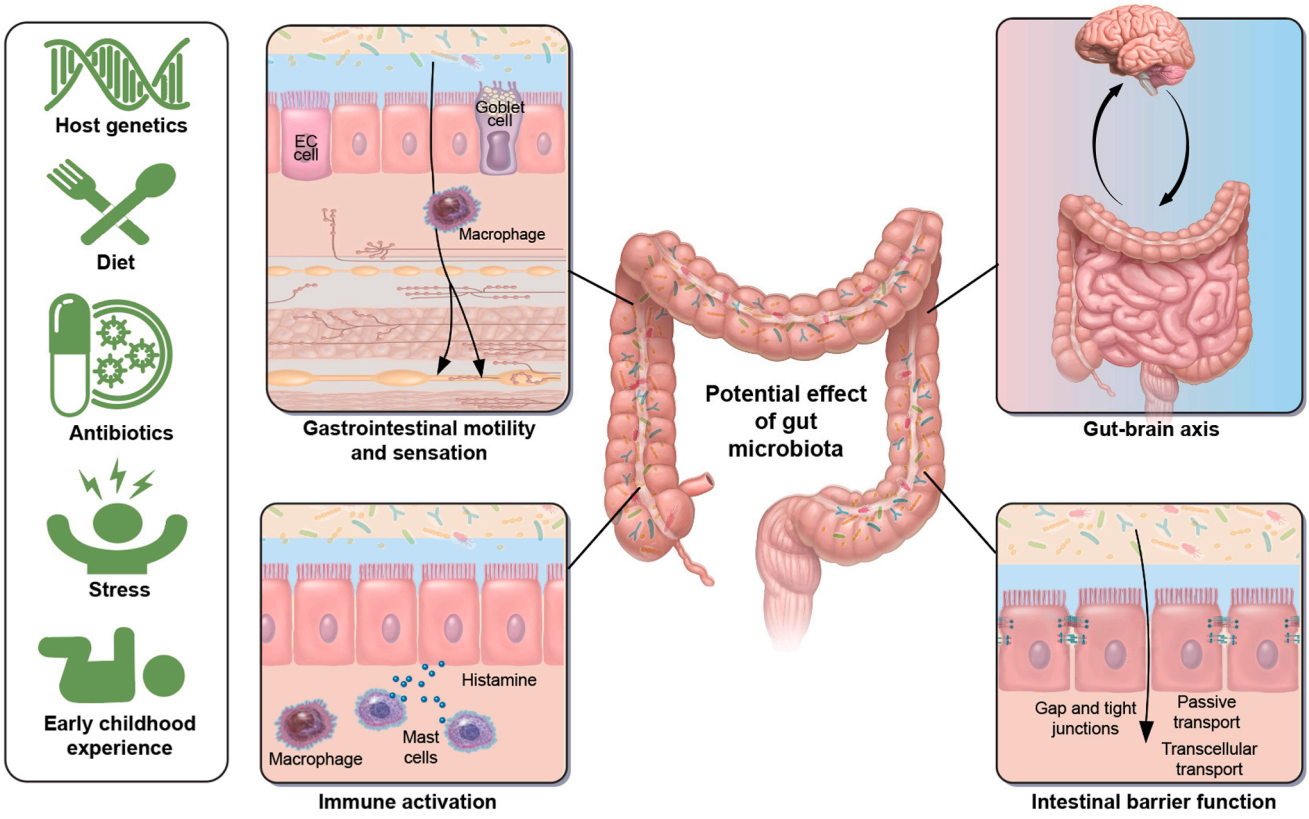

Figure 1. Schematic representation of the key role of gut microbiota involved in the pathophysiology of DGBI.

Microbiota and Associated Mediators

Intestinal microbiota has a multifaceted influence on DGBI pathophysiology, which is likely mediated by peripheral as well as systemic and central mechanisms through what is referred to as the microbiome–gut–brain axis. Furthermore, diet, and environmental (psychological stress, medications) factors critically influence microbiota (Figure 1). Changes in GI physiology related to DGBI can, in turn, influence microbial ecology; in the Belgian Flemish Gut Flora Project, for example, stool form was among the leading covariates that explain interindividual variations in microbiota composition.[29] In European studies, the identification of certain microbiome clusters referred to as enterotypes was thought to be linked to subtle variations in dietary patterns.[30] Short-term dietary changes, whether prescribed or patient-initiated, need to be considered when performing and evaluating clinical and translational microbiome studies on patients with DGBI.[31] In addition, methodological (eg, 16s high-throughput sequencing vs metagenomics; small intestinal vs colonic vs fecal or luminal vs mucosal sampling) complicate the interpretation of microbiome studies in DGBI. Last, DGBI are typically long-term problems leaving single-point-in-time studies prey to the influences of symptom severity, diet, and environmental factors.[32]

The Microbiome in Esophageal Disorders

In health, gram-positive bacteria such as *Streptococcus* dominate the esophageal microbiome, whereas in gastroesophageal reflux disease, Barrett's esophagus, and esophageal adenocarcinoma, gram-negative species are frequently isolated,[33] a change that has been proposed to play a role in the pathogenesis of esophagitis and its sequelae.[34] Information on the microbiome in functional heartburn and noncardiac chest pain is scanty, with one study noting a similar shift toward gram-negative species and linked to toll-like receptor (TLR) 2 activation and dilation of intercellular spaces.[35] In another study in nonerosive reflux disease, alterations in the microbiome were linked to impaired esophageal motor function.[36] Proton pump inhibitor (PPI) effects on esophageal microbiome composition seem limited.[37]

Microbiome in Functional Dyspepsia

There are limited data to suggest that the oral microbiome is altered in FD.[38] Despite past assertions that the stomach was a microbiological desert, recent studies have revealed a complex gastric microbiome. Data on the gastric microbiome in FD are limited, with 2 studies reporting a relative abundance of Proteobacteria.[39] Data on the gastric microbiome in relation to PPI use are not consistent and suffer from a lack of longitudinal studies. Most recent data on the duodenal microbiome in FD are derived from the study of the mucosa-associated microbiome and have revealed an association between abundance of members of the phyla Firmicutes, Bacteroidetes, and Fusobacteria with symptom severity and of *Streptococcus*, *Prevotella*, and *Veillonella* with gastric emptying rate.[40] Other studies of

the duodenal luminal or mucosal microbiomes have revealed variable alterations with some predictive of symptom severity[41] and others being similar to IBS.[42] A hypothesis has emerged that links an altered duodenal microbiome and its metabolites to disruption of the epithelial barrier, certain host immune responses (with eosinophils featuring prominently), activation of the gut–brain axis, and, ultimately, the generation of symptoms that typify FD. Like celiac disease and NCGS (perhaps more appropriately termed nonceliac wheat sensitivity), alterations of microbial-derived peptidases also have been described in FD.[43]

Microbiome in Irritable Bowel Syndrome

Small intestinal microbiome. Pending the application of emerging technologies that permit dynamic and direct sampling of the microbiome along the length of the small intestine,[44] discussions on the contributions of microbes to the pathogenesis of IBS symptoms have largely centered on the controversial topic of small intestinal bacterial overgrowth (SIBO). To what extent one accepts that SIBO plays a role in IBS is, in turn, based on one's thoughts on the relative merits and limitations of the various methodologies employed to diagnose this disorder.[45,46] For example, in many of the positive hydrogen breath tests reported in IBS, lactulose was the preferred substrate, an approach fraught with a high false positive rate.[46] Although more accurate and associated with a much lower level of positivity in IBS, breath tests using the alternative substrate, glucose, are also not without their limitations. The time-honored, although scarcely gold standard, test for SIBO, the quantitation of bacteria obtained from an aspirate of the duodenum or jejunum, is also subject to limitations. It is invasive, prone to contamination, may underestimate obligate anaerobes, and its diagnostic threshold continues to be debated (ie, is the cutoff $>10^3$ or $>10^5$ colony-forming units/mL).[47] SIBO has been proposed to explain other phenomena, such as "leaky gut" and "brain fog," which some contend to be associated with IBS; all suffer from the issue that has bedeviled the SIBO-IBS relationship—the accuracy of the diagnosis of SIBO. Until agreement can be reached on what constitutes a normal small intestinal microbiome (and its metabolic products), the relationship between IBS and SIBO will remain contentious.

Saffouri and colleagues[48] compared results of aspiration and culture of small intestinal contents with an analysis of microbial community composition using 16s ribosomal RNA sequencing in symptomatic individuals and healthy controls. On quantification, SIBO rates were 52% for the symptomatic group and 0% for the controls. Results from sequencing also differed between the 2 groups and could accurately differentiate between the 2 groups but did not correlate with the presence of SIBO. In contrast, Leite and colleagues[49] found that results of quantitative cultures performed on MacConkey medium compared well with 16s and shotgun sequencing of duodenal aspirates obtained from consecutive subjects with upper GI symptoms.

Colonic and fecal microbiome. The phenomenon of PI-IBS is now well established, and its development linked to a prolonged reduction in gut microbial diversity, which can be slow to recover.[50,51] Although it has been proposed that cross-reactivity between antibodies directed against the cyto-lethal distending toxin produced by *Campylobacter jejuni* and vinculin, an important cytoskeletal protein, could lead to injury to the enteric nervous system, rates of positivity for these antibodies in IBS have provided mixed results.[52,53] Evidence for an increased risk for IBS among adults who were prescribed antibiotics in the past provides further, albeit indirect, evidence for a role for microbial disruption in IBS.[54]

Although relatively easy to collect, fecal samples provide limited insights into the status of the gut microbiome. The interpretation of fecal and colonic mucosal microbiome studies in IBS is further complicated by the variable impacts of diet, sex, IBS subtype, symptom activity and severity, geography, and exercise. It is not surprising, therefore, that fecal microbiome studies in IBS have failed to produce a reproducible IBS signature. Indeed, the colonic mucosal microbiome may correlate better with IBS phenotype and symptoms.[55–57] In one intriguing study, IBS pain severity was associated with the presence of proteolytic bacteria and staphylococcal superantigens.[58]

The study of the microbiome in IBS has lately witnessed a shift from composition to function, facilitated by the application of various multiomics platforms. In a longitudinal study, Mars and colleagues[59] revealed the variability of microbiome composition over time and demonstrated differences between IBS subtypes and healthy controls. Metabolomic data revealed reduced levels of hypoxanthine and a state of purine starvation in IBS; when combined with mucosal physiological testing, metabolomic findings nicely separated irritable bowel syndrome with diarrhea (IBS-D) and irritable bowel syndrome with constipation (IBS-C). In another study, patients with IBS-D demonstrated stress-induced xanthine upregulation in CD4+ T cells; these stress-induced responses were attenuated by AdorA2B receptor inhibition. *Lactobacillus murinus*–derived spermidine directly triggered visceral hypersensitivity.[60] Other studies attest to the ability of these approaches to provide greater insights into symptom pathogenesis.[61,62]

Changes in the virome have been described in IBS and, in one study, a virome signature characteristic of IBS was identified.[63,64] Although it has been hypothesized that certain fungi, such as *Candida* species, may play a role in the pathogenesis of visceral pain, clinical studies, to date, have failed to identify a mycobiome signature of IBS.[65]

Microbiome in Chronic Idiopathic Constipation

There is relatively little information on the status of microbiome in chronic constipation. As in IBS, several factors may impact interpretation of microbiome data, such as dietary fiber intake, physical exercise, and psychological comorbidity. Constipation subtype, that is, whether slow or normal transit or pelvic floor dyssynergia, influences

microbiome composition, begging the question as to whether it is slow transit and not constipation per se that results in an altered microbiome.[66,67] As in IBS, the colonic mucosal microbiome may be more informative being able to differentiate chronic idiopathic constipation from healthy controls with 94% accuracy.[68] More advanced methods such as metagenomics and metabolomics are beginning to provide valuable insights.[69–71]

Microbiota-Associated Mediators

Bile acids. Bile acids have a variety of physiological effects of relevance to DGBI, including impacts on motility, intestinal secretion, mucosal permeability, and visceral sensation, as well as acting as signaling molecules with effects well beyond the GI tract.[72] Bile acid malabsorption has been described in 20% to 30% of individuals with functional diarrhea or IBS-D. It has been linked to increased colonic permeability, more severe and urgent diarrhea, and a negative impact on quality of life.[73,74] In IBS-D, fecal primary bile acids correlated with pain severity,[75] suggesting an effect of bile acids on visceral sensation; others, however, failed to document any difference in colonic sensation between IBS-D subjects with or without bile acid malabsorption.[76] The observation that rectal instillation of taurocholic acid promoted rectal urgency through release of insulin-like peptide 5 provides yet another mechanism to explain the diarrheagenic effects of bile acids.[77]

Short-chain fatty acids. Short-chain fatty acids (SCFAs) could influence gut function and generate symptoms in DGBI through several mechanisms, with effects on motility well documented. SCFA effects on the gut barrier, the gut–brain axis, and motility, are both SCFA-specific and dose-dependent.[78,79] Equally important are the difficulties of correlating fecal and intraluminal levels of SCFAs. Various changes in SCFAs have been reported in IBS; Gargari and colleagues[80] suggested that individuals with non-constipated IBS and elevated fecal levels of SCFAs may represent a distinct phenotype. However, a meta-analysis concluded that there was no convincing evidence to support the presence of alterations in SCFA in subjects with IBS.

Other metabolites. A number of bacterial metabolites have been linked to symptom pathogenesis in DGBI, most notably histamine[15] and calcitonin gene–related peptide[81] with visceral hypersensitivity and tryptophan[82,83] and polyamine metabolites[84] with gut function.

Epithelial Interactions

The intestinal tract has a multilayered and multicomponent barrier system that maintains homeostatic interactions with the luminal contents facilitating absorption and immune responses and preventing systemic exposure to noxious or allergenic stimuli. This is broadly composed of intestinal secretions (containing antimicrobial peptides, immunoglobulins), an adherent mucus layer, mucus-associated microbiota, the epithelium itself with tight junctions between epithelial cells and submucosal immune and nervous systems. Measurement of intestinal barrier properties has been of significant interest in DGBI. In vivo permeability studies rely on measuring the excretion of minimally absorbed compounds, predominantly saccharides (lactulose, mannitol, sucralose), after the oral administration of a standardized dose. Because sucrose is absorbed rapidly in the proximal small bowel, it is thought to be a better marker of gastric or gastroduodenal permeability. In contrast, the artificial disaccharide, sucralose, is not broken down by the microbiota, making it a more suitable marker for colonic permeability.[85]

Changes in Irritable Bowel Syndrome

Intestinal permeability. The literature on permeability in IBS is very heterogeneous, undoubtedly a consequence of variations in patient selection and methodology employed (tracers used, timing of collection, dietary protocol). Up to 60% of patients with IBS have demonstrated increased in vivo permeability.[86] However, not all studies find increased permeability in IBS, especially those studies involving patients with IBS-C.[87] In addition, the signal for impaired permeability is much stronger in PI-IBS. Ex vivo studies have relied on endoscopically obtained mucosal biopsy samples, mainly from the rectosigmoid colon and, rarely, from the duodenum or proximal colon. Most studies, and particularly those in IBS-D, demonstrated lower transmucosal resistance and increased macromolecular flux across the biopsies. Small intestinal ultrastructural and gene expression changes have been associated with alterations in bowel frequency in IBS.[88] Most studies failed to associate increased paracellular permeability with abdominal pain and overall IBS symptom severity.[86]

Mucosal biofilms have emerged as of potential pathological significance in GI disorders; in one study, mucosal biofilms were evident in the terminal ileum and ascending colon in almost 60% of a group of patients with IBS.[89] These biofilms featured an overgrowth of *Escherichia coli* and *Ruminococcus gnavus* along with an accumulation of bile acids. The latter correlated with an increase in fecal excretion of bile acids. Another study showed that increased intake of lactose and fructooligosaccharides disrupted the mucus barrier in the colon with an associated increase in mast cell counts and advanced glycation end products.[90] A recent study showed that, in addition to epithelial permeability, blood vessel density and vascular permeability were also increased in the colonic mucosa in IBS.[91]

Molecular studies. Among microRNAs, miR-29a has been shown to modulate visceral hypersensitivity. Zhou et al[92] found overexpression of miR-29a in blood microvesicles, as well as in small bowel and colon tissues of patients with IBS-D, which reduced expression of glutamate-ammonia ligase, which plays a role in conversion of glutamate and ammonia into glutamine. miR-29a/b also could increase intestinal permeability in patients with IBS-D by targeting nuclear factor (NF)-κB–repressing factor and claudin 1 expression. miR-199 was significantly decreased, and TRPV1 expression increased in blood microvesicles of patients with IBS with visceral and thermal hypersensitivity.[93] Additional studies have also delineated roles for miRs 219a-5p, 338-3p, 495, 200, and 148b-5p.[94,95] In a recent

study, *Prevotella*, overrepresented in individuals with loose stools, was found to be negatively associated with the expression of SMC5, which is involved in the sumoylation pathway. Also, the expression of PLA2G4A, a host gene that plays an important role in both arachidonic acid metabolism and modulation of epithelial homeostasis, positively correlated with the abundance of *Bacteroides massiliensis* in IBS.[96] There is evidence of an altered antibacterial gene expression profile in patients with IBS compared with healthy controls and patients with ulcerative colitis.[97] In a study on the colonic RNA protein-protein–based interactome, investigators discovered key pathways associated with MAPK, PI3K/Akt, and NF-κB signaling.[98] The small bowel transcriptomic profile of subjects with IBS-D performed using a 91-gene microarray panel showed upregulation of genes encoding INADL, MAGI1, PPP2R5C, MAPKAPK5, TLR3, and interleukin (IL)-15.[99] Baseline expression of duodenal TRPV1 and 3 was increased in IBS-D, and TRPV3 in IBS-C.[7] Duodenal TRPV1 expression correlated with abdominal pain, and inversely with first rectal sensation and pain thresholds in response to rectal distension. Connectivity analysis in colonic mucosa showed clustering of barrier regulation and PDZ domains. In rectosigmoid biopsies from patients with IBS-D, messenger RNA expressions of 21 genes were changed: P2RY4, VIP, CCL20, C4BPA complement cascade, IFIT3, TFF1, retinol binding protein, fibronectin (FN1), ion channel functions (GUCA2B), and PDZ domain-containing protein 3 (PDZD3).[100] The concentration of β-defensin-2 was found to be greater in children with IBS and functional abdominal pain compared with healthy controls, and more so in female compared with male individuals.[101] In addition, levels correlated with abdominal pain and intestinal permeability.

Sucrase-isomaltase deficiency. Traditionally regarded as a rare condition, with a prevalence estimate of 0.2% to 5% in the general population, it has become apparent of late that the true prevalence of sucrase-isomaltase deficiency (SID) may be higher than previously reported and its contribution to DGBI-like symptoms underappreciated, especially in adults. This deficit in recognition may relate to several factors, including the existence of multiple gene variants with diverse clinical phenotypes (some may overlap with DGBI) and variations in testing methodologies.[102] Congenital SID is the consequence of mutations in the SI gene located on chromosome 3q25.1. Some mutations in the SI gene result in decreased expression or activity of the SI enzyme, leading to reduced ability to digest sucrose and starch. To date, more than 70 SI gene mutations have been identified, and most of these mutations were missense or nonsense mutations.[103,104] In addition, studies have demonstrated that heterozygous carriers of SI gene variants may also experience symptoms. Recent literature has highlighted overlap between SID and IBS and FD, as well as functional abdominal pain.[105]

Changes in functional dyspepsia. A lower transmucosal resistance and increased fluorescein isothiocyanate–dextran flux have been reported in FD.[106] Studies also have demonstrated that PPI use and acute cold thermal stress resulted in small bowel barrier disruption.[107,108] In addition, duodenal eosinophilia has been reported in a subset of patients with FD in some, but not all, studies, and in at least one study correlated with increased permeability.[109,110] In a recent study, infiltration of eosinophils in duodenal villi was associated with FD but not *Helicobacter pylori* infection.[111] Duodenal goblet cell numbers and mucin exocytosis were reduced in patients with FD compared with control subjects.[112]

Enteroendocrine System

The GI tract has the largest endocrine system in the body and consists of enteroendocrine (EECs), neuroendocrine, and enterochromaffin (EC) cells that make up only 1% of total GI epithelial tissue. Along with microbiota and luminal contents, these cells coordinate homeostatic enteroendocrine-neuro-immune responses and mediate responses to inflammatory stimuli. These responses may involve impacts on motility, secretion, visceral sensation, absorption, vascular tone, microcirculation, local immune defense, and cellular proliferation (Figure 2). Recently, specific EECs, termed neuropod cells, were found to provide a glutamatergic synaptic connection with vagal neuronal afferents and to mediate fast (in milliseconds) and precise transduction of signals from components of the luminal microenvironment to the brain.[113] In a mouse model, flagellin was found to stimulate colonic neuropod cells, thereby promoting release of peptide YY onto vagal nodose neurons to activate central circuits and reduce feeding. This microbiota–gut–brain sensory circuit, termed "neurobiotic sense" provides a direct link between luminal microbes and the brain.[114] Whether other gut microbiota–derived signaling molecules and metabolites can rapidly transduce gut to brain signals via vagal afferents through these neuropod cells remains to be investigated.

Serotonin and Serotonin Transporter

Serotonin, or 5-hydroxytryptamine (5-HT), is a key GI mucosal paracrine and neurocrine signaling molecule regulating GI function, specifically motility and secretion; it also acts as a neurotransmitter in the central nervous system. Prolonged EC activation was associated with distension-mediated hypersensitivity in mice.[115] Interestingly, the sensitizing effect of the SCFA, isovalerate, associated with GI inflammation, was dependent on the presence of ECs. Other microbial-derived metabolites, including SCFAs (ie, butyrate and acetate) and secondary bile acids (specifically deoxycholate), were shown to increase transcription of tryptophan hydroxylase 1 (Tph1), promoting 5-HT synthesis, and the neuroendocrine secretory protein chromogranin A, leading to increased stimulation of myenteric neurons and gut motility. Other microbial metabolites, including α-tocopherol, tyramine, and p-aminobenzoate, promote Tph1 expression and the downstream synthesis of 5-HT. For example, a small amount of 5-HT is synthesized by a deconjugation process of glucuronide-conjugated 5-HT by bacterial β-glucuronidases, and these enzymes are depleted in a subset of patients with IBS.[116,117] Recently, it was also shown that the

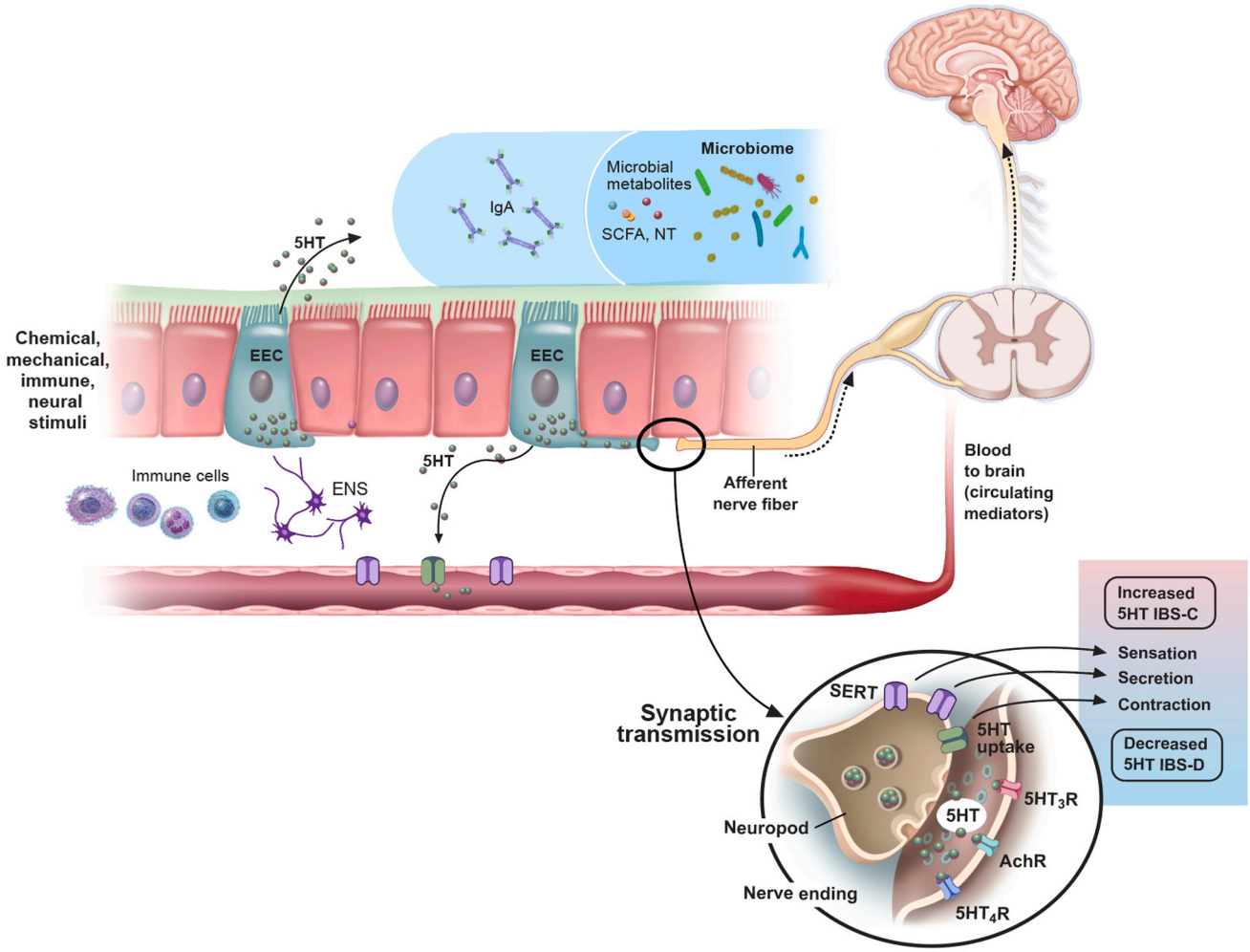

Figure 2. Nutrient sensory transduction, gut microbiota, and immune factors on endocrine to brain serotonin signaling. EECs, translating physiological luminal signal into biochemical signals, and specialized ECs, neuropods, facilitate rapid transduction of sensory signals from nutrients and microbes in the lumen of the gut to the brain, influence serotonin (5-HT) secretion from ECs, and thereby modulate sensation and motility. NT, neurotransmitter.

bacterium *Edwardsiella tarda* produced indole derivatives of tryptophan and activated human, mouse, and zebrafish EECs through the receptor transient receptor potential ankyrin A1 (Trpa1), resulting in 5-HT release.[118] Moreover, microbiota-mediated indoles can activate the aryl hydrocarbon receptor and induce expression of IL-22, which subsequently impacts epithelial integrity and immunity; this signaling was found to be altered in a PI-IBS model.[119,120]

Functional polymorphisms and the presence of the short S allele result in reduced serotonin transporter (SERT) expression and accumulation of 5-HT with consequent hypercontractility, hypersensitivity, and increased mucosal secretion leading to diarrhea and pain.[121,122] The homozygous S genotype of the SLC6A4 gene has been implicated in the pathophysiology of IBS and has been reported to be more common among women with IBS-D. In contrast, a meta-analysis showed a significant association between a SERT insertion polymorphism and the risk of IBS-C in Asian individuals.[121] Functional relevance of single nucleotide polymorphisms (SNPs) in the P2 promoter have also been shown in females with IBS-C.[123] The rs2020938 SNP was found to be associated with differential SLC6A4 expression in the jejunum and with stool consistency.

Serotonin in Functional Dyspepsia

Studies suggested that patients with FD exhibited decreased basal and postprandial plasma levels of 5-HT; other data revealed differences between FD subtypes, with basal 5-HT levels being higher in the epigastric pain syndrome subtype.[124] Although further studies are needed, these findings might explain the reduction in gastric phase III activity identified in FD. A few studies showed that the 5-HTTLPR S/L genotype is more common than the S/S polymorphism in FD, in general, and in the postprandial distress syndrome (PDS) subtype, in particular, suggesting that it is the L allele of the 5-HTTLPR gene that influences susceptibility to PDS.[125]

Serotonin in Irritable Bowel Syndrome

Studies suggested that IBS-D is associated with elevated, and IBS-C with reduced levels of 5-HT in the gut. A

functional polymorphism in the SERT gene has been reported in women with IBS-D.[122] In a study from India, the frequency of the SLC6A4 polymorphism and higher levels of 5-HT were associated with IBS, particularly IBS-D and PI-IBS.[126] However, in a recent meta-analysis of 27 studies, including 3443 patients with IBS and 3359 controls, the authors concluded that the 5-HTTLPR mutation is associated with IBS-C, but not with IBS-D or IBS-M, particularly among East Asian populations.[121] Patients with PI-IBS displayed higher levels of 5-HT in the rectal mucosa than subjects with IBS-D who did not have a prior history of infectious gastroenteritis and those with nondiarrheal IBS. Alterations in both enteric mucosal and blood 5-HT signaling have been demonstrated in children and adults with IBS. Most IBS research has focused on the 5-HT_3 and 5-HT_4 receptors.

Immune System and Neuroimmune Interactions

Initial studies focused on low-grade colonic mucosal inflammation, particularly in patients with IBS-D and PI-IBS. Over the years, the studies have extended to other IBS subtypes as well as FD and involve examination of the small bowel, in addition to the colon.

Functional Dyspepsia

Emerging evidence suggests the involvement of T cells, mast cells, and eosinophils in the pathophysiology of FD, although findings remain inconsistent. Small studies have reported increased CD8+ T-cell infiltration and upregulation of proinflammatory cytokines like IL-1 and IL-6 in postinfection FD.[127] Mast cell activity also appears elevated in FD, as indicated by higher cell counts, signs of degranulation, and increased release of histamine, serotonin, and tryptase.[128] These activated mast cells often reside near mucosal nerves, possibly facilitating enhanced signal transmission to the brain via upregulated TRPV1 and TRPV2 receptors.[129,130] Duodenal eosinophils have attracted significant interest in both PDS and epigastric pain syndrome subsets of patients with FD, especially of post-infection origin. Newer research highlights that eosinophil activation, measured by degranulation, rather than cell count alone, may be more relevant to the development of symptoms, such as early satiety.[109] Furthermore, elevated production of the Th2 cytokines IL-5 and IL-13 by stimulated peripheral blood mononuclear cells (PBMCs) from patients with FD suggests a broader immune dysregulation involving eosinophil recruitment and activation.[131]

Irritable Bowel Syndrome

Elevated plasma IL-6 and IL-8 levels, and an abnormal IL-10/IL-12 ratio in stimulated PBMCs, suggested a proinflammatory Th1 response in IBS. A meta-analysis found that tumor necrosis factor-α levels were consistently higher in patients with IBS, whereas a reduction in IL-10 levels only achieved significance in male individuals. However, these findings are limited by small sample sizes and methodological variability. While genetic variants affecting IL-10 production may influence IBS susceptibility, the evidence remains inconclusive. The origin and relevance of altered cytokines in IBS remain unclear, especially because elevated cytokine levels (eg, IL-6, IL-8) have been observed primarily in patients with concurrent psychiatric conditions. Of particular interest was a report of increased production of the Th2 cytokines, IL-5 and IL-13, by stimulated PBMCs, which aligns with findings of elevated eosinophils and mast cells in both FD and IBS. Cytokine levels differed between male and female individuals using hormonal contraceptives, but not between male and normally cycling female individuals.[132]

Infiltration of CD3+ T cells and mast cells, particularly in the rectosigmoid and descending colon, has been reported in IBS. A novel single cell transcriptomic study in a small number of patients with IBS-D revealed abnormalities in T-cell signaling pathways (HIF-1α pathway, NF-κB pathway, IL-17 signaling pathway, and FOS).[133] In a meta-analysis of more than 700 patients with IBS, mast cell infiltration was linked to both IBS-C and IBS-D.[134] However, the field remains controversial because of variability in findings, partially owing to small sample sizes, demographic variations, inconsistent cell counting techniques, and the patchy nature of immune activation. More reliable data have emerged when functional assessments, such as immune cell activation and mediator release are performed. For instance, electron microscopy has revealed mast cell degranulation, and mucosal biopsies from patients with IBS have demonstrated increased release of bioactive compounds, such as histamine, tryptase, prostaglandins, and cytokines. In a recent study assessing colonic mast cells in IBS, the MRGPRX2 pathway was found to be functionally upregulated, supporting its role in abdominal pain in IBS.[135] In another study, patients with IBS-D exhibited improvement in colonic barrier dysfunction, mast cell recruitment, and activation following institution of a low-FODMAP diet. FODMAP-mediated barrier dysfunction in IBS-D is mediated by direct activation of the TLR4 receptor on colonic mast cells by fecal lipopolysaccharide.[136] However, contamination from mucus-associated microbiota may confound the origin of these mediators, highlighting the need for more refined methodologies.

Immune activation in DGBI is driven by multiple overlapping mechanisms. For example, the reported increase in gut-homing T cells could be based on genetic predisposition, local allergic reactions to food antigens, bile acid malabsorption, gut microbiota alterations, and/or increased intestinal permeability. In a small study of 108 patients with IBS, confocal laser endomicroscopy (CLE) showed that more than half demonstrated epithelial barrier disruption and eosinophil degranulation on exposure to food antigens.[137] Another key driver of immune activation is gut microbiota dysbiosis. Multiple studies demonstrated that microbial products—like flagellin and bacterial DNA—can cross the intestinal barrier, stimulating mucosal immune responses. Evidence includes the presence of elevated levels of antiflagellin antibodies and fecal beta-defensin-2, as well as upregulation of bacterial recognition

receptors like TLR4 and TLR5.[138] Fecal transplants from patients with IBS into mice replicate immune cell activation and mast cell infiltration, further confirming the role of the microbiota.[139] Moreover, bacterial production of histamine, facilitated by histidine decarboxylase, has been implicated in visceral hypersensitivity via histamine 4 receptor signaling.[15] These findings underscore a complex interplay among food antigens, microbiota, and the host immune system in perpetuating DGBI-associated gut dysfunction.

Neuro-Immune Interactions

In patients with IBS, activated mast cells have been identified to lie close to mucosal nerves, and proximity correlated with abdominal pain severity and frequency. Studies using human biopsy supernatants and adoptive transfer models in rodents have shown that immune-derived mediators—such as histamine, proteases, and polyunsaturated fatty acid metabolites can directly excite sensory neurons. These mediators activate proteinase-activated receptors, Histamine 1 receptors, and transient receptor potential (TRP) channels (TRPV1, TRPV4, TRPA1). Both histamine and serotonin stimulated translocation of TRPV4 to the membrane in nociceptors, resulting in heightened neuron sensitivity and visceral pain.[140] Interestingly, mast cells in the mucosa of patients with IBS were found to produce higher levels of nerve growth factor, which was, in turn, associated with a heightened density of nerve fibers.[141] Similarly, patients with IBS exhibiting elevated levels of brain-derived neurotrophic factor also displayed increased nerve sprouting.[142] Moreover, there was a positive correlation between brain-derived neurotrophic factor levels and abdominal pain scores in these individuals. Together, these findings underscore the importance of interactions between the immune and nervous systems in DGBI that amplify sensory signaling with effects on symptom severity (Figure 3).

Diagnostic and Clinical Implications

Food-Related Diagnostic and Provocation Testing

Provocations have ranged from capsaicin to elicit visceral sensitivity in FD and nutrient drink tests to assess gastric accommodation and nutrient sensitivity. In a double-blind randomized crossover trial in patients with IBS, fructose and fructans, but not glucose, exacerbated symptoms.[143] Others reported triggering of symptoms by fructans, galactooligosaccharides, and mannitol.[144,145]

Probe-Based Confocal Laser Endomicroscopy (pCLE)

CLE allows for real-time, high-resolution imaging of tissues at a cellular level during endoscopy. The number of epithelial gaps in the ileum and colon was found to be increased in patients with IBS.[146] Similarly, in patients with FD (vs controls), significantly higher epithelial gap density on CLE was identified in the distal duodenum.[147] In patients with IBS and self-reported food sensitivities, a large proportion of patients was found to have a positive reaction to 1 of the 5 different foods applied to the duodenal epithelium.[148] In CLE+ patients, intraepithelial lymphocytes increased, epithelial leaks/gaps formed, and intervillous spaces widened. CLE+ patients but not CLE− patients, treated with an exclusion diet experienced symptom improvement. In another, larger follow-up study from the same group, patients with IBS and no evidence of classic IgE-mediated food allergies underwent separate duodenal challenges with 4 common food components, followed by CLE. Seventy percent of patients were CLE+, with more than 60% reacting to wheat. In duodenal biopsies, CLE+ subjects had a higher number of intraepithelial lymphocytes, increased claudin-2 expression, and increased eosinophil degranulation. Duodenal fluid revealed higher levels of eosinophilic cationic protein.[137] However, in a blinded study led by a different group, a positive CLE response to wheat did not predict who benefited from subsequent wheat elimination.[149] In contrast, in an international multicenter validation study, intraobserver agreement was found to be substantial to perfect and interobserver agreement substantial.[150]

Antibody Tests

Although many so-called food sensitivity tests lack adequate validation, a recent trial of IBS subjects with $\geq$1 positive response on an 18-food IgG assay and a moderate average daily abdominal pain showed that those randomized to an exclusion diet guided by the antibody response were more likely to experience a reduction in abdominal pain.[5] However, the adherence to diet was limited and in per-protocol analysis with only adherent patients, the outcomes were less impressive. Furthermore, there was an imbalance between the 2 study groups in the extent to which FODMAPs were excluded.

Colonoscopic Allergen Provocation Test

This technique involves exposing colonic mucosa to a food item and assessing wheal and flare reactions. In the study by Aguilera-Lizarraga et al,[4] most patients with IBS developed wheals in the rectosigmoid in response to the submucosal injection of food mixtures. The exact etiology of these mucosal colonoscopic allergen provocation test responses needs to be clarified. In addition, the distal intestine does not see food in its native form, nor does food reach the submucosal space in the absence of frank loss of the epithelial surface, a phenomenon that has not been observed in DGBI.

Magnetic Resonance Imaging and Magnetic Resonance Imaging Water Content

In adults with IBS-D, magnetic resonance imaging scanning identified the ascending colon as less able to accommodate postprandial inflow and thereby account for more rapid colonic transit.[151] In healthy adults, fructose led to greater increases in small bowel water content while fructans (ie, inulin) substantially increased colonic gas.[152] In adults with FD (vs healthy controls), cine gastric

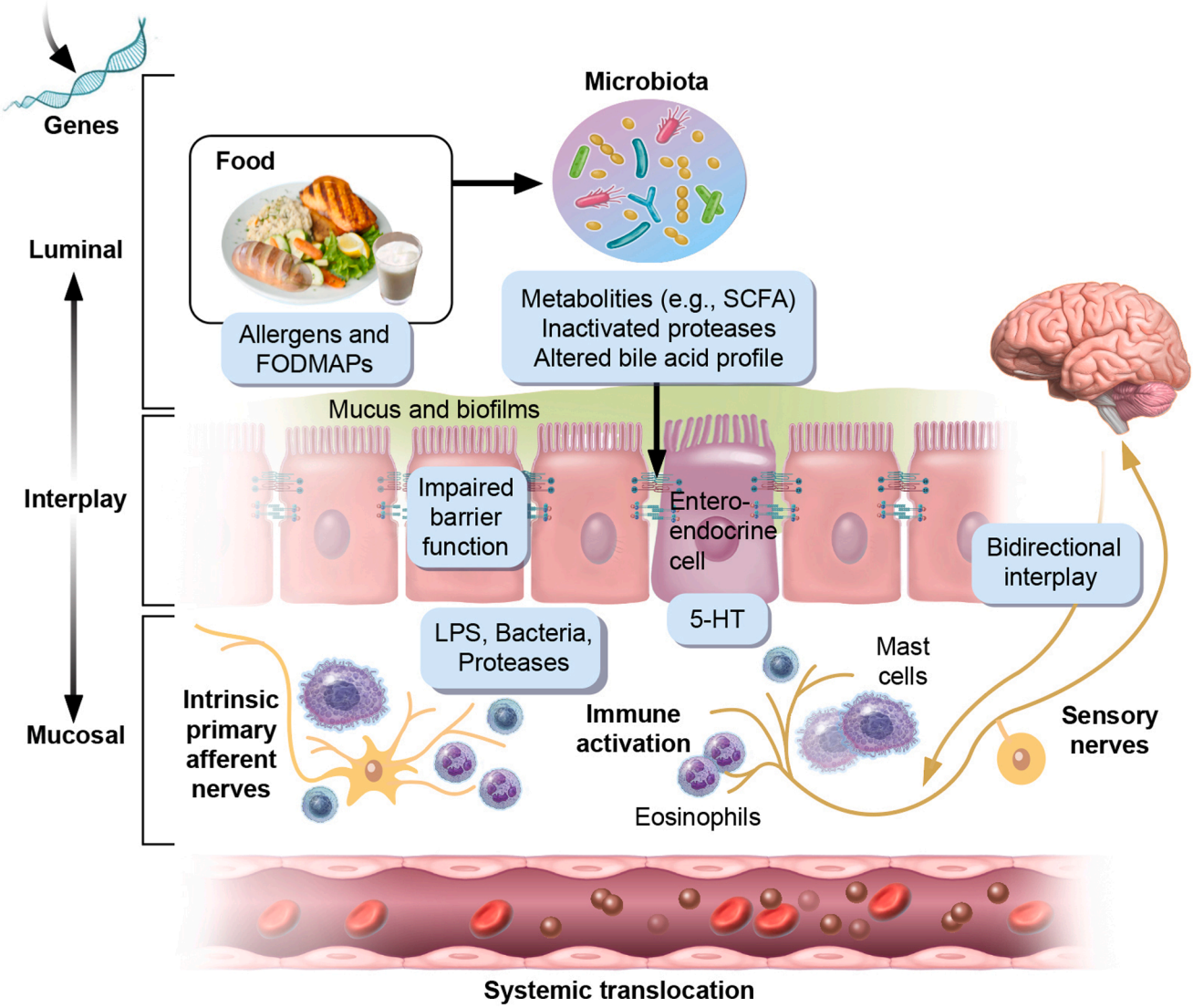

Figure 3. Schematic representation of putative microenvironmental factors involved in the pathophysiology of DGBI. The figure highlights the interplay between luminal and mucosal factors. Mucosal barrier weakness (eg, changes in mucus, increased epithelial barrier) may allow amplification of signaling from the lumen to the neural and immune elements. LPS, lipopolysaccharide.

magnetic resonance imaging identified that the propagation velocity of antral peristalsis was significantly lower.[153]

Microbiome and Metabolites

Although microbiome composition and activity can now be assessed in an increasingly rapid and standardized fashion, commercial microbiome composition testing is not currently recommended.[154] A saccharolytic microbiome has been shown to predict the response to a low-FODMAP diet.[155,156] A study integrating data from multiple studies suggested that those enriched in microbial metabolic pathways related to colonic methane and SCFA production were more likely to benefit from a low-FODMAP diet.[157] However, attempts to predict responses to a low- FODMAP diet based on specific SCFA profiles have to date met with mixed results. Similarly, although volatile organic compound profiles have also predicted a response to low-FODMAP diet, a single predictive volatile organic compound has yet to be identified.[158] Reference has already been made to the limited diagnostic potential of anti-CdtB and anti-vinculin antibodies.

Novel capsule strategies are now allowing pan-intestinal sampling of contents without the need for bowel preparation.[44] Serial sampling strategies are already allowing us to appreciate the complexity as well as the variability of microbiota in healthy individuals and the influence of diet, sleep, etc.[159] Other capsules allow assessment of luminal pH, temperature, gas production, and motility patterns. The role of these technologies in clinical practice continues to be evaluated.

Fecal Bile Acids and Serum Testing for Bile Acid Malabsorption

The gold standard ^{75}selenium-homotaurocholic acid (SeHCAT) scan is not widely available. However, combination of serum C4 along with fecal total and primary bile acid

concentration has been shown to have high predictive value for diagnosis of bile acid diarrhea.[160]

Breath Testing

The limited diagnostic potential of breath testing and its implication for a role of SIBO in DGBI pathophysiology[46] have already been discussed.

Intestinal Barrier

Although various entities already market saccharide excretion–based testing assessment of barrier function, these tests are unvalidated. Studies have demonstrated effects of dietary contamination, intraindividual variability[85] on standardized diets as well as effect of sex.[161]

Disaccharide Deficiency

The current gold standard for diagnosing congenital or acquired SID is endoscopic duodenal mucosal biopsy for quantitative disaccharidase assay. Patients with genetic (or congenital) SID have significantly reduced sucrase activity with normal small bowel histology. The disaccharidase assay requires scrupulous attention to sample collection and access to a specialty laboratory. Although the measurement of hydrogen and methane in the breath following the ingestion of sucrose for SID is easier to perform and is more accessible, its specificity for SID has been questioned.[162] Of concern are false positive results due to rapid orocecal transit or bacterial overgrowth, and false negative results in patients recently treated with antibiotics. In the future, the incorporation of ^{13}C, a stable isotope, may improve specificity specifically and overcome the confounding effects of by diet and other variables.[163]

Genetic and Pharmacogenomic Testing

In one study of 125 adults with IBS-D, stool consistency responders (reduction in loose stools) to ondansetron were more than twice as likely to carry the CC genotype of the SNP p.N163K rs6766410 of the HTR3C gene.[122] In a study of 42 adults with IBS-D, frequencies of the TPH1 rs453773G allele in linkage disequilibrium with TPH1 alleles (rs7130929 T allele and rs211105 C allele) were significantly lower in ramosetron responders.[164] Variations in Carbohydrate-Active enZYme (hCAZyme) genes were tested in relationship to the response to a low-FODMAP diet in the DOMINO study.[165] In the dietary arm, the number of hypomorphic hCAZyme genes positively correlated with treatment response rate and in the IBS-D group, hCAZyme carriers were 6 times more likely to respond to the diet than noncarriers. In addition, such testing can allow prediction of adverse outcomes, as demonstrated in a recent study.[166]

Novel Fecal Biomarkers

Fecal levels of calprotectin, lactoferrin, and β-defensin-2 have been used to differentiate IBS from inflammatory bowel disease with generally encouraging results.[167] Indeed, calprotectin has been recommended as a first-line test in the assessment of the patient with chronic diarrhea. In interpreting these tests, one needs to be very mindful of cutoff levels that have varied between studies and will obviously significantly affect test sensitivity and specificity. For example, a low cutoff may increase the likelihood of finding elevated levels among children and adults with functional abdominal pain or IBS. The detection of elevated fecal levels of epithelial colorectal markers (such as EDN, HNL (pab/765), and HPLBII-P) in IBS suggests epithelial but not immune dysregulation.[168] Fecal myeloperoxidase and serum neutrophil gelatinase–associated lipocalin are promising novel biomarkers, which in addition to fecal calprotectin, may refine differentiation between inflammatory bowel disease and IBS.[169]

Figure 4 summarizes microenvironment-based diagnostic strategies for assessing GI mechanisms relevant to DGBIs.

Future Directions

Every study of a component of the luminal microenvironment in DGBI must confront the challenges faced by the heterogeneity of the phenotype and of the multiple intrinsic and environmental factors that may confound interpretation. The first step toward definitive incrimination of any luminal factor must, therefore, be a complete definition of the phenotype, including the delineation of clinically and pathophysiologically meaningful subtypes. The latter has, to date, relied on relatively simple symptom categories, be it predominant upper GI symptom pattern or usual stool form, but attempts to provide more detailed and coherent subtyping are under way and show promise in differentiating between patient groups whose symptoms may be of predominantly central or peripheral origin.[170] How these subgroups relate to changes in the microbiome, immune response, epithelial integrity, or enteroendocrine responses has yet to be determined.

Attention to the role of diet in DGBI has been long overdue and its importance validated by the clinical impact of dietary strategies such as the low-FODMAP diet. Recently, a study from the United Kingdom also found that the Mediterranean diet had a favorable response over traditional dietary advice in IBS.[171] Although intolerance rather than allergy is the predominant mechanism underlying food-triggered DGBI symptoms, novel immunologically mediated effects have been postulated and their relevance to DGBI deserves further explanation. Food-induced symptoms, regardless of etiology, are undoubtedly complex and likely multifactorial involving yet to be fully delineated interactions with all the other components of the luminal microenvironment. The ongoing debate and controversy surrounding gluten- or wheat-induced symptoms in DGBI are illustrative. Which component(s) of wheat is(are) the culprit(s), is this an intolerance or a subtle immune-mediated or enteroendocrine response, are interactions with the microbiome involved, could the barrier be impaired and contribute to the systemic symptoms that some implicate in this poorly defined entity, NCGS? These

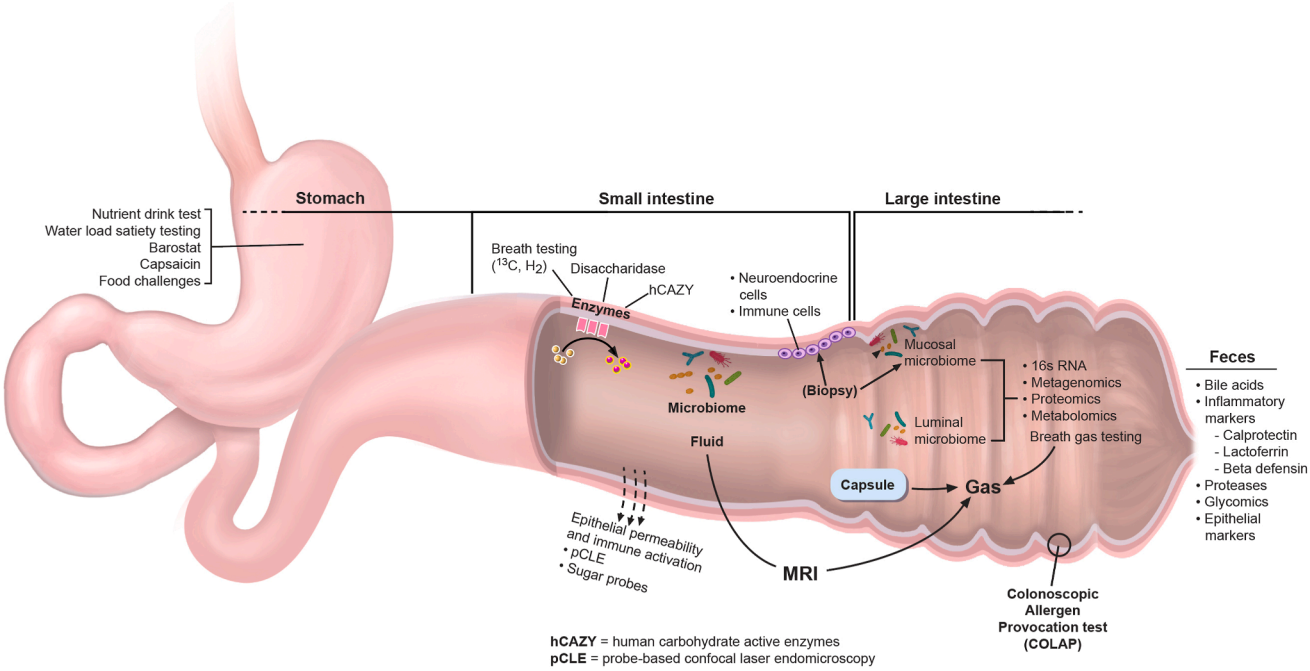

Figure 4. Microenvironment-based diagnostic strategies for assessing gastrointestinal mechanisms relevant to DGBIs. Targeted diagnostic strategies based on luminal and mucosal studies of gastric, small intestinal, and colonic function are proposed. MRI, magnetic resonance imaging.

same questions could and should be applied to other food-related symptoms.

Studies of the microbiome in DGBI are characterized more than anything else by their inconsistency. To expect that small, cross-sectional studies that failed to correct for important confounding factors would identify a microbiome signal for any DGBI was unrealistic. Furthermore, it is becoming apparent in this area, as in all areas of gut microbiome research, that microbial composition alone may not provide the answer, with metabolomics and other omics beginning to show promise by revealing the production of neuromodulatory molecules by certain bacteria, for example. Such endeavors may well discover metabolites or metabolite profiles more causally related to symptoms and, therefore, serving as better biomarkers and targets for intervention.

Epithelial properties such as barrier integrity and disaccharidase expression are already attracting novel targeted interventions. The accurate detection of barrier dysfunction in human subjects is not without its challenges; one looks forward to the validation of tests that align with phenotype and prove predictive of responses to novel interventions. Related molecules, such as proteases, are also attracting attention and deserve further study.

In terms of the neuroendocrine axis, there is much to be done to disentangle the complex interactions that take place between microbiome, enteroendocrine cells and the enteric and central nervous systems. Although considerable excitement greeted the suggestion that certain polymorphisms related to serotonin biology might serve as genetic markers for IBS or certain IBS subtypes, the translation of these findings into an actionable diagnostic test or therapeutic intervention has yet to be realized.

The suggestion that the immune system might play a role in DGBI is now supported by a considerable volume of research data. Here again the focus has shifted from relatively simplistic approaches such as counting cells in biopsies or running assays of cytokines in serum to a much more detailed examination of immune triggers, immune cell activation, and interactions among the immune, nervous, and enteroendocrine systems. It is to be hoped that such studies will finally define whether there is a state of "inflammation" or "immune activation" in DGBI or in very specific subsets thereof and lead the way to targeted interventions against eosinophils or mast cells, for example. Recent studies demonstrating benefits from ebastine (H1 receptor antagonist) and with mesalamine (anti-inflammatory 5-amino salicylate), albeit only in a subset of patients,[172,173] provide encouragement.

A brief look at the history of drug discovery and clinical application in DGBI over the past several years attests to the relative futility of testing any intervention in "all" IBS or FD or even in currently delineated subsets such as IBS-D or PDS. On the other hand, the examination of incident DGBI cases, such as postinfection IBS (and, perhaps, post-COVID DGBI), can provide a novel paradigm for understanding disease pathobiology and a platform for testing targeted therapies. Larger-scale and multisite efforts at patient recruitment and sample collection will not only add power to these studies but also will provide an understanding of the disorder across geographic, cultural, and socioeconomic spectra. Emerging analytic tools, such as artificial intelligence, may prove invaluable in making sense of the mountains of data generated by studies that combine measurements of omics, immune mediators, and genomes,

as well as environmental factors in heterogeneous populations. Evidence has already emerged from a randomized controlled trial to suggest that an artificial intelligence–generated personalized diet (based on microbiome data) may be more effective than a low-FODMAP diet in improving microbial diversity and reducing symptom severity.[174]

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Conflicts of interest
These authors disclose the following: Madhusudan Grover has consulted for Alfasigma Pharmaceuticals. Giovanni Barbara has consultancies, business interests or sources of honoraria payments from Aboca, AB Biotics, Agave, Alfa Sigma, AGPharma, Bayer, Biocodex, Boeringer, Bromatech, Cadigroup, Danone, Diadema, Falk Pharma, GE Healthcare, Giuliani, Mayoly, Malesci, Sanofy, Sofar, and Yakult. William Chey has served as consultant for Ardelyx, Atmo, Biomerica, Gemelli, Kohler Ventures, Phathom, Redhill, Bausch/Salix/Valeant, Takeda, and Vibrant. He receives stock options from Coprata, Evinature, FoodMarble, Kiwi BioSciences, and Modify Health. His patents include My Nutrition Health, Digital Manometry, and Rectal Expulsion Device. Bruno P. Chumpitazi is consultant for Ironwood Pharmaceuticals. Eamonn M.M. Quigley is a consultant for Novonesis, Vibrant, Atmo Biosciences, Biocodex, Farmasierra, and Atlantia. The remaining authors disclose no conflicts.

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