Immune Activation in Functional Gastrointestinal Disorders

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Abstract: There is growing appreciation that functional gastrointestinal disorders (FGIDs) such as functional dyspepsia and irritable bowel syndrome are heterogeneous conditions linked by subtle inflammation within the gastrointestinal (GI) tract. The literature suggests that while the symptoms of these diseases may manifest with similar clinical presentations, there are significant differences in triggers and disease severity among patients classified into the same subtype. It is hypothesized that the subtle inflammation observed in these patients is related to an imbalance in GI homeostasis. Disruption of the delicate homeostatic balance within the GI tract can result from any number or combination of factors, including dysbiosis, loss of barrier integrity, genetic predisposition, or immune responses to dietary or luminal antigens. This article discusses the interplay between the immune system, microbiota, and luminal environment in FGIDs. In addition, the article proposes emerging immune pathways, including those involving T-helper type 17 response and innate lymphoid cells, as potential regulators of the subtle inflammation characteristic of FGIDs that warrant investigation in future studies.

Functional gastrointestinal disorders (FGIDs) are conditions of the gastrointestinal (GI) tract for which there are no overt structural pathologies. Rather, FGIDs are conditions of disordered GI function.[1] These disorders, including irritable bowel syndrome (IBS) and functional dyspepsia (FD), are diagnosed by patient-reported symptoms and a lack of clinical pathology.[2] It is estimated that over 40% of the general population reports such unexplained abdominal symptoms.[3] Although associated with immune activation and an influx of effector cells such as eosinophils and mast cells into the GI tract,[4] no widely accepted or definable profile of immune activation has been determined for FGIDs. This is largely due to variations in reported findings between studies and a lack of subtyping of patients into recognized disease subgroups, such as epigastric pain syndrome (EPS) or postprandial distress syndrome (PDS), in FD.[5] Although the range of GI tract symptoms defining FGIDs is limited, it is likely that FGIDs represent a

multitude of conditions distinguished by their causative agents or other environmental factors not yet elucidated. This heterogeneity complicates efforts to determine immune profiles of these conditions for diagnostic and therapeutic potential. Results of such studies are affected by the degree of characterization of the study cohort, and sample sizes of most published cohorts are likely too small to accurately phenotype smaller subpopulations.[5]

Recent advances in the field have suggested involvement of imbalances in the microbiota, immune responses against dietary components, and a link between FGIDs and atopy, as well as a possible association between FGIDs and nonceliac wheat sensitivity (NCWS). In addition, there is indirect evidence in the literature that T-helper type (Th) 17, in conjunction with Th2, immune responses are involved in the pathogenesis of FGIDs.[5]

Gastrointestinal Homeostasis and Functional Gastrointestinal Disorders

Within the GI tract, homeostasis describes the interplay between the epithelial barrier, immune system, and microbiota in the maintenance of tolerance toward both the commensal bacteria of the gut and luminal food antigens. From early infancy, an individual's GI immune system evolves in conjunction with his or her microbiome, resulting in conjoint functions, ranging from protecting mucosal surfaces from pathogenic invasion and recognition of self molecules to stimulating a cell-mediated immune response.[6] In homeostasis, the microbiota is compartmentalized due to epithelial antimicrobial secretions, as well as the physical barrier the epithelium provides through secretion of mucus. This restricts contact between the epithelium and potentially harmful microbes, preventing translocation of organisms into host tissues.[7]

Regulatory T cells are immune mediators of GI tract homeostasis, and the depletion or absence of these mediators results in inflammation within the GI tract.[8] It has been recognized that the ability of regulatory T cells to maintain homeostasis involves the suppression of γδ T cells,[9] which are thought to link innate and adaptive immune responses.[10] *Clostridium* species and *Bacteroides* species have the capacity to increase the concentration of regulatory T cells within the GI tract in animal studies,[11,12] providing confirmation of a liaison between the microbiome and the immune system. Furthermore, secretory immunoglobulin (Ig) A released onto GI tract mucosal surfaces prohibits the binding of pathogens and related products to the epithelium. Interestingly, previous animal research has demonstrated that resident microorganisms are involved in regulating this IgA secretion.[13] It is thought that the microbiota's role in IgA regulation is to provide continuous stimulus for the maturation of the host immunity against foreign pathogens while concurrently encouraging tolerance toward commensals.[14,15] In this manner, the relationship between host and microbial components contributes to the maintenance of GI homeostasis. Accordingly, a modification to the diversity, number, and/or functionality of the microbiota commonly results in a compromised host defense system or an ultimate loss of homeostasis. Consideration of the current literature suggests an overarching disease model of FGIDs as a group of heterogeneous conditions linked by a loss of homeostatic balance in the gut. This imbalance is likely driven by alterations in the relationship between luminal antigens, the immune system, and the microbiota. This article presents recent evidence on immune parameters that may be implicated in the subtle inflammation recognized in these conditions.

Subtypes of Functional Gastrointestinal Disorders

FD and IBS represent the 2 most common FGIDs and will be the focus of this article. FD affects the upper GI tract and is characterized by PDS and EPS, the 2 currently recognized subtypes. Although the Rome III and IV criteria describe these subtypes as having distinct symptom profiles, the literature suggests the overlap of these profiles to be as high as 66% in clinical practice,[16] limiting the utility of such subtyping in predicting response to therapy and management. A study by Carbone and colleagues[17] reported an EPS/PDS overlap of 51%, but proposed that this overlap could be mitigated by reclassifying postprandial epigastric pain as a symptom of PDS, suggesting that with further investigation, the onset of symptoms following ingestion of a meal may be important in the clinical distinction of patients with FD. Associated with symptoms in the lower GI tract, IBS subtypes are based on the stool profile: diarrhea (IBS-D), constipation (IBS-C), mixed (IBS-M), or unknown. As with FD, recognition of overlap highlights the need for more objective classifications for patients with symptoms that may include abdominal pain, bloating, and excessive gas.

Increases in peripheral T-cell populations expressing gut homing–associated signals (α4+β7+ or β7+) have been reported in both FD[18] and IBS.[19] The low-grade mucosal inflammation characteristic of these FGIDs consists of alterations in lymphocyte populations (identified in both FD and IBS), eosinophils (FD), and mast cells (IBS).[4,20] It is important to consider that while these cell types are commonly associated with type 2 immune responses, these effector cells also have described roles in the regulation of innate immunity.[21,22] Altered cytokine levels in FD and IBS are also reported within the literature[23-25]; however, no distinct profile has been

reliably reproduced.[5] This lack of consensus suggests heterogeneous immunopathologies within cohorts that result in similar symptoms, which understandably limits the success of therapeutic trials for these patients. Given the complexity of maintaining homeostatic balance within the GI tract, reviewed in detail elsewhere,[26,27] there are numerous factors that may result in the symptoms considered characteristic of these disorders. Links between the luminal environment and FGIDs are best exemplified through reported cases of FGIDs developing following an episode of acute gastroenteritis.[28,29] Additionally, food components[30] and atopy have been linked to FGIDs,[31,32] suggesting that further investigation into the immune cascades known to be activated in response to such stimuli is warranted.

Genetic Predispositions and Altered Immune Responses in Functional Gastrointestinal Disorders

Based on the results of twin and familial studies, it is accepted that there is a familial heredity pattern in FD and IBS.[33,34] A population study from Sweden on first- to third-degree relatives demonstrated that heritability of IBS is not restricted to first-degree relatives, and also highlighted that genetics do not account for all IBS cases.[35] However, according to a review of this topic,[36] reports of single nucleotide polymorphism (SNP) associations in FGIDs have been found in small cohorts, which likely contribute to a lack of consistency regarding the significance of these associations and prevent definitive conclusions regarding the role of such mutations in the development of FGIDs.

Pathophysiologic associations between FD and SNPs in the G-protein subunit 825 have been reported,[37] and a rare mutation in the *SCN5A* gene, which encodes a sodium channel, has been shown to be linked to abdominal pain in IBS patients.[38] In addition, polymorphisms in genes encoding epithelial barrier function and serotonin signaling have been linked to FGIDs.[39] In terms of genetic variation in immune pathways, a polymorphism in tumor necrosis factor (TNF) α occurs in a higher frequency of IBS patients than in controls.[40] Although this study found no significant difference in interleukin (IL) 10 polymorphism frequencies, it was reported that IBS patients were more likely to have a genotype of high TNF-α production with low IL-10 production, suggestive of an imbalance between inflammatory TNF-α and immunomodulatory IL-10. Arisawa and colleagues[41] examined the frequencies of polymorphisms in the genes for IL-17A, IL-17F, and macrophage migration inhibitory factor (MIF) in a Japanese population, and found no associations with risk for FD. However, the authors did report an association between a polymorphism in MIF and the EPS FD subtype. The chemokine RANTES (regulated upon activation, normal T cell expressed and secreted) is a potent chemoattractant for monocytes and memory T cells, and polymorphisms in the gene encoding this peptide have been reported to be associated with greater risk for EPS, but not PDS.[42] Evidence of genetic risk for FGID development conferred by SNPs in immunogenes identified through genome-wide association studies is controversial. A meta-analysis of 16 SNPs in genes for immune factors previously linked to IBS found only a moderate association between the rs4263839 SNP in the *TNFSF15* gene and IBS.[43] Additional associations between SNPs in genes linked with barrier proteins and channels[44] may suggest that individuals with this genetic risk may be more susceptible to GI tract immune responses driven by altered barrier function; however, validation of such associations must be confirmed in larger cohorts.

Although genetic-focused studies indicate a role for genetic variation in promoting immune dysfunction in a subset of FGIDs through altered immune signaling, it is unlikely that genetic risk alone is capable of driving FGID pathophysiology. External factors are likely to be implicated, and the contribution of genetic susceptibility to disease burden remains to be elucidated. Mahurkar and colleagues[45] identified alterations in DNA methylation in peripheral blood mononuclear cells (PBMCs) of IBS patients and controls, particularly in pathways associated with oxidative stress and neuronal function. Additionally, genes encoding neuropeptide hormones were differentially methylated between patients and controls in this study, consistent with the dysregulation of gut-brain axis interactions reported in FGIDs. Methylation links interactions of genes and the environment and has implications for regulation of cell development and differentiation.[45] Such epigenetic alterations may explain some links between environmental factors (including diet and the microbiota) and FGIDs with further investigation.

The Link Between Atopy and Functional Gastrointestinal Disorders

Given the presence of duodenal eosinophilia in FD and mast cells in IBS, it is postulated that FGIDs are associated with Th2 responses, such as those seen in allergy and atopy. Development of an allergy is a 2-step process involving sensitization to a specific antigen followed by a secondary encounter. Sensitization is the process by which allergen-specific Th2 lymphocytes secrete IL-4, IL-5, and IL-13 to promote class switching of B cells to produce specific IgE antibodies. These antibodies then mediate degranulation of mast cells and eosinophils upon

re-encountering the same antigen, resulting in the appearance of symptoms associated with allergic reactions, such as shortness of breath, nausea, urticaria, or, with severe allergy, anaphylaxis.[46] Kindt and colleagues[47] reported increased production of IL-5 and IL-13 and decreased interferon (IFN) γ levels following lymphocyte stimulation in both FD and IBS patients compared to controls. These findings were accompanied by a decrease in IL-12 production from stimulated monocytes. This reduction in Th1-associated cytokines and increase in Th2-associated cytokines suggest a disruption to the Th1/Th2 cytokine balance in FGIDs; however, when combined with other studies of circulating and local cytokines in these conditions, no clear profile is discernible.[18,23-25,48,49]

A population-based study from the United Kingdom reported an association between atopic conditions and IBS, FD, and chronic idiopathic constipation.[31] This study also reported that patients with multiple FGIDs had the highest prevalence of atopic conditions when compared to patients with a single FGID. Cow's milk allergy has also been reported as a risk factor for pediatric FGIDs.[50] A later study of 3542 Australians[51] found that FD and IBS had associations with asthma and allergies to food, animal, and pollen antigens. When the FGID populations were separated by subtype, IBS-C, IBS-M, PDS, and EPS were associated with asthma. Although food allergy was significantly associated with IBS-C, IBS-D, IBS-M, PDS, EPS, and postinfectious (PI) FD, there was no significant association with PI-IBS. These associations were independent of psychological distress, and the variations in association by subtype suggest that allergy or atopy may predispose patients to FGIDs.

Interestingly, studies have suggested that 85% to 93% of patients who self-reported food hypersensitivity as the cause of their GI complaints met the Rome II criteria for an FGID, and less than 5% of these cohorts tested positive for an IgE-mediated allergy following the gold standard double-blind, placebo-controlled food challenge.[32,52] Conversely, FGID symptoms have been associated with higher levels of total IgE in both patient and general populations.[53] In the patient population, mild to moderate IBS symptom scores appeared to drive this association. Expanding on this work, Vara and colleagues[54] found no significant association between intestinal or extraintestinal symptom severity score reported by IBS patients and total IgE level, and, as such, the clinical significance of elevated IgE levels in FGID populations is still unclear. In addition, it is plausible that a subset of FGID patients have their symptoms driven by a non-IgE–mediated hypersensitivity reaction (or intolerance) to food antigens, and this could explain the mixed success of dietary interventions in some patient cohorts.[55-57]

Nonceliac Wheat Sensitivity and Functional Gastrointestinal Disorders

There is significant evidence to suggest that dietary components such as wheat are capable of triggering FGIDs in certain cases. NCWS is a condition characterized by the occurrence of GI or extraintestinal symptoms following ingestion of gluten- or wheat-based foods, and is estimated to affect 4% to 13% of the general population.[58] NCWS is distinct from celiac disease or wheat allergy, and currently has no well-defined pathologic profile. Celiac disease is an autoimmune condition characterized by a T-cell–mediated response to specific gluten peptides in genetically predisposed individuals, whereas wheat allergy is distinguished by an IgE antibody–mediated inflammatory response to a variety of wheat-based antigens.[59] NCWS is diagnosed in the absence of either of these conditions; is confirmed with a negative double-blind, placebo-controlled gluten challenge; and could be considered an intolerance to wheat or wheat components.[60] There is an overlap of symptoms between reported NCWS and many FGIDs, leading to the suggestion that NCWS may be a subtype of FGIDs. In an Italian survey of 486 patients suspected of having NCWS, 52% recognized that they experienced epigastric pain,[61] the main symptom of the EPS subtype of FD. Furthermore, an Australian population-based study found a significant association between patients reporting NCWS and FGIDs.[58] A systematic review of human studies in the field reported a link between ingestion of gluten-containing foods and the onset of FD-like symptoms.[30] It has been suggested that NCWS-associated inflammation results from innate, as opposed to adaptive, immune pathways. A study of mucosal biopsy specimens showed increased expression of Toll-like receptors (TLRs) in NCWS patients when compared to celiac disease patients or controls.[62] TLRs are innate antigen sensors, initiating inflammatory responses upon activation, and the lack of adaptive immunity markers identified in the study supports the suggestion that NCWS involves innate pathways. Host-derived proteases in the small intestine are unable to fully metabolize gluten, so it is possible that the remaining undigested gluten peptides are recognized by the immune system[63] and gluten components may be immunogenic or detrimental to intestinal barrier function. For instance, gliadin peptides are associated with barrier dysfunction in eosinophilic esophagitis and can disrupt epithelial tight junctions.[64,65] Adherence to a gluten-free diet has had some success in alleviating symptoms for patients with FGIDs. A study of IBS patients found significantly altered symptom responses between gluten-free and gluten challenge groups.[66] Moreover, following a study of 134 patients identified as having either IBS

or FD according to the Rome III criteria, 75% reported improvement of symptoms while following a gluten-free diet, although only 14% described a relapse of symptoms after rechallenge with gluten.[67] Despite the occurrence of reported symptom improvement by NCWS patients on a gluten-free diet, it appears that a majority of patients with NCWS do not have measurable or reproducible responses to gluten with challenge. A study of 1312 adults reporting NCWS found that only 16% had responses specific to gluten following a double-blind, placebo-controlled gluten challenge.[68] This suggests that gluten is not the component of wheat-based food that is driving symptom onset in this subset of patients.

Fructans are the main carbohydrate constituent of wheat and are poorly absorbed by the small intestine, where they may lead to increased short-chain fatty acid production by the microbiota and increased water transport in the colon.[69] Fructans fall under the classification of fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs), and IBS patients with self-reported NCWS have significant improvement in GI symptoms when placed on a diet low in FODMAPs.[70] In the same study, only 8% of patients had responses specific to gluten. Fructans were investigated by Skodje and colleagues[71] in a double-blind crossover challenge of self-reported NCWS patients. While gluten challenge presented no significant effect on these patients, fructans were observed to induce worsening of symptoms.[71] Although the current evidence linking sensitivity to dietary components, including gluten and fructose, to FGIDs is largely observational, it is plausible that these antigens are stimulating the immune response in a situation of altered homeostatic balances in the GI tract of FGID patients. Supporting this possibility, animal research suggests that fructans have immunomodulatory properties, which may be dependent, at least in part, on digestion by the microbiota.[72] This is consistent with the observation that fructans of different chain length may have different effects on GI physiology, and further studies are warranted to examine these wheat components as potential antigenic triggers in FGIDs.

Alterations in Barrier Function and Implications for Immune Activation

Impairment of the intestinal barrier has been previously described in both FD and IBS[73-76] through measurement of permeability[77,78] and altered expression of tight junction proteins, including zonula occludens (ZO-1) and claudins 1 to 4.[79] This barrier dysregulation has been correlated with the degree of subtle inflammation in a cohort of FD patients.[73] A more recent study by Komori and colleagues[80] reported decreased expression of ZO-1 in FD patients when compared to a control group with non-FD abdominal symptoms, and an association between ZO-1 expression and duodenal permeability. Patients with IBS-D have also been reported to have decreased ZO-1 expression and protein levels, significantly associated with mast cell activation and symptoms.[81] Interestingly, ZO-1 is also lower in patients with celiac disease[82] due to the upregulation of zonulin, the mammalian analogue of zonula occludens toxin, by gliadin peptides.[64] Zonulin has been shown to reduce ZO-1 expression, resulting in reduced integrity of ZO-1 in the tight junction complex in humans and rats.[83] This relationship may implicate some antigenic dietary peptides as a cause of barrier dysfunction in some subsets of FGIDs, where disruption of the barrier integrity allows for stimulation of the immune system with infiltrating antigens or components of the microbiota.

Dysregulation of the Microbiota in Functional Gastrointestinal Disorders

The microbiota is critical for the regulation of homeostatic conditions in the GI tract through the role that it plays in mediating metabolism and digestion, regulating immune system maturation, and defending the luminal environment against pathogens.[7] Consequently, dysbiosis is a feature of a number of GI diseases, including inflammatory bowel disease (IBD)[84] and allergy.[85] Alterations in the composition of the GI tract microbiota have been reported in both FD and IBS[86,87]; however, the difficulties associated with sampling and culturing species from tissues of the GI tract and the inherent variation in the microbial community from individual to individual have limited the identification of a specific dysbiosis signature in these conditions. There have been reports of higher levels of known bacterial fermentation products in FGID patients compared to controls, suggesting altered microbial metabolism in some patients. For example, IBS patients have higher levels of organic acids, including propionic acid and acetic acid, when measured against controls, which could potentially contribute to the visceral hypersensitivity observed in some FGID patients.[88]

Dysbiosis in this context may be modulated by a loss or reduction in commensal species that regulate the delicate balance between immune/microbiota tolerance, consequently impairing homeostasis. Broad-spectrum antibiotic use has been observationally associated with IBS development within 12 months[89]; however, further work to characterize this link is required. Animal research using antibiotics to eliminate commensals, including *Clostridium* species, has shown such species to be crucial for modulation of homeostatic T-cell and innate lymphoid

cell (ILC) responses, by decreasing production of IL-22 from ILCs and regulatory T-cell populations.[90]

Whether the observed reduction in diversity and increased instability of the microbiota over time in GI disease are causal or consequential is unknown,[91] but may suggest a situation where homeostatic regulation is challenged by the opportunistic expansion of pathobionts. The newfound dominance of such species may provoke the immune system and potentiate disease. In this hypothesized scenario, recognition of bacterial components by innate pattern recognition receptors, such as TLRs, on dendritic cells initiates the activation of intracellular signaling to result in secretion and activation of inflammatory cytokines such as IL-1β and IFNs.[92] These molecules signal for recruitment and activation of effector cell populations, resulting in low-grade inflammation such as that observed in FGIDs. Elevated levels of antimicrobial β-defensin 2 have been reported in IBS patients,[93] suggesting activation of the innate immune pathway. Furthermore, the expression of TLR4, TLR5, and TLR9 was reported to be upregulated in the small intestine of IBS patients.[94] TLR4 mediates recognition of gram-negative bacteria through sensing lipopolysaccharide, TLR5 detects bacterial flagellin, and TLR9 responds to bacterial DNA.[95] The downstream cytokines released by this pathway have also been reported to be altered between FGID patients and controls. For example, increased IL-1β and IFN-γ have been related to FGIDs[18,24,96]; however, it is important to note that these are not consensus findings across all studies in the area.[5] When considered together, there is a suggestion that unknown host factors in some FGID subsets contribute to dysbiosis, which may then subsequently stimulate the immune system through TLRs, initiating an inflammatory cascade leading to symptom presentation.

Nonclassical T-Helper Type 2 Responses in Functional Gastrointestinal Disorders

Recent advances in cell phenotyping and identification methods have allowed for the recognition of noncanonical immune response pathways beyond adaptive Th1 and Th2 responses. These pathways, including those involving ILC and adaptive Th17 responses, have been implicated in GI diseases, including IBD and celiac disease, and may represent potential drivers of the subtle inflammation observed in FGIDs. Homeostatic disruption in the GI tract may drive loss of regulatory T cells and ILC3s in favor of a Th17/ILC2-mediated inflammatory state.

Although traditionally associated with protection from extracellular pathogens, Th17 immune responses can also induce inflammation and autoimmune conditions. Th2-independent recruitment of eosinophils and mast cells has been reported through Th17 pathways, and a Th17/regulatory T-cell balance exists in homeostatic conditions.[97] Secretion of IL-23 induces naive $CD4^+$ T cells to differentiate into Th17 cells, capable of producing IL-17, TNF-α, and IL-6 in animal models.[97,98] Of note, TNF-α and IL-6 have been reported to be increased in the periphery of FGID patients.[18,25] In addition, Futagami and colleagues[99] identified increased proportions of $CD68^+$ $CCR2^+$ macrophages in patients with PI-FD compared to controls. Interestingly, IL-17 has been shown to induce production of IL-1β and TNF-α from human macrophages,[100,101] and levels of these cytokines have been reported to be increased in PBMC supernatants in an FD cohort.[18] Th17 responses have been demonstrated in asthma patients, where Th17 lymphocytes are induced by antigen-presenting cells to release IL-17, which is able to act on the airway epithelium.[102,103] This results in the activation of macrophages and recruitment of eosinophils via release of factors, including IL-5 and granulocyte-macrophage colony-stimulating factor.[102] Given the heterogeneity of the immune factors described thus far in FGIDs, it is plausible that a subset of patients may have symptoms that result from a Th17-driven immune response initiated by antigen presentation.

ILCs are now recognized as potent innate regulators of GI tract homeostasis, with the capacity to drive inflammation in response to stimuli. There are 3 major subtypes of ILCs—ILC1, ILC2, and ILC3—and their function largely mirrors that of Th1, Th2, and Th17 cells, respectively.[104] Under homeostatic conditions, ILC3 actively suppresses innate and adaptive responses. This ILC subset is characterized by expression of the transcription factor retinoic acid receptor–related orphan receptor γ t and modulates the Th cell response to the commensal microbiota of the small intestine.[105] A loss of homeostasis can result in a switch from ILC3-mediated homeostasis to an ILC2-dominant response. Inflammatory ILC2 responses can be stimulated by cytokines, including IL-33, IL-25,[106] IFN-γ,[107] and IL-1,[108] as well as in response to serine protease released by mast cells.[109] In this context, ILC2s secrete IL-5 and IL-13 to drive eosinophil recruitment and subsequent inflammation (Figure).[110]

Of novel interest in the field of FGIDs is evidence that ILC2 populations can respond to neurotransmitters, including vasoactive intestinal peptide (VIP), implicated in the maintenance of the circadian rhythm. In addition, VIP is released by neurons in allergic airway inflammation.[111] Research in mice also demonstrated correlations between eosinophil number, serum IL-5 level, and circadian rhythm.[112] Given the expression of the receptors for VIP on ILC2s, it is hypothesized that postprandial VIP secretion can activate expansion of ILC2[112] and if the homeostatic balance of the GI tract is disturbed, then this

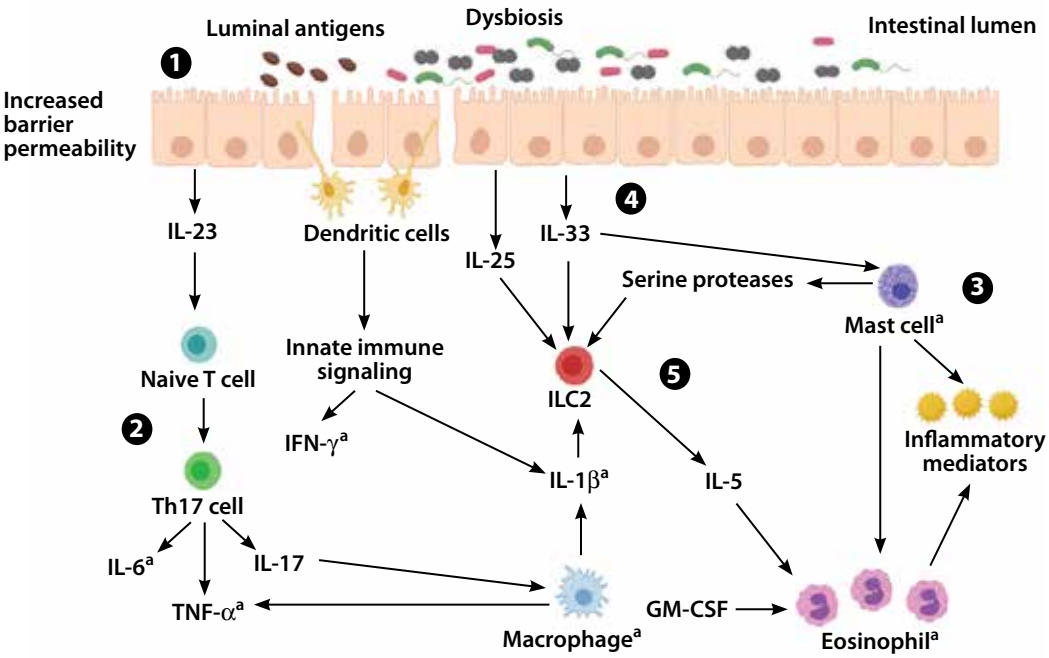

Figure. Hypothesized immune responses and links to FGIDs. The hypothesis of FGIDs as disorders of gastrointestinal homeostatic imbalance suggests that Th17 and ILC2 pathways warrant investigation as mediators of underlying immune activation. In homeostasis, Th17 cells exist in a balance with regulatory T cells, and ILC3s are involved in suppression of immune responses. Stimulation of the immune system by antigens or alterations in the microbiota (1) contributes to increased barrier permeability. This interruption drives the maturation of Th17 cells (2), which produce IL-6, TNF-α, and IL-17. IL-17 has been shown to induce macrophages in patients with asthma and is capable of secreting IL-1β. IL-33 facilitates increased mast cell activity, which is capable of driving eosinophil recruitment (3). Additionally, IL-25, IL-33, IL-1β, and serine proteases influence the expansion of ILC2 populations (4), disrupting ILC3 homeostasis. ILC2 populations drive further eosinophil recruitment through activity of IL-5 (5). This inflammatory environment, driven by cytokine activity and release of inflammatory mediators, may contribute to the symptoms reported by FGID patients.

[a]Denotes factor identified in the literature as altered in FGID cohorts.

FGID, functional gastrointestinal disorder; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; ILC, innate lymphoid cell; Th, T helper type; TNF, tumor necrosis factor.

may result in eosinophilia. Considering the links between sleep disturbances in FGIDs[113] and reports of altered VIP in IBS patients,[114] if demonstrated in humans, this theory may explain the postprandial nature of symptom onset in some FGID subsets and help to explain duodenal eosinophilia in FD subjects reporting postprandial distress.[115] This indirect evidence warrants investigation, particularly in patients reporting meal-induced symptom onset. It is plausible that as yet unknown factor(s) mediating the loss of GI tract homeostasis promote effector cell recruitment through VIP secretion and ILC2 expansion.

Conclusion

The identification of underlying immune activation in FGIDs represented a significant advance in the understanding of these conditions. Current evidence in the field indicates that these conditions are manifestations of homeostatic imbalance in the GI tract, as opposed to traditional activation of inflammatory cascades seen in organic diseases; however, the evidence to support this as a linking hypothesis is lacking.

The disparity within the literature with regard to the activity of specific immune mediators and the heterogeneity inherent to FGIDs has limited the understanding of how these conditions develop, and, therefore, hampered improvements in patient management. In addition, most studies reporting alterations in cytokine levels and immune signaling factors are observational in nature and have not investigated the known immune pathways associated with observed changes in such factors. The deficiency of evidence to support the assumption of

these conditions as driven by Th2 immune activation provides a basis to investigate other pathways more recently identified as capable of recruiting effector cells such as mast cells and eosinophils independently of classical Th2 mechanisms. If homeostasis is interrupted in FGID patients, there is potential for regulatory T cells and ILC3 populations to be suppressed by the expansion and inflammatory action of Th17 and ILC2 populations. The literature suggests that the Th17/regulatory T-cell axis warrants investigation due to reports of altered levels of TNF-α, IL-1β, and IL-6 in FGID studies, all of which are implicated in Th17 pathways. The emerging field of ILC biology also merits study in FGID patients due to the role of ILC3 in mediating homeostasis. In addition, both Th17 and ILC2s have the capacity to recruit eosinophils and mast cells.

The development of a single hypothesis to describe the nature of immune activation in FGIDs will rely on the mechanistic identification of unique FGID phenotypes driven by specific signaling cascades and inflammatory signals. This approach will provide opportunities for specific diagnostic, management, and therapeutic options for these patient groups.

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