In the ZOne: How Impedance Facilitates Progress in Functional Dyspepsia Research

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Non-ulcer or functional dyspepsia (FD), one of the most common chronic gastrointestinal conditions, is a major health burden, particularly in developed countries [1]. Symptoms generally manifest as one of two subtypes: epigastric pain syndrome (EPS) or post-prandial distress syndrome (PDS), with anxiety and depression as common comorbidities [1]. While the worldwide prevalence of FD is reported to be about one in 10 people, there is considerable regional variation that may be attributable to reporting practices, since rates of uninvestigated dyspepsia are generally high [1]. Despite these high prevalence rates, relatively little is known about the etiology or biological basis for FD; at present, it remains classified as an unexplained or ‘functional GI disorder,’ defined by chronic gastrointestinal symptoms in the absence of obvious structural or biochemical pathology. This classification has been challenged by the observation of subtle but distinct immune abnormalities in patients with functional dyspepsia, including eosinophilia of the duodenal mucosa and increases in the number of circulating gut-homing T cells [1, 2]. Though it is unclear as to what provokes this immune response, factors such as an altered gut microbiome [3] or dietary components such as gluten peptides and fructans may contribute [4].

In this issue of *Digestive Diseases and Sciences*, Komori et al. [5] aimed to expand the understanding of FD pathogenesis, identifying a link between reduced duodenal, but not gastric impedance, a surrogate marker of epithelial paracellular permeability, and decreased zonula occludens-1 (*ZO-1*) gene expression. As a key tight junction component, *ZO-1* is an appropriate marker of tight junctional integrity; the association between loss of *ZO-1* and increased paracellular

permeability builds on previous reports of increased duodenal permeability in patients with FD [6]. Loss of *ZO-1* expression is common to many gastrointestinal disorders, but notably, disruption of *ZO-1* integrity is a key mechanism in the pathogenesis of celiac disease, whereby gliadin peptides upregulate the production of zonulin, a mammalian analogue of zonula occludens toxin [7]. In vitro and in vivo studies have demonstrated that zonulin decreases *ZO-1* expression and dysregulates *ZO-1* protein integrity in intercellular tight junctions, which complements the observation that decreased *ZO-1* expression is present in celiac disease [7]. These findings are pertinent since significant associations between FD and both celiac and non-celiac gluten sensitivity (NCGS) have been reported [4]. There are some questions remaining as to whether NCGS is in itself a new entity, or, given the high degree of overlap that NCGS and celiac disease have with FD, they might rather represent different manifestations of a similar disease process. Moreover, zonulin has been proposed as a surrogate marker of intestinal permeability [8] and has been associated with anxiety and depression, although findings such as this remain somewhat controversial; conflicting reports on the utility of zonulin as a biomarker of intestinal paracellular permeability may arise from technical issues such as the differing use of plasma and serum versus the sampled tissue (Fig. 1).

Alternatively, loss of tight junctional integrity may arise from disruption of the structure of the gut microbiota. Studies in animal models have demonstrated that antibiotic-induced disruption of the microbiota increases intestinal paracellular permeability [9]. These findings suggest that key commensal flora are necessary to maintain intestinal homeostasis and that loss of certain species disrupts the balance between interleukin (IL)-22 and IL-17, depleting innate sources of IL-22 and facilitating a T-helper (Th)17 response. Recent advances in endoscopic technology have enabled aseptic biopsy sampling of the duodenum providing the opportunity to study the duodenal microbiota in isolation [3]. Studies utilizing this aseptic

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Fig. 1 Hypothetical context for changes observed by Komori et al. (red bold) in the disrupted homeostasis of the functional dyspepsia duodenal mucosa. Alterations in the microbiota can drive Th17 responses, reducing tight junction integrity and promoting IL-1β

secretion from macrophages and eosinophil recruitment. Alternatively, gliadin-induced secretion of zonulin can decrease tight junction integrity through reduction of ZO-1 and drive IL-1β secretion through antigen exposure

biopsy device point to an altered duodenal microbiome in FD patients, when compared with non-FD controls. Whether these changes in the microbiota are a cause or consequence of FD remains to be determined; however in FD patients, increased bacterial load is associated with more severe GI symptom responses to standardized nutrient challenge [3].

While the evidence supports FD-associated dysbiosis, there have been no studies examining Th17 responses in FD patients. We have previously discussed several lines of indirect evidence suggestive of Th17 involvement in FD [10]. For instance, IL-17 induces production of IL-1β and tumor necrosis factor (TNF)-α from blood mononuclear cells isolated from FD patients, with both factors significantly increased compared with healthy individuals. Furthermore, pre-clinical models have demonstrated that Th17 pathways can drive eosinophil recruitment independently of Th2 cytokines; despite the consensus that FD has the hallmarks of a Th2 response, no empirical evidence exists to support this assertion [2]. In the context of the findings of Komori et al, IL-1β mRNA levels significantly correlated with intestinal permeability as determined by reduced mucosal impedance. While there was no association between TNF mRNA expression and mucosal permeability, these measurements were at the gene, rather than at the protein level. Nevertheless, increased *IL1β* expression and intestinal permeability in FD are consistent with previous findings, and support

further investigations of microbiota-immune homeostasis are warranted.

As mentioned above, FD generally manifests as either EPS- or PDS-dominant symptoms. Much discussion has focussed on whether these are two manifestations of the same disorder or are entirely different entities, and how these subtypes might relate to the immune pathology associated with FD. In that sense, there was no difference between EPS and PDS subtypes in terms of mucosal impedance, *ZO-1* or *IL-1β* gene expression in the study by Komori et al. [5]. While this may simply be a question of study power, with only 24 FD patients, it may also suggest that intestinal paracellular permeability is a common downstream consequence of the etiological factors driving EPS and PDS FD. Enrichment of specific microbiota populations or differential dietary antigens may provoke different symptoms but lead to the same downstream pathology of altered permeability.

While the work of Komori et al. makes significant advances in associating the loss of ZO-1 as the basis of increased duodenal permeability in FD, there is much work to be done in understanding the pathways driving this pathology. For instance, many of the changes that regulate intestinal permeability are post-translational [7], and thus protein level studies will be informative of mechanisms leading to FD-associated mucosal permeability. Similarly, IL-1β signaling is largely driven by caspase-1 mediated cleavage of pro-IL-1β into its active form, and while *IL1β* mRNA levels

are indicative, they are not an accurate measure of IL-1 β signaling per se [2]. Most importantly, ex vivo and animal studies may be useful in understanding which environmental factors, such as diet or dysbiosis, drive these changes.

In summary, it is becoming clearer that FD is not an unexplained functional disorder, but rather a condition of altered mucosal homeostasis. Multiple independent studies suggest an underlying immune dysregulation that may be driven by dietary antigens, microbes or a combination of both, and many of the pathologies driven by such immune dysregulation would lead to increased intestinal paracellular permeability. Future work should focus on studies of immune homeostasis, particularly whether host-microbiota imbalance drives loss of mucosal integrity in FD.

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