***Bin1*: A New Player in IBD Barrier Dysfunction**

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The ability of the host to regulate permeability of the intestinal barrier is a critical determinant of host defense. Regulation of permeability occurs at the level of epithelial tight junctions (TJ). TJ are composed of transmembrane proteins, such as occludin, claudin, and JAM family proteins. Plaque proteins, such as zonula occludins (ZO), connect the TJ complex to F-actin and actomyosin rings regulating cytoskeletal reorganization (Fig. 1, reviewed in [1]). Several signaling pathways, such as myosin light chain kinase (MLCK), protein kinase C (PKC), mitogen-activated protein kinases (MAPK), and the Rho family of small GTPases, control the remodeling and maintenance of TJ. TJ not only regulate paracellular transport of nutrients and water, but they also provide a barrier against enteric microbes. Although the exact etiology is unknown, there is ample evidence that epithelial barrier function is compromised in inflammatory bowel disease (IBD) patients. Data from various studies using inert non-metabolized probes indicate that paracellular permeability is increased in Crohn's Disease (CD) [2]. Furthermore, increased permeability may precede the onset of inflammation [3]. Researchers also found that enteric permeability is increased in 10–54 % of healthy, first-degree relatives of IBD patients [4, 5]. To date, several studies have associated IBD with mutations in the *NOD2* gene [6, 7]. However,

NOD2 penetrance of the most at-risk genotypes is low [8], and it is likely that cooperation with other genetic factors, such as TJ defects, is required for disease development. Interestingly, Buhner et al. detected increased permeability in IBD patients and family members with *NOD2* mutations [9]. Altogether, these findings support the speculation that altered permeability constitutes one of several genetically determined variables that enhances the risk for developing IBD.

The notion that altered epithelial permeability predisposes patients to IBD is supported by data from several animal models with transmucosal barrier defects. Using transgenic mice that express constitutively active MLCK, Su et al. demonstrated that, while insufficient to fully induce experimental colitis alone, TJ defects exacerbated disease in the adoptive transfer colitis model [10]. Other models of barrier disruption, such as dominant negative N-cadherin expression, resulted in spontaneous inflammation [11], further enforcing the link between barrier dysfunction and immune homeostasis. Altered permeability may also occur due to effects of local cytokines. Tumor necrosis factor, a cytokine critical for the pathogenesis of IBD, activates MLCK, resulting in TJ disruption via internalization of occludin from the cell membrane [12]. Also, interleukin-13, a cytokine upregulated in ulcerative colitis, induces expression of the pore-forming TJ protein claudin-2, leading to barrier dysfunction [13]. Altogether, these data represent the dynamic relationship between inflammation and permeability and how their imbalance may result in the development of IBD.

In this issue of *Digestive Diseases and Sciences*, Chang et al. present data that further support the tie between TJ permeability and IBD pathogenesis via the protein Bridging integrator 1 (*Bin1*) [14]. *Bin1* is a member of the Bin amphiphysin 1 (BAR) adaptor family with myriad

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biological functions, including cytoskeletal organization, tumor suppression, transcriptional regulation, and DNA repair [15, 16]. *Bin1* homologs are found in yeast and *Caenorhabditis elegans*, supporting the argument for evolutionary conservation of function (reviewed in [17]). Chang et al. also describe a novel function of *Bin1* in regulating TJ permeability during experimental colitis. The authors previously generated *Bin1* mosaic knockout (KO) mice in which genetic deletions occur throughout the tissue with intermixing of cells harboring recombinant (*Bin1*^{-/-}) or wild-type (*Bin1*^{+/+}) alleles. *Bin1* attenuation had no effect on baseline histology, but it did enhance resistance to dextran sodium sulfate (DSS)-induced colitis. *Bin1* attenuation increased survival and tempered disease activity, as manifested by decreases in diarrhea, bloody stools, and weight loss. Decreased *Bin1* also enhanced epithelial barrier function, measured by increased basal transepithelial electrical resistance (TER) and decreased paracellular mannitol flux. Importantly, the resistant phenotype was specific for inflammation-induced stimuli, as no difference was observed in chemically induced permeability by phorbol ester (PBDU) or sodium caprate. Chang et al. suggest that enhanced barrier function mitigated epithelial damage and inflammatory cell infiltration, possibly by preventing bacterial translocation and/or diffusion of pro-inflammatory cytokines. Thus, these findings suggest that *Bin1* enhances permeability shifts in the setting of inflammation.

Thus far, most IBD therapies heavily rely on immunosuppressive drugs, including corticosteroids and antibodies against pro-inflammatory cytokines. Despite advances in immunosuppressive therapy, IBD remains a challenging disease to treat. The current study by Chang et al. proposes epithelial *Bin1* as a novel therapeutic target without relying on immunosuppression. The data presented suggest that inhibition of *Bin1* could enhance intestinal barrier function and thereby attenuate disease. Another potential benefit of *Bin1* blockade is enhanced epithelial regeneration after inflammation-induced damage (Fig. 1). Within the mammalian system, *Bin1* interacts with c-myc to restrict oncogenic and transcriptional properties [15]. Reductions in *Bin1* expression are observed at high frequencies in lung, prostate, and metastatic cancer [18]. In the intestine, *Bin1* deletion exacerbates Ras-mediated carcinogenesis, and attenuated expression correlates with tumor progression [18, 19]. Although it is unclear whether *Bin1* is involved in c-myc regulation in colitis, *Bin1* inhibition may enhance c-myc functions. Cellular proliferation is a key step in mucosal regeneration (reviewed in [20]). As c-myc participates in cellular proliferation, *Bin1* inhibition may further benefit IBD patients by promoting wound healing. This is especially important because clinicians consider mucosal healing to be a reliable indicator of clinical

Fig. 1 Bridging integrator (*Bin1*) positively regulates intestinal permeability: potential sites of action. Shown are sites where *Bin1* may affect mucosal permeability. *Bin1* may interact directly with epithelial tight junction (TJ) proteins and/or occludin endocytic recycling. It is also possible that *Bin1* impacts c-myc transcriptional activities that affect proteins involved in proliferation and permeability. *MLCK* myosin light chain kinase, *ZO* zonula occludins. Figure is adapted from Ulluwishewa et al. [1]

efficacy of therapeutic agents [21]. Despite the therapeutic potential of *Bin1* inhibition, however, one must consider the potential risk for dysplasia development, as *Bin1* is an intestinal tumor suppressor and colitis increases the risk for colorectal cancer.

It remains to be determined precisely how *Bin1* participates in the regulation of barrier function during colitis. Immunohistochemical analysis revealed cytosolic and membrane-associated staining patterns of *Bin1* in colonic epithelial cells [18]. One speculation is that *Bin1* regulates endocytic recycling and subcellular rearrangement of TJ proteins, such as occludin, which is a critical step in TJ regulation. While the authors found no significant change in occludin expression upon *Bin1* attenuation, it is plausible that subcellular localization of occludin and other members of the TJ complex were altered. Several studies have linked *C. elegans*, mammalian *Bin1* [22], and other members of the BAR protein family to endocytosis and endocytic recycling. Endocytosis of occludin is a critical step in TJ remodeling, as demonstrated by the attenuation of cytokine-induced intestinal permeability following pharmacological inhibition of endocytosis [12]. Thus, the effects of *Bin1* on mucosal permeability may be due, in part, to alterations in occludin endocytic recycling (Fig. 1).

IBD has long been considered to result from the combination of immune dysregulation and altered mucosal permeability. Data presented by Chang et al. further support the hypothesis that compromised permeability predisposes the host to IBD. The authors present a novel

animal model that points to targeting *Bin1* as a means of strengthening mucosal barrier function in the colon. As combination therapies become increasingly common, it is attractive to speculate that the topical administration of *Bin1* small molecule inhibitors may serve as a useful adjunct to systemic immunomodulatory agents.

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