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Commentary

The tight junction protein claudin-2 is upregulated in inflammatory bowel disease, and yet its deficit worsens infectious and chemical colitis. In this issue of the *JCI*, Raju and Shashikanth et al. examined the contribution of claudin-2 to immune-mediated colitis. The authors used transgenic mouse models to show that claudin-2 deficiency attenuated colitis progression as well as a leak barrier defect, albeit at the risk of intestinal obstruction. Further, inhibition of claudin-2 by targeting casein kinase 2 (CK2) also ameliorated colitis. The findings reveal unsuspected links between the pore and leak pathways of intestinal permeability and immune responses leading to colitis. They additionally suggest potential targets for therapeutic intervention in inflammatory bowel disease.

Claudin-2 pore causes leak that breaches the dam in intestinal inflammation

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Intestinal barrier

The intestinal epithelium maintains a semipermeable barrier between the gut lumen and the body as a physiological imperative. This barrier allows the uptake of needed nutrients and reclamation of the water and electrolytes used in the course of digestion, and also resists the passage of toxins, undesirable metabolites, and the bacteria and other microorganisms that comprise the intestinal microbiota within the lamina propria. The selectivity of the barrier is provided not only by the epithelial cells themselves and the array of specific transporters they express, but also by the permeability properties of the intercellular tight junctions that are critical contributors to overall barrier function. Ample evidence suggests that what's referred to as a "leaky gut" can cause or result from intestinal disorders (1). Diminished intestinal barrier function allows microorganisms and their metabolites to inappropriately stimulate components of the mucosal immune system, often perpetuating inflammation and barrier dysfunction in a vicious cycle (2).

Tight junction permeability is determined, in part, by the properties of a family of transmembrane proteins known as claudins. At least 27 family members have been identified and their expression varies within the intestinal epithelium (as well as other epithelial tissues). Claudin homomeric and heteromeric dimers that form by binding to partners on adjacent cells define overall barrier properties (3, 4). In a general sense, claudin isoforms have been subdivided into those that provide a seal between adjacent cells and those that form specific (typically cation-selective) pores through the junction (3, 5). In the setting of intestinal inflammation, such as occurs in inflammatory bowel diseases (IBDs), the dogma had been that expression of sealing claudins was downregulated while that of pore-forming claudins was increased, accounting for an accompanying loss of overall barrier function. The former effect would increase macromolecular flux across the epithelium (the leak pathway), while the latter would permit backflow of water and sodium into the intestinal lumen. Among other effects, this combination of changes would worsen accompanying diarrhea by allowing so-called "leak-flux" diarrhea (2, 6, 7).

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A role for claudin-2

Claudin-2 is a pore-forming claudin whose expression has been shown to be elevated in patients with various inflammatory diseases of the gut, as well as in various models of IBD employing epithelial cells exposed to inflammatory mediators (8). Because expression of claudin-2 correlates with disease severity, it had been assumed to play a pathogenic role in IBD (6). This hypothesis was, however, called into question by findings that both chemical and infectious colitis were in fact worsened when claudin-2 was knocked out (9–11). However, the role played by claudin-2 in the immune-mediated colitis typical of IBD had not been established.

In this issue of the *JCI*, Raju and Shashikanth et al. (12) confirm a pathogenic rather than protective role for claudin-2, and reveal, in fact, that the pore pathway established by claudin-2 overexpression promotes colitis induced by the transfer of pathogenic T cells into immunodeficient *Rag1*-knockout mice. Claudin-2 overexpression also accounts for the deleterious effect of the inflammatory cytokine, interleukin 13 (IL-13), on epithelial barrier function. Furthermore, not only did claudin-2 expression account for the IL-13–associated increase in pore pathway permeability to sodium ions and other small cations, but it was also required to hasten the progression of immune-mediated colitis and subsequent development of leak pathway defects.

Raju, Shashikanth and coauthors (12) posit that claudin-2 may be a viable therapeutic target to limit colitis progression. However, a note of caution is needed; a subset of mice lacking claudin-2 that were subjected to immune-mediated colitis subsequently died as a result of intestinal obstruction and ischemic injury, despite

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reduced inflammation. Because fibrosis and altered motility were excluded as causes of this obstruction, the authors hypothesized that adequate hydration of the intestinal contents may require some level of claudin-2 expression, particularly in the face of other molecular events occurring in the setting of immune injury to the bowel (and analogous to intestinal obstruction seen in cystic fibrosis). Indeed, adding polyethylene glycol to the drinking water of claudin-2–knockout mice induced a mild osmotic diarrhea and abrogated the intestinal obstruction and mortality.

Prior work from the Turner lab implicated casein kinase 2 (CK2) as a key regulator of various tight junction components, including claudin-2; in vitro, CK2 inactivates claudin-2 pore function (13). Raju, Shashikanth and coauthors, therefore, tested whether an orally bioavailable CK2 inhibitor could benefit their immune colitis model and showed that the compound reduced inflammation in a manner dependent on claudin-2 expression, but without the untoward effects of claudin-2 knockout on intestinal obstruction and mortality (12). Overall, these encouraging findings suggest that CK2 and claudin-2 are promising targets in IBD to reduce disease progression.

Conclusions and future directions

Despite the progress made in this study, important questions remain. For example, it is unclear why promotion of a tight junction pore that selectively permits passage of sodium ions and water through the paracellular pathway would subsequently predispose to mucosal immune activation, generation of a leak pathway of intestinal barrier dysfunction, and the progression of experimental colitis. Further, while the claudin-2 pore pathway clearly contributes to luminal hydration and averts intestinal obstruction, reproducing this effect alone failed to affect colitis severity. Rather, the authors speculate that increases in claudin-2 expression are accompanied by increases in sodium ion concentrations in the lamina propria that may drive the development of pathogenic T cells. This hypothesis should be testable, and could further indicate a role for manipulating dietary sodium in the management of IBD. The authors also did not establish why downregulation of claudin-2 function with a CK2 inhibitor benefitted colitis without the liability of intestinal obstruction seen with a total claudin-2 knockout. This discrepancy could reflect the degree of claudin-2 inhibition, but clarification is important to justify further efforts to target the CK2/claudin-2 axis therapeutically. In any event, the findings of Raju and Shashikanth et al. (12) clearly suggest different avenues for the treatment of intestinal inflammation. Such approaches may avert newly diagnosed disease and/or prevent relapse if activation of the epithelial barrier pore pathway is indeed a critical early contributor to disease initiation and progression, as the studies in animal models suggest. Because available treatments for IBD are not uniformly effective, these alternative approaches may offer much-needed options for patients.

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