Control the epithelial barrier: A pivotal first line of defense

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ARTICLE

Scott KG-E, Meddings JB, Kirk DR, Lees-Miller SP, Buret AG. Intestinal infection with *Giardia* spp. reduces epithelial barrier function in a myosin light chain kinase-dependent fashion. *Gastroenterology* 2002;123:1179-90.

COMMENTARY

Lumen-derived material gains access to the mucosa by permeating between adjacent epithelial cells (ie, paracellular pathway), by transcytosis across the apical and basolateral cell membranes (ie, transcellular pathway) or by exploiting breaks or erosions in the epithelium that may, for example, result from inflammation. Increased epithelial permeability (or decreased barrier function) has repeatedly been demonstrated in a variety of gut disturbances; notably, in inflammatory bowel disease (IBD). There has been an exponential increase in our knowledge of the structural elements that comprise the epithelial barrier, and of the intrinsic factors (eg, cytokines) and external stimuli (eg, bacterial toxins) that can either perturb or enhance epithelial permeability. Canadian researchers have been very active in the study of epithelial permeability and have been responsible for major advances in the field, documenting increased permeability in patients with ulcer disease and IBD and some of their first degree relatives (as well as before onset of overt inflammation), and elucidating mechanisms of stress-induced and cytokine-induced increases in permeability (1-8). A recent study from Scott et al (9) continues this impressive tradition.

Juxtaposing murine in vivo and in vitro epithelial cell culture studies, Scott et al (9) assessed if, and then how, infection with the protozoan parasite, *Giardia* spp, might affect epithelial permeability. Earlier work by this group had shown that the barrier function of human nontransformed small bowel-derived epithelial cell (SCBN) monolayers was perturbed by exposure to *G lamblia* (10), a parasite that causes malabsorption and diarrhea. In the present study they demonstrated that mice infected with 2×10^5 live *G muris* trophozoites display increased small intestinal (but not gastric or colonic) permeability, as assessed by nonabsorbable sugars. In addition, this effect was T cell-independent (ie, it also occurred in athymic mice that lack thymus-educated T cells), implying a direct effect of the parasite on the epithelium. Adopting a cell culture approach, they

subsequently showed that SCBN cells cultured with *G lamblia* displayed an increase in myosin light chain kinase (MLCK) phosphorylation, a concomitant increase in epithelial permeability (as least to markers the size of 3000 Da fluorescein isothiocyanate-dextran (FITC), which predominantly cross the epithelial via paracellular pathways) and altered distribution of the filamentous (F)-actin cytoskeleton and tight-junction associated protein, zona occludens 1 (ZO-1). These events were all inhibited by an inhibitor of MLCK phosphorylation, ML-9 (Sigma Chemical Co, USA). These in vitro data provide a mechanism by which *Giardia* spp infection could directly cause increased epithelial permeability, and support the postulate that arose from studies using T cell-deficient mice that the in vivo increase in permeability could be due to direct effects of the parasite on the enterocyte.

The idea that microbes could alter epithelial barrier function is not new (11) nor is the observation that MLCK is a controller of paracellular permeability (12), so why is the report by Scott et al (9) important? First, epithelial permeability is increased in human giardiasis (13) and so the murine and in vitro models are directly relevant to understanding the human condition. *Cryptosporidium parvum* and *Entamoeba histolytica* also increase epithelial permeability, leading to speculation that data from the *Giardia* model might be applicable to protozoon-induced barrier disruption in general. Indeed, *Giardia*-induced enteropathy is similar to that observed in, for example, bacterial enteritis and celiac disease, which led Scott et al (9) to suggest that *Giardia*-induced enteropathy/barrier dysfunction could serve as a model for other human diseases.

Second, by adding to the models in which MLCK activation has been implicated as the cause of epithelial barrier disruption, this study lends further credence to the hypothesis that targeting of MLCK could ameliorate disorders due to, or associated with, increased epithelial permeability. Clearly, targeting of a single molecule to treat conditions with different etiologies represents an attractive therapeutic option.

Third, the same researchers have also shown that increases in epithelial monolayer permeability evoked by *G lamblia* are due to enhanced apoptosis and could be mitigated by inhibiting caspase-3 activation (14). Likewise activation of proteinase-activated receptor 1 on SCBN cells results in altered ZO-1 distribution and decreased barrier function that were prevented by pretreatment with inhibitors of caspase-3 or MLCK activation (15). This begs the question: is the increase in epithelial permeability observed following *Giardia*

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infection due to a parasite-derived proteinase? An intriguing possibility! Moreover, these observations show that several mechanisms contribute to the maintenance or disruption of epithelial barrier function – in this instance, physiological contraction of the enterocyte cytoskeleton and induction of programmed cell death – and also suggest that common mechanisms of barrier control might be mobilized in a variety of physiological and pathophysiological instances. This could allow physicians to modulate disease by controlling epithelial barrier function.

This is an elegant study integrating in vivo with in vitro work and will leave the reader with no doubt that *Giardia* infection can directly affect epithelial permeability via activation of

MLCK (and caspase-3 [14]). The outstanding issues are: can the data gleaned from the SCBN cell culture model be applied to *G. muris*-induced barrier disruption in mice and, if so, will the observations hold true for the human giardiasis and perhaps other enteropathies? This work both complements and extends that of other investigators in the field, and highlights MLCK and caspase-3 as potential targets for pharmacological modulation of epithelial permeability.

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