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COMMENTARY

Silence Please!

siRNA Approaches to Tighten the Intestinal Barrier in Vivo

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The dynamic nature of the intestinal epithelium is central to its roles in both intestinal homeostasis and human disease. Although often referred to as the mucosal or intestinal barrier for its ability to limit harmful molecules or antigens in the lumen from entering the body, the intestinal epithelium also allows for paracellular transport of fluid, ions, and nutrients that are crucial for normal gut function. A key structural component of the mucosal barrier that contributes to intestinal epithelial permeability is the intracellular tight junction, and defects in the tight junction barrier have been implicated in the etiology of chronic intestinal inflammation. In this issue of *The American Journal of Pathology*, Al-Sadi et al[1] report on the molecular mechanisms and intracellular pathways involved in increased intestinal tight junction permeability mediated by the pro-inflammatory cytokine, tumor necrosis factor-α (TNF-α).

The hypothesis that increased intestinal permeability contributes to the pathogenesis of inflammatory bowel disease (IBD) was first described by Shorter et al[2] in the early 1970s. Although several studies followed showing an association with decreased barrier function in patients with Crohn's disease,[3–5] it was Hollander et al[6] who first demonstrated that both Crohn's disease patients and a subset of their healthy relatives have a primary defect in intestinal permeability. Other groups expanded these findings and provided a genetic link (*NOD2* 3020insC) for the increased intestinal permeability observed in first-degree relatives of patients with Crohn's disease.[7–9] Collectively, these studies support the concept that increased intestinal permeability may predispose one to develop chronic intestinal inflammation through increased transport of luminal antigens.

TNF-α has been shown to induce intestinal epithelial cell permeability through the increased expression and activation of myosin light-chain kinase (MLCK).[10–14] Monoclonal antibodies directed against TNF-α have shown improvement in clinical aspects of the disease,[15–17] as well as a reduction in mucosal pro-inflammatory cytokine production.[18] Furthermore, the work of Suenaert et al[19] showed that the intestinal tight junction barrier in patients with active Crohn's disease could be restored with anti-TNF treatment. Although monoclonal antibodies to TNF-α, such as infliximab, have shown promise in the treatment of intestinal inflammation, there is limited knowledge regarding the intracellular mechanisms involved in the TNF-α–mediated increase in intestinal epithelial cell permeability.

Bridging the Gap between TNF-α Signaling and Tight Junction Function *in Vitro*

In this issue of *AJP*, Al-Sadi et al[1] reveal the counterparts of a signaling pathway initiated in the inflamed intestinal epithelium due to a rise in TNF-α. The group established the relevance of their *in vitro* model, colonic epithelial CaCo2 cells, for the study of TNF-α–induced tight junction dysfunction by showing that treatment of CaCo2 monolayers with this pro-inflammatory cytokine causes increased transepithelial flux. Moreover, the downstream target of TNF-α involved in epithelial barrier function, MLCK, was also increased in TNF-α treated CaCo2 monolayers.

Because TNF-α is known to activate mitogen-activated protein (MAP) kinases, the group went on to investigate the

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role of MAP kinases in epithelial barrier function. They showed that TNF-α activates the MAP kinases ERK1/2, but does not affect p38. Pharmacological inhibition or siRNA silencing of ERK1/2 reversed the effect of TNF-α on *MLCK* gene expression and subsequently restored transepithelial resistance in CaCo2 monolayers, suggesting that ERK1/2 has a key role in the regulation of intestinal barrier function.

In an effort to connect ERK1/2 signaling with the regulation of *MLCK* gene expression, this study focused on the transcription factor Elk-1, a known downstream target of MAP kinases. With the use of *in silico* analysis, the authors identified two Elk-1 binding motifs on the promoter region of the *MLCK* gene, with only one of them being adequate and sufficient to promote *MLCK* gene expression in CaCo2 cell monolayers. TNF-α induces Elk-1 activation in an ERK1/2-dependent manner, whereas both ERK1/2 and Elk-1 are necessary for *MLCK* gene expression and the reduction of tight junction function in CaCo2 monolayers, thus confirming the importance of this signaling pathway in the regulation of tight junction permeability *in vitro*. However, how relevant and important is this molecular pathway for the regulation of intestinal barrier function *in vivo*?

Confirming the Importance of the TNF-α/ERK1/2/Elk/MLCK-Dependent Barrier Function *in Vivo*: Tools for Future Therapeutic Targets

The final parts of this study include experiments confirming *in vitro* findings in an *in vivo* model. Here, the authors used a relatively new and sophisticated technique to silence intracellular molecules *in vivo*. An initial evaluation of the mouse model for relevance was performed to show that intestinal permeability was increased after intraperitoneal administration of TNF-α. The authors also showed that ERK1/2 and Elk-1 activity, as well as *MLCK* gene expression, were upregulated after TNF-α treatment.

Perhaps the most important findings of this study involve the application of an *in vivo* silencing approach using siRNA. This group has knocked down target molecules after *in vivo* transfection of siRNA with similar efficacy in previous studies.[20,21]

In these experiments, the authors selectively knocked down the expression of ERK1/2 or Elk-1 in the intestine with siRNA *in vivo*, thus inhibiting the effect of TNF-α on intestinal barrier function.

Today, the pro-inflammatory effects of TNF-α are commonly treated with anti−TNF-α antibodies that have taken the lead in development of immune-modulating drugs because TNF-α is known to be an important cytokine in gut diseases such as Crohn disease and ulcerative colitis.[17,22–24]

As intravenous administration of any drug presents with a number of limitations, a more localized approach could potentially optimize treatment options. Al-Sadi et al[1] were able to block downstream targets of TNF-α and reverse pro-inflammatory effects in a localized manner, suggesting an alternative approach for treatment without the side effects of a systemically administered drug. In particular, the authors restored intestinal barrier function by targeting two separate TNF-α downstream molecules, directly to the mouse intestine. As shown in Figure 1, development of drugs that silence the expression of ERK1/2 or Elk-1 would prevent *MLCK* gene expression, thus restoring tight junction and intestinal barrier function.

An important question that needs to be addressed after this study is whether *in vivo* silencing of ERK1/2 and/or Elk-1 ameliorates the colitis phenotype in relevant colitis mouse models. Both the trinitrobenzene sulfonic acid (TNBS) and the dextran sodium sulfate (DSS) colitis mouse models, as well as the immune models of intestinal inflammation, are widely accepted models for the study of inflammatory bowel disease. For example, future studies should address the extent of colitis in mice treated with siRNA against ERK1/2 or Elk-1 targeted to the intestine *in vivo*.

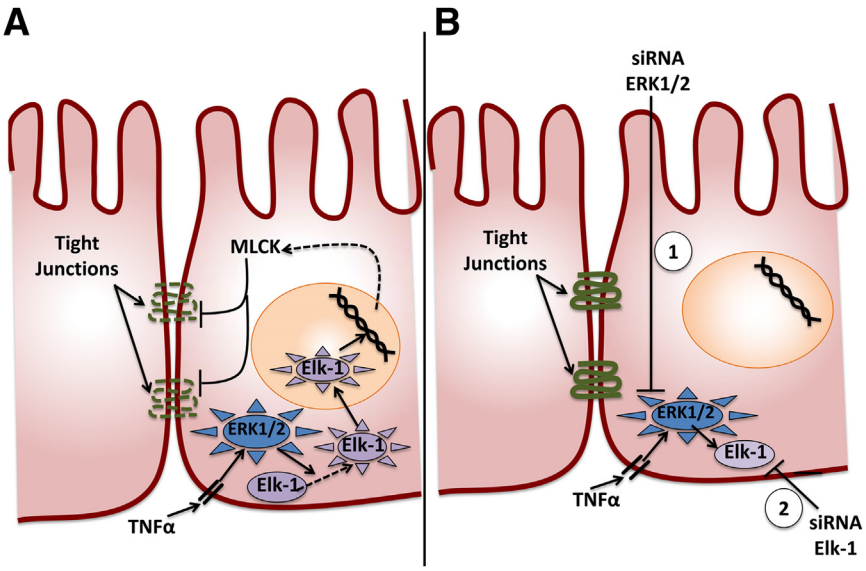

Figure 1 **A:** Schematic representation of the intracellular pathways involved in increased intestinal tight junction permeability mediated by the pro-inflammatory cytokine, TNF-α. **B:** Targeted silencing of ERK1/2 or Elk-1 expression prevents *MLCK* gene expression, thus restoring tight junctions and intestinal barrier function.

More importantly, the findings in the line of work discussed in this commentary suggest that similar *in vivo* silencing may be applied on other intracellular molecules exerting pro-inflammatory effects in the gut. The development of drugs that administer such silencing agents to the site of injury in the intestine should become a major focus in the field of gastroenterology.

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