Mechanisms and functional implications of intestinal barrier defects

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Increased permeability predicts relapse in *asymptomatic* Crohn's disease patients

Wyatt *et al.*, Lancet (1993)
Increased permeability (loss of barrier function) is present in Crohn's disease patients and some of their relatives.

May et al., Gastroenterology (1993)
Interpretation

Barrier dysfunction contributes to CD pathogenesis and/or reactivation

or

Barrier dysfunction is a sensitive biomarker of immune activation and impending disease
How is barrier function reduced in inflammatory bowel disease?

aphthous ulcer (active disease)

inactive Crohn’s disease
What defines intestinal barrier function?
Tight junction barrier function decreases following myosin II regulatory light chain (MLC) phosphorylation.

Atisook et al., Am J Physiol (1990)
Berglund et al., Am J Physiol Gastrointest Liver Physiol (2001)
Atisook & Madara, Gastroenterology (1991)
T cell activation causes TNF-dependent water secretion and increased paracellular protein flux

Ultrastructural tight junction changes caused by T cell activation

MLCK\(^{-/-}\) mice are protected cytokine-mediated
MLC phosphorylation, occludin internalization

\[\text{Water movement out of lumen (μl x cm}^{-1} \times \text{h}^{-1})\]

-50 0 50 100 150 200

\[\text{BSA flux (μg x cm}^{-1} \times \text{h}^{-1})\]

0.0 0.1 0.2 0.3 0.4 0.5 0.6

MLCK expression and activity are increased in active Crohn’s disease.

Blair et al. Lab Invest. 2006
Epithelial barrier dysfunction induced by acute T cell activation is not associated with epithelial loss (erosion) or tight junction disassembly.

MLCK inhibition, either genetic or pharmacologic, prevents acute T cell activation- (or cytokine-) induced diarrhea.

Intestinal epithelial MLCK is activated in human disease (CD).

Is MLCK-dependent barrier loss sufficient to cause disease?

Should it be?
Expression of constitutively-active MLCK (CA-MLCK) in mouse intestinal epithelium

**constitutively active MLCK**

villin promoter

WT

CA-MLCK

Barrier loss is insufficient to cause disease

May et al., Gastroenterology (1993)
CA-MLCK mice have altered mucosal cytokine balance

Transient epithelial damage causes similar changes in LPMC cytokine production

In vitro production by isolated colonic LPMCs

Boirivant et al., Gastroenterology (2008)
Induction of experimental colitis by CD4\(^+\)CD45Rb\(^{hi}\) adoptive transfer
Onset of CD4^+CD45Rb^{hi} adoptive transfer-induced colitis is accelerated and disease is more severe in CA-MLCK mice

• MLCK-dependent epithelial barrier dysfunction is insufficient to induce chronic disease.

• Barrier dysfunction activates both proinflammatory and immunoregulatory events

• MLCK-dependent epithelial barrier dysfunction predisposes to development of chronic enteritis.

**Can MLCK inhibition limit disease progression?**
**In vitro cytokine production in colon organ culture**

Onset of colitis is delayed after adoptive transfer of LIGHT-transgenic T cells into MLCK\(^{-/-}\) mice

Su et al, unpublished
Might MLCK be an effective target for Crohn's disease maintenance therapy?

**Percent of patients without relapse**

- PI < 0.03, n=35
- PI > 0.03, n=37

**Time (days)**

- 0
- 60
- 90
- 120
- 180
- 240
- 300
- 360

**Drug increased permeability**

**Normal permeability**

**Troubled permeability?**

**X**
MLCK-dependent tight junction barrier dysregulation is one of several “hits” that can contribute to pathogenesis and progression of inflammatory bowel disease.
Here's leaking at you, kid