Webinar Q&A Report

Conversations Between Mucosal Immunity and the Gut Barrier

Questions in this Q&A Report were submitted during the live webinar, Conversations Between Mucosal Immunity and the Gut Barrier.

Answers were provided by Jerrold Turner, MD, PhD, Professor of Pathology at Harvard Medical School and Professor of Medicine at Brigham and Women's Hospital.

There is evidence of pancreatic enzyme leakage out of the intestinal lumen in acute disease and experimental studies of circulatory shock in rats have shown improved survival with enteral infusion of protease inhibitors suggesting a role of pancreatic proteases: what is the possible role of pancreatic proteases in contributing to local injury and disease?

I don't think specific proteases have been identified. But it is clear that proteases can activate signaling pathways, e.g., the proteinase activated receptor PAR-2,[1-4] and degrade cell surface proteins, including tight junction proteins. There's also some very interesting work related to luminal proteases in the context of IBS-associated microbiota.[5] Overall, there are still more questions than answers, but I anticipate that we will learn a great deal about this in the next few years.

Is there evidence of direct immune cell interactions with CL-2?

This is a really interesting idea that, to my knowledge, has not been explored. However, changes in claudin-2 expression have been associated with almost any inflammatory process in the intestines, so it is possible. This is true for other tight junction proteins including JAM-A and occludin, which are critical for transmigration of several types of leukocytes across the epithelium[6, 7] as well as intraepithelial lymphocyte migration.[8] In the case of claudin-2 I think is more likely that the increased paracellular Na^+ permeability resulting from claudin-2 expression modulates immune cell differentiation.[9, 10] It is also important to remember that several cytokines, including IL-13 and IL-22 can induce transcriptional activation of claudin-2 within the distal epithelium.[11, 12]

In addition to occludin endocytosis mechanism, is there any other defects of barrier-forming tight junction protein cause leak pathway?

I imagine there must be. For example, we know that ZO-1 knockdown in vitro increases leak pathway permeability,[13] and unpublished work from my group indicates that the same is true in vivo. Studies from the Nusrat-Parkos group have also shown that JAM-A knockout increases both pore and leak pathway permeability and, possibly, also unrestricted pathway permeability.[14] The changes in pore pathway permeability appears to be, at least in part, secondary to increased expression of claudin 10 and 15. The relevance of these changes to homeostasis and pathobiology are not well understood.

Intestinal epithelial cells lacking PTPN2 can drive macrophage M1-like polarization that can feed back (via IL-6) to further increase claudin-2 expression. This supports the idea of a feed-forward, self-amplifying loop in disease pathogenesis.

Yes, excellent point. There are many studies that promote this idea, and it is not unique to inflammatory bowel disease. A similar vicious cycle has been proposed in disorders ranging from environmental enteric dysfunction to graft-versus-host disease, and our work using long myosin light chain kinase knockout mice shows that blocking myosin light chain kinase-driven leak pathway of barrier loss prevents propagation of graft-versus-host disease. The same is true of experimental inflammatory bowel disease, either through genetic myosin light chain kinase deletion or pharmacological diversion of the enzyme away from the region of the tight junction.[15, 16] With regard to PTPN2, which has been genetically linked to inflammatory bowel disease, there is a number of excellent papers.[17-19] The story here is a bit more complex, PTPN2 is a regulator of cytokine signaling in many cell types. For example, recent work using intestinal epithelial-specific PTPN2 knockout mouse makes it clear that there is an epithelial-intrinsic effect of PTPN2 dysfunction.[20]

Activation of myosin light chain kinase could be an adaptive response to infection, if we target myosin light chain kinase aren't we interfering with the adaptive response by the body? Have you checked the long-term effects of myosin light chain kinase inhibition? Given the normal physiological role of MLCK is it a viable target?

These are important questions. My opinion is that physiological Na^+-nutrient cotransport-induced TJ regulation was important evolutionarily, when it was critical to absorb all available nutrients, no matter how great the portion size, as there might be a long delay before food was available again. In contrast, food is now generally available and portions can be managed. As a result, the myosin light chain kinase-dependent paracellular amplification of transcellular nutrient absorption is not essential and is more of an evolutionary relic. As evidence, our experiments using divertin, which prevents myosin light chain kinase-dependent barrier regulation did not detect any effect on weight gain of young mice with ad lib access to chow. Divertin did not cause weight loss in adult mice or have any other apparent toxicities.[16] In addition, long MLCK KO mice grow and gain weight normally.[15]

In terms of myosin light chain kinase in other cell types, inhibition would have severe toxicities. First, the gene that encodes epithelial myosin light chain kinase is the same gene that encodes smooth muscle myosin light chain kinase.[21, 22] We know that inhibition of smooth muscle myosin light chain kinase results in hypotension, visceral paralysis, and death.[23] In addition, the enzymatic domains of cardiac muscle and skeletal muscle myosin light chain kinases are very

similar to that of the smooth muscle/non-muscle myosin light chain kinase and any enzymatic inhibitors of one would likely inhibit the others. Thus, enzymatic myosin light chain kinase inhibition is not a therapeutic fidelity that should be considered. Even if this could be accomplished specifically in intestinal epithelial cells, they would be toxicities, as myosin light chain kinase is necessary for intestinal epithelial wound repair.[24] This is the reason we took the alternative approach of preventing myosin light chain kinase-dependent tight junction regulation by blocking myosin light chain kinase recruitment to the perijunctional actomyosin ring with divertin.[16] In the process of developing divertin, we actively excluded small molecules that inhibited myosin light chain kinase enzymatic activity, smooth muscle contraction, or epithelial migration.[16]

Finally, although we've shown that claudin-2-mediated pore pathway upregulation is beneficial in *C. rodentium* colitis,[12] our preliminary experiments suggest that this is not true of myosin-chain kinase-mediated leak pathway permeability increases. In fact, it seems that inhibition of myosin light chain kinase-mediated pathway permeability increases may be beneficial in infectious colitis as it is in experimental inflammatory bowel disease and graft-versus-host disease.[15, 16, 25, 26]

So, I am confident that targeting myosin light chain kinase-dependent tight junction regulation will be safe as long as we use a selective approach that does not interfere with other essential functions of myosin light chain kinase.

What is mucosal immunity? Does it include short- or long-term immunity?

Mucosal immunity is a general term that can be applied in many contexts.

What can intestinal permeability in healthy individuals be attributed to aside from genetic predisposition? Are there any diet-related factors associated with this phenomenon as well?

Diet, environmental factors, and mucosal immune status can all affect barrier function. For example, in first-degree relatives, NOD2 polymorphisms have been linked to increased permeability (Buhner et al. Gut. 2006). Dietary emulsifiers could also be considered (Chassaing et al. Nature. 2015).

What treatments improve gut barrier in Celiac Disease?

Some work has been done using the drug larazotide, but eliminating gluten is really the best answer.

Is it known if pathogens directly disrupt the cldn-2 interacting with another cldn-2?

That isn't known. However, C. perfringens enterotoxin causes barrier loss by binding to claudins 3 and 4.

What impact on gut barrier would chronic immune activation have?

It would be expected to cause chronic permeability increases. This could lead to a self-amplifying cycle of immune activation leading to increased permeability leading to further immune

activation. In the absence of immunoregulation-dependent suppression of this process, it could theoretically culminate in disease.

Does pH affect regulation of TJ permeability pathways?

Extracellular acidification can reduce paracellular conductance in some cell types (Hein et al. Chem Phys Lipids. 1992). Intracellular pH can also affect tight junction permeability (Turner et al. Am J Phys. 2000).

Rather than having anatomically separate pore and leak pathways might claudin2 mediate both pore and leak at the same cellular/junction site by simply alternating between two molecular conformations?

It's a reasonable hypothesis, but Alan Yu's work carefully mapping the claudin-2 channel (e.g., Li et al. J Biol Chem, 2014 and Rosenthal et al. Acta Physiol, 2017), Anderson and van Itallie's work showing that claudin-2 upregulation specifically enhances pore, but not leak, pathway permeability (van Itallie et al. J Cell Sci, 2008), and my group's patch clamping of claudin-2 channels (Weber et al. eLife, 2015) all suggest that this is not the case. In contrast, other (e.g., Yu et al. Am J Physiol, 2005; Buschmann et al. Mole Bio Cell, 2013; van Itallie et al. Mol Biol Cell, 2009; Marchiando et al. J Cell Biol, 2010) indicate that occludin and ZO-1 are more critical for leak pathway regulation.

Great presentation Jerry. What happens to the MLCK that is removed from the junction in response to divertin? Is it degraded, does it accumulate in the cytosol, or get recycled to the membrane once divertin wears off?

We haven't looked at this carefully, but our data suggest that MLCK1 diverted from the junction stays in the cytoplasm (like MLCK2).

What happens to intestinal macrophages when claudin 2 or MLCK pathways are active?

In the constitutively active MLCK transgenic mice, lamina propria CD11c-positive cells are concentrated closer to the surface than in wild type mice. We don't know if this reflects activation, but similar shifts in location have been reported in IBD patients.

Does increased claudin-2 by cytokine treatment or transgenics replace endogenous claudins or is it simply added to tight junctions?

Great question. We don't know the answer to this. We would love to better understand junctional organization.

Do you think that the mechanisms responsible for injury and the described promotion of disease with barrier loss and mediation of immune activation are the same in acute disease?

We know that some of the signaling pathways are the same. However, chronic processes always trigger secondary events. There must, therefore, be differences as well.

Thank you for the great talk Dr. Turner. Given the developmental changes in the pore pathway (i.e. with weaning) do you suspect that the effect of the benefit or harm context would be different with age?

Really interesting question. I don't think there are meaningful data to address this. However, I think the answer to your question must be yes, there are differences. If they were not, it's hard to understand why this would have developed and been retained evolutionarily. For instance, in the weaning example you gave, the switch between claudin-2 and claudin-15 is a mystery. They seem to form nearly identical Na^+ channels. I suspect that claudin-2 may have a greater open probability or higher throughput, but that has not been studied.

Can you comment of the role of the nervous system and neuromediators on intestinal permeability? Thank you.

This is a really important area that we just don't understand. It is clear that there is a role for the nervous system. However, other than showing that some neural mediators can affect permeability, primarily in vitro, little is known.

Does IL-13 regulate CK-2 activity?

I am not aware of any specific to IL-13. However, Koch et al. (Mucosal Immunology, 2006) did publish work showing that CK2 is activated in IBD. They concluded that it is essential for homeostasis. Our work using the inhibitor suggests that that is not entirely accurate but does not exclude other roles for CK2.

Is it possible that intestinal contractility is also altered in these experimental models?

Yes, I'm sure that it must be. It is also important to recognize that in vivo permeability measurements are affected by transit rate. This is one of the reasons it is essential to include normalizing controls, such as 70 kDa dextran in our experiments, in such assays.

How do you protect barrier function from necrosis factor?

Myosin light chain kinase is the primary driver of tumor necrosis factor -induced barrier loss. This leads to occludin internalization and, likely, degradation. Preventing myosin light chain kinase activity at the perijunctional actomyosin ring or overexpressing occludin have both been shown to block or at least reduce tumor necrosis factor -induced barrier loss in vivo.

What is the contribution of macrophages in the tight junction?

Macrophages are an important source of many cytokines, including tumor necrosis factor. I don't know of a direct physical interaction between macrophages and epithelial cells that regulates tight junctions.

Can bacterial/viral infection cause increased permeability and development of IBD?

Infection can definitely increase permeability. Although mechanisms are not known, there are several studies showing that enteric infection with Salmonella or Campylobacter increases subsequent risk of developing IBD (Gradel et al., Gastroenterology, 2009).

What happens in cirrhosis to claudins and MCLK?

There is a long-standing hypothesis that intestinal permeability increases allow LPS to enter the portal circulation that promotes cirrhosis. There are data both supporting and contradicting this hypothesis. I'm not aware of work in the other direction, i.e., cirrhosis affecting intestinal tight junctions. There are, however, some very interesting studies of canalicular tight junctions within the liver in the context of cirrhosis. Some of these suggest that cholangiocytes regulate tight junctions by mechanisms similar to those I have described in this presentation. It is also interesting to note that claudin-1 and occludin play critical roles in hepatitis C infection. Whether this relates to barrier function or not is unknown.

I am working on the role of proteolytic processing occluding junctions in regulating mucosal barrier destruction. In Trichinella spiralis challenge mouse model, what proteases are involved in occluding junction destruction?

I don't think specific proteases have been identified. But it is clear that proteases can activate signaling pathways, e.g., the proteinase activated receptor PAR-2, and degrade cell surface proteins, including tight junction proteins. There's some very interesting work in the context of IBS from Dr. Madhu Grover at the Mayo Clinic that begins to get at this issue from a clinical/translational perspective. I anticipate that he will teach us a great deal about this in the next few years.

Thank you. Is there any information on the role of tissue fibrosis in the deterioration of barrier function?

Great question. I don't know of any data directly linking fibrosis to barrier function but I think it's safe to assume that when there is fibrosis secondary to disease, barrier function must be altered.

Has pregnancy been studied in relation to the IBD? Can pregnancy be somehow related to its onset?

IBD during pregnancy is definitely an issue and has been studied. There is little information on IBD developing in pregnancy. Koslowsky et al. (IBD J, 2018) wrote one nice study that would give you a broader perspective.

Is there any difference in response to inflammatory stimuli among men and women?

Yes, there can be. The details are complicated and not entirely understood. In the context of these studies, it is notable that claudin-2 is X-linked.

Related to the point about differences between claudin-2 and claudin-15, can Claudin-15 also allow water to pass (as Claudin-2 can)?

Yes, see Rosenthal et al. (Acta Physiol, 2020).

Is it only the tight junction that loses its integrity? Can the membrane lipid bilayer also be perturbed by inflammation?

The inflammatory process can definitely damage cells and cell membranes. In terms of permeability, this is the unrestricted pathway. Your question brings up a separate interesting issue regarding the structure of the lipid bilayers at the tight junction barrier. This remains a mystery despite having been described in 1963 (Farquhar, J Cell Biol).

Did you see any sex differences in your mechanistic studies in mice?

We don't see any mechanistic differences. The rate of disease progression can vary and, for that reason, individual experiments are done using only one sex. However, all terms are repeated at least once in each sex. We haven't seen major phenotypic differences between sexes. However, sex has been described as a factor in disease development in some IBD models.

How does permeability affect other tissue like muscle?

I'm not entirely sure what you're asking. Vascular endothelial mobility can be an issue in muscle, but there are no epithelial cells or epithelial tight junctions. If you are asking about the effect of intestinal epithelial tight junction permeability in other tissues, there certainly are systemic effects of intestinal barrier regulation. In the most general terms, increased intestinal permeability promotes immune disease and has been hypothesized to be a factor in other disorders ranging from autism to rheumatoid arthritis.

Do you know of any evidence that gut microbiota associated with malnutrition are also associated with these kinds of changes in mucosal barrier integrity?

There are some data. For example, there is increased permeability, or loss of barrier integrity, in environmental enteric dysfunction, which has also been associated with an altered microbiome.

References

1. Cenac N, Chin AC, Garcia-Villar R, et al. PAR2 activation alters colonic paracellular permeability in mice via IFN-gamma-dependent and -independent pathways. J Physiol 2004; 558:913-25.
2. Roka R, Demaude J, Cenac N, et al. Colonic luminal proteases activate colonocyte proteinase-activated receptor-2 and regulate paracellular permeability in mice. Neurogastroenterol Motil 2007; 19:57-65.
3. Caminero A, McCarville JL, Galipeau HJ, et al. Duodenal bacterial proteolytic activity determines sensitivity to dietary antigen through protease-activated receptor-2. Nat Commun 2019; 10:1198.

4. Chin AC, Lee WY, Nusrat A, et al. Neutrophil-mediated activation of epithelial protease-activated receptors-1 and -2 regulates barrier function and transepithelial migration. J Immunol 2008; 181:5702-10.
5. Edogawa S, Edwinson AL, Peters SA, et al. Serine proteases as luminal mediators of intestinal barrier dysfunction and symptom severity in IBS. Gut 2020; 69:62-73.
6. Martin-Padura I, Lostaglio S, Schneemann M, et al. Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. J Cell Biol 1998; 142:117-27.
7. Ostermann G, Weber KS, Zernecke A, et al. JAM-1 is a ligand of the beta(2) integrin LFA-1 involved in transendothelial migration of leukocytes. Nat Immunol 2002; 3:151-8.
8. Edelblum KL, Shen L, Weber CR, et al. Dynamic migration of gammadelta intraepithelial lymphocytes requires occludin. Proc Natl Acad Sci U S A 2012; 109:7097-102.
9. Kleinewietfeld M, Manzel A, Titze J, et al. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. Nature 2013; 496:518-22.
10. Wu C, Yosef N, Thalhamer T, et al. Induction of pathogenic TH17 cells by inducible salt-sensing kinase SGK1. Nature 2013; 496:513-7.
11. Heller F, Florian P, Bojarski C, et al. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. Gastroenterology 2005; 129:550-64.
12. Tsai PY, Zhang B, He WQ, et al. IL-22 Upregulates Epithelial Claudin-2 to Drive Diarrhea and Enteric Pathogen Clearance. Cell Host Microbe 2017; 21:671-681 e4.
13. Van Itallie CM, Fanning AS, Bridges A, et al. ZO-1 stabilizes the tight junction solute barrier through coupling to the perijunctional cytoskeleton. Mol Biol Cell 2009; 20:3930-40.
14. Laukoetter MG, Nava P, Lee WY, et al. JAM-A regulates permeability and inflammation in the intestine in vivo. J Exp Med 2007; 204:3067-76.
15. Su L, Nalle SC, Shen L, et al. TNFR2 activates MLCK-dependent tight junction dysregulation to cause apoptosis-mediated barrier loss and experimental colitis. Gastroenterology 2013; 145:407-15.
16. Graham WV, He W, Marchiando AM, et al. Intracellular MLCK1 diversion reverses barrier loss to restore mucosal homeostasis. Nat Med 2019; 25:690-700.
17. Spalinger MR, Sayoc-Becerra A, Santos AN, et al. PTPN2 Regulates Interactions Between Macrophages and Intestinal Epithelial Cells to Promote Intestinal Barrier Function. Gastroenterology 2020; 159:1763-1777 e14.
18. Spalinger MR, Schmidt TS, Schwarzfischer M, et al. Protein tyrosine phosphatase non-receptor type 22 modulates colitis in a microbiota-dependent manner. J Clin Invest 2019; 129:2527-2541.
19. Marchelletta RR, McCole DF. T-Cell Protein Tyrosine Phosphatase Protects Intestinal Barrier Function by Restricting Epithelial Tight Junction Remodeling. J Clin Invest 2021; In press.
20. Marchesi F, Martin AP, Thirunarayanan N, et al. CXCL13 expression in the gut promotes accumulation of IL-22-producing lymphoid tissue-inducer cells, and formation of isolated lymphoid follicles. Mucosal Immunol 2009; 2:486-94.
21. Kamm KE, Stull JT. Dedicated myosin light chain kinases with diverse cellular functions. J Biol Chem 2001; 276:4527-30.

22. Graham WV, Wang F, Clayburgh DR, et al. Tumor necrosis factor-induced long myosin light chain kinase transcription is regulated by differentiation-dependent signaling events. Characterization of the human long myosin light chain kinase promoter. J Biol Chem 2006; 281:26205-15.
23. He WQ, Peng YJ, Zhang WC, et al. Myosin light chain kinase is central to smooth muscle contraction and required for gastrointestinal motility in mice. Gastroenterology 2008; 135:610-20.
24. Russo JM, Florian P, Shen L, et al. Distinct temporal-spatial roles for rho kinase and myosin light chain kinase in epithelial purse-string wound closure. Gastroenterology 2005; 128:987-1001.
25. Nalle SC, Turner JR. Menetrier's disease therapy: rebooting mucosal signaling. Sci Transl Med 2009; 1:8ps10.
26. Nalle SC, Zuo L, Ong M, et al. Graft-versus-host disease propagation depends on increased intestinal epithelial tight junction permeability. J Clin Invest 2019; 129:902-914.

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