of valopicitabine. Antimicrob Agents Chemother 2006;50: 3444–3446.
19. Sarrazin C, Kieffer TL, Bartels D, Hanzelka B, Muh U, Welker M, Wincheringer D, Zhou Y, Chu HM, Lin C, Weegink C, Reesink H, Zeuzem S, Kwong AD. Dynamic HCV genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir (VX-950). Gastroenterology 2007;132:(In Press)
20. Pawlotsky JM. Hepatitis C virus population dynamics during infection. Curr Top Microbiol Immunol 2006;299:261–284.
21. Richman DD. The impact of drug resistance on the effectiveness of chemotherapy for chronic hepatitis B. Hepatology 2000;32: 866–867.
22. Darling JM, Fried MW. Optimizing treatment regimens in hepatitis C. Clin Liver Dis 2006;10:835–850.
23. Del Rio RA, Post AB, Singer ME. Cost-effectiveness of hematologic growth factors for anemia occurring during hepatitis C combination therapy. Hepatology 2006;44:1598–1606.
24. Thevenot T, Cadranel JF, Di Martino V, Pariente A, Causse X, Renou C, Hagege H, Denis J, Lunel-Fabiani F. A national French survey on the use of growth factors as adjuvant treatment of chronic hepatitis C. Hepatology 2007;45:377–3383.
25. Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, Vinelli F, Scotto G, Bacca D, Annese M, Romano M, Zechini F, Sogari F, Spirito F, Andriulli A. Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. N Engl J Med 2005;352:2609–2617.
26. Zeuzem S, Buti M, Ferenci P, Sperl J, Horsmans Y, Cianciara J, Ibranyi E, Weiland O, Noviello S, Brass C, Albrecht J. Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. J Hepatol 2006;44:97–103.
27. Jensen DM, Morgan TR, Marcellin P, Pockros PJ, Reddy KR, Hadziyannis SJ, Ferenci P, Ackrill AM, Willems B. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. Hepatology 2006;43: 954–960.
28. Berg T, von Wagner M, Nasser S, Sarrazin C, Heintges T, Gerlach T, Buggisch P, Goeser T, Rasenack J, Pape GR, Schmidt WE, Kallinowski B, Klinker H, Spengler U, Martus P, Alshuth U, Zeuzem S. Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. Gastroenterology 2006;130:1086–1097.
29. Rodriguez-Torres M, Lawitz E, Muir AJ, Keane J, Kieffer TL, McNair L, McHutchison J. Current status of subjects receiving peginterferon alfa-2a (PEG-IFN) and ribavirin (RBV) follow-on therapy after 28-day treatment with the hepatitis C protease inhibitor telaprevir (VX-950), PEG-IFN and RBV. Hepatology 2006;44 (suppl 2):532A.

Address requests for reprints to: Prof. Jean-Michel Pawlotsky, MD, PhD, Department of Virology, Hôpital Henri Mondor, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France. e-mail: jean-michel.pawlotsky@hmn.aphp.fr.

0016-5085/07/$32.00
doi:10.1053/j.gastro.2007.03.014

Got Guts? Need Nerve!

See "Enteric glia regulate intestinal barrier function and inflammation via release of s-nitrosoglutathione," by Savidge T, Newman P, Pothoulakis C, Ruhl A, Neunlist M, Bourreille A, Hurst R, Sofroniew MV, on page 1344.

Studies of inflammatory bowel disease (IBD) pathogenesis have primarily focused on the mucosal immune system and, more recently, the epithelium. Studies of experimental IBD also suggest a potential role for enteric glia in disease pathogenesis.[1,2] Together with neurons, glia are intrinsic components of the enteric nervous system. Within the mucosa, the enteric glia, which encase nerve fibers, form a dense mesh surrounding the crypt bases and experimental ablation of these enteric glia results in severe hemorrhagic enteritis.[1,2] New data reported in this issue of GASTROENTEROLOGY suggest that disruption of intestinal epithelial barrier function may be one mechanism by which glial ablation causes enteritis (Figure 1).[3]

Disruption of the intestinal epithelial barrier, which can be measured as increased permeability, is common in gastrointestinal disease and was reported in Crohn's disease (CD) >25 years ago.[4] These increases in permeability might simply be due to mucosal ulceration, but are also present in patients with inactive CD, in which ulceration is not present. In addition, the presence of increased permeability during clinical remission is associated with greater rates of relapse to active CD.[5,6] Correlative data also suggest that barrier defects may be related to initial CD pathogenesis; a subset of healthy first-degree relatives of CD patients are known to have increased permeability.[7] Although anecdotal, the report of CD in a previously healthy first-degree relative with increased permeability further supports the idea that barrier loss may be a contributing factor in IBD development.[8] There are also emerging data that immune-mediated barrier defects are prevalent in diarrhea-predominant irritable bowel syndrome (IBS).[9,10] Together with increased appreciation of permeability defects in animal models over the past decade,[11,12] these observations have fueled interest in the role of intestinal permeability in disease. Many believe that improved understanding of the mechanisms of barrier loss has great potential to define pathogenesis of IBD, and IBS, and may drive development of novel treatments.

Inflammatory activity and cytokine stimulation may be responsible for some cases in which increased intestinal

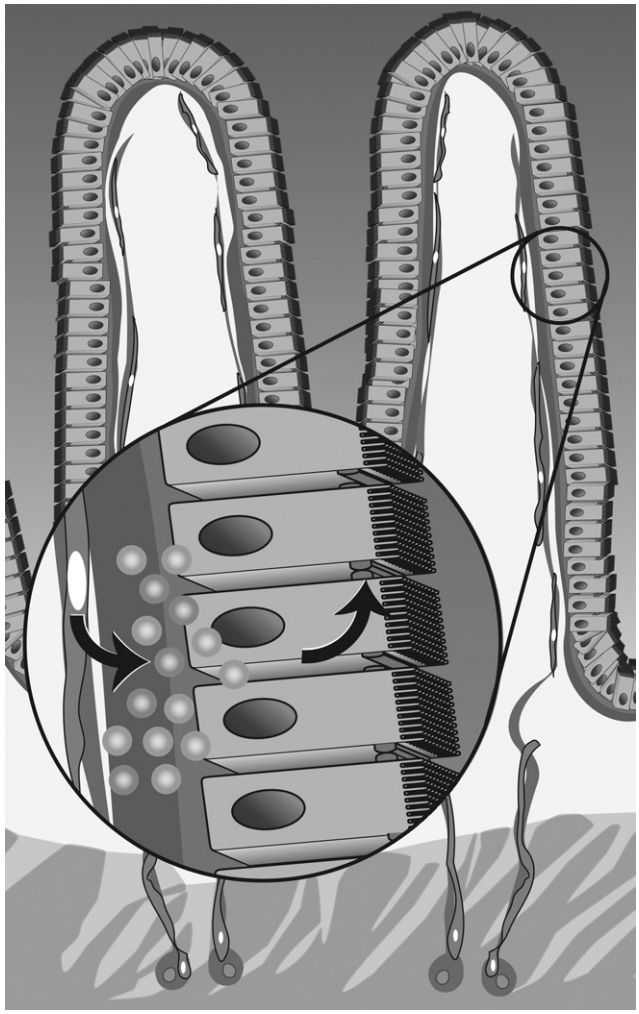

Figure 1. Intestinal epithelial barrier regulation by glial products. Enteric glia encircle enteric neurons as neuronal processes extend from Auerbach's and Meissner's plexi to the mucosa. As shown in the inset, these glia release many products, including glial-derived neurotrophic factor and GSNO. Savidge et al. show that GSNO can prevent epithelial barrier loss, perhaps by signaling to the epithelial tight junction.

permeability is present despite the absence of mucosal erosion. This is likely because the intestinal epithelial tight junction, which defines permeability in intact mucosa, can be regulated by inflammatory cytokines.[13–18] In cell culture and animal models, acute tumor necrosis factor (TNF)-α treatment causes increased permeability of intestinal epithelial tight junctions within hours.[19] Prevention of these barrier defects by blocking elements of the signal transduction pathway prevents TNF-induced diarrhea in vivo.[19,20] In CD patients, anti-TNF therapy is able to restore intestinal barrier function,[21] and genetic analyses of CD families suggests that increased intestinal permeability may be linked to a specific mutation of the *NOD2/CARD15* CD susceptibility gene.[22] Thus, many stimuli can trigger intestinal barrier dysfunction.

Despite growing interest in, and understanding of, mechanisms of barrier loss by TNF, it is likely that other mechanisms also contribute to barrier dysfunction. For example, TNF neutralization is unable to completely restore normal barrier function responses in CD patients.[23] Thus, the possibility remains that a primary epithelial defect contributes to CD pathogenesis. Alternatively, a primary defect separate from both immune and epithelial cells may also participate in this complex regulatory process.

In this issue of GASTROENTEROLOGY, Savidge et al.[3] use a genetic model of glial ablation-induced enteritis to better characterize the mechanisms of this disease and, remarkably, find a potential role for barrier defects. This model uses transgenic mice in which the glial fibrillary acidic protein (GFAP) promoter directs expression of herpes simplex virus thymidine kinase.[1] Administration of ganciclovir for 2 weeks causes marked ablation of glia within the jejunal and ileal enteric nervous system. Similar to immune-mediated glial ablation,[2] ganciclovir-induced glial loss triggers a TH1-polarized immune response.[3] Although GFAP expression, a measure of glial abundance, was reduced by about 50% after 7 days of ganciclovir treatment, neither obvious intestinal pathology nor marked elevation in mucosal cytokine expression were noted until 14 days of ganciclovir treatment, when GFAP expression was reduced by approximately 90%.[3] Despite this lag in development of gross pathology, intestinal permeability to small probes was increased within 7 days of ganciclovir treatment, a phenomenon also reported after immune-mediated glial destruction.[24] This suggests that enteric glia may provide a critical homeostatic influence that helps to maintain intestinal epithelial barrier function. This idea is not without precedent, as mucosal glial-derived neurotrophic factor has been reported to inhibit epithelial apoptosis and be upregulated in CD.[25] Moreover, in the central nervous system, glia are thought to play a critical role in maintaining the endothelial blood–brain barrier.[26,27]

To better characterize the potential role of enteric glia in epithelial barrier function, Savidge et al.[3] created a reductionist system by culturing intestinal epithelial (Caco-2) cells and isolated enteric and central glia. Their data show that glia release a soluble substance that interacts with the basolateral surface of Caco-2 cells to enhance barrier function. This was associated with increased expression of the tight junction proteins ZO-1 and occludin. Biochemical analysis identified the presence of reduced and nitrosylated forms of glutathione in the fraction of glial secretions including the barrier-inducing activity, and direct addition of these compounds to Caco-2 cultures showed that *s*-nitrosoglutathione (GSNO) had similar activity. Moreover, intraperitoneal GSNO administration prevented barrier defects, cytokine induction, and gross enteritis during ganciclovir-induced glial ablation.[3] These data therefore suggest that GSNO may play a pivotal homeostatic role in maintaining the mucosal barrier and/or suppressing inflammation. Consistent with this, GSNO also reduced paracellular perme-

ability increases seen in colon biopsies from CD patients, but had no effect on permeability of biopsies from control subjects.[3]

How then does this relate to human IBD? Is there evidence supporting changes in glial function in CD? This remains a controversial topic, with available data suggesting that absolute numbers of glia may be increased in IBD but that synthesis of glia-derived molecules may be either increased or decreased.[2,28,29] In some cases, this may reflect the ability of glia to respond to cytokines,[29,30] thereby providing a link between mucosal immune status and the enteric nervous system. In addition, it seems that glia-derived GSNO is able to modulate the immune system, at least partly by inhibiting nuclear factor κB.[31,32] Thus, as noted in other available models of IBD with increased intestinal permeability,[11,12] it remains unclear if glial ablation triggers a mucosal inflammatory response in which an early event includes barrier disruption or if the barrier disruption triggers subsequent inflammation. In either case, emerging data suggest that barrier disruption and mucosal cytokine release can establish a self-amplifying feed-forward loop that results in disease.[33]

Further study of this glial ablation model may provide important additional information. For example, most IBD models require the presence of luminal bacteria.[34] It would be of interest to know if this is also true in the case of glial ablation. Moreover, because compromised mucosal barrier function is now well-documented in chronic disease models with TH1 immune polarization,[11,12] it would be of interest to determine the mechanisms of barrier loss after glial ablation. For example, does loss of glial products, such glial-derived neurotrophic factor, cause barrier loss via increased epithelial apoptosis?[25,35] Alternatively, does the effect of glial ablation represent in vivo tight junction regulation, and, if this is the case, what signaling events are important for this to occur?[14–16,18] Finally, it will be critical to extend these observations beyond experimental models of disease to rigorously assess enteric glia and GSNO production in IBD and IBS and, ultimately, to determine if GSNO can prevent or alleviate disease in patients. All of these questions will require further study, but the work by Savidge et al. represents an important step forward and underscores the fact that, if you've got guts, you need nerve (or at least glia).

LIPING SU
JERROLD R. TURNER
Department of Pathology
The University of Chicago
Chicago, Illinois

References

1. Bush TG, Savidge TC, Freeman TC, Cox HJ, Campbell EA, Mucke L, Johnson MH, Sofroniew MV. Fulminant jejuno-ileitis following ablation of enteric glia in adult transgenic mice. Cell 1998;93:189–201.
2. Cornet A, Savidge TC, Cabarrocas J, Deng WL, Colombel JF, Lassmann H, Desreumaux P, Liblau RS. Enterocolitis induced by autoimmune targeting of enteric glial cells: a possible mechanism in Crohn's disease? Proc Natl Acad Sci U S A 2001;98:13306–13311.
3. Savidge T, Newman P, Pothoulakis C, Ruhl A, Neunlist M, Bourreille A, Hurst R, Sofroniew MV. Enteric glia regulate intestinal barrier function and inflammation via release of s-nitrosoglutathione. Gastroenterology 2007;132:1344–1358.
4. Sundqvist T, Magnusson KE, Sjodahl R, Stjernstrom I, Tagesson C. Passage of molecules through the wall of the gastrointestinal tract. II. Application of low-molecular weight polyethyleneglycol and a deterministic mathematical model for determining intestinal permeability in man. Gut 1980;21:208–214.
5. Wyatt J, Vogelsang H, Hubl W, Waldhoer T, Lochs H. Intestinal permeability and the prediction of relapse in Crohn's disease. Lancet 1993;341:1437–1439.
6. D'Inca R, Di Leo V, Corrao G, Martines D, D'Odorico A, Mestriner C, Venturi C, Longo G, Sturniolo GC. Intestinal permeability test as a predictor of clinical course in Crohn's disease. Am J Gastroenterol 1999;94:2956–2960.
7. Hollander D, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. Ann Intern Med 1986;105:883–885.
8. Irvine EJ, Marshall JK. Increased intestinal permeability precedes the onset of Crohn's disease in a subject with familial risk. Gastroenterology 2000;119:1740–1744.
9. Tibble JA, Sigthorsson G, Foster R, Forgacs I, Bjarnason I. Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. Gastroenterology 2002;123:450–460.
10. Dunlop SP, Hebden J, Campbell E, Naesdal J, Olbe L, Perkins AC, Spiller RC. Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. Am J Gastroenterol 2006;101:1288–1294.
11. Olson TS, Reuter BK, Scott KG, Morris MA, Wang XM, Hancock LN, Burcin TL, Cohn SM, Ernst PB, Cominelli F, Meddings JB, Ley K, Pizarro TT. The primary defect in experimental ileitis originates from a nonhematopoietic source. J Exp Med 2006;203:541–552.
12. Madsen KL, Malfair D, Gray D, Doyle JS, Jewell LD, Fedorak RN. Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. Inflamm Bowel Dis 1999;5:262–270.
13. Weber CR, Turner JR. Inflammatory bowel disease: is it really just another break in the wall? Gut 2007;56:6–8.
14. Prasad S, Mingrino R, Kaukinen K, Hayes KL, Powell RM, MacDonald TT, Collins JE. Inflammatory processes have differential effects on Claudins 2, 3 and 4 in colonic epithelial cells. Lab Invest 2005;85:1139–1162.
15. Zeissig S, Burgel N, Gunzel D, Richter J, Mankertz J, Wahnschaffe U, Kroesen AJ, Zeitz M, Fromm M, Schulzke JD. Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. Gut 2007;56:61–72.
16. Heller F, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, Mankertz J, Gitter AH, Burgel N, Fromm M, Zeitz M, Fuss I, Strober W, Schulzke JD. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. Gastroenterology 2005;129:550–564.
17. Blair SA, Kane SV, Clayburgh DR, Turner JR. Epithelial myosin light chain kinase expression and activity are upregulated in inflammatory bowel disease. Lab Invest 2006;86:191–201.
18. Wang F, Schwarz BT, Graham WV, Wang Y, Su L, Clayburgh DR, Abraham C, Turner JR. IFN-gamma-induced TNFR2 upregulation is required for TNF-dependent intestinal epithelial barrier dysfunction. Gastroenterology 2006;131:1153–1163.

19. Clayburgh DR, Musch MW, Leitges M, Fu YX, Turner JR. Coordinated epithelial NHE3 inhibition and barrier dysfunction are required for TNF-mediated diarrhea in vivo. J Clin Invest 2006;116:2682–2694.
20. Clayburgh DR, Barrett TA, Tang Y, Meddings JB, Van Eldik LJ, Watterson DM, Clarke LL, Mrsny RJ, Turner JR. Epithelial myosin light chain kinase-dependent barrier dysfunction mediates T cell activation-induced diarrhea in vivo. J Clin Invest 2005;115:2702–2715.
21. Suenaert P, Bulteel V, Lemmens L, Noman M, Geypens B, Van Assche G, Geboes K, Ceuppens JL, Rutgeerts P. Anti-tumor necrosis factor treatment restores the gut barrier in Crohn's disease. Am J Gastroenterol 2002;97:2000–2004.
22. Buhner S, Buning C, Genschel J, Kling K, Herrmann D, Dignass A, Kuechler I, Krueger S, Schmidt HH, Lochs H. Genetic basis for increased intestinal permeability in families with Crohn's disease: role of CARD15 3020insC mutation? Gut 2006;55:342–347.
23. Suenaert P, Bulteel V, Vermeire S, Noman M, Van Assche G, Rutgeerts P. Hyperresponsiveness of the mucosal barrier in Crohn's disease is not tumor necrosis factor-dependent. Inflamm Bowel Dis 2005;11:667–673.
24. Aube AC, Cabarrocas J, Bauer J, Philippe D, Aubert P, Doulay F, Liblau R, Galmiche JP, Neunlist M. Changes in enteric neurone phenotype and intestinal functions in a transgenic mouse model of enteric glia disruption. Gut 2006;55:630–637.
25. Steinkamp M, Geerling I, Seufferlein T, von Boyen G, Egger B, Grossmann J, Ludwig L, Adler G, Reinshagen M. Glial-derived neurotrophic factor regulates apoptosis in colonic epithelial cells. Gastroenterology 2003;124:1748–1757.
26. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 2006;7:41–53.
27. Janzer RC, Raff MC. Astrocytes induce blood-brain barrier properties in endothelial cells. Nature 1987;325:253–257.
28. di Mola FF, Friess H, Zhu ZW, Koliopanos A, Bley T, Di Sebastiano P, Innocenti P, Zimmermann A, Buchler MW. Nerve growth factor and Trk high affinity receptor (TrkA) gene expression in inflammatory bowel disease. Gut 2000;46:670–679.
29. von Boyen GB, Steinkamp M, Reinshagen M, Schafer KH, Adler G, Kirsch J. Proinflammatory cytokines increase glial fibrillary acidic protein expression in enteric glia. Gut 2004;53:222–228.
30. von Boyen GB, Steinkamp M, Geerling I, Reinshagen M, Schafer KH, Adler G, Kirsch J. Proinflammatory cytokines induce neurotrophic factor expression in enteric glia: a key to the regulation of epithelial apoptosis in Crohn's disease. Inflamm Bowel Dis 2006;12:346–354.
31. Khan M, Sekhon B, Giri S, Jatana M, Gilg AG, Ayasolla K, Elango C, Singh AK, Singh I. S-Nitrosoglutathione reduces inflammation and protects brain against focal cerebral ischemia in a rat model of experimental stroke. J Cereb Blood Flow Metab 2005;25:177–192.
32. Prasad R, Giri S, Nath N, Singh I, Singh AK. GSNO attenuates EAE disease by S-nitrosylation-mediated modulation of endothelial-monocyte interactions. Glia 2007;55:65–77.
33. Clayburgh DR, Shen L, Turner JR. A porous defense: the leaky epithelial barrier in intestinal disease. Lab Invest 2004;84:282–291.
34. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 2006;3:390–407.
35. Gitter AH, Bendfeldt K, Schulzke JD, Fromm M. Leaks in the epithelial barrier caused by spontaneous and TNF-alpha-induced single-cell apoptosis. FASEB J 2000;14:1749–1753.

Address requests for reprints to: Jerrold R. Turner, MD, PhD, Department of Pathology, The University of Chicago, 5841 South Maryland Avenue, MC 1089, Chicago, Illinois 60637. E-mail: jturner@bsd.uchicago.edu.

0016-5085/07/$32.00
doi:10.1053/j.gastro.2007.03.015

It's Chickens and Eggs All Over Again: Is Central Reorganization the Result or Cause of Persistent Visceral Pain?

See "Pain in chronic pancreatitis: The role of reorganization in the central nervous system," by Dimcevski G, Saber AK, Funch–Jensen P, Le Pera D, Valeriani M, Arendt-Nielsen L, and Drewes on page 1546.

Since peripheral and central contributions to visceral hypersensitivity were last reviewed formally in this journal,[1] there has been growing appreciation that visceral disorders characterized by persistent discomfort and pain reveal, at least in part, a dysregulated central nervous system. Brain imaging and related experimental strategies have contributed to this concept, although such studies have not yet revealed aspects of visceral pain processing in brain distinct from nonvisceral pain processing.[2] Generally, it has been found that brain areas activated by experimental visceral stimuli, either in naïve subjects or patients with visceral disorders, are similar to those activated by nonvisceral stimulation. In addition to activation of the insula, anterior cingulate gyrus, and somatosensory areas, brainstem areas have been shown to be deactivated in some studies in patients, a result that has been interpreted to indicate reduced activity (and dysregulation) in endogenous pain modulatory systems. As advanced in the report by Dimcevski et al,[3] functional reorganization within the central nervous system can accompany chronic visceral disorders and contribute to pain.

They examined event-related brain potentials in healthy subjects and in a group of chronic pancreatitis patients (mean, 5.4 years duration), electrically stimulating, sequentially, above the gastroesophageal junction, the stomach and then the horizontal part of duodenum. At each site of stimulation, they measured event-related evoked potentials from surface electrodes on the scalp and assessed sensation. Finding that neuronal sources in