"Interstitial cells of Cajal integrate excitatory and inhibitory neurotransmission with intestinal slow-wave activity," in which we propose a new integrated model for inhibitory nitrergic neurotransmission in the gastrointestinal tract (Nat Commun 2013;4:1630). Some important statements and conclusions of this summary need to be clarified.

It is well established that interstitial cells of Cajal (ICC) are pacemaker cells that form a network that integrates and coordinates intestinal peristalsis throughout the whole gastrointestinal tract (Annu Rev Physiol 2006;68:307–343). ICC generate slow waves of depolarization and thus rhythmic contractions of the gut, which are essential for normal gut homeostasis. However, their role in enteric neurotransmission is highly controversial. This controversy is based on apparently contradictory findings in different experimental settings using diverse in vivo and ex vivo models. To gain insights into ICC-mediated enteric neurotransmission, we have generated a new mouse model for time-specific genetic manipulation of ICCs in adult animals (*c-Kit*^{CreERT2}). By using this new model, we clearly show that ICC mediate excitatory enteric neurotransmission as evidenced by a complete loss of excitatory junction potentials and contractions of intestinal smooth muscle cells (SMC) after acute ICC depletion (Nat Commun 2013;4:1630). Regarding nitric oxide (NO)-dependent inhibitory neurotransmission, we propose a new, unifying concept in which ICC play a prominent role. This new concept explains and integrates major discrepancies and controversies in the field; it shows, by inducible gene targeting, that nitrergic neurotransmission is a complex and fine-tuned system with sophisticated backup mechanisms. We have demonstrated that ICC depletion has no effect on inhibitory neurotransmission, whereas ICC-specific deletion of *Prkg1* (cGK1, PKG1), a downstream effector of the NO signaling cascade, results in disturbed gastrointestinal motility and a complete loss of NO-dependent neurotransmission to intestinal SMC (Nat Commun 2013;4:1630). This clearly indicates that ICC, which form close contacts with enteric nerve endings and express greater amounts of the NO-receptor NO-sensitive guanylyl cyclase (NO-GC) than SMC (Gastroenterology 2011;140:1608–1617), are the primary targets of NO in the gut, which act as scavengers of NO and mediate nitrergic neurotransmission via Prkg1 to SMC (Nat Commun 2013;4:1630). Upon ICC depletion, this NO-capture function is lost and NO diffuses to and acts on intestinal SMC (NO spillover) with subsequent smooth muscle relaxation. Similar spillover mechanisms have been shown for neurotransmitters in the central nervous system before, supporting our new concept (Math Biosci 2012;240:169–186). Therefore, intestinal SMC represent a second-level backup system that is able to respond to NO in the absence of ICC and thus can compensate for loss of ICC.

Recent findings cited in Drs Goyal and Sullivan's summary support our new model of intestinal neurotransmission. First, cell-specific deletion of the NO receptor NO-GC in intestinal SMC or ICC does not abolish nitrergic enteric neurotransmission, respectively (Gastroenterology 2011; 140:1608–1617; Gastroenterology 2013;145:188–196). Because the NO receptor is needed to scavenge NO, deletion of NO-GC in ICC phenocopies ICC depletion which enables NO spillover to SMC; subsequently, only the combined deletion of NO-GC in ICC and SMC blocks nitrergic signaling completely (Gastroenterology 2013;145:188–196). Second, in contrast to ICC, cell specific deletion of *Prkg1* in intestinal SMC does not affect nitrergic neurotransmission substantially (Nature Communications 2013;4:1630). Because ICC, which express all components of the NO signaling cascade, are the primary targets of NO that mediate SMC relaxation, disruption of the NO signaling pathway in SMC by *Prkg1* deletion has no major effect on enteric nitrergic neurotransmission. Third, expression of bovine Prkg1 from the smooth muscle-specific *Sm22α* locus in globally *Prkg1* deleted animals (*Prkg1* SMC rescue animals) restores SMC relaxation ex vivo in response to exogenous applied 8-Br-cGMP in organ bath experiments (Circ Res 2007;101:1096–1103). This is in agreement with our study showing that Prkg1-proficient SMC are able to respond to NO in ICC-depleted animals. However, enteric neurotransmission in a physiologically relevant setting must be investigated by direct nerve stimulation (eg, electrical field stimulation) to release endogenous NO from enteric nerve terminals, which was not investigated in the cited study (Circ Res 2007;101:1096–1103). Therefore, it is not possible to exclude a role of ICC in nitrergic enteric neurotransmission based on these findings. Fourth, in *W/W^v* mice, which lack subtypes of ICC, the lower esophageal and the pyloric sphincter are hypotensive with normal nitrergic relaxation in line with a selective lack of excitatory cholinergic input from enteric neurons (Gastroenterology 2001;121:34–42). However, we have not investigated *W/W^v* animals in our study as suggested in Drs Goyal and Sullivan's comment. Fifth, in contrast with *W/W^v* mice, both sphincters are hypertensive in global *nNOS* knockout mice owing to the selective lack of NO-dependent inhibitory innervation (Gastroenterology 2001;121:34–42).

Taken together, these data address the concerns raised by Drs Goyal and Sullivan and support our novel model of an integrated and multilayered system of nitrergic enteric neurotransmission in which ICC play a dominant role.

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ACTIVATED INTESTINAL MACROPHAGES IN PATIENTS WITH CIRRHOSIS RELEASE NO AND IL-6 THAT MAY DISRUPT INTESTINAL BARRIER FUNCTION

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NO and IL-6 that may disrupt intestinal barrier function. J Hepatol 2013;58:1125–1132.

Bacterial infections commonly complicate decompensated cirrhosis and are thought to be associated with increased bacterial translocation across the intestinal barrier (Gastroenterology 2011;141:1220–1230; Hepatology 2006;44:633–639). Alterations in circulating bacterial DNA, even in the presence of negative cultures, increases the risk of hepatic decompensation, variceal hemorrhage, and hepatorenal syndrome (Gastroenterology 2007;133: 818–824). In health, the gut epithelial barrier provides an effective barrier against microorganisms while simultaneously providing a semipermeable membrane for nutrient absorption (Nat Rev Immunol 2009;9:799–809). In cirrhosis, studies have confirmed that intestinal bacterial flora is altered (Hepatology 2006;45:744–757). However, it is unclear why the intestinal epithelial barrier fails, facilitating translocation of bacterial products and DNA (Hepatology 2008;48:1924–1931; Hepatology 2010;52:2044–2052).

The authors of this study hypothesize that, similar to other inflammatory states, intestinal macrophage activation occurs in cirrhosis. They therefore determined the intestinal macrophage phenotype in decompensated cirrhosis and whether these macrophages are capable of modulating intestinal permeability.

This study included South African patients with nonalcoholic steatohepatitis or alcohol-related cirrhosis aged 18–80 with either decompensated (n = 29) or compensated (n = 15) disease. Patients underwent peripheral blood sampling and gastroscopy for variceal screening, where duodenal biopsies were taken. Controls (n = 19) were patients undergoing gastroscopy for reflux or dyspepsia.

Plasma levels of circulating endotoxin (lipopolysaccharide [LPS]) and LPS-binding protein were used as a surrogate marker of bacterial translocation. Both LPS and LPS-binding protein levels were significantly increased in patients with cirrhosis.

Mucosal mononuclear cells were isolated and examined for activation status (expression of CD68, CD14, CD16, Trem-1), monocyte/macrophage lineage (CD33, CD14), costimulatory molecules (CD80, CD86), and surface expression of Toll-like receptors -2 and -4 using FACS analysis and immunohistochemistry. This demonstrated a significant increase in an activated macrophage phenotype (expressing $CD68^+$) with increased frequency of CD33/CD14 expression and coexpression of innate immune receptors for LPS (CD14), CD33, and Trem-1 in cirrhosis compared with controls. This demonstrates that intestinal macrophages in cirrhosis have an activated phenotype and express innate immune receptors for bacterial translocation. There was no difference in the prevalence of dendritic cells, natural killer cells, or activated B cells.

To analyze the transcriptional profile of the intestinal mucosa, analysis of inflammatory and housekeeping gene expression was performed. Quantitative reverse transcriptase polymerase chain reaction confirmed up-regulation of inducible nitrous oxide synthase (iNOS) and other genes associated with inflammatory cell and monocyte recruitment, including interleukin (IL)-8, chemokine ligand (CCL)2, and CCL13. Increased levels of IL-6, IL-8, and CCL2/monocyte chemotactic protein (MCP)-1 were found in the supernatant of biopsy cultures. IL-8 and CCL2/MCP-1 are secreted by activated $CD14^+$Trem-1^+ macrophages in response to microbial stimuli in inflammatory bowel disease, which may be important in the pathogenesis of Crohn's disease (J Clin Invest 2008;118:2269–2280; J Immunol 2010;184:4069–4073). Furthermore, IL-8 production is known to be increased by *Escherichia coli* in Crohn's disease (Gastroenterology 2004;127:80–93), suggesting that increased IL-8 observed at the transcriptional and protein levels in cirrhosis may reflect a response to bacterial induced stimulation.

The relevance of increased iNOS mRNA at the transcription level was further assessed by immunohistochemistry. This demonstrated increased iNOS synthesis, with increased iNOS-activated macrophages in cirrhotics.

Because intestinal macrophages that express innate response receptors such as $CD14^+$ and TREM-1 have been associated with inflammation and pro-inflammatory cytokine production, colocalization studies were performed. These demonstrated that $CD14^+$ cells were iNOS positive, thus confirming the presence of classically activated intestinal macrophages. Both increased numbers of IL-6–positive cells in decompensated cirrhosis and colocalization of IL-6 and IL-8 with $CD68^+$ and $iNOS^+$ macrophages were demonstrated by immunohistochemistry, indicating that macrophages are the major source of intestinal IL-6 released in cirrhosis. IL-6 was predominantly present in $CD11c^-$ cells, which are not dendritic cells, thus indicating that dendritic cells are not a major source of this pro-inflammatory cytokine. Interestingly, Trem-1^+ macrophages and intestinal epithelial cells have been shown as a major source of IL-6 in Crohn's disease. IL-8 colocalized in $iNOS^+$ cells, but only in a subpopulation of $CD68^+$ cells, suggesting that other inflammatory cells also produce IL-8.

Histopathologic and ultrastructural analysis of duodenal wall and epithelial barrier was performed by light microscopy and transmission electron microscopy. There was no difference in morphology of the interepithelial junctions between groups. However, functional analysis demonstrated a reduced transepithelial electrical resistance and higher transepithelial passage of FITC-dx4 in cirrhosis compared with controls, indicating impaired duodenal barrier function. Structural tight junction proteins (ZO-1,11 Occludin, and claudin-1) and gap junction protein (connexion-43) were not different at the mRNA and protein levels between groups, but increased levels of claudin-2, a known pore-forming tight junction protein, were observed via Western blot, and immunohistochemistry documented a vesicular staining pattern of the protein on the apical pole of epithelial cells in decompensated cirrhosis, suggesting that it is responsible for increased intestinal permeability. Indeed, epithelial barrier dysfunction and elevated claudin-2 expression associated with