Published in final edited form as:

Gastroenterology. 2013 December ; 145(6): 1193–1196.

The tuning of the gut nervous system by commensal microbiota

Nobuhiko Kamada and John Y. Kao

Division of Gastroenterology, Department of Internal Medicine, University of Michigan Medical School

The gastrointestinal (GI) tract, particularly the lower gut, is colonized by numerous symbiotic microorganisms that are collectively referred to as the gut microbiota. The resident gut microbiota contributes to various vital processes within the host, including the adaptation of nutrients, the development of lymphoid structures and immune system, and the prevention of pathogenic microorganism outgrowth^{1, 2}. Complex processes that are controlled by the healthy gut microbiota generally benefit the host. However, a perturbed gut microbial community (a.k.a., dysbiosis) may affect a number of physiological functions of the gut leading to the development of GI disorders. Conditions that are affected by dysbiosis include inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and intestinal infections¹⁻³. Another important contribution of the gut microbiota is the regulation of gut motility⁴. Germ-free animals exhibit delayed gastric emptying, reduced intestinal transit, and a decreased expression of neuromodulators compared to conventional animals⁴⁻⁶. Moreover, the reconstitution of germ-free animals with certain bacterial strains or conventional flora from mice or humans restores the perturbed gut physiological functions^{4, 7}. Intestinal motility also contributes to the maintenance of healthy microbiota by eliminating pathogenic microorganisms⁸. Thus, it is possible that dysbiosis may impact disease severity through its affect on intestinal motility. In fact, dysmotility has been reported in patients with IBD⁸⁻¹⁰.

Unlike other organs of the body, intestinal motility is regulated by an intrinsic nervous system, referred to as the enteric nervous system (ENS), in addition to regulation by the central nervous system (CNS). Although the ENS normally communicates with the CNS to regulate GI motility, the ENS is capable of operating autonomously even if signals from the CNS are absent. The ENS is composed of enteric neurons and enteric glial cells (EGC). Morphological and functional abnormalities of the ENS have been reported in many GI disorders, implicating possible causal role of gut motility defects in disease pathogenesis^{11, 12}. It is possible that the gut microbiota directly activates the ENS, although the precise mechanism has yet to be elucidated. However, the ENS is separated from the luminal content by the mucosal cell layers and embedded deeply within the wall of the GI tract making them inaccessible to the luminal commensal microbes. Therefore, communication between luminal microbes and ENS is likely to require factors such as

Address requests for reprints to: Nobuhiko Kamada, Division of Gastroenterology, Department of Internal Medicine, University of Michigan Medical School, 1150 W. Medical Center Drive, Ann Arbor, MI, 48109. nkamada@med.umich.edu; John Y. Kao, Division of Gastroenterology, Department of Internal Medicine, University of Michigan Medical School, 1150 W. Medical Center Drive, Ann Arbor, MI, 48109 jykao@med.umich.edu.

The authors disclose no conflicts

bacterial byproducts, such as cell wall components and/or their metabolites. Indeed, bacterial lipopolysaccharide or bacterial metabolites, such as short-chain fatty acids, has been shown to affect ENS functions^{4, 13}. But the exact mechanism by which gut microbiota communicates with the ENS is poorly understood.

In this issue of *Gastroenterology*, Brun et al. demonstrated that commensal microbiota fine-tunes gut homeostasis by regulating ENS integrity and function via TLR2 signaling¹⁴. The authors found a significant reduction in the expression and number of HuC/D⁺ and neuronal nitric oxide (nNOS)⁺ enteric neurons, S100 β ⁺ EGC, neurofilament protein peripherin, and glial fibrillary acidic protein (GFAP) in the myenteric plexus of TLR2^{-/-} mice compared to wild-type mice. In the submucosal plexus, β III-tubulin⁺ neurons and fibers were significantly reduced, while peripherin⁺ fibers and GFAP⁺ glial bundles are intact in TLR2^{-/-} mice. These findings suggested that TLR2-dependent signaling in the intestine regulates structural integrity in both the myenteric and submucosal plexuses. Consistent with these structural perturbations in the intestinal neuromuscular tissue, TLR2^{-/-} mice displayed functional anomalies in GI motility. TLR2^{-/-} mice exhibited a higher contraction frequency and amplitude in spontaneous rhythmic activity, and enhanced electric field stimulation-elicited contractions. Together with the enhanced contractility, TLR2^{-/-} mice displayed significant increases in gastric emptying and GI transit. Taken together, TLR2 signaling regulates the GI motility functions through influencing the neuronal network integrity.

How does TLR2 signaling regulate GI motility? At first, Brun et al. showed the expression of TLR2 in smooth muscle layers of the mouse ileum. They also detected the expression of TLR2 in neurons, glia, endothelial cells, and macrophages. It is noteworthy that functional integrity of the ENS was not altered in bone-marrow chimeric mice reconstituted with TLR2^{-/-} mice-derived hematopoietic cells. This suggests that TLR2 signaling in non-hematopoietic cells contributes to the ENS homeostasis, although hematopoietic-derived cells in the smooth muscle layer, such as macrophages, express TLR2 (Figure 1). It has been reported that TLRs are expressed in the ENS and play pivotal roles in the regulation of GI homeostasis, including gut motility^{13, 15}. However, the precise mechanisms by which TLR signaling affects ENS homeostasis are still largely unknown. In their study, Brun et al. demonstrated the link between TLR2 signaling and a glial cell line-derived neurotrophic factor (GDNF) that promotes the development and survival of many types of neurons. In TLR2^{-/-} mice, the expression of GDNF was significantly decreased in the intestinal muscle layer. In accordance with this result, signaling downstream of GDNF was impaired in the isolated longitudinal smooth muscle-myenteric plexus (LMMP). Ex vivo stimulation of LMMP demonstrated that TLR2 ligands, such as Pam₃CSK4 (TLR2/1 ligand) and FSL1 (TLR2/6 ligand) increased the mRNA expression of GDNF. Thus, TLR2 signaling likely stimulates non-hematopoietic cells in the intestinal smooth muscle layer directly and promotes the neurotrophic factor GDNF expression (Figure 1). As mentioned above, the ENS is separated from the luminal content and inaccessible by the luminal microbes. Although it is possible that the bacterial products “leak” through the epithelium and reach the smooth muscle layer, the precise delivery mechanisms of the bacterial ligands to the smooth muscle layer remain to be undetermined.

Brun et al. further demonstrated the axis of the gut microbiota-TLR2-GDNF in fine tuning the ENS¹⁴. The gut microbiota depleted mice displayed marked reduction of GDNF expression as well as maldevelopment of enteric neurons when treated with broad-spectrum antibiotics. Importantly, these anomalies in the ENS of the microbiota-depleted mice could be corrected by the administration of either a TLR2 ligand or the GDNF. These findings clearly indicated that gut microbiota regulates the ENS development/maintenance through activation of TLR2 signaling (Figure 1). Finally, the authors addressed the link between the ENS anomalies driven by impaired TLR2 signaling and GI diseases. Consistent with a previous report¹⁶, TLR2^{-/-} mice exhibited increased susceptibility to inflammation in two different experimental colitis models (DSS and DNBS colitis). Administration of GDNF reduced the severity of the experimental colitis models.

The study by Brun et al.¹⁴ highlighted the importance of gut microbiota in regulating ENS function leading to the prevention of experimental colitis. Although the authors clearly demonstrated the importance of TLR2 signaling in sensing gut microbiota, the contribution of the other TLRs¹⁵ in this process remains to be investigated. Indeed, it is been reported that TLR4 signaling regulates gut motility¹³, although TLR4 stimulation did not enhance the expression of GDNF in the smooth muscle. This implies that gut microbiota modulates ENS function through multiple TLRs and signals from each individual TLR might activate different pathways resulting in either excitatory or inhibitory signals on ENS. These considerations may be important when designing a therapeutic approach to manage gut dysmotility using bacterial agents, such as probiotics and prebiotics. Further study is required to better understand how TLR2-dependent GDNF regulates gut immune homeostasis and whether intestinal motility participates in this immune regulatory process. Other critical human studies are also needed to determine the relevance of this finding in patients with IBS or IBD. The current demonstration of a gut microbiota and ENS connection has important clinical implications to clinicians managing patients with intestinal dysmotility and provides the much needed rationale for using probiotics in their symptom management. Thus, this study offers important additional insight into the mutualism between our gut bacteria and us.

References

1. Kamada N, Seo SU, Chen GY, Nunez G. Role of the gut microbiota in immunity and inflammatory disease. *Nat Rev Immunol*. 2013; 13:321–35. [PubMed: 23618829]
2. Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol*. 2010; 10:159–69. [PubMed: 20182457]
3. DuPont AW, DuPont HL. The intestinal microbiota and chronic disorders of the gut. *Nat Rev Gastroenterol Hepatol*. 2011; 8:523–31. [PubMed: 21844910]
4. Barbara G, Stanghellini V, Brandi G, Cremon C, Di Nardo G, De Giorgio R, Corinaldesi R. Interactions between commensal bacteria and gut sensorimotor function in health and disease. *Am J Gastroenterol*. 2005; 100:2560–8. [PubMed: 16279914]
5. Abrams GD, Bishop JE. Effect of the normal microbial flora on gastrointestinal motility. *Proc Soc Exp Biol Med*. 1967; 126:301–4. [PubMed: 6066182]
6. Iwai H, Ishihara Y, Yamanaka J, Ito T. Effects of bacterial flora on cecal size and transit rate of intestinal contents in mice. *Jpn J Exp Med*. 1973; 43:297–305. [PubMed: 4580917]
7. Kashyap PC, Marcobal A, Ursell LK, Larauche M, Duboc H, Earle KA, Sonnenburg ED, Ferreyra JA, Higginbottom SK, Million M, Tache Y, Pasricha PJ, Knight R, Farrugia G, Sonnenburg JL.

- Complex interactions among diet, gastrointestinal transit, and gut microbiota in humanized mice. *Gastroenterology*. 2013; 144:967–77. [PubMed: 23380084]
8. Lomax AE, Fernandez E, Sharkey KA. Plasticity of the enteric nervous system during intestinal inflammation. *Neurogastroenterol Motil*. 2005; 17:4–15. [PubMed: 15670258]
 9. Neunlist M, Van Landeghem L, Bourreille A, Savidge T. Neuro-glial crosstalk in inflammatory bowel disease. *J Intern Med*. 2008; 263:577–83. [PubMed: 18479256]
 10. Manichanh C, Borruel N, Casellas F, Guarner F. The gut microbiota in IBD. *Nat Rev Gastroenterol Hepatol*. 2012; 9:599–608. [PubMed: 22907164]
 11. 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. [PubMed: 9568712]
 12. Gulbransen BD, Bashashati M, Hirota SA, Gui X, Roberts JA, MacDonald JA, Muruve DA, McKay DM, Beck PL, Mawe GM, Thompson RJ, Sharkey KA. Activation of neuronal P2X7 receptor-pannexin-1 mediates death of enteric neurons during colitis. *Nat Med*. 2012; 18:600–4. [PubMed: 22426419]
 13. Anitha M, Vijay-Kumar M, Sitaraman SV, Gewirtz AT, Srinivasan S. Gut microbial products regulate murine gastrointestinal motility via Toll-like receptor 4 signaling. *Gastroenterology*. 2012; 143:1006–16 e4. [PubMed: 22732731]
 14. Brun P. Toll-like receptor 2 tunes gut inflammation by ensuring enteric nervous system integrity. *Gastroenterology*. 2013
 15. Barajon I, Serrao G, Arnaboldi F, Opizzi E, Ripamonti G, Balsari A, Rumio C. Toll-like receptors 3, 4, and 7 are expressed in the enteric nervous system and dorsal root ganglia. *J Histochem Cytochem*. 2009; 57:1013–23. [PubMed: 19546475]
 16. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell*. 2004; 118:229–41. [PubMed: 15260992]

Figure 1. Tuning of the enteric nervous systems by the gut microbiota

In the gastrointestinal tract, resident microbes translocate to the lamina propria (LP) from the intestinal lumen through the microfold (M) cells and/or uptake by LP dendritic cells. Alternatively, secreted bacterial components, such as peptidoglycans (PGN) directly translocate to the LP. Bacteria or their secreted components access the intestinal smooth muscle layer through an unknown pathway and activate TLR2 signaling in non-hematopoietic cells, such as enteric neurons, glia, and smooth muscle cells, in both the myenteric and submucosal plexuses. TLR2 stimulation by the commensal bacterial ligands induces expression of a neurotrophic factor glial cell line-derived neurotrophic factor (GDNF). GDNF increases the number of enteric neurons and glial cells by promoting the development and survival of these cells, thereby enhancing structural and functional development of the enteric nervous system (ENS). The gut microbiota-mediated fine-tuning of the ENS functions decreases the susceptibility of the host to chronic inflammatory gastrointestinal disorder, such as inflammatory bowel disease.