and inflammatory bowel disease. It remains to be established how IgAs are selecting the microbiota. One speculation is that IgAs allow the microbiota to attach to the mucus layer, thus avoiding bacterial wash out in the intestinal bolus and allowing access to the nutrients released by the epithelium.

Hence, IgAs play a major role in shaping rather than in eliminating the microbiota.

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Macrophages Have a Grip on the Gut

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We host a world inside, and every day, new evidence reveals how relevant our microbiota is for daily living. In the most recent issue of *Cell*, Muller and colleagues demonstrate that microbiota commensals also influence colon peristalsis via a direct effect of muscolaris externae macrophages (Muller et al., 2014).

Influence of inflammatory reactions on gut peristalsis is common knowledge. A dramatic example of this is represented by ileus, a well-known dangerous complication of abdominal surgery. Muscularis externa-residing macrophages have been recognized as key players in the second long-lasting phase of this phenomenon, and pharmacological or genetic depletion of resident macrophages results in a decrease of inflammatory mediators and normalized muscle function and gastrointestinal transit in the wake of surgical manipulation (Kalff et al., 1999; Wehner et al., 2007). Under these inflammatory conditions, several mechanisms have been implicated in macrophage activation, including sensing of danger-associated or pathogen-associated molecular patterns, inflammatory cytokines, and direct translocation of bacteria or their products (Boeckxstaens and de Jonge, 2009). Interestingly, ileus hypomotility is a generalized functional reaction involving areas of the intestine not directly affected by the inflammatory reaction, and evidence indicates that this generalized paralysis is sustained by neural pathways activated by immune cells (de Jonge et al., 2003).

It is also common knowledge that intestine peristalsis is influenced by physiological parameters, including diet and luminal microbiota, and evidence in germ-free and gene-targeted animals indicates that gut-flora sensing by the immune system has profound effects on gastrointestinal motility (Anitha et al., 2012). However, aside from controlling gastrointestinal motility under pathologic conditions, the cellular and molecular mechanisms involved in physiological effects of microbiota on gastrointestinal transit are far less understood.

Muller and colleagues now provide a first key element in this setting by showing that gastrointestinal peristaltic activity is regulated by muscularis externa macrophages, which represent a distinct macrophage population organized in layers between the serosa and the longitudinal muscle, the longitudinal and circular muscles, and the outer and the inner circular muscles, and extending from the stomach to distal colon both in humans and mice (Mikkelsen, 2010) (Figure 1). These macrophages are known to contribute to ileus by releasing inflammatory mediators, but their role in physiological conditions is unknown. Muller et al. (2014) have shown that under homeostatic conditions, this macrophage population stimulated enteric neuronal activity via release of bone morphogenetic protein 2 (BMP2). They have also provided evidence that BMP2 expression is reduced following antibiotic treatment, thus suggesting that muscularis externa macrophages tune intestinal motility as a function of luminal microbiota content. Though the authors have not provided information on the molecular mechanisms allowing microbiota sensing by the muscularis externa macrophages,

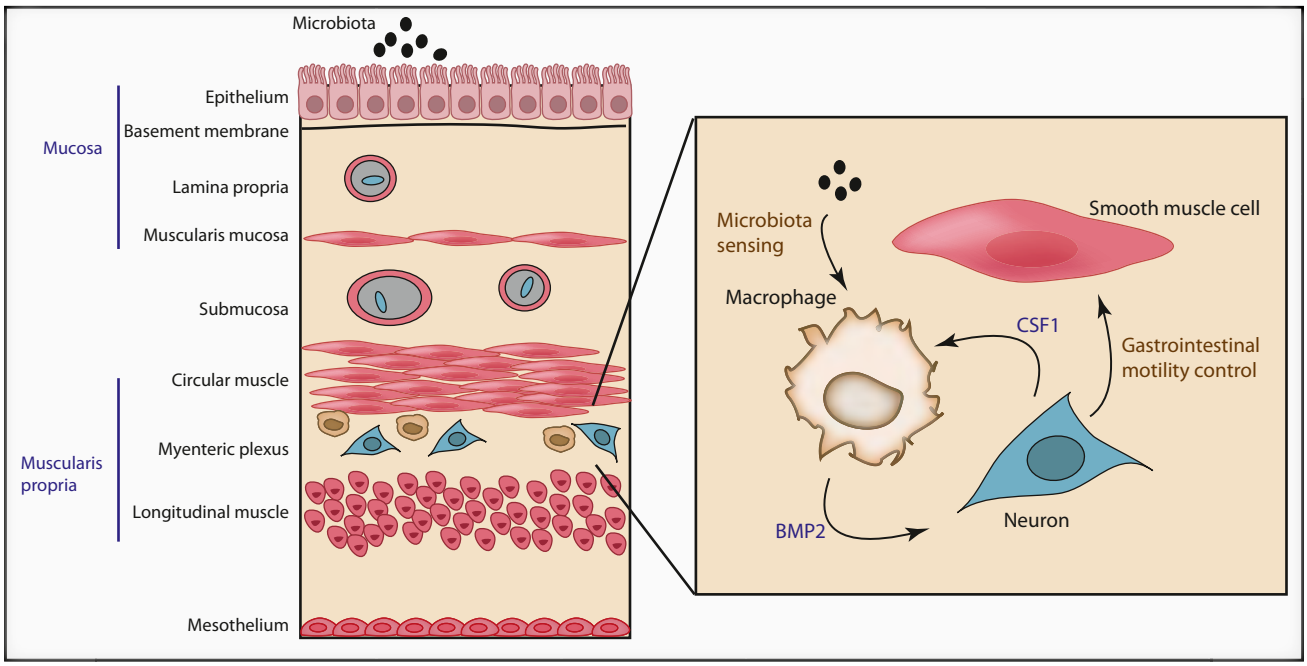

Figure 1. Crosstalk of Muscularis Mucosae Macrophages and Enteric Neurons
In the gut, muscularis propria macrophages are organized in layers between the longitudinal and circular muscles and the outer and the inner circular muscles. In these areas, macrophages establish a bidirectional interaction with enteric neurons, being sustained in their development by neuronal-derived CSF1 and by converse stimulating neuronal activity via BMP2 secretion. Enteric neurons then control gastrointestinal motility acting on smooth-muscle cells. Changes in the microbiome composition influence muscularis propria macrophages, which accordingly tune intestinal peristalsis acting on this network. Adapted from Barrett, K.E., Barman, S.M., Boitano, S., Brooks, H. (2010). Ganong's Review of Medical Physiology, 23rd Edition (New York: McGraw Hill Companies).

these results reveal an unexpected role of BMP2 in physiological regulation of gastrointestinal motility. Similarly, further investigation will be required to define whether BMP2 also plays a role in regulating motility under inflammatory conditions.

BMP2 is known to play a role in enteric smooth muscle and neuronal differentiation (Chalazonitis et al., 2008). However, both the enteric nervous system and the pacemaker interstitial cells of Cajal (which control peristaltic contractions) and serotonin-expressing enteroendocrine cells (which activate peristaltic reflexes in response to luminal signals) were not affected by acute depletion of muscularis externa macrophages. These observations would rule out their role as BMP2 cell targets. Conversely, muscularis externa macrophages were found to line up with nerve fibers and often showed close proximity with βIII-tubulin$^+$ enteric neurons, which constitutively express the BMP2 receptor. This receptor is composed of BMPRIa and BMPRII serine kinases and signals through SMAD phosphorylation. Muller and colleagues (Muller et al., 2014) have provided evidence for the presence of nuclear phosphorylated SMAD1/5/8 complex in ex vivo isolated primary enteric neurons after exposure to BMP2 and also detected evidence of BMP2 activity on enteric neurons in vivo. The authors also have shown that *Csf1*$^{op/op}$ mice, which are severely compromised in macrophage development, have reduced number of enteric neurons positive for nuclear pSMAD1/5/8 complex, architectural abnormalities in the enteric nervous system, and cecum dilation, all evidence consistent with a role of macrophages in the development of the enteric nervous system. Thus, BMP2 now emerges as a key mediator used by muscularis externa macrophages to sustain the development of the enteric nervous system and also constitutively activate enteric neurons, thus regulating colonic contractility under physiological conditions.

Indeed, the interaction of enteric neurons with macrophages also has ontogenic implications operating in the other direction, as enteric neurons were found to produce the macrophage growth factor CSF1 and by this mean to sustain maintenance of muscularis externa macrophages, which are indeed absent in *Csf1*$^{-/-}$animals and in chimeric animals reconstituted with *Csf1r*$^{-/-}$ bone marrow (Mikkelsen and Thuneberg, 1999). Of note, muscularis externa macrophages were also efficiently depleted by treating animals with a blocking anti-CSF1 monoclonal antibody. Interestingly, this effect was already evident as soon as 24 hr after CSF1 blockade and was completely reverted in the following 7 days. This finding implies that muscularis externa macrophage homeostasis depends on a continuous CSF1-mediated support from enteric neurons, similarly to what has also been previously shown for CD103$^-$ CD11b$^+$ lamina propria macrophage homeostasis (Bogunovic et al., 2009). Thus, macrophages and enteric neurons provide each other support and guarantee homeostatic function of the gut, while commensal microbiota sensing tunes this bidirectional crosstalk.

Muscularis externa macrophages were found to be more strictly dependent on CSF1 signaling than lamina propria macrophages because a single injection of low-dose blocking anti-CSF1R monoclonal antibody resulted in efficient

depletion of muscularis externa macrophages, with no significant effects on lamina propria macrophages. Taking advantage of this, the authors were able to evaluate the effect of selective depletion of muscularis externa macrophages on the peristaltic reflex, which was tested in an ex vivo model evaluating intestinal contractions in response to stepwise distension of colonic rings. Colonic rings depleted of muscularis externa macrophages revealed evidence for hyperreactivity to stretch-induced contractions, which were more rapid and frequent and induced at shorter duration of stretch. A similar phenotype was observed when colonic rings from control animals were treated with the BMP2 receptor inhibitor dorsomorphin. Conversely, administration of exogenous BMP2 decreased stretch-induced hypercontractions observed in colonic rings depleted of muscularis externa macrophages and accelerated colonic transit time when injected intraperitoneally in control animals. These results confirm that macrophage-derived BMP2 regulates intestinal contraction. Interestingly, however, though muscularis externa macrophage depletion resulted in increased intestinal reactivity, colonic transit time was increased. Taken together, these results are consistent with a role of muscularis externa macrophages in physiological regulation of gastrointestinal motility, though the observation that their depletion delayed transit time in face of an increased intestinal reactivity suggests that they might play a role mostly in coordinating muscle contraction, an aspect that was not investigated in this study.

Overall, this study provides evidence for an intimate interaction of macrophages in muscolaris externa in the gut wall with enteric neurons, which is required for a normal development and function of the gut, thus providing a nice example of the essential role of macrophages as regulators of tissue homeostasis (Wynn et al., 2013). Furthermore, effects of microbiota sensing on muscularis externa macrophages and the consequent indirect impact on enteric neuron and smooth-muscle cell functions provide the basis for a better understanding of how peristaltic activity of the colon is regulated under steady-state conditions. Muller now provides clarity on how macrophages can have a grip on the gut.

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