Modulation of Gut Microbiota Composition by Serotonin Signaling Influences Intestinal Immune Response and Susceptibility to Colitis

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SUMMARY
Serotonin directly regulates bacterial growth in a species-dependent manner and indirectly via β-defensins. Higher gut mucosal serotonin levels select for a more colitogenic microbiota, resulting in increased severity of colitis. We show that serotonin-microbiota axis plays an important role in gut inflammation.

BACKGROUND & AIMS: Serotonin (5-hydroxytryptamine [5-HT]) is synthesized mainly within enterochromaffin (EC) cells in the gut, and tryptophan hydroxylase 1 (Tph1) is the rate-limiting enzyme for 5-HT synthesis in EC cells. Accumulating evidence suggests the importance of gut microbiota in intestinal inflammation. Considering the close proximity of EC cells and the microbes, we investigated the influence of gut-derived 5-HT on the microbiota and the susceptibility to colitis.

METHODS: Gut microbiota of Tph1−/− and Tph1+/+ littermates were investigated by deep sequencing. Direct influence of 5-HT on bacteria was assessed by using in vitro system of isolated commensals. The indirect influence of 5-HT on microbiota was assessed by measuring antimicrobial peptides, specifically β-defensins, in the colon of mice and HT-29 colonic epithelial cells. The impact of gut microbiota on the development of dextran sulfate sodium-induced colitis was assessed by transferring gut microbiota from Tph1−/− mice to Tph1+/+ littermates and vice versa.

RESULTS: A significant difference in microbial composition between Tph1−/− and Tph1+/+ littermates was observed. 5-HT directly stimulated and inhibited the growth of commensal bacteria in vitro, exhibiting a concentration-dependent and species-specific effect. 5-HT also inhibited β-defensin production by HT-29 cells. Microbial transfer from Tph1−/− to Tph1+/+ littermates and vice versa altered colitis severity, with microbiota from Tph1−/− mice mediating the protective effects. Furthermore, germ-free mice colonized with microbiota from Tph1−/− mice exhibited less severe dextran sulfate sodium-induced colitis.

CONCLUSIONS: These findings demonstrate a novel role of gut-derived 5-HT in shaping gut microbiota composition in relation to susceptibility to colitis, identifying 5-HT–microbiota axis as a...
Serotonin, also known as 5-hydroxytryptamine (5-HT), is a biogenic amine that has been widely studied for its neuropsychological and cognitive roles in the central nervous system. What is often underappreciated is that the vast majority of 5-HT in the body is found in the gastrointestinal (GI) tract. The GI tract contains about 95% of the body’s 5-HT, which is synthesized mainly within the enteric endocrine cells (EECs). Enterochromaffin (EC) cells are the best characterized subset of EECs and are the main source of 5-HT in the gut. EC cells are dispersed among epithelial cells in the mucosal layer of the GI tract and release 5-HT apically into the gut lumen, as well as basolaterally, in response to various mechanical and chemical stimuli. EC cells synthesize 5-HT from its precursor hydroxytryptophan. Tryptophan hydroxylase (Tph) catalyzes the synthesis of 5-HT. Two isoforms of Tph enzymes regulate 5-HT synthesis; these are Tph1, mainly present in EC cells, whereas Tph2 predominates in the brain stem and enteric neurons.

Changes in EC cell numbers and intestinal 5-HT content have been observed in experimental colitis and the 2 major forms of inflammatory bowel disease (IBD), Crohn’s disease (CD) and ulcerative colitis (UC). In a seminal study, we demonstrated that Tph1-deficient (Tph1/-) mice, which have significantly reduced 5-HT amount in the gut, exhibit reduced severity of colitis in 2 well-defined models of colitis (dextran sulfate sodium [DSS] and dinitrobenzene sulfonic acid [DNBS]). We also revealed that 5-HT plays a key role in the activation of immune cells to produce proinflammatory cytokines. These findings are supported by findings that the severity of chemical-induced colitis or spontaneous colitis associated with interleukin (IL) 10 deficiency is increased when combined with 5-HT enhancing effects of serotonin reuptake transporter deficiency, highlighting 5-HT as an important signaling molecule in the pathogenesis of colitis. However, the precise mechanisms by which 5-HT influences the disease pathogenesis remain to be determined.

The mammalian GI tract is colonized by a complex, heterogeneous, and dynamic microbial ecosystem, and in humans, the GI tract contains up to 1 x 10^{14} colony-forming units of bacteria with colonization occurring soon after birth. Commensal microorganisms within the GI tract play crucial roles in GI physiology, aid in digestion, provide competitive barriers to pathogen invasion, and contribute to the development of the host immune system. In addition, gut microbiota are located at the complex interface of the epithelial barrier, and they are sensitive to changes in response to environmental factors, such as diet and drugs, and signals derived from the intestinal immune system, such as antimicrobial peptides (AMPs).

There is now growing evidence that gut microbiota plays an important role in the pathophysiology of IBD. Fecal and intestinal mucosa-associated microbiota of IBD patients are characterized by decreased biodiversity and disruption of the microbe-host equilibrium. Contribution of intestinal microbiota in the disease pathogenesis is further demonstrated by using gnotobiotic mice, whereby colitis is not induced in the absence of microbes. Because of strategic location of EC cells in the epithelial lining of the mucosa and the emerging role of 5-HT in gut pathology, it is very likely that 5-HT from EC cells plays an important role in the modulation of gut microbiotal composition in the context of gut pathology and pathophysiology. However, little is known regarding the precise relationship between 5-HT signaling and gut microbiota.

In this study, by using in vitro system of commensal bacteria culture and in vivo system using Tph1/- and germ-free (GF) mice, we investigate the role of gut-derived 5-HT in the regulation of gut microbiota composition and highlight a key role for 5-HT-microbiota axis in the pathogenesis of experimental colitis. Our study demonstrates that 5-HT selects for a more colitogenic microbiota directly by regulating the growth of bacteria in a species-dependent manner as well as indirectly by inhibiting β-defensin production from colonic epithelial cells, which altogether leads to perpetuation of gut inflammation.

Results

**Tph1/- Mice Have Altered Gut Microbiota**

To determine whether mucosal 5-HT plays a role in selecting the microbiota, we analyzed the microbial composition of Tph1/- and Tph1+/+ mice, which have different levels of 5-HT in gut, with Tph1/- mice having the lower amount. To minimize the genetic influence, we used Tph1/- mice. We compared the cecal bacterial profiles of Tph1/- and Tph1+/+ offspring (F1 mice) from crosses of Tph1/- offspring parents, as well as Tph1/- mice from a breeding colony of Tph1/- mice (Inbred). The 3 groups of mice were separated into distinct clusters as shown by

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Authors share co-first authorship.

Abbreviations used in this paper: Abx, antibiotics; AMP, antimicrobial peptide; CD, Crohn’s disease; DMSO, dimethyl sulfoxide; DNBS, dinitrobenzene sulfonic acid; DSS, dextran sulfate sodium; EC, enterochromaffin; EEC, enteric endocrine cell; ELISA, enzyme-linked immunosorbent assay; ERK1/2, extracellular signal-regulated kinase-1 and -2; FDR, false discovery rate; GF, germ-free; GI, gastrointestinal; GIL, gut isolate library; hBD, human β-defensin; IBD, inflammatory bowel disease; IL, interleukin; mBD, mouse β-defensin; MPO, myeloperoxidase; OD, optical density; OTU, operational taxonomic unit; PBS, phosphate-buffered saline; PDI, principal coordinate ordination; PPAR-γ, peroxisome proliferator-activated receptor gamma; qRT-PCR, quantitative real-time polymerase chain reaction; SCFA, short-chain fatty acid; Tph, tryptophan hydroxylase; UC, ulcerative colitis; WT, wild-type; 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan.

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visualization of Bray-Curtis diversity by principal coordinate ordination (PCoA) (Figure 1A). Although the 2 groups of Tph1−/− mice (Inbred and F1 offspring of Tph1+/− crosses) separated into distinct clusters, they appeared more similar in composition (Figure 1B). To confirm the functional effect of the altered microbiota in Tph1−/− mice, we next analyzed the short-chain fatty acid (SCFA) concentrations within the feces of naïve Tph1+/− and Tph1−/− mice by using gas chromatography–mass spectrometry. We observed lower levels of acetate, butyrate, and propionate in Tph1−/− mice (Figure 1C).

To predict microbiota that is likely to be strongly influenced by the Tph1−/− genotype, we identified those taxa that were significantly different from the Tph1+/− heterozygous mice shared by both groups of Tph1+/− mice. Using Kruskal-Wallis non-parametric test, 32 operational taxonomic units (OTUs) are significantly different (P < .025, adjusted for false discovery rate [FDR]). This included 10 of the top 50 most abundant OTUs. Nine of 10 of these differences were increased in the relative abundance on the Tph1+/− mice relative to the Tph1−/− mice, including an increase in the 5 OTUs within the Bacteroidetes (OTUs representing Prevotella, Bacteroides, and 3 OTUs classified only to Bacteroidales), 2 taxa in the Firmicutes (Oscillibacillus, Lachnospiraceae), and 1 Proteobacteria OTU (Helicobacter). Within the Tenericutes, one OTU of Allobaculum was increased and another decreased (Figure 2). Further investigations will be required to validate these findings and determine the mechanisms that alter these taxa in the knockout mice.

5-Hydroxytryptamine Directly Influences Gut Bacteria Growth In Vitro

EC cells release 5-HT apically into the gut lumen as well as basolaterally. To explore whether EC cell–derived 5-HT can directly modulate gut microbial composition, we next sought to explore the direct effect of 5-HT on the growth of gut bacteria. We assessed the growth rate by using in vitro growth of 12 bacterial strains representing the major gut phyla groups: Bacteroidetes, Firmicutes, and Proteobacteria (Figure 3). According to the literature, 0.01 mg/L is the physiological concentration of 5-HT in the gut lumen. Among the 12 strains tested, we observed a concentration-dependent modulation of bacterial growth by 5-HT, and the effect was species-specific in 10 strains. In general, the anaerobic Bacteroides were more sensitive to 5-HT than the facultative anaerobes, although specific strains may exhibit enhanced growth at low concentrations. No significant effect on the growth of the strain of Clostridium bolteae or C ramosum was observed. These findings demonstrate that 5-HT can directly alter gut microbiota composition.

5-Hydroxytryptamine Attenuates β-defensin Production From Colonic Epithelial Cells

On the basis of our previous finding that Tph1+/− mice exhibit attenuated severity of DSS-induced colitis, we explored whether 5-HT can influence gut microbiota indirectly via AMPs. We found total β-defensin levels in naïve Tph1+/− mice, which have reduced 5-HT in gut, are higher, compared with wild-type (WT) (Tph1+/−) littersmates (Figure 4A). Restoration of 5-HT levels by 5-HT precursor, 5-hydroxytryptophan (5-HTP), in Tph1+/− mice reduced β-defensin production in the colon (Figure 4A). Total β-defensin levels were also higher in the colon of Tph1−/− mice as compared with WT littermates post-DSS (57.8215 ± 2.970896 and 35.70425 ± 2.672975, respectively).

Because mouse β-defensin (mBD)-1 and mBD-3 levels were decreased on 5-HTP administration in the colon of Tph1−/− mice (Figure 4B), we decided to further investigate the role of 5-HT in β-defensin production by using HT-29 colonic epithelial cells. Human β-defensin (hBD)-1 (human orthologue of mBD-1) and hBD-2 (human orthologue of mBD-3) have been investigated extensively, which are expressed constitutively or induced under inflammatory conditions, respectively. In addition, we have previously found reduced severity of DSS-induced colitis in mice on inhibition of 5-HT7 receptor activation by a selective antagonist (SB-269970). In the present study, treatment with SB-269970 prevented 5-HT–induced down-regulation of β-defensin 1 and 2 (Figure 4C and D). We also used 5-HT7 receptor deficient (5-HT7R−/−) mice and found that these mice exhibit higher levels of mBD-1 and mBD-3 in the colon (Figure 4E).

5-HT inhibits pereoxisome proliferator-activated receptor gamma (PPAR-γ) expression in various cells whereas PPAR-γ activates β-defensin in human colonic epithelial cells. We investigated PPAR-γ expression in the colon, whereas 5-HT administration attenuated the expression (Figure 4F). In HT-29 cells, 5-HT inhibited PPAR-γ expression, whereas SB-269970 restored the expression (Figure 4G). To examine whether an increased expression of mBD-1 and mBD-3 is mediated through PPAR-γ, Tph1+/− mice were intraperitoneally treated with either vehicle (dimethyl sulfoxide [DMSO]) or PPAR-γ antagonist, GW-9662. The antagonist-treated mice showed lower levels of both β-defensins (Figure 4H). It has been shown that 5-HT inhibits PPAR-γ expression through extracellular signal-regulated kinase-1 and -2 (ERK1/2) pathway in pulmonary artery smooth muscle cells. We observed similar finding in HT-29 cells that pre-treatment with MEK inhibitor (PD98059) masked the inhibitory effect of 5-HT (Figure 4I). These findings altogether suggest that 5-HT down-regulates PPAR-γ via 5-HT7 receptors and subsequently inhibits the production of β-defensins from colonic epithelial cells.

Mucosal 5-Hydroxytryptamine Induced Changes in Gut Microbiota Alter Susceptibility to Colitis

Tph1+/− mice exhibit reduced severity of colitis. We thus hypothesized that differences in gut microbiota composition between Tph1+/− and Tph1+/− mice play a role in the altered susceptibility to colitis, and that Tph1+/− microbiota confer a colitogenic effect. To study the effect of gut microbiota changes induced by 5-HT, we gavaged cecal contents from naïve Tph1+/− into Tph1+/− mice and...
vice versa and induced colitis with 5% DSS (Figure 5A). Adoptive transfer of microbiota from Tph1\(^{+/−}\) to Tph1\(^{-/-}\) mice increased the severity of colitis of the recipient mice as reflected by increase in macroscopic (Figure 5B) and histologic damage scores (Figure 5C and D) and myeloperoxidase (MPO) activity (Figure 5E), compared with Tph1\(^{+/−}\) mice that received microbiota from Tph1\(^{-/-}\) mice. Notably, IL1\(β\) is a mucosal inflammatory marker in IBD, and recent studies reveal caspase-8, in addition to caspase-1, is involved in IL1\(β\) regulation.\(^{29–32}\) We found an increased cleavage of caspase 8, when microbiota from Tph1\(^{+/−}\) mice was transferred to the recipient mice (Figure 5F), which correlated to an increase in IL1\(β\) levels (Figure 5G). Moreover, there were higher levels of IL6 and IL17A in the colon.

**Figure 1.** (See previous page). Tph1\(^{+/−}\) mice have an altered gut microbiota compared with heterozygous Tph1\(^{+/−}\) mice. 16S partial sequencing profiling analysis of cecal content of 3 groups of mice was carried out. Heterozygous mice (Tph1\(^{+/−}\) (F1)) bred from heterozygous parents were compared with homozygous knockout mice (Tph1\(^{-/-}\) (F1)) also bred from the same colony of heterozygous parents and mice from a breeding colony of knockout mice (Tph1\(^{-/-}\) (inbred)). (A) PCoA of Bray-Curtis dissimilarity revealed each group of mice had distinct microbiota. The 2 groups of Tph1\(^{-/-}\) mice separated from Tph1\(^{+/−}\) mice along the PCoA1 axis and from each other along the PCoA2 axis. (B) Taxonomic summaries (average of each group) at the genus level revealing greater similarity between the 2 groups of Tph1\(^{+/−}\) mice. (C) Concentration of acetate, butyrate, propionate, and lactate in the feces of Tph1\(^{+/−}\) and Tph1\(^{-/-}\) mice. Data are from 1 representative experiment of 2 independent experiments performed. Data are presented as mean ± standard deviation from 4 mice per group; \(^*P<.05\) by Student\(t\) test.
of the recipient mice that received microbiota from \textit{Tph1\(^{+/-}\)} mice (Figure 5G).

To further confirm the colitogenic effect of \textit{Tph1\(^{+/-}\)} microbiota in increasing the susceptibility to DSS-induced colitis, we treated both \textit{Tph1\(^{+/-}\)} and \textit{Tph1\(^{-/-}\)} mice with broad-spectrum antibiotics (Abx) in drinking water for 10 days before the induction of colitis (Figure 6A). Abx treatment abrogated the differences in the colitis susceptibility between these mice as shown by similar macroscopic (Figure 6B) and histologic scores (Figure 6C and D), MPO activity (Figure 6E), as well as proinflammatory cytokine levels (Figure 6F). Altogether, these findings suggest that 5-HT perturbs and configures microbiota to a colitogenic microbiota, which subsequently increases host susceptibility to colitis.

**Transfer of Gut Microbiota From \textit{Tph1\(^{-/-}\)} Mice Exhibits Up-regulation of Gut Barrier Integrity and Down-regulation of Inflammation in Germ-free Mice**

To further elucidate the role of gut 5-HT–microbiota axis in the pathogenesis of colitis, we transferred microbiota from either \textit{Tph1\(^{+/-}\)} or \textit{Tph1\(^{-/-}\)} littermates into GF mice and examined the development of DSS-colitis (Figure 7A). Although there was no difference in EC cell number and 5-HT levels on day 5 post-DSS (Figure 7B and C), investigations on the parameters of colitis revealed lower macroscopic scores, histologic damage score, MPO activity, and proinflammatory cytokines (IL1\(\beta\) and IL6) in GF mice colonized with microbiota from the \textit{Tph1\(^{+/-}\)} mice, as compared with those colonized with microbiota from \textit{Tph1\(^{-/-}\)} mice (Figure 7D–H). Recently, it has been shown that IL17C produced by epithelial cells plays an important role in the protection of DSS-colitis by inducing hBD-2.\(^{\text{23}}\)

There was an increase in IL17C levels in GF mice with microbiota from \textit{Tph1\(^{+/-}\)}, supporting a protective role of IL17C in DSS-induced colitis (Figure 7I). However, we did not observe difference in IL23 levels (Figure 7J). Next, we investigated the expression of gut barrier components. There was a higher expression of mBD-3 (Figure 8A), ZO-1 (Figure 8B), but not occludin (Figure 8C), in GF mice with \textit{Tph1\(^{-/-}\)} microbiota, as compared with the GF mice with microbiota from \textit{Tph1\(^{+/-}\)} littermates. GF mice with \textit{Tph1\(^{-/-}\)} microbiota exhibited up-regulated Muc2 and Muc5ac expression, compared with GF mice with \textit{Tph1\(^{+/-}\)} microbiota (Figure 8D). Together, these findings reveal microbiota of \textit{Tph1\(^{+/-}\)} mice have the ability not only in maintaining the gut barrier integrity but also in reducing the severity of colitis.

**\textit{Tph1\(^{+/-}\)} and \textit{Tph1\(^{+/-}\)} Microbiota Transferred to Germ-free Mice Result in Distinct Microbiota Before and After Dextran Sulfate Sodium Administration**

Analysis of microbial composition in GF mice colonized with gut microbiota from \textit{Tph1\(^{+/-}\)} and \textit{Tph1\(^{-/-}\)} mice revealed distinct microbiota before and after DSS treatment. Principal coordinate analysis (PCoA) of Bray-Curtis dissimilarity revealed that the microbiota of \textit{Tph1\(^{+/-}\)} and \textit{Tph1\(^{-/-}\)} mice is separated into 2 distinct groups (permutational multivariate analysis of variance, \(P < .01\)). DSS administration shifted the microbial communities as expected, but GF mice colonized by \textit{Tph1\(^{+/-}\)} microbiota still exhibited distinct microbiota, compared with that in GF mice colonized by microbiota from \textit{Tph1\(^{-/-}\)} mice (Figure 9A). At the phylum level, DSS administration induced an expansion of Proteobacteria in both groups and reduction in Bacteroidetes/ expansion of Firmicutes in the GF mice receiving microbiota from \textit{Tph1\(^{+/-}\)} mice, but GF mice receiving \textit{Tph1\(^{-/-}\)} microbiota were protected from this microbial shift (Figure 9B). In addition, investigations at the OTU level revealed reduction of 2 distinct Bacteroidales OTUs in the GF mice colonized by \textit{Tph1\(^{+/-}\)} microbiota compared with the GF mice colonized by \textit{Tph1\(^{-/-}\)} microbiota, whereas the GF mice colonized by the \textit{Tph1\(^{+/-}\)} microbiota exhibited less \textit{Akkermansia} (Figure 9C). The increased abundance of \textit{Akkermansia} in the GF mice colonized by \textit{Tph1\(^{+/-}\)} microbiota was further confirmed by testing the direct effect of 5-HT on the growth rate of the bacterium, whereby 5-HT inhibited the growth in a concentration-dependent manner (Figure 9D).

**Discussion**

5-HT is a key enteric mucosal signaling molecule influencing gut physiology (motor and secretory function) and thus maintaining GI homeostasis. Dysregulated 5-HT signaling is observed in many GI diseases including IBD, functional disorders such as irritable bowel syndrome, colorectal cancer, and in various enteric infections.\(^{\text{2,9–14}}\)

During the past decade, more studies are enlightening gut function as well as pathology rely on interactions with gut microbiota. Healthy microbiota is thought to collaborate with host to maintain the intestinal barrier, and disruption of this relationship can compromise the gut function. Because of close proximity of gut microbiota and 5-HT producing EC cells in the gut mucosal layer, cross-talk between them is likely to play a critical role in maintaining intestinal homeostasis. Whereas recently gut bacteria have been shown to stimulate the release of 5-HT from EC cells,\(^{\text{34}}\) the converse effect of 5-HT on microbiota remained to be determined. This study illustrates that 5-HT plays a key role in the regulation of gut microbial composition and that the direct and indirect influence of 5-HT on microbial composition affect the susceptibility to experimental colitis.

In recent years, gut microbiota has emerged as a topic of great interest in biomedical research. Many studies have demonstrated that disruption of the balanced composition of the gut microbiota is associated with both GI and non-GI diseases.\(^{\text{35–37}}\) In general, gut microbiota performs several vital functions for host health, including digestion of complex host-indigestible polysaccharides, pathogen displacement, synthesis of vitamins, and development of immune system.\(^{\text{36}}\) Two major bacterial phyla, Firmicutes and Bacteroidetes, and 5 minor bacterial phyla, Proteobacteria, Actinobacteria, Fusobacteria, Cyanobacteria, and Verrucomicrobia, comprise the gut microbiota in adult humans.\(^{\text{25}}\) EC cells, which are dispersed among the epithelial cells, lie in...
close proximity to gut microbiota and react to changes in gut contents by releasing biologically active molecules including 5-HT. Recently, it has been shown that microbial-derived metabolites, such as SCFAs (ie, butyrate and acetate) or secondary bile acids, especially deoxycholate, act on EC cells and up-regulate the expression of \textit{Tph1}. In addition, bacterial toxins including cholera toxin and \textit{Escherichia coli} lipopolysaccharide have been shown to stimulate 5-HT release from EC cells. Taken together, there is now evidence to postulate a role of microbiota in 5-HT production from EC cells. In addition to the effect of gut microbiota on 5-HT production from EC cells, it is also possible that 5-HT may influence microbiota in relation to gut function. Indeed, in our studies, we observed gut microbial composition differs between \textit{Tph1}\textsuperscript{-/-} and \textit{Tph1}\textsuperscript{+/+} littermates, which have different levels of gut 5-HT, with \textit{Tph1}\textsuperscript{-/-} mice having the lower amount.

On the basis of previous studies that revealed importance of littermates in defining host genetic effect on the gut microbiota composition as well as subsequent microbial effect on the host susceptibility of DSS-induced colitis, we controlled for non-genetic confounders by generating littermates from \textit{Tph1}\textsuperscript{+/+} parents to investigate whether the impact of \textit{Tph1} genotype on gut microbiota is dominant over both parentage and housing conditions. Here we observed altered microbial composition in \textit{Tph1}\textsuperscript{-/-} mice, along with altered SCFA concentrations. Interestingly, we observed lower acetate, butyrate, and propionate levels in the feces of \textit{Tph1}\textsuperscript{-/-} mice compared with \textit{Tph1}\textsuperscript{+/+} mice. The precise reason for these lower levels is not clear, but it seems possible that the lower levels in \textit{Tph1}\textsuperscript{-/-} mice reflect the differences in microbial composition between the \textit{Tph1}\textsuperscript{-/-} and \textit{Tph1}\textsuperscript{+/+} mice.

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**Figure 3. Direct effect of serotonin (5-HT) on gut microbial communities.** In vitro growth of 10 gut commensals in the presence of serotonin (5-HT) at 0.01, 0.1, and 1 mg/mL was measured by OD at 650 nm relative to control (without 5-HT) at 24 and 72 hours for aerobic and anaerobic bacteria, respectively. Concentration-dependent stimulation and inhibition of bacterial growth by 5-HT are species-specific. Data are from 1 representative experiment of 3 independent experiments with quadruplicates. Data are represented as mean ± standard error of the mean. *P < .05 by Student t test. Significant differences from negative control (no 5-HT) are indicated by *.
Increased mucosal 5-HT content and EC cell hyperplasia are associated with experimental colitis and IBD. Moreover, IBD patients have dysbiotic microbiota with a decrease in obligate anaerobes. In our in vitro study using diverse commensal bacterial strains, there was a significant growth inhibition in most of the bacteria tested. According to the literature, 0.01 mg/L is the physiological concentration of 5-HT in the gut lumen. When we used higher concentration, there was significant growth inhibition in most of the bacteria tested, and when affected, the obligate anaerobes were more sensitive to 5-HT. This provides evidence that high levels of 5-HT can directly alter the configuration of gut microbiota.

AMPs shape the composition of the microbiota and help maintain gut homeostasis. Defensins constitute a major class and are necessary to fend off microorganisms in the mucosal layer. Abnormal β-defensin production has been implicated in a number of GI disorders including IBD, whereby diminished antimicrobial activity due to attenuated hBD-1 and hBD-2 expression is associated with colonic CD patients. In addition, 5-HT receptor expression is increased in the inflamed regions of CD patients. 5-HT receptor is also up-regulated in the murine intestine post-DSS, compared with the controls, whereas blocking 5-HT signaling with a selective 5-HT-R antagonist or genetic deletion of the receptor alleviates colitis in DSS- and DNBS-colitis. Previously, Guseva et al found that blocking 5-HT receptor exacerbates severity of DSS-colitis. The authors state that the dose, route of administration, and housing of animals may account for the difference in the results.

In our study, inhibition of 5-HT receptors by selective antagonist restored β-defensin production in HT-29 cells. 5-HT-R mice also expressed higher levels of mBD-1 and mBD-3 in the colon. Recently, it has been demonstrated that 5-HT4 receptor stimulation via enema administration has a protective effect in the experimental colitis but not via intraperitoneal injection, which is shown to be associated with increased motility. Further studies are warranted to elucidate the role of other 5-HT receptors expressed on intestinal epithelial cells in β-defensin production.

PPAR-γ is essential for maintaining β-defensin expression in the colon. There is now substantial evidence from experimental models of colitis and IBD patients that PPAR-γ agonists play a role as a key inhibitor of colitis by regulating immune activation and inflammation. We found GW-9662 reduced mBD-1 and mBD-3 expression in Tph1/- mice, whereas 5-HT inhibited β-defensin production by attenuating PPAR-γ via 5-HT7 receptors through ERK1/2-dependent pathway in HT-29 cells. There are studies showing that Bacteroides thetaiotaomicron and Enterococcus faecalis activate intestinal epithelial PPAR-γ, which decreases IL8 and increases IL10 production, respectively. Our in vitro study using bacterial strains illustrated the concentration- and species-dependent effect of 5-HT on the growth of B thetaiotaomicron and E faecalis, providing further support that 5-HT can also inhibit PPAR-γ in a microbiota-dependent manner. Altogether, these findings suggest 5-HT released from EC cells directly and indirectly (via modulation of β-defensin production) plays a crucial role in regulation of gut microbial composition. These findings are further supported by the observations of different gut microbial composition in Tph1/- and Tph1/+ mice.

There is now abundant evidence to postulate a link between gut microbiota and IBD. In CD, fecal stream diversion reduces inflammation and induces mucosal healing in the excluded intestinal segment, whereas infusion of intestinal contents reactivates the disease. In UC, short-term treatment with broad-spectrum Abx rapidly reduces mucosal inflammation. Recently, a randomized controlled trial has shown that fecal microbiota transplantation induces remission in a significantly greater percentage of patients with active UC than placebo, with no difference in adverse events. Adoptive transfer of microbiota from Tph1/- to Tph1/+ and vice versa and the studies using GF mice provide evidence for the important role of 5-HT–microbiota axis in the pathogenesis of colitis, with Tph1/- microbiota mediates protective effect in GF mice. GF mice express Tph1 and secrete 5-HT at a much lower level than SPF mice. Our finding that there was no difference in EC cell number and 5-HT levels after transfer of either Tph1/+ or Tph1/- microbiota into GF mice post-DSS suggests that the difference in the disease severity can be attributed to the difference in the microbiota composition.

Figure 4. (See previous page). 5-HT down-regulates PPAR-γ via 5-HT7 receptors and subsequently inhibits β-defensin production. (A) Levels of total β-defensins in the colon of Tph1+/+, Tph1−/−, and 5-HTP–treated Tph1−/− mice. (B) Quantification of mDefb1 (left) and mDefb3 (right) mRNA expression in the colon of Tph1+/+, Tph1−/−, and 5-HTP–treated Tph1−/− mice. (C) Quantification of defb1 mRNA expression (left) and measurement of hBD-1 peptides (right) after 5-HT7 receptor antagonist (SB-269970; 1 μmol/L) treatment in HT-29 cells. (D) Quantification of defb4 mRNA expression (left) and measurement of hBD-2 peptides (right) after 5-HT7 receptor antagonist treatment. (E) Quantification of mDefb1 (left) and mDefb3 (right) mRNA expression in the colon of 5-HT-R mice. (F) Quantification of PPAR-γ mRNA expression after 5-HT7 receptor antagonist (SB-269970; 1 μmol/L) treatment in HT-29 cells. (H) Quantification of mDefb1 (left) and mDefb3 (right) mRNA expression in Tph1−/− mice treated with PPAR-γ antagonist (GW-9662; 2 mg/kg intraperitoneally per day for 5 days), compared with vehicle (DMSO)-treated mice. (I) Quantification of defb1 (left) and defb4 (right) mRNA expression in HT-29 cells treated with MEK inhibitor (PD98059; 40 μmol/L). In vitro qRT-PCR data are representative of 3 individual experiments with quadruplicates. 5-HT concentration is 10−7 mol/L. In vitro data are represented as mean ± standard error of the mean, whereas in vivo data are represented as mean ± standard deviation from 4 to 6 mice per group; *P < .05, **P < .01, and ****P < .0001 by Student t test or 1-way analysis of variance, with Bonferroni multiple comparison test.
Consistent with our finding that \( Tph1^{+/+} \) and \( Tph1^{-/-} \) mice carry different microbiota composition, we found distinct differences in microbiota between GF mice with \( Tph1^{-/-} \) littermates pre- and post-DSS. Deep sequencing at the genus level revealed that GF mice colonized by \( Tph1^{+/+} \) microbiota exhibit low abundance of \( A\) muciniphila\) on DSS treatment, compared with that in GF mice with microbiota from \( Tph1^{-/-} \) mice. We also observed concentration-dependent direct inhibitory effect of 5-HT on the growth rate of the \( A\) muciniphila\. Although the role of \( A\) muciniphila in the pathogenesis of colitis is still unclear, it has been shown that \( A\) muciniphila\)–derived extracellular vesicles mediate protective effects in the development of DSS-induced colitis.\(^62\) In addition, \( A\) muciniphila\) adheres to colonic epithelial cells and strengthens an impaired gut barrier\(^63\) by stimulating enterocyte proliferation and promoting wound restitution,\(^64\) thereby suggesting an important role of this bacterium in mediating protection in intestinal inflammation. Higher abundance of \( A\) muciniphila\) in \( Tph1^{+/+} \) microbiota along with the inhibitory effect of high 5-HT in the growth of the bacterium further provide evidence to postulate the influence of 5-HT–microbiota axis in the pathogenesis of colitis. Altogether, these results highlight the importance of 5-HT in regulating gut microbial composition and ultimately altering susceptibility to DSS-induced colitis.

In summary, this study not only provides novel information on 5-HT–microbiota axis in relation to intestinal immune responses and the pathogenesis of colitis but also shed light on the bidirectional relationship between EC cells and microbiota in gut function. Identifying the specific bacterial species associated with alteration in gut 5-HT levels in inflammation may ultimately lead to improved therapeutic strategies using the bacterial species or targeting 5-HT signaling in various intestinal inflammatory disorders including IBD.

**Methods**

All authors had access to the study data and reviewed and approved the final manuscript.

**Mice**

All mice used in this study were male and 6–8 weeks old, except for GF mice, which were male and 10–12 weeks old. Breeding pairs of \( Tph1^{+/+} \) (WT) and \( Tph1^{-/-} \) mice on C57BL/6 background were obtained from CNRS, Paris, France. \( Tph1^{+/+} \) mice on C57BL/6 background were originally produced by gene mutation as previously described.\(^65\) Briefly, \( Tph1^{-/-} \) mice have been generated by substituting exon 2 of the \( Tph1 \) locus by the nlslacZneopolyA cassette. These mice are viable, express normal amounts of 5-HT in the brain, and show no observed differences in food intake or body weight as compared with \( Tph1^{+/+} \) mice. \( Tph1^{+/+} \) and \( Tph1^{-/-} \) offspring (F1 mice) were generated from crosses of \( Tph1^{+/+} \) offspring parents, as well as mice from a breeding colony of \( Tph1^{-/-} \) mice (Inbred). Breeding pairs of 5-HT\(^{-}\) receptor–deficient (5-HT\(^{-}\)-R\(^{−/−}\)) mice on C57BL/6 background, originally generated as described by Hedlund et al,\(^66\) were provided by Peter B. Hedlund (Scripps Research Institute, La Jolla, CA). C57BL/6 mice were purchased from Taconic Biosciences, Rensselaer, NY. GF mice on the C57BL/6 background were derived and maintained under gnotobiotic conditions in the Axenic/ Gnotobiotic Unit at McMaster University. All experiments were approved by the McMaster University animal ethics committee and conducted under the Canadian guidelines for animal research.

**Experimental Protocol**

As previously described,\(^12\) 5-HTP (Cat. # H9772; Sigma-Aldrich, St Louis, MO) was administered subcutaneously at a dosage of 50 mg/kg twice a day for 8 days; control mice received saline. \( Tph1^{+/+} \) mice received daily intraperitoneal injection of GW-9662 at 2 mg/kg/day for 5 days, and control mice received DMSO. DSS (molecular mass 40 kDa; Cat. # 02160110; MP Biomedicals Incorporated, Solon, OH) was added to drinking water at a final concentration of 5% (w/v) and 2.5% (w/v) for SPF and GF mice, respectively, for 5 days. Mean DSS consumption was noted per cage each day. Mice were killed 5 days after the beginning of DSS administration. Macroscopic damage scores were performed by using a previously published scoring system for DSS-colitis.\(^67\) Colonic damage was scored on the basis of a published scoring system that considers architectural derangements, goblet cell depletion, edema/ulceration, and degree of inflammatory cell infiltrate.\(^67\) MPO (an index of granulocytes infiltration and inflammation) activity was determined by using a published protocol.\(^67\) \( Tph1^{+/+} \) and \( Tph1^{-/-} \) mice were administered ad libitum with broad-spectrum Abx, consisting of neomycin (0.5 g l\(^{-}\)), ampicillin (0.5 g l\(^{-}\)), vancomycin (0.5 g l\(^{-}\)), and metronidazole

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**Figure 5.** (See previous page). Microbiota from \( Tph1^{-/-} \) mice attenuates DSS-induced colitis. (A) Schematic of cecal microbiota transfer, followed by colitis induction with 5% DSS in sterile drinking H\(2\)O. (B) Macroscopic scores on day 5 post-DSS. (C) Representative photomicrographs of H&E stained colon cross sections on day 5 post-DSS visualized using Nikon Eclipse 80i microscope. Original magnification, \( \times 10 \). (D) Histologic damage scores. (E) MPO activity on day 5 post-DSS. (F) Western blot analysis performed on protein extracts obtained from the colon homogenates of the recipient mice administered with DSS. Representative Western blot with \( \beta\)-actin is presented as loading controls. Pro-Casp8, pro-caspase-8; Casp-8, caspase-8. Each lane represents an individual mouse. Results are the representative of 3 independent experiments performed on at least 3 mice per group. (G) Cytokine levels in the colon of \( Tph1^{+/+} \) and \( Tph1^{-/-} \) littermates on day 5 post-DSS after transfer of gut microbiota from \( Tph1^{+/+} \) mice to \( Tph1^{+/+} \) mice and vice versa. Data are from 1 representative experiment of 2 independent experiments performed. Data are represented as mean ± standard deviation from 8 to 10 mice per group; \( *P < .05 \) by Student t test. (→) denotes microbiota transfer.
(0.5 g L⁻¹) in sterile drinking water for 10 days before the start of DSS (5%)-colitis and continuing until day 5 post-colitis; their control groups received sterile drinking water before the induction of colitis.

**Adoptive Microbiota Transfer**

For adoptive microbiota transfer experiments, 200-μL cecal samples from Tph1⁺/⁻ mice were diluted in sterile phosphate-buffered saline (PBS) and gavaged to Tph1⁻/⁻.
Figure 7. GF mice colonized by microbiota from Tph1−/− mice are resistant to DSS-induced colitis. (A) Schematic of cecal microbiota transfer, followed by DSS treatment. (B) 5-HT expressing EC cells on day 5 post-DSS. (C) 5-HT levels in the colon on day 5 post-DSS. (D) Macroscopic scores on day 5 post-DSS. (E) Representative photomicrographs (left) of H&E stained colon sections on day 5 post-DSS visualized using Nikon Eclipse 80i microscope, original magnification, ×10, and histologic scores (right) on day 5 post-DSS. (F) MPO activity on day 5 post-DSS. (G–J) Cytokines (IL1β, IL6, IL17C, and IL23) in the colon on day 5 post-DSS, respectively. Data are from 1 representative experiment of 2 independent experiments performed. Data are represented as mean ± standard deviation from 5 mice per group; *P < .05 and **P < .01 by Student t test. (→) denotes microbiota transfer.
littermates and vice versa for 7 days beginning 2 days before induction of DSS (5%)-colitis and continuing until day 5 post-colitis. GF mice received microbiota (cecal) by oral gavage from $Tph1^{-/-}$ or $Tph1^{+/+}$ littermates 7 days before the induction of DSS (2.5%) colitis.

**Bacterial Culture**

To determine the effect of 5-HT on bacterial growth, the growth rate of diverse commensal bacterial strains from human gut isolate library (GIL) in Dr Michael Surette lab were studied in the presence of 5-HT at varying concentrations (0.01 mg/mL, 0.1 mg/mL, 1 mg/mL). Obligate anaerobic strains included *Akkermansia muciniphila* (ATCC BAA-835), *Bacteroides fragilis* (GIL83), *Bacteroides intestinalis* (GIL98), *Bacteroides thetaiotaomicron* (GIL179), *Clostridium bolteae* (GIL94), *Clostridium ramosum* (GIL107), *Eubacterium limosum* (GIL141), *Flavonifractor plautii* (GIL193), and *Ruminococcus gnavus* (GIL116). Facultative anaerobic strains included *Enterococcus faecalis* (GIL6), *Streptococcus salivarius* (GIL9), and *Streptococcus australis* (GIL58). *Escherichia coli* DH5α was used as a control. Strains were grown in brain-heart infusion broth overnight and diluted 1:100 into 96-well microplates containing 150 μL of media supplemented with 5-HT as indicated. Anaerobes...
were incubated at 37°C in an anaerobic environment (5% CO₂, 5% H₂, 90% N₂) for 72 hours, whereas facultative anaerobes were incubated at 37°C in 5% CO₂ for 24 hours. Microbial growth was measured by optical density (OD) at 650 nm and normalized to control culture (no 5-HT).

**Microbiome Profiling and Analysis**

Bacterial profiling was carried out by amplification of the V3 region of the 16SrRNA gene as described previously.⁶⁸,⁶⁹ Amplification products were sequenced on an Illumina MiSeq (Farncombe Institute) with 2 × 250 nt
Table 1. Quantitative Real-time Polymerase Chain Reaction Human Primers

|         | Forward (5’-3’)                      | Reverse (5’-3’)                      |
|---------|--------------------------------------|--------------------------------------|
| Gapdh   | CTTAGCACCCTGCGCAAG                    | TGGTCATGAGTCTCTCAGG                   |
| Pparg   | AAGGCCATTITCTCAGAAG                   | AGGAGTGGGAGTCTCTCC                   |
| Defb1   | Bio-Rad qHsaCID0015106, PrimePCR SYBR Green Assay | Bio-Rad qHsaCID0015106, PrimePCR SYBR Green Assay |
| Defb4   | Bio-Rad qHsaCID0038951, PrimePCR SYBR Green Assay | Bio-Rad qHsaCID0038951, PrimePCR SYBR Green Assay |
The data were analyzed according to the 2-ΔΔCT method and IL23, Cat. # M2300) were determined according to the manufacturer's instructions (Quantikine Murine; R&D Systems, Minneapolis, MN). IL17C levels were measured by using commercially available ELISA kit (Cat. # SED347Mu; Cloud Clone Corp, Katy, TX). Levels of mouse total β-defensins, human β-defensin 1 and 2 peptide were measured by using commercially available ELISA kits (Cat. # MBS9315750, MBS052463, and MBS703403, respectively; Mybiosource, Cedarlane, Burlington, Canada).

Quantitative Real-time Polymerase Chain Reaction Mouse Primers

| Mouse Primers | Forward (5'-3') | Reverse (5'-3') |
|---------------|----------------|----------------|
| 18S           | GTAACCGGTGAACCCCATT | CCATCCAATCGGTAGTACG |
| Defb1         | GGTTGGGCTTCTCACAAG | ACAAGCCATCGGTGCTTTATG |
| Defb3         | GGATCCATACCTTCTGTTGCC | ATTTGAGGAAAGGAATCCAC |
| Ocln          | ATGTCGGGCGATGCTTCTC | CTTTGCGTCTGGTGCTGTAT |
| Pparg         | CTGCTCAAGTATGGTGCATGA | ATGGAGACTCAGTTATCA |
| Tjp1          | ACCCGAAACTGCTGCTGGATAG | AAATGCCGGCGAAACTTGTGA |

Western Blot

Colonized mice were homogenized in Tris-buffered saline containing protease inhibitor (Cat. # P8340; Sigma-Aldrich). Equal amounts of protein homogenates from each group were loaded and electrophoresed onto 7-20% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane by using Transblot turbo transfer system (Bio-Rad) as per manufacturer's instructions. Membranes were blocked with 3% bovine serum albumin blocking buffer for 1 hour at room temperature and incubated with primary antibodies against Pro caspase-8 (1:1000) (Cat. # 4927; Cell Signaling Technology), Cleaved Caspase-8 (1:1000) (Cat. # 8592; Cell Signaling Technology), Muc2 (0.2 μg/mL) (Cat. # sc-15334; Santa Cruz Biotechnology), Muc5ac (1:1000) (Cat. # M5293; Sigma-Aldrich) for overnight at 4°C. Membranes were washed, incubated with either anti-rabbit horseradish peroxidase–linked antibody (1:5000, Cat. # 7074; Cell Signaling Technology) or anti-mouse horseradish peroxidase–linked antibody (0.08 μg/mL, Cat. # sc-2318; Santa Cruz Biotechnology) for 1 hour at room temperature. Proteins were visualized by use of SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific). β-actin was used as a loading control. Densitometric analysis was performed on Western blots with ImageJ software (version 1.48), normalized to total actin. Total protein concentration of homogenized tissue was determined by using DC Protein Assay Kit (Bio-Rad).

Enzyme-Linked Immunosorbert Assay

5-HT levels were measured by using commercially available enzyme-linked immunosorbert assay (ELISA) kits (Cat. # IM1749; Beckman Coulter, Fullerton, CA). Briefly, colonic tissues were weighed and were homogenized in 0.2 N perchloric acid. After centrifugation at 10,000 g for 5 minutes, the supernatants were collected, and the pH was neutralized by using 1 mol/L borate buffer. The supernatants were used for analysis of 5-HT levels using commercially available ELISA kit (Beckman Coulter). 5-HT content was expressed as a function of tissue weight (ng/mg). For intestinal cytokine and defensin measurement, colonic tissues were homogenized in Tris-buffered saline containing a protease inhibitor mixture (Cat. # P8340; Sigma-Aldrich, Oakville, Canada). Samples were centrifuged for 5 minutes at 3300g, and the resulting supernatants were frozen at −80°C until use. Total protein levels were quantified in the colon homogenates by using DC Protein Assay Kit (Cat. # 5000111; Bio-Rad Laboratories). Cytokine levels (IL1β, Cat. # SMLB00C; IL6, Cat. # SM6000B; IL17A, Cat. # SM1700; and IL23, Cat. # M2300) were determined according to the manufacturer’s instructions (Quantikine Murine; R&D Systems, Minneapolis, MN). IL17C levels were measured by using a commercially available ELISA kit (Cat. # SED347Mu; Cloud Clone Corp, Katy, TX). Levels of mouse total β-defensins, human β-defensin 1 and 2 peptide were measured by using commercially available ELISA kits (Cat. # MBS9315750, MBS052463, and MBS703403, respectively; Mybiosource, Cedarlane, Burlington, Canada).

Statistical Analysis

Data are represented as means ± standard deviation or means ± standard error of the mean. Where appropriate, data were analyzed by using unpaired Student t test, 1-way analysis of variance, followed by Newman-Keuls, Bonferroni multiple comparison post hoc tests, or Mann-Whitney tests using GraphPad Prism version 5 (GraphPad Software, La Jolla, CA). Bacterial community structures were assessed by

Table 2. Quantitative Real-time Polymerase Chain Reaction Mouse Primers

| Mouse Primers | Forward (5'-3') | Reverse (5'-3') |
|---------------|----------------|----------------|
| 18S           | GTAACCGGTGAACCCCATT | CCATCCAATCGGTAGTACG |
| Defb1         | GGTTGGGCTTCTCACAAG | ACAAGCCATCGGTGCTTTATG |
| Defb3         | GGATCCATACCTTCTGTTGCC | ATTTGAGGAAAGGAATCCAC |
| Ocln          | ATGTCGGGCGATGCTTCTC | CTTTGCGTCTGGTGCTGTAT |
| Pparg         | CTGCTCAAGTATGGTGCATGA | ATGGAGACTCAGTTATCA |
| Tjp1          | ACCCGAAACTGCTGCTGGATAG | AAATGCCGGCGAAACTTGTGA |
using Bray-Curtis beta diversity measures after rarefaction to normalize for variable number of reads per sample. Permutational multivariate analysis of variance was used to analyze statistical differences in beta diversity using the vegan package in R. Results were visualized using by using PCoA plots. Calculations of taxa that differed significantly between mice groups were computed by using DESeq2 (considered significant, if the P value was <.01 after adjustment for multiple testing via DESeq2’s implementation of the Benjamini-Hochberg multiple testing adjustment procedure). An associated P value <.05 was considered statistically significant in this study.

References

1. Kim DY, Camilleri M. Serotonin: a mediator of the brain–gut connection. Am J Gastroenterol 2000; 95:2698–2709.
2. Gershon M. Roles played by 5-hydroxytryptamine in the physiology of the bowel. Aliment Pharmacol Ther 1999; 13:15–30.
3. Lundgren O. Enteric nerves and diarrhoea. Basic Clin Pharmacol Toxicol 2002;90:109–120.
4. Racke K, Reimann A, Schwörer H, Kilbinger H. Regulation of 5-HT release from enterochromaffin cells. Behav Brain Res 1995;73:83–87.
5. Fitzpatrick PF. Tetrahydropterin-dependent amino acid hydroxylases. Annu Rev Biochem 1999;68:355–381.
6. Margolis KG, Stevanovic K, Li Z, Yang QM, Oraveca T, Zambrowicz B, Jhaver KG, Diacou A, Gershon MD. Pharmacological reduction of mucosal but not neuronal serotonin opposes inflammation in mouse intestine. Gut 2013;63:928–937.
7. Walther DJ, Bader M. A unique central tryptophan hydroxylase isoform. Biochem Pharmacol 2003; 66:1673–1680.
8. Walther DJ, Peter JU, Bashammakh S, Hörtinqal H, Voits M, Fink H, Bader M. Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science 2003; 299:76.
9. Ahonen A, Kyösola K, Penttilä O. Enterochromaffin cells in macrophages in ulcerative colitis and irritable colon. Ann Clin Res 1976;8:1–7.
10. Belal A, Boulos P, Robson T, Burnstock G. Neurochemical coding in the small intestine of patients with Crohn’s disease. Gut 1997;40:767–774.
11. Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszky H, Crowell MD, Sharkey KA, Gershon MD, Mawe GM. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome 1. Gastroenterology 2004;126:1657–1664.
12. Ghia JE, Li N, Wang H, Collins M, Deng Y, El–Sharkawy RT, Côté F, Mallet J, Khan WI. Serotonin has a key role in pathogenesis of experimental colitis. Gastroenterology 2009;137:1649–1660.
13. Linden DR, Chen J-X, Gershon MD, Sharkey KA, Mawe GM. Serotonin availability is increased in mucosa of guinea pigs with TNBS-induced colitis. Am J Physiol Gastrointest Liver Physiol 2003;285:G207–G216.
14. Manocha M, Khan WI. Serotonin and GI disorders: an update on clinical and experimental studies. Clin Transl Gastroenterol 2012;3:e13.
15. Li N, Ghia JE, Wang H, McClemens J, Cote F, Suehiro Y, Mallet J, Khan WI. Serotonin activates dendritic cell function in the context of gut inflammation. Am J Pathol 2011;178:662–671.
16. Haub S, Ritze Y, Bergheim I, Pabst O, Gershon M, Bischoff S. Enhancement of intestinal inflammation in mice lacking interleukin 10 by deletion of the serotonin reuptake transporter. Neurogastroenterol Motil 2010; 22:826–834.
17. Liévin-Le Moal V, Servin AL. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. Clin Microbiol Rev 2006;19:315–337.
18. Hill DA, Artis D. Intestinal bacteria and the regulation of immune cell homeostasis. Annu Rev Immunol 2009; 28:623–667.
19. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JL. Human nutrition, the gut microbiome and the immune system. Nature 2011;474:327.
20. Manichanh C, Borruel N, Casellas F, Guarner F. The gut microbiota in IBD. Nat Rev Gastroenterol Hepatol 2012; 9:599–608.
21. Kennedy R, Hoper M, Deodhar K, Erwin P, Kirk S, Gardiner K. Interleukin 10-deficient colitis: new similarities to human inflammatory bowel disease. Br J Surg 2000;87:1346–1351.
22. Tsukamoto K, Ariga H, Mantyh C, Pappas TN, Yanagi H, Yamamura T, Takahashi T. Luminally released serotonin stimulates colonic motility and accelerates colonic transit in rats. Am J Physiol Regul Integr Comp Physiol 2007; 293:R64–R69.
23. O’Neil DA, Porter EM, Elewaut D, Anderson GM, Eckmann L, Ganz T, Kagnoff MF. Expression and regulation of the human β-defensins hBD-1 and hBD-2 in intestinal epithelium. J Immunol 1999;163:6718–6724.
24. Kim JJ, Bridle BW, Ghia JE, Wang H, Syed SN, Manocha MM, Rengasamy P, Shajib MS, Wan Y, Hedlund PB. Targeted inhibition of serotonin type 7 (5-HT7) receptor function modulates immune responses and reduces the severity of intestinal inflammation. J Immunol 2013;190:4795–4804.
25. Ke R, Xie X, Li S, Pan Y, Wang J, Yan X, Zang W, Gao L, Li M. 5-HT induces PPARY reduction and proliferation of pulmonary artery smooth muscle cells via modulating GSK-3β/β-catenin pathway. Oncotarget 2017; 8:72910–72920.
26. Liu Y, Tian XY, Mao G, Fang X, Fung ML, Shyy JJ-Y, Huang Y, Wang N. Peroxisome proliferator-activated receptor-γ ameliorates pulmonary arterial hypertension by inhibiting 5-hydroxytryptamine 2B receptor. Hypertension 2012;60:1471–1478.
27. Peyrin-Biroulet L, Beisner J, Wang G, Nuding S, Oommen ST, Kelly D, Parmentier-Decruq E, Dessein R, Merou E, Chavatte P. Peroxisome proliferator-activated receptor gamma activation is required for maintenance of innate antimicrobial immunity in the colon. Proc Natl Acad Sci U S A 2010;107:8772–8777.
28. Han X, Chen C, Cheng G, Liang L, Yao X, Yang G, You P, Shou X. Peroxisome proliferator-activated receptor γ attenuates serotonin-induced pulmonary artery smooth muscle cell proliferation and apoptosis inhibition involving ERK1/2 pathway. Microvasc Res 2015; 100:17–24.

29. Becker C, Watson AJ, Neurath MF. Complex roles of caspases in the pathogenesis of inflammatory bowel disease. Gastroenterology 2013;144:283–293.

30. Gringhuis SI, Kaptein TM, Wevers BA, Theelen B, Van Der Vlist M, Boekhout T, Geijtenbeek TB. Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1β via a noncanonical caspase-8 inflammasome. Nat Immunol 2012;13:246–254.

31. Gurung P, Kanneganti TD. Novel roles for caspase-8 in IL-1β and inflammasome regulation. Am J Pathol 2015; 185:17–25.

32. Monie TP, Bryant CE. Caspase-8 functions as a key mediator of inflammation and pro-IL-1β processing via both canonical and non-canonical pathways. Immuno Rev 2015;265:181–193.

33. Ramirez-Carrozzi V, Sambandam A, Luis E, Lin Z, Jeet S, Lesch J, Hackney J, Kim J, Zhou M, Lai J. IL-17C regulates the innate immune function of epithelial cells in an autocrine manner. Nat Immunol 2011; 12:1159–1166.

34. Yano JM, Yu K, Donaldson GP, Shasti GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 2015;161:264–276.

35. Buttó LF, Hailer D. Dysbiosis in intestinal inflammation: cause or consequence. Int J Med Microbiol 2016; 306:302–309.

36. Sekirov I, Russell SL, Antunes LCM, Finlay BB. Gut microbiota in health and disease. Physiol Rev 2010; 90:859–904.

37. Umbrello G, Esposito S. Microbiota and neurologic diseases: potential effects of probiotics. J Transl Med 2016; 14:298.

38. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JJ. Host-bacterial mutualism in the human intestine. Science 2005;307:1915–1920.

39. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JJ. Host-bacterial mutualism in the human intestine. Science 2005;307:1915–1920.

40. Khan W, Ghia J. Gut hormones: emerging role in immune activation and inflammation. Cln Physiol Behav 2010; 161:19–27.

41. Sharkey KA, Mawe GM. Neuroimmune and epithelial interactions in intestinal inflammation. Curr Opin Pharmacol 2002;2:669–677.

42. Fukumoto S, Tatemaki M, Yamada T, Fujimiya M, Mantyh C, Voss M, Eubanks S, Harris M, Pappas TN, Takahashi T. Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. Am J Physiol Regul Integr Comp Physiol 2003;284: R1269–R1276.

43. Reigstad CS, Salmonson CE, Rainey JF, Szurszewski JH, Linden DR, Sonnenburg JL, Farrugia G, Kashyap PC. Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. FASEB J 2015; 29:1395–1403.

44. Bearcroft C, Perrett D, Farthing M. 5-hydroxytryptamine release into human jejunum by cholera toxin. Gut 1996; 39:528–531.

45. Kidd M, Gustafsson B, Drozdov I, Modlin I. IL1β- and LPS-induced serotonin secretion is increased in EC cells derived from Crohn’s disease. Neurogastroenterol Motil 2009;21:439–450.

46. Lemire P, Robertson SJ, Maughan H, Tatollo I, Streutker CJ, Platnich JM, Muruve DA, Philpott DJ, Girardin SE. The NLR protein NLRP6 does not impact gut microbiota composition. Cell Rep 2017;21:3653–3661.

47. Mamanpoulos M, Ronchi F, Van Hauwermeiren F, Vieira-Silva S, Yilmaz B, Martens L, Saeyes Y, Drexl SK, Yazdi AS, Raes J. Nlrp6- and ASC-dependent inflammasomes do not shape the commensal gut microbiota composition. Immunity 2017;47:339–348.

48. Bishop A, Pietroletti R, Taat C, Brummelkamp W, Polak J. Increased populations of endocrine cells in Crohn’s ileitis. Virchows Arch 1987;410:391–396.

49. Frank DN, Amand ALS, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A 2007;104:13780–13785.

50. Ott S, Musfeldt M, Wenderoth D, Hampe J, Brant O, Fölsch U, Timmis K, Schreiber S. Reduction in diversity of the colonic mucosa associated bacterial microbiota in patients with active inflammatory bowel disease. Gut 2004;53:685–693.

51. Muniz LR, Knosp C, Yeretsgian G. Intestinal antimicrobial peptides during homeostasis, infection, and disease. Frontiers in Immunology 2012;3:310.

52. Wehkamp J, Harder J, Weiichenthal M, Mueller O, Herrlinger KR, Fellermann K, Schroeder JM, Stange EF. Inducible and constitutive β-defensins are differentially expressed in Crohn’s disease and ulcerative colitis. Inflamm Bowel Dis 2003;9:215–223.

53. Guseva D, Holst K, Kaune B, Meier M, Keubler L, Glage S, Buettner M, Bleich A, Pabst O, Bachmann O. Serotonin 5-HT7 receptor is critically involved in acute inflammation and pro-IL-1β processing via extracellular pathogen sensor for the induction and processing of IL-1β via a noncanonical caspase-8 inflammasome. Nat Immunol 2012;13:246–254.

54. Bishop A, Pietroletti R, Taat C, Brummelkamp W, Polak J. Increased populations of endocrine cells in Crohn’s ileitis. Virchows Arch 1987;410:391–396.

55. Frank DN, Amand ALS, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A 2007;104:13780–13785.

56. Ott S, Musfeldt M, Wenderoth D, Hampe J, Brant O, Fölsch U, Timmis K, Schreiber S. Reduction in diversity of the colonic mucosa associated bacterial microbiota in patients with active inflammatory bowel disease. Gut 2004;53:685–693.

57. Muniz LR, Knosp C, Yeretsgian G. Intestinal antimicrobial peptides during homeostasis, infection, and disease. Frontiers in Immunology 2012;3:310.
57. Are A, Aronsson L, Wang S, Greicius G, Lee YK, Gustafsson J-A, Pettersson S, Arulampalam V. Enterococcus faecalis from newborn babies regulate endogenous PPARγ activity and IL-10 levels in colonic epithelial cells. Proc Natl Acad Sci U S A 2008; 105:1943–1948.

58. Kelly D, Campbell JI, Grant G, Jansson EA, Terao S, Chiba T, Mabe K. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-γ and RelA. Nat Immunol 2004;5:104–112.

59. Campieri M, Gionchetti P. Bacteria as the cause of ulcerative colitis. Gut 2001;48:132–135.

60. Ohkusa T, Kato K, Terao S, Chiba T, Mabe K, Kishida T, Nakamura Y. Newly developed antibiotic combination therapy for ulcerative colitis: a double-blind placebo-controlled multicenter trial. Am J Gastroenterol 2010; 105:1820–1829.

61. Moayyedi P, Surette MG, Kim PT, Libertucci J, Wolfe M, Mabe K, Akkermansia muciniphila adheres to enterocytes and strengthens the integrity of epithelial cell layer. Appl Environ Microbiol 2015; 81:3655–3662.

62. Kang CS, Ban M, Choi EJ, Moon HG, Jeon JS, Kim DK, Park SK, Jeon SG, Roh T-Y, Myung SJ. Extracellular vesicles derived from gut microbiota, especially Akkermansia muciniphila, protect the progression of dextran sulfate sodium-induced colitis. PloS One 2013; 8:e76520.

63. Reunanen J, Kainulainen V, Huuskonen L, Ottman N, Belzer C, Huhtinen H, de Vos WM, Satokari R. Akkermansia muciniphila protects against endotoxin-induced colitis. PloS One 2013; 8:e76520.

64. Alam A, Leoni G, Quiros M, Wu H, Desai C, Nishio H, Kim JJ, Shajib MS, Manocha MM, Khan WI. Investigating intestinal inflammation in DSS-induced model of IBD. J Vis Exp 2012;3678.

65. Côté F, Thévenot E, Filgny C, Fromes Y, Darmon M, Ripoche M-A, Bayard E, Hanoun N, Saurini F, Lechat P. Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function. Proc Natl Acad Sci U S A 2003;100:13525–13530.

66. Hedlund P, Danielson P, Thomas E, Slanina K, Carson M, Stearns JC, Davidson CJ, McKeon S, Whelan FJ, Stearns K, J-E.G., L.R., M.S., S.B., M.S.S., and S.B. performed experiments. Y.H.K., H.W., E.D., and W.I.K: conception and design of research. Y.H.K., H.W., E.D., and W.I.K: interpretation of results of experiments. Y.H.K., H.W., E.D., and W.I.K: drafting the manuscript. Y.H.K., S.M.C., M.G.S., and W.I.K. edited and revised manuscript.