Serotonin Has a Key Role in Pathogenesis of Experimental Colitis

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See editorial on page 1562.

BACKGROUND & AIMS: Mucosal changes in inflammatory bowel disease are characterized by ulcerative lesions accompanied by a prominent infiltrate of immune cells as well as alteration in serotonin (5-hydroxytryptamine [5-HT])–producing enterochromaffin cells. We investigated the role of 5-HT in colonic inflammation in mice. METHODS: Colitis was induced with dextran sulfate sodium or dinitrobenzene sulfonic acid in tryptophan hydroxylase 1–deficient (TPH1−/−) mice, which have markedly reduced 5-HT in the gastrointestinal tract, and in mice given the 5-HT synthesis inhibitor parachlorophenylalanine. RESULTS: Delayed onset, decreased severity of clinical disease, and significantly lower macroscopic and histologic damage scores were observed in TPH1−/− mice, compared with wild-type mice, and in mice given parachlorophenylalanine after induction of colitis by dextran sulfate sodium. This was associated with down-regulation of macrophage infiltration and production of proinflammatory cytokines. 5-HT stimulated production of proinflammatory cytokines from macrophages collected from the peritoneal cavity of wild-type mice; this process was inhibited by a nuclear factor κB inhibitor, indicating a critical role for nuclear factor κB signaling in 5-HT–mediated activation of immune cells. Restoration of 5-HT levels in TPH1−/− mice by the 5-HT precursor 5-hydroxytryptophan increased the severity of DSS-induced colitis. We also observed significant reduction in severity of colitis in TPH1−/− mice after induction of dinitrobenzene sulfonic acid–induced colitis. CONCLUSIONS: 5-HT is involved in the pathogenesis of inflammation in experimental colitis. These findings provide insight into the mechanisms of gastrointestinonal inflammation and could lead to new therapeutic strategies for inflammatory disorders.

The inflammatory bowel diseases (IBDs) are idiopathic, chronic, recurrent intestinal disorders of complex pathogenesis that are represented mainly by Crohn’s disease and ulcerative colitis. IBD is the most common and serious chronic inflammatory condition of the human bowel.1,2 Mucosal changes in IBD are characterized by ulcerative lesions accompanied by a prominent infiltrate of activated cells from both the innate and adaptive immune systems. In addition, inflammation in the gut is associated with an alteration in serotonin (5-hydroxytryptamine [5-HT])–producing enterochromaffin (EC) cells. The best characterized subset of gut endocrine cells, which are dispersed throughout the gut mucosa and are the main source of 5-HT in the gut,3–5 5-HT can also be produced by mast cells in rodents, which are also increased in inflammation.6 EC cells produce about 95% of 5-HT in the body and respond to luminal stimuli directly via the enzymes and transporters present on the apical part and indirectly by mediators from the surrounding cells.7 EC cells synthesize 5-HT from its precursor L-tryptophan. Tryptophan hydroxylase (TPH) catalyzes the rate-limiting step in the synthesis of 5-HT from tryptophan and has been detected prominently in EC cells.8 Recent studies show that there are 2 isoforms of TPH enzymes regulating 5-HT synthesis. TPH1 is mainly present in EC cells and spleen, whereas TPH2 is predominantly present in the brainstem and also in myenteric plexus neurons.9

5-HT is an important enteric mucosal signaling molecule influencing gut physiology (motor and secretory function) following inflammation and is considered important in maintaining intestinal homeostasis. Changes in EC cell numbers and in 5-HT content have been reported in association with both Crohn’s disease and ulcerative colitis.10–13 In addition, an alteration in EC cell numbers and in amount of 5-HT is observed in infection-induced inflammation in the gut,14–17 functional disorders such as irritable bowel syndrome,12,18,19 and colon carcinoma.20 The association between alteration in EC

Abbreviations used in this paper: CRP, C-reactive protein; DAI, disease activity index; DNBS, dinitrobenzene sulfonic acid; DSS, dextran sulfate sodium; EC, enterochromaffin; 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan; IL, interleukin; LPS, lipopolysaccharide; MPO, myeloperoxidase; NF-κB, nuclear factor κB; PCPA, parachlorophenylalanine; SERT, serotonin reuptake transporter; TNBS, trinitrobenzene sulfonic acid; TNF, tumor necrosis factor; TPH, tryptophan hydroxylase.

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cell numbers and 5-HT production and various gastrointestinal diseases highly emphasizes the significance of 5-HT in intestinal homeostasis. In the experimental models of colitis induced by trinitrobenzene sulfonic acid (TNBS), dinitrobenzene sulfonic acid (DNBS), and dextran sodium sulfate (DSS), increases in 5-HT content have been observed.\textsuperscript{21–23} By using the DNBS model of colitis, we have shown an amelioration of colonic inflammation in monocyte chemoattractant protein 1-deficient mice in association with a reduction in the numbers of EC cells.\textsuperscript{21} In addition, Bischoff et al.\textsuperscript{24} showed an increase in the severity of TNBS-induced colitis in serotonin reuptake transporter (SERT)-deficient mice in which failure of inactivation of 5-HT due to lack of plasmalemmal SERT increases the availability of 5-HT. Recently we have shown an important immunoendocrine axis in the gut in an enteric infection–induced model of gut inflammation, where secretory products from CD4\textsuperscript{+} T cells interact with EC cells or their precursors to enhance 5-HT production in the gut.\textsuperscript{17} In addition, we observed that EC cell and 5-HT responses in inflammation are influenced by the immunologic profile of the inflammatory response.\textsuperscript{25} Although these studies have shown an association between EC cells/5-HT content and intestinal inflammation, it is not clear whether 5-HT by itself has any role in regulating gut inflammation. Nonetheless, due to the strategic location of EC cells in gut mucosa, it is very likely that 5-HT plays an important role in immune activation in relation to gut pathology and pathophysiology in various gastrointestinal disorders, including IBD.

In the present study, we investigated the role of 5-HT in the development of colonic inflammation in 2 different models of experimental colitis using TPH1-deficient (TPH1\textsuperscript{−/−}) mice, which have a markedly reduced amount of 5-HT in the gut, and mice treated with the 5-HT synthesis inhibitor parachlorophenylalanine (PCPA). To gain further mechanistic insight, we conducted in vitro studies on the production of proinflammatory cytokines from macrophages after 5-HT stimulation with or without the presence of nuclear factor \( \kappa \) B (NF-\( \kappa \)B) inhibitor.

We show that 5-HT plays a critical role in the generation of inflammation in both DSS- and DNBS-induced colitis and that 5-HT is critical in the pathogenesis of colitis in these models.

\textbf{Figure 1.} Effects of lack of 5-HT in the gut in the development of DSS-induced colitis. TPH1\textsuperscript{+/+} and TPH1\textsuperscript{−/−} mice were given 5\% DSS solution in the drinking water to induce colitis. Control mice received water without DSS. (A) DAI. (B) Macroscopic damage score in DSS-induced colitis on day 5 after DSS-induced colitis and in mice without colitis. (C) Histologic scores on day 5 after administration of DSS. (D) (a) Light micrograph of an H&E-stained colonic section from a TPH1\textsuperscript{+/+} mouse on day 5 after administration of DSS. (b) Light micrograph of an H&E-stained colonic section from a TPH1\textsuperscript{−/−} mouse on day 5 after administration of DSS. Original magnification 10\( \times \). Each value represents the mean ± SEM from 12 mice. *Significantly lower (\( P < .05 \)) in DSS-treated TPH1\textsuperscript{−/−} mice compared with DSS-treated TPH1\textsuperscript{+/+} mice.
Materials and Methods

Animals

TPH1−/− mice on C57BL/6 background were originally produced by gene mutation as described by Côté et al.26 Briefly, exon 2 of the TPH1 locus has been substituted by the nlslacZneo/polyA 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 from wild-type mice. Breeding pairs of TPH1−/− mice and their wild-type (TPH1+/+) littermates were obtained from Université Pierre et Marie Curie, CNRS, France, and were kept and bred under specific pathogen-free conditions. C57BL/6 were purchased from Taconic Farms Suppliers (Albany, NY). All experiments were approved by the McMaster University animal ethics committee and conducted under the Canadian guidelines for animal research.

Induction of DSS and DNBS Colitis

DSS (mol wt, 40 kilodaltons; ICN Biomedicals Inc, Soho, OH) was added to the drinking water in a final concentration of 5% (wt/vol) for 5 days.25 Mean DSS consumption was noted per cage each day. For the DNBS study, mice were anesthetized with isoflurane (Abbott, Toronto, Canada). A 10-cm-long PE-90 tubing (ClayAdams, Parsippany, NJ), attached to a tuberculin syringe, was inserted 3.5 cm into the colon. Colitis was induced by administration of 100 μL of 5 mg of DNBS solution (ICN Biomedicals Inc) in 50% ethanol and left for 3 days.23 Mice with colitis were supplied with 6% sucrose in drinking water to prevent dehydration.

Experimental Protocol

TPH1−/− and TPH1+/+ mice were exposed to DSS (5%) for 5 days. In separate experiments, TPH1−/− mice were treated with PCPA (Sigma–Aldrich, Mississauga, Ontario, Canada) and TPH1+/+ mice were given 5% DSS solution in the drinking water to induce colitis and killed on day 5 after administration of DSS. (A) MPO activity, (B) serum CRP levels, (C) TNF-α levels in colonic tissues, (D) IL-1β levels in colonic tissues, and (E) IL-6 levels in colonic tissues. Each value represents the mean ± SEM from 12 mice. *Significantly lower (P < .05) in DSS-treated TPH1−/− mice compared with DSS-treated TPH1+/+ mice.

Figure 2. Effects of the lack of 5-HT on MPO activity, CRP, and proinflammatory cytokines (TNF-α, IL-1β, and IL-6) in DSS-induced colitis. TPH1−/− and TPH1−/− mice were given 5% DSS solution in the drinking water to induce colitis and killed on day 5 after administration of DSS. (A) MPO activity, (B) serum CRP levels, (C) TNF-α levels in colonic tissues, (D) IL-1β levels in colonic tissues, and (E) IL-6 levels in colonic tissues. Each value represents the mean ± SEM from 12 mice. *Significantly lower (P < .05) in DSS-treated TPH1−/− mice compared with DSS-treated TPH1+/+ mice.
Canada) or with vehicle (saline) intraperitoneally at a dosage of 300 mg/kg per day for 6 days starting 1 day before the induction of colitis. As previously described, 5-hydroxytryptophan (5-HTP) (Sigma–Aldrich) was administered subcutaneously at a dosage of 50 mg/kg twice a day for 8 days starting 3 days before the induction of colitis. Control mice received saline as vehicle. Mice were exposed to DNBS (5 mg) for 3 days.

**Evaluation of Inflammation**

Disease activity index (DAI), macroscopic scores, and colonic damage were assessed using a previously described scoring system for DSS-induced and DNBS-induced colitis. Formalin-fixed colon segments were stained with H&E and scored. Myeloperoxidase (MPO) activity was determined following an established protocol.

**C-Reactive Protein Assay in Serum**

Blood was collected 5 or 3 days after the beginning of DSS or DNBS treatment, respectively, by intracardiac puncture in anesthetized (isoflurane) mice. C-reactive protein (CRP) levels were determined using an enzyme-linked immunosorbent assay commercial kit (R&D Systems, Minneapolis, MN).

**Cytokine Tissue**

Colonic samples were prepared as previously described. Cytokine levels (interleukin [IL]-1β, IL-6, tumor necrosis factor [TNF]-α) were determined using an enzyme-linked immunosorbent assay commercial kit (R&D Systems).

**Table 1. Effects of PCPA Treatment on the Inflammatory Markers in DSS-Induced Colitis in Wild-type (TPH1+/−) Mice**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (saline)</th>
<th>PCPA (300 mg/kg)</th>
</tr>
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<tbody>
<tr>
<td>MPO (U/mg tissue)</td>
<td>2.25 ± 0.88</td>
<td>0.93 ± 0.14</td>
</tr>
<tr>
<td>CRP (ng/mL serum)</td>
<td>31.34 ± 2.12</td>
<td>22.22 ± 1.69</td>
</tr>
<tr>
<td>TNF-α (pg/mg protein)</td>
<td>17.35 ± 6.95</td>
<td>1.88 ± 1.88</td>
</tr>
<tr>
<td>IL-6 (pg/mg protein)</td>
<td>58.45 ± 18.24</td>
<td>5.00 ± 0.68</td>
</tr>
<tr>
<td>IL-1β (pg/mg protein)</td>
<td>39.26 ± 4.98</td>
<td>16.96 ± 3.19</td>
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NOTE. Mice were given 5% DSS solution in the drinking water to induce colitis in parallel to continued PCPA (300 mg/kg per day) or vehicle (saline) treatment and were killed on day 5 after administration of DSS to assess MPO activity, serum CRP level, and proinflammatory cytokines TNF-α, IL-1β, and IL-6. The values are shown as means ± SEM from 12 mice.

*Significantly different compared with vehicle saline.
Determination of 5-HT Level

Determination of 5-HT levels was performed 5 or 3 days after the beginning of DSS or DNBS treatment, respectively, following a previous method.25 Full-thickness specimens were taken from the colon; after extraction, the 5-HT content in the supernatant was analyzed by enzyme immunoassay using a commercially available kit (Beckman Coulter, Fullerton, CA). The 5-HT content

Figure 4. Effects of 5-HTP treatment in DSS-induced colitis. TPH1+/− and TPH1−/− mice were given 5% DSS solution in the drinking water to induce colitis in parallel to continued 5-HTP (50 mg/kg twice a day) or vehicle (saline) treatment. (A) 5-HT-expressing EC cells on day 5 after administration of DSS. (B) DAI in 5-HTP− and vehicle (saline)-treated mice. (C) Macroscopic scores on day 5 after administration of DSS. (D) Histologic scores on day 5 after administration of DSS. (E) (a) Light micrograph of an H&E-stained colonic section from a 5-HTP−treated TPH1+/− mouse on day 5 after administration of DSS and (b) from a 5-HTP−treated TPH1−/− mouse on day 5 after administration of DSS. Original magnification 10×. Each value represents the mean ± SEM from 10 mice. *Significantly higher in 5-HTP-treated mice compared with vehicle-treated mice.
of the tissue was expressed as a function of wet weight (nanogram per milligram tissue).

**Colonic Immunohistochemistry and Culture of Peritoneal Macrophages**

5-HT–expressing EC cells and F4/80-expressing macrophages in colonic sections were stained according to previously reported methods. For macrophage culture, resident peritoneal cells were collected and cultured as described previously. Macrophage cultures were then exposed to 5-HT (10\(^{-11}\) mol/L) (Sigma–Aldrich) with or without the presence of lipopolysaccharide (LPS) (100 ng/mL; Sigma–Aldrich), which acts as an inhibitor of NF-κB activation. Supernatants were collected after 24 hours for assessment of IL-1β and IL-6 amounts by enzyme-linked immunosorbent assay.

**Statistical Analysis**

Results are presented as means ± SEM. Statistical analysis was performed using one-way or 2-way analysis of variance followed by Student–Newman–Keuls multiple comparisons post hoc analysis, and a P value of <.05 was considered significant.

**Results**

**Reduced Availability of 5-HT in the Gut Decreases the Severity of DSS-Induced Colitis**

Administration of DSS in wild-type (TPH1\(^{+/+}\)) mice induced colitis characterized by weight loss and frequent stools, which was evident by day 3 post-DSS. In TPH1\(^{-/-}\) mice, the onset of DSS-induced colitis was delayed, as reflected in the DAI shown in Figure 1A. The DAI was significantly lower in TPH1\(^{-/-}\) mice compared with TPH1\(^{+/+}\) mice on days 3, 4, and 5 after DSS treatment. In addition, the macroscopic and histologic scores were significantly decreased in TPH1\(^{-/-}\) mice as compared with those in TPH1\(^{+/+}\) mice on day 5 after DSS treatment (Figure 1B–D). This reduction in the severity of DSS-induced colonic inflammation in TPH1\(^{-/-}\) mice was associated with down-regulation in MPO activity in colonic tissues, serum CRP level, and the amount of proinflammatory cytokines (TNF-α, IL-1β, and IL-6) in the colon (Figure 2).

To further validate the role of 5-HT in DSS-induced colitis, we examined whether interfering with 5-HT synthesis in the gut with a pharmacologic agent can modulate DSS-induced colonic inflammation. Treatment with PCPA (300 mg/kg intraperitoneally) in TPH1\(^{+/+}\) mice significantly decreased the level of 5-HT in the colon (5-HT amount was 28.2 ± 4.4 ng/mg of tissue and 17.67 ± 1.62 ng/mg of tissue in TPH1\(^{+/+}\) mice that received vehicle and PCPA, respectively; \(P < .05\)). This depletion of 5-HT in the gut significantly reduced the macroscopic score and histologic damage score in TPH1\(^{+/+}\) mice as compared with that in saline-treated TPH1\(^{+/+}\) mice with DSS-induced colitis (Figure 3). This decrease in severity of DSS-induced colitis by PCPA treatment was also associated with significant down-regulation of MPO activity, CRP level, and TNF-α, IL-6, and IL-1β amounts (Table 1).

| Table 2. Effects of 5-HT Precursor 5-HTP Treatment on the Inflammatory Markers in DSS-Induced Colitis in Wild-type (TPH1\(^{+/+}\)) and TPH1\(^{-/-}\) Mice |
|------------------|------------------|------------------|------------------|------------------|
|                  | TPH1\(^{+/+}\)   |                  | TPH1\(^{-/-}\)   |                  |
|                  | Vehicle          | 5-HTP            | Vehicle          | 5-HTP            |
| MPO (U/mg tissue)| 2.25 ± 0.88      | 2.52 ± 0.12\(^a\)| 0.83 ± 0.34      | 2.39 ± 0.49\(^a\)|
| CRP (ng/mL serum)| 31.34 ± 2.12     | 36.53 ± 1.02\(^a\)| 20.34 ± 1.47     | 43.08 ± 8.55\(^b\)|
| TNF-α (pg/mg protein)| 17.35 ± 6.95 | 24.3 ± 1.75\(^a\)| 0.75 ± 0.35      | 20.6 ± 1.61\(^a\)|
| IL-6 (pg/mg protein)| 58.45 ± 18.24 | 82.5 ± 7.34\(^a\)| 5.35 ± 1.24      | 45.35 ± 4.93\(^b\)|
| IL-1β (pg/mg protein)| 39.26 ± 4.98 | 50.2 ± 5.3\(^a\)| 7.25 ± 2.10      | 55.02 ± 9.16\(^b\)|

**Administration of 5-HT Precursor Enhances the Severity of DSS-Induced Colitis in Mice Lacking 5-HT**

To substantiate whether the attenuated inflammation in DSS-induced colitis in TPH1\(^{-/-}\) mice was in fact due to the lack of 5-HT, we restored 5-HT amount in TPH1\(^{-/-}\) mice by administration of 5-HTP, the product of TPH1 and an immediate precursor of 5-HT, and examined the effect on DSS-induced colitis. Administration of 5-HTP enhanced the severity of DSS-induced colitis in mice lacking 5-HT in the gut. We observed significantly higher numbers of 5-HT–expressing EC cells from TPH1\(^{-/-}\) mice treated with 5-HTP (50 mg/kg subcutaneously) compared with those treated with saline (Figure 3).
4A). Consistent with increased 5-HT–expressing EC cells, we also observed significantly higher amounts of 5-HT in the colon of TPH1−/− mice treated with 5-HTP (5-HT amount was 1.4 ± 0.8 ng/mg and 25.59 ± 1.7 ng/mg of tissue in TPH1−/− mice that received saline and 5-HTP, respectively; *P < .05). Investigations on the effect of 5-HTP treatment in TPH1−/− mice with DSS-induced colitis revealed a significant increase in the DAI during the 4 last days of DSS-induced colitis (Figure 4B) in TPH1−/− mice that received 5-HTP as compared with mice that received saline. 5-HTP treatment also significantly increased the macroscopic and

**Figure 5.** Macrophage staining and accumulation of F4/80+ cells in DSS-induced colitis in TPH1+/+ and TPH1−/− mice. Colitis was induced by DSS (5%), and F4/80+ cells were evaluated on day 5 after the induction of colitis. (A) F4/80+ area. Data are expressed as the percentages of the positive staining areas of the total area. Each bar represents the mean ± SEM. (A) Significantly lower (P < .05) in TPH1−/− mice compared with TPH1+/+ mice after administration of DSS. (B) (a) F4/80 immunostaining of colon tissue of TPH1+/+ mice on day 5 after administration of DSS. (b) F4/80 immunostaining of colon tissue of TPH1−/− mice on day 5 after administration of DSS. (C) Effect of 5-HT stimulation on IL-1β and IL-6 production from macrophages in vitro. Peritoneal macrophages (3 × 105 cells/mL) were harvested from wild-type (TPH1+/+) mice and cultured with medium or with 5-HT (10−10 mol/L) with or without the presence of LPS (100 ng/mL) and with or without the presence of NF-κB inhibitor (10 µmol/L). The levels of IL-1β present in the culture supernatant were investigated by enzyme-linked immunosorbent assay. *Significantly higher compared with medium-treated cells and #significantly different between the groups tested.
histologic damage scores in DSS-induced colitis in TPH1−/− mice compared with TPH1+/− mice that were not treated with 5-HTP (Figure 4C and D). This was associated with an up-regulation of MPO activity and CRP level and an increase in TNF-α, IL-6, and IL-1β amounts in 5-HTP-treated TPH1−/− mice with DSS-induced colitis (Table 2).

5-HT Regulates Macrophage Infiltration in DSS-Induced Colitis and Stimulates Production of Proinflammatory Cytokines

Macrophages are critical in many immune responses, including those associated with IBD. To elucidate the mechanism by which 5-HT influences the development of colitis, we next investigated the role of 5-HT in macrophage function in relation to gut inflammation. First we assessed macrophage infiltration in the colonic wall in TPH1+/+ and TPH1−/− mice in DSS-induced colitis by immunostaining of F4/80+ macrophages in colonic tissues. In TPH1+/+ mice, there was massive transmural infiltration of F4/80+ macrophages after induction of DSS colitis. As shown in Figure 5A and B, F4/80+ cells were evident in mucosa, submucosa, and muscle layers. In contrast, there were significantly fewer F4/80+ macrophages in TPH1−/− mice with DSS-induced colitis. There was no significant difference in F4/80 staining between control TPH1+/+ and TPH1−/− mice (data not shown). These findings show a significant down-regulation of macrophage infiltration in DSS-induced colitis in TPH1−/− mice.

The role of 5-HT in macrophage function in inflammation was further studied by examining the ability of 5-HT to stimulate macrophages to produce proinflammatory cytokines by harvesting macrophages from the peritoneal cavity of TPH1+/+ mice and assessing IL-1β and IL-6 production after LPS stimulation. We observed significantly higher amounts of IL-1β and IL-6 in the culture supernatant of LPS-stimulated macrophages after incubation with 5-HT (Figure 5C). Addition of NF-κB inhibitor in the culture media significantly decreased the production of 5-HT-stimulated proinflammatory cytokines by the macrophages in response to LPS. Per se 5-HT alone significantly increased the levels of the 2 cytokines, and this effect was inhibited by the addition of NF-κB inhibitor.

Reduced Availability of 5-HT in the Gut Decreases the Severity of DNBS-Induced Colitis

To determine whether the previously described changes were restricted to the DSS-based model, we performed studies in the DNBS-based model of experimental colitis. There was a significant reduction in the severity of colonic inflammation in TPH1−/− mice as compared with that in TPH1+/+ mice in DNBS-induced colitis. Macroscopic evaluation of the colon of TPH1+/+ mice after administration of DNBS revealed massive ulceration, thickening of the colonic wall, hyperemia, and severe adhesions between the colon and other organs. There was a significant increase in macroscopic damage in TPH1+/+ mice on day 3 after administration of DNBS. In contrast, TPH1−/− mice after DNBS administration developed significantly less mucosal damage, less thickening of the colonic wall, and fewer adhesions (Figure 6A). Histologic examination in TPH1+/+ mice in DNBS-induced colitis revealed an intense granulocyte infiltrate in the mucosa and submucosa, often involving the muscularis propria. There was also marked mucosal damage associated with goblet cell depletion. The microscopic score of TPH1−/− mice was significantly less as compared with that in TPH1+/+ mice on day 3 post-DNBS, and this was accompanied by less mucosal damage, goblet cell depletion, and cellular infiltration into the mucosa and submucosa (Figure 6B and C).

Reduced macroscopic and microscopic scores in TPH1−/− mice were associated with a down-regulation of MPO activity and IL-1β amount in colonic tissues. We observed significantly less MPO activity and IL-1β amount after DNBS administration in TPH1−/− mice as compared with that in TPH1+/+ mice (Figure 6D and E).

Discussion

Inflammation of the gut has traditionally been viewed as a process in which activated immune cells cause the destruction of other mucosal cells that behave as passive bystander targets. Progress in understanding the process of intestinal inflammation has led to a much broader and more integrated picture of the various mucosal components, a picture in which epithelial, endocrine, and nerve cells display an active role in gut inflammation. Mucosal inflammation in conditions ranging from infective acute enteritis or colitis to IBD is accom-
Gut inflammation is characterized by mucosal recruitment of macrophages. Macrophages are a major source of proinflammatory cytokines IL-1β, IL-6, and TNF-α and perform a key role in activation of immune response and generation of gut inflammation. Serotoninergic receptors have been characterized in immune cells, including macrophages. Due to the strategic location of EC, it is likely that 5-HT may play an important role in inflammation and activation of macrophages in the context of gut inflammation. In this study, we observed a higher amount of IL-1β and IL-6 in the culture supernatant of macrophages harvested from the peritoneal cavity of mice following 5-HT stimulation with or without LPS stimulation. This 5-HT-stimulated production of proinflammatory cytokines by macrophages was inhibited by the addition of NF-κB inhibitor, implying a crucial role of NF-κB signaling in this hormone-mediated activation of immune cells. These findings of 5-HT-mediated macrophage function corroborate the study of Freire-Garabal et al., in which it was shown that 5-HT up-regulates phagocytic activity of macrophages through the 5-HT1A receptor via the NF-κB signaling pathway. These findings, along with the observations of attenuation in MPO activity and serum CRP level, suggest that 5-HT has an important role in the generation of colonic inflammation by regulating the infiltration of inflammatory cells such as macrophages and the production of proinflammatory mediators in the colon. In addition to the role of 5-HT in macrophage function, recently we also observed that reduced severity of colitis in TPH1−/− mice is associated with the impaired ability of dendritic cells to produce proinflammatory cytokines and sequential activation of CD4+ cells (data not shown). Taken together, these observations suggest that 5-HT plays an important role in gut inflammation by influencing the production of cytokines from immune cells.

We also investigated the role of 5-HT in a DNBS-based model of experimental colitis to see whether the role of 5-HT seen in the DSS model is specific for this model. DNBS-induced colitis is a well-characterized Th1-driven transmural inflammation of the colon and may be considered a model of Crohn’s disease, which is also characterized by transmural inflammation and with the development of a Th1-type immune response. Similar to DSS-induced colitis, we observed significant attenuation in colonic inflammation in TPH1−/− mice after DNBS administration. The amelioration of DNBS-induced colonic inflammation in TPH1−/− mice was observed in macroscopic and microscopic indexes as well as MPO activity and IL-1β production.

Taken together, the results of the present study reveal a novel function of 5-HT in regulation of gut inflammation in relation to the recruitment of inflammatory cells...
and activation of proinflammatory cytokine production. Utilizing a unique genetic model of 5-HT deficiency in the gut and adopting a strategy to inhibit 5-HT synthesis by a pharmacologic agent, we provide evidence that lack of 5-HT causes reduction in the severity of inflammation in experimental colitis and this reduction can be inverted by reconstitution of 5-HT synthesis in the gut. In addition, this study revealed an NF-κB–dependent molecular mechanism of 5-HT–mediated activation of immune cells in the context of inflammation. Alteration in 5-HT signaling in response to chemical stimuli such as DSS or DNBS can take part in generation of inflammation in the gut by influencing the infiltration and activation of immune cells and by increasing production of inflammatory mediators. In addition to enhancing our understanding of the pathogenesis of experimental colitis, this study provides novel information on 5-HT in the context of immunoenocrine interactions in the gut and in intestinal homeostasis. This is very important not only due to the alteration in 5-HT amount observed in various intestinal inflammatory conditions but also in light of serious and unforeseen complications caused by the use of 5-HT receptor agonists and antagonists in clinical practice. Recently it was also shown that 5-HT deficiency causes slower growth of colon cancer allografts in vivo, and more importantly these results are consistent with a recent study that showed up-regulation of inflammation in SERT–/– mice, which exhibit elevated mucosal 5-HT availability. In SERT–/– mice, markedly enhanced severity of colitis both macroscopically and histologically together with an increased mortality was observed after TNBS administration as compared with wild-type mice, and this was correlated with an up-regulation of MPO activity. It has been shown that SERT expression is down-regulated in TNBS-induced colitis and in rectal biopsy specimens of patients with ulcerative colitis. Thus, the reduction in SERT resulting in enhancement of 5-HT availability can contribute to the up-regulation of intestinal inflammatory process. Our present data in TPH1–/– mice and the study in SERT–/– mice together provide strong evidence in favor of a proinflammatory role for 5-HT. In a wider context, because a beneficial effect with treatment with a 5-HT receptor antagonist has been shown in both clinical and experimental arthritis, these data may have implications in understanding the role of gut hormone in the pathogenesis of nongastrointestinal inflammatory diseases such as arthritis, which may ultimately lead to improved therapeutic strategies in both gastrointestinal and nongastrointestinal inflammatory disorders.

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Conflicts of interest
The authors disclose no conflicts.

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