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Colitis Induces Enteric Neurogenesis Through a 5-HT₄–dependent Mechanism

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Abstract

Background: The intestine is known to contain enteric neuronal progenitors, but their precise identity and the mechanisms that activate them remain unknown. Based on the evidence for the neurogenic role of serotonin (5-HT) in the postnatal gut and the observation of enteric neuronal hyperplasia in inflammatory bowel disease, we hypothesized that colitis induces a neurogenic response through 5-HT₄ receptor signaling.

Methods: We examined the effects of 5-HT₄ agonism on colonic neurogenesis and gliogenesis in vitro and in vivo in adult mice using dextran sodium sulfate to experimentally induce colitis.

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Results: In vitro, 5-HT₄ agonism led to increased neuronal proliferation and density. Induction of experimental colitis in vivo similarly resulted in increased numbers of myenteric neurons, and this was inhibited by 5-HT₄ antagonism. Interestingly, both in vitro and in vivo, 5-HT₄ signaling increased glial cell proliferation but did not increase glial cell numbers, leading us to hypothesize that glia may give rise to neurons. After induction of colitis in normal, Nestin-GFP and Sox2-GFP transgenic mice, it was revealed that multiple glial markers (Sox2, Nestin, and CD49b) became strongly expressed by enteric neurons. Immunoselected enteric glia were found to give rise to neurons in culture, and this was inhibited in the presence of 5-HT₄ blockade. Finally, isolated glia gave rise to a neuronal network upon transplantation into aganglionic embryonic avian hindgut.

Conclusions: These results show that colitis promotes enteric neurogenesis in the adult colon through a serotonin-dependent mechanism that drives glial cells to transdifferentiate into neurons.

Keywords

enteric nervous system; neurogenesis; enteric glial cells; serotonin; colitis

The enteric nervous system (ENS) is comprised of enteric neurons and glial cells that regulate multiple aspects of gastrointestinal function, including motility, absorption, and secretion. Throughout life, this neuroglial network is repeatedly subject to injury from infections, toxins, mechanical stress, and aging.¹⁻³ Little is known about how the ENS responds to injury and whether it is able to generate new enteric neurons to compensate for those that are injured or lost. In the setting of inflammatory bowel disease in humans, enteric neuronal hyperplasia has been described,^{4,5} suggesting that the mature gut is capable of generating new neurons. Recently, evidence supports this regenerative capacity^{6,7} but the stimuli that induce enteric neurogenesis remain elusive, and the underlying mechanisms are largely unknown.

In rodents, constitutive neurogenesis continues during early postnatal life but then stops.⁸ Interestingly, enteric neuronal stem/progenitor cells (ENSCs) remain present in the adult mouse and human intestine.⁹⁻¹³ However, they do not give rise to new neurons under steady-state conditions in vivo. Adult enteric neurogenesis has only been demonstrated after specific perturbations, and even this has been inconsistently observed. Neurogenesis has been demonstrated after chemical ablation of myenteric neurons with benzalkonium chloride,^{7,14} suggesting that the role of adult ENSCs may be to respond to injury and replace injured or absent neurons. However, a recent study failed to detect neurogenesis despite a variety of insults, including benzalkonium chloride.¹⁵

Serotonin (5-HT) has been shown to promote adult enteric neurogenesis by signaling through the 5-HT₄ receptor.^{6,16} However, whether 5-HT mediates injury-induced neurogenesis is unknown. Moreover, although the cellular origin of newly born enteric neurons remains uncertain, recent evidence^{7,15} suggests that they may arise from enteric glia. These findings are reminiscent of the neurogenic potential of radial glia in the central nervous system^{17,18} and in the carotid body, where glial-like cells give birth to neurons after hypoxia.¹⁹ Understanding the mechanisms that govern the birth of new neurons in the adult gut has important therapeutic implications for altering the pathologic response of ENS injury

in gastrointestinal disease and also for harnessing that neurogenic potential to replace damaged or missing enteric neurons.

In this study, we show that colitis stimulates enteric neurogenesis in vivo, and that this effect is abrogated in the presence of a serotonin 5-HT₄ receptor antagonist. Interestingly, glial, but not neuronal, proliferation is dramatically increased in the setting of colitis, yet total glial cell numbers do not increase. We find that enteric glial cells are able to generate neurons both in vitro and in vivo through a 5-HT₄-dependent mechanism. These results suggest that colonic inflammation, acting through 5-HT₄ signaling, can stimulate glia to undergo neurogenesis, shedding light on the pathophysiology of inflammatory bowel diseases and offering new possibilities for modifying the ENS response to injury and potentially for replacing absent or abnormal neurons in neurointestinal diseases.

MATERIALS AND METHODS

Isolation and Expansion of ENSCs

All animal experiments were performed with institutional approval. C57BL/6 mice were killed on postnatal day 7 to 21 (P7–21), and the dissected colon was dissociated with dispase (250 µg/mL; StemCell Technologies, Vancouver, BC, Canada) and collagenase XI (1 mg/mL; Sigma Aldrich, St. Louis, MO) at 37°C for 1 hour with gentle pipetting. The cell suspension was passed through a 40-µm cell strainer and cultured at a density of 50,000 cells per milliliter of NeuroCult proliferation medium (StemCell Technologies) containing 20 ng/mL epidermal growth factor (StemCell Technologies) and 10 ng/mL basic fibroblast growth factor (bFGF; StemCell Technologies) for 7 to 10 days on fibronectin-coated dishes to form neurosphere-like bodies (NLBs). NLBs were dissociated with Accutase (StemCell Technologies) at 37°C for 30 minutes with gentle pipetting. The cell suspension was passed through a 40-µm cell strainer and plated on fibronectin-coated glass-bottom chamber slides (Biomedical Technologies, Ward Hill, MA). Cells were cultured for 7 days in neural differentiation medium (NeuroCult; Stem-Cell Technologies), then processed for immunohistochemistry. 5-HT₄ receptor agonism or antagonism was achieved using 100 nM RS67506 (Tocris Bioscience, Bristol, United Kingdom) or 100 nM GR125487 (Tocris Bioscience), respectively.

Cell Proliferation and Apoptosis

To detect cell proliferation, dissociated NLBs were cultured in the presence of 10 µM 5-ethynyl-2-deoxyuridine (EdU) for 24 hours. EdU incorporation was detected using the Click-iT EdU Imaging Kit (Invitrogen, Carlsbad, CA). Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay was used to detect apoptosis, using the in situ cell death fluorescein detection kit (Roche Diagnostics, Penzberg, Germany).

Isolation of CD49-immunoreactive Cells

The longitudinal muscle–myenteric plexus layer was harvested from the small and large intestine of adult C57BL/6 mice, minced with scissors, and dissociated enzymatically with dispase (250 µg/mL), collagenase XI (1 mg/mL), and DNase I (1 mg/mL; Sigma Aldrich).

Cells were stained with Alexa Fluor 647 conjugated anti-mouse CD49b and with the following lineage (Lin) markers: phycoerythrin-conjugated anti-mouse CD31, anti-mouse CD45, and anti-mouse TER-119 antibodies (BioLegend, San Diego, CA). Flow cytometry was performed on an FACS Vantage SE/DiVa SORP (BD Biosciences, San Jose, CA). Purified CD49b⁺/lin⁻ cells were cultured for 5 to 7 days on fibronectin-coated glass chamber slides at 40,000 cells per milliliter in serum-free neural differentiation medium (Neurocult; StemCell Technologies).

In Vivo Colitis

Colitis was induced by adding 3% dextran sulfate sodium (DSS; Reagent Grade Cat. #160110, mol weight 40,000; MP Biomedicals, Santa Ana, CA) to the drinking water of 4 month-old C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) for 7 days. For mice receiving 5-HT₄ antagonist, 100 nM GR125487 was added to the drinking water during the 7 days of DSS treatment and for 48 hours after completion. Nestin-GFP transgenic mice²⁰ on the C57BL/6 genetic background obtained as a generous gift from Jorg Dietrich's laboratory were used. C57BL/6 wild-type mice were used as the control. Mice were killed 48 hours after colitis and the distal colon examined by immunohistochemistry. Sox-GFP transgenic mice²¹ were obtained as a generous gift from the Konrad Hochedlinger laboratory and similarly were killed 48 hours after DSS colitis.

Cell Proliferation In Vivo

Intraperitoneal injection with 50 mg/kg EdU was performed every 48 hours during DSS administration, every 12 hours for the next 48 hours, and 1 and 2 hours before killing. The distal colon was removed, fixed in 10% formalin, and EdU incorporation detected using the ClickiT EdU Imaging Kit as above.

Clinical Phenotype and Histologic Scoring of Colitis

Mice were weighed weekly and occult blood in the stool tested using the Hemocult system (SmithKline Diagnostics, San Jose, CA). Fecal water content was calculated as follows: $([\text{wet stool weight} - \text{dry stool weight}]/\text{wet stool weight}) \times 100$. A blinded reader scored the severity of colitis on H&E-stained slides using a grading scale modified from Mizoguchi.²² A mean colitis score (range, 0–6) was calculated based on 20 random fields covering the entire colon.

Immunohistochemistry

Immunohistochemistry was performed as previously described.¹³ The following primary antibodies were used: rabbit anti-Nestin (1:200; Abcam, Cambridge, MA), mouse anti-Tuj1 (1:100; Covance, Dedham, MA), goat anti-glial fibrillary acidic protein (GFAP) (1:500; Abcam), rabbit anti-CD49b (1:100; Novus Biologicals, Littleton, CO), mouse anti-HuC/D (1:100; Molecular Probes, Carlsbad, CA) rabbit anti-Sox10 (1:500; Abcam), and CN (chick neuron antibody) was kindly provided by Dr. Hideaki Tanaka (Kumamoto University, Japan).²³ Secondary antibodies included: goat anti-mouse immunoglobulin G Alexa Fluor 546, goat anti-rabbit Alexa Fluor 488, donkey anti-goat Alexa Fluor 488, donkey anti-goat

Alexa Fluor 546, and donkey anti-mouse Alexa Fluor 546, all from Invitrogen. Cell nuclei were stained with DAPI (Vector Labs, Burlingame, CA).

Murine CD49b glial transplants to aneural chick hindgut. Immunoselected CD49b⁺ glia were cultured at a density of 20,000 cells per well in 96-well chambers. The cells formed NLBs when grown in Neurocult proliferation medium (StemCell Technologies) containing 20 ng/mL epidermal growth factor (StemCell Technologies) and 10 ng/mL basic fibroblast growth factor (bFGF; StemCell Technologies) for 7 days. The NLBs were stained with DiI (Life technologies, Grand Island, NY) before transplantation.

Chick hindgut was removed on embryonic day 5 (E5) before colonization by enteric neural crest cells. One or 2 NLBs were implanted into the wall of the proximal hindgut using fine forceps under microscopic visualization. The gut was then placed onto the chorioallantoic membrane of an E9 host chick embryo. After 7 to 10 days on the chorioallantoic membrane, the gut was removed and processed for immunohistochemistry.

Statistical Methods

Data are expressed as mean $\pm$ SD. Paired *t* tests were used to evaluate the statistical significance between 2 groups. When more than 2 groups were compared, mean values were statistically compared by one-way analysis of variance with Tukey post hoc multiple comparison tests of differences. Statistical significance was considered at a cutoff of $P < 0.05$.

RESULTS

5-HT₄ Receptor Agonism Promotes Enteric Neurogenesis In Vitro

NLBs were prepared from 1 to 3 week-old mice. After dissociation, the cells were cultured in differentiation conditions in the presence or absence of the 5-HT₄ receptor agonist, RS67506 (RS). Neurons and glial cells were detected by immunoreactivity to Tuj1 and GFAP, respectively. In control conditions, $4.2\% \pm 1.0\%$ of ENSCs gave rise to neurons, whereas neuronal density increased to $15.1\% \pm 7.0\%$ ($P < 0.01$) in the presence of RS (Fig. 1A, C). To determine whether the RS-mediated increase in neuronal density was associated with increased neuronal cell proliferation, the incorporation of the thymidine analog, EdU, by ENSCs was measured. In the presence of RS, significantly more Tuj1⁺ enteric neurons incorporated EdU as compared with control (Fig. 1D, F; $25.1\% \pm 3.5\%$ versus $10.2\% \pm 2.2\%$).

The presence of 5-HT₄ agonism also significantly increased the rate of GFAP⁺ glial cell proliferation (Fig. 1F), but this was not associated with an increase in glial cell density (Fig. 1C), raising the question of whether glial cell apoptosis was occurring. Using a TUNEL assay, we found that the proportion of apoptotic glial cells in the presence of the 5-HT₄ agonist ($8.7\% \pm 2.7\%$) was not statistically different from that observed in control conditions ($12.0\% \pm 2.3\%$).

DSS-induced Colitis Promotes Enteric Neurogenesis In Vivo

Mice were fed with 3% DSS for 7 days to induce colitis, with or without the addition of a 5-HT₄ antagonist GR125487 (GR) to the drinking water. Mice from both experimental groups were significantly smaller than controls, losing approximately 10% of body weight by day 7 (Fig. 2A). By 21 days, weight returned to normal in both groups (Fig. 2A). After completion of DSS on day 7, fecal water content in both DSS and DSS + GR mice was significantly higher than controls (Fig. 2B). Blood was present in the stool in both experimental groups during DSS treatment and for an additional 7 days, after which the blood resolved. A blinded pathologist scored distal colon samples obtained 48 hours after completing DSS treatment for colitis severity. The colitis score was statistically similar in the presence and absence of GR (Fig. 2C), with a trend to worse colitis in the presence of the 5-HT₄ antagonist.

Compared with controls, DSS treatment induced a 68% increase in the number of myenteric neurons in the distal colon, whereas treatment with a 5-HT₄ antagonist abolished that increase (Fig. 3A). In DSS-treated mice, we found a significant positive correlation between neuronal density and the histologic colitis score (correlation coefficient, $r = 0.71$). This correlation was not observed in mice treated with GR ($r = 0.50$), in whom colitis was present but neuronal density was normal. The rate of neuronal proliferation in vivo was significantly increased in DSS-treated colon (1.2% of neurons were EdU positive), whereas no neuronal proliferation was observed in controls (Fig. 3B, C).

Glial cell density, measured by GFAP-immunoreactivity, was not significantly different among the 3 groups of mice (Fig. 3A). Despite this, however, EdU incorporation by enteric glial cells was increased 5-fold after DSS treatment (Fig. 3B, D), suggesting a significant colitis-induced increase in gliogenesis. Interestingly, this increase was abrogated in the presence of the 5-HT₄ antagonist (Fig. 3B).

Enteric Glial Cells Give Rise to Neurons

Given the significant increase in glial cell proliferation without a concomitant increase in glial cell number and the significant increase in neuronal density with only a modest increase in neuronal proliferation (Fig. 3A, B), we hypothesized that colitis may promote neurogenesis from glial cells. We first tested this hypothesis by inducing colitis in Sox2-GFP and Nestin-GFP transgenic mice. In the postnatal gut, Sox2 is a transcription factor specifically expressed by glial cells (Fig. 4A), and not by enteric neurons.²⁴ After colitis, however, approximately 8% of colon HuC/D+ neurons were Sox2-GFP+ (Fig. 4A). Similarly, Nestin, an intermediate filament protein, is predominantly a marker of glial cells²⁵ (Fig. 4B) and is expressed by only a very small subpopulation of HuC/D+ enteric neurons. Of 362 myenteric ganglia counted in the normal adult mouse colon, only 0.6% of neurons expressed Nestin. After DSS treatment, however, the proportion of enteric neurons expressing Nestin increased significantly to 1.8% (Fig. 4B). Additionally, CD49b, (integrin $\alpha 2$) is exclusively expressed by glial cells in the postnatal ENS (Fig. 4C). However, CD49b expression was activated in some enteric neurons after DSS treatment (Fig. 4C). The appearance of these 3 glial markers in enteric neurons after colitis suggests a glial-to-neuronal lineage switch in response to bowel inflammation.

To confirm the ability of enteric glial cells to differentiate into neurons, we isolated CD49b⁺Lin⁻ glial cells from the longitudinal muscle–myenteric plexus layer of the adult mouse intestine by flow cytometry. CD49b⁺ cells, which represent enteric glial cells by immunofluorescence (Fig. 5A), comprise $5.9\% \pm 2.5\%$ of all unfractionated cells in the longitudinal muscle–myenteric plexus layer (Fig. 5B). Immunofluorescent staining of cells immediately after sorting revealed that the CD49b⁻ fraction contained a mixed population, including neurons (Fig. 5C), whereas the CD49b⁺ cells comprised almost exclusively enteric glial cells expressing Sox10, GFAP (Fig. 5D), and S100 β (not shown), but not HuC/D (Fig. 5E).

After culturing CD49b-selected cells for 5 to 7 days, many GFAP⁺ and Tuj1⁺ cells were seen, displaying typical glial and neuronal morphology, respectively (Fig. 5F). Interestingly, 33% of cells expressed both Tuj1 and GFAP (Fig. 5F) and possessed an indeterminate morphology (Fig. 5F, arrowheads). Interestingly, while many glial cells and biphenotypic neuroglial cells incorporated EdU (Fig. 6A, open arrows), neuronal cells did not (Fig. 6A, solid arrow). CD49b-selected cells were then cultured in the presence of the 5-HT₄ antagonist, GR. In control cultures, 44% of cells were Tuj1⁺GFAP⁻ enteric neurons. This proportion decreased to 20% in the presence of GR (Fig. 6B–D). Glial cell density remained essentially unchanged with GR treatment (Fig. 6B–D). However, 5-HT₄ antagonism significantly increased the proportion of Tuj1⁺GFAP⁺ double immunoreactive cells from 33% to 58% (Fig. 6B–D).

To test whether CD49b-selected enteric glial cells could give rise to neurons *in vivo*, we transplanted NLBs derived from CD49b⁺ cells into the aneural embryonic chick hindgut. These guts were cultured on the chorioallantoic membrane of E9 host embryos for 7 to 10 days and subsequently analyzed by immunofluorescence. An extensive neuroglial network comprised mouse-derived cells was observed (Fig. 7). The neuroglial plexi formed from the transplanted cells were distributed along the submucosal and myenteric areas where the endogenous ENS would normally form. Importantly, the neurons were not immunoreactive to CN, a chick-specific neuronal antibody, confirming they are mouse-derived (Fig. 7).

DISCUSSION

Although enteric neuronal progenitors are present in the postnatal intestine,^{9–13} new enteric neurons are not born in the normal adult gut. The stimuli capable of generating new enteric neurons and the mechanisms through which this occurs are largely unknown. In this study, we find that experimentally induced colitis is associated with enteric neurogenesis. This observation has several important implications. First, recent evidence suggests that increases in neuronal density can exacerbate mucosal inflammation.²⁶ Importantly, enteric neuronal hyperplasia has been observed in inflammatory bowel disease,^{4,5} which may thereby worsen the underlying inflammatory process. Second, identifying the mechanisms underlying postnatal neurogenesis will help us to identify potential targets to promote the birth of new neurons in intestinal neuropathic disease or to limit pathologic hyperplasia of the ENS.²⁶

Although postnatal neurogenesis occurs constitutively in the adult brain,^{27,28} this does not seem to be the case in the gut, where neurogenesis, at least in rodents, stops within the first

few months of life.⁷ However, recent data suggest that specific stimuli can induce neurogenesis in the adult intestine. Chemical injury to the myenteric plexus or oral supplementation with a serotonin agonist has both been shown to stimulate neurogenesis.^{6,7} However, Joseph et al¹⁵ failed to identify neurogenesis after multiple injury models, including DSS colitis and chemical injury. We find that DSS colitis consistently promotes neurogenesis soon after peak inflammation. It is possible that both the specific regions of the gut interrogated and the timing of the analysis may account for the differences observed between our study and that of Joseph et al,¹⁵ who examined different regions of the gut and at much later time points.

We find that DSS-induced colitis results in a significant increase in neuronal density in the distal colon in vivo. Based on the incorporation of the thymidine analog, EdU, we find no neurogenesis in normal adult colon, consistent with previous studies.^{6,7,15} However, robust neurogenesis does occur after experimental colitis. Interestingly, despite a 68% increase in the number of enteric neurons, only 1.2% incorporated EdU suggesting that another cell type may be giving rise of new neurons. In contrast, DSS induced robust enteric gliogenesis. Although 1% of glial cells actively incorporate EdU, in normal adult colon, DSS-treated mice exhibited a 5-fold increase in the rate of gliogenesis. However, a concomitant increase in total glial cell numbers was not observed. Of note, both the neurogenic and gliogenic response to colitis are dependent on serotonin signaling, as feeding mice an antagonist to the 5-HT₄ receptor eliminated these responses. This result was consistent with our in vitro data, where addition of a 5-HT₄ agonist resulted in increased glial proliferation without an increase in glial cell number, despite the absence of glial apoptosis. Based on these results and recent published data,^{7,15,29} we hypothesized that glial cells may be giving rise to new neurons. To test this hypothesis, we used Sox2-GFP and Nestin-GFP transgenic mice, both of which express GFP in enteric glia. After colitis, Hu-immunoreactive enteric neurons turned on GFP expression, suggesting that inflammation may stimulate glial cells to give rise to neurons.

CD49b, a known cell surface marker of enteric glia,¹⁵ was also expressed by enteric neurons after colitis. To confirm the neurogenic potential of enteric glial cells, we isolated CD49b⁺ cells from the intestine by flow cytometry. Although the cells were predominantly glia after immunoselection, they formed neurons after 5 to 7 days in culture. In the central nervous system, radial glia give rise to neurons,¹⁷ and in the retina, a similar glial-to-neuronal transdifferentiation has been described.³⁰ In the intestine, evidence also supports a neurogenic role for glia.^{7,15,29} Our results confirm this finding. Interestingly, CD49b⁺ cells give rise not only to neurons but also to biphenotypic cells with both neuronal and glial immunophenotypes. Although these cells require further characterization, their existence suggests a possible transitional state during their transdifferentiation. The addition of a 5-HT₄ antagonist blocked the formation of neurons from cultured CD49b⁺ cells. Together, our findings suggest that enteric neurogenesis occurs in response to inflammation in the adult colon, and these new neurons arise from enteric glia through a 5-HT₄-dependent pathway.

DSS colitis increases mucosal serotonin by inducing the expression of tryptophan hydroxylase 1, the rate-limiting enzyme for serotonin biosynthesis in enterochromaffin cells.^{31,32} Both serotonin and tryptophan hydroxylase 1 have essential roles in the pathogenesis of

mucosal inflammation in colitis.³³ Importantly, serotonin has been shown to promote enteric neurogenesis in the postnatal mouse intestine.⁶ Our results are consistent with this and also demonstrate that the effects of serotonin on the mucosa and the enteric ganglia are distinct.³⁴ We find that 5-HT₄ receptor antagonism inhibits the neurogenic effect of serotonin but not the severity of the mucosal inflammation. This is consistent with the observation that selective depletion of enteric 5-HT in the mucosa protects the bowel from inflammation but has no effect on gastrointestinal motility.³⁵ Interestingly, although DSS-induced colitis is associated with increased 5-HT₃ receptor-expressing nerve fibers in the mucosa, it reduces the expression of the 5-HT₄ expressing nerve fibers.³² We speculate that this downregulation of 5-HT₄ expression may represent a protective response intended to limit the extent of neurogenesis that would otherwise occur with colitis.

The compelling data from Liu et al,⁶ which demonstrated serotonin-dependent postnatal enteric neurogenesis, showed the incorporation of BrdU in new neurons that migrated from “germinal centers” outside the myenteric ganglia. These results and ours suggest the possibility that multiple mechanisms may exist for enteric neurogenesis, and additional studies are needed to characterize the stimuli that evoke the neurogenic response and the pathways responsible.

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FIGURE 1.

5-HT₄ enhances enteric neurogenesis in vitro. Dissociated ENSCs were cultured in control media (A and D) or in the presence of 100 nM RS, a 5-HT₄ receptor agonist (B and E). Arrow in (E) highlights a proliferating neuron, enlarged in inset. Addition of RS to the cultures increased the density of neurons but not glial cells (C). RS led to a significant increase in the rate of both neuronal (TuJ1) and glial cell (GFAP) proliferation (F). * $P < 0.05$, ** $P < 0.01$ comparing experimental to control.

FIGURE 2.

5-HT₄ inhibition does not alter severity of DSS-induced colitis. Adult mice (n = 4 per group) treated with DSS with or without GR for 7 days demonstrated significant weight loss (A) and an increase in fecal water content on day 7 (B). Histologic colitis scores were statistically similar in the DSS and DSS + GR groups (C). **P* < 0.05 comparing experimental to control.

FIGURE 3.

Colitis induces 5-HT₄-dependent enteric neurogenesis in vivo. Mice (n = 4 per group) were killed 2 days after completing DSS treatment. The number of neurons (HuC/D⁺) was increased in DSS-treated mice, whereas the number of glial cells (GFAP⁺) was not (A). Treating mice with the 5-HT₄ antagonist, GR125487, abrogated the increase in neuron number (A). Proliferation of both enteric neurons and glial cells was significantly increased by DSS treatment (B). Examples of EdU-positive neurons (C) and glial cells (D) are shown (cells marked with arrows are enlarged in the insets). **P* < 0.05; ***P* < 0.01 comparing experimental to control.

FIGURE 4.

Colitis alters enteric glial immunophenotype. Sox2 is not expressed by enteric neurons in control mice (A). After DSS treatment, HuC/D⁺Sox2⁺ cells were observed in the colonic myenteric ganglia (Aⁱ⁻ⁱⁱⁱⁱ, asterisks). Nestin is rarely expressed by enteric neurons in control mice (B). After DSS treatment, a significant increase in HuC/D⁺Nestin⁺ cells was found in the myenteric ganglia (Bⁱ⁻ⁱⁱⁱⁱ, blue arrows). Similarly, colonic neurons do not normally express CD49b (C, arrows), whereas DSS treatment led to a significant increase in cells coexpressing CD49b⁺ and HuC/D⁺ (Cⁱ⁻ⁱⁱⁱⁱ, arrows).

FIGURE 5.

Enteric glial cells form neurons in culture. CD49b specifically marks glia not neurons (A). CD49b⁺ cells were immunoselected by flow cytometry and comprised $5.9\% \pm 2.5\%$ of unfractionated longitudinal muscle-myenteric plexus layer cells (B). The CD49b⁻ fraction contained many neurons (C), whereas the CD49b⁺ population was immunoreactive for GFAP and Sox10 (D) but not HuC/D (E). After 5 to 7 days in culture, neuronal (Tuj1, open arrows), glial (GFAP, closed arrow), and double immunoreactive cells (arrowheads) were seen (F).

FIGURE 6.

Serotonin promotes a glial-to-neuronal fate change in vitro. A, EdU incorporation was observed in glial and double immunoreactive cells (open arrows) but not neurons (solid arrow). Although control cultures contained many neurons (B and D), addition of a 5-HT₄ antagonist (GR) decreased neuronal density and increased the proportion of biphenotypic neuroglial cells (C and D). * $P < 0.05$.

FIGURE 7.

CD49b-immunoselected glial cells give rise to neurons ex vivo. A, E5 chick midgut (MG) and hindgut (HG) containing 2 transplanted Dil-labeled NLBs (arrows). After 7 days, CN⁻ (C and E) mouse-derived Tuj1⁺ neurons (B, arrow) form an extensive neuronal network (D) in the submucosal (SM) and myenteric (M) regions. GFAP⁺ glial cells (F, arrow, magnified view in (G)) are also present. epith = epithelium, delineated by dashed line in (B).