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Crosstalk between Toll-like receptor 4 and enteric serotonergic pathways in a mouse model of dinitrobenzene sulfonic acid-induced colitis

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Background: Changes in serotonin (5-HT) levels, anomalies in serotonergic and cholinergic machinery and altered Toll-like receptor 4 (TLR4) expression have been shown in IBD in patients and related animal models. Thus, we aimed to assess the crosstalk of enteric serotonergic system and TLR4 signalling in a mouse model of dinitrobenzene sulfonic acid (DNBS)-induced colitis.
Methods: Male C57/Bl6 (WT) and TLR4$^{-/-}$ mice (9 ± 2 weeks old; N = 10 mice) were presensitised with 1% dinitrobenzene sulfonic acid (DNBS), and after 1 week was intrarectally instilled with 2.5% DNBS. Small intestine inflammation was measured by disease activity index and histological analysis. Changes in ileal muscle tension were isometrically recorded following: (1) cumulative addition of carbachol (CCh; 0.1–100 μM); (2) electric field stimulation (EFS, 0–40 Hz); (3) 60 mM KCl; (4) 30 μM 5-HT addition with or without 0.1 μM ondansetron (5-HT3R antagonist). Immunofluorescence distribution of the neuronal HuC/D and nNOS and glial GFAP markers were determined in longitudinal-muscle-myenteric plexus whole mounts (LMMPs) by confocal microscopy.
Results: In WT mice, DNBS treatment altered receptor and not-receptor mediated responses (+120% of Emax to CCh and +103% of contraction to KCl, respectively; $p < 0.001$, $N = 5$ mice/group) together with an altered cholinergic neurotransmission (–50% at 10 Hz; $p < 0.01$, $N = 5$ mice/group) and 2-fold increase to 30 μM 5-HT-mediated response ($p < 0.001$, $N = 5$ mice/group). After DNBS treatment TLR4$^{-/-}$ mice showed a significant increase in excitatory-mediated response (+98% of Emax to CCh; +80% of contraction to KCl; +120% at 10 Hz; $p < 0.001$, $N = 5$ mice/group) together with a significant reduction of 30 μM 5-HT-mediated response (–50%, $p < 0.001$, $N = 5$ mice/group). These changes were associated to a significant decrease of the total number of HuC/D^{+} neurons (–44% and –19% for WT DNBS and TLR4 DNBS mice, respectively) together with a 1.3-fold increase in S100b immunofluorescence in WT mice after DNBS treatment.
Conclusion: These findings not only suggest an important role of TLR4 in small intestine neuromuscular dysfunction during colitis but also provide novel information on the potential benefits of targeting TLR4 in various gut disorders that exhibit aberrant cholinergic and 5-HT signalling.

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Differences in expression of PPARγ in small intestine vs. colon impact the effect of 5-aminosalicylates in inflammatory bowel disease

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Background: The nuclear receptor peroxisome proliferation-activated receptor γ (PPARγ) harbours anti-inflammatory effects. There is evidence that PPARγ mediates the effect of 5-aminosalicylic acid (5-ASA). 5-ASA is the first-line drug in ulcerative colitis (UC, while its use in Crohn`s disease (CD) is debated and not recommended according to guidelines. We hypothesise that the inconsistent therapeutic effect of 5-ASA in Crohn's disease is caused by different expression of PPARγ between the large and small intestine.
Methods: Levels of *PPARγ* mRNA were measured by RNASeq in mucosal biopsies from (1) active/inactive ileal CD (n = 14/17) and healthy controls (n = 9) and (2) colonic biopsies from patients with active/inactive UC (n = 24/24) and active/inactive CD (n = 24/21) and healthy controls (n = 20). Subsets of ileal and colonic biopsies were examined by western blot, immunohistochemistry (IHC) and *in situ* hybridisation (ISH). The effects of 5-ASA on *PPARγ* expression and TNF/IL17/polyI:C induced cytokine release were examined in the colonic cell-line HT29 and primary human IECs (colonoids) using RNASeq and ELISA in.
Results: *PPARγ* mRNA was strongly downregulated in colonic biopsies from inflamed mucosa of UC (log2 = –1.65, fold change 0.319 ($p < 0.001$)) and CD (log2 = –1.3, fold change 0.406 ($p < 0.001$)) compared with healthy controls. In ileal biopsies from CD and controls, *PPARγ* mRNA was not differentially expressed between inflamed and non-inflamed or healthy mucosa. These findings were confirmed by ISH in a subset of biopsies. Western blot analysis and IHC revealed almost undetectable levels of PPARγ protein in ileum, in the colon however, PPARγ was strongly expressed in healthy controls and inactive IBD; with significantly lower levels in active IBD. *PPARγ* was downregulated by TNF, TNF+IL17 and TNF + poly(I:C) in colonoids derived from IBD-patients (n = 3) and non-IBD controls (n = 3). 5-ASA attenuated the release of TNF-induced proinflammatory cytokines such as CXCL1, CXCL8 and CXCL10 from both HT29 cells and colonoids. This effect was reversed by the PPARγ antagonist GW9662.
Conclusion: 5-ASA harbours anti-inflammatory effects on intestinal epithelial cells mediated by epithelial PPARγ. We suggest that the previously observed lack of effect of 5-ASA in CD is related to differences in PPARγ expression in small intestine vs. colon. These results suggest that patients with Crohn's colitis may benefit from 5-ASA similarly to UC patients and challenge the current view on use of 5-ASA in CD.

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PD-1 expressing T cells in patients with different types of colitis

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