SMALL INTESTINE NEUROMUSCULAR DYSFUNCTION IN A MOUSE MODEL OF DINITROBENZENE SULFONIC ACID-INDUCED COLITIS: INTERACTION BETWEEN TOLL-LIKE RECEPTOR-4 AND SEROTONIN

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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 between the enteric serotonergic system and TLR4 signaling 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 presensitized with 1% DNBS, and after 1 week were intrarectally instilled with 2.5% DNBS. Small intestine inflammation was assessed by measuring the indices of disease activity and by performing histological analysis of ileal tissue samples. Changes in ileal muscle tension were isometrically recorded following: i) carbachol cumulative addition (CCh; 0.1-100 μM); ii) electric field stimulation (EFS, 0-40 Hz); iii) 60 mM KCl; iv) 30 μM 5-HT addition with or without 0.1 μM ondansetron (5-HT3R antagonist). Immunofluorescence distribution of the neuronal HuC/D and nNOS markers or the glial GFAP marker were determined in longitudinal muscle myenteric plexus whole-mounts (LMMPs) preparations by confocal microscopy.
RESULTS: In WT mice, DNBS treatment affected receptor and non-receptor mediated excitatory responses (+120% of Emax to CCh and +103% of contraction to KCl, respectively; P<0.001, N=5 mice/group) as well as the cholinergic neurotransmission (-50% at 10 Hz; P<0.01, N=5 mice/group) and 5-HT-mediated response (2-fold increase to 30 μM 5-HT,P<0.001, N=5 mice/group). After DNBS treatment TLR4-/- mice showed a significant increase in the excitatory response (+98% of Emax to CCh; +80% of contraction to KCl; +120% at 10 Hz; P<0.001, N=5 mice/group) and 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 S100- immunofluorescence in WT mice after DNBS treatment.
CONCLUSIONS: These findings suggest an important role of TLR4 in small intestine neuromuscular dysfunction during colitis and provide novel information on the potential benefits of targeting TLR4 in gut disorders that exhibit aberrant cholinergic and 5-HT signaling.