Changes of small bowel inhibitory neurotransmission in mice lacking TLR4 signaling

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Background. Toll-like receptors (TLRs) play a crucial role in ensuring the homoeostatic microflora-host crosstalk (Okun et al., 2011; Kabouridis and Pachnis, 2015; Barbara et al., 2016). Limited information has been reported on TLR4-mediated modulation of both motility and enteric neuronal survival on small bowel motor function (Anitha et al., 2012). This study aimed to assess the role of TLR4 signaling at controlling ileal inhibitory neuromuscular contractility. Methods. Male TLR4 knockout (TLR4-/-, 9±1 weeks old) and sex- and age-matched wild-type (WT) C57BL/6J mice were used for these experiments. In distal ileum segments, mounted longitudinally in organ baths, the relaxant response was evaluated as changes in muscle tension recorded by isometric transducers following electric field stimulation (EFS, 0-40 Hz) in non-adrenergic non-cholinergic (NANC) conditions (EFS=10 Hz, 1 µM guanethidine and 1 µM atropine). These experiments were carried out with or without i) 0.01 mM 1400W (inhibitor of inducible nitric oxide synthase, iNOS), ii) 0.1 mM Nω-nitro-L-arginine methyl ester (L-NAME; pan-NOS inhibitor); iii) 0.1 mM theophilline (P1 receptor antagonist); iv) 0.1 mM suramin (P2 receptor antagonist). To evaluate the involvement of P2Y1R in ileal relaxation, segments from WT and TLR4-/- mice were exposed to 0.001-1 mM ADP. In ileal frozen sections, immunoreactivity of glial fibrillary acidic protein (GFAP) and S100β was determined by confocal microscopy. The integrity of ENS neurochemical profile was assessed by immunohistochemistry of HuC/D, neuronal NOS (nNOS), iNOS, vasoactive intestinal peptide (VIP) in ileal longitudinal muscle-myenteric plexus (LMMP) whole mount preparations. P2X7R expression was also studied in ileal LMMP preparations. Results. In the ENS of TLR4-/- mice, the total number of HuC/D+ and nNOS+ neurons was significantly reduced (by 33±2% and 47±2%, $P<0.05$, respectively), with a proportional increase of VIP+ neurons ($P<0.05$). Loss of TLR4 induced gliosis in the submucosal and myenteric plexi, as shown by changes in S100β immunoreactivity ($P<0.01$) and by a 3.1-fold increase in process length of GFAP+ gliofilaments ($P<0.01$) in TLR4-/- mice. In vitro contractility studies showed a higher NANC relaxation in TLR4-/- mice, which was partly mediated by purinergic signaling, as suggested by the 1.43-fold increase ($P<0.05$) in the amplitude of ileal relaxant response following cumulative addition of ADP. Furthermore, increases in iNOS and P2X7R expression in LMMPs from TLR4-/- were revealed by immunohistochemical analyses compared to controls Conclusion. Our study provides evidence that in small intestine, TLR4 signaling fine-tunes ENS circuitry modulating the inhibitory component of neuromotor activity. This action is mediated by nitrergic and purinergic co-transmission, both being essential for maintaining a proper bidirectional neural-glial communication and gut motility.

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