Toll-like receptor 2 and 4 signaling regulates integrity of enteric nervous system and intestinal function

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Intestinal microbiota influence host physiology either directly through their metabolic products or indirectly interacting with microbial associated molecular patterns receptors such as Toll Like receptors (TLRs; Rakoff-Nahoum et al.,2004; Kawai and Akira, 2010; Sloane et al., 2010; Collins et al., 2012). Recently it has been shown that TLR-derived signaling is required for proper development of both central nervous system and enteric nervous system (Okun et al., 2011; Anitha et al., 2012). This study was undertaken to assess whether TLR2 and TLR4 signaling is required to preserve ENS integrity and function. Male C57Bl/6 mice (2 weeks old) were daily treated subcutaneously with OxPAPC, a mixture of oxidized phospholipids that blocks both TLR2 and TLR4 signaling (1.5 μg/g body weight) for 7 days. Ileum integrity was assessed by histological examination. Contractile activity of isolated ileum segments, mounted vertically in organ baths, was evaluated as changes in isometric muscle tension following electric field stimulation (EFS, 1-40 Hz) in presence or absence of 1 μM tetrodotoxin or atropine. Expression of neuronal markers such as HuC/D, BetaIII-tubulin, peripherin, and of glial marker such as S100β were determined by double labeling immunofluorescence and western blotting in longitudinal muscle-myenteric plexus preparations (LMMPs). The integrity of ENS neurochemical code was assessed by performing acetylcholinesterase biochemical staining and by evaluating the expression levels of neuronal nitric oxide synthase (nNOS) and choline acetyltransferase (ChAT). Mice treated with OxPAPC for 7-days didn't evidence any sign of illness or ileal histological anomalies. In vitro contractility studies showed an altered neuronal cholinergic transmission (-52±8% at 20 Hz). In the ENS of OxPAPC-treated mice HuC/D immunoreactivity was drastically decreased and associated to an irregular BetaIII-tubulin and peripherin staining and reduced number of S100β positive glial cells. A reduced staining of acetylcholinesterase fibers together with a loss of nNOS immunopositive neurons (-33±3%) was found after TLR2 and TLR4 signaling inhibition. Furthermore, a significant decrease in the expression of HuC/D, peripherin, nNOS and ChAT (-19±3%, -31±2%, -43±5%, and -33±6%, respectively) was revealed by western blotting analyses in LMMPs from control and treated animals. Our study provides evidence that TLR2 and TLR4 signaling is necessary for ENS homeostasis. The absence or a defective activity of these innate immunity receptors, *per se* or as a consequence of gut dysbiosis, could determine complex anomalies in ENS architecture, neurochemical coding and function leading to the onset of gut diseases.

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