Intestinal expression of glial-derived neurotrophic factor is dependent on Toll-like receptor 2 signaling

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Numerous gastrointestinal disorders are associated with structural and functional alterations of enteric nervous system (ENS; Mawe et al., 2009). Toll-like receptors (TLRs) mediate inflammatory responses toward pathogens and activate beneficial signals for ensuring tissue integrity under physiological conditions (Turer EE et al., 2008; Rakoff-Nahoum et al., 2004; Rakoff-Nahoum et al., 2006). The finding of TLR2 in the central nervous system (CNS) and its ability to recognize endogenous molecules produced by injured tissues, has pointed out that its influence extends well beyond the induction of host defense responses (Okun et al., 2011; Kawai and Akira, 2010). The aim of the study was to evaluate the expression and the functional role of TLR2 in the ENS. Male $TLR2^{-/-}$ (4 weeks old) and age-matched wild-type (WT) C57BL/6J mice were used. Ileal primary cultures were generated from enzymatically dissociated preparations of ileal longitudinal smooth muscle-myenteric plexus (LMMP). In WT mice $TLR2^{+}$ cells were characterized by immunohistochemistry of ileal frozen section and multiparameter flow cytometry staining of cells dissociated from LMMP using antibodies against HuC/D, glial fibrillary acidic protein (GFAP) and alpha-smooth muscle actin (alfa-SMA). The number of $nNOS^{+}$ and HuC/D^{+} neurons was determined in LMMP primary cell cultures of WT and $TLR2^{-/-}$ mice by confocal immunofluorescence whereas the expression of nNOS and glial derived-neurotrophic factor (GDNF) was evaluated by western blot analyses and quantitative RT-PCR, respectively. In ileal organ cultures of WT and $TLR2^{-/-}$ mice GDNF mRNA levels were evaluated by RT-PCR and immunocytochemistry after incubation with Pam3CysSerLys4 (TLR2 agonist) or conditioned medium-derived from WT LMMP primary cell cultures, respectively. In WT mice TLR2 expression was observed in neurons (5.54±2%), glia (12±3%) and smooth muscle cells (11±4%). The number and expression of $nNOS^{+}$ neurons were significantly reduced (-36±8% and -22±2%, respectively) in myenteric ganglia of $TLR2^{-/-}$ mice compared to WT mice. GDNF expression quantified by quantitative RT-PCR was found to be reduced in $TLR2^{-/-}$ mice. Treatment with the specific TLR2 ligand Pam3CysSerLys4 augmented the expression of GDNF in WT ileal primary cultures. Stimulation with conditioned medium derived from WT ileal primary cultures induced increased expression of nNOS in $TLR2^{-/-}$ ileal primary cultures as revealed by immunoblot analyses. In $TLR2^{-/-}$ ileal primary cultures GDNF supplementation increased nNOS expression to levels comparable to those found in WT ileal primary cultures. Our study provides evidence that TLR2 signaling is required for adequate GDNF expression in the ENS.

Kawai and Akira (2010). Nat Immunol. 11, 373-84.
Mawe et al. (2009). Neurogastroenterol Motil 21, 481-91.
Okun et al. (2011). Trends Neurosci. 34, 269-81.
Rakoff-Nahoum et al. (2004). Cell 118, 229-41.
Rakoff-Nahoum et al. (2006). Immunity 25, 319-29.
Turer EE et al. (2008). J Exp Med. 205, 451-64.