

Adrenergic innervation regulates intestinal microbiota diversity via generation of cholinergic Th17 lymphocytes.

S Dhawan^a, G De Palma^b, RA Willemze^a, JMM Duarte^a, FW Hilbers^a, EF Verdu^b, P Bercik^b, WJ de Jonge^a

a) Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, The Netherlands

b) Farncombe Family Digestive Health Research Institute, McMaster University, Canada

Background. The vagal anti-inflammatory reflex is mediated by non-neuronal immune cells that express Choline Acetyl Transferase (ChAT). ChAT catalyses the biosynthesis and secretion of acetylcholine. In this study we characterize a novel population of ChAT⁺ Th17 cells found in the intestine.

Methods. We used ChAT(BAC)-eGFP reporter mice, expressing eGFP under the control of ChAT promoter. CD45⁺CD4⁺ cells from small intestine and colon were analysed by flow cytometry. In an *in-vitro* antigen specific dendritic cell-T-cell assay, stimulation with an adrenergic receptor agonist norepinephrine (NE) was tested. CD4-specific ChAT^{-/-} mice were generated by crossing CD4^{cre} into ChAT^{fl/fl}. Intestinal microbiota was analysed using Illumina sequencing.

Results. Sorted intestinal CD4⁺ChAT⁺ cells expressed elevated levels of IL-17, IL-22 and RORC compared with their CD4⁺ChAT⁻ counterparts, indicating that ChAT⁺ T-cells reflect Th17 cells. The generation of these cells is stimulated *in-vitro* after activation of adrenergic receptors on dendritic cells. Interestingly, CD4ChAT^{-/-} mice displayed a reduced expression of antimicrobial peptides compared to ChAT^{fl/fl} mice leading to increased microbial diversity and richness in the small intestine.

Conclusion. Our data provide evidence of cholinergic Th17 cells in the intestine, which (1) respond to sympathetic input by up-regulating acetylcholine production, and (2) drive antimicrobial peptide secretion, affecting microbial richness and diversity. We provide evidence that autonomic dysfunction, as seen in patients with IBD, can lead to intestinal dysbiosis through altered production of antimicrobial peptides and thus contribute to pathophysiology of inflammatory disorders.