Neural regulation of innate and adaptive immunity in the gut
Dhawan, S.

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Outline of the thesis
This thesis investigates the role of neurotransmitters acetylcholine (ACh) and norepinephrine (NE), in modulating the innate and adaptive immune function in the intestine, during physiological and pathophysiological conditions. Furthermore, this thesis attempts to advance our current understanding of the gut-brain immune axis, also known as the cholinergic anti-inflammatory pathway, coined largely due to the cholinergic nature of the vagus nerve (1,2). In chapter 2 we discuss the evidences for and against the vagal cholinergic anti-inflammatory pathway. In light of current neuroanatomical evidences, we hypothesise that managing inflammatory bowel diseases (IBD) with nutritional interventions which might drive intrinsic cholinergic signalling the in the intestine might be of clinical relevance.

The gut harbours an estimate of 100 trillion microbes, and intestinal epithelium forms the first line of defence against microbial permeability (3). Postoperative ileus (POI) is a condition whereby prolonged impairment of gastrointestinal motility and impaired barrier function activates resident immune cells of the innate and adaptive immune system (4). Classical theory suggests that invading leukocytes and activated resident macrophages release inflammatory cytokines and chemokines, impairing contraction and motility of the small intestine (5). More recently, it has been shown that IFNγ producing CCR9+ T cells are activated at the site of inflammation (6). However, the mechanisms underlying the inhibition of GI motility in response to this local acute inflammation can be best described as an outcome of a complex and highly intertwined neuroimmunological response, involving activation of neural inhibitory pathways that affect the entire gut (7). In chapter 3, we identify and describe a cellular mediator of epithelial permeability and bacterial translocation in a mouse model of POI. In this study we delineate a part of this neuroimmune response by investigating the role of mast cells in IM induced barrier dysfunction by using two mast cell deficient mouse strains. Despite evidences that vagus nerve stimulation attenuates the disruption of tight junction in intestinal epithelium in endotoxemic mice (8), it remains debateable whether vagus nerve derived ACh reaches immune cells in the mucosa (9). Moreover, the observed maintenance of epithelial barrier function may be an
indirect effect of cholinergic signalling, or be a consequence of reduced TNFα and IL6 production by macrophages as widely documented (10). In chapter 4 we describe the role of cholinergic receptors in maintaining and restoring the epithelial barrier function by regulating the paracellular flux of antigens. To better understand the clinical relevance of cholinergic signalling, we examined the expression levels of choline acetyltransferase (ChAT), (an enzyme primarily responsible for ACh synthesis) in the intestinal epithelium of patients with ulcerative colitis (UC) and Crohn’s Disease (CD).

With respect to innervation of the intestine, sympathetic/adrenergic nerve fibres innervate both the mucosa and gut associated lymphoid tissue. Although it is widely accepted that sympathectomy reduces migration of T cells into Peyer patches (11,12), studies have reported contrasting effects of β adrenergic receptors on APC-T cell interaction (13,14). In chapter 5 we describe the role of adrenergic and cholinergic receptors in modulating the dendritic cell response. To this end, we examine changes in DC function such as endocytosis, cytokine production and DC induced T cell differentiation. Findings of our in-vitro experiments revealed a ‘cholinergic drive’ in a subset of T cell, which was observed to be tightly regulated by adrenergic receptors. In chapter 6 we identify and localise these cholinergic T cells (ChAT T cells) in in mice and human tissues. To validate our in vitro findings, we enhanced (salbutamol injections) or depleted (surgical sympathectomy) the adrenergic input to the intestinal tissue in mice, and observed its effects on ChAT T cells. On successfully identifying drivers of ChAT T cells, we set out to investigate their role in intestinal immunity. To this end, we co-cultured organoids of primary intestinal epithelium (Sato et al., 2009) with sorted ChAT+ T cells. In addition, we investigated the effect of T cell specific ChAT deletion in the mouse intestine. Taking cues from our in-vitro organoid-Tcell co-culture data, we examined the effects of ChAT T cell depletion on microbial diversity in mouse intestines.

Numerous studies show that ACh (and other neurotransmitters) can in fact stimulate and activate various cholinergic receptors, either in an autocrine or by affecting neighbouring cells in a paracrine manner (15,16). By this virtue, T-cells may also be affected by some of their endogenously produced and exogenously secreted ACh, in an autocrine and paracrine manner. In chapter 7 we assessed
the changes in human T cell function i.e. polarization and cytokine release \textit{in vitro}, after manipulating the lymphocyte-derived, autocrine or paracrine ACh bioavailability. Therefore, we blocked muscarinic and nicotinic receptors, acetylcholine esterase (AChE) and choline high affinity transporter (CHT-1) with pharmacological antagonists in CD3/CD28 stimulated T cells. In the context of inflammatory bowel diseases (IBD), we assessed transcriptional changes in lymphocytes associated with cholinergic signalling in PBMC’s and LPMC’s isolated from inflamed and non-inflamed patients. Finally, in \textbf{chapter 8} we discuss the findings of this thesis in a wider perspective, with a special emphasis on its potential clinical relevance.
REFERENCES


