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CHAPTER 1

Introduction and outline of the thesis

The aim of this thesis is to further unravel the working mechanism of the so-called 'cholinergic anti-inflammatory pathway', specifically in intestinal inflammation. The gut homes our largest collection of microbes, with up to 10^{12} organisms packed together per milliliter of luminal content. Therefore, especially in the intestine, inflammatory reactions need to be tightly regulated since exaggerated inflammation may lead to tissue damage and morbidity, while at the same time the tissue needs to be protected from invading pathogens. The control of inflammation is realized by two major mechanisms: self-controlling innate immune mechanisms and brain-derived immunoregulatory output. Evidence is mounting that the parasympathetic nervous system comprised by the vagus nerve is a potent player in neuro-immune inflammation. The afferent vagus system is known to detect and subsequently regulate the inflammatory response via activation of the hypothalamic pituitary adrenal axis. However, more recent evidence reveals that efferent vagus nerve cholinergic activity exerts quite potent immuno-modulatory potential as well[1].

A decade ago, Borovikova et al reported that acetylcholine, the principle neurotransmitter of the vagus nerve, can attenuate pro-inflammatory cytokine release in macrophages[1]. Moreover, they demonstrated that electrical stimulation of the vagus nerve attenuates the systemic inflammatory response to endotoxin. This implies an immune regulating role for the vagus nerve, and is referred to as 'the cholinergic anti-inflammatory pathway'. In line, in several experimental animal models of inflammatory disease, such as ischaemia-reperfusion injury[2], post-operative ileus[3], haemorrhagic shock [4], peritonitis[5], pancreatitis[6], and experimental arthritis[7], activation of the cholinergic nervous system inhibits inflammatory processes and ameliorates disease. Especially in the gastrointestinal tract, which is under strict control of the vagus nerve[8,9], vagus nerve stimulation has been extensively studied as a novel approach to treat intestinal inflammatory conditions. **Chapter 2** is a review in which the role of the vagus nerve as a regulator of intestinal inflammation is comprehensively discussed.

Acetylcholine, the principle neurotransmitter released by the vagus nerve, can exert its anti-inflammatory effect via binding to nicotinic acetylcholine receptors (nAChRs), which are expressed on macrophages and other immune cells[10, 11]. Wang et al published that acetylcholine or nicotine specifically interact with α7 cholinergic receptors to inhibit TNFα production[12], but the exact intracellular mechanism remained unclear. In **chapter 3**, we further investigated via which intracellular signaling pathways nicotine exerts its anti-inflammatory effect on peritoneal macrophages. Moreover, we corroborated our *in vitro* findings using an *in vivo* mouse model of postoperative ileus. We conclude that stimulation of the vagus nerve attenuates macrophage activation by activating the intracellular JAK2-STAT3 signaling pathway. In **chapter 4**, we further analyzed the role of the JAK2-STAT3 pathway in the anti-inflammatory potential of nAChR activation.

The immune system of the gut faces the challenge of discriminating between self and non-self in order to elicit a proper response against pathogens, but must at the same time tolerate mutual beneficial organisms and food antigens. Antigen presenting cells such as macrophages and dendritic cells are thought to be crucial in maintaining this balance. Intestinal macrophages are the first phagocytic cells of the immune system to interact with microorganisms that have breached the epithelium. Smythies et al showed that human resident intestinal macrophages are rather phagocytes than cytokine producers[13]. Therefore, in **chapter 5**, we examined whether the anti-inflammatory potential of vagus nerve activity in intestinal inflammation solely rests on reduced macrophage cytokine production, or if other important macrophage functions such as endocytosis and phagocytosis are affected. Furthermore, we examined the nicotinic acetylcholine receptor type and signaling pathways involved in these processes. Finally, we studied the effects of vagal signaling on phagocytosis and uptake *in vivo*.

In **chapter 6**, we tried to analyze how vagus nerve activity can modulate immune cells *in vivo*. In chapter 3, we showed that cholinergic nerve fibers are in close anatomical apposition to macrophages in the small intestine. However, since the half-life of acetylcholine is very short, one may question if acetylcholine that is released at the vagal termini actually reaches the immune cells in a quantity that could explain the *in vitro* effects. We hypothesized that, next to the direct anti-inflammatory effects of vagus nerve derived acetylcholine on nAChR on tissue macrophages, vagus nerve stimulation could affect immune cells via post-ganglionic mechanisms that lead to the release of alternative neurotransmitters, such as neuropeptides. Vasoactive intestinal polypeptide (VIP) and substance P (SP) are neuropeptides that are abundantly expressed in the gut, and elicit important immunomodulatory functions in the intestine[14-16]. Therefore, in **chapter 6,** we investigated whether vagus nerve released acetylcholine negatively regulates macrophage reactivity directly, or by modulating the responses to co-released VIP or SP.

All previous data were conducted in cell lines, mouse macrophages and experimental mouse models. Data from human studies considering cholinergic immunomodulation are limited. Is has been long known that cigarette smoking is an important environmental factor in ulcerative colitis, as smoking appears to have a protective effect in the development of disease and reduces its severity[17]. However, clinical trials of nicotine treatment in ulcerative colitis have shown variable outcomes[18]. As previous data suggest that nicotinic receptor α7 may specifically participate in the inflammatory response of monocytes[12], in **chapter 7**, we evaluated whether smoking, or repeated nicotine exposure affected nAChR α7 monocyte expression, and whether this renders human monocytes more susceptible to cholinergic immune-modulation. Luyer et al demonstrated in a hemorrhagic shock rat model, that high fat enteral nutrition could inhibit the inflammatory response by way of efferent vagal fibers[19].

Hence, the second aim of **chapter 7** was to establish the effect of oral olive oil diet on human whole blood LPS-induced cytokine production.

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