Neuro-immunity in intestinal disease: in vivo studies of postoperative ileus and colitis
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SUMMARY AND CONCLUSIONS

In this thesis I have addressed the role of the extrinsic and enteric nervous system in the complex interplay of immune homeostasis of the gastrointestinal tract, the mediators, cell types and transduction pathways involved, based on experimental data obtained from models of intestinal surgery and colitis. The interplay within the gastrointestinal wall between neurons and mast cells, residential macrophages and dendritic cells, involves epithelial barrier function, Toll-like receptors and a myriad of neuroactive substances. Multidirectional signaling between the different components occurs in the gastrointestinal wall, and spinal and central neuronal pathways impact inflammation and its consequences.

Particularly in one clinically important phenomenon, postoperative ileus (POI), these neuro-immune pathways play a prominent role. It is important to realise that POI is a common clinical condition that arises after almost every abdominal surgical procedure. In POI, the normal coordinated propulsive motor activity of the gastrointestinal tract is disrupted and this increases patient morbidity and as a consequence, the length of hospital stay and involved costs. It has been shown that an inflammatory response in the muscularis externa of the small bowel underlies the impairment of gut motility after abdominal surgery. In this thesis the mechanism behind this muscular inflammation was investigated in a mouse model of POI. More specifically, it was described how innate immune cells that reside in the muscularis externa may be activated and through what pathways these cells affect immune responses and gut epithelial barrier function.

Within the interaction of the nervous and immune “super-systems” the vagus nerve has been recently put forward to play a prominent role. This has let me to study the role of the vagus nerve in modulation of immune functions of resident innate immune cells, but also of important functional characteristics of the gut such as the maintenance of the intestinal epithelial barrier. Within this theme I investigated the potential of activation of nicotinic acetylcholine receptors (nAChRs) that are expressed by gut immune cells, to modulate intestinal macrophage function and dampen inflammation in experimental colitis. The pathogenesis of POI and neuro-immune regulation of the intestinal barrier is further reviewed in Chapter 1.

During abdominal surgery in animal models and patients undergoing abdominal surgery, it has been shown that epithelial barrier is disturbed and this may affect the course of POI. In turn, mast cells are implicated in the regulation of the epithelial barrier in gut disease. In Chapter 2 we described how mast cell activation contributes to the pathogenesis of POI by eliciting a disturbance of intestinal barrier function. To this end we performed our experiments in a mouse model for POI in two mast cell deficient mouse strains. We show that intestinal manipulation during abdominal surgery in mice resulted in a mast cell dependent inflammation and barrier dysfunction. These data underscore the importance of mast cells in the pathogenesis of POI and the potential of mast cell stabilizers in the clinical setting to shorten the period of POI.

In addition to activation of mast cells, the pathogenesis of POI involves the activation of macrophages and dendritic cells that reside in the muscularis externa. How these cells are activated is topic of investigation of Chapter 3. The influx of bacteria and their antigens across the epithelial barrier following intestinal manipulation may activate these cells. Alternatively, IL-1β that is quickly released as a response to tissue damage that may occur during abdominal surgery, may be of importance in IM induced inflammation. Therefore we investigated whether TLR- and/or IL-1R- signaling is involved in the inflammatory cascade after abdominal surgery. In this chapter we concluded that recognition of bacteria by TLRs is not a major factor in
IM induced POI while signaling through IL-1R and Myd88 is crucial in POI. Because these experiments indicate that the inflammasome formation is not required, our data point towards a mechanism in which IL-1β activation is mediated by mast cell derived proteases. Based on these results we speculate that intestinal manipulation elicits a local immune response that involves an early production of mast cell mediators and IL-1β that initiates the inflammatory cascade following intestinal manipulation. Barrier disturbances caused by mast cell mediators and immune responses lead to incoming bacteria that further contribute to IM induced inflammation. This cascade of events results in a major inflammatory response leading to impaired gastrointestinal motility and POI.

In order to maintain barrier function and homeostasis in the gut, the regulated phagocytosis and processing of bacteria is of great importance. Stimulation of the vagus nerve has been shown to reduce immune responses and innate immune signaling through nAChRs that are expressed by macrophages and dendritic cells. In the gastrointestinal tract, immune cells are localized in close proximity of neuronal fibers and are therefore ideal targets for immune modulation by the so-called ‘cholinergic anti-inflammatory’ pathway. In Chapter 4, the modulation of intestinal macrophage functions, mainly phagocytic properties, by nAChR signaling and the receptor subtype that mediates these effects is assessed. We show here that nAChR α4/β2- rather than α7-activation enhances the phagocytic potential in mouse macrophages, while NF-κB activity and pro-inflammatory cytokine production is inhibited. Also, in mice, electrical stimulation of the vagus nerve increases the epithelial permeability for luminal bacteria. Therefore, a tempting and controversial hypothetical interpretation of these results could be that vagus nerve activity may stimulate surveillance in the intestinal mucosa and peritoneal compartment. Whether or not such a mechanism is in place in the healthy gastrointestinal tract deserves further attention in future studies.

Although it has been shown in Chapter 4 that vagal or cholinergic modulation of macrophage functions is likely modulated by nAChR α4/β2, rather than nAChR α7, treatment with nAChR α7 agonists has been widely advocated to be beneficial in animal models of inflammatory disease including POI. In Chapter 5 we therefore further explored the potential of nAChR α7 activation in the treatment of intestinal inflammation. In this chapter we tested two selective nAChR α7 agonists in mouse models of acute colitis. The results of this study are actually controversial because: although this study showed that nicotine moderately improved intestinal inflammation; both selective nAChR α7 agonists worsen DSS colitis or were ineffective in TNBS colitis. These effects were seemingly independent of inflammatory events and possibly caused by side effects due to the wide spread expression of nAChR α7 peripherally and centrally. The results of this study are important and warrant caution because nAChR α7 agonists are widely evaluated for clinical use to treat excess inflammation. Clearly the efficacy and outcome of nAChR α7 agonists is highly dependent on the inflammatory context and cell type to be targeted.

Concluding, the results from the research performed in this thesis provide further evidence for targeting mast cells in POI and show that blocking IL-1R activation may prevent early inflammatory events and subsequent POI after abdominal surgery. Also, the data obtained here indicate that the expression of nAChRs and cellular mechanisms that lead to dampening of immune responses through nAChR activation need to be further investigated before evaluating nAChR agonists in a clinical setting.