Neuro-immunity in intestinal disease: in vivo studies of postoperative ileus and colitis
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IMPLICATIONS AND FUTURE PERSPECTIVES

In this thesis, new mechanisms in the course of postoperative ileus (POI) and other models for intestinal inflammation were revealed, which has led to the identification of cellular targets for treatment. Intestinal manipulation (IM) during surgery activates mast cell-induced barrier dysfunction and is likely to involve TLR activation and MyD88 signalling pathways. These data provide more evidence for the potential of mast cell stabilizers in a clinical setting. Recently, in a pilot study in humans, perioperative treatment with the mast cell stabilizer ketotifen showed a dose-dependent restoration of gastric emptying in patients that underwent abdominal surgery. However, other parameters including recovery of colonic transit and clinical end points such as nausea, pain and abdominal cramping were not improved. Possibly, non-histamine receptor (HR)-related properties of ketotifen such as inhibitory effects on motility may have affected the outcome of these parameters. Nonetheless, this pilot study shows potential of the use of mast cell stabilization in patients undergoing abdominal surgery, albeit the use of more selective mast cell stabilizers may improve efficacy in POI.

We show that mast cell activation following IM induces barrier dysfunction. We investigated the role of barrier dysfunction in POI by using a variety of toll-like receptor (TLR) KO mice. In TLRKO’s, MPO influx and IL-1β and IL-6 production was affected in TLR2 and TLR9 KO mice. These data, together with reported data showing that an influx of bacteria into the muscularis externa starts 6 hours after manipulation, indicate that bacterial recognition contributes to POI but may not be crucial in its initiation. Probably, luminal bacteria will play a role in the perpetuation of IM induced inflammation. However, to draw definitive conclusions, signalling of all TLRs should be inhibited or gut bacteria should be eliminated. The latter has been done in one experimental POI study, where treatment with antibiotics prevented loss of muscle contractility after colonic manipulation. However, inflammatory parameters were not determined, therefore this study showed no link between gut bacteria and the severity of inflammation. Antibiotics are being used in patients undergoing (abdominal) surgery, although POI has not been assessed as the main endpoint in most of these studies.

The clinical significance of bacterial translocation during surgery is under debate. The “gut origin of sepsis” hypothesis refers to the translocation of bacteria across the intestinal epithelial barrier and could induce sepsis. This may occur during abdominal as well as non-abdominal surgery. Recent studies point towards a correlation between bacterial translocation and increased septic morbidity in surgical patients and therefore prevention of bacterial translocation during surgery may reduce the incidence of septic complications.

Since Myd88 is crucial in the induction of inflammation in after IM while TLR signalling is a minor factor, we looked further into activation of another receptor that is dependent on Myd88 signalling, the IL-1 receptor (IL-1R). Here we show that blocking of IL-1 receptor (IL-1R) signalling has potential in the treatment of POI. Importantly, the recombinant IL-1R antagonist (IL-1RA, anakinra) we used in our studies has proven to be safe and effective in treatment of IL-1β mediated diseases such as gout. IL-1RA may be very effective in treatment of IM induced inflammation since IL-1β is possibly one of the first cytokines produced after IM as it is stored as a pro-form that does not need to be transcribed after activation. Simultaneously, IL-1β production is increased through NF-kappaB dependent transcription. Furthermore, IL-1β is implicated in initiation of factors that are upregulated early after IM, including synthesis of cyclooxygenase type 2 (COX-2), inducible nitric oxide synthase (iNOS), production of IL-6, and expression of adhesion molecules ICAM-1 and VCAM-1. The link to IL-1beta is particularly interesting with respect to intestinal barrier function.
We show here that IL-1β increases epithelial permeability. So blocking IL-1R signalling during abdominal surgery may be effective in POI by preventing early events after surgery and reducing bacterial translocation.

In the gut, cholinergic fibres are located in close apposition to immune cells and is therefore the ideal site for neuro-immune modulation. In the last decade, vagus nerve stimulation has been shown to dampen immune responses in a number of disease models; this is referred to as the ‘cholinergic anti inflammatory pathway’. Also in POI, electrical vagus nerve stimulation reduces inflammation and improves gastric emptying. However, electrical stimulation of the vagus nerve may not be an appropriate treatment option in patients. Nutritional stimulation (through high-lipid enteral nutrition) of the vagus nerve has been shown to attenuate inflammation and promote gastrointestinal motility in experimental models of POI and is a potentially well feasible strategy to prevent POI. Some studies show a beneficial effect of early feeding and even chewing gum on the course of POI. Whether these effects are mediated by the vagus nerve remains to be established.

As an alternative route to stimulate vagus nerve output pharmacologically, nAChRα7 agonists are also effective in treatment of experimental POI. In order to therapeutically use nAChR agonists in patients, we have to consider the potential side effects because of widespread expression of nAChRs, including in the brain. This is probably the cause of disease aggravation by treatment with nAChRα7 agonists in experimental colitis, presented in this thesis. Importantly, treatment with nicotine in much lower doses than nAChRα7 agonists moderately reduced inflammatory parameters. This indicates that other receptor subtypes besides the nAChRα7 may mediate the effects of nicotine. Higher doses would be more effective but this is not possible, because of unwanted side effects in the brain and the periphery. This has been shown in several studies where treatment with nicotine induced disease remission in UC patients, but adverse effects such as light-headedness and nausea make the use of nicotine inferior to the standard medical therapy patients receive. Therefore, selective agonists designed to be unable to pass the blood brain barrier and act on certain nAChR subtypes and cell types have potential in the treatment of intestinal inflammation.

In POI, anti-inflammatory strategies can interfere with wound healing and defence against bacteria and therefore recovery of patients. The effects of anti inflammatory therapies could be investigated on wound healing in animal models before use in the clinic. Also it is important that treatment with anti-inflammatory mediators is short and directed at prevention of acute muscular inflammation.
Future perspectives

In the last decade, in animal models of POI, experimental interventions including administration of carbon monoxide, pretreatment with blocking antibodies against adhesion molecules, and mast cell stabilization have been shown to be effective preventing inflammation and motility disturbances in animal models of POI. In addition, activation of the cholinergic anti-inflammatory pathway by electrical or nutrition stimulation of the vagus nerve and administration of cholinergic agonists have been successful in the prevention of experimental POI. Here we add a potential new target to the list: the IL-1R. However, despite the successful prevention of POI in animal models, there are still few studies conducted to evaluate the potential of anti-inflammatory strategies in patients. In addition, although it has been shown that inflammatory mechanisms take place in human POI, the correlation between muscular inflammation and the occurrence of POI still needs to be established. Therefore it is now important that new studies will be performed to indicate that the pathophysiological mechanism found in animal studies is similar in patients. In addition to developing new anti-inflammatory therapies for prevention of POI, the results from these studies will also show how relevant the POI animal model is for testing new treatment strategies for POI. Nevertheless, as in animals, in patients it has been shown that intestinal handling is the main trigger for inflammation and POI and should thus be prevented as much as possible. Hence, in addition to the existing measures taken to reduce the duration of POI, laparoscopy and perioperative, short treatment with anti-inflammatory mediators will be likely to be successful in shortening the duration of POI.