Neuro-immunity in intestinal disease: in vivo studies of postoperative ileus and colitis
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General introduction
GENERAL INTRODUCTION

Inflammatory mechanisms in postoperative ileus
Postoperative ileus (POI) is a common clinical condition that arises after almost every abdominal surgical procedure and refers to the disruption of the normal coordinated propulsive motor activity of the gastrointestinal tract (1). Hence, this increases patient morbidity and as a consequence, the length of hospital stay and involved costs. It has been shown that an inflammatory influx of neutrophils and monocytes into the muscularis externa of the small bowel underlies the impairment of gut motility after intestinal manipulation (IM) (2-4). This inflammatory reaction is initiated by activation of mast cells (5) and other innate immune cells that reside in the muscularis externa including macrophages (6) and dendritic cells (7).

Barrier dysfunction during abdominal surgery
Mast cell activity has been linked to disruption of intestinal barrier integrity and disease progression in several animal models of gut disease (8-11). Also, after abdominal surgery barrier dysfunction occurs, and has been associated with increased postoperative septic morbidity (12). In an animal model for POI it has shown that orally administered beads appear in local mesenteric vessels and the muscularis externa following IM (13). The existence of this pathway was confirmed in another study where fluorescent labelled beads and LPS were instilled into the colon and emerge in the small intestine muscularis layer after colonic manipulation (14). Given the potential of mast cells to regulate epithelial barrier, they may mediate the occurrence of the barrier dysfunction during abdominal surgery. This is investigated in Chapter 2 of this thesis.

Activation of muscularis externa phagocytes

Luminal microbiota
How IM induced barrier dysfunction contributes to IM induced POI has not been shown so far. Luminal bacteria may activate the muscularis externa network of macrophages and dendritic cells, thereby playing a role in the pathogenesis of POI. It has been demonstrated that small fluorescent beads that given orally before IM, were ingested by monocytes that extravasated into the muscularis externa (13). However, monocyte loaded beads only started to appear in the muscularis externa 6h after intestinal manipulation (13) while the inflammatory cascade starts earlier after intestinal manipulation (3). For example ICAM-1 mRNA is expressed within 15 minutes of manipulation (15) and subsequent influx of immune cells within 3h after IM (3). Therefore, translocated bacteria may be involved at later stages after IM, but are probably not the initial trigger for the IM induced inflammatory cascade.

DAMPS
An early trigger may be the release of damage associated molecular patterns (DAMPs) (16). DAMPs are endogenous intracellular molecules such as reactive oxygen species (ROS) or ATP that are upregulated upon tissue damage (16) that is likely to occur during bowel handling. It has been shown that ATP is a potent activator of muscular phagocytes (17). Activation of innate immune cells by DAMPs results in the assembly of an intracellular protein complex called the ‘inflammasome’ leading to activation of caspase-1. This enzyme cleaves cytosolic pro-IL-1β into mature IL-1β that is subsequently secreted (16). In this way, IL-1β may be released quickly after intestinal manipulation and initiate the inflammatory cascade via IL-1R-
Myd88 signalling in resident phagocytes. In this thesis, both the role for bacterial recognition through TLRs and IL-1R signalling in IM induced POI is explored.

The maintenance of intestinal barrier function
To maintain barrier function and homeostasis in the gut, the regulated phagocytosis and processing of bacteria is of great importance. Mucosal immune cells are well adapted to deal with the great load of antigens that is present in the gut lumen. For instance, intestinal mucosal macrophages display strong phagocytic and bactericidal activities but do not produce cytokines upon ingestion of bacteria (18). Also gut dendritic cells are well programmed to maintain tolerance to self-antigens and immunity to pathogens (19). In addition to the regulated uptake and processing of incoming antigens by the gut immune system, the integrity of the epithelial barrier is crucial in maintaining intestinal homeostasis. During inflammation, mediators released by activated immune cells reduce intestinal barrier function by affecting the epithelial tight junctions (TJs) (20). Tight junctions are intraepithelial protein complexes that function as a selective barrier to paracellular transport. For instance, the cytokines TNF and IL-1β cause TJ rearrangements thereby increasing paracellular permeability of the epithelium (20).

Neuro-immune regulation of barrier function

ENS Modulation of the epithelial integrity
The extensive intestinal network of neurons that comprises the enteric nervous system (ENS) modulates immune mediated intestinal barrier function but also acts on the epithelial cells directly. For instance, the neurotransmitter VIP affects epithelial barrier function by regulating the organization of the tight junction protein complex (21). Enteric glial cells (EGC) are also part of the ENS and are located the mucosa and the plexuses of the ENS. EGCs are required to maintain epithelial barrier function, partly through their secretion of S-nitrosoglutathione (GSNO) and also through regulation of gut immune responses (22).

The vagus nerve in regulation of intestinal immunity
In the gut, cholinergic fibres are located in close apposition to immune cells (23), and would thus be the ideal site for neuro-immune modulation. It has been shown that activation of the vagus nerve negatively regulates macrophage immune responses via the peripheral release of acetylcholine (ACh) (24;25). In particular, signalling through the nicotinic acetylcholine receptor α7 (nAChRα7) has been implicated in mediating the effects of ACh (24). Activation of this so-called 'cholinergic anti-inflammatory pathway' has been shown to ameliorate disease in various models of inflammation, including sepsis (24), ischemia reperfusion (26) haemorrhage (27) and POI (23). In mouse models of colitis, enhanced parasympathetic output is involved in the negative regulation of intestinal inflammation via efferent activity of the vagus nerve (28;29). Vagal activity may lead to peripheral release of its principal neurotransmitter ACh, but the vagal nerve particularly projects to other post-ganglionic enteric neurons. In this way the vagus nerve regulates gut immune function and barrier through neuropeptides and neurotransmitters released by the ENS.
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Outline of this thesis
OUTLINE OF THIS THESIS

In this thesis the mechanism of muscular inflammation that underlies the paralysis of the gastrointestinal tract after abdominal surgery was investigated. More specifically, it was studied how innate immune cells that reside in the muscularis externa may be activated and through what pathways they affect immune responses and gut epithelial barrier function. Also ENS regulation of the intestinal barrier and intestinal immune responses, in particular by the vagus nerve is investigated.

In Chapter 1, background is given on the clinical aspects and pathogenesis of POI. In particular, the role of mast cells and macrophages is reviewed. Also in this chapter, the composition of the epithelial barrier is described and how this barrier is affected by immune mediators released from mast cells and other immune cells residing in the gut. In addition, the ENS functions in modulating immune responses and barrier integrity with focus on the cholinergic anti-inflammatory pathway is described in this chapter.

It has not been clarified yet how mast cell activation contributes to the pathogenesis of POI. The local release of mediators may directly activate the inflammatory cascade. Alternatively, it has been shown that barrier disturbances occur in patients during abdominal surgery and also in a mouse model of POI there are indications of a transient increase in epithelial permeability shortly after intestinal manipulation. Thus, mast cells may be involved in the pathogenesis of POI by modulation of the epithelial barrier. Therefore, in Chapter 2 the role of mast cells in IM induced barrier disruption is investigated by using two mast cell deficient mouse strains. Also in this chapter the possibility that a decrease in blood pressure during IM may lead to barrier disturbances is explored.

The phagocytes that reside in the muscularis externa may be activated by incoming bacterial antigens. In Chapter 3 we studied the role of bacterial recognition through TLR signalling. Alternatively, the release of damage associated patterns (DAMPs) due to local tissue damage may initiate the inflammatory cascade via induction of IL-1β production. Therefore, also in this chapter, the role of IL-1β in the inflammatory response following intestinal manipulation was assessed.

In the intestinal tract, immune cells are localized in close proximity of cholinergic fibers and are therefore ideal targets for immune modulation by ENS. Cholinergic inhibition of pro-inflammatory cytokine production by macrophages has been firmly established. However, besides an effect on cytokine secretion, the cholinergic nervous system may also affect macrophage phagocytic properties. Therefore, in Chapter 4, we studied the modulation of intestinal macrophage functions, mainly phagocytic properties, by ACh mediated signalling. In addition we aimed to identify the nAChR subtype that mediates the effects of ACh on macrophages.

In mouse models of inflammation, it has been shown that the anti-inflammatory effects of ACh released from the vagus nerve depend on signalling through the nAChRα7 and administration of selective nAChRα7 agonists proved to be beneficial in disease including POI. Whether this pathway could also be used to treat inflammatory bowel disease has not been shown yet. Therefore our aim in Chapter 5 was to study the effects of nicotine and selective nAChRα7 agonists in colonic inflammation. To this end we performed an in vivo study in two mouse models of acute colitis.