Nutritional stimulation of the autonomic nervous system

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Abstract
Disturbance of the inflammatory response in the gut is important in several clinical diseases ranging from inflammatory bowel disease to postoperative ileus. Several feedback mechanisms exist that control the inflammatory cascade and avoid collateral damage. In the gastrointestinal tract, it is of particular importance to control the immune response to maintain the balance that allows dietary uptake and utilization of nutrients on one hand, while preventing invasion of bacteria and toxins on the other hand. The process of digestion and absorption of nutrients requires a relative hyporesponsiveness of the immune cells in the gut to luminal contents which is not yet fully understood. Recently, the autonomic nervous system has been identified as an important pathway to control local and systemic inflammation and gut barrier integrity. Activation of the pathway is possible via electrical or via pharmacological interventions, but is also achieved in a physiological manner by ingestion of dietary lipids. Administration of dietary lipids has been shown to be very effective in reducing the inflammatory cascade and maintaining intestinal barrier integrity in several experimental studies. This beneficial effect of nutrition on the inflammatory response and intestinal barrier integrity opens new therapeutic opportunities for treatment of certain gastrointestinal disorders. Furthermore, this neural feedback mechanism provides more insight in the relative hyporesponsiveness of the immune cells in the gut. Here, we will discuss the regulatory function of the autonomic nervous system on the inflammatory response and gut barrier function and the potential benefit in a clinical setting.

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Key words: Inflammation; Nutrition; Acetylcholine; Inestinal barrier; Innate immunity; Autonomic nervous system; Cholecystokinin

INTRODUCTION
Disturbance of the inflammatory response in the gut is important in several clinical diseases ranging from inflammatory bowel disease (IBD) to postoperative ileus (POI). Although a fierce response to pathogens, ischemia, trauma and other forms of injury is necessary, the inflammatory cascade needs to be tightly controlled to avoid local tissue damage or systemic effects such as shock, organ failure or even death. Especially in the gastrointestinal tract a delicate equilibrium is required; on one hand there needs to be a state of relative hyporesponsiveness to commensal bacteria, dietary antigens and biological toxins that are ingested along with nutrients, and on the other hand potential pathogens must be recognized and neutralized when necessary.
An unrestrained inflammatory response is not desired since it may cause local damage to healthy tissue, cause acute and chronic inflammatory diseases and even evolve into critical systemic inflammatory syndromes such as sepsis.

Controlling the inflammatory response has been a therapeutic target for many gastrointestinal diseases for a long time. For IBD, control of the intestinal inflammatory response via an anti-inflammatory therapy using tumour necrosis factor-α (TNF-α) antibodies has been proven to be very successful in the clinical situation.[8,9] For other syndromes such as POI, only experimental evidence is available that shows a positive effect of an anti-inflammatory therapy.[10]

In the last decade, a new anti-inflammatory pathway has been discovered involving the autonomic nervous system. Several experimental studies have shown that stimulation of the autonomic nervous system can dampen the inflammatory response and prevent loss of gut barrier integrity.[10,11] In this way, activation of the autonomic nervous system can potentially ameliorate inflammation-based diseases and prevent complications. Both IBD and POI have been shown to be reduced effectively in experimental models using activation of the autonomic nervous system.[8,12] A more physiological and less invasive way of activating this anti-inflammatory vagal pathway is by administration of nutrition.[13-15]. Nutrition and specifically dietary lipids activate the autonomic nervous system via afferent vagal nerve fibres and release of neuro-endocrine hormones.[16,17] In this way nutrition may be used as therapy to prevent excessive inflammation and the accompanying local tissue damage. Although the beneficial effect of nutrition on inflammation has been recognized by many for a long time in several clinical settings, this vagal feedback mechanism gives more insight in the mode of action of nutrition and individual food components and helps to develop new nutritional therapies.

### THE INFLAMMATORY RESPONSE

Release of proinflammatory cytokines by macrophages and neutrophils is essential in the initial and rapid innate immune response. Additionally, exposure to bacterial ligands elicits a complex orchestrated network of responses in which complement, chemokines, adhesion molecules, heat shock proteins and other late mediators play a role.[18] One of the key mediators released early after exposure to bacterial structures is TNF-α.[19] This pleiotropic cytokine causes activation of macrophages and stimulates neutrophils. Inhibition of this cytokine has been shown to be therapeutic in IBD and rheumatoid arthritis, although it may also be detrimental in patients carrying certain pathogens while also in sepsis.[20] Other rapidly released pro-inflammatory mediators include interleukin-6, interleukin-1β and interleukin-8.

The inflammatory response is often triggered by bacteria or bacterial products that have distinctive characteristics called pathogen-associated molecular patterns that are recognized by immune cells via Toll-like receptors (TLRs).[21] There are several ligands for TLRs including endotoxin, the major constituent of Gram-negative bacteria (TLR4), bacterial DNA (TLR9) and peptidoglycans (TLR2). Activation of TLRs triggers several intracellular signalling pathways leading to translocation of the transcription factor nuclear factor-κB (NF-κB) to the nucleus and ultimately resulting in the release of inflammatory mediators.[22] To prevent an exaggerated inflammatory response and to manage the collateral damage caused by release of proinflammatory mediators, several control systems are activated at all levels. This so-called “anti-inflammatory response”, consists of numerous anti-inflammatory mediators, including cytokines, neutromediators, hormones and stress molecules released to restore homeostasis. Interleukin-10 (IL-10) and transforming growth factor-β are important anti-inflammatory cytokines. For example, IL-10 has been shown to play a pivotal role in the intestinal recovery following surgery.[23] Another important pathway in the anti-inflammatory response is triggered by the catabolism of heme by the enzyme heme-oxygenase 1. This results in induction of biliverdin which has been shown to protect against polymicrobial sepsis in cecal ligation and puncture.[24] Furthermore, carbon monoxide is formed which has been shown to ameliorate development of postoperative ileus via reduction of the inflammatory response and induction of IL-10.[25]

To prevent an exaggerated inflammatory response several control systems are activated at all levels. This so-called “anti-inflammatory response”, consists of numerous anti-inflammatory mediators, including cytokines, neutromediators, hormones and stress molecules. IL-10 and transforming growth factor-β are the main identified anti-inflammatory cytokines. The complex of these counter-regulatory mechanisms to severe infection of injury is also called the “compensatory anti-inflammatory response syndrome” (CARS). CARS was at first considered to be a global deactivation of the immune system following systemic inflammatory response syndrome. However, new insights suggest that it is rather a reprogramming of leukocytes leading to a compartmentalized control to prevent excessive inflammation upon infection and injury.[26]

### INTESTINAL BARRIER INTEGRITY

The intestinal lumen is an important reservoir of bacteria that is strictly separated from the sterile environment of the host via a physical/anatomical and immunological barrier. Changes or defects in certain components of the intestinal barrier may lead to activation of the inflammatory system potentially leading to known gastrointestinal diseases such as inflammatory bowel disease.[27] The physical/anatomical barrier of the gut is formed by a monolayer of epithelial cells, originating from multipotent stem cells present in the crypt. These cells differentiate into several subclasses, amongst which are absorptive enterocytes, that make up > 80% of all
small intestinal epithelial cells, Paneth cells and goblet cells\cite{28,29}. The intestinal cells are bound together with several protein complexes including occluding, claudin and zona occludens proteins, also called tight-junctions\cite{30}. Breakdown of this barrier potentially leads to the translocation of luminal antigens, bacteria and their toxic products into the circulation\cite{31,32}. In the case of transmural damage to all intestinal layers (mucosa, submucosa, muscularis and serosa) luminal content may pass into the abdominal cavity leading to detrimental effects as sepsis.

The immunological barrier is formed by enterocytes which are considered to actively participate as innate immune sensors of microbial pathogens and commensal organisms. Host recognition of microbial components is achieved by pattern recognition receptors, like the cytoplasmic NOD-like receptors and membrane-bound TLRs. Crypt Paneth cells secrete defensins (e.g., antimicrobial peptides) into the villous crypt, thereby maintaining its sterility and regulating microbial homeostasis\cite{30}. It has also been shown that Paneth cells are equipped with the proper molecules to recognize and signal endotoxin, the major component of Gram-negative bacteria\cite{34}. Goblet cells secrete mucus (composed of glycoproteins and water) providing a filter overlying the intestinal epithelium and secrete trefoil peptides; small proteins needed for epithelial growth and repair. Gut-associated lymphoid tissue is present in the lamina propria and provides immune surveillance. Finally, sampling of luminal antigens occurs by M-cells and dendritic cells, which present antigens to T and B cells, thereby inducing the acquired immune system. This response includes secretion of large amounts of IgA by plasma cells. This secretary IgA covers the mucosal surface and has a major role in excluding antigen from passing the epithelium\cite{35}.

Interestingly, the process of digestion and metabolism of nutrients requires a physiological breach of the intestinal barrier, without noticeable activation of the immune response. This relative hyporesponsiveness to luminal contents during the process of food uptake is not fully understood. The autonomic nervous system may be important in regulation of this process.

THE CENTRAL NERVOUS SYSTEM AND INNATE IMMUNITY

Excessive release of inflammatory mediators following activation of inflammatory cells by bacterial products is (amongst other pathways) controlled by the central nervous system. Supposedly, inflammatory mediators such as TNF-α and interleukins activate afferent vagal pathways leading to a variety of responses. The hypothalamic-pituitary-adrenal signalling pathway is activated causing an instantaneous release of serum corticosteroids that leads to inhibition of (excessive) inflammation. In addition to this afferent or sensory function during inflammation, the efferent vagal system is also involved in regulation of the inflammatory response\cite{36}. Previous studies have shown that stimulation of efferent vagal fibres increases release of acetylcholine, the principal neurotransmitter of the vagus nerve. Acetylcholine subsequently binds to specific alpha 7 nicotinic receptors (α7 nAChR) leading to activation of an intracellular pathway\cite{37}. It has been shown that the transcription factor STAT3 is phosphorylated by tyrosine kinase Jak2, leading to the anti-inflammatory effect via NF-κB\cite{38}. Ultimately this results in a decreased release of both early inflammatory mediators such as TNF-α but also late mediators such as HMGB-1\cite{39,40}. Stimulation of this neural feedback loop is efficient in reducing the inflammatory response in several experimental models. Direct electrical stimulation or pharmacological stimulation of nicotinic receptors via agents such as CNI-1493 significantly reduces the systemic inflammatory response to endotoxic shock\cite{41}. Furthermore, activation of this neural anti-inflammatory pathway, the so-called cholinergic anti-inflammatory pathway reduces the inflammatory response and its sequelae during septic peritonitis and following hemorrhagic shock\cite{42}. Interestingly, recent evidence indicates that vagus nerve signalling has a dual effect in macrophages. Besides inhibiting cytokine secretion, the cholinergic nervous system also enhances endocytosis and phagocytosis of bacteria by macrophages\cite{43}. This effect on phagocytosis is caused by stimulation of the nAChR α4/β2 rather than α7 nAChR. The spleen has also been implicated in the anti-inflammatory cholinergic pathway; however the underlying mode of action at this stage is elusive. The vagus nerve conveys signals from the brain to the immune cells residing in the spleen via the celiac-superior mesenteric plexus ganglia and the splenic nerve\cite{44}. The requirement of the nicotinic acetylcholine receptor α7 in this process is not yet clear.

NUTRITIONAL STIMULATION OF THE AUTONOMIC NERVOUS SYSTEM

Besides electrical or pharmacological stimulation, the autonomic nervous system can also be stimulated in a physiological way via nutrition, more specifically via dietary lipids and proteins/peptides. Ingestion of dietary lipids, proteins and peptides triggers release of cholecystokinin (CCK)\cite{45}. CCK binds to CCK-1 and CCK-2 receptors, leading to a rapid activation of the autonomic nervous system via the afferent vagal pathway\cite{46}. Subsequently the efferent vagal pathway is activated resulting in release of acetylcholine. Binding of acetylcholine to α7 nicotinic receptors on inflammatory cells then leads to a decreased release of inflammatory cytokines. Such a nutritional intervention with dietary fat has been proven to be very efficient in reducing the systemic inflammatory response, ameliorate tissue damage and preserve intestinal barrier function\cite{13-15}. Interestingly, the protective effects of dietary lipids were shown to be efficient when given before an inflammatory trigger. Even when the nutritional intervention is started after the inciting event it still is effective\cite{46}. This is especially of interest in...
acute clinical settings such as trauma in which changes in intestinal barrier integrity are clinically important and may be related to associated complications. The fact that such a negative feedback mechanism exists seems logical and functional during feeding. During digestion and absorption of vital nutrients, a fierce immune response to temporarily present bacterial toxins, antigens and destructive endogenous lysozymes accompanying nutrition needs to be avoided. Next to the dampening effect of lipid enriched nutrition on the inflammatory response, intestinal barrier integrity is also preserved in various experimental models. This beneficial effect on gut barrier integrity may be explained twofold. First of all, release of acetylcholine can prevent intestinal damage via a decreased release of inflammatory cytokines. Both TNF-α and IFN-γ have long since been known to modulate the epithelial barrier in the intestine. Interestingly, lipid rich nutrition preserves intestinal barrier function early on, suggesting that local inflammation may be of importance. Another route may be through enteric glia cells since ablation of these enteric glia cells has been shown to directly affect gut barrier function and may result in inflammation. The effects of lipid rich nutrition on these cells however needs to be further investigated.

NEW THERAPEUTIC STRATEGIES

Stimulation of the autonomic nervous system may be useful as a therapeutic target in acute inflammatory based conditions. There have been many experimental studies showing a beneficial effect of electrical or pharmacological stimulation of the autonomic nervous system. However, general activation of nicotinic receptors may also have a wide scope of unwanted effects on other cells or cell systems besides inflammatory cells, which have yet to be determined.

Enteral administration of dietary fat provides a physiologic way to stimulate the cholinergic anti-inflammatory pathway and administration of nutrition is considered to be safe. Early enteral nutrition is successfully implemented in fast-track programs such as the Enhanced Recovery After Surgery program in which both hospital stay and the complication rate following colorectal surgery are reduced.

Surgical patients are an interesting intervention group since the timing of surgery (and therefore the trigger for the immune response) is predetermined. Dampening the postoperative local and inflammatory response is expected to have important effects on related complications such as POI, but potentially also on other parameters. Future clinical trials with dietary lipids as a means to stimulate the anti-inflammatory cholinergic pathway will provide more clarity to these questions.

CONCLUSION

The autonomic nervous system is an endogenous pathway that dampens the inflammatory response and regulates intestinal barrier function. Further delineation of this pathway may help to understand the relative hyporesponsiveness in the intestine to luminal contents. Furthermore, nutritional stimulation of this anti-inflammatory neurogenic feedback may be used as an important therapeutic target in several inflammatory based diseases.
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