Transient Receptor Potential Channels in Intestinal Inflammation: What Is the Impact of Cigarette Smoking?

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Key Words

Transient receptor potential channels · Cigarette smoking · Intestinal inflammation

Abstract

Inflammatory bowel disease (IBD) is characterized by severe gastrointestinal inflammation and results from a complex interplay between genetic and environmental factors. IBD includes two prominent subtypes: Crohn's disease (CD) and ulcerative colitis (UC). One of the main risk factors for the development of CD is cigarette smoking, while UC is rather a disease of ex-smokers. To date, many of the mechanisms underlying the immune imbalance in IBD and the involvement of cigarette smoke (CS) are incompletely understood. Transient receptor potential (TRP) proteins are non-selective cation channels that, upon activation, lead to plasma membrane depolarization and, in general, to Ca^{2+} influx. TRP channels of the ankyrin and vanilloid family, expressed by sensory neurons in the central and enteric nervous systems, have been extensively studied in the context of intestinal inflammation. Moreover, recent advances made on the role of non-neuronal expressed TRP channels shed light on the involvement of epithelial cells in inflammatory processes.

This review focuses on how CS may impact TRP channel function in intestinal inflammation. Firstly, we discuss the current knowledge on neuronal TRP channels, known to be linked to IBD, in health, immune homeostasis and intestinal inflammation. Subsequently, we address how TRP channels are activated by CS and its components in other organ systems and also hypothesize on the potential implications for CS-mediated TRP channel activation in gut inflammation.

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Introduction

Inflammatory bowel disease (IBD) is characterized by severe gastrointestinal inflammation and results from a complex interplay between genetic and environmental factors, of which cigarette smoking is the most prominent. IBD includes two main subtypes: Crohn's disease (CD) and ulcerative colitis (UC). The hallmarks of and differences between CD and UC are listed in table 1.

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Table 1. Hallmarks of and differences between CD and UC

Crohn's disease	Ulcerative colitis
<i>Clinical Features</i>	
Starts in the terminal ileum	Confined to the colon
Can extend to any part of the gastrointestinal tract	Progress from distal to proximal
Discontinuous (skip lesions, cobble stones)	Continuous
<i>Histology</i>	
Transmural inflammation	Superficial inflammation
Acute and chronic inflammatory cell infiltrate	Acute and chronic inflammatory cell infiltrate
Crypt architectural irregularity, ulcer (loss of crypts), cryptitis	Cryptitis, crypt abscesses, branched and shortened crypts, crypt regeneration + Paneth cell metaplasia
Granulomas	Mucin granulomas (histiocytic aggregates, giant cells)
–	Decreased mucus, decreased goblet cells
<i>Immunological response</i>	
Th1/17 response	Th2/17 response
<i>Oxidative stress</i>	
Infiltration of neutrophils (source of ROS)	Infiltration of neutrophils (source of ROS)
	Increase of 4-HNE-modified proteins in the mucosa
<i>Important differential inflammatory mediators</i>	
CD8+ T cell transcriptional signature as a potential immunological marker	LTB ₄ as a major chemotactic factor for inflammatory cells
Higher levels of CRP in serum	Higher levels of IL-13, IL-17 and CRP in serum
CRP levels in CD are even higher than in UC	Disease activity correlates with amount of IL-13 and IL-17
	IL-17 as a progress marker for disease
<i>Important shared inflammatory mediators in CD and UC</i>	
Increased IL-8 in the mucosa	
Increased MCP-1 (mainly produced by macrophages, dendritic cells and monocytes)	
<i>Differential risk factors</i>	
Genetic defects: processing of intracellular bacteria, autophagy, innate immunity	Genetic defects: barrier function
Antibiotic exposure, especially early in life	Antibiotic exposure
Cigarette smoking: detrimental	Cigarette smoking: protective
Diet: meats, fatty foods, desserts	Diet: linoleic acid
Stress, depression	–
<i>Shared risk factors in CD and UC</i>	
Geographic location, Western lifestyle	
Hygiene hypothesis	
Bacterial gastroenteritis	
NSAID use	
Vitamin D deficiency	
Diet: high intake of mono- and disaccharides, total fats	

For additional information on shared and differential cytokines in IBD, see the review by Neurath [4]. ROS = Reactive oxygen species.

In CD, the whole intestinal wall is affected, resulting in transmural inflammation which can extend to any part of the intestine and is hallmarked by a complex set of phenotypes (ileal and/or colonic involvement) [1]. There is increasing interest in the use of immunological markers

for prognosis. A potential immunological marker would be the CD8+ transcriptional signature [2]. In the serum of CD patients, higher levels of C-reactive protein (CRP) are detected [3]. Inflammation in CD is characterized by a T helper (Th)1/17 response [4].

In UC, inflammation is usually limited to the mucosal lining of the colon and rectum, and starts at the distal part of the gut, after which progression to more proximal regions occurs. The inflammatory mediator leukotriene B₄ (LTB₄) is suggested to be a major chemotactic factor for inflammatory cells in UC [5]. In the serum of UC patients, higher levels of IL-13, IL-17 and CRP are found and disease activity is correlated with the amount of IL-13 and IL-17. IL-17 appears to be a major marker of disease progression in UC [6]. Inflammation in UC is characterized by a Th2/17 response [4].

In both CD and UC, enhanced chemokine expression (e.g. IL-8) is correlated with increased disease activity in the colon of active patients, the amount of cells expressing monocyte chemoattractant protein (MCP)-1 is increased and IL-8 is increased in the mucosa [4–10].

It is being increasingly recognized that transient receptor potential (TRP) channels are implicated in bowel disorders and IBD [11, 12]. These channels, named after the role of its founding member in *Drosophila* phototransduction [13, 14], are important sensors and transducers in the digestive system. They are cation-permeable channels and mediate the depolarization of cells upon activation by exogenous and local endogenous stimuli. Except for TRPM4 and TRPM5, all TRP channels are Ca²⁺ permeable, hence their activation impacts intracellular Ca²⁺ signaling [15]. Several TRP channels were first described as receptors sensitive to temperature changes and pungent or cooling spices. These channels function as molecular sensors for specific chemical entities, among which are painful toxins, playing a role in chemesthesis [16–18]. For example, capsaicin, a component of hot peppers, is a potent agonist of the TRP vanilloid 1 (TRPV1) channel [19, 20]. Functional TRP channels consist of tetramers which open and close due to conformational changes in the protein structure [21–25]. To date, about 27 distinct TRP subunit genes are found to be encoded in the human genome. Five of the six identified subfamilies are implicated in chemo-, thermo- and/or mechanosensation, namely vanilloid TRP (TRPV), melastatin TRP (TRPM), ankyrin TRP (TRPA), polycystin TRP (TRPP) and canonical TRP (TRPC). The potential of TRP channels as a drug target in disease of the digestive system has been extensively reviewed before [26].

Cigarette smoke (CS) contains chemical irritants, e.g. nicotine and acrolein, which are known to activate TRP channels. In this way, CS may affect a diverse set of cellular pathways. Also, TRP channels are able to modulate the function of immune cells, e.g. CD4⁺ T cells [27, 28]. This review aims to provide an overview of what is cur-

rently known about the role of three TRP channels (TRPA1, TRPV1 and TRPV4) in the gut, their link with the immune system and their implications in inflammatory diseases of the intestine such as IBD. We hypothesize that a noxious substance like CS, which is able to interfere with the function of TRP channels in, for example, the lung, also impacts on TRP channel function in the gut, thereby providing a mechanistic link between cigarette smoking and gut disease.

Function of TRP Channels in the Healthy Gut

As cation entry pathways, TRP channels modulate many physiological processes. They play a role in the control of the membrane potential and excitability of neurons, epithelial cells, muscle cells and the interstitial cells of Cajal. TRP channels are also involved in the absorption of Ca²⁺ and Mg²⁺, and in the maintenance of blood flow, pacemaker activity, motor activity, secretion processes and mucosal homeostasis in the gut [26]. The main functions of TRP channels are molecular sensing of chemical and physical stimuli, downstream or secondary signal transduction via G-protein-coupled receptors (GPCRs) and ion transport. In primary afferent sensory neurons, TRP channels transduce signals, leading to the local release of neuropeptides such as calcitonin gene-related peptide (CGRP), substance P and somatostatin, which modulate local tissue function. In addition, TRP channel activation affects the central nervous system, eventually causing autonomic reflex responses and sensations [29, 30].

The gut is extensively innervated and its function is controlled by both the extrinsic (autonomous) nervous system and its own local nervous system (called enteric or intrinsic nervous system). The enteric nervous system contains motor neurons that control digestive tract motility and primary afferent sensory nerves that detect chemical, mechanical or osmolarity changes in the gut. The gut lumen gets in contact with multiple chemicals, toxins and irritants that can be present in the ingested food, released by the intestinal tissue, produced by the microbial population or just present in the digestive fluids [26]. Due to their broadly tuned chemical sensitivities, sensory TRP channels play a role in the detection of these compounds (fig. 1). For example, although first being identified as a cold-activated channel, it was found that TRPA1 acts as a sensor for spices (e.g. mustard, horseradish and wasabi). In addition, TRPA1 is activated by endo- and exogenous chemical irritants such as ozone, tear gas, nicotine and acrolein, among others [26, 31], and by al-

Fig. 1. Colocalization of TRP channels in the gut. The gut lumen contains a cocktail of irritating chemicals (e.g. nicotine, acrolein, ozone), toxins (e.g. from tropical plants and venoms), food constituents (e.g. pepper components), bacteria, etc. TRP channels are able to sense chemical and physical stimuli in the gut, among which are irritating chemicals, toxins and food constituents. The

figure depicts the architecture of the intestinal wall, with the presence of intestinal epithelium including enteroendocrine cells, CD45+ immune cells and CD4+ T cells, muscle layer and neurons, and shows the location of TRPA1, TRPV1 and TRPV4 in the intestinal environment. HEV = high endothelial venule; Mph = macrophage; EEC = enteroendocrine cell.

kalosis [32]. Another example is TRPV4, the activation of which is elicited by moderate heat, cell swelling and arachidonic acid metabolites [33]. A third example is TRPV1, which is a sensor for several spices, noxious heat, acidosis and endogenous stimuli (e.g. lipid mediators in the arachidonic acid metabolism) [34, 35].

Localization of TRP Channels in the Gut

The expression of TRP channels has been investigated using immunohistochemistry combined with retrograde tracing, with functional assays and by RT-qPCR and Western blotting. The channel TRPV1, belonging to the vanilloid subfamily, is found in extrinsic spinal and vagal primary afferent sensory neurons in the myenteric plexus (table 2), as shown in the mouse, rat and guinea-pig [11,

36–46]. The amount of TRPV1-expressing neurons depends on the gut region, higher in visceral than somatic afferent neurons [41, 47–49]. In rodents and human, TRPV1-expressing spinal and vagal neurons are present in the muscles and myenteric nerve plexuses, are associated with the arterioles and mucosa of the intestine and colocalize with CGRP, substance P, somatostatin and other neuropeptides and messenger molecules [11, 36–42, 48, 50–58]. Some reports also suggest the existence of TRPV1-expressing neurons in the enteric nervous system, but this is still controversial [54]. An explanation might be the existence of distinct TRPV1 splice variants that show different immunoreactivity [59–61].

In addition to the nerves, non-neuronal cells can also express TRPV1 (fig. 1), e.g. the serous acinar and ductal cells of the human submandibular gland, the gastrin and parietal cells of the stomach, the epithelial cells of the

Table 2. Expression of TRPA1, TRPV1 and TRPV4 on neuronal and non-neuronal cell types in the gut

	Neuron	Epithelial cell	Enteroendocrine cell	Muscle cell	Immune cell ¹
Channel
TRPA1	+	+	+		
TRPV1	+	+	+		+
TRPV4	+	+		+	+

¹ CD4+ T cell or CD45+ cell.

esophagus, stomach and ileum and the enteroendocrine cells, macrophages, CD4+ T cells and CD45+ cells in the gastrointestinal tract [53, 60, 62–71].

TRPV4, another vanilloid TRP family member, has been reported to be expressed by primary spinal afferent sensory neurons and colocalizes with CGRP in the epithelial, submucosal and muscle cells in the intestine [72, 73]. In human tissue, TRPV4 has also been reported in intestinal epithelial, glial and infiltrated inflammatory cells [74]. In the human epithelial cell line Caco2, TRPV4 was found to be expressed at the basolateral side, but also weakly at the apical side [75].

Another example, from the ankyrin TRP family, is TRPA1. This channel has been shown to be expressed by extrinsic primary afferent and intrinsic enteric neurons and endocrine cells in the gut mucosa [26]. The activation of TRPA1, both in the esophagus and intestine, results in the excitation of primary afferent neurons in the vagal, splanchnic and pelvic nerves [76].

Implication of TRP Channels in Immune Homeostasis of the Gastrointestinal Tract

Upon activation of TRPA1- and TRPV1-expressing neurons, neurotransmitters are released, leading to changes in vascular, immune and smooth muscle functions in the intestine [38, 77–80]. Importantly, TRPA1 and TRPV1 are implicated in the maintenance of immune homeostasis in the gastrointestinal tract through interaction with the microbiome.

Although we focus on the lower gastrointestinal tract (ileum and colon) in this review, we include some insights on the upper gastrointestinal tract since these may also be of importance for other gut regions. For example, it has been shown that the function of TRPV1 in trigeminal neurons innervating the oral cavity can be modulated by the presence of endotoxin (lipopolysaccharide, LPS), an abundant outer wall glycolipid of Gram-negative bacteria [81]. Sensory neurons innervating the oral mucosa that express CGRP and TRPV1 also express immune receptors, such as the LPS receptor, Toll-like receptor 4 (TLR4) [81–84]. It has been reported that LPS as such cannot activate TRPV1 [81], but can increase the neuronal sensitivity towards TRPV1 agonists, yielding an increased release of CGRP (fig. 2) [81, 82]. CGRP not only increases neuronal sensitivity in the gut [84], but also has an anti-inflammatory role by binding to the CGRP receptor, which is expressed by immune cells, leading to a downregulation of TNF- α production by macrophages [85] and dendritic cells (DCs) [86].

For TRPA1, it has been shown that it can be activated by LPS in nociceptive neurons in a TLR4-independent manner, and that this channel mediates at least some acute LPS effects, including pain, inflammation, vasodilation and CGRP release (fig. 3) [87]. In addition, LPS-induced activation of TRPA1 is enhanced by 4-hydroxynonenal (4-HNE), an endogenous TRPA1 agonist produced by lipid peroxidation, indicating that this channel has increased sensitivity to LPS during inflammation [87]. To date, the involvement of TRPV4 in gut immune homeostasis remains unknown. Based on research on ciliated epithelial

Fig. 2. Stimulation of the trigeminal ganglia inhibits LPS-induced production of TNF- α by macrophages.

Fig. 3. Involvement of TLR4 in LPS-mediated activation of TRPV1, but not TRPA1.

cells (e.g. airways), this channel could be implicated in cell volume homeostasis and the regulation of ciliary activity [88]. However, gut and lung epithelia are very different, with lung epithelial cells bearing cilia, while gut epithelial cells have a characteristic brush border. Also, due to the functional differences between the ileum and colon, the role of TRP channels differs between these gut compartments. One may speculate that TRPV4 is involved in the evolution of immune homeostasis towards the onset of inflammation, as the channel's expression and function is modified by intestinal inflammation and its activation gives rise to proinflammatory signals [12].

TRP Channels in the Inflamed Gut

TRPV1

In addition to its role in gut homeostasis, TRPV1 is also involved in gut inflammation, pain and hyperalgesia [11]. Inflammation causes an upregulation and sensitization of TRPV1. In functional disorders such as irritable bowel syndrome (IBS) and quiescent UC and CD, a correlation has been shown between the amount of mucosal TRPV1-expressing neurons and pain severity [11]. Induction of TRPV1 protein in whole-gut tissue has been denoted in disorders of the lower gastrointestinal tract. Both CD and UC patients appear to have more TRPV1-positive neurons in the rectosigmoid colon. The same observation has been made in IBS patients and patients with quiescent IBD showing IBS-like symptoms [11, 51]. In addition, a study outside the gut showed that LTB₄, a major inflammatory mediator in UC, induces itching of skin via TRPA1 and TRPV1 [89].

The TRPV1 channel exerts a nociceptive role, which is why its activation on primary afferent neurons causes the

induction of pain. Many proalgesic factors affect TRPV1, and it seems that gut inflammation might be involved in the emergence of chemical and mechanical hyperalgesia [61, 90–92]. In inflammatory hypersensitivity, TRPV1 mainly functions as a secondary transducer, as its activity is boosted by mechanisms dependent on the receptors for inflammatory mediators and neurotrophins. IBS patients strongly suffering from diarrhea show hypersensitivity to food containing TRPV1 agonists (e.g. chili), resulting in painful and burning sensations [93]. In an experimental IBD mouse model like dextrane sodium sulfate (DSS) colitis, activated TRPV1 channels enhanced clinical symptoms, histological inflammation and neutrophil accumulation [94].

In cases of tissue irritation and injury, the activity of TRPV1 on vasculature, immune system and smooth muscles contributes to the development of neurogenic inflammation. CGRP, somatostatin and the tachykinins, substance P and neurokinin A, are the messengers involved [95, 96]. TRPV1 appears to have a dual role at the level of the gut mucosa. On the one hand, activation of TRPV1 protects the mucosa against injurious insults [77], and on the other hand, its activation causes stronger inflammation in models of colitis in wild-type mice compared to TRPV1 knock-out mice [94]. Also, the TRPV1 agonist capsaicin causes intestinal inflammation and the release of substance P in a similar way to *Clostridium difficile* toxin A. These effects can be abolished by the TRPV1 antagonist capsazepine [97].

TRPV4

TRPV4 is particularly known for its role in the pain pathway, as TRPV4 activation induces somatic and visceral pain [26, 73, 98]. TRPV4 is found to be increased in the inflamed gut tissue of human IBD patients (both UC

and CD) in comparison to healthy gut tissue, and its activation leads to inflammation [74, 75].

In the mouse, during DSS-induced colitis, TRPV4 mRNA and protein increases in colonic epithelium [75]. The increase of TRPV4 in the mouse colon during ongoing inflammation indicates a potential role for TRPV4 in intestinal inflammation [12]. TRPV4 function is regulated by inflammatory mediators. It has been shown that anti-inflammatory molecules such as plant cannabinoids decrease TRPV4 mRNA expression in the mouse jejunum (upper part of the ileum) [99], while proinflammatory molecules such as histamine, serotonin and proteases enhanced the neuronal response to TRPV4 activation in the mouse colon [98]. These proinflammatory mediators can induce the production of arachidonic acid metabolites, resulting in the activation of TRPV4 [98]. Also, selective blockade of TRPV4 in an IBD mouse model attenuated colitis and pain [74]. Intracolonic administration of TRPV4 agonists in wild-type mice causes inflammation-like tissue damage in the colon, with edema, hyperemia and prominent mucus production. This is accompanied with upregulated cytokines and chemokines such as IL-6, keratinocyte-derived chemokine (KC), MCP-1 and RANTES [75]. Furthermore, there is increased TRPV4 mRNA expression in colon biopsies of CD and UC patients, with the highest increase in UC patients [74].

Activation of TRPV4 by the synthetic agonist 4 α -phorbol-12,13-didecanoate (4 α PDD) elicits inflammation via a neurogenic mechanism, which includes the release of neuropeptides (substance P and CGRP) by sensory neurons. In the gut, intraluminal administration of TRPV4 agonists results in activation of nociceptors, visceral hyperalgesia and allodynia [73, 98].

Many studies have investigated the role of neuronal TRPV4 in intestinal inflammation. Recent research is also focusing on epithelial TRPV4, mainly via in vitro studies. For example, it has been shown that TRPV4 activation triggers IL-8 production in esophageal epithelial cells [100]. In the intestinal epithelial cell lines, Caco2 and T84, TRPV4 activation by 4 α PDD leads to a dose-dependent increase in intracellular Ca²⁺ concentration and chemokine release [75]. These findings in epithelial cell lines suggest that TRPV4 may play a role in intestinal inflammation via a non-neurogenic mechanism, leading to the production of chemokines such as IL-8.

TRPA1

TRPA1 is upregulated in colitis in mice and in colon biopsies of human UC and CD patients [69]. The activa-

tion of TRPA1 on visceral sensory neurons causes the release of neuropeptides, which results in vasodilatation, local inflammation and sustained mechanical hyperalgesia in the gut [101–103]. In the literature, both pro- and anti-inflammatory effects have been attributed to TRPA1. For example, it has been shown that TRPA1 agonists are able to elicit colitis, associated with an increased TRPA1 protein expression in sensory neurons [104]. DSS colitis was found to be less severe in mice deficient for TRPA1, although DSS is not a direct activator of sensory neurons; it seems that the colitis was maintained due to a sustained activation of TRPA1 by endogenous inflammatory stimuli (e.g. prostaglandins, cytokines and oxidative stress mediators like 4-HNE and acrolein) [105]. In contrast, another study showed that the TRPA1 agonist AITC causes a protective effect in experimentally induced gastritis in rats, probably via endogenous prostaglandins [106]. Moreover, the IL-6 signal transducer gp130 is indispensable for expression of the TRPA1 ion channels in the dorsal root ganglia, suggesting that TRPA1 is prone to proinflammatory signals like IL-6 [107]. TRPA1 mRNA expression is increased in whole colon tissue in murine colitis and in human biopsies of active CD and UC patients. Furthermore, a protective role has been attributed to TRPA1 in the gut, as its ablation ameliorates the disease activity index and histological score in DSS colitis, and TRPA1 mediates the decrease in proinflammatory neuropeptides, cytokines and chemokines [69].

Other TRP Channels

Besides TRPV1, TRPV4 and TRPA1, knowledge about other TRP channels in gut disease is limited. TRPV6, mainly expressed by intestinal epithelial cells and the colon cell line, Caco2, is highly selective for Ca²⁺ transport and is therefore important for Ca²⁺ absorption in the gut [108–110]. This channel is regulated by dietary factors and calcitropic hormones [108, 111–114]. Disturbed Ca²⁺ homeostasis is associated with a CD-like pathology and may lead to osteoporosis [115].

Interaction between TRP Channels and CS in the Lung

In Belgium, 27% of the population smokes, with the highest percentages being amongst men, the unemployed and workers [116]. CS is amongst the three leading risk factors for global disease burden [117]. In comparison to never-smokers, current smokers have a 2–3 times higher

Table 3. TRP activation in non-neuronal cell types

Cell type	TRP channel	Activating component	Effect	Reference
Human lung fibroblasts	TRPA1	whole CS acrolein	induction of IL-8/KC	[110]
Human esophageal cells	TRPV1	4-HNE	induction of IL-8	[117]
Human intestinal epithelial cells	TRPV4	4aPDD	induction of IL-8	[61]

mortality rate. The major causes of death include lung cancer, chronic obstructive pulmonary disease (COPD), ischemic heart disease and total stroke [118]. In the lung, CS causes an increase in inflammatory cells, including DCs, CD8+ and CD4+ T cells, in the airways and pulmonary tissue, leading to the development of lymphoid neogenesis and COPD. Many cytokines, chemokines and their receptors are involved in this immunological response, such as MCP-1, IL-8 and CCR6 [119].

TRP channels have been linked to pathological conditions of the lung. For example, they are often related to cough and wheezing in asthma patients, e.g. TRPV1 is increased in airway nerves and airway smooth muscle cells of individuals with cough [120]. Single nucleotide polymorphisms (SNPs) in the TRPV1 gene are also linked to cough [120]. TRPA1 has been shown to play a role in neuro-immune interactions in the airways, giving rise to asthmatic airway inflammation after allergen challenge [121]. Furthermore, gene polymorphisms of TRPV4 have been associated with COPD [88].

The interaction between the chemical irritants contained in CS and TRP channels has been studied in the lung-innervating neurons. For instance, TRPA1 is known to be sensitive to various irritants and chemicals, among which are air pollutants and prominent CS components [122]. In addition, nicotine is able to activate TRPA1, although at concentrations higher than those found in CS, but relevant for smoking cessation therapies [123].

TRP channels are expressed in a subpopulation of primary sensory neurons in the airways. Upon activation, these neuronal TRPs may give rise to neurogenic inflammation, which implies the release of proinflammatory neuropeptides, such as substance P and CGRP [46, 87]. However, TRP channels are also expressed by non-neuronal cells (table 3), among which are immune and epithelial cells [70, 124], thus contributing to acute inflammatory responses in the lung in response to CS or one of its components. Indeed, the TRPA1 agonists acrolein and CS were able to induce release of IL-8/KC by epithelial cells in primary cell culture [124]. Furthermore, TRPV1

and TRPV4 mRNA levels are increased in lung tissue from COPD patients and both channels mediate ATP release from bronchial epithelial cells [125].

Impact of CS on TRP Channels in the Gut?

Few studies have probed the effect of CS on TRP channels in the gut. There are important similarities in the molecular effects of CS in the lungs and the gut (fig. 4), which makes it interesting to investigate whether it similarly affects the TRP channels in the gut and lungs. Nevertheless, many differences exist between lung and gut epithelia, not in the least in their function. In both the lungs and gut, the epithelium acts as a barrier, but lung epithelial cells play a role in, e.g. mucociliary transport, while gut epithelial cells, with their characteristic brush border, are rather involved in digestion. TRP channels are expressed and play a role in both the lungs and gut, CS affects TRP channels in the lung and the expression of TRPs is increased in the gut of IBD patients [69, 124]. Therefore, it is likely that CS also affects TRP channels in the gut.

Cigarette smoking is a major modulating factor in the development of IBD. First, it worsens CD, necessitating increased need for steroids and immunosuppressive drugs, more frequent relapses and surgery [126]. Moreover, current smokers have a two times higher risk and ex-smokers have a 1.8 times higher risk of developing CD in comparison to never-smokers. In contrast, UC occurs in ex-smokers, as shown by several meta-analyses [127–135], and the risk of developing UC is three times higher in the first five years after smoking cessation [127].

Several mechanisms are suggested to be responsible for the negative effect of cigarette smoking in CD patients. It is known that CS causes an increased expression of IL-8, CCR6, CCL20 and mucins, induction of autophagy and apoptosis, increased recruitment of immune cells and a decrease in IFN- γ (fig. 4) in the ileum. Furthermore, the oxidizing chemicals contained in CS can have prothrombotic effects, and thereby trigger microvasculature

Fig. 4. Effects of cigarette smoking on the gut and lungs.

abnormalities and ischemia in the gut. Also, the impaired bacteria-host response, which is a hallmark of CD, can be worsened by smoking, as it exerts an immunosuppressive effect on macrophages [136, 137]. Different mechanisms might be at the base of the differential smoke-induced effects in CD and UC patients. Indeed, the main sites of inflammation differ between these two diseases: CD mainly occurs in the terminal ileum while UC is usually initiated in the distal colon. The fundamental difference between the ileum and the colon can possibly explain why CS may exert its effect on CD and UC via very different mechanisms. The situation is more complex in the colon than in the ileum due to the numerously present microbiota. The effect of CS on the colon is ambiguous, even though epidemiological evidence clearly shows a protective effect of CS on UC. The most important effect of CS on the colon is a prominent shift in microbiome composition and activity [138]. To date, the exact mechanisms which underlie the differential effects of CS on CD and UC remain a matter of debate. For example, it has been suggested that differential DC responses to CS between CD and UC may play a role [139]. Another study suggests that CS induces barrier dysfunction in the ileum, but not

in the colon [140]. IL-8 is also known to be increased in the mucosa of UC and CD patients [7–10]. A study reported that CS affects IL-8 levels in the mucosa of UC and CD patients [141]. Of course, the gut is a very complex organ system with complex functions (e.g. the interaction immune system-microbiome). The effect of CS on the gut is probably a combination of several mechanisms.

In a mouse model, we have demonstrated that, similarly to the lung, CS affects the gut immune system by enhancing the recruitment of CD11b+ DCs, CD4+ and CD8+ T cells to the mouse ileal Peyer's patches, which are the main lymphoid organs in the gut [142]. Furthermore, CS alters the production of immune factors in the mouse ileum (increased CXCL2, an IL-8 homolog) and in the proximal colon (increased IL-6) [138]. Therefore, it is likely that CS exerts its effect on gut inflammation via mechanisms similar to the lung, with the levels of similar cytokines (IL-6 and IL-8), chemokines (MCP-1) and receptors (CCR6) being increased by CS [119, 143].

We speculate that CS and its components activate TRP channels in the gut, which can lead to increased cytokine production and, in this way, predispose the gut environment for the development of inflammation. How CS has

a differential effect on TRP channels in UC and CD is unknown; however, different hypotheses can be put forward. First, differences in innervation between the ileum and the colon could contribute to a different role for TRP channels in CD and UC. Whereas both the ileum and colon are supplied with autonomic and sensory fibers from the celiac and superior mesenteric plexuses, the colon is additionally innervated by the pelvic nerves. Differences in the distribution of TRP-expressing sensory neurons between the ileum and colon as well as the degree of TRP expression could maybe contribute to the different responses in CD and UC towards CS. For example, a study has shown that TRPV1 expression (in the submucosa, smooth muscle layer and myenteric plexus) is elevated in the distal part of the colon in comparison to the proximal part [144]. Secondly, differential TRP expression and activation on epithelial cells and/or inflammatory cells in different parts of the gastrointestinal tract could be implicated in the two pathologies. According to a study by Kun et al. [69], mainly TRPA1 and TRPV1-immunopositive macrophages are detected in UC, while TRPA1- and TRPV1-immunopositive infiltrating plasma cells are more prominent in CD. Finally, differences in microbial composition between distinct gut regions and the potential interaction of bacterial components with TRP channels could play a role. CS differentially affects CD and UC, and prominent effects of CS on the microbial composition in the colon have been reported [138, 145]. This is particularly interesting as LPS (a component of the microbial cell wall)-TLR4 interactions are able to sensitize e.g. TRPV1. Gut epithelial cells express both TRPV1 and TLRs. However, probiotics such as *Lactobacillus* are able to reduce TRPV1 activation. It may be that CS indirectly affects the TRP channels in the colon via the induction of changes in the microbiome, but these effects will strongly depend on which species and niches are changed and whether microbial metabolism and the resulting metabolite pool are affected. Taken together, the differential effects of CS in CD and UC could originate from the CS-induced changes in the microbiome in the colon, which can modulate TRP channel activity.

It is particularly interesting that activation of the TRP channels can boost IL-8 expression (e.g. in the lungs). Furthermore, as ion channels, TRP channels can also affect cellular processes (e.g. via Ca^{2+} entry) and their activation can have far reaching consequences for the cell. It might be interesting to examine whether CS affects Ca^{2+} inflow and Ca^{2+} -dependent cellular processes. An example is autophagy, which is known to be induced by CS in the epithelium of both the lungs and ileum [11, 12, 146–

148]. The activation of immune cells can also be modulated through Ca^{2+} inflow. In CD4+ T cells, TRPV1 is associated with T cell receptor (TCR) co-receptor CD4 and contributes to TCR-induced Ca^{2+} inflow [27]. Altogether, these data suggest that TRP channels may contribute to inflammation in the ileum, and they possibly play a role in the detrimental effect of CS on CD.

Surprisingly, in IBD genome-wide association studies (GWAS), no SNPs in TRP genes have been found to be associated with IBD [149]. Nevertheless, TRP function is often associated with factors that do show significant polymorphisms in IBD GWAS, e.g. TLR4, ATG16L1 [81, 150]. However, in lung disease, SNPs in TRP channels are found. Trpv4 gene polymorphisms are associated with COPD [151] and Trpv1 genetic variants are linked with a lower risk of active childhood asthma [152]. It might be that the IBD GWAS did not detect any Trp polymorphisms, because the studies did not focus on specific risk factors such as CS. A specific genetic study taking CS into account as a cofactor would be needed to verify this hypothetical statement.

In addition, it is known that CS affects extraintestinal manifestations of IBD, specifically rheumatoid arthritis. Interestingly, TRP channels also play a role in rheumatoid arthritis, with TRPA1 and TRPV1 involved in the anti-inflammatory effects of N-acylethanolamines in rheumatoid arthritis synovial cells [153].

Furthermore, the link with functional disorders such as IBS might be of interest. A study has shown that TRPV1 is increased in rectosigmoid biopsies of patients with IBS and quiescent IBD. TRPV1 expression clearly correlated with increased pain perception [11]. This may point to a link between IBS and colonic IBD.

Neuronal TRPs have been thoroughly studied in the gut [26], but not in relation to CS. The number of studies regarding the effects of CS on epithelial TRP channel expression and function in the gut is scarce. Several studies have probed the effects of CS components on TRPV1 and TRPV4 in in vitro cell culture models (table 3). In human esophageal epithelial cells, the activation of TRPV1 also leads to the induction of IL-8, and this induction is further enhanced by 4-HNE, which is released upon CS- and inflammation-induced oxidative stress. One of the stress-inducing capacities of 4-HNE is the modification of proteins. Moreover, the TRPV1 protein itself is modified by 4-HNE [154]. TRPV1's cytokine-inducing capacity is modulated by oxidative stress, due to reactive oxygen species which are present in both the particulate and gaseous phase of CS [69].

In conclusion, there are indications that CS may affect TRPs in the gut in a similar way to how it affects TRPs in the lung. This concerns an immunomodulatory role via the induction of cytokines like IL-8, e.g. in the case of epithelial TRPs, but this may even extend to cellular processes like autophagy. This CS-TRP interaction may provide the mechanistic link between CS and IBD. Of course, further studies, taking into account the substantial differences between the distinct gut compartments (ileum and colon), are needed to unravel the role of TRP channels in the pathogenesis of gut disease.

Acknowledgements

Liesbeth Allais is supported by a doctoral grant from the Special Research Fund of Ghent University (01D41012). This work was funded by the Belgian Federal Government (IAP P7/30, IAP P7/13), FWO Flanders (G.A022, G.0702.12, G.0896.12 and G.0C77.15), a Ghent University Concerted Research Action (BOF14-GOA-027) and the Research Council of the KU Leuven (GOA/14/011 and PF-TRPLe).

Disclosure Statement

No author has an ethical or financial conflict of interest.

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