REVIEW

Channels and Transporters in Immunity

Ion channel regulation of gut immunity

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Mounting evidence indicates that gastrointestinal (GI) homeostasis hinges on communications among many cellular networks including the intestinal epithelium, the immune system, and both intrinsic and extrinsic nerves innervating the gut. The GI tract, especially the colon, is the home base for gut microbiome which dynamically regulates immune function. The gut's immune system also provides an effective defense against harmful pathogens entering the GI tract while maintaining immune homeostasis to avoid exaggerated immune reaction to innocuous food and commensal antigens which are important causes of inflammatory disorders such as coeliac disease and inflammatory bowel diseases (IBD). Various ion channels have been detected in multiple cell types throughout the GI tract. By regulating membrane properties and intracellular biochemical signaling, ion channels play a critical role in synchronized signaling among diverse cellular components in the gut that orchestrates the GI immune response. This work focuses on the role of ion channels in immune cells, non-immune resident cells, and neuroimmune interactions in the gut at the steady state and pathological conditions. Understanding the cellular and molecular basis of ion channel signaling in these immune-related pathways and initial testing of pharmacological intervention will facilitate the development of ion channel–based therapeutic approaches for the treatment of intestinal inflammation.

Introduction

The gut is the largest immune organ in the body (Chassaing et al., 2014). Like in the skin and airways, the mucosal surfaces in the gastrointestinal (GI) tract constitute the largest barrier surface in the body. The intestinal mucosal surfaces are lined by intestinal epithelial cells (IECs) that interact with their external environments to regulate nutrient absorption and protection from harmful stimuli in the gut lumen (Turner, 2009). Among these harmful stimuli are pathogens, xenobiotics, and food antigens that have the potential to disrupt the intestinal barrier and subsequently promote systemic inflammation and tissue damage, especially in individuals with genetic and immune predisposition. Besides maintaining intestinal barrier integrity, the IECs also respond to both chemical and physical stimuli in the gut lumen, and subsequently release immunological factors including cytokines and chemokines to regulate host immune responses (Chen et al., 2022).

The gut microbiota actively regulates the integrity and function of the intestinal barrier, and changes in the composition and function of the gut microbiota contribute to intestinal barrier dysfunction (Takiishi et al., 2017). Emerging evidence demonstrates that there is an imbalance in both intestinal microbiota and mucosal immunity as well as intestinal barrier

integrity in many autoimmune diseases such as inflammatory bowel diseases (IBD), suggesting that the gut microbiota–immune system–intestinal barrier axis plays a crucial role in controlling gut immune homeostasis and tolerance at the steady state and regulating abnormal autoimmune responses (Antonini et al., 2019). The mucosal immune system comprises both innate immune cells, such as macrophages and DCs and the majority of the body's lymphocytes population which are embedded throughout the epithelial layers and exposed to the lumen (Kaymak et al., 2021). There is evidence that both innate and adaptive immune cells can contribute to the regulation of the gut microbiota's biogeographical distribution along the GI tract, suggesting a bi-directional regulation between the gut microbiota and immune system (Gu et al., 2022).

Besides resident and infiltrated cells in the gut epithelium, the intestinal wall is densely innervated by both extrinsic and intrinsic nerves (Phillips and Powley, 2007). Autonomic nervous system (ANS) comprising the sympathetic and parasympathetic nervous systems receives commands from the central nervous system (CNS) and regulates various GI functions (Browning and Travagli, 2014). Primary sensory nerves extensively project to the GI tract and mediate interoception, the perception of

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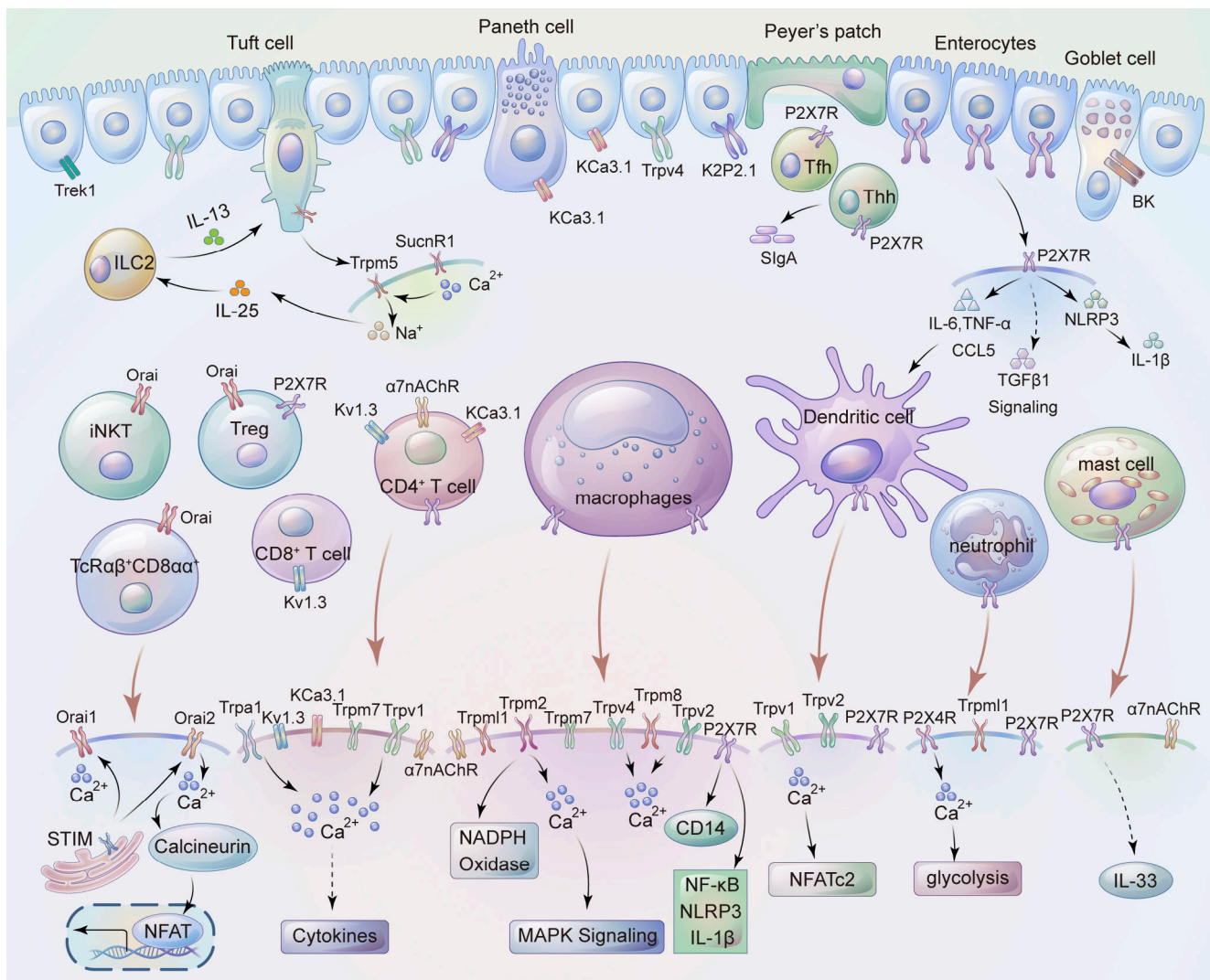
Figure 1. Summary of cell types and associated ion channels involved in gut immunity based on current literatures. Distinct cell types present in the intestinal epithelium, such as enterocytes, tuft cell, Paneth cell, Peyer's patch and goblet cell, sense either internal or external stimuli through potassium channel, TRP channel, and P2X receptors, and maintain the integrity of the intestinal epithelium. Immune cell-expressing ion channels regulate immune activation and cytokine/chemokine release in the intestinal inflammation through provoking Ca^{2+} influx and intracellular Ca^{2+} mobilization.

sensations from inside the body (Maniscalco and Rinaman, 2018; Bai et al., 2019). Moreover, the GI tract is the only abdominal organ that has evolved with its own nervous system fully contained within the gut wall, known as the enteric nervous system (ENS), also known as the "second brain" in the gut (Furness, 2012; Wood, 2016; Gershon and Margolis, 2021). Interactions between extrinsic and intrinsic nervous system maintain normal GI motility through regulating the GI smooth muscle tone (Kulkarni et al., 2018). In addition to motility regulation, both intrinsic and extrinsic nerves in the gut interact with immune cells in the epithelium and contribute to the immune homeostasis at steady state and autoimmune responses under pathological conditions (Wang et al., 2022).

Accumulating evidence suggests that ion channels are expressed by both excitatory and non-excitatory cells throughout the GI tract (Fuentes and Christianson, 2016; Fig. 1). Various ion

channels are involved in regulating neurotransmission and neurotransmitter release in both intrinsic and extrinsic nerves, ion secretion and absorption in IECs, production and release of cytokines from immune cells, and intestinal smooth muscle contraction (Fuentes and Christianson, 2016; Fig. 2). Channelopathies resulting from abnormal expression and function can often impact GI functions including motility, secretion, visceral pain, immune dysregulation, and gut–brain communications (Beyder and Farrugia, 2016). This Review focuses on recent progresses in understanding the roles of various ion channels in intestinal immune cells, non-immune resident cells, and neuroimmune interactions. Understanding the molecular and cellular mechanisms underlying ion channel regulation of GI immunity will facilitate the identification and development of ion channel-based therapeutic approaches for the treatment of immune disorders in the GI tract.
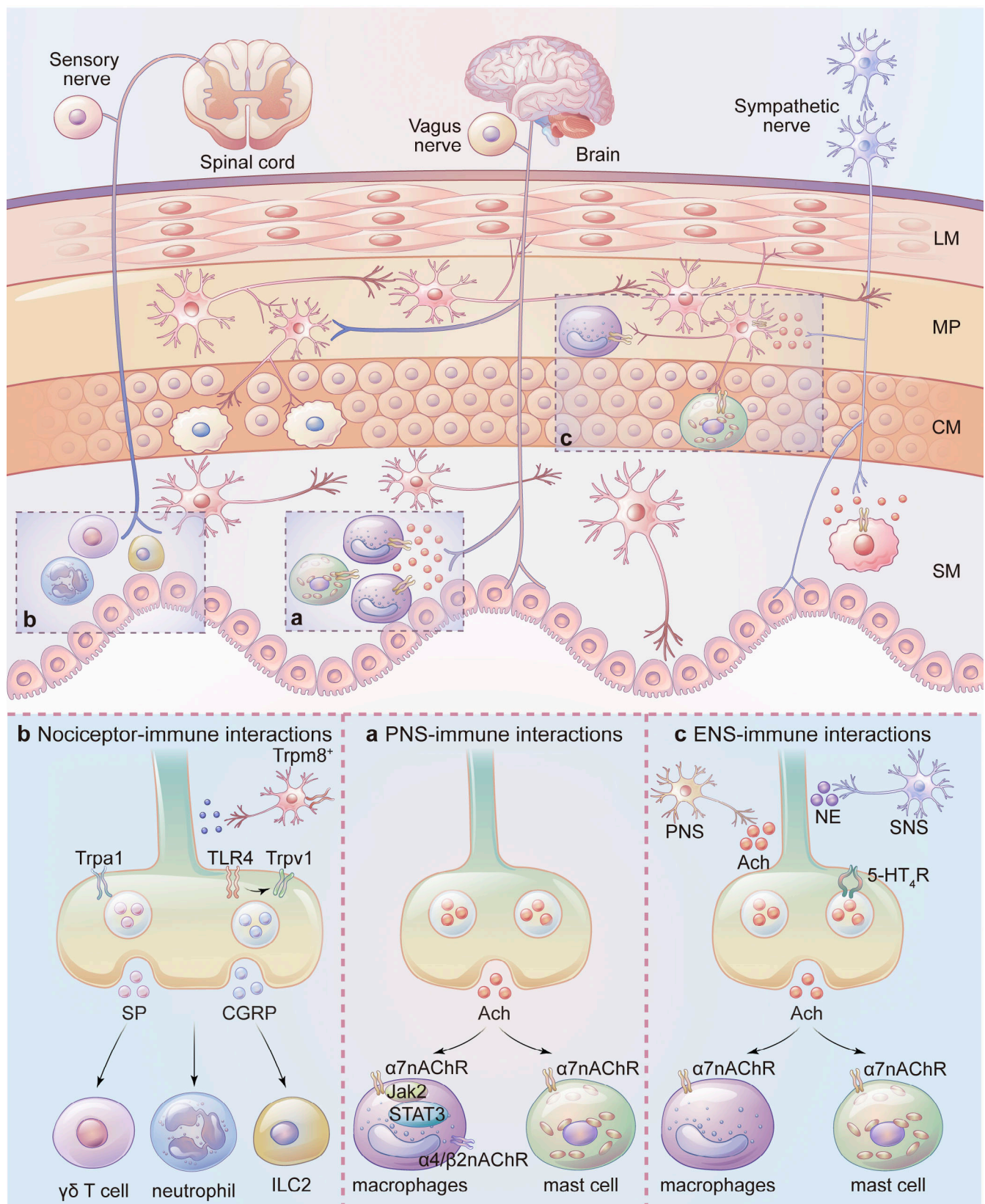
Figure 2. **Ion channels in gut immunity involving crosstalk between gut-innervating neurons and resident immune cells.** The GI tract is innervated by spinal cord sensory neurons, brainstem originating vagus neuron and intrinsic enteric neurons. The VNS-immune interaction (a) is mediated by the release of ACh from the vagus nerve, which suppresses the activation of mast cells and macrophages. TRP channels expressed by nociceptors control the release of neurotransmitters such as SP and CGRP, which may modulate the activities of immune cells and contribute to the nociceptor-immune interaction (b). ENS

Feng et al.
Ion channels and gut immunity

Journal of General Physiology   3 of 17
https://doi.org/10.1085/jgp.202113042

activation causes acetylcholine release and subsequently modulates the function of monocytes/macrophages in the setting of intestinal inflammation (c). It should be noted that both sympathetic and parasympathetic nerve systems can act indirectly through the ENS. LM, longitudinal muscle; MP, myenteric plexus; CM, circular muscle; SM, submucosa.

Ion channel functions in immune cells in the gut
Ca^{2+} release-activated Ca^{2+} (CRAC) channels

Ca^{2+} ions are essential to the development, activation, and maintenance of the immune system (Vig and Kinet, 2009). It is well established that both activation and proliferation of T cells require Ca^{2+} entry (Cahalan and Chandy, 2009; Feske et al., 2015). This is nicely reflected by the findings that at steady-state human intestinal lamina propria T cells exhibit minimal proliferation upon stimulation of antigen receptor when compared with T cells in the blood. This low proliferation activity is correlated with reduced Ca^{2+} signaling in isolated lamina propria T cells from non-inflamed tissue. Strikingly, GI inflammation promotes the proliferation of lamina propria T cells and normalizes their Ca^{2+} signaling to the levels in the blood T cells in IBD patients (Schwarz et al., 2004). Moreover, Ca^{2+} influx is required for T-cell proliferation in both peripheral blood and intestinal lamina propria, further highlighting the essential role of Ca^{2+} signaling in T-cell immunity (Schwarz et al., 2004).

The most studied Ca^{2+} entry pathway in T cells is store-operated calcium entry (SOCE) mediated by CRAC channels, which are encoded by the ORAI1 gene and regulated by stromal interaction molecules STIM1 and STIM2 within endoplasmic reticulum (ER)–plasma membrane (PM; Lewis, 2001; Oh-Hora et al., 2013; Vaeth et al., 2020). The pore of the CRAC channel is formed by PM protein Orai1 and Orai2 while stromal interaction molecules (STIM) sense the drop of cytosolic Ca^{2+} concentration and subsequently engage with the Orai proteins to drive Ca^{2+} influx (Vaeth et al., 2020). CRAC channels are the major player to trigger immune responses in T cells, while other ion channels such as K^+ channels modulate Ca^{2+} signaling through altering membrane potential of T cells to provide the driving force for Ca^{2+} entry (Cahalan and Chandy, 2009). Repetitive or prolonged increase in Ca^{2+} influx through CRACs activates NFAT, thereby promoting T-cell proliferation and cytokine gene expression through the Ca^{2+}–calcineurin–NFAT signaling pathway (Park et al., 2020). Interestingly, STIM-induced SOCE is specifically required for the development of agonist-selected T cells (regulatory T cells, invariant natural killer T cells, and $TCR\alpha\beta^+$ $CD8\alpha\alpha^+$ intestinal intraepithelial lymphocytes) but not for thymic development of conventional $TCR\alpha\beta^+$ T cells, suggesting CRAC-mediated Ca^{2+} signaling regulates maturation of distinct T-cell lineages (Oh-Hora et al., 2013; Fig. 1).

T and B cells from Orai1 knock-in mice (Orai1[KI/KI]) expressing a nonfunctional ORAI1-R93W protein display severely impaired SOCE and CRAC channel function, resulting in a strongly reduced expression of several key cytokines including IL-2, IL-4, IL-17, interferon-γ (IFNγ), and TNFα in $CD4^+$ and $CD8^+$ T cells. In addition, T cells from the Orai1[KI/KI] mice fail to develop colitis in an adoptive transfer model of IBD (McCarl et al., 2010). Pharmacological inhibition of SOCE using the CRAC channel inhibitor BTP2 also selectively reduces the production of pathogenic cytokines by human colonic T cells and innate lymphoid cells

(ILCs), which alleviates the clinical course of colitis in mice. Moreover, T-cell-specific genetical ablation of Orai1 and Stim1 attenuates the severity of intestinal inflammation in a mouse model of IBD. Most importantly, treatment with a selective CRAC channel inhibitor CM4620 attenuates IBD severity and colitogenic T-cell function in mice (Letizia et al., 2022). These findings reaffirm the requirement of Orai/Stim signaling for T-cell function and provide important insights into the in vivo functions of CRAC channels in the immune response underlying IBD pathogenesis. Therefore, CRAC channels have been considered as drug targets of T cells in immune-mediated disorders, including IBD. Indeed, a selective CRAC inhibitor Synta 66 (GSK1349571A) inhibits CRAC current in rat basophilic leukemia cells and thapsigargin-induced Ca^{2+} influx in Jurkat T cells in a concentration-dependent manner. When used to treat lamina propria mononuclear cells and biopsy specimens from inflamed areas of patients with IBD, Synta 66 reduces T-bet expression and inflammatory cytokine production (Di Sabatino et al., 2009). Interestingly, urolithin A, one of the urolithins of microbiota-derived metabolites from ellagic acid, can inhibit SOCE in murine $CD4^+$ T cells and downregulates the expression of Orai1 and STIM1/2 through enhancing the expression of miR-10a-5p (Zhang et al., 2019; Fig. 1). Despite these promising results in modulating T-cell activation by CRAC inhibitors, no clinical trial has been conducted on any classes of CRAC blockers in IBD patients (Tian et al., 2016; Giuffrida and Di Sabatino, 2020). Besides IBD, CRAC channels are also involved in helicobacter pylori (H. pylori)-induced GI pathology (Kern et al., 2015). H. pylori secrets vacuolating toxin (VacA), which is colocalized with STIM1 in the ER and may restrict STIM1 interaction with membrane bound Orai1 upon Ca^{2+} store depletion, thereby inhibiting T-cell activation and proliferation and suppressing host immune response (Kern et al., 2015). Moreover, SOCE is essential for the cytotoxic function of cytotoxic T lymphocytes (CTLs) both in vivo and in vitro by regulating the degranulation of CTLs, their expression of Fas ligand and production of TNFα and IFNγ. The presence of SOCE in CTLs can prevent the engraftment of colon carcinoma cells and control tumor growth. In contrast, patients with loss-of-function mutations in either Orai1 or STIM1 are immunodeficient and are prone to develop virus-associated tumors (Weidinger et al., 2013; Fig. 1).

Transient receptor potential (TRP) channels

TRP channels constitute a superfamily of Ca^{2+}-permeable, non-selective cation channels (Wu et al., 2010). Among TRP channels, TRPM4 and TRPM5 are permeable to monovalent cations but impermeable to divalent cations. Although other TRP channels are Ca^{2+} permeable, the selectivity varies greatly due to their distinct pore structures. For instance, P_{Ca}/P_{Na} ratio is <1 for TRPM1 but >100 for TRPV5 and TRPV6 (Gees et al., 2010; Yelshanskaya et al., 2021). TRP channels are traditionally considered as molecular sensors for temperature, pain, itch, vision,

sound, and taste; however, recent studies indicated that they may also be involved in various other physiological functions including immune regulation (Inada et al., 2006; Parenti et al., 2016; Froghi et al., 2021). Both innate and adaptive immune cells express various types of TRP channels which actively regulate immune response primarily by activating intracellular Ca^{2+} responses and related intracellular-signaling pathways, thereby affecting immune cell proliferation/differentiation, cytokine production, and cellular cytotoxicity (Inada et al., 2006; Parenti et al., 2016; Froghi et al., 2021). Not surprisingly, several TRP channels are closely related to GI inflammation (Parenti et al., 2016; Froghi et al., 2021).

TRPA1
TRPA1 is primarily expressed by primary nociceptors and serves as a critical mediator of pain and itch sensations (Xie and Hu, 2018). TRPA1 is activated by diverse electrophilic irritants which covalently modify intracellular reactive cysteine residues (Hinman et al., 2006; Macpherson et al., 2007). Activated TRPA1 possesses high Ca^{2+} permeability, while increased intracellular free Ca^{2+} can also actively regulate TRPA1 function through an intracellular Ca^{2+}-binding pocket (Zhao et al., 2020). Although TRPA1 along with the capsaicin receptor TRPV1 is exclusively expressed by the dorsal root ganglion (DRG) neurons, recent studies raised the possibility that TRPV1 and TRPA1 could be functionally expressed by the intestinal $CD4^+$ T cells, although with opposite functions: TRPV1 is shown to be related to TCR-mediated Ca^{2+} influx, T-cell activation, and differentiation into Th1-effector cells, while TRPA1 is reported to modulate these pro-inflammatory T-cell responses (Bertin et al., 2014; Bertin et al., 2017). This functional difference is also reflected by a TRPV1-mediated promotion but a TRPA1-mediated inhibition of experimental colitis in mice (Bertin et al., 2014; Bertin et al., 2017). Moreover, TRPA1 expression is upregulated in inflamed colon tissues from both humans and mice (Kun et al., 2014; Bertin et al., 2017; Fig. 1). It is intriguing that although both TRPV1 and TRPA1 mediate Ca^{2+} influx that initiates intracellular Ca^{2+} signaling in a similar manner, loss of function of TRPV1 and TRPA1 show opposite phenotypes in mouse models of experimental colitis (Bertin et al., 2014; Bertin et al., 2017). These contradictory findings suggest that TRP channel action in GI immunity may be more complicated than we thought. Moreover, although it was shown that TRPV1 and TRPA1 function in $CD4^+$ T cells (Bertin et al., 2014; Bertin et al., 2017), mRNA encoding these channels are barely detectable according to available gene expression databases (such as https://www.immgen.org or https://biogps.org). It should also be noted that these functional studies are dependent on the use of global knockout (KO) mice. Since both TRPV1 and TRPA1 are expressed by primary nociceptors, the potential involvement of neuro-immune interactions in the setting of chemical-induced intestinal inflammation cannot be excluded. Future studies with immune cell-specific TRPA1 and TRPV1 KO mice should be employed to further support the role of TRP channels in gut immunity.

TRPM2
TRPM2 is a Ca^{2+}-permeable channel that activated by warm temperatures, oxidative stress, and NAD^+-related metabolites such as ADP-ribose (ADPR; Huang et al., 2018). TRPM2 is functionally expressed by macrophages and TRPM2-deficient mice have enhanced gastric inflammation and decreased bacterial colonization when chronically infected with *H. pylori*, compared with wild-type mice. This is because TRPM2-deficient macrophages are unable to control intracellular Ca^{2+} levels, resulting in Ca^{2+} overload which subsequently enhances activities of both mitogen-activated protein kinase (MAPK) and NADPH-oxidase upon *H. pylori* stimulation (Beceiro et al., 2017). In a mouse model of dextran sulphate sodium (DSS)-induced colitis, TRPM2 deficiency reduces the production of the CXCL2 chemokine, resulting in diminished neutrophilic influx to the colon, and protects against colon inflammation, suggesting that pharmacological inhibition of TRPM2 might be a new therapeutic strategy for treating IBD (Yamamoto et al., 2008; Knowles et al., 2013; Fig. 1).

TRPM7
TRPM7 chanzyme harbors both a cation channel and a serine/threonine kinase and has been implicated in regulating thymopoiesis and cytokine expression. Silencing TRPM7 function promotes the development of thymic Treg cells through the downstream IL-2–IL-2R and FOPX3 signaling (Mendu et al., 2020). TRPM7 kinase activity also contributes to TGF-β–induced CD103 expression and pro-inflammatory Th17 cell differentiation and colonization in the gut (Romagnani et al., 2017). Interestingly, loss of TRPM7-mediated cation influx (such as Ca^{2+} and Mg^{2+}), but not its kinase activity, was shown to prevent LPS-TLR4 signaling-induced macrophage activation (Schappe et al., 2018), increase apoptosis of B cell precursors, and significantly reduce the number peripheral B cells (Krishnamoorthy et al., 2018). Surprisingly, TRPM7 kinase-dead (KD) K1646R knock-in mice, which have a similar TRPM7-mediated currents with wild-type T cells, show reduced store-operated Ca^{2+} entry in T lymphocytes, resulting in reduced T-cell proliferation and enlarged spleen in mice (Beesetty et al., 2018). Although rare evidence was shown that TRPM7 is involved in the intestinal homeostasis, a possibility was raised that TRPM7 may participate in both IBD-related and sporadic colorectal cancer, as TRPM7 is overexpressed in human IBD-related and sporadic colorectal cancer (Pugliese et al., 2020). Future studies on the role of TRPM7 in the GI tract may shed new light on the dual functions of TRPM7 chanzyme in gut immunity.

TRPM8
TRPM8 is expressed by primary sensory neurons playing a central role in detecting environmental cold temperatures and natural compounds including menthol (McKemy et al., 2002). Unexpectedly, with in vitro molecular techniques and Ca^{2+} imaging assay, TRPM8 expression and function were also detected in both peritoneal and bone marrow-derived macrophages (Khalil et al., 2016). TRPM8 is required for macrophage phagocytosis and maintaining an anti-inflammatory cytokine profile (Khalil et al., 2016). Pharmacological activation of TRPM8 by repeated applications of menthol enemas protects against DSS-induced colitis while global TRPM8 KO aggravates colitis severity, which is correlated with the profile of macrophage-derived TNFα

Feng et al.
Ion channels and gut immunity

Journal of General Physiology   5 of 17
https://doi.org/10.1085/jgp.202113042

(inflammatory) and interleukin-10 (anti-inflammatory) cytokine production (Khalil et al., 2016; Fig. 1). However, these in vivo data were generated from global TRPM8 KO mice, which could not exclude the possibility that the TRPM8 expressed by sensory neurons may modulate the macrophage functions through neuroimmune crosstalk. Conditional immune cell-specific TRPM8 KO mice are needed to validate the role of TRPM8 in immunomodulation in the gut.

TRPV1 and TRPV2

Although nociceptor-expressed TRPV1 is essential to the generation of inflammatory thermal pain (Julius, 2013), CD4^{+} T cells were also reported to express functional TRPV1 which was shown to play a pro-inflammatory role in a mouse model of colitis (Bertin et al., 2014), which is in marked contrast to TRPA1 activation of which was shown to attenuate colitis (Bertin et al., 2017). Notably, pharmacological inhibition of TRPV1 using BCTC also restrains pro-inflammatory T-cell responses (Bertin et al., 2014). However, functional expression of TRPV1 in CD4^{+} T cells needs to be further validated as capsaicin does not seem to activate significant whole-cell currents and intracellular Ca^{2+} responses in normal CD4^{+} T cells (Bertin et al., 2014) but evokes robust activation in sensory neurons (Stein et al., 2006). Moreover, the use of high concentrations of capsaicin is a concern as capsaicin activates native TRPV1 in sensory neurons with an EC_{50} around 50 nM (Stein et al., 2006) while 16 µM capsaicin is used to stimulate CD4^{+} T cells (Bertin et al., 2014). It should also be noted that capsaicin can alter membrane ion flux independent of TRPV1 function (Braga Ferreira et al., 2020) and a recent report showed no expression of TRPV1 protein in CD4^{+} T cells in the skin (Kemeny et al., 2018).

Besides CD4^{+} T cells, TRPV1 function was also reported in dendritic cells (DCs) as gain of function of TRPV1 promotes DC activation, enhances activation of NFATc2 signaling, and promotes cytokine production induced by inflammatory stimuli in in vitro tests (Duo et al., 2020). In vivo, TRPV1 gain of function causes excessive recruitment of DCs and enhances Th17 immune responses in lamina propria of colon, thereby increasing the susceptibility of mice to experimental colitis (Duo et al., 2020). However, no TRPV1-mediated membrane currents or intracellular Ca^{2+} responses have been recorded on DCs and an indirect action through neuroimmune interactions cannot be excluded in the gain of function mutants either. Genetic ablation of TRPV2 function was shown to attenuate DSS-induced colitis at the clinical, histopathological and immunohistochemical levels. This result is likely caused by a reduction of macrophage recruitment to the inflammation site (Issa et al., 2014), which is consistent with a report that TRPV2 engages in macrophage particle binding and phagocytosis (Link et al., 2010). These studies suggest that immune cells might possess intrinsic functions of TRPV2 (Fig. 1).

TRPML1

TRPML1 is predominantly localized on the membranes of late endosomes and lysosomes (LELs). Functionally, TRPML1 mediates the release of Ca^{2+} and heavy metal Fe^{2+}/Zn^{2+} ions into the cytosol from the LEL lumen, thus regulating the ingestion of large particles by promoting the maturation of phagosome (Dayam et al., 2015; Dayam et al., 2017) and providing the membrane for the cell surface (Samie et al., 2013), suggesting a potential role of TRPML1 in macrophage/neutrophil phagocytosis. Moreover, inhibition of TRPML1-mediated Ca^{2+} signaling in endolysosome reduces the transportation of single-stranded RNA (ssRNA) into lysosomes in TLR-mediated response to pathogens while chemical activation of TRPML1 by the TRPML channel agonist ML-SA1 enhances this process (Li et al., 2015).

P2X7 receptor

Extracellular adenosine triphosphate (ATP) is an important intercellular signaling molecule that binds to both ionotropic P2X receptors and G-protein-coupled P2Y receptors. Both P2X and P2Y receptors are widely distributed through the nervous, cardiovascular, and immune systems, and play critical roles in synaptic transmission, somatosensation, and inflammation. Among them, P2X7 purinergic receptor (P2X7R), which mediates transmembrane K^{+} efflux and Ca^{2+} influx in immune cells, engages with extracellular ATP released by mucosal immune cells and microbiota in the gut and is critically involved in intestinal homeostasis through eliciting diverse immune responses (Cheng et al., 2021). Besides P2X7R, genetic manipulation of neutrophil-expressing P2X4R showed that the ATP–P2X4R signaling axis in myeloid cells causes Ca^{2+} influx that promotes glycolysis in response to an increase in microbiota-derived luminal ATP, thereby regulating intestinal inflammation (Tani et al., 2021; Fig. 1).

P2X7R is expressed by both lymphocytes and macrophages as well as IECs in the small bowel although these cells displayed different sensitivity to ATP (de Campos et al., 2012). In colonoscopy samples from Crohn's disease (CD) patients, P2X7R expression is upregulated primarily in DCs and macrophages, which is correlated with enhanced tissue inflammation and apoptosis. Moreover, autocrine activation of P2X receptors by Pannexin-1–mediated ATP release stimulates IL-2 expression and T-cell proliferation and exacerbates inflammation in murine models of type 1 diabetes and IBD (Schenk et al., 2008). Strikingly, P2X7R KO animals do not develop colitis in response to TNBS, DSS, or oxazolone, suggesting that P2X7R signaling is an important component in the pathogenesis of chemical-induced intestinal inflammation (Neves et al., 2014; Figliuolo et al., 2017). Mechanistically, P2X7R activation triggers regulatory T-cell death and inhibits regulatory T-cell migration to the colon. Pharmacological inhibition or genetic ablation of the P2X7R function increases survival and migration of regulatory T cells which promotes immune system tolerance in the gut (Figliuolo et al., 2017; Fig. 1). Consistent with genetic ablation results, inhibition of P2X7R by orally administered Brilliant blue G or $MgCl_2$ attenuates the severity of DSS-induced colitis, which is correlated with decreased accumulation of P2X7R^{+} mast cells in the colon (Ohbori et al., 2017). Moreover, both in vitro and in vivo data showed that the pro-inflammatory cytokine IL-6 increases ATP synthesis and P2X7R-mediated signaling in Tregs, resulting in the conversion of Tregs to Th17 cells, suggesting that inhibition of P2X7R in Tregs may ameliorate tissue inflammation (Schenk et al., 2011). Accordingly, pretreatment with P2X7R inhibitor A740003 was

Feng et al.
Ion channels and gut immunity

Journal of General Physiology
https://doi.org/10.1085/jgp.202113042

6 of 17

shown to prevents rats from developing TNBS-induced colitis while intraperitoneal administration of A740003 or brilliant blue G is effective in attenuating the severity of TNBS-induced colitis, and reducing myeloperoxidase (MPO) activity, collagen deposition, densities of lamina propria T-cells and macrophages (Marques et al., 2014; Fig. 1).

Interestingly, deficiency in ATP–P2X7R signaling could also disrupt mucosal immune system, leading to intestinal inflammation. For instance, in Peyer's patches, the organized lymphoid follicles in the intestinal mucus membrane, P2X7R function was shown to control the number of T follicular helper cells (Tfh) that secret high-affinity secretory IgA (SIgA) which binds and depletes mucosal bacteria, resulting in reduced mucosal colonization of commensals (Proietti et al., 2014). P2X7R deficiency in Tfh cells enhances germinal center reactions to produce more Tfh cells to actively eliminate commensals. Since mucosal commensals are critical in shaping host immune system, P2X7R-deficient mice are more susceptible to polymicrobial sepsis (Proietti et al., 2014). Besides controlling the production of SIgA in the Peyer's patches, P2X7R signaling also helps to maintain elevated concentrations of circulating CD14 during infection to clear sepsis (Alarcon-Vila et al., 2020; Fig. 1). Since both over activation and deficiency of P2X7R can disrupt intestinal immunity, it is critical to selectively manipulate the P2X7R function in the settings of distinct intestinal immune disorders.

P2X7R deficiency in macrophages in the GI tract also increases the worm burdens in murine *Trichinella spiralis* infection (Guan et al., 2021). Mechanistically, *T. spiralis* infection upregulates the expression of P2X7R which subsequently activates the NF-κB/NLRP3/IL-1β pathway in macrophages, which initiates an innate immune program to protect the host from *T. spiralis* infection by increasing the capacity of macrophages to kill newborn *T. spiralis* larvae (Guan et al., 2021). A recent study also showed that P2X7R mediates the activation of mast cells by ATP released from apoptotic IECs upon infection by *Heligmosomoides polygyrus* (Hp) nematode in mice lack Spi-B, the Ets transcription factor regulating myeloid differentiation (Shimokawa et al., 2017). Activated mast cells produce IL-33 which drives the production and activation of the IL-13–producing group 2 innate lymphoid cells (ILC2), which subsequently increases the number of goblet cells to produce mucin to help clearance of Hp infection at the early phase (Shimokawa et al., 2017; Fig. 1).

Besides regulating intestinal inflammation and parasitic infection, P2X7R-mediated signaling also suppresses the development of colitis-associated cancer (CAC; Hofman et al., 2015). In loss of function studies using both genetic and pharmacologic tools, blockade of P2X7R function increases proliferation of IECs and protected them from apoptosis through enhanced TGFβ1 signaling. Moreover, P2X7R blockade also alters immune cell infiltration and promotes Treg accumulation within lesions in the intestines (Hofman et al., 2015). Like other ion channels, specificity is one of the critical issues regarding genetic and pharmacological manipulations of P2X7R. As P2X7R is expressed by diverse cell types, global P2X7R KO approach does not have cellular specificity. Some of the commonly used P2X7R inhibitors such as Brilliant blue G and MgCl$_2$ likely also have many

off-target actions when given in vivo. Recently, some potent P2X7R antagonists have been developed by several pharmaceutical companies. For example, A-438079 is very appealing for its efficacy in reverting allodynia in the neuropathic pain model. Nevertheless, it has short half-life and low bioavailability (Letavic et al., 2017). JNJ-47965567, which is one of the most used ligands in basic and preclinical research, displays poor oral bioavailability (Bhattacharya et al., 2013). Although high throughput screening identified AZD9056 as a selective orally active inhibitor of P2X7R, it lacks effect on inflammatory biomarkers in a phase IIa study assessing its potential in the treatment of moderately to severely active CD (Eser et al., 2015). Moreover, there are many types of purinergic receptors that can be activated by extracellular ATP and might also be involved in extracellular ATP-induced immune regulation besides P2X7R. Thus, although P2X7R is becoming an exciting target for immune cell-related chronic inflammatory disorders, these potential complications need be taken into consideration in experimental data interpretation and clinical trial design.

nAChR

Nicotinic acetylcholine receptors (nAChRs) are cholinergic receptors that form ligand-gated ion channels. Both α7 nicotinic acetylcholine receptor (α7nAChR) and α9nAChR subunits were identified in macrophages and B cells (Wang et al., 2003; Zhang et al., 2020). α7nAChR subunit is also expressed by human lamina propria T cells. Chronic nicotine treatment in cell cultures only upregulates the expression of T-bet mRNA (Th1-dominant) in human lamina propria T cells but shows negligible effect on the expression of GATA-3 mRNA (Th2-dominant) through α7nAChRs (Kikuchi et al., 2008), which might explain why chronic nicotine stimulation, such as smoking, modulates immune response of mucosal T cells in IBD, and nicotine has a beneficial influence on ulcerative colitis (UC; Th1-dominant) but not CD (Th2-dominant; Fig. 1).

The α7nAChR protein is also markedly increased in the inflamed colon in a mouse model of DSS-induced colitis and demonstrates a protective role in DSS-induced inflammation as the α7nAChR agonist PNU282987 suppresses DSS-induced colon inflammation in vivo and reduces LPS-induced increase in cytokine production and ROS levels in α7nAChR-expressing macrophages in vitro (Xiao et al., 2020). Similarly, when systemically applied, another α7nAChR partial agonist encenicline attenuates the severity of TNBS- and DSS-induced colitis in mice through the α7nAChRs. Mechanistically, encenicline increases the number of FoxP3+ regulatory T cells (immunosuppressive) but reduces the number of IL-17A–producing T cells (pro-inflammatory) in DSS-treated mice, suggesting that activation of α7nAChR by encenicline alleviates colitis through altering the balance of immunosuppressive/pro-inflammatory T cells in the gut (Salaga et al., 2016).

Interestingly, chemical activation of the α7nAChRs expressed by the gastric muscular macrophages of Parkinson's disease rats by NU-282987 or GTS-21 reduces the number of macrophages, expression of pro-inflammatory mediator, and improves gastric motility (Zhou et al., 2021). GTS-21 also reduces the levels of IL-6 in blood and colon, enhanced colonic permeability, and

Feng et al.
Ion channels and gut immunity

Journal of General Physiology
https://doi.org/10.1085/jgp.202113042

7 of 17

inflammation in mice subjected to cecal ligation and puncture. In marked contrast, splenectomized animals and α7nAChR KO mice display a more severe septic phenotype, which can also be improved by GTS-21 (Nullens et al., 2016). Although these findings support that direct chemical stimulation of the α7nAChRs is effective in attenuating GI inflammation, Snoek et al. (2010) reported that systemic applications of nicotine and two selective α7nAChR agonists AR-R17779 and GSK1345038A have no significant beneficiary effect on disease severity in two mouse models of experimental colitis. Instead, treatment with AR-R17779 or GSK1345038A worsens disease severity and increases pro-inflammatory cytokine levels in the colon in DSS colitis. These conflicting experimental results regarding the functions of α7nAChR need to be addressed before conclusions are drawn for the role of nAChRs in gut immunity.

Besides expression in T cells and macrophages, nAChRs are also expressed by mucosal mast cells (MMCs) and play an essential role in food allergy (FA). For instance, in a mouse model of FA associated with severe allergic diarrhea, both exaggerated Th2 cell-mediated immune responses and MMC hyperplasia take place in the colon. Vagal stimulation by 2-deoxy-D-glucose and application of nAChR agonist nicotine or GTS-21 is sufficient to attenuate the allergic symptoms in the FA mice by inhibiting MMC hyperplasia and T-cell-mediated immune responses (Yamamoto et al., 2014; Fig. 1). Although emerging evidence supports the involvement of nAChRs in gut immunity, most of these studies rely on pharmacological inhibition and global KO approaches, which do not provide information regarding specific cellular functions. Future studies using cell-specific genetic manipulations are needed to provide more mechanistic studies to validate these findings.

K+ channels

It is well-established that calcium-activated potassium channel KCa3.1 plays an important role in modulating Ca^{2+} signaling through the control of the membrane potential in T lymphocytes (Wulff and Castle, 2010). In the ileum, KCa3.1 expression is primarily detected in Paneth cells and T cells in inflamed lamina propria (Simms et al., 2010). Interestingly, single nucleotide polymorphism mapping has identified KCNN4, a human gene encoding the KCa3.1 protein, as a susceptibility gene in the development of CD in Australian and New Zealand populations (Simms et al., 2010). KCa3.1 mRNA and protein levels were also reported to be upregulated in IECs from CD and UC patients but not in non-IBD intestinal inflammation (Suss et al., 2020). In mouse DSS colitis model, both expression and function of the KCa3.1 channel are upregulated in the CD4+ T lymphocytes in the mesenteric lymph node (Ohya et al., 2014), which is a driving force of the enhanced production of inflammatory cytokines, such as IFNγ. The upregulation of KCa3.1 expression is caused by HDAC2- and HDAC3-mediated epigenetic modifications as a pan-HDAC inhibitor vorinostat and selective inhibitors of HDAC2 and HDAC3 suppress the increase in transcriptional expression of KCa3.1 in the splenic CD4+ T cells in the DSS colitis model (Matsui et al., 2018; Fig. 1). Moreover, KCa3.1 channel blocker TRAM-34 reduces the severity of DSS-induced colitis and normalizes the

expression of KCa3.1, NDPK-B, and Th1 cytokines in the mesenteric lymph node CD4+ T cells (Ohya et al., 2014). Similar to TRAM-34, another KCa3.1 blocker NS6180 also suppresses colon inflammation and improved body weight loss in the TNBS colitis model (Strobaek et al., 2013). Moreover, KCa3.1 KO mice also display impaired Ca^{2+} influx and cytokine production in CD4+ Th1 and Th2 cells, whereas T-regulatory and Th17 cell functions are not affected. Consistent with pharmacological inhibition studies, KCa3.1 KO mice are protected against developing severe colitis in mouse models of colitis induced by either T-cell transfer or TNBS (Di et al., 2010), further supporting a causative role of KCa3.1 in intestinal inflammation.

Besides KCa3.1, voltage-gated Kv1.3 channel is also expressed by human T cells and controls T-cell activation, proliferation, and cytokine production by maintaining the driving force for Ca^{2+} influx through CRAC channels. In fact, both KCa3.1 and Kv1.3 cooperatively and compensatorily regulate antigen-specific memory T cell functions (Chiang et al., 2017). In mucosal biopsies from patients with active UC, the expression level of Kv1.3 in both infiltrated CD4+ and CD8+ T cells is markedly increased, which is associated with the production of pro-inflammatory IL-17A and TNF-α (Koch Hansen et al., 2014). As Kv1.3 loss-of-function in mice is lethal, functional studies are mainly dependent on pharmacological tools. Inhibition of Kv1.3 expressed by CD4+ T cells with a small molecule inhibitor DES1 suppresses intestinal inflammation in a humanized mouse model of UC (Unterweger et al., 2021). Another Kv1.3 blocker PAP-1 also protects animals against DSS-induced colitis possibly through inhibition of NLRP3 inflammasome pathway downstream of Kv1.3 signaling (Mei et al., 2019). It should be noted that the specificity of these inhibitors should be carefully evaluated in the preclinical studies. Moreover, cell specific ablation of distinct K+ channels is also needed for mechanistic studies regarding functions of KCa3.1 and Kv1.3 in distinct cell types.

Ion channels in intestinal immune-epithelial crosstalk

Intestine immune cells are embedded in different layers of gut lumen surrounded by numerous resident non-immune cells such as IECs, chemosensory tuft cells, and mucin-secreting goblet cells. Immune-epithelial crosstalk at the intestinal barrier surface is a central mechanism of host defense. Ion channels are broadly expressed by diverse resident non-immune cells in the GI tract which play a critical role in gut homeostasis and intestinal inflammation.

CRAC

IEC-derived antimicrobial peptides are critical components of intestinal innate immunity (Muniz et al., 2012). A recent study demonstrated that Orai1 expressed by the pancreatic acini plays an essential role in the control of intestinal microbiota and immunity through releasing antimicrobial peptides (Ahuja et al., 2017; Tilg and Adolph, 2017). Conditional KO of Orai1 from pancreatic acini of adult mice impairs Ca^{2+} signaling and reduces the total level of cathelicidin-related peptide CRAMP. This reduction in secretion of antimicrobial peptides from pancreas causes intestinal bacterial outgrowth and dysbiosis, ultimately leading to systemic bacterial translocation, inflammation, and

Feng et al.
Ion channels and gut immunity

Journal of General Physiology 8 of 17
https://doi.org/10.1085/jgp.202113042

death, representing a novel mode of cross organ immune regulation. These dire consequences can be rescued by purified liquid diet and broad-spectrum antibiotics treatments which inhibit bacterial outgrowth and supplement of synthetic CRAMP (Ahuja et al., 2017; Tilg and Adolph, 2017).

Interestingly, the CRAC channel inhibitor BTP2 or CM4620 was shown to significantly reduce the production of inflammatory cytokines (such as IFNγ, TNF, IL-17A, IL-13, and IL-4) from human intestinal T cells, B cells, ILCs and myeloid cells, and improve colon inflammation in a mouse model of IBD (Letizia et al., 2022). Note that CM4620 is in an ongoing phase I/II clinical trial for children and young adults with acute pancreatitis (Letizia et al., 2022). Although pharmacological inhibition of SOCE does not affect the functions of human or mouse IECs in colonic organoid cultures, epithelium-expressed STIM1 regulates intestinal homeostasis through promoting the loss of goblet cells in IBD patients (Liang et al., 2022). Taken together, these studies implicated that CRAC expressed by either epithelia or immune cell may serve as an important regulator of intestinal immune function and could be potential drug targets for the treatment of IBD.

TRP channels

TRPM5 is a cation channel mediating sweet, umami, and bitter tastes upon activation by intracellular Ca^{2+} resulting from activation of upstream G-protein-coupled taste receptors in taste buds (Dutta Banik et al., 2018). Like the taste bud cells, enteric tuft cells are chemosensory IECs expressing TRPM5 and succinate receptor 1 (SUCNR1), a GPCR activated by a microbiota-derived metabolite succinate. Upon infections, tuft cells sense succinate secreted from pathogens such as protists tritrichomonas, bacteria, and helminths, through SUCNR1 activation resulting in an increase in intracellular Ca^{2+} that subsequently activates TRPM5. Both in vivo and in vitro data showed that TRPM5 activation is critical to modulating Na^+ influx and membrane depolarization in the tuft cells, driving the release of IL-25 that activates IL-25 receptor expressed by lamina propria ILC2s which subsequently promotes IL-13 production and release, and further induces tuft cell expansion. These findings place TRPM5 at the center of tuft cell-ILC2 signaling circuit that mediates epithelia immune interactions to fight intestinal parasite infections (Howitt et al., 2016; Nadjsombati et al., 2018; Schneider et al., 2018; O'Leary et al., 2019; Fig. 1).

TRPV4 is a non-selective cation channel-sensing cell volume dynamics and many inflammatory mediators (Toft-Bertelsen and MacAulay, 2021). Besides dominant expression in the skin and kidney, TRPV4 is also expressed by IECs and immune cells, especially macrophages (Luo et al., 2018; Rizopoulos et al., 2018). TRPV4 mRNA transcripts are significantly increased in colon biopsies from both CD and UC patients when compared with healthy subjects (Rizopoulos et al., 2018). TRPV4 mRNA expression is also significantly increased in the colon of TNBS-treated mice compared with control animals. Administrations of a selective TRPV4 antagonist RN1734 suppresses the severity of TNBS-induced colitis and visceral pain (Fichna et al., 2012). Another study showed increased TRPV4 expression in human colon samples, human IEC cell lines (Caco-2 and T84), and

inflamed colons of mice induced by intracolonic administration of a TRPV4 activator 4α-phorbol-12,13-didecanoate (4αPDD), further supporting a role of TRPV4 in intestinal inflammation (D'Aldebert et al., 2011; Fig. 1). However, these pharmacological manipulations lack specificity and have potential off-target actions. Other studies using both pharmacological and genetic approaches showed that TRPV4 is upregulated in vascular endothelial cells and contributes to increased vascular permeability promoting DSS-induced colon inflammation (Matsumoto et al., 2018). Further studies showed that TRPV4 expressed by vascular endothelial cells and potentially macrophages facilitates the progression of colitis-associated cancer induced by azoxymethane/DSS (Matsumoto et al., 2020). It should be noted that TRPV4 is also expressed by muscularis macrophages in the gut (Luo et al., 2018) which are shown to be required for maintain normal GI motility. Activation of TRPV4 promotes GI motility through direct interaction between the muscularis macrophages with intestinal smooth muscles involving macrophage-derived prostaglandin E2. Moreover, genetic or pharmacological inhibition of TRPV4 can improve chemotherapy-induced GI hypermotility in mice (Luo et al., 2018). Therefore, immune cell-expressed ion channels likely have a broader function in the GI tract beyond gut immunity.

P2X7 receptor

Human fetal small intestinal IECs express P2X7R that is activated during *Toxoplasma gondii* infection (Quan et al., 2018), which promotes the production and release of IL-1β through NLRP3 inflammasome activation. In vitro siRNA knockdown of P2X7R reduces *T. gondii*-induced IL-1β secretion and substantially increased parasite proliferation in *T. gondii*-infected FHs 74 Int epithelial cells, which is correlated with a reduction of the NLRP3 inflammasome activation, providing mechanistic insights into mucosal immune mechanisms of *T. gondii* infection (Quan et al., 2018). Interestingly, another study reported that in vitro and in vivo infections of IECs by *T. gondii* and *T. spiralis* do not promote inflammasome-associated IL-1β secretion. Instead, these parasites cause a P2X7R-dependent production of CCL5, TNF-α, and IL-6 from IECs, which recruits CD11C+/CD103+ DCs into the epithelial layer (Huang et al., 2017). Although these results suggest that P2X7R might engage multiple signaling pathways in the intestinal epithelium to mount innate immune response upon parasite infections (Fig. 1), more selective genetic and pharmacological tools are needed to validate these findings.

K+ channels

Many types of K+ channels are expressed in the intestinal epithelium and participate in maintaining normal intestinal secretion and barrier integrity (Cosme et al., 2021). For instance, deficiency of Trek1 (K2P2.1), a two-pore-domain background potassium channel, causes barrier dysfunction of the human colon epithelial cell line T84 monolayer. Barrier dysfunction in a mouse model of ovalbumin-induced intestinal allergy is also found to be related to reduced Trek1 expression caused by activation of the MAPK pathways and HDAC1, which is rescued by HDAC1 inhibitors (Huang et al., 2016; Fig. 1).

GI inflammation also involves K+ channels. For instance, the K (ATP) channel Kir6.1, SUR2B is upregulated in the mesenteric

Feng et al.
Ion channels and gut immunity

Journal of General Physiology   9 of 17
https://doi.org/10.1085/jgp.202113042

lymphatic vessels in a mouse model of TNBS-induced ileitis, and the K (ATP) channel blocker glibenclamide improves lymphatic pumping through attenuating TNBS-induced membrane hyperpolarization in the mesenteric lymphatic vessels, suggesting the involvement of K (ATP) channels in TNBS-induced lymphatic contractile dysfunction (Mathias and von der Weid, 2013). Moreover, LRRC26, a regulatory subunit of the Ca^{2+}- and voltage-activated BK channel, is functionally expressed by the mucin-secreting goblet cells in the GI tract and LRRC26-associated BK channels contribute to the resting transepithelial current across mouse distal colonic mucosa. LRRC26 deficiency renders inactivity of normally expressed BK channels at physiological conditions. Deficiency in LRRC26 or BK pore-forming α-subunit dramatically exacerbates DSS-induced colitis, suggesting a protective role of LRRC26-associated BK channels against chemical-induced colitis in mice, potentially through maintaining normal function of the goblet cells and mucin secretion (Gonzalez-Perez et al., 2021; Fig. 1).

Ion channels in intestinal neuroimmune interactions

The GI tract is innervated by both extrinsic and intrinsic nervous systems (Phillips and Powley, 2007). ANS receives signals from the CNS to regulate GI function while sensory neurons from dorsal root and vagal ganglia detect danger signals in the gut lumen to initiate protective responses. Moreover, ENS, the second brain positioned along the gut wall, comprises a full repertoire of sensory neurons, interneurons, motor neurons, and glial cells which collectively detect luminal contents, drive secretory function and intestinal motility, and maintain immune homeostasis and GI barrier integrity (Adamantidis et al., 2014; Camilleri, 2021; Niesler et al., 2021; Wang et al., 2022). These distinct neuronal components and their signal transducers participate in regulating GI inflammation and host defense (Fig. 2).

Vagal-immune interactions

Sympathetic input suppresses colon motility by acting on intrinsic myenteric neurons while regulates gut immune function through releasing noradrenaline that acts on G-protein-coupled α and β adrenergic receptors expressed by enteric neurons and immune cells (Populin et al., 2021; Smith-Edwards et al., 2021). The vagus nerve, on the other hand, modulates intestinal inflammation through the key mediator of the cholinergic anti-inflammatory pathway, acetylcholine (ACh), acting primarily on nAChRs and driving Na^+ influx and membrane depolarization (Van Der Zanden et al., 2009a), regulating cytokine release from immune cells (Rueda Ruzafa et al., 2021). Most of the vagus nerve-mediated anti-inflammatory responses in the GI tract are mediated by α7nAChR (Cailotto et al., 2014; Ji et al., 2014; Matteoli et al., 2014; Rana et al., 2018; Bosmans et al., 2019; Yang et al., 2021), although α4/β2 nAChRs are also reported to promote macrophage phagocytosis in vitro and potentially increases uptake of luminal bacteria by lamina propria macrophages in response to vagus nerve stimulation (VNS) in mice (van der Zanden et al., 2009b; Fig. 2).

In mouse models of IBD, VNS modulates intestinal inflammation through a centrally mediated vagal modulation mechanism (Cheng et al., 2020). Besides VNS, chemical stimulation of the central cholinergic pathway has also been used to drive ACh–nAChR signaling in the GI tract (Ji et al., 2014) to reduce mucosal inflammation in mouse models of colitis induced by DSS or TNBS. This modulatory effect is abolished in mice with vagotomy, splenic neurectomy, or splenectomy, suggesting the involvement of a vagus nerve-to-spleen circuit and α7nAChR signaling pathway (Ji et al., 2014). Innate immune cells such as resident macrophages and mast cells are critical targets for the vagus nerve-driving anti-inflammatory response in the GI tract, for instance, VNS dampens intestinal inflammation in a murine model of experimental FA in a α7nAChR-independent manner, possibly through dampening mast cell activity and increasing phagocytosis of ovalbumin by CX3CR1+ macrophages (Bosmans et al., 2019). In a mouse model of postoperative ileus (POI), VNS driven by electroacupuncture causes the release of ACh that activates the α7nAChR-mediated JAK2/STAT3 signaling pathway in macrophages, suppressing the production of inflammatory cytokines (Yang et al., 2021). VNS attenuates surgery-induced intestinal inflammation and improves postoperative intestinal transit in wild-type, splenic denervated, and T-cell-deficient mice, suggesting that the vagal nerve-mediated anti-inflammatory effect in the intestine is likely independent of the spleen and T cells (Matteoli et al., 2014).

Nociceptor-immune interactions

Visceral sensory neurons residing in the DRG that have sensory nerve endings project to the gut wall and mesentery mediating mechanosensory and chemosensory responses in the GI tract (Spencer and Hu, 2020). Accumulating evidence indicates that there is a bi-directional communication between these sensory nerve endings and immune cells, driving aberrant neuroimmune interactions which can lead to visceral hypersensitivity and GI inflammation (Boeckxstaens and Wouters, 2017). This neuro-immune regulation is largely orchestrated through nociceptor-expressed TRP channels and nociceptor-derived neuropeptides (Lai et al., 2017).

In DSS-induced colitis model, the TRPV1/CGRP-positive nerve fiber density increases in the distal colon wall and TRPV1 activation-induced release of CGRP/SP from the distal colon is greater than that from the proximal colon, which is further enhanced in the setting of colitis. TRPV1 expression is increased in DRGs projecting to the distal colon when compared to that in the proximal colon, which is also further enhanced during colitis (Engel et al., 2012). This expression pattern is correlated with the phenomenon showing the proximodistal aggravation of inflammation in the DSS colitis model. Consistent with the importance of TRP channels and neuropeptides in GI inflammation, SP deficiency and RTX-mediated ablation of the TRPV1+ fibers improve DSS colitis without the presence of the proximodistal gradient of inflammation, suggesting substance P released from the TRPV1-expressing sensory neurons is required for this phenomenon (Engel et al., 2012). Genetic ablation of TRPV1 or TRPA1 significantly attenuates the severity of colitis (Szitter et al., 2010; Utsumi et al., 2018). Moreover, DSS treatment up-regulates both CGRP+ and SP+ sensory nerve fibers and TRPA1 or TRPV1 KO mice have less upregulation of SP+ but not

Feng et al.
Ion channels and gut immunity

Journal of General Physiology   10 of 17
https://doi.org/10.1085/jgp.202113042

the CGRP[+] sensory nerve fibers, suggesting that DSS-induced colitis is driven by TRPA1- and TRPV1-mediated release of SP (Utsumi et al., 2018; Fig. 2). On the other hand, another study reported that capsaicin-induced denervation of the TRPV1^{+} extrinsic sensory fibers in neonatal mice exacerbates oxazolone-induced colitis without significant changes in the expression of CGRP[+] or SP[+] nerve fibers in the colon, suggesting a dispensable role of neuropeptides and the involvement of additional nociceptor-derived neurotransmitters in the pathogenesis of the colitis (Lee et al., 2012).

TRPA1 is reported to be a major mediator of the TNBS-induced colitis (Engel et al., 2011). Mechanistically, TNBS directly activates TRPA1 through covalent modification of cysteine and lysine residues in the cytoplasmic N-terminus of the channel. Application of TNBS activates inward currents, promotes Ca^{2+} influx and release of SP from cultured DRG neurons in a TRPA1-dependent manner. Importantly, genetic or pharmacological ablation of TRPA1 function suppresses both TNBS- and DSS-induce colitis, supporting a critical role of TRPA1-mediated SP release in the pathogenesis of colitis (Engel et al., 2011; Fig. 2).

In addition to chemical-induced colitis, TRPV1^{+} neurons also protect against infection caused by an enteric bacterial pathogen *Citrobacter rodentium* as RTX-induced ablation of the TRPV1^{+} fibers increases fecal and colonic adherent *C. rodentium* on day 10 post-infection (p.i.) until day 29 p.i., which is recapitulated by genetic ablation of the TRPV1 channel function (Ramirez et al., 2020). It is proposed that bacteria-mediated activation of TLR4 receptor sensitizes TRPV1 channels and subsequently causes release of CGRP which plays an anti-inflammatory role in maintaining mucosal homeostasis in the GI tract (Assas et al., 2014; Fig. 2).

Besides TRPA1/TRPV1, TRPM8 is also functionally expressed by the colon-innervating DRG neurons and TRPM8 expression is upregulated in inflamed colon samples from both human and mouse models of DSS- and TNBS-induced colitis (Harrington et al., 2011). TRPM8 KO mice display enhanced levels of inflammatory neuropeptides in the colon although the inflammation is not significantly altered when compared with wild-type mice. Systemic activation of TRPM8 by icilin suppresses intestinal inflammation in wild-type mice but not in TRPM8 KO mice in chemically induced colitis models. Icilin also reduces the levels of inflammatory cytokines in the colon of TNBS-treated mice. The level of CGRP in the colon of DSS-treated TRPM8 KO mice is increased when compared with that in the wild-type mice. Interestingly, application of icilin can block capsaicin-induced CGRP release in ex vivo colon tissues. These findings suggest that TRPM8 activation suppresses CGRP release from the TRPV1^{+} nociceptors to execute an anti-inflammatory response (Ramachandran et al., 2013; Fig. 2). However, it is not understood why icilin activation of TRPM8 does not promote CGRP release since published studies showed that activation of TRPM8 is correlated with CGRP release from DRG neurons and TRPM8 protects against intestinal injury and mucosal inflammation through CGRP (de Jong et al., 2015; Fouad et al., 2021). It is also important to distinguish the function of TRP channels in immune cells from primary nociceptors in the setting of intestinal inflammation as both cell types are critically involved in the pathogenesis of chemical-induced GI inflammation. Selective ablation of ion channel function in distinct nociceptors and immune components using conditional KO approaches can be used to address this issue.

ENS-immune interactions

Emerging evidence suggests that enteric neurons expressing choline acetyltransferase (ChAT) play a critical role in gut immunity and inflammation as evidenced by a reduction of both cholinergic neurons and ACh in IBDs (Zheng et al., 2021). Direct activation of the ChAT^{+} enteric neurons triggers the release of ACh that subsequently acts on the α7nAChR expressed by monocytes/macrophages to inhibit intestinal inflammation (Tsuchida et al., 2011). Mechanistically, ACh activates nAChRs on monocytic myeloid-derived suppressor cells to promote the release of anti-inflammatory cytokine IL-10 through the ERK signaling (Zheng et al., 2021). The involvement of cholinergic enteric neuron-α7nAChR-expressing macrophage signaling axis has been proposed to mediate the beneficial effects of 5-hydroxytryptamine receptor 4 (5-HT₄R) agonists such as mosapride citrate (MOS), CJ-033466, and prucalopride on GI inflammation, especially after abdominal surgery in a mouse model of POI (Tsuchida et al., 2011; Stakenborg et al., 2019). This action of 5-HT₄R agonists can be recapitulated by the inhibitory effect of electrical field stimulation (EFS) on macrophage function, which is attenuated by the neurotransmission blocker tetrodotoxin (TTX) and the N-type VGCC inhibitor ω-conotoxin (Stakenborg et al., 2019; Fig. 2). However, if selective activation of 5-HT₄R-expressing cholinergic enteric neurons in vivo by 5-HT₄R agonists or EFS is sufficient to inhibit POI is not known as these manipulations are not specific considering the widespread expression of 5-HT₄R and non-specific activation of diverse types of enteric neurons by EFS. Future studies using neurogenetic techniques to gain genetic access to distinct enteric neuron populations for both gain- and loss-of-function manipulations are required to validate these findings.

More interestingly, recent studies have identified an indirect vagal nerve-cholinergic myenteric neurons–muscularis macrophages axis in the modulation of GI inflammation. Histological studies showed that vagal nerve endings surround enteric neurons expressing nNOS, vasoactive intestinal peptide, and ChAT (Cailotto et al., 2014). Moreover, vagus nerve interacts with cholinergic myenteric neurons which are in close contact with the α7nAChR-expressing muscularis macrophages (Matteoli et al., 2014; Fig. 2). In a mouse model of FA produced by repeated oral ovalbumin administration, VNS and cholinergic activation with α7nAChR agonist GTS-21, or nicotine can suppress the allergic symptoms through dampening mast cell hyperplasia and expression of Th1 and Th2 cytokines in the colon. These beneficial effects are not mediated by systemic immune modulation, further supporting the involvement of a localized α7nAChR-mediated cholinergic neuroimmune interaction between VNS, ENS activation, and mucosal mast cell function (Matteoli et al., 2014). On the other hand, these histological studies are indirect evidence for potential interactions between vagal and enteric neurons in the gut. Direct evidence of functional interactions based on electrophysiological recordings

Feng et al.
Ion channels and gut immunity

Journal of General Physiology
https://doi.org/10.1085/jgp.202113042

11 of 17

and neurotransmitter release are needed to support these conclusions.

Conclusions

In the past decades, significant progresses have been made in understanding the cells, molecules, and cell–cell interactions in gut immunity. It is clear that ion channels play an essential role in maintaining gut immune homeostasis under physiological conditions as well as inflammatory GI disorders including IBD. Although expression and function of some specific ion channels such as TRP channels in intestinal immune cells need to be re-evaluated, convincing evidence has been provided to demonstrate that many ion channels, especially Orai channels, Kv1.3, P2X7R, and nAChR, are extensively expressed by CD4+, CD8+, and Tregs and play critical roles in expansion and differentiation of distinct immune cell subsets as well as immune tolerance at the steady state in gut immunity. Dysregulation of these ion channels in distinct immune cell subsets also critically contribute to intestinal inflammatory disorders.

However, there are two important questions that need to be answered by future studies. First, can we use ion channel expression profiles as biomarkers for intestinal inflammatory disorders, such as IBD? Ion channels could be a good candidate to assess the risk and prognosis and evaluate treatment options for inflammatory diseases in the GI tract based on their expression patterns and functions in gut immunity. The use of ion channel marker-assisted diagnosis and targeted therapies derived from an individual's molecular profile of ion channels should facilitate the application of personalized medicine. Therefore, it will be important to perform a big data-based comparison of ion channel properties between different conditions (male versus female, aging versus adult, physiology versus pathology) to fine-tune the correlation between ion channel expression and function with GI inflammatory disorders.

Secondly, can the basic ion channel mechanisms in gut immunity unveiled in animal studies be translated into clinical trials using specific ion channel blockers? In general, the translation of findings from animal models to clinical practice has not been very successful. Therefore, experimental designs of preclinical animal studies need to be improved and humanized animal models of GI disorders might enhance translational potentials (Unterweger et al., 2021). This is especially exciting because ongoing high throughput drug screening has identified numerous activators and inhibitors that have the potential to be used in the treatment of GI inflammatory disorders by targeting ion channels such as Orai channels, Kv1.3, P2X7R, and nAChR, which are the most important channels in gut immunity that have emerged over the past decades. Among them, Orai channels, Kv1.3 are the most promising ion channel drug targets for the treatment of IBD as shown by recent preclinical studies (Unterweger et al., 2021; Letizia et al., 2022). On the other hand, it remains a big challenge to target distinct cell types when the same ion channels are expressed by various tissues in the gut to avoid any off-target side effects and toxicity issues associated with ion channel activators and blockers. Alternatively, VNS produced by electrical stimulation and acupuncture points is a promising alternative treatment approach for IBD through nAChR-mediated cholinergic anti-inflammatory pathways (de Araujo and de Lartigue, 2020; Lei and Duan, 2021).

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