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VIEWPOINT

Cyclic nucleotides, gut physiology and inflammation

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Misregulation of gut function and homeostasis impinges on the overall well-being of the entire organism. Diarrheal disease is the second leading cause of death in children under 5 years of age, and globally, 1.7 billion cases of childhood diarrhea are reported every year. Accompanying diarrheal episodes are a number of secondary effects in gut physiology and structure, such as erosion of the mucosal barrier that lines the gut, facilitating further inflammation of the gut in response to the normal microbiome. Here, we focus on pathogenic bacteria-mediated diarrhea, emphasizing the role of cyclic adenosine 3',5'-monophosphate and cyclic guanosine 3′,5′-monophosphate in driving signaling outputs that result in the secretion of water and ions from the epithelial cells of the gut. We also speculate on how this aberrant efflux and influx of ions could modulate inflammasome signaling, and therefore cell survival and maintenance of gut architecture and function.

Introduction

Cyclic adenosine 3',5'-monophosphate (cAMP) and cyclic guanosine 3′,5′-monophosphate (cGMP) are key nucleotide second messengers that are exploited by various pathogenic bacteria during the course of infection and persistence in host cells [1–3]. Levels of cyclic nucleotides are increased markedly during some bacterial infections, and because they orchestrate fluid and electrolyte imbalance, these signaling molecules may have a broader role in virulence [3–5]. In contrast, recent studies demonstrated that cyclic nucleotides, particularly cGMP, can have a protective role in gut infection [6,7]. Here, we discuss important insights that have been gained into the role of cyclic nucleotides in the context of three common bacterial gut infections, namely cholera, enterotoxigenic *Escherichia coli* (ETEC) infection, and salmonellosis. As the actions of cyclic nucleotides in the gut often lead to ion imbalances, we speculate on how this

Abbreviations
AC, adenylyl cyclase; AJ, adherens junction; ASC, apoptotic speck-containing protein with caspase activation and recruitment domain; CARD, caspase activation and recruitment domain; CaSR, calcium-sensing receptor; CFTR, cystic fibrosis transmembrane conductance regulator; CL, cardiolipin; CNG, cyclic nucleotide-gated channels; CTX, cholera toxin; DJ, desmosome junction; DRA, downregulated in adenoma; EE, early endosome; EFS, electrical field stimulation; EPEC, enteropathogenic *Escherichia* coli; ER, endoplasmic reticulum; ETEC, enterotoxigenic *Escherichia* coli; GN, guanylin; GSDMD, gasdermin D; GUCY2C/GC-C, guanylyl cyclase C; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; KCNK6, K^+ channel subfamily K member 6; LPS, lipopolysaccharide; LT, heat-labile toxin; MC, mitochondria; mtROS, mitochondrial reactive oxygen species; NHE3, Na^+/H^+ exchanger isoform 3; NLRP3, NOD, leucine-rich repeat and pyrin domain containing protein 3; NOD, nucleotide-binding oligomerization domain; P2X7R, P2X purinoceptor 7; PDE3, cAMP-specific phosphodiesterase; PFT, pore-forming toxin; PKA, protein kinase A; PKGII, cGMP-dependent protein kinase II; ST, heat-stable toxin; TJ, tight junction; UGN, uroguanylin.

may impinge on the innate immune system via the inflammasome.

Cholera

Cholera is an important public health issue worldwide. Recent estimates indicate high burden of cholera in 69 endemic countries with ~ 2.9 million cases and 95 000 deaths annually [8]. Cholera toxin (CTX), a virulence factor produced by *Vibrio cholerae*, was discovered by Sambhu Nath De in Kolkata in 1959 by demonstrating that a bacteria-free culture filtrate can result in profound fluid accumulation in ligated rabbit ileal loops [9]. A decade later, Goldberg *et al*. reported that CTX specifically induces cAMP production, but not cGMP production, in canine intestinal mucosa [10,11]. Arguably, the pathogenesis of cholera mediated by cAMP signaling is one of the most thoroughly studied and best understood diarrheal disease mechanisms.

Vibrio cholerae is transmitted by the fecal–oral route, whereupon it colonizes intestinal crypts and releases CTX, an enterotoxin with a monomeric A (enzymatic) subunit divided into two domains (A1 and A2) inside a pore formed by the pentameric B (binding) subunit [12]. The B subunit ring of the CTX binds to GM1 ganglioside receptor on the plasma membrane of the intestinal epithelial cells (IECs), triggering endocytosis and retrograde trafficking of the toxin to endoplasmic reticulum (ER) via the *trans*-Golgi network [5]. In the ER, the A1 chain unfolds and mimics an ER-associated degradation substrate to get access to the cytoplasm, where it refolds and escapes ubiquitination and degradation. Constitutive activation of adenylyl cyclase by A1 chain-mediated ADP ribosylation of Gs alpha subunit (Gαs) protein results in pathological increases in cAMP [13].

One of the key downstream effectors of cAMP is protein kinase A (PKA), which in turn phosphorylates and stimulates the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel. Sustained activation of CFTR by CTX causes hypersecretion of anions, chloride and bicarbonate into the intestinal lumen [4,5]. To preserve electroneutrality and osmotic balance, rapid loss of sodium ions and water follows, resulting in acute watery diarrhea in the form of rice-water stool of up to 1 L·hour^{-1}, severe dehydration, and electrolyte imbalance that can be fatal within hours if left untreated [4,5,14]. Consistent with this model of CTX action, strong linear correlation was observed between fecal cAMP levels and fluid loss in cholera patients [15]. Furthermore, CFTR-null mice showed reduced fluid secretion in the intestine in response to CTX, despite intracellular accumulation of cAMP, providing evidence for the crucial role of CFTR in cholera pathophysiology (Fig. 1) [16].

In addition to its role in CFTR activation, cAMP could contribute to cholera pathogenesis through additional mechanisms. For instance, attenuation of Na^+ absorption due to reduced expression of Na^+/H^+ exchangers (e.g., NHE3) and enhancement of HCO^{3-} secretion due to stimulation of $Cl^-/HCO3^-$ exchangers (e.g., DRA/downregulated in adenoma) could act in parallel to cause diarrhea [17,18]. Furthermore, CTX-driven cAMP increase was reported to inhibit autophagy by suppressing autophagosome maturation, which might help bacteria escape host defense [19]. CTX was shown to inhibit phagocytosis and interleukin-12 (IL-12) cytokine production [20,21], and CTX-mediated cAMP increase was shown to disrupt intestinal barrier function by inhibiting exocyst-mediated trafficking of proteins, including E-cadherin and Notch signaling components, to cell–cell junctions [22].

Enterotoxigenic *Escherichia coli* infection

Enterotoxigenic *E. coli* strains cause diarrheal disease that is widespread in developing countries and a leading cause of pediatric morbidity and mortality worldwide. ETEC is also the most common causative agent isolated in traveler's diarrhea [23]. Following ingestion via the fecal–oral route, ETEC colonizes and adheres to the small intestinal mucosa and elaborates two enterotoxins, a heat-labile toxin (LT) and a heat-stable toxin (ST) [24]. Evans *et al*. first described the mechanism of LT-mediated adenylate cyclase activation using pigeon erythrocyte membranes [25]. Soon afterward, Hughes *et al*. and Field *et al*. independently reported the ability of ST to increase cGMP by activation of the receptor guanylate cyclase C in IECs [26,27]. Consistent with these observations, patients infected with *E. coli* expressing only ST had markedly lower fecal cAMP as compared to infection with *E. coli* expressing both LT and ST [28]. The ST peptide elicits a rapid rise in secretory response followed by a gradual decay. On the other hand, LT results in a slow and sustained increase in fluid accumulation [29].

Labile toxin is strikingly similar to CTX, in terms of structure, function, and immune response [30]. Studies determining evolutionary origins of the toxins indicated that LT is horizontally acquired from *V. cholerae* [31]. Like CTX, LT is composed of a single A subunit incorporated within a ring of five B subunits [32]. The catalytically active A1 fragment of LT activates adenylate cyclase causing an increase in cAMP levels, which in turn results in CFTR activation and

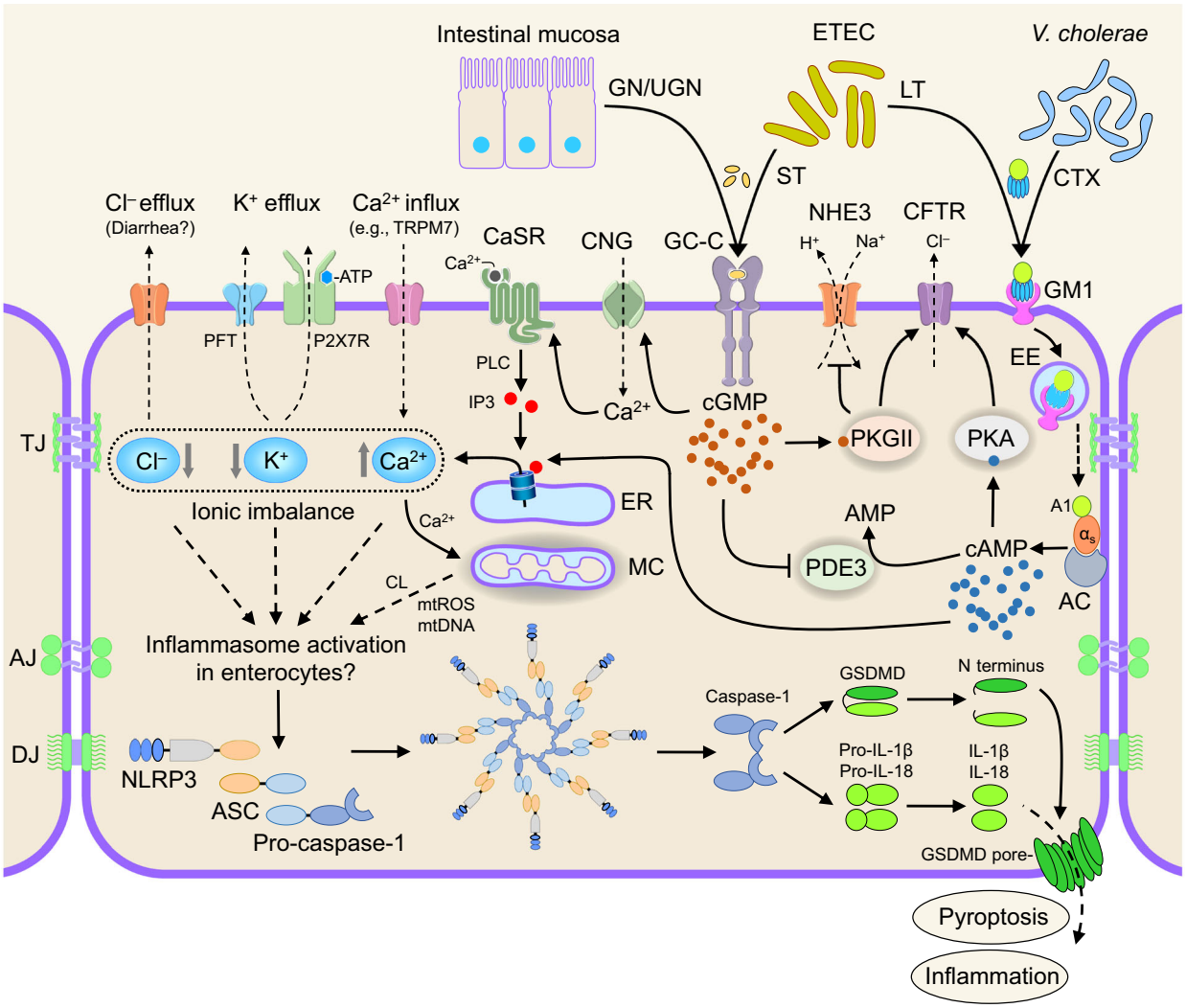

Fig. 1. Proposed model for ionic regulation of inflammasomes in enterocytes. *Vibrio cholerae*, ETEC, and *Salmonella* modulate ionic balance through various mechanisms. The LT produced by ETEC and the closely related CTX act on $G\alpha_s$ proteins and increase cAMP levels. Endogenous hormones GN and UGN or ST produced by ETEC binds to GC-C and stimulates the production of cGMP. cGMP directly stimulates the CFTR, CNG, and PKGII and inhibits PDE3. CNG stimulates CaSR surface expression and signaling, which results in the activation of phospholipase C (PLC) and increased production of IP3 and release of intracellular Ca^{2+} from the ER. Elevated intracellular cAMP levels could also increase intracellular Ca^{2+} by modulating IP3 receptors. NLRP3 inflammasome activation could proceed through ionic imbalances and cyclic nucleotide signaling as suggested in the text and depicted in the figure. Note that the evidence for activation of NLRP3 inflammasome by disturbances in intracellular ion fluxes has been suggested largely from studies on professional immune cells, such as macrophages. AC: adenylyl cyclase; AJ: adherens junction; CL: cardiolipin; DJ: desmosome junction; EE: early endosome; MC: mitochondria; mtROS: mitochondrial reactive oxygen species; TJ: tight junction.

Cl^- secretion in host cells (Fig. 1) [30,32]. Although both LT and ST have been documented to cause hypersecretion of fluid and electrolytes, activation of adenylate cyclase and cAMP production by LT is recognized as the major contributor to ETEC pathogenesis and mediates principal virulence functions such as bacterial adhesion and disruption of intestinal fluid homeostasis [33,34]. The exact mechanism by which LT enhances bacterial adhesion to host cells remains unclear. One potential scenario is that cAMP released by the host cells into the intestinal lumen is sensed by ETEC to promote adherence to host cells [35]. Of note, microaerophilic conditions and media rich in glucose and salt induce LT expression [36,37]. Thus, glucose-regulated enhancement of ETEC adhesion might be mediated, in part, by LT production and increase in cAMP release by IECs [38]. On the other hand, exposure of ETEC to short-chain fatty acids in the

colon may reduce the production of LT to aid shedding of bacteria into the feces to continue the chain of infection [39,40]. Most insights concerning cAMP-regulated cellular phenotypes have come from cell culture studies. In addition to enhancing bacterial adhesion and secretory response, both LT and CTX induce morphological alterations of cells in multiple cell models, but the underlying molecular mechanisms remain unknown [41–43]. Furthermore, *in vitro* studies in the Caco-2 cell line suggested that the increase in intracellular cAMP level during ETEC infection could inhibit intestinal vitamin B1 (thiamin) uptake and contribute to malnutrition in diarrhea [44].

The heat-stable enterotoxin (ST) is an 18-amino acid peptide with three disulfide bridges and is structurally and functionally similar to endogenous hormones, guanylin (GN) and uroguanylin (UGN) [2]. Studies *in vitro* and *in vivo* have established the guanylyl cyclase C (GUCY2C or GC-C), a protein predominantly expressed on the apical surface of IECs, as the bona fide ST receptor [2,45–47]. GC-C is a multidomain protein and, like other members of the receptor guanylyl cyclase family, consists of an extracellular ligand-binding domain, a transmembrane domain, a juxtamembrane domain, a pseudokinase domain, and a C-terminal guanylyl cyclase domain [48]. Binding of endogenous paracrine hormones (GN and UGN) or the superagonist ST peptide to GC-C results in elevated guanylyl cyclase activity and production of cGMP. The cGMP signaling cascade in the intestine has two downstream elements: activation of cGMP-dependent protein kinase II (PKGII) and inhibition of cAMP-specific phosphodiesterase (PDE3). PKGII activation leads to the stimulation of CFTR and concurrent inhibition of NHE3. On the other hand, cGMP-mediated inhibition of PDE3 might also stimulate CFTR through the cross-activation of cAMP-dependent protein kinase (PKA) [2]. The net effect is increase in chloride secretion and reduction in sodium absorption, resulting in watery diarrhea (Fig. 1) [2]. In addition, GC-C stimulation enhances duodenal bicarbonate secretion and increases $Ca2^{+}$ influx through cyclic nucleotide-gated channels (CNG) [49,50]. The GC-C/cGMP axis can also activate cyclin-dependent kinase inhibitor p21 leading to cytostasis and cellular senescence [51]. The model of ST-induced, GC-C-mediated diarrhea was tested by examining wild-type and GC-C knockout mice orally challenged with ST peptide or infection with ETEC. Under both conditions, GC-C-null mice were resistant to ST-mediated diarrhea [45,46]. The recent identification of a familial diarrhea syndrome caused by gain-of-function mutations in GC-C further supports the mechanism of GC-C activation in ST-mediated diarrhea [52,53].

Salmonellosis

Salmonellosis is a common foodborne illness caused by *Salmonella enterica* sp., with serovars Typhi, Paratyphi, and Typhimurium exemplifying important human pathogens. Recent global estimates of disease burden reported 90 300 deaths from nontyphoidal and 178 000 deaths from typhoidal salmonellosis in 2015 [54]. Given that *Salmonella* is an invasive pathogen, the pathogenesis of salmonellosis is different from that of cholera and ETEC. Studies using ligated rabbit ileal loops infected with *Salmonella* Typhimurium strains showed fluid accumulation to a similar extent as that of cholera loops. *Salmonella*-challenged loops, however, contained more mucus than *V. cholerae*-infected loops. More importantly, both *S.* Typhimurium and *V. cholerae* infection exhibited similar potential to increase mucosal cAMP levels [55]. Apart from causing secretory diarrheal phenotypes, the increase in cAMP levels in salmonellosis could impact multiple cellular pathways, including inhibition of autophagy to facilitate intracellular bacterial survival and replication [19].

Three potential mechanisms could explain the induction of cAMP in salmonellosis. The first could be the presence of a putative enterotoxin, similar to CTX and LT, that activates adenylate cyclase causing an increase in intracellular cAMP concentration [56]. Independent studies demonstrating that a cell-free lysate of *S.* Typhimurium can induce cAMP levels in isolated intestinal cells and fluid accumulation in ligated ileal loops support an enterotoxin-mediated mechanism [57,58], although the molecular nature of this toxin remains to be elucidated. Second, activation of adenylate cyclase may be due to prostaglandins released upon tissue damage during *Salmonella* infection. This mechanism is supported by the fact that indomethacin, an anti-inflammatory drug that acts primarily through the inhibition of prostaglandin synthesis, inhibited *Salmonella*-induced adenylate cyclase activity and cAMP accumulation [59]. Third, *S.* Typhimurium infection releases a large quantity of extracellular ATP, in amounts similar to those found in enteropathogenic *E. coli* (EPEC) infection, which is broken down to adenosine in the intestinal lumen [60]. Extracellular adenosine is an important mediator of inflammation of the intestinal mucosa and acts by interacting with adenosine receptor 2B, causing a marked rise in cAMP levels in host cells [61]. Adenosine and cAMP are reported to be potent regulators of *S.* Typhimurium infection-triggered pro-inflammatory

IL-6 cytokine production [62]. In addition, adenosine-driven rise in cAMP could activate CFTR and result in Cl^- secretion and secretory diarrhea [61]. Although the later mechanism has not been explored in the context of *Salmonella* infection, there are reports in the literature describing potent Cl^- secretory response mediated by adenosine that could contribute to watery diarrhea in EPEC infection [63]. In this context, it is worth noting that the two transport proteins well documented to play a role in EPEC diarrhea, namely NHE3 and DRA, are also regulated by cyclic nucleotides [4,5]. Cyclic GMP signaling is known to play a key role in diverse cellular functions, and their contribution to the pathogenesis of enteroinvasive infection has been recognized. For instance, apart from inducing cAMP levels in the host, enteroinvasive bacteria such as *Salmonella* serovar Dublin and enteroinvasive *E. coli* were shown to increase cGMP concentration [64]. Likewise, the increase in nitrated cGMP (8-nitro-cGMP) during infection with *S.* Typhimurium is thought to mediate nitric oxide (NO)-regulated cytoprotective and antimicrobial host defense [65]. Stronger evidence emerged from recent studies discovering a critical role for receptor guanylyl cyclase C (GC-C)/cGMP axis in protecting the intestinal mucosa from invasive *S.* Typhimurium infection [6].

Studies in the GC-C-null mice documenting accelerated mortality during oral *S.* Typhimurium, but not by intraperitoneal infection, provided most definitive evidence for the role for GC-C in providing protection against enteroinvasive infections [6]. Consistent with this finding, *in vitro* and *in vivo* studies indicated that GC-C deficiency enhanced *S.* Typhimurium invasion of the intestinal epithelium [7]. General stress response markers including raised serum cortisol, thymic atrophy, and depletion of CD4/CD8 thymocytes were exacerbated in *Salmonella*-infected GC-C-null mice [6]. Quantitative real-time PCR analysis of the ileum showed elevated cytokine and chemokine expression, and histopathological examination showed marked epithelial damage, submucosal edema, thinning of mucus lining, loss of goblet cells, focal tufting, and distortion of the crypt–villus architecture in GC-C-null mice following oral *S.* Typhimurium infection [6,7]. Mechanistically, *Salmonella* infection downregulates GC-C-cGMP axis and suppresses IL-22-mediated host antimicrobial defenses and alters the gut microbiome [6].

Guanylyl cyclase C-null mice infected with *Citrobacter rodentium*, the natural rodent pathogen closely related to EPEC and enterohemorrhagic *E. coli*, also showed enhanced susceptibility and systemic dissemination, indicating compromised immune defenses against an attaching–effacing enteric bacterial pathogen [66]. Taken together, the unique mechanism linking cGMP signaling, mucosal homeostasis, and host immunity established GC-C as a key player guarding host intestine from some enteric pathogens. Given the importance of GC-C signaling in intestinal barrier function, and the use of linaclotide and plecanatide, two recently FDA-approved oral GC-C agonists for managing irritable bowel syndrome with constipation [67], this opens up new possibilities for treating enteroinvasive diseases.

Ionic regulation of inflammasomes and pyroptotic cell death

The activation of inflammatory and immune pathways during infection, for example, by Toll-like receptors, is well understood. Here, we discuss pathways called the inflammasomes which have emerged as important mediators of inflammation, and potential regulation of their function by ionic imbalances induced by bacterial enterotoxins.

The inflammasome is an intracellular multiprotein complex that detects pathogenic microorganisms and sterile stressors, resulting in the production of the pro-inflammatory cytokines IL-1β and IL-18 and subsequent cell death by pyroptosis. Inflammasomes play a central role in mucosal innate immunity and provide the first-line host defense against enteric infections [68,69]. Inflammasomes assemble through the actions of sensor proteins that include the family of nucleotide-binding oligomerization domain (NOD), leucine-rich repeat domain proteins containing a pyrin domain or caspase activation and recruitment domain (CARD), absent in melanoma 2-like receptors, or the protein pyrin. These proteins oligomerize to recruit and activate caspase-1 through an adaptor protein called apoptotic speck-containing protein with CARD (ASC). Upon activation, inflammasomes form single 'foci' or 'specks' which contain the sensor, ASC, and caspase-1. The catalytic activation of caspase-1 results in proteolytic maturation of pro-IL-1β and pro-IL-18 and the cleavage of gasdermin D (GSDMD). The N terminus of cleaved GSDMD forms pores that result in loss of ions and influx of water, resulting in cell swelling and lysis during pyroptosis.

Studies have shown that ionic imbalances can trigger pyroptosis through the activation of inflammasomes. For instance, altered K^+ or Cl^- ion fluxes activate the NOD, leucine-rich repeat and pyrin domain containing protein 3 (NLRP3) inflammasome sensor [70]. NLRP3 inflammasome assembly is also mediated by physicochemically distinct cues that include endogenous

danger signals (e.g., ATP) or metabolites (e.g., fatty acids) [71]. The ubiquitously expressed two-pore domain K^+ channel subfamily K member 6 (also known as TWIK2) has been implicated in K^+ efflux during NLRP3 activation in macrophages [72]. Pannexin-1 channels indirectly activate NLRP3 by promoting the release of ATP which activates its P2X purinoceptor 7 (P2X7R), a large nonspecific channel, stimulating NLRP3 through loss of ions, including K^+. Recombinant ASC protein can form inflammasome-like aggregates in the presence of low K^+ *in vitro*, which suggests that features inherent in its structure may facilitate the response to low K^+ [73].

In the gut, P2X7R and pannexin-1 are expressed in enteric neurons. P2X7R–pannexin-1 signaling and ASC-dependent death of neurons and glia have been studied in a mouse model of Crohn's disease [74]. The inhibition of pannexin-1 with probenecid *in vivo* reduced glial responses. Inhibition of caspases with zVAD in cultured enteric neurons prevented cell death, suggesting a role for inflammasomes in these processes. In colitic mice, the loss of enteric neurons was correlated with decreased electrical field stimulation (EFS)-driven colon relaxation and elevated EFS-driven contractions. Despite the role of P2X7R in mouse models of inflammatory bowel disease and its increased expression in Crohn's disease mucosa [75], P2X7R antagonists (e.g., AZD9056) have shown little efficacy in preventing symptoms of Crohn's disease; however, the reduction in abdominal pain indicates a more important role for P2X7R in nociception [76,77].

During infection, NLRP3 assembly and caspase-1 activation can be induced directly by K^+ efflux driven by bacterial pore-forming toxins (PFTs), including those produced by enteric pathogens [78–80]. Gram-negative bacteria can directly activate caspase-4 or caspase-5, which serve as cytosolic receptors for bacterial lipopolysaccharide (LPS). EPEC [81], *Salmonella*, and other enteric Gram-negative bacteria [82] can activate caspase-4/5, which cleave GSDMD, leading to pore formation and K^+ efflux. This caspase-4/5-dependent, 'noncanonical' NLRP3-activation pathway is unique to Gram-negative bacteria and distinct from 'canonical' NLRP3 activation by PFTs, P2X7R, and pannexin-1 [70]. Blocking K^+ efflux, for example, by antagonists of P2X7R (e.g., A438079, AZ11645373, AZ11648720, AZ10573295), pannexin-1 (e.g., probenecid), or ATP-sensitive K^+ channels (e.g., glibenclamide) inhibits NLRP3 activation and inflammation [83]. Thus, the reduction of cellular K^+ is a common and widespread mechanism of NLRP3 inflammasome activation that can be impaired by high extracellular K^+ concentrations [70].

In addition to K^+, the loss of Cl^- ions can trigger ASC focus formation in an NLRP3-dependent manner [84]. The exposure of LPS-treated macrophages to culture medium lacking both K^+ and Cl^- (e.g., by using Na-gluconate for ionic and osmotic balance) results in the spontaneous assembly of inflammasomes containing NLRP3 and ASC specks and activation of caspase-1. However, loss of Cl^- alone triggers NLRP3-dependent assembly of ASC specks, a reduction in K^+-triggered NLRP3 assembly, interaction with the activator NEK7, and caspase-1 activation. It is plausible that reduced intracellular Cl^- primes inflammasomes and reduces the threshold for full activation by a second trigger that stimulates K^+ efflux. This would increase the risk of inflammation in conditions where luminal Cl^- is constitutively elevated, for example, in familial chloride diarrheas that arise from inactivating mutations in the $Cl^-/HCO3^-$ exchanger DRA (SLC26A3) expressed on the apical surface of IECs [85,86]. Inflammasome activation can therefore be blocked with Cl^- channel blockers such as 4-(2-butyl-6,7-dichloro-2-cyclopentylindan-1-on-5-yl)oxybutyric acid, flufenamic acid (an nonsteroidal anti-inflammatory drug), and 5-nitro-1-(3-phenylpropylamino)benzoic acid [84].

An alternative mechanism mediated by K^+-induced translocation of chloride intracellular channels to the plasma membrane and consequent Cl^- efflux has been demonstrated [87]. Further studies are required to demonstrate whether basolaterally secreted K^+ ions in IECs, the key to fluid release during diarrhea [4], affect NLRP3 activation.

Cyclic nucleotides, Ca^{2+}, and NLRP3: a potential link?

Ca^{2+} is also an important regulator of the NLRP3 inflammasome. Murine G protein-coupled Ca-sensing receptor (CaSR; encoded by G protein-coupled receptor family C group 2 member A) activates NLRP3 through the generation of inositol-1,4,5-triphosphate (IP3), which releases Ca^{2+} from the ER [88]. In addition to Ca^{2+}, CaSR responds to cations such as Gd^{3+} and the allosteric agonist R568. In human monocytes, both CaSR and the related G protein-coupled receptor family C group 6 member A, which signals via $G\alpha_q$, trigger NLRP3 activation in response to high extracellular Ca^{2+} [89]. High intracellular Ca^{2+} that can be induced, for example, by thapsigargin (which inhibits the sequestration of cytosolic Ca^{2+} into the ER) may trigger NLRP3 activation. Uptake of Ca^{2+} by mitochondria, leading to elevated ROS, cardiolipin exposure, and release of mtDNA can all increase NLRP3

activation in macrophages. Plasma membrane calcium channels such as transient receptor potential melastatin 2, transient receptor potential melastatin 7, and transient receptor potential cation channel subfamily V member 2 have also been linked to NLRP3 inflammasome activation, for example, in response to cell swelling [90,91]. In summary, these studies indicate that elevated cytosolic Ca^{2+} can trigger NLRP3 inflammasomes, inflammatory cytokine release, and pyroptosis.

Importantly, CTX, which increases cytoplasmic cAMP and Ca^{2+} (via modulation of IP3 receptors; Fig. 1), triggers activation of inflammasomes [79,92,93]. However, the exact mechanisms by which cAMP regulates inflammasome activity remain to be clarified. For example, both adenosine receptor-mediated increases in cAMP levels [94] and CaSR-mediated reduction in cAMP increased inflammasome activity [88].

No less fascinating, though far less understood, is how and if cGMP signaling could impact inflammasome pathways. Notably, NO, an activator of soluble guanylyl cyclase and inducer of cGMP levels, was found to suppress activation of the NLRP3 inflammasome. However, this effect was found to be independent of cGMP [95]. Furthermore, unlike cAMP, no interaction was observed between cGMP and endogenous NLRP3 [88]. However, studies on GC-C have provided insights into the potential role of cGMP signaling in inflammasome activation. The GC-C/cGMP axis stimulates CNG, resulting in an increase in Ca^{2+} influx and post-transcriptional induction of CaSR surface expression and signaling [50]. Thus, cGMP-mediated effects on cytoplasmic Ca^{2+} and CaSR expression could represent a previously unrecognized mechanism of cGMP-mediated regulation of inflammasome pathways (Fig. 1).

Activation of inflammasomes during enteric infections could act as a barrier to limit the bacterial burden and systemic spread of pathogens. Indeed, the inflammasome is a major component of the innate immune system that is induced early in the intestinal mucosa in patients with cholera [96]. Therefore, it is not surprising that the genetic resistance to cholera in humans involved natural selection of genes of the inflammasome pathway and K^+ channels involved in cyclic AMP-mediated chloride secretion [97].

Studies conducted using mouse models also support a key role for inflammasome activation in response to *V. cholerae* infection [98]. Toxins secreted by *V. cholerae* can activate the inflammasome in a variety of ways. For example, CTX has been shown to specifically induce release of IL-1β through an ASC-dependent but NLRP3-independent pathway, whereas the PFT hemolysin, secreted by El Tor biotype strains, triggers the NLRP3–ASC-dependent inflammasome [79]. Other Gram-negative enteric pathogens, such as *E. coli* (including ETEC), *C. rodentium*, and *S.* Typhimurium, have been shown to activate the inflammasome through cytosolic sensing of their LPS [81,82,98,99].

It is important to note that certain enteric bacteria have also adopted inflammasome-evasion strategies crucial for virulence. For example, following cellular invasion, *Salmonella* downregulates expression of flagellin and type 3 secretion system to attenuate caspase-1 responses [100]. Thus, taken together, inflammasome activation is common to enteric infections where cAMP and cGMP play crucial roles, but molecular links between these second messengers and the inflammasome are as yet unknown.

Conclusions

Gut microbiota play an important role in protecting against diarrheal infection as well contributing to inflammation in chronic gut diseases [101,102]. Ion transport across IECs may play an important role in the establishment and maintenance of the gut microbiota [103]. Indeed, prominent depletion of *Faecalibacterium* and *Bifidobacterium* and an increase in *Enterobacteriaceae* have been described in patients with an activating mutation in GC-C that is characterized by dysregulated intestinal ion transport, secretory diarrhea, and chronic mucosal inflammation [104]. Independent studies have also reported alterations in the gut microbiota composition in GC-C-null mice and a decrease in the relative abundance of *Lactobacillus* spp., members of which have been implicated in inflammasome activation through caspase-1-dependent processing and release of IL-1β by macrophages [6,66,105]. These findings support an alternate model in which cyclic nucleotide signaling in enterocytes could regulate inflammasome activation by shaping the gut microbiota and modulating host–microbe cross talk. Thus, prolonged inflammasome activation may explain why chronic inflammation is seen in patients with activating mutations in GC-C, with a considerable number of them diagnosed with Crohn's disease [104].

In summary, it is attractive to speculate that cyclic nucleotides may play a complex role in the regulation of inflammasomes in the context of enteric infections. The host has evolved redundant systems to detect and respond to pathogens, and on the other hand, pathogens can subvert inflammasome signaling to increase virulence. The effects of ionic imbalances, which underlie diarrhea, on inflammation need to be better understood to appreciate the broader impact of

infection on physiological and immune homeostasis in the gut.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

All authors wrote the paper and finalized the manuscript.

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