cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

The Role of Ion Transporters in the Pathophysiology of Infectious Diarrhea

Soumita Das,[1] Rashini Jayaratne,[2] and Kim E. Barrett[2]

[1]Department of Pathology, [2]Department of Medicine, University of California San Diego School of Medicine, La Jolla, California

SUMMARY

Intestinal ion transporters ensure fluid and electrolyte homeostasis. Several are modulated during enteric infections, potentially contributing to diarrhea. This review surveys changes in the abundance and/or regulation of transporters that occur in these conditions, pointing to possible novel targets for therapy.

Every year, enteric infections and associated diarrhea kill millions of people. The situation is compounded by increases in the number of enteric pathogens that are acquiring resistance to antibiotics, as well as (hitherto) a relative paucity of information on host molecular targets that may contribute to diarrhea. Many forms of diarrheal disease depend on the dysregulation of intestinal ion transporters, and an associated imbalance between secretory and absorptive functions of the intestinal epithelium. A number of major transporters have been implicated in the pathogenesis of diarrheal diseases and thus an understanding of their expression, localization, and regulation after infection with various bacteria, viruses, and protozoa likely will prove critical in designing new therapies. This article surveys our understanding of transporters that are modulated by specific pathogens and the mechanism(s) involved, thereby illuminating targets that might be exploited for new therapeutic approaches. ***(Cell Mol Gastroenterol Hepatol 2018;6:33–45; https://doi.org/10.1016/j.jcmgh.2018.02.009)***

Keywords: Ion Transport; Diarrhea; Enteric Pathogen; Epithelium.

The intestinal epithelium is responsible for absorbing nutrients, such as sugars and peptides, as well as electrolytes and water.[1] Most water absorption occurs in the small intestine, with residual water absorption occurring in the colon. Absorptive processes are predominant in villi whereas secretory processes are predominant in the crypts. To facilitate solute and water absorption, the intestines rely on transporters that permit the movement of solutes through the cell membrane. Water then follows passively via both paracellular and transcellular routes. The transporters that mediate solute uptake or secretion are expressed differentially throughout the intestines, and have a wide range of substrates. Under normal conditions, the various transporters work together to provide an optimum balance between absorption and secretion, with absorption predominating to reclaim the 8–9 L of fluid that are used daily during digestion and absorption of meals in human beings. However, during pathologic states, such as infections with diarrheal pathogens, this balance is disrupted, with either increased secretion, loss of absorption, or both.[1] Although the gut has a substantial reserve capacity for absorption, ultimately this imbalance can cause diarrhea.

Diarrhea is an almost ubiquitous sign of enteric infection, leading to the question of what benefit it provides for the microbe or the host. For the microbe, diarrhea presumably facilitates the colonization of additional hosts, particularly in settings in which sanitation is compromised. For the host, the diarrheal response, although potentially harmful in terms of dehydration, also may represent a primitive host defense mechanism, reducing microbial colonization and perhaps restricting cellular entry by invasive species.[2] Because of its risks, diarrhea often calls for treatment in serious cases and/or particularly vulnerable hosts. However, most currently available antidiarrheal agents may have side effects, target motility rather than transport processes themselves, and often are relatively ineffective, particularly in the setting of life-threatening infectious diarrhea. There is therefore a need for new therapies, for which it is important to understand the underlying mechanism(s) of diarrhea.

Overview of Epithelial Transport Function

The transport of ions across the plasma membrane is crucial for cellular homeostasis. There are 3 major

Abbreviations used in this paper: **ATP, adenosine triphosphate; ATPase, adenosine triphosphatase; cAMP, adenosine 3′,5′-cyclic monophosphate; CDI,** ***Clostridium difficile*** **infection; CFTR, cystic fibrosis transmembrane conductance regulator; CLCA1, chloride channel accessory 1; CT, cholera toxin; CXCR2, C-X-C motif chemokine receptor 2; DRA, down-regulated in adenoma; ENaC, epithelial sodium channel; EPEC, enteropathogenic** ***Escherichia coli*****; EspG, Escherichia coli secreted protein G; ETEC, enterotoxigenic** ***Escherichia coli*****; GPR39, G-protein coupled receptor 39; KCC, potassium-chloride cotransporter; LPA, lysophosphatidic acid; LT, heat-labile toxin; NHE, sodium/hydrogen exchanger; NHERF2, sodium/hydrogen exchanger regulatory factor 2; NKCC, sodium-potassium-2 chloride cotransporter; ORT, oral rehydration therapy; PKC, protein kinase C; SGLT1, sodium-glucose cotransporter 1; SLC, solute carrier; ST, heat-stabile toxin; Tcd,** ***Clostridium difficile*** **toxin; TNF, tumor necrosis factor; ZnR, zinc sensing receptor.**

Most current article

2352-345X
https://doi.org/10.1016/j.jcmgh.2018.02.009

mediators of ion transport: (1) transporters (both cotransporters and exchangers), (2) ion channels, and (3) pumps.

Transporters are transmembrane proteins that mediate the transport of ions and sometimes other solutes, such as glucose or amino acids. Some also may transport drugs or metabolites. Cotransporters bind to their substrates on one side of the membrane, causing a conformational change that releases the substrates on the other side of the membrane. Exchangers transfer a solute into the cell in exchange for one that is secreted out of the cell. In either case, the activity of transporters is driven by the prevailing combined electrochemical gradients for the solutes in question.

Ion channels are pore-forming transmembrane proteins that open as gates in response to a variety of cellular signals, allowing high-capacity solute passage. The direction of ion movement depends on the electrochemical gradient for that solute across the membrane.

Pumps expend cellular energy, in the form of adenosine triphosphate (ATP) hydrolysis, and allow for uphill transport of one or more of their substrates. An example is the Na^+,K^+ adenosine triphosphatase (ATPase), which exports 3 sodium ions for every 2 potassium ions taken up into the cell, maintaining a low intracellular sodium concentration and sustaining the negative membrane potential.

Intestinal epithelial cells control the secretion and absorption of electrolytes through various arrangements of the ion transporters described earlier, which function together to maintain fluid balance; this fluid balance is impaired during diarrhea.[1] Impairments in transporter function can occur during infections and in inflammatory diseases, or may be caused by genetic mutations.

Major Transporters Implicated in Infectious Diarrhea

Although the intestines express a large array of distinct transport proteins, only a subset have been examined for their possible contributions to infectious diarrhea (Table 1). Thus, we focus on those transporters here (Figures 1 and 2). They include the following.

1. Sodium/hydrogen exchangers (NHEs): NHE3 (solute carrier [SLC]9A3) (and to a lesser extent, NHE2 [SLC9A2]) are responsible for electroneutral NaCl absorption in the small intestine and colon, by functioning in partnership with a chloride/bicarbonate exchanger.[3]

2. Sodium/glucose cotransporter (SGLT1, SLC5A1): this transporter is responsible for the absorption of both glucose and sodium ions postprandially.[4]

3. Down-regulated in adenoma (DRA [SLC26A3]): this transporter is a Cl^-/HCO_3^- exchanger, and is responsible for Cl^- absorption (and also transports SO_4^{2-}). DRA functions in concert with NHEs in the electroneutral absorption of NaCl.[5]

4. Epithelial sodium channel (ENaC): this channel mediates electrogenic Na^+ absorption and is localized to the distal colon.[6]

5. Ca^{2+}-activated chloride channels[7]: these channels mediate the efflux of chloride ions and are activated by increases in intracellular Ca^{2+} concentration. Their precise molecular identity in the gut still is controversial, although one candidate is chloride channel accessory 1 (CLCA1).[8] Other studies have implicated transmembrane protein 16A[9,10] (anoctamin 1), although the precise relative roles, if any, for both channels is still under investigation.

6. Sodium/potassium/chloride cotransporter 1 (NKCC1 [SLC12A2]): this transporter mediates the uptake of Na^+, K^+, and $2Cl^-$ ions across the basolateral membrane, and thereby supplies chloride for secretion.[11]

7. Cystic fibrosis transmembrane conductance regulator (CFTR): this is an adenosine 3′,5′-cyclic monophosphate (cAMP)- and guanosine 3′,5′-cyclic monophosphate–regulated chloride channel present primarily at the apical surfaces of epithelial cells, and mediates chloride efflux as part of the chloride secretory mechanism.[1] It also can transport bicarbonate.

8. Na^+,K^+ ATPase: This establishes and maintains a low intracellular Na^+ concentration that is a driving force for several different transport mechanisms, both secretory and absorptive.[12]

Regulation of Transport

The transporters discussed earlier can be regulated in 3 main ways to effect changes in overall levels of epithelial transport.[1] First, changes in transcription/translation of a given transporter will result in changes in its abundance, and associated changes in the capacity of the epithelium for transport function. Second, transport activity may be controlled by trafficking of a given transporter into or out of the plasma membrane. Finally, transporter activity may be acutely regulated by post-translational modifications, such as phosphorylation by various kinases, or may be modulated directly by intracellular second messengers such as free cytosolic calcium. Each of these mechanisms has been implicated in dysregulated transport in the setting of infection.

Epithelial Dysfunction in Diarrhea: Relative Roles of Secretion and Absorption

Intestinal epithelial cells play the key role in diarrheal pathogenesis. The epithelium is the first line of defense and host-microbe interactions are crucial in the development of infectious diarrhea. In addition to its transport functions, moreover, the epithelium also forms a barrier that may protect the host from the intrusion of microbial pathogens or toxins. Intestinal barrier dysfunction also may play a significant role in diarrheal disease (so-called leak-flux diarrhea).[13–15] However, in this review, we have focused mostly on the role of ion transporters in infectious diarrhea.

A classic view of infectious diarrhea implicated direct stimulation of epithelial chloride secretion, with associated

Table 1. Major Ion Transporters Targeted by Enteric Infections

Transport function	Transporter	Location	Examples of regulation by enteric pathogens
Absorption	NHEs	Apical membrane of small intestinal villus and surface epithelial cells in colon	Function of NHE2 and NHE3 decreased in response to cholera toxin[29,50] EHEC toxin Stx2 shown to prevent trafficking of NHE2 to the apical membrane[40] Rotavirus decreases NHE3[34,61] EPEC increases NHE2 activity[24,25] *C difficile* TcdB decreases NHE3 activity[57,58]
	SGLT1	Apical membrane of small intestinal villi[108]	Function decreased by EPEC infection[29,30] Rotavirus decreases SGLT1[34,61]
	ENaC	Apical membrane of surface cells in distal colon	Decreased by *Salmonella* infection in mice[17]
	DRA	Apical membrane of small intestinal villous cells and surface cells in colon[109–111]	Decreased by EPEC, *C rodentium*, and *Salmonella* infection[19,21,29,34,112]
Secretion	CaCC	For CLCA1 in human beings, apical membrane of small intestinal and colonic crypt epithelial cells (and goblet cells)[113]	Possibly stimulated by *E histolytica*, *G lamblia*, cholera (ACE toxin), *V parahaemolyticus*, and rotavirus[34,53]
	NKCC1	Basolateral membrane of small intestinal and colonic crypt epithelial cells[114]	In cell lines and tissue ex vivo, expression increased by enteroinvasive *E coli* and *Salmonella Dublin*[43]
	CFTR	Apical membrane of small intestinal and colonic epithelial cells with expression decreasing from crypt to villus[115]	Increased activity after infection with ETEC, enteroinvasive *E coli*, or *Salmonella Dublin*; the latter also may increase expression in cell lines[8,32] Redistributed into epithelial cytosol without a change in expression after *Salmonella* infection in mice[17] *E histolytica* and norovirus increase chloride secretion[34,116,117]
Absorption and secretion	Na^+, K^+ ATPase	Basolateral membrane of epithelial cells throughout the small intestine and colon	*Salmonella* infection in mice accompanied by redistribution from basolateral to apical membrane[17] Activated by the magnesium transporter C (MgtC) virulence factor of *Salmonella*[118]

ACE, accessory cholera enterotoxin; CaCC, Ca^{2+}-activated chloride channel; CLCA1, chloride channel accessory 1; EHEC, enterohemorrhagic *E coli*; Stx2, Shiga toxin 2.

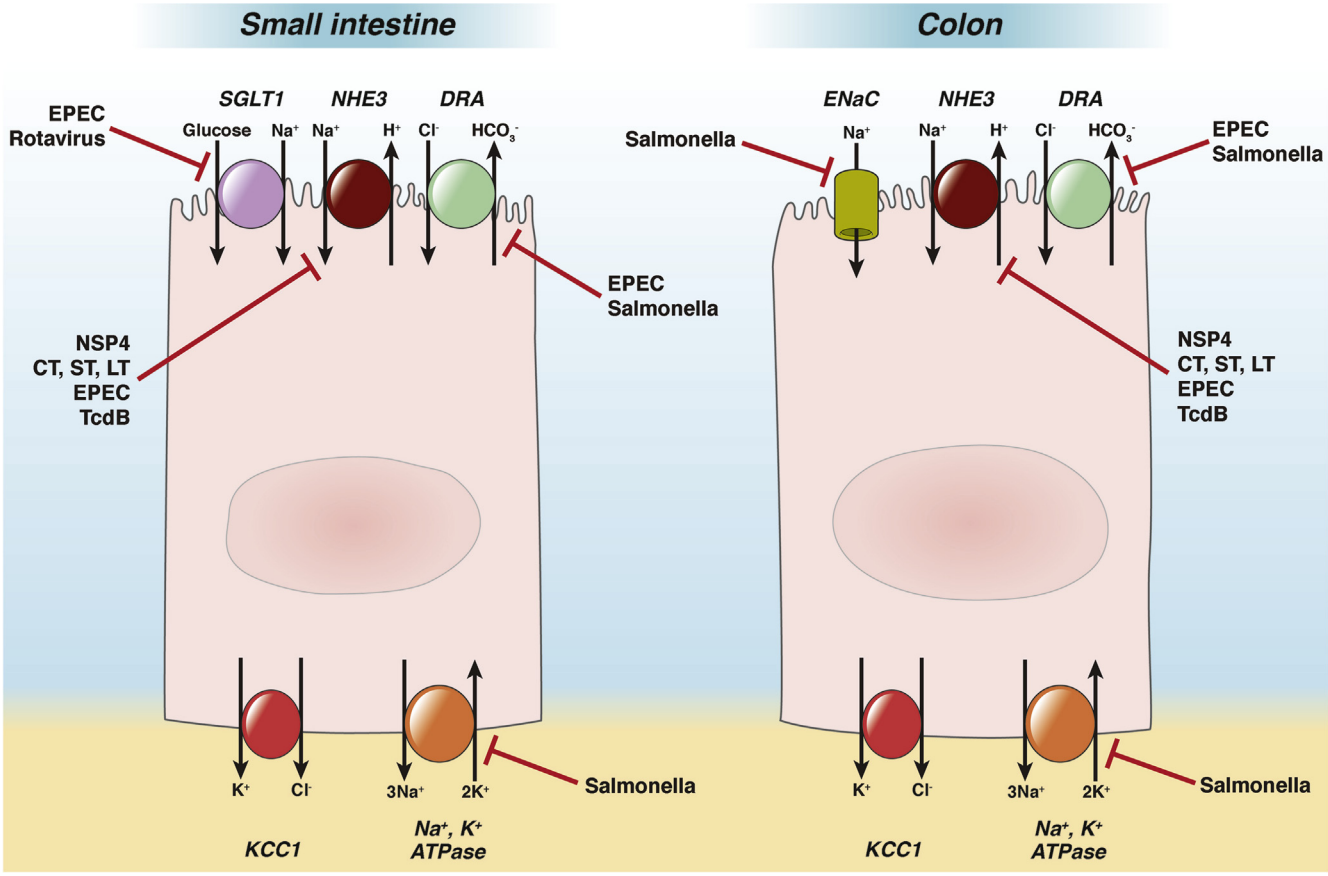

Figure 1. Localization of absorptive ion transporters (discussed in text) in the small intestine and colon, and their regulation by pathogens or their secreted toxins. The figure is not intended to imply that the illustrated transporters are necessarily expressed in the same cells. Note particularly that ENaC is present only in the distal colon. The *red bars* indicate inhibitory effects. The effect shown for *Salmonella* on the Na^+, K^+ ATPase consists of mislocalization to the apical membrane that would be expected to disrupt absorptive transport; however, note that a stimulatory effect of a *Salmonella* effector on the ATPase also has been reported[118] (not shown). CT, cholera toxin; DRA, down-regulated in adenoma; ENaC, epithelial sodium channel; EPEC, enteropathogenic *E. coli*; KCC1, potassium chloride cotransporter-1; NHE, sodium hydrogen exchanger; NSP4, Rotavirus non-structural protein 4; SGLT1, sodium glucose cotransporter-1; ST, heat-stable toxin of *E. coli*; LT, heat-labile toxin of *E. coli*; TcdB, *C. difficile* toxin B.

loss of fluid, as the primary driving force for diarrheal symptoms (eg, in the setting of infection with *Vibrio cholerae* or enterotoxigenic strains of *Escherichia coli* [ETEC]). However, it has become increasingly obvious that changes in electrolyte absorptive processes also are involved in disease pathogenesis. For example, an increase in epithelial cAMP not only activates chloride secretion, but also inhibits electroneutral NaCl absorption.[16] Furthermore, studies with invasive pathogens such as nontyphoidal *Salmonella* species or enteropathogenic *E coli* (EPEC) have failed to uncover any evidence of active anion secretion in the setting of infection.[17,18] Rather, diarrheal disease may result from the specific suppression of absorptive transport mechanisms.[17,19]

Specific Transporters Implicated in the Pathogenesis of Infectious Diarrhea

In this section, we summarize evidence for the modulation of transporters by selected bacteria, protozoa, and viruses that cause diarrheal illness. Of note, studies to date have used a variety of models, including colon cancer cell lines, tissue explants, xenografts, and whole animals (typically mice), which raises questions about the extent to which all conclusions can be extrapolated to human patients. The recent introduction of organoid models, as well as monolayers derived from these, should offer benefits in developing an enhanced understanding of diarrheal mechanisms.[20]

Bacterial Diarrhea

A major cause of diarrheal diseases in developing countries is infection by bacteria, such as enterotoxigenic and enteropathogenic *E coli*, *Salmonella*, *Shigella*, and *V cholerae*. Bacterial pathogens remain important causes of foodborne illness in developed countries as well.

E coli

Although many strains of *E coli* are harmless commensals, several are diarrheagenic, albeit with distinct mechanisms. For example, EPEC can be distinguished from other diarrheagenic *E coli* by its ability to form attaching/effacing

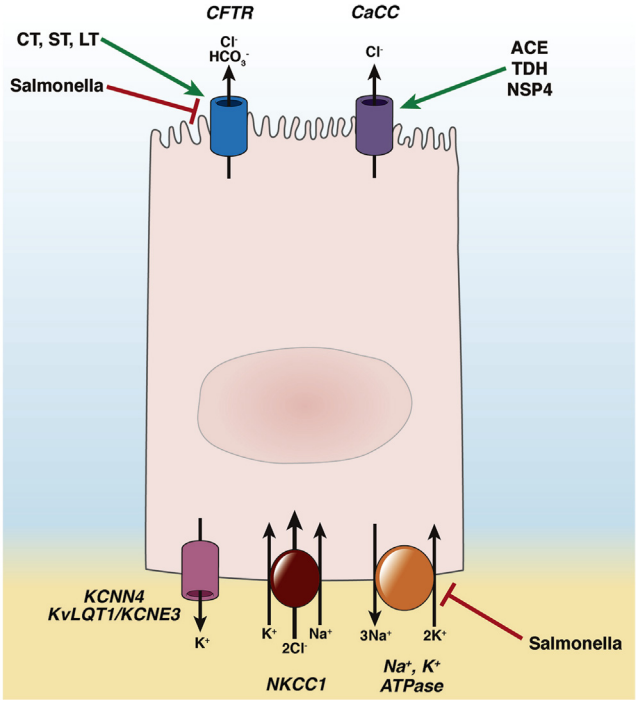

Figure 2. Chloride secretory mechanism in the small intestine and colon, and regulation of its constituent transporters by pathogens or their secreted toxins. The *green arrows* and *red bars* represent stimulatory and inhibitory effects, respectively. CaCC, calcium-activated chloride channel; ACE, accessory cholera enterotoxin; TDH, thermostable direct hemolysin of *V parahemolyticus*; NSP4, rotavirus nonstructural protein-4; KCNN4, calcium-activated potassium channel; KvLQT1/KCNE3, cAMP-activated potassium channel.

lesions at its sites of attachment to the epithelium, resulting in loss of microvilli. Infection decreases the abundance of absorptive transporters in the epithelial apical membrane secondary to this disruption, but also by more rapid effects that are independent of the loss of microvilli. In particular, EPEC causes internalization of DRA from the apical surface of enterocytes and presumably causes subsequent diarrhea by inhibiting electroneutral NaCl absorption.[21] EPEC uses a type III secretion system to inject bacterial effectors into host cells. The effectors E coli secreted protein G (EspG) and EspG2 cause disruption of the host microtubule network and are necessary for the internalization of DRA, although the exact mechanism is not yet known.[19] EPEC also disrupts tight junctions and thereby impairs barrier function, which in turn provokes infiltration by inflammatory cells.[22,23]

EPEC infection also acutely increases NHE2 activity, but decreases that of NHE3 (of note, NHE3 is the major isoform contributing to NaCl absorption in vivo in association with DRA).[24,25] Protein kinase C (PKC) is thought to be involved in EPEC-mediated up-regulation of NHE2, and the kinase also can suppress NHE3 activity, although whether this accounts for the action of EPEC needs to be investigated.[26] DRA and NHE3 are thought to be coupled via NHE regulatory factor 2 (NHERF2), but the bacterial effectors required to decrease DRA after infection (EspG and EspG2) are not required for the decrease in NHE3; rather, another effector, EspF, has been implicated.[27,28] Unlike many other enteric pathogens, EPEC not only inhibits electrolyte absorptive mechanisms, but also is capable of inhibiting the activity of SGLT1.[29,30] This has been advanced as an explanation for the fact that diarrhea associated with EPEC infection is poorly responsive to oral rehydration therapy (ORT).

ETEC is a major cause of traveler's diarrhea as well as childhood diarrhea in developing countries,[31] and causes disease by elaborating toxins: heat-labile toxins (LTs), heat-stable toxins (STs), or both. LTs activate an increase in Cl^- secretion mediated by CFTR via their ability to increase cAMP. STs, on the other hand, stimulate CFTR in a guanosine 3′,5′-cyclic monophosphate–dependent manner.[32] The toxin also inhibits NHE3 activity by dysregulating trafficking of the transporter.[33] Some studies have suggested that ST causes net secretion, whereas others have suggested that it is only antiabsorptive.[34,35] ST also may stimulate HCO_3^- secretion via a mechanism independent of CFTR, but with possible involvement of DRA or putative anion transporter 1.[36] LT-expressing strains of ETEC, which functionally can be considered to cause diarrhea in a manner analogous to that occurring in the setting of cholera, presumably also cause a cAMP-dependent decrease in the activity and trafficking of NHEs.[29]

Enterohemorrhagic *E coli* is a highly pathogenic strain capable of causing severe bloody diarrhea as well as systemic sequelae such as endothelial injury and renal failure. Many of the effects of enterohemorrhagic *E coli* infection relate to its ability to elaborate Shiga toxins 1 and 2, which are known to cause epithelial apoptosis and barrier dysfunction with an associated inflammatory response, although some effects of the pathogen on tight junctions are independent of Shiga toxins.[37–39] The inflammatory infiltrate is most likely to mediate the diarrheal response rather than a direct effect of infection on transporters per se, although Shiga toxin 1 also has been shown to prevent NHE2 trafficking to the apical membrane secondary to depleting intracellular galectin-3.[40]

Salmonella

Nontyphoidal *Salmonella* species, such as *S enterica* serovar Typhimurium, are invasive bacteria that use a type III secretion system to deliver a variety of effectors into intestinal epithelial cells. They are leading causes of foodborne diarrhea in many settings.[41] They were long considered to trigger diarrhea predominantly via their ability to trigger a host inflammatory response, although they likely also exert direct effects on epithelial transport function.[42] Studies in cell lines and human intestinal xenografts suggested up-regulation of both the activity and expression of NKCC1 and CFTR after infection, secondary to the ability of the bacteria to induce cyclooxygenase-2 and inducible nitric oxide synthase.[17,43] More recent studies in a murine model of *Salmonella* diarrhea, however, failed to show an increase in CFTR or chloride secretion.[17] Rather, infection resulted in diarrhea that was associated with a reduction in DRA and ENaC expression in the proximal and distal colon, respectively, redistribution of CFTR into the epithelial cytosol, mislocalization of Na^+, K^+ ATPase to the apical membrane,

and an expansion of the compartment expressing NKCC1.[17] Furthermore, both diarrheal symptoms and down-regulation of DRA in infected mice were independent of neutrophil infiltration.[44] Thus, at least initially, diarrhea in the setting of *Salmonella* infection may reflect a direct inhibition of electrolyte absorption in the gut, perhaps secondary to epithelial immaturity induced by effectors delivered by the type III secretion system.

Shigella

The mechanism of fluid loss after Shigella infection differs from toxigenic diarrheas caused by *V cholerae* and ETEC. *Shigella* is an invasive pathogen that also generates Shiga toxins and stimulates an inflammatory infiltrate and watery and/or bloody diarrhea. Most of the diarrheal response is caused by the toxin as well as bacterial effectors that activate inflammatory cytokines, attract polymorphonuclear cells, and activate chloride secretion.[45] Infection with *Shigella flexneri* may generate acute dysentery, watery diarrhea, or both.[46] At least in monkeys, dysentery alone was associated with diminished colonic absorption or even net colonic secretion, whereas although these were also present in the setting of diarrhea, jejunal secretion additionally was seen.[47] However, the specific transporters that account for these effects have not been identified. Shiga toxin also decreased water absorption in the human colon without changing short circuit current, implying it influences an electroneutral process such as NaCl absorption,[48] but the mechanism is unknown.

Vibrio

V cholerae causes diarrhea predominantly by activating net secretion of chloride ions. Its major virulence factor is cholera toxin (CT), which binds to apical GM1 receptors on host epithelial cells, thereby allowing translocation of the toxin into the cell.[49] CT adenosine diphosphate-ribosylates and thereby up-regulates the activity of a guanosine triphosphatase that governs adenylate cyclase, resulting in irreversible increases in cAMP production. In turn, protein kinase A is activated and increases CFTR activity and subsequent chloride secretion.[32] CT also inhibits Na^+ absorption by down-regulating both NHE2 and NHE3, although through different mechanisms (post-translational vs post-transcriptional).[50] cAMP is responsible for this decrease in NHE abundance, and also has an acute effect on NHE activity mediated via NHERF2.[50,51] In addition, the accessory cholera toxin stimulates CLCA.[34] The overall result is an increase in both sodium and chloride ions in the lumen, leading to diarrhea. However, SGLT1 activity is unaffected, accounting for the efficacy of ORT in cholera.[52] Similarly, *Vibrio parahaemolyticus*, frequently acquired from contaminated seafood, elaborates a thermostable direct hemolysin toxin that activates CLCA via activation of PKC and thus Ca^{2+}-dependent Cl^- secretion.[53]

Clostridium difficile

C difficile infection (CDI) causes often debilitating diarrhea, frequently is precipitated by the systemic use of antibiotics or acquired in health care settings, and generates health costs of $1 billion per year.[54] The bacteria elaborate toxins A and B (TcdA and TcdB), as well as an additional toxin called binary toxin.[55] Most of the diarrheal effects of CDI have been attributed to the ability of TcdA to trigger epithelial injury, barrier dysfunction, an inflammatory infiltrate, and activation of subepithelial elements such as nerve endings and mast cells, rather than effects on transport function.[56] However, TcdB causes a pronounced inhibition of NHE3 activity in cell lines.[57] Furthermore, patients with CDI have decreased NHE3 in the apical membranes of their enterocytes.[58] In fact, bezlotoxumab, a monoclonal antibody that neutralizes TcdB, recently received Food and Drug Administration approval for the prevention of CDI recurrence.[59]

Viral Diarrhea

Important viral pathogens that cause diarrhea include rotavirus, norovirus, sapovirus, adenovirus, and astrovirus. Among these, rotavirus is the most common cause of severe diarrhea and diarrheal mortality in infants and young children worldwide.[60] Rotavirus invades enterocytes of the small intestine. It produces a toxin, rotavirus nonstructural protein 4, that increases intracellular Ca^{2+} levels, and thereby activates Cl^- secretion and decreases activity of NHE3.[61] Rotavirus infection also results in malabsorption of sodium and glucose owing to decreases in SGLT1.[34,61] Interestingly, rotavirus infection also disrupts cellular Na^+ and K^+ homeostasis, which in theory could impair intestinal absorption, but without altering abundance of the Na^+, K^+ ATPase.[62] However, the involvement of ion transport dysregulation in the pathogenesis of the other viral infections mentioned remains to be examined.[34]

Parasite-Mediated Diarrhea

Entamoeba histolytica, *Giardia lamblia*, and *Cryptosporidium parvum* are common causes of water-borne diarrhea. *Giardia* tropozoites strongly adhere to the epithelial surface of the intestine via a ventral adhesive disc. *Giardia* causes a loss of the absorptive surface similar to EPEC. It decreases NaCl and glucose absorption owing to this loss of absorptive surface area.[34,63,64] *Giardia* also apparently directly stimulates intestinal chloride secretion secondary to activation of PKC.[34,64,65] *Entamoeba histolytica*, on the other hand, may be directly toxic to epithelial cells, resulting in barrier defects and consequent diarrhea.[66] There is also evidence that the parasites can produce serotonin and prostaglandin E_2, which are direct chloride secretagogues.[67,68] Finally, *C parvum* causes a loss of absorptive villous enterocytes in the small intestine with an associated reduction in SGLT1 abundance and function, which has been assumed to underlie diarrheal pathogenesis along with host cell prostaglandin production and diminished barrier function.[69–71]

Inflammatory Diarrhea

As indicated earlier, some pathogens, particularly invasive bacteria, alter the function of ion transporters by virtue of their ability to provoke a mucosal inflammatory response. In

fact, there is substantial evidence that inflammation alone is sufficient to modify ion channels and other transporters.[72,73] For example, inflammatory cytokines/chemokines can influence cell proliferation and the census of ion transporters varies in less vs more differentiated epithelial cells, with a predominance of secretory transporters in the former cells. Many inflammatory cytokines also impact tight junction integrity, which indirectly alters ion transport. In ulcerative colitis, the expression of ENaC is reduced, perhaps secondary to an effect of tumor necrosis factor (TNF)-α.[74] Inflammatory mediators present in Crohn's disease also decrease ENaC expression.[75] Furthermore, mice lacking the interleukin 8 receptor, C-X-C motif chemokine receptor 2 (CXCR2), are prone to severe diarrhea in the setting of infection with *Citrobacter rodentium*. This finding has been attributed to the role that CXCR2 plays in neutrophil recruitment; when CXCR2 signaling is absent, the numbers of luminal bacteria increase owing to impaired host defense. There is an accompanying down-regulation of CFTR and DRA, and an exacerbation of infection-mediated diarrhea.[76] On the other hand, the ability of *Salmonella* to induce down-regulation of DRA as well as diarrhea in mice was independent of a neutrophilic infiltrate because these responses were intact in animals lacking CXCR2.[44] The exact mechanisms of DRA regulation are not fully understood. Nevertheless, it is of interest that TNF-α−overexpressing (TNF+/Δ AU-rich elements (ARE)) transgenic mice with high levels of interleukin 1β and interferon-γ in both the ileum and colon showed decreased expression of DRA.[77] The effect of TNF-α on DRA also has been investigated in human intestinal epithelial cells. In these studies, TNF-α activated nuclear factor-κB, which in turn reduced expression of DRA secondary to direct binding of p65 to the DRA promoter.[78] This has been advanced as a contributing mechanism in inflammatory bowel disease–associated diarrhea, although the relevance of this mechanism in infectious diarrhea has yet to be studied.

Taken together, these findings therefore illustrate the complex cross-talk that may occur between infection, inflammation, and epithelial function. They likewise underscore the fact that inflammation may not always be associated with triggering a diarrheal response in the setting of infection, but rather may be protective in certain circumstances.

Implications for Treatment of Diarrhea

The discussion thus far implies that efforts to reverse infection-associated changes in transporter expression and/or function may offer new and more specific approaches to much-needed therapies for infectious diarrhea, particularly because conventional antidiarrheal agents that impair intestinal motility may be counterindicated and/or may have side effects. We discuss how this applies to various existing treatments as well as modalities that could be repurposed for therapies or are currently under development (Table 2).

Oral Rehydration Therapy

The use of prepackaged mixtures of glucose and salt that can be dissolved and delivered orally to patients with severe, dehydrating diarrhea was one of the simplest but most impactful clinical advances of the past century, and has doubtless saved countless lives in developing countries where provisions for intravenous rehydration are absent. ORT drives water reabsorption in diseases such as cholera by taking advantage of the fact that although the electroneutral NaCl absorptive process is impaired by the disease, the function of SGLT1 is intact and can mediate sodium ion and fluid absorption if glucose is provided. This addresses acute water loss caused by diarrhea, even if it does not combat the root cause of the diarrheal episode (which is often self-limiting). Newer forms of ORT include those that incorporate starch and/or zinc.[79] Starch-based ORT drives Na^+ absorption by providing short-chain fatty acids in the colon,[79] and has been shown to be more effective than conventional ORT. Zinc-based ORT also has been proven to be more effective than conventional ORT, but the mechanism is not fully understood. In vitro studies have suggested that zinc inhibits basolateral K^+ channels,[80,81] which would prevent chloride secretion. The zinc-sensing receptor (ZnR/G-protein coupled receptor 39 (GPR39)) also may be involved in the effect of zinc and is another possible drug target.[82,83] In Caco-2 cells, activation of ZnR/GPR39 increased Cl^- absorption by up-regulating K^+/Cl^- cotransporter (KCC1) activity.[83] Conversely, knockdown of ZnR/GPR39 decreased barrier function in colonic epithelial cells.[82,84]

Ion Transporters as Drug Targets

Agents currently used to combat diarrhea include antimotility agents and probiotics. Antimotility agents are useful for the traveler in combating the most distressing phase of an acute diarrheal attack, but often are contraindicated in severe infectious diarrhea because of potential adverse side effects such as paralytic ileus and ischemic colitis. Furthermore, they have no direct impact on any ion transport defects. There is, on the other hand, high-quality clinical evidence that probiotics are of value in the treatment of acute infectious diarrhea.[85] There are likely several possible mechanisms underlying this beneficial effect. However, based on in vitro and animal studies, certain strains of probiotics have the potential to reduce the incidence of diarrhea via effects on ion transport, including by restoring the expression of transporters down-regulated by infection, such as DRA.[86] *Lactobacillus acidophilus* also up-regulates intestinal NHE3 expression and function.[87,88]

One strategy for antidiarrheal drug discovery is to look for active constituents of traditional remedies. Cocoa-derived flavonoids inhibit chloride secretion by blocking CFTR.[89] Other examples of natural products that block enterotoxin-induced secretory diarrheas are lysophosphatidic acid[90] (egg yolk, cabbage, tomato) and tannins or tannic acid[91] (grape seed, oak, and tea), which inhibit smooth muscle contraction and intestinal chloride secretion.[92] Similarly, 3-acyl-2-aminothiophene, tannic acid, and red wine extract also are Ca^{2+}-activated chloride channel inhibitors derived from natural compounds that potentially could be useful in the treatment of diarrhea.[93]

It also remains attractive to consider that infectious diarrhea might be effectively targeted by small molecules that

Table 2. Examples of Therapeutics Targeting Ion Transporters That Are in Use or in Development for Infectious Diarrhea

Treatment	Transporters	Targeted pathway	Status
Natural products: cocoa-derived flavonoids	CFTR	Flavonoids inhibit chloride secretion by blocking CFTR[89]	Preclinical in vitro study
Lysophosphatidic acid	CFTR, DRA, NHE3	LPA inhibits intestinal chloride secretion,[92] up-regulates DRA expression,[104] and stimulates NHE3 activity[102]	Preclinical in vitro studies as well as in mice with DSS colitis or treated with CT
Red wine and green tea extracts, tannins (gelatin tannate)	CaCC	Red wine and green tea extracts, resveratrol dimer, and tannic acid inhibit CaCC[92,93,119]	Preclinical in vitro studies as well as a neonatal mouse model of rotaviral diarrhea Gelatin tannate in clinical trials
L acidophilus	NHE3 DRA	Up-regulates NHE3[87,88] Up-regulates DRA[86]	Preclinical in vitro studies Preclinical in vitro studies
(R)-Benzopyrimido-pyrrolo-oxazine-dione-27	CFTR	Inhibits CFTR[95]	Preclinical studies in mice treated with CT or ST, and in human enteroids
Thiazolidione, pyrimido-pyrrolo-quinoxalinedione/ benzopyrimido-pyrrolo-oxazinedione, and glycine hydrides	CFTR	CFTR inhibitors[120]	Preclinical in vitro and mouse studies
Crofelemer	CFTR, CaCC	Partial antagonist of CFTR, relatively strong inhibitor of CaCCs[98]	Approved
Clotrimazole	KCNN4	Blocks basolateral K^+ channels, which prevents chloride secretion[100]	Approved for antifungal use but no trials in diarrheal disease
Zinc	KCC1	ZnR activation stimulates chloride absorption[83]	Approved as supplement to ORS
Antisecretory factor, Salovum (AS-Faktor AB, Stockholm)	Undefined	Prevents intestinal fluid secretion induced by CT, ST, TcdA[121]	Shown to be effective against diarrhea in small trials

CaCC, Ca^{2+}-activated chloride channel; DSS, dextran sulfate sodium; KCNN, calcium-activated potassium channel.

act specifically on transporters implicated in the disease. For example, small-molecule modulators of CFTR function could be useful not only in the treatment of cystic fibrosis, but also in secretory diarrhea.[94] (R)-Benzopyrimido-pyrrolo-oxazinedione-27 inhibits CFTR and was shown to be effective in animal models of secretory diarrhea caused by cholera and *E coli* enterotoxins.[95] Thiazolidione, pyrimido-pyrrolo-quinoxalinedione (PPQ)/benzopyrimido-pyrrolo-oxazinedione, and glycine hydrides are CFTR inhibitors that currently are being explored in this context.[96,97] Similarly, although it has not reportedly been used in infectious diarrhea in human beings, the antisecretory agent crofelemer is approved for the treatment of human immunodeficiency virus–induced diarrhea and appears to act by inhibiting chloride secretion via CFTR and CLCA.[98] It also is being evaluated for the treatment of diarrhea occurring in the setting of cancer chemotherapy.[99] The antifungal agent clotrimazole blocks basolateral K^+ channels, which then prevents apical chloride secretion.[100] NKCC1 is a potential target for antidiarrheal agents, but selective agents would be difficult to develop because of the need for the inhibitor to target NKCC1 in the intestines as opposed to NKCC2 present in the kidneys; inhibition of NKCC2 would result in a diuretic action. It also may be a drawback that NKCC1 is expressed basolaterally, and may not be readily accessible to agents delivered orally, because oral administration is attractive to avoid systemic side effects.

Finally, a novel approach would be to activate transport by NHE and/or DRA, which are targeted frequently by diarrheal pathogens, or to increase their abundance. In this regard, the probiotic *L acidophilus* has been shown to increase messenger RNA for DRA in colonic epithelial cell lines by transcriptional activation of its promoter.[86] Similarly, lysophosphatidic acid (LPA) is a small, bioactive glycerophospholipid that regulates intestinal electrolyte transport. It stimulates NHE3 trafficking to the apical membrane and its activity,[101,102] inhibits CFTR-dependent Cl^- secretion,[103] and stimulates Cl^-/OH^- exchange, and both expression of and apical abundance of DRA in Caco-2 cells.[104,105] The actions of LPA are mediated via LPA receptors and it also increases the apical expression of the epidermal growth factor receptor.[106] LPA thereby activates the mitogen-activated protein kinase (MAPK)/ERK-extracellular signal-regulated kinases (MEK-ERK) pathway, RhoA and Rho-associated kinase, and proline-rich tyrosine kinase 2. Knockdown of proline-rich tyrosine kinase 2 blocked activation of NHE3 by LPA. NHE3 activation by LPA is attributable to the LPA5 receptor and was abolished in the absence of NHERF2.[102,107] Similarly, LPA increases DRA promoter activity, which involves the LPA2 receptor and the phosphatidylinositol 3-kinase/AKT serine/threonine kinase 1 (PI3K/AKT) pathway.[104] In total, these results suggest that LPA, or agents that target its receptor, might be useful

as antidiarrheal agents. Indeed, LPA inhibits intestinal fluid accumulation in response to cholera toxin in mice.[103]

Conclusions

In conclusion, numerous enteric pathogens cause diarrhea, at least in part, by specifically targeting the expression, abundance, and/or function of ion transporters. An emerging theme in this area of research is the importance of down-regulated absorptive pathways, such as NHE3 and DRA, in diarrheal pathogenesis, in addition to the classic up-regulation of active chloride secretion that is produced by infections such as cholera. Knowledge about the molecular mechanisms of infectious diarrheal disease is already yielding possible advances in targeted therapies, as well as a rationale to repurpose existing agents. Although there may be challenges in bringing new antidiarrheal drugs to market in light of the predominant incidence of diarrheal diseases in developing countries, the societal value of such agents is clear to address the ongoing burden of diarrheal illness and its long-term sequelae.

References

1. Barrett KE, Keely SJ. Integrative physiology and pathophysiology of intestinal electrolyte transport. In: Johnson LR, ed. Physiology of the Gastrointestinal Tract. Vol. 1 and 2. 4th ed. San Diego: Academic Press, 2006:1931–1951.
2. Tsai PY, Zhang B, He WQ, Zha JM, Odenwald MA, Singh G, Tamura A, Shen L, Sailer A, Yeruva S, Kuo WT, Fu YX, Tsukita S, Turner JR. IL-22 upregulates epithelial claudin-2 to drive diarrhea and enteric pathogen clearance. Cell Host Microbe 2017;21:671–681 e4.
3. Kato A, Romero MF. Regulation of electroneutral NaCl absorption by the small intestine. Ann Rev Physiol 2011; 73:261–281.
4. Wright EM, Loo DD, Hirayama BA. Biology of human sodium glucose transporters. Physiol Rev 2011; 91:733–794.
5. Schweinfest CW, Spyropoulos DD, Henderson KW, Kim JH, Chapman JM, Barone S, Worrell RT, Wang Z, Soleimani M. slc26a3 (dra)-deficient mice display chloride-losing diarrhea, enhanced colonic proliferation, and distinct up-regulation of ion transporters in the colon. J Biol Chem 2006;281:37962–37971.
6. Kunzelmann K, Mall M. Electrolyte transport in the mammalian colon: mechanisms and implications for disease. Physiol Rev 2002;82:245–289.
7. Pauli BU, Abdel-Ghany M, Cheng HC, Gruber AD, Archibald HA, Elble RC. Molecular characteristics and functional diversity of CLCA family members. Clin Exp Pharmacol Physiol 2000;27:901–905.
8. Roussa E, Wittschen P, Wolff NA, Torchalski B, Gruber AD, Thevenod F. Cellular distribution and subcellular localization of mCLCA1/2 in murine gastrointestinal epithelia. J Histochem Cytochem 2010;58:653–668.
9. Ousingsawat J, Mirza M, Tian Y, Roussa E, Schreiber R, Cook DI, Kunzelman K. Rotavirus toxin NSP4 induces diarrhea by activation of TMEM16A and inhibition of Na+ absorption. Pflugers Arch 2011;461:579–589.
10. Ousingsawat J, Martins JR, Schreiber R, Rock JR, Harfe BD, Kunzelmann K. Loss of TMEM16A causes a defect in epithelial Ca2+-dependent chloride transport. J Biol Chem 2009;284:28698–28703.
11. D'Andrea L, Lytle C, Matthews JB, Hofman P, Forbush B 3rd, Madara JL. Na:K:2Cl cotransporter (NKCC) of intestinal epithelial cells. Surface expression in response to cAMP. J Biol Chem 1996;271:28969–28976.
12. Robinson JD, Flashner MS. The (Na^+ + K^+)-activated ATPase. Enzymatic and transport properties. Biochim Biophys Acta 1979;549:145–176.
13. Camilleri M, Sellin JH, Barrett KE. Pathophysiology, evaluation, and management of chronic watery diarrhea. Gastroenterology 2017;152:515–532 e2.
14. Guttman JA, Finlay BB. Tight junctions as targets of infectious agents. Biochim Biophys Acta 2009; 1788:832–841.
15. Turner JR. Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application. Am J Pathol 2006;169:1901–1909.
16. Clarke LL, Harline MC. CFTR is required for cAMP inhibition of intestinal Na^+ absorption in a cystic fibrosis mouse model. Am J Physiol 1996;270:G259–G267.
17. Marchelletta RR, Gareau MG, McCole DF, Okamoto S, Roel E, Klinkenberg R, Guiney DG, Fierer J, Barrett KE. Altered expression and localization of ion transporters contribute to diarrhea in mice with Salmonella-induced enteritis. Gastroenterology 2013;145:1358–1368.
18. Hecht G, Koutsouris A, Enteropathogenic E. coli attenuates secretagogue-induced net intestinal ion transport but not Cl^- secretion. Am J Physiol 1999; 276:G781–G788.
19. Gill RK, Borthakur A, Hodges K, Turner JR, Clayburgh DR, Saksena S, Zaheer A, Ramaswamy K, Hecht GA, Dudeja PK. Mechanism underlying inhibition of intestinal apical Cl/OH exchange following infection with enteropathogenic E. coli. J Clin Invest 2007; 117:428–437.
20. Singh V, Yang J, Chen TE, Zachos NC, Kovbasnjuk O, Verkman AS, Donowitz M. Translating molecular physiology of intestinal transport into pharmacologic treatment of diarrhea: stimulation of Na^+ absorption. Clin Gastroenterol Hepatol 2014;12:27–31.
21. Gujral T, Kumar A, Priyamvada S, Saksena S, Gill RK, Hodges K, Alrefai WA, Hecht GA, Dudeja PK. Mechanisms of DRA recycling in intestinal epithelial cells: effect of enteropathogenic E. coli. Am J Physiol Cell Physiol 2015;309:C835–C846.
22. Michail SK, Halm DR, Abernathy F. Enteropathogenic Escherichia coli: stimulating neutrophil migration across a cultured intestinal epithelium without altering transepithelial conductance. J Pediatr Gastroenterol Nutr 2003;36:253–260.
23. Spitz J, Yuhan R, Koutsouris A, Blatt C, Alverdy J, Hecht G. Enteropathogenic Escherichia coli adherence to intestinal epithelial monolayers diminishes barrier function. Am J Physiol 1995;268:G374–G379.

24. Gawenis LR, Stien X, Shull GE, Schultheis PJ, Woo AL, Walker NM, Clarke LL. Intestinal NaCl transport in NHE2 and NHE3 knockout mice. Am J Physiol Gastrointest Liver Physiol 2002;282:G776–G784.
25. Hecht G, Hodges K, Gill RK, Kear F, Tyagi S, Malakooti J, Ramaswamy K, Dudeja PK. Differential regulation of Na^+/H^+ exchange isoform activities by enteropathogenic E. coli in human intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol 2004;287:G370–G378.
26. Hodges K, Gill R, Ramaswamy K, Dudeja PK, Hecht G. Rapid activation of Na^+/H^+ exchange by EPEC is PKC mediated. Am J Physiol Gastrointest Liver Physiol 2006; 291:G959–G968.
27. Cha B, Donowitz M. The epithelial brush border Na^+/H^+ exchanger NHE3 associates with the actin cytoskeleton by binding to ezrin directly and via PDZ domain-containing Na^+/H^+ exchanger regulatory factor (NHERF) proteins. Clin Exp Pharmacol Physiol 2008; 35:863–871.
28. Hodges K, Alto NM, Ramaswamy K, Dudeja PK, Hecht G. The enteropathogenic Escherichia coli effector protein EspF decreases sodium hydrogen exchanger 3 activity. Cell Microbiol 2008;10:1735–1745.
29. Viswanathan VK, Hodges K, Hecht G. Enteric infection meets intestinal function: how bacterial pathogens cause diarrhoea. Nat Rev Microbiol 2009;7:110–119.
30. Dean P, Maresca M, Schuller S, Phillips AD, Kenny B. Potent diarrheagenic mechanism mediated by the cooperative action of three enteropathogenic Escherichia coli-injected effector proteins. Proc Natl Acad Sci U S A 2006;103:1876–1881.
31. Kotloff KL. The burden and etiology of diarrheal illness in developing countries. Pediatr Clin North Am 2017; 64:799–814.
32. Golin-Bisello F, Bradbury N, Ameen N. STa and cGMP stimulate CFTR translocation to the surface of villus enterocytes in rat jejunum and is regulated by protein kinase G. Am J Physiol Cell Physiol 2005; 289:C708–C716.
33. Chen T, Kocinsky HS, Cha B, Murtazina R, Yang J, Tse CM, Singh V, Cole R, Aronson PS, de Jonge H, Sarker R, Donowitz M. Cyclic GMP kinase II (cGKII) inhibits NHE3 by altering its trafficking and phosphorylating NHE3 at three required sites: identification of a multifunctional phosphorylation site. J Biol Chem 2015; 290:1952–1965.
34. Hodges K, Gill R. Infectious diarrhea: cellular and molecular mechanisms. Gut Microbes 2010;1:4–21.
35. Vaandrager AB. Structure and function of the heat-stable enterotoxin receptor/guanylyl cyclase C. Mol Cell Biochem 2002;230:73–83.
36. Sellers ZM, Mann E, Smith A, Ko KH, Giannella R, Cohen MB, Barrett KE, Dong H. Heat-stable enterotoxin of Escherichia coli (STa) can stimulate duodenal HCO_3^- secretion via a novel GC-C- and CFTR-independent pathway. FASEB J 2008;22:1306–1316.
37. Roxas JL, Koutsouris A, Bellmeyer A, Tesfay S, Royan S, Falzari K, Harris A, Cheng H, Rhee KJ, Hecht GA. Enterohemorrhagic E. coli alters murine intestinal epithelial tight junction protein expression and barrier function in a Shiga toxin independent manner. Lab Invest 2010;90:1152–1168.
38. Philpott DJ, McKay DM, Mak W, Perdue MH, Sherman PM. Signal transduction pathways involved in enterohemorrhagic Escherichia coli-induced alterations in T84 epithelial permeability. Infect Immun 1998; 66:1680–1687.
39. Hurley BP, Thorpe CM, Acheson DW. Shiga toxin translocation across intestinal epithelial cells is enhanced by neutrophil transmigration. Infect Immun 2001; 69:6148–6155.
40. Laiko M, Murtazina R, Malyukova I, Zhu C, Boedeker EC, Gutsal O, O'Malley R, Cole RN, Tarr PI, Murray KF, Kane A, Donowitz M, Kovbasnjuk O. Shiga toxin 1 interaction with enterocytes causes apical protein mistargeting through the depletion of intracellular galectin-3. Exp Cell Res 2010;316:657–666.
41. Wadamori Y, Gooneratne R, Hussain MA. Outbreaks and factors influencing microbiological contamination of fresh produce. J Sci Food Aric 2017; 97:1396–1403.
42. Santos RL. Pathobiology of salmonella, intestinal microbiota, and the host innate immune response. Front Immunol 2014;5:252.
43. Resta-Lenert S, Barrett KE. Enteroinvasive bacteria alter barrier and transport properties of human intestinal epithelium: role of iNOS and COX-2. Gastroenterology 2002;122:1070–1087.
44. Marchelletta RR, Gareau MG, Okamoto S, Guiney DG, Barrett KE, Fierer J. Salmonella-induced diarrhea occurs in the absence of IL-8 receptor (CXCR2)-dependent neutrophilic inflammation. J Infect Dis 2015; 212:128–136.
45. Sansonetti PJ, Phalipon A, Arondel J, Thirumalai K, Banerjee S, Akira S, et al. Caspase-1 activation of IL-1beta and IL-18 are essential for Shigella flexneri-induced inflammation. Immunity 2000;12:581–590.
46. Kotloff KL, Riddle MS, Platts-Mills JA, Pavlinac P, Zaidi AKM. Shigellosis. Lancet 2018;391:801–812.
47. Rout WR, Formal SB, Giannella RA, Dammin GJ. Pathophysiology of Shigella diarrhea in the rhesus monkey: intestinal transport, morphological, and bacteriological studies. Gastroenterology 1975; 68:270–278.
48. Creydt VP, Miyakawa MF, Martin F, Zotta E, Silberstein C, Ibarra C. The Shiga toxin 2 B subunit inhibits net fluid absorption in human colon and elicits fluid accumulation in rat colon loops. Braz J Med Biol Res 2004;37:799–808.
49. Muanprasat C, Chatsudthipong V. Cholera: pathophysiology and emerging therapeutic targets. Future Med Chem 2013;5:781–798.
50. Subramanya SB, Rajendran VM, Srinivasan P, Nanda Kumar NS, Ramakrishna BS, Binder HJ. Differential regulation of cholera toxin-inhibited Na-H exchange isoforms by butyrate in rat ileum. Am J Physiol Gastrointest Liver Physiol 2007;293:G857–G863.
51. Murtazina R, Kovbasnjuk O, Chen TE, Zachos NC, Chen Y, Kocinsky HS, Hogema BM, Seidler U, de Jonge HR, Donowitz M. NHERF2 is necessary for basal

activity, second messenger inhibition, and LPA stimulation of NHE3 in mouse distal ileum. Am J Physiol Cell Physiol 2011;301:C126–C136.
52. Alam NH, Ashraf H. Treatment of infectious diarrhea in children. Paediatr Drugs 2003;5:151–165.
53. Takahashi A, Sato Y, Shiomi Y, Cantarelli VV, Iida T, Lee M, Honda T. Mechanisms of chloride secretion induced by thermostable direct haemolysin of Vibrio parahaemolyticus in human colonic tissue and a human intestinal epithelial cell line. J Med Microbiol 2000; 49:801–810.
54. Kyne L, Hamel MB, Polavaram R, Kelly CP. Health care costs and mortality associated with nosocomial diarrhea due to Clostridium difficile. Clin Infect Dis 2002; 34:346–353.
55. Kuehne SA, Cartman ST, Heap JT, Kelly ML, Cockayne A, Minton NP. The role of toxin A and toxin B in Clostridium difficile infection. Nature 2010; 467:711–713.
56. Pothoulakis C, Lamont JT. Microbes and microbial toxins: paradigms for microbial-mucosal interactions II. The integrated response of the intestine to Clostridium difficile toxins. Am J Physiol Gastrointest Liver Physiol 2001;280:G178–G183.
57. Hayashi H, Szaszi K, Coady-Osberg N, Furuya W, Bretscher AP, Orlowski J, Grinstein S. Inhibition and redistribution of NHE3, the apical Na^+/H^+ exchanger, by Clostridium difficile toxin B. J Gen Physiol 2004; 123:491–504.
58. Engevik MA, Engevik KA, Yacyshyn MB, Wang J, Hassett DJ, Darien B, Yacyshyn BR, Worrell RT. Human Clostridium difficile infection: inhibition of NHE3 and microbiota profile. Am J Physiol Gastrointest Liver Physiol 2015;308:G497–G509.
59. Kufel WD, Devanathan AS, Marx AH, Weber DJ, Daniels LM. Bezlotoxumab: a novel agent for the prevention of recurrent Clostridium difficile infection. Pharmacotherapy 2017;37:1298–1308.
60. Lanata CF, Fischer-Walker CL, Olascoaga AC, Torres CX, Aryee MJ, Black RE. Child Health Epidemiology Reference Group of the World Health Organization and UNICEF. Global causes of diarrheal disease mortality in children <5 years of age: a systematic review. PLoS One 2013;8:e72788.
61. Halaihel N, Lievin V, Ball JM, Estes MK, Alvarado F, Vasseur M. Direct inhibitory effect of rotavirus NSP4(114-135) peptide on the Na^+-D-glucose symporter of rabbit intestinal brush border membrane. J Virol 2000; 74:9464–9470.
62. del Castillo JR, Ludert JE, Sanchez A, Ruiz MC, Michelangeli F, Liprandi F. Rotavirus infection alters Na^+ and K^+ homeostasis in MA-104 cells. J Gen Virol 1991; 72:541–547.
63. Buret AG. Pathophysiology of enteric infections with Giardia duodenalius. Parasite 2008;15:261–265.
64. Troeger H, Epple HJ, Schneider T, Wahnschaffe U, Ullrich R, Burchard GD, Jelinek T, Zeitz M, Fromm M, Schulzke JD. Effect of chronic Giardia lamblia infection on epithelial transport and barrier function in human duodenum. Gut 2007;56:328–335.
65. Gorowara S, Ganguly NK, Mahajan RC, Walia BN. Study on the mechanism of Giardia lamblia induced diarrhoea in mice. Biochim Biophys Acta 1992;1138:122–126.
66. Leroy A, Lauwaet T, De Bruyne G, Cornelissen M, Mareel M. Entamoeba histolytica disturbs the tight junction complex in human enteric T84 cell layers. FASEB J 2000;14:1139–1146.
67. McGowan K, Kane A, Asarkof N, Wicks J, Guerina V, Kellum J, Baron S, Gintzler AR, Donowitz M. Entamoeba histolytica causes intestinal secretion: role of serotonin. Science 1983;221:762–764.
68. Dey I, Keller K, Belley A, Chadee K. Identification and characterization of a cyclooxygenase-like enzyme from Entamoeba histolytica. Proc Natl Acad Sci U S A 2003; 100:13561–13566.
69. Zhang XT, Gong AY, Wang Y, Chen X, Lim SS, Dolata CE, Chen XM. Cryptosporidium parvum infection attenuates the ex vivo propagation of murine intestinal enteroids. Physiol Rep 2016;4:24.
70. Laurent F, Kagnoff MF, Savidge TC, Naciri M, Eckmann L. Human intestinal epithelial cells respond to Cryptosporidium parvum infection with increased prostaglandin H synthase 2 expression and prostaglandin E2 and F2alpha production. Infect Immun 1998;66:1787–1790.
71. Kapel N, Huneau JF, Magne D, Tome D, Gobert JG. Cryptosporidiosis-induced impairment of ion transport and Na^+-glucose absorption in adult immunocompromised mice. J Infect Dis 1997;176:834–837.
72. Yang H, Jiang W, Furth EE, Wen X, Katz JP, Sellon RK, Silberg DG, Antalis TM, Schweinfest CW, Wu GD. Intestinal inflammation reduces expression of DRA, a transporter responsible for congenital chloride diarrhea. Am J Physiol 1998;275:G1445–G1453.
73. Sugi K, Musch MW, Field M, Chang EB. Inhibition of Na^+, K^+-ATPase by interferon gamma down-regulates intestinal epithelial transport and barrier function. Gastroenterology 2001;120:1393–1403.
74. Greig E, Sandle GI. Diarrhea in ulcerative colitis. The role of altered colonic sodium transport. Ann N Y Acad Sci 2000;915:327–332.
75. Zeissig S, Bergann T, Fromm A, Bojarski C, Heller F, Guenther U, Zeitz M, Fromm M, Schulzke JD. Altered ENaC expression leads to impaired sodium absorption in the noninflamed intestine in Crohn's disease. Gastroenterology 2008;134:1436–1447.
76. Spehlmann ME, Dann SM, Hruz P, Hanson E, McCole DF, Eckmann L. CXCR2-dependent mucosal neutrophil influx protects against colitis-associated diarrhea caused by an attaching/effacing lesion-forming bacterial pathogen. J Immunol 2009;183:3332–3343.
77. Xiao F, Juric M, Li J, Riederer B, Yeruva S, Singh AK, Zheng L, Glage S, Kollas G, Dudeja P, Tian DA, Zhu J, Bachmann O, Seidler U. Loss of downregulated in adenoma (DRA) impairs mucosal HCO_3^- secretion in murine ileocolonic inflammation. Inflamm Bowel Dis 2012; 18:101–111.
78. Kumar A, Chatterjee I, Gujral T, Alakkam A, Coffing H, Anbazhagan AN, Borthakur A, Saksena S, Gill RK, Alrefai WA, Dudeja PK. Activation of nuclear factor-kappaB by tumor necrosis factor in intestinal epithelial

cells and mouse intestinal epithelia reduces expression of the chloride transporter SLC26A3. Gastroenterology 2017;153:1338–1350 e3.
79. Binder HJ, Brown I, Ramakrishna BS, Young GP. Oral rehydration therapy in the second decade of the twenty-first century. Curr Gastroenterol Rep 2014;16:376.
80. Hoque KM, Rajendran VM, Binder HJ. Zinc inhibits cAMP-stimulated Cl secretion via basolateral K-channel blockade in rat ileum. Am J Physiol Gastrointest Liver Physiol 2005;288:G956–G963.
81. Hoque KM, Sarker R, Guggino SE, Tse CM. A new insight into pathophysiological mechanisms of zinc in diarrhea. Ann N Y Acad Sci 2009;1165:279–284.
82. Cohen L, Sekler I, Hershfinkel M. The zinc sensing receptor, ZnR/GPR39, controls proliferation and differentiation of colonocytes and thereby tight junction formation in the colon. Cell Death Dis 2014;5:e1307.
83. Sunuwar L, Asraf H, Donowitz M, Sekler I, Hershfinkel M. The Zn^{2+}-sensing receptor, ZnR/GPR39, upregulates colonocytic Cl(-) absorption, via basolateral KCC1, and reduces fluid loss. Biochim Biophys Acta 2017; 1863:947–960.
84. Sunuwar L, Gilad D, Hershfinkel M. The zinc sensing receptor, ZnR/GPR39, in health and disease. Front Biosci (Landmark Ed) 2017;22:1469–1492.
85. Guandalini S. Probiotics for prevention and treatment of diarrhea. J Clin Gastroenterol 2011;45:S149–S153.
86. Raheja G, Singh V, Ma K, Boumendjel R, Borthakur A, Gill RK, Saksena S, Alrefai WA, Ramaswamy K, Dudeja PK. Lactobacillus acidophilus stimulates the expression of SLC26A3 via a transcriptional mechanism. Am J Physiol Gastrointest Liver Physiol 2010; 298:G395–G401.
87. Kumar A, Anbazhagan AN, Coffing H, Chatterjee I, Priyamvada S, Gujral T, Saksena S, Gill RK, Alrefair WA, Borthakur A, Dudeja PK. Lactobacillus acidophilus counteracts inhibition of NHE3 and DRA expression and alleviates diarrheal phenotype in mice infected with Citrobacter rodentium. Am J Physiol Gastrointest Liver Physiol 2016;311:G817–G826.
88. Singh V, Raheja G, Borthakur A, Kumar A, Gill RK, Alakkam A, Malakooti J, Dudeja PK. Lactobacillus acidophilus upregulates intestinal NHE3 expression and function. Am J Physiol Gastrointest Liver Physiol 2012; 303:G1393–G1401.
89. Schuier M, Sies H, Illek B, Fischer H. Cocoa-related flavonoids inhibit CFTR-mediated chloride transport across T84 human colon epithelia. J Nutr 2005;135:2320–2325.
90. Tanaka T, Kassai A, Ohmoto M, Morito K, Kashiwada Y, Takaishi Y, Urikura M, Morishige J, Satouchi K, Tokamura A. Quantification of phosphatidic acid in foodstuffs using a thin-layer-chromatography-imaging technique. J Agric Food Chem 2012;60:4156–4161.
91. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. Crit Rev Food Sci Nutr 1998; 38:421–464.
92. Namkung W, Thiagarajah JR, Phuan PW, Verkman AS. Inhibition of $Ca2^{+}$-activated Cl^{-} channels by gallotannins as a possible molecular basis for health benefits of red wine and green tea. FASEB J 2010;24:4178–4186.
93. Ko EA, Jin BJ, Namkung W, Ma T, Thiagarajah JR, Verkman AS. Chloride channel inhibition by a red wine extract and a synthetic small molecule prevents rotaviral secretory diarrhoea in neonatal mice. Gut 2014; 63:1120–1129.
94. Verkman AS, Lukacs GL, Galietta LJ. CFTR chloride channel drug discovery–inhibitors as antidiarrheals and activators for therapy of cystic fibrosis. Curr Pharm Des 2006;12:2235–2247.
95. Cil O, Phuan PW, Gillespie AM, Lee S, Tradtrantip L, Yin J, Tse M, Zachos NC, Lin R, Donowitz M, Verkman AS. Benzopyrimido-pyrrolo-oxazine-dione CFTR inhibitor (R)-BPO-27 for antisecretory therapy of diarrheas caused by bacterial enterotoxins. FASEB J 2017;31:751–760.
96. Mayol JM, Arbeo-Escolar A, Alarma-Estrany P, Adame-Navarrete Y, Fernandez-Represa JA. Progesterone inhibits chloride transport in human intestinal epithelial cells. World J Surg 2002;26:652–656.
97. Thiagarajah JR, Broadbent T, Hsieh E, Verkman AS. Prevention of toxin-induced intestinal ion and fluid secretion by a small-molecule CFTR inhibitor. Gastroenterology 2004;126:511–519.
98. Tradtrantip L, Namkung W, Verkman AS. Crofelemer, an antisecretory antidiarrheal proanthocyanidin oligomer extracted from Croton lechleri, targets two distinct intestinal chloride channels. Mol Pharmacol 2010; 77:69–78.
99. Gao JJ, Tan M, Pohlmann PR, Swain SM. HALT-D: a phase II evaluation of crofelemer for the prevention and prophylaxis of diarrhea in patients with breast cancer on pertuzumab-based regimens. Clin Breast Cancer 2017; 17:76–78.
100. Rufo PA, Merlin D, Riegler M, Ferguson-Maltzman MH, Dickinson BL, Brugnara C, Alper SL, Lencer WI. The antifungal antibiotic, clotrimazole, inhibits chloride secretion by human intestinal T84 cells via blockade of distinct basolateral K^{+} conductances. Demonstration of efficacy in intact rabbit colon and in an in vivo mouse model of cholera. J Clin Invest 1997; 100:3111–3120.
101. Lee-Kwon W, Kawano K, Choi JW, Kim JH, Donowitz M. Lysophosphatidic acid stimulates brush border Na^{+}/H^{+} exchanger 3 (NHE3) activity by increasing its exocytosis by an NHE3 kinase a regulatory protein-dependent mechanism. J Biol Chem 2003;278:16494–16501.
102. Lin S, Yeruva S, He P, Singh AK, Zhang H, Chen M, Lamprecht G, de Jonge HR, Tse M, Donowitz M, Hogema BM, Chun J, Seidler U, Yun CC. Lysophosphatidic acid stimulates the intestinal brush border Na^{+}/H^{+} exchanger 3 and fluid absorption via LPA(5) and NHERF2. Gastroenterology 2010;138:649–658.
103. Li C, Dandridge KS, Di A, Marrs KL, Harris EL, Roy K, Jackson JS, Makarova NV, Fujiwara Y, Farrar PL, Nelson DJ, Tigyi GJ, Naren AP. Lysophosphatidic acid inhibits cholera toxin-induced secretory diarrhea through CFTR-dependent protein interactions. J Exp Med 2005; 202:975–986.
104. Singla A, Kumar A, Priyamvada S, Tahniyath M, Saksena S, Gill RK, Alrefai WA, Dudeja PK. LPA

stimulates intestinal DRA gene transcription via LPA2 receptor, PI3K/AKT, and c-Fos-dependent pathway. Am J Physiol Gastrointest Liver Physiol 2012; 302:G618–G627.
105. Singla A, Dwivedi A, Saksena S, Gill RK, Alrefai WA, Ramaswamy K, Dudeja PK. Mechanisms of lysophosphatidic acid (LPA) mediated stimulation of intestinal apical Cl^-/OH^- exchange. Am J Physiol Gastrointest Liver Physiol 2010;298:G182–G189.
106. Yoo BK, He P, Lee SJ, Yun CC. Lysophosphatidic acid 5 receptor induces activation of Na^+/H^+ exchanger 3 via apical epidermal growth factor receptor in intestinal epithelial cells. Am J Physiol Cell Physiol 2011; 301:C1008–C1016.
107. Lin R, Murtazina R, Cha B, Chakraborty M, Sarker R, Chen TE, Lin Z, Hogema BM, de Jong HR, Seidler U, Turner JR, Li X, Kovbasnjuk O, Donowitz M. D-glucose acts via sodium/glucose cotransporter 1 to increase NHE3 in mouse jejunal brush border by a Na^+/H^+ exchange regulatory factor 2-dependent process. Gastroenterology 2011;140:560–571.
108. Wood IS, Trayhurn P. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. Br J Nutr 2003;89:3–9.
109. Field M. Intestinal ion transport and the pathophysiology of diarrhea. J Clin Invest 2003;111:931–943.
110. Silberg DG, Wang W, Moseley RH, Traber PG. The down regulated in adenoma (dra) gene encodes an intestine-specific membrane sulfate transport protein. J Biol Chem 1995;270:11897–11902.
111. Jacob P, Rossmann H, Lamprecht G, Kretz A, Neff C, Lin-Wu E, Gregor M, Groneberg DA, Kere J, Seidler U. Down-regulated in adenoma mediates apical Cl^-/HCO_3^- exchange in rabbit, rat, and human duodenum. Gastroenterology 2002;122:709–724.
112. Petri WA Jr, Miller M, Binder HJ, Levine MM, Dillingham R, Guerrant RL. Enteric infections, diarrhea, and their impact on function and development. J Clin Invest 2008;118:1277–1290.
113. Gruber AD, Elble RC, Ji HL, Schreur KD, Fuller CM, Pauli BU. Genomic cloning, molecular characterization, and functional analysis of human CLCA1, the first human member of the family of Ca^{2+}-activated Cl^- channel proteins. Genomics 1998;54:200–214.
114. Jakab RL, Collaco AM, Ameen NA. Physiological relevance of cell-specific distribution patterns of CFTR, NKCC1, NBCe1, and NHE3 along the crypt-villus axis in the intestine. Am J Physiol Gastrointest Liver Physiol 2011;300:G82–G98.
115. Strong TV, Boehm K, Collins FS. Localization of cystic fibrosis transmembrane conductance regulator mRNA in the human gastrointestinal tract by in situ hybridization. J Clin Invest 1994;93:347–354.
116. Lejeune M, Moreau F, Chadee K. Prostaglandin E2 produced by Entamoeba histolytica signals via EP4 receptor and alters claudin-4 to increase ion permeability of tight junctions. Am J Pathol 2011;179:807–818.
117. Troeger H, Loddenkemper C, Schneider T, Schreier E, Epple HJ, Zeitz M, Fromm M, Schulzke JD. Structural and functional changes of the duodenum in human norovirus infection. Gut 2009;58:1070–1077.
118. Gunzel D, Kucharski LM, Kehres DG, Romero MF, Maguire ME. The MgtC virulence factor of Salmonella enterica serovar Typhimurium activates Na^+, K^+-ATPase. J Bacteriol 2006;188:5586–5594.
119. Yu B, Jiang Y, Zhang B, Yang H, Ma T. Resveratrol dimer trans-ε-viniferin prevents rotaviral diarrhea in mice by inhibition of the intestinal calcium-activated chloride channel. Pharmacol Res 2018;129:453–461.
120. Verkman AS, Synder D, Tradtrantip L, Thiagarajah JR, Anderson MO. CFTR inhibitors. Curr Pharm Design 2013; 19:3529–3541.
121. Lange S, Lonnroth I. The antisecretory factor: synthesis, anatomical and cellular distribution, and biological action in experimental and clinical studies. Int Rev Cytol 2001; 210:39–75.

Received January 2, 2018. Accepted February 26, 2018.

Correspondence
Address correspondence to: Kim E. Barrett, Department of Medicine, University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093-0063. e-mail: kbarrett@ucsd.edu; fax: (858) 246-1788.

Author contributions
All authors participated in drafting the manuscript as well as critically reviewing the manuscript for important intellectual content.

Conflicts of interest
The authors disclose no conflicts.