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REVIEW

The Role of Ion Transporters in the Pathophysiology of Infectious Diarrhea

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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 anti-diarrheal 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-stable toxin; Tcd, *Clostridium difficile* toxin; TNF, tumor necrosis factor; ZnR, zinc sensing receptor.

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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 $\text{Cl}^-/\text{HCO}_3^-$ 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 channels⁷: 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} <i>C difficile</i> TcdB decreases NHE3 activity ^{57,58} Function decreased by EPEC infection ^{29,30} Rotavirus decreases SGLT1 ^{34,61}
	SGLT1	Apical membrane of small intestinal villi ¹⁰⁸	Decreased by <i>Salmonella</i> infection in mice ¹⁷
	ENaC	Apical membrane of surface cells in distal colon	Decreased by EPEC, <i>C rodentium</i> , and <i>Salmonella</i> infection ^{19,21,29,34,112}
	DRA	Apical membrane of small intestinal villous cells and surface cells in colon ¹⁰⁹⁻¹¹¹	
Secretion	CaCC	For CLCA1 in human beings, apical membrane of small intestinal and colonic crypt epithelial cells (and goblet cells) ¹¹³	Possibly stimulated by <i>E histolytica</i> , <i>G lamblia</i> , cholera (ACE toxin), <i>V parahaemolyticus</i> , 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 <i>E coli</i> and <i>Salmonella Dublin</i> 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 <i>E coli</i> , or <i>Salmonella Dublin</i> ; the latter also may increase expression in cell lines ^{8,32} Redistributed into epithelial cytosol without a change in expression after <i>Salmonella</i> infection in mice ¹⁷ <i>E histolytica</i> 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	<i>Salmonella</i> infection in mice accompanied by redistribution from basolateral to apical membrane ¹⁷ Activated by the magnesium transporter C (MgtC) virulence factor of <i>Salmonella</i> ¹¹⁸

ACE, accessory cholera enterotoxin; CaCC, Ca²⁺-activated chloride channel; CLCA1, chloride channel accessory 1; EHEC, enterohemorrhagic *E coli*; Stx2, Shiga toxin 2.

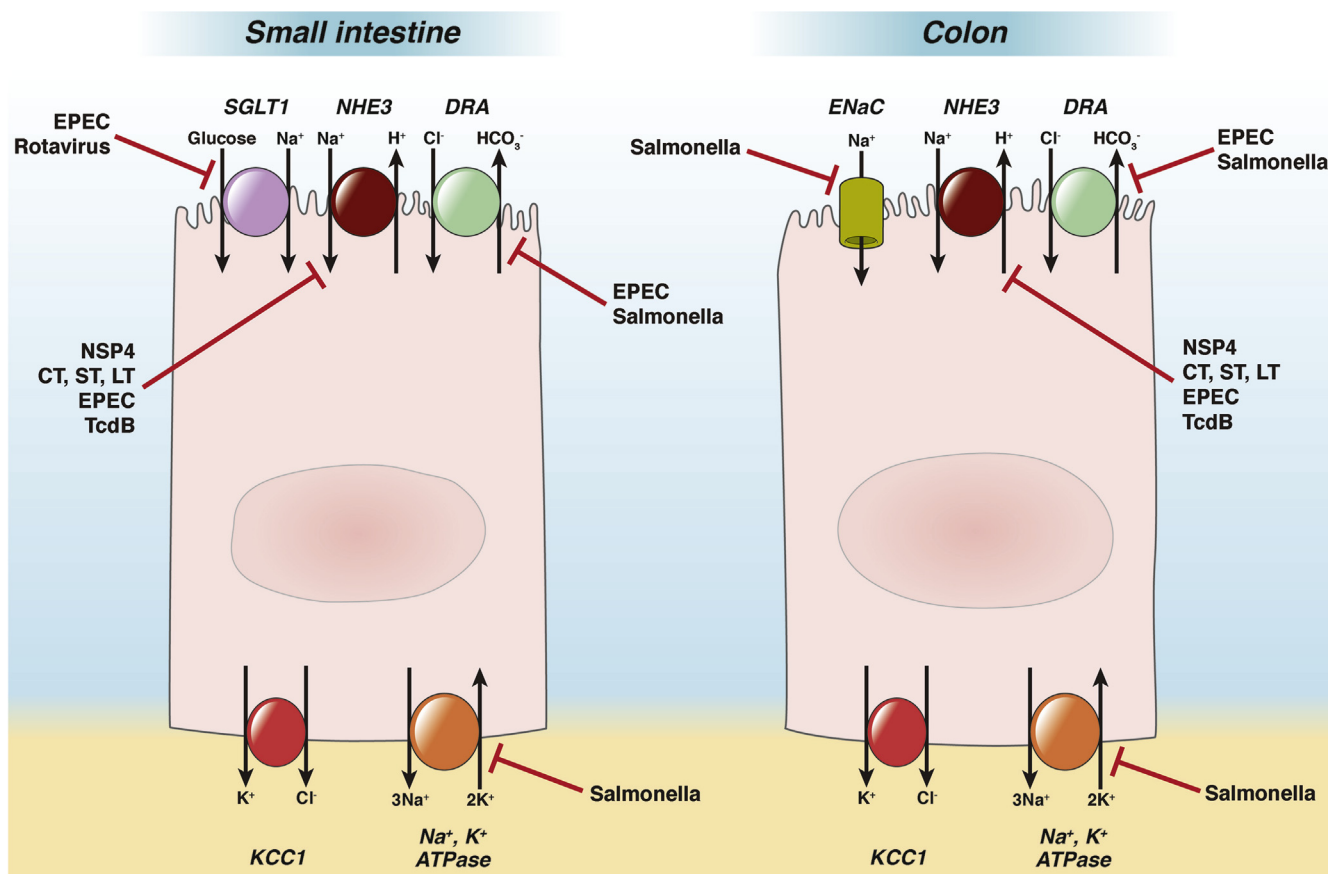


Figure 1. Localization of absorptive ion transporters (discussed in text) in the small intestine and colon, and their regulation by pathogens or their secreted toxins. The figure is not intended to imply that the illustrated transporters are necessarily expressed in the same cells. Note particularly 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^{17,18} (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

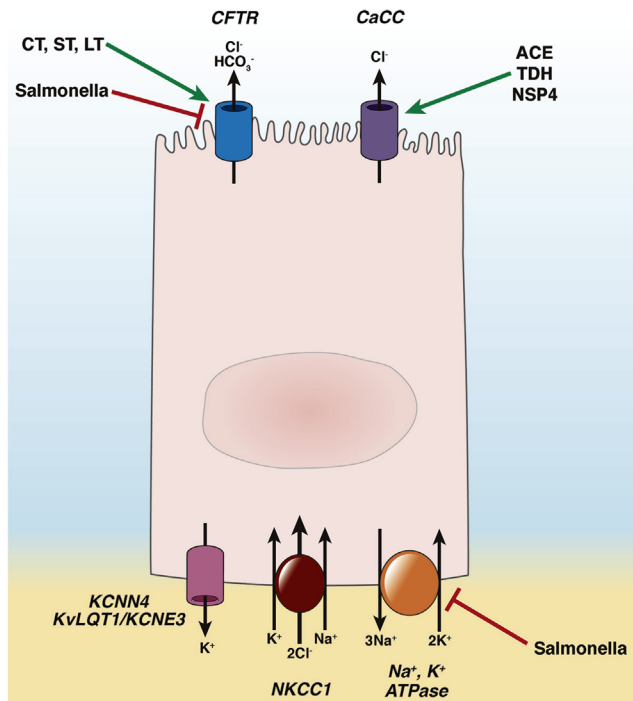


Figure 2. Chloride secretory mechanism in the small intestine and colon, and regulation of its constituent transporters by pathogens or their secreted toxins. The green arrows and red bars represent stimulatory and inhibitory effects, respectively. CaCC, calcium-activated chloride channel; ACE, accessory cholera enterotoxin; TDH, thermolabile 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).

EPEC 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 anti-absorptive.^{34,35} ST also may stimulate HCO₃⁻ secretion via a mechanism independent of CFTR, but with possible involvement of DRA or putative anion transporter 1.³⁶ LT-expressing strains of EPEC, 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 food-borne 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 Ca²⁺-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 Ca²⁺ 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* trophozoites 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 E₂, 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 1 β 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- κ B, 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 electro-neutral 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 acid⁹⁰ (egg yolk, cabbage, tomato) and tannins or tannic acid⁹¹ (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 Ca²⁺-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
<i>L. acidophilus</i>	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, Ca²⁺-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 anti-diarrheal 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.

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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.