Mechanisms Underlying Dysregulation of Electrolyte Absorption in Inflammatory Bowel Disease–Associated Diarrhea

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Abstract: Inflammatory bowel diseases (IBDs), including Crohn’s disease and ulcerative colitis, are chronic relapsing inflammatory disorders of the gastrointestinal tract. Chronic inflammation of the intestine affects the normal fluid and electrolyte absorption leading to diarrhea, the hallmark symptom of IBD. The management of IBD-associated diarrhea still remains to be a challenge, and extensive studies over the last 2 decades have focused on investigating the molecular mechanisms underlying IBD-associated diarrhea. These studies have shown that the predominant mechanism of diarrhea in IBD involves impairment of electroneutral NaCl absorption, with very little role if any played by anion secretion. The electroneutral NaCl absorption involves coupled operation of Na+/H+ exchanger 3 (NHE3 or SLC9A3) and Cl-/HCO3− exchanger DRA (Down Regulated in Adenoma, or SLC26A3). Increasing evidence now supports the critical role of a marked decrease in NHE3 and DRA function and/or expression in IBD-associated diarrhea. This review provides a detailed analysis of the current knowledge related to alterations in NHE3 and DRA function and expression in IBD including the mechanisms underlying these observations and highlights the potential of these transporters as important and novel therapeutic targets.

Keywords: intestinal inflammation, NaCl absorption, NHE3, DRA

The inflammatory bowel diseases (IBDs) are multifactorial complex diseases that include the entities of ulcerative colitis (UC) and Crohn’s disease (CD) and are emerging as a major global health concern. Europe has the highest IBD incidence closely followed by North America1 from 21 to 246 per 100,000 for UC and 8 to 214 per 100,000 for CD.2 The pathogenesis of IBD involves complex interactions between various environmental stimuli, luminal microflora, and hereditary predisposition leading to gut immune dysregulation resulting in chronic mucosal inflammation and diarrhea.3,4 The medical management of IBD is challenging; however, the primary therapeutic goals are still centered on maintaining remission and treating diarrhea. According to recent data, majority of the patients with UC and CD present with diarrhea at the onset of disease.5 However, the severity of this diarrhea varies widely and is linked to the location, extent, and degree of intestinal inflammation and can be used as an index of the disease activity and in determination of appropriate treatment strategies.5

The mechanisms underlying IBD-associated diarrhea include (1) altered expression and/or function of ion transporters and channels; (2) increased paracellular permeability; and (3) increased intestinal transit time (dysmotility) leading to inadequate water and solute absorption.5–10 The retention of electrolytes in the intestinal lumen leads to accumulation of water and initiation of diarrhea. Substantial evidence now supports the notion that dysregulation in electrolyte absorption rather than secretion is the major contributing factor to IBD-associated diarrhea.6,11,12 The decrease in electrolyte absorption has been attributed to impaired function or reduced expression of Na+/H+ exchanger 3 (NHE3 or SLC9A3) and Cl-/HCO3− exchanger DRA (Down Regulated in Adenoma or SLC26A3), the key molecular species involved in electroneutral NaCl absorption in the ileum and colon.5,13

This review attempts to highlight the recent advances in our understanding of the role played by Na+ and Cl− transport dysfunction in the pathogenesis of diarrhea in IBD. Major emphasis will be placed on the molecular mechanisms underlying downregulation of ion transporters involved in electroneutral sodium chloride absorption (NHE3 and DRA). An understanding of the mechanisms of NHE3 and DRA dysregulation is important for identification of novel therapeutic targets for the treatment of IBD-associated diarrhea.
MECHANISMS UNDERLYING DIARRHEA

Fluid Homeostasis in Intestine

Of 8 to 10 L/d of fluid presented to the intestinal lumen (representing sum total of ingested food and biological secretions), the small intestine absorbs the majority of this fluid content and the remaining ~1.5 to 1.9 L of the fluid is absorbed by large intestine. Therefore, under normal conditions, <0.1 to 0.2 L/d of fluid volume is excreted in the stool. It is important to note that although it is the small intestine that absorbs most of this volume, the maximal absorptive capacity of the large intestine (4.5–5 L/d) is enough to compensate for an increased fluid delivery to the colon that may occur due to dysregulated absorption or motility in the small intestine. In IBD, however, the absorptive capacity of the colon is significantly reduced leading to diarrhea.

Mechanism(s) of Electrolyte Transport in Intestine and Diarrhea

The gastrointestinal tract exhibits segmental heterogeneity in the expression pattern of various ion transporters and channels, which work in conjunction and determine the electrolyte content and fluid volume in the lumen. The movement of solutes, particularly sodium and chloride across the intestinal epithelium, sets up an osmotic gradient along which water is absorbed. It follows that the nature and severity of diarrhea seen in IBD depends on which segment of the intestine is inflamed. In the intestine, Na+ and/or Cl− transport occurs through (1) nutrient-coupled Na+ absorption, (2) electroneutral NaCl absorption, (3) electrogenic Cl− secretion by cystic fibrosis transmembrane conductance regulator, and (4) electroneutral Na+ absorption by ENaC. Nutrient-coupled sodium absorption involves the cotransport of sugars (glucose and galactose) and amino acids along with Na+, where the concentration gradient of sodium established by Na+/K+ ATPase facilitates the absorption of solute. Oral rehydration therapy, which is a life-saving treatment in diarrhea, exploits the Na-glucose cotransport mechanism to promote water absorption that occurs osmotically along with solute and salt absorption.

Electroneutral NaCl absorption is the predominant mechanism for Na+, Cl−, and fluid absorption in the ileum and colon of the mammalian gastrointestinal tract. This process occurs through coupled functioning of Na+/H+ and Cl−/HCO3− exchangers and is not associated with the generation of transepithelial current. Recent studies in various animal models of IBD and biopsies from patients with UC and CD report impairment of active sodium and chloride absorption as the prime feature of diarrhea in IBD. Electrogenic sodium absorption is also reduced under inflammatory conditions, secondary to decreased expression of apical sodium channel ENaC (epithelial sodium channel) and basolaterally located cation transporter, Na+/K+ ATPase.

Ion transport mechanisms involved in Cl− secretion include (1) the apical cystic fibrosis transmembrane conductance regulator–type Cl− channel, (2) the basolateral Na+/K+−2Cl− cotransport system (NKCC1), (3) the basolateral K+ channels, and (4) the Na+/K+ ATPase, which energizes Cl− transport. In this regard, results obtained from electrophysiological studies conducted in the colonic mucosa from patients with UC and CD show a lack of electrogenic Cl− secretion in response to secretagogues indicating that Cl− secretory pathway does not play any significant role in IBD-associated diarrhea. The focus of the current review is, therefore, restricted to the alterations in the expression and/or activity of molecular species involved in electroneutral NaCl absorption under inflammatory conditions.
Molecular Basis for Electroneutral Sodium Chloride Absorption in Intestine

Na\(^+\)/H\(^+\) Exchangers

The Na\(^+\)/H\(^+\) exchangers play a central role in Na\(^+\) and water absorption, maintenance of intracellular (cytosolic and organelar) pH, and cell volume. There are 11 NHE isoforms identified in mammals (NHE1-11). In the intestine NHE1 (SLC9A1), NHE2 (SLC9A2), NHE3 (SLC9A3), and NHE8 (SLC9A8) have been shown to be present in the intestinal epithelium.\(^{18}\) NHE1 is located on the basolateral membrane of the intestinal epithelial cells and as such does not contribute to luminal ion and water absorption.\(^{19}\) NHE3 is predominantly expressed during early life. NHE8 knockout (KO) mice do not have diarrhea probably because of compensatory increase in NHE2 and NHE3 in the small intestine.\(^{20}\) NHE3 (SLC9A3) has received the most attention and is believed to be the major transporter for Na\(^+\) absorption in the intestine\(^{13}\) (Fig. 1). This has been proven by studies in NHE3 KO mice, demonstrating that the lack of functional NHE3 transporter manifests with altered intestinal Na\(^+\) and water absorption and a diarrheal phenotype.\(^{21,22}\) NHE2 dysfunction does not cause such changes, and double KO mice lacking both NHE3 and NHE2 do not have a more severe diarrheal phenotype than single NHE3 KO.\(^{23}\) Interestingly, recent evidence alludes to the multiple mechanisms by which NHE3 dysfunction might play a role in IBD-associated diarrhea as discussed in “Mechanisms Underlying IBD-associated Diarrhea”.

Cl\(^-\)/HCO\(_3\)\(^-\) Exchangers

Two members of the SLC26 gene family have been identified as candidate genes representing apical Cl\(^-\)/HCO\(_3\)\(^-\) exchangers in mammalian intestinal epithelial cells namely DRA (SLC26A3) and PAT-1 (putative anion transporter, SLC26A6).\(^{24,25}\) DRA is predominantly expressed in the colon and duodenum, and its expression is less abundant in jejunum and ileum, whereas the expression pattern of PAT-1 is the inverse of DRA, except for the robust expression of both in the duodenum.\(^{26}\) Several lines of evidence suggest that NHE3 couples with DRA (the major luminal intestinal Cl\(^-\)/HCO\(_3\)\(^-\) exchanger) to mediate electroneutral NaCl absorption in the ileum and colon (Fig. 1). The role of DRA in mediating the vectorial Cl\(^-\) absorption is evident from congenital chloride diarrhea, a rare genetic disorder caused by mutations in DRA, characterized by voluminous diarrhea, massive loss of Cl\(^-\) in stool, and metabolic alkalosis.\(^{27,28}\) Similar to congenital chloride diarrhea phenotype, DRA KO mice develop severe diarrhea, metabolic alkalosis, and serum electrolyte imbalance.\(^{29}\) In contrast, PAT-1–deficient mice do not exhibit diarrheal phenotype suggesting that it is less important in mediating bulk intestinal Cl\(^-\) absorption.\(^{24}\) Another study with hepatocyte nuclear factor KO mice exhibiting diarrheal phenotype also showed that expression of ileal and colonic DRA were substantially reduced in mice deficient in either intestinal HNF1\(\alpha\) or HNF1\(\beta\), and DRA was almost abolished in mice deficient in both factors in the intestine. This complete loss of DRA in double KOs correlated with severe diarrhea.\(^{30}\) Borenshtein et al\(^{31}\) using *Citrobacter rodentium*–induced colitis model in mice also reported complete loss of DRA expression associated with severe diarrhea. Taken together, these studies indicate that loss of DRA function results in development of severe diarrheal phenotype.

**MECHANISMS UNDERLYING IBD-ASSOCIATED DIARRHEA**

A substantial body of literature derived from patients with IBD, KO models, mouse models of IBD, and in vitro models of inflammation has provided strong evidence for dysregulation of Na\(^+\) and Cl\(^-\) absorption in intestine mediated through NHE3 and DRA in IBD-associated diarrhea. These findings are discussed below in detail.

**NHE3 in IBD-associated Diarrhea**

**Alterations in NHE3 Function or Expression in Patients with UC**

Studies investigating alterations in NHE3 function and/or expression in patients with UC have indicated that decreased activity of NHE3 in inflamed mucosa may underlie diarrhea associated with UC. However, there are contradictory reports regarding the mechanisms causing the loss of NHE3 function in patients with UC, with some studies showing decreased NHE3 activity despite correct cellular NHE3 localization and unaltered expression, while several others demonstrating reduced gene expression, as presented in Table 1 and outlined below.

A number of studies have shown a decrease in NHE3 expression in human IBD.\(^{32,33}\) In a study by Sullivan et al\(^{33}\) quantitative analysis by Western blot in patients with CD and UC showed a decrease in NHE3 protein in 87% of sigmoid colon biopsies (n = 42) and 100% of ileal biopsies (n = 8). No decrease was found in intestinal alkaline phosphatase in inflamed samples, ruling out confounding effects due to epithelial cell loss secondary to inflammation. Siddique et al\(^{32}\) also found that NHE3 protein and Na\(^+\) pump activity was decreased in UC and CD patient biopsies (n = 13, treated IBD patients and n = 13, untreated IBD patients), whereas NHE3 mRNA was decreased only in CD and not in UC biopsies.

Furthermore, Farkas et al\(^{12}\) using human sigmoid and rectal biopsies from patients with UC (n = 69) showed a decrease in NHE3 activity in the apical and middle region of the colonic crypts. However, a corresponding decrease in NHE3 mRNA by quantitative PCR in the UC biopsies was not observed. Yeruva et al\(^{14}\) also used human UC biopsies (n = 40) with varying severity of inflammation. A significant decrease in Na\(^+\) absorption in moderately inflamed colon was seen, in parallel with a reduction in NHE3 activity. However, this decrease in NHE3 activity was not associated with a decrease in NHE3 gene expression or a change in membrane localization of NHE3 molecules in the crypts of the sigmoid colon biopsies in patients with UC.\(^{34}\)
Lohi et al\(^{35}\) showed that there was no significant difference in NHE3 mRNA levels between inflamed and noninflamed colonic biopsies from patients with UC (n = 10).

From above studies, it seems that the differences in NHE3 function and expression may be partly attributed to segmental variations along the length of the intestine or to differences in disease severity between the various studies. The functional impairment in NHE3, despite normal expression and localization also suggested the potential involvement of other factors in UC-associated diarrhea, such as regulatory proteins, discussed under “Role of NHE3 Regulatory Factors”.

**Alterations in NHE3 Function or Expression in Murine Inflammatory Models**

Yeruva et al\(^{36}\) studied NHE3 activity and mRNA levels in 3 different murine colitis models having differing segmental disease and severity of inflammation (Tnf\(\Delta ARE^{+/−}\), Rag2\(^{−/−}\) CD4\(^{+}\) CD45\(^{RBhigh}\) and DSS-induced IL-10\(^{−/−}\) model). NHE3 mRNA expression was unchanged in the distal ileum of Tnf\(\Delta ARE^{+/−}\) mouse model and the colon of IL-10\(^{−/−}\) mouse model compared with their respective controls and was increased in Rag2\(^{−/−}\) CD4\(^{+}\) CD45\(^{RBhigh}\) colon, but brush border localization of NHE3 was preserved in the mucosa in all 3 models. NHE3 activity was, however, decreased in all 3 murine models. This evidence again points to a functional defect in NHE3, possibly due to alterations in the PDZ domain-containing protein PDZK1 (NHERF3), discussed later in this review. Another study using IL-2 KO mice, which develop spontaneous colitis, showed a reduction in colonic Na\(^{+}\) absorption only partly due to the downregulation of NHE3 protein and mRNA levels.\(^{38}\) It was surprising to note that although net Na\(^{+}\) absorption was reduced by 80%, NHE3 mRNA and protein expression were reduced by only 41% and 24%, respectively.\(^{38}\) This pointed to other factors operating in the pathogenesis of diarrhea such as decreased Cl\(^{−}\) anion exchange (coupled to Na\(^{+}\)), decreased basal Na\(^{+}/\)K\(^{+}\)-ATPase, other defective Na\(^{+}\) transporters, or defects in NHE3 endocytosis. In a study by Sullivan et al\(^{33}\) in dextran sulfate sodium (DSS)- and TNBS-induced colitis in mice, downregulation of NHE3 protein expression was found to occur in the mouse colon. Thus, there is strong evidence to suggest decreased NHE3 transporter activity in the presence of mucosal inflammation.

**Lessons from NHE3 KO Mice**

Studies using KO mice suggest that deficiency of NHE3 may be a critical contributor to dysregulated immunomodulation, loss of barrier function, and dysbiosis observed in IBD. For example, deletion of NHE3 in mice manifests with spontaneous colitis-associated with mild diarrhea, occasional rectal prolapse, and reduced body weight. Histological examination showed crypt hyperplasia, diffuse neutrophilic infiltrate with increase in matrix metalloproteinase 8 expression, a marked decrease in PAS-positive goblet cells and induction of inducible nitric oxide synthase and tumor necrosis factor (TNF)-\(\alpha\). These findings suggested a possible immunomodulatory role of NHE3 and its essential role in the maintenance of epithelial barrier integrity. Another significant observation supporting this theory was the increased bacterial adhesion and translocation found in the distal colon of NHE3\(^{−/−}\) mice and the fact that the colitis was ameliorated by oral administration of antibiotics.\(^{41}\)

Along the same lines, Kiela et al\(^{32}\) showed that NHE3 KO mice, when exposed to even low concentrations of DSS, developed severe colitis resulting in early death secondary to intestinal bleeding, hypovolemic shock, and sepsis. They also showed evidence of small intestinal injury in these mice by microarray analysis with an induction of numerous proinflammatory genes in small intestinal mucosa in response to DSS. This was surprising because DSS is not known to cause inflammation in the small intestine in wild-type mice. Furthermore, treatment of the NHE3 KO mice with broad-spectrum antibiotics resulted in decreased

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**TABLE 1. NHE3 in IBD**

<table>
<thead>
<tr>
<th>Patients with IBD</th>
<th>mRNA</th>
<th>Protein</th>
<th>Function</th>
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<td>⇧ (32,33)</td>
<td>⇧ (17,32,34)</td>
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<td>⇧ (36)</td>
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<td>Rag2(^{−/−}) CD4(^{+}) CD45(^{RBhigh}) (mice)</td>
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⇧ indicates increase, ⇧ indicates decrease, and ⇧ indicates no effect. Number in parenthesis indicate citation number.
cytokine expression in the small intestinal mucosa, suggesting a possible role played by intestinal microbiome in this process. Another study showed that NHE3 KO mice when housed in a conventional facility spontaneously developed distal colitis with mild diarrhea, whereas the KO mice in an ultraclean facility showed improvement in colitis symptoms. This indicates the important role played by altered gut microbiome in colitis.

Further evidence for the immunomodulatory effect of NHE3 downregulation in IBD in addition to its transporter function defect was shown in the following study. Larmonier et al created double KOs of NHE3 and IL-10 in mice and compared the immunohistochemistry and chemokine profile of the colitis that occurred in these mice with either NHE3 or IL-10 single KO mice. Double KO mice displayed a more severe degree of inflammation with a significant increase in neutrophils and mononuclear cell infiltrate possibly due to increased expression of chemokines.

In summary, NHE3 deficiency in IBD seems to contribute to the pathogenesis of diarrhea in multiple ways including decreased Na⁺ absorption, disruption of barrier integrity, immunomodulation, and dysbiosis.

Mechanisms Underlying NHE3 Dysregulation in Experimental Models of Colitis

Role of proinflammatory cytokines. With respect to mechanisms, in vitro cell culture models and experimental models of colitis have provided valuable insights into the possible mediators that decrease NHE3 function and/or expression in IBD. Rocha et al demonstrated a decrease in NHE2 and NHE3 protein and mRNA expression in both human C2/bbe cells and in rat intestine (ileum and colon) after treatment with interferon (IFN)-γ, a cytokine known to be increased in IBD-affected intestine. Another study by our group showed that TNF-α and IFN-γ decreased NHE3 transcription in C2/bbe cells by repressing NHE3 promoter activity through a protein kinase A (PKA)-mediated phosphorylation of Sp1 and Sp3. In addition, there is evidence showing overproduction of nitric oxide (NO), another inflammatory mediator, in patients with IBD and experimental models of colitis. This regard, we have earlier demonstrated that NO decreased NHE3 function by the activation of a soluble guanylate cyclase and protein kinase G (PKG) in intestinal epithelial cells. These studies indicated that the proinflammatory mediators (cytokines and NO) decrease NHE3 function and expression mainly via PKA and PKG pathways, respectively.

Role of NHE regulatory factors. In the context of IBD, recent studies have focused on the role played by NHERF family members in NHE3 dysfunction. To date, 4 NHERF proteins have been identified: NHERF1 (EBP50), NHERF2 (E3KARP), PDZK1 (NHERF3), and IKEPP. Each of these proteins harbors 2 to 4 PDZ domains, and while they are homologous, they each contribute in a unique fashion to regulation of NHE3 or other substrate proteins. This uniqueness partly depends on their differing localization in the intestinal epithelium and different binding partners of these 4 proteins. The NHERF proteins colocalize with NHE3 and serve as scaffolding proteins to help form NHE3 containing signaling complexes, thereby affecting NHE3 function in a signal-dependent and cell-specific manner.

Sullivan et al showed that along with NHE3 protein, NHERF1 mRNA and protein were also downregulated in sigmoid mucosal biopsies in most of the patients of UC (n = 13) and/or CD (n = 8). Similar decrease in NHE3, NHERF1, and NHERF2 protein expression was also demonstrated in DSS- and TNBS-treated mice colon. However, Lenzen et al using IL-10 KO mice showed diminished mRNA and protein of NHERF2 and PDZK1, whereas NHERF1 expression was unchanged.

Yeruva et al found a significant downregulation of PDZK1 mRNA and protein expression in colon biopsies from patients with UC, as well as in inflamed murine ileum and colon. In addition, as described above in “Alterations in NHE3 Function or Expression in Patients with UC” and “Alterations in NHE3 Function or Expression in Murine Inflammatory Models”, NHE3 activity was also decreased in both murine and human biopsies despite correct brush border localization and unaltered protein expression of NHE3. This finding indicates that PDZK1 downregulation might be a contributing factor to the functional defect of NHE3 observed in intestinal inflammation.

Overall, it seems that either decreased NHE3 function and/or expression results in decreased Na⁺ absorption in IBD. The decreased function could be secondary to altered signaling pathways, and/or NHERF expression, which may involve altered localization of NHE3 on enterocyte surface membrane. In addition, altered expression of NHE3 is attributed to the effects of proinflammatory cytokines through PKA, Sp1, and Sp2 transcription factors. Also, the direct effect of NHE3 loss on the disruption of gut barrier integrity and dysbiosis cannot be ruled out.

Altered Cl⁻ Absorption in IBD

As the inflammatory mediators are predominantly prosecretory in nature, increased net chloride secretion (electrogenic) was initially thought to play a major role in the development of diarrhea in IBD. However, recent studies pertaining to dysregulated electrolyte transport in IBD have indicated that decreased sodium and chloride absorption is the major contributor to diarrhea associated with gut inflammation. This was attributed to several important studies obtained from both animal and human models. For example, in vitro flux studies conducted in colonic mucosa and rectum of patients with UC exhibited a significant reduction in net chloride absorption rather than enhanced chloride secretion. Furthermore, transmucosal electrical potential difference was reported to be either significantly low or absent in the mucosa of patients with UC. A decrease in electrical potential difference is consistent with defective chloride absorption. Roediger et al reported that in patients with UC, Cl⁻ uptake by the colon is significantly diminished, which goes well with the elevated levels of colonic and fecal Cl⁻ content. Along the same lines,
Farkas et al. reported significantly lower Cl⁻/HCO₃⁻ exchange activity in the colonic crypts isolated from patients with UC. Collectively, all these studies reflect the notion that impaired Cl⁻ absorption in IBD represents one of the critical events in IBD-associated diarrhea.

**DRA in IBD-associated Diarrhea**

**Alterations in DRA Function or Expression in Patients with IBD**

An early study of Yang et al. provided the first evidence of altered DRA expression in patients with UC. Although limited in size to only 3 specimen pairs of healthy and moderately inflamed UC tissue, a significant reduction in DRA mRNA expression and complete absence of DRA protein expression in the inflamed surface epithelium was reported. In another study by Lohi et al., preoperative colonic samples were taken from 10 patients with UC and 4 control subjects, and the mRNA and protein expression of DRA was examined. Cases of UC with severe disease symptoms and markedly reduced survival. These studies collectively indicate that diminished Cl⁻ absorption in patients with UC as compared with normal individuals or patients with CD and ischemic colitis. This suggests impairment in membrane-targeting event(s) that could reduce DRA surface expression leading to a decrease in Cl⁻ absorption. In this regard, a recent study correlated the alterations in DRA expression with Cl⁻/HCO₃⁻ exchange activity in crypts of 128 healthy individuals and 69 patients with IBD (with active UC and diarrheal symptoms). In this study, a robust reduction in Cl⁻ absorption with a parallel decrease of ~50% in DRA mRNA expression was demonstrated in the surface cells of the colonic crypts in patients with UC. These studies collectively indicate that diminished Cl⁻ absorption in patients with UC can be attributed to decreased DRA expression.

Overall, the patient data clearly pinpoint to the downregulation of DRA function and/or expression as one of the critical features which underlies the pathogenesis of diarrhea associated with UC (Table 2). However, there is still very limited information on abnormalities in DRA function and expression in patients with CD. In addition to the role of DRA in diarrhea, the upcoming evidence now suggests that downregulation of DRA expression may also be involved in the pathogenesis of IBD. For example, a 2-stage genome-wide association studies conducted in Japan, using 1384 patients with UC and 3057 control individuals identified single nucleotide polymorphism in SLC26A3 gene, which was associated with lower DRA expression, constituting a risk factor for the development of UC. However, direct correlation of genetic deficiency in inducing inflammatory diarrhea needs further investigation.

**Alterations in DRA Function or Expression in Mouse Models of IBD**

Studies in different murine inflammatory models have further advanced our current knowledge about the role of DRA in IBD pathophysiology. In this regard, Yang et al. showed a significant reduction (5 to 7 fold) in DRA mRNA expression in cecum, proximal and distal colon of HLA-B27/β2m transgenic rats as compared with the wild-type rats. In addition, a similar decrease in DRA mRNA expression was also shown in IL-10 KO mice housed in conventional conditions after developing colonic inflammation. It should be noted that DRA expression was preserved in IL-10 KO mice housed in germ-free environment as these mice failed to develop colonic inflammation. This phenomenon clearly indicated that downregulation of DRA expression is the direct consequence of intestinal inflammation and is not secondary to the absence of IL-10 expression.

In another model of colitis induced by DSS, we have reported ~50% to 60% decrease in DRA mRNA and protein expression in the distal colon (but not in ileum or proximal colon). Also, immunofluorescence staining revealed substantial reduction in DRA expression on the apical membrane in the colonic sections of DSS mice. This study used 3% DSS which resulted in mild colitis with no significant loss of surface epithelium, suggesting that decreased DRA expression is the direct consequence of intestinal inflammation (as observed in patients with UC, “Alterations in DRA Function or Expression in Patients with IBD”). Along the same lines, Xu et al. also demonstrated a decrease in intestinal DRA protein expression in DSS mice associated with diarrheal phenotype.

In another study, a significant decrease in DRA mRNA and protein expression in both ileum and mid distal colon of TNF⁻/ARE mice (TNF-α overexpressing mouse model that closely resembles CD and displays strong expression of TH1 cytokines) was demonstrated by Xiao et al. In another model of C. rodentium-induced colitis in mice, a dramatic decrease in DRA mRNA and protein expression was observed. This was associated with significant increase in fecal chloride levels, hypo-chloremia, and fatal diarrhea.

Therefore, the above studies from the animal models of IBD further attest to the conclusion derived from patients with IBD showing that a downregulation of DRA clearly underlies IBD-associated diarrhea.

**Lessons from DRA KO Mice**

Studies with DRA KO mice demonstrated a significantly reduced apical Cl⁻/HCO₃⁻ exchange activity, higher chloride levels and water content in the stool (diarrhea), distended large intestine, retarded growth and a short life span of ~4 to 5 months. Furthermore, Xiao et al. reported that DRA KO mice were extremely susceptible to DSS-induced colitis, exhibited severe disease symptoms and markedly reduced survival. These studies in DRA KO mice highlighted other important roles played by DRA in the pathogenesis of IBD, outside of its transporter function. In this regard, Schweinfest et al. reported a striking
TABLE 2. DRA in IBD

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<th>Patients with IBD</th>
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⇓ indicates decrease, and ⇩ indicates no effect. Number in parenthesis indicate citation number.

feature of “conjoined crypts” in the colon of DRA KO mice (wherein the crypt orifices were merged) with an enhanced rate of proliferation. In contrast to the wild-type mice where the proliferative cells were confined only at the bottom of the crypts, the proliferative zone in these KO mice occupied 30% to 50% of the crypt axis. Thus, loss of DRA expression in the KO mice altered the overall proliferative homeostasis of the colonic crypts, similar to the pattern observed in colitis patients.71

Also, the absence of an adherent inner mucus layer in the colon of DRA KO mice with no significant difference in the number of goblet cell count as compared with the wild-type mice was reported.70 This study suggested that although the machinery involved in mucus production was intact in DRA KO mice, the low luminal pH in the KO mice (due to reduced bicarbonate secretion) inhibited the expansion and sheet formation of the mucin granules secreted from the goblet cells. The absence of protective colonic mucus layer, such as observed in DRA KO mice, is considered as a potential mechanism in the development of colonic inflammation in both murine colitis models and patients with IBD.72 Furthermore, improper formation of the adherent mucus layer may also result in dysbiosis due to “ease of penetration” by pathogenic bacteria in absence of a barrier.52 Based on the above observations, it can be stated that DRA deficiency not only results in decreased absorptive efficiency of colon but also in impaired mucus layer formation resulting in aggravated inflammation and diarrhea associated with IBD.

Mechanisms Underlying DRA Dysregulation in IBD

Role of inflammatory mediators. To better understand the mechanisms directly affecting DRA function and/or expression in IBD, few in vitro studies have investigated the direct modulation of DRA by proinflammatory mediators. The study by Yang et al demonstrated a 4-fold decrease in DRA mRNA expression in response to IL-1β treatment in Caco-2 cells. The results obtained from nuclear run on assays indicated that IL-1β-mediated inhibition of DRA expression occurred at the transcriptional level. Furthermore, our group showed that IFN-γ directly decreased DRA function and expression by downregulating DRA promoter activity through a JAK/STAT1 pathway in Caco-2 cells.66 In addition, the study identified that IFN-γ responsive region was located in the −933 to −925 bp region harboring a gamma-activated site cis element. These studies provided a mechanistic link between increased expression and activation of STAT1 in colonic mucosa of patients with UC and diminished DRA function and expression. Our group also demonstrated a significant decrease in DRA mRNA and protein expression in response to direct TNF-α treatment in vitro.57 These studies further showed the involvement of TNF-α induced activation of NF-κB pathway in inhibiting DRA expression at the transcriptional level, which may partly contribute to IBD-associated diarrhea.67

Besides proinflammatory cytokines, chronic intestinal inflammation is also associated with augmented production of reactive oxygen and nitrogen species. An increased production of reactive oxygen and nitrogen species has been shown to play a critical role in pathophysiology of IBD in both human and animal models of IBD.73 In this regard, we earlier reported an inhibition of apical Cl−/OH− exchange activity by H2O2, a highly reactive oxygen metabolite.68 However, this inhibition of Cl−/OH− exchange activity was found to be independent of changes in the surface expression of DRA and may have involved other posttranslational modifications such as phosphorylation, lipid raft-dependent pathways or involvement of accessory proteins such as NHERFs, which remain to be studied. Furthermore, pathologically elevated NO levels have been demonstrated in IBD.49,74 In this context, we have previously shown that nitric oxide inhibited apical Cl−/OH− exchange activity through a cGMP-PKG and PKC-mediated pathway in Caco-2 cells.75 This
study indicated that dysregulated chloride absorption associated with IBD could be partly secondary to observed increase in NO in the inflamed intestine. Although the direct involvement of DRA in this study was not examined, however, it is well established that DRA is the key anion transporter involved in chloride absorption in intestinal epithelial cells.

In summary, loss of DRA function and/or expression in IBD leads to decreased chloride absorption and associated diarrhea in IBD. This decrease in DRA function/expression during gut inflammation can be attributed to direct modulation of surface DRA levels involving posttranslational mechanisms or activation of proinflammatory pathways that can alter DRA expression at the transcriptional level. In addition, DRA also seems to play an important role in maintaining a protective mucus barrier; however, identification of DRA as a critical intermediate in development of dysbiosis warrants further detailed investigations.

CONCLUSIONS

Understanding the pathophysiological basis of IBD-associated diarrhea has evolved significantly during the past decade due to many advances in our knowledge about the molecular mechanisms underlying NaCl absorption in the gut in health and disease. This has been possible due to the identification of molecular isoforms of SLC9 and SLC26 gene families involved in gut NaCl absorption. It is now well established that a coupled operation of NHE3 (SLC9A3) and DRA (SLC26A3) underlies the electroneutral NaCl absorption in the intestine. In addition, molecular studies with patients with UC and CD and with various animal models of IBD in mice including those with NHE3 and DRA KO mice have further increased our understanding of the role of NHE3 and DRA in IBD-associated diarrhea. The studies reviewed here clearly show that a decrease in NHE3 and DRA function and/or expression are the fundamental defects that explain the basis of defective electrolyte and fluid absorption in IBD-associated diarrhea, whereas the anion secretion plays very limited role if any in this process. Therefore, these transporters represent important and novel therapeutic targets for IBD-associated diarrhea. Overwhelming evidence now suggests that gut inflammation–mediated downregulation of DRA expression secondary to the potential effect of cytokines to be the predominant mechanism involved in reduction in chloride absorption in IBD. In contrast, the effects of gut inflammation on NHE3 seem to be mainly affecting its function that could be secondary to the alterations in regulatory NHERF proteins. However, the role of repressing NHE3 expression could not be ruled out. In addition, it seems that the basis for some of the contradictory reports in the literature with respect to a decrease in DRA and NHE3 function and/or expression may also be due to the involvement of different segments of the intestine and severity of inflammation. Studies from NHE3 and DRA KO mice further indicate that in addition to their respective roles in intestinal Na⁺ and Cl⁻ absorption, these transporters may have additional roles in the pathogenesis of IBD which include effects on gut barrier, immunomodulation, and dysbiosis. Further studies are needed to understand these other than the “transporter roles” of NHE3 and DRA. Also, strategies to upregulate function and/or expression of these key transporters would represent novel therapeutic approaches for IBD-associated diarrhea.

REFERENCES


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