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ORIGINAL ARTICLE

Ion Transport Basis of Diarrhea in a Mouse Model of Adoptive T Cell Transfer Colitis

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Abstract

Background Diarrhea, a major pathological hallmark of inflammatory bowel disease, is characterized by a significant reduction in the expression and function of key intestinal ion transporters. The adoptive naïve $CD4^+$ T cell transfer colitis is an immune-based, chronic colitis mouse model which resembles human Crohn's disease. Although mice with T cell transfer colitis demonstrate diarrhea, the ion transporter basis of this phenotype has not been explored.

Aims/Methods In the current studies, we aimed to determine the mRNA and protein levels of the key NaCl transporters DRA and NHE3 along with the mRNA expression of other transporters in the inflamed intestine.

Results Naïve $CD4^+$ T cells, transferred to Rag2 knockout mice, induced severe colonic inflammation characterized by histological damage and increased mRNA levels of cytokines in the colon with no effect in the ileum. Diarrheal phenotype was a key feature of the excised colons of mice where loose stools were evident. Our results demonstrated that the key chloride transporter DRA, mRNA, and protein levels were significantly reduced in the inflamed colon. However, expression of the key sodium hydrogen exchanger NHE3 was unaffected. The mRNA expression of other important transporters was also determined; in this regard, the sodium channel ENACα and the monocarboxylate transporters MCT1 and SMCT1 mRNA levels were also significantly lower compared to control mice. However, CFTR mRNA was not altered in the colon or ileum.

Conclusions The studies conducted herein for the first time demonstrate the downregulation of important intestinal ion transporters in proximal and distal colon in T cell transfer colitis mouse model, providing valuable evidence for the ion transporter basis of diarrhea in this chronic model of inflammation.

Keywords Ion transporters · T-cell transfer colitis · Diarrhea · DRA · Inflammatory bowel disease

Introduction

Many mouse models of colitis provide valuable insights into the pathogenesis of human IBD. Although models of chemical injury-induced acute colitis (e.g., dextran sulfate sodium or DSS, oxazolone, and 2,4,6-trinitrobenzene sulfonic acid or TNBS) are easier to develop (within 5–7 days), these models mainly recapitulate mucosal inflammation associated with ulcerative colitis (UC). However, the more widespread Crohn's disease (CD), where transmural inflammation is involved is more challenging to study. In this regard, multiple models of chronic colitis and ileitis have been developed, where the progression of inflammation occurs in a delayed manner, sometimes taking as long as 5–6 months (e.g., IL-10 KO and SAMP/Yit) [1, 2]. Comparatively, one major advantage of the adoptive T cell transfer colitis model is its relatively quick development within 4–6 weeks and the

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presence of transmural inflammation in colon and sometimes also in the ileum [3].

The adoptive T cell transfer colitis mouse model has emerged as an important tool to study chronic inflammation and the early immunological characteristics of inflammatory bowel disease (IBD) [3]. The basis for the development of intestinal inflammation in this model is by disrupting T cell homeostasis, which has been shown to be important in the pathogenesis of gut inflammation [4]. The key characteristic of this model is the fact that it is an immune-based model which can be reproducibly induced in various strains of mice within a short period of time (weeks) [5]. Since diarrhea is a major pathological hallmark of human IBD, and is persistently observed in mouse models of IBD, it is of significance to investigate the ion transporter basis for this phenotype in this model of IBD.

Ion transporters play a pivotal role in fluid and electrolyte homeostasis of the intestine. Key mechanisms by which diarrhea is perpetuated in the gut include lack of absorption and/or increase in secretion. Electroneutral NaCl absorption is mediated mainly via coupling of intestinal epithelial luminal membrane NHE3 (Na^+/H^+ exchanger 3) and SLC26A3 or DRA (downregulated in adenoma, a Cl^-/HCO_3^- exchanger). It has previously been established that one of the predominant mechanisms of IBD-associated diarrhea involves downregulation of DRA and NHE3.

Previously, we and others have demonstrated that pro-inflammatory cytokines including interleukin-1 beta (IL-1β), interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) can affect the reduction in function and/or expression of the key intestinal Na^+ and Cl^- ion transporters (NHE3 and DRA) [6–10]. Several other studies have also shown the dysregulation of ion transporters in intestinal inflammation in patients and in mouse models of colitis, including the epithelial sodium channel (ENAC) [11]. In addition, the monocarboxylate transporter 1 (MCT1) has also been shown to be downregulated in intestinal inflammation associated with IBD [12]. The putative anion transporter-1 (PAT1) was also shown to be transcriptionally regulated by IFN-γ, a key pro-inflammatory cytokine involved in gut inflammation [6]. Although the adoptive transfer of T cells to induce colitis has been widely utilized for decades, the ion transporter basis of diarrhea in this model has not yet been investigated. Therefore, in the current study, the ion transporter basis of the pathophysiology of diarrhea in the T cell transfer colitis model was investigated, to understand the molecular mechanisms involved in the development of diarrhea.

Male and female Rag2 knockout mice were transferred either with naïve CD4 T cells isolated from C57BL/6 wild-type (WT) mice or with PBS at 8 weeks and were observed 4 weeks post-transfer; when signs and symptoms of colitis were prevalent, the expression of various ion transporters was investigated. Our results showed that the colon, both proximal and distal, demonstrated inflammation characterized by histopathological damage and cytokine mRNA upregulation. In addition, colonic diarrheal phenotype observed was directly associated with the reduced expression of the key transporters, DRA; however, NHE3 expression remained unchanged. The mRNA expression of PAT1, ENACα, MCT1, and SMCT1 was also significantly lowered with no apparent effect on CFTR. Therefore, the study conducted herein provides important evidence of the ion transporter basis for the pathogenesis of diarrhea in this model of chronic inflammation.

Methods

Mice

C57BL/6 (WT) and $Rag2^{-/-}$ mice on the C57BL/6 background were purchased from the Jackson Laboratories and maintained at the Vanderbilt University Medical Center (VUMC) animal facilities in accordance with the Institutional Animal Care and Use Committee guidelines at the VUMC.

Reagents for Flow Cytometry

Fluorochrome-coupled anti-mouse CD4, CD19, CD45RB, CD8α, and TCRβ were purchased from Thermo Fisher, BD Biosciences (San Jose, CA) or Tonbo Biosciences (San Diego, CA). 7AAD was purchased from BD Biosciences. All cell staining was performed following conventional techniques.

Development of Adoptive Transfer Model of Colitis

T cell adoptive transfer was conducted as indicated before [13]. Briefly, single-cell suspensions of red blood cell (RBC)-depleted splenocytes were isolated from 8-week-old WT C57BL/6 mice. $CD4^+$ T cells were enriched by depleting $CD19^+$ and $CD8^+$ cells using magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). $CD4^+CD45RB^{high}$ T cells were sorted from the enriched cells using a FACSAria III instrument at the Flow Cytometry Shared Resource facility at the VUMC. Sorted cells were washed twice in sterile PBS. $CD4^+CD45RB^{hi}$ cells were adoptively transferred via i.p. injection (1×10^5 cells in 100 µl of PBS) into 8-week-old $Rag2^{-/-}$ recipient mice. Age- and sex-matched control $Rag2^{-/-}$ mice received only PBS. At 28 days post-transfer, mice were killed, and segments of the ileum, and proximal and distal colons were excised for various analysis.

RNA Extraction and RT PCR

RNA was extracted from mucosal scrapings of mice ileum, and proximal and distal colons with the use of the RNeasy Mini Kit from Qiagen (Valencia, CA) per the manufacturer's protocol. The extracted RNA was reverse-transcribed and amplified using SYBR green reagent with gene-specific primers listed in Table 1.

Protein Extraction and Western Blotting

Small amounts of distal and proximal colonic mucosal scrapings were harvested in RIPA Lysis buffer (Cell signaling, Danvers, MA) with protease inhibitor (Roche, Basel, Switzerland) and phosphatase inhibitor (Sigma-Aldrich, St. Louis, MO). These samples were then homogenized using a gun and sonicated until a homogenous lysate was prepared. These samples were then pelleted down by centrifugation at 7000 rpm for 7 min, and the supernatants were used for determining protein concentration with Bradford assay (Bio-Rad, Hercules, CA). 25 µg of protein from each sample was loaded onto a 7.5% precast SDS PAGE gel (Bio-Rad), then transferred to a PVDF membrane (Bio-Rad) and probed with the respective primary antibodies based on the protein of interest at 4 °C overnight after blocking in 5% PBS milk for 1 h at RT. The primary antibodies include; DRA rabbit polyclonal antibody, 1:500 in 1% PBS milk, DRA antibody was raised against the C-terminal amino acid (745–764) sequence: INTNGGLRNRVYEPVETKF of SLC26A3 (accession number: BC025671) at Research Resource Centre (RRC), NHE3 rabbit antibody, 1:3000 in 1% PBS milk (gift from Dr. Chris Yun, Emory University), and GAPDH rabbit antibody (Sigma), 1:8000 in 1% PBS milk. The membranes were then washed and probed with secondary HRP-conjugated antibodies at a ratio of 1:5000 (Promega, Madison, WI) for 1 h at RT. Following washing the membranes were processed as per the manufacturer's protocol (Bio-Rad) for visualizing protein bands via enhanced chemiluminescence.

Table 1 Gene-specific primer sequences

Gene	Sequence (5′ → 3′)
Mouse IL-1β	F-GCAACTGTTCCTGAACTCAACT R-ATCTTTTGGGGTCCGTCAACT
Mouse TNF-α	F-TACTGAACTTCGGGGTGATTGGTCC R-CAGCCTTGTCCCTTGAAGAGAACC
Mouse IL-10	F-ATTTGAATTCCCTGGGTGAGAAG R-CACAGGGGAGAAATCGATGACA
Mouse IL-22	F-ATGAGTTTTTCCCTTATGGGGAC R-GCTGGAAGTTGGACACCTCAA
Mouse DRA	F-TGGTGGGAGTTGTCGTTACA R-CCCAGGAGCAACTGAATGAT
Mouse NHE3	F-GGCCTTCATTCGCTCCCCAAG R-ATGCTTGTACTCCTGCCGAGG
Mouse GAPDH	F-TGTGTCCGTCGTGGATCTGA R-CCTGCTTCACCACCTTCTTGAT
Mouse CFTR	F-CTGGACCACACCAATTTTGAGG R-GCGTGGATAAGCTGGGGAT
Mouse ENACα	F-ATCGGCTTCCAACTGTGCA R-CCAGGGCTTCTTCCTCTAGAGC
Mouse SMCT1	F-TGCCATTTCCTTATGGGTAGG R-AGTGGAGTCCTTTCCGCATTA
Mouse MCT1	F-TGTTGTTGCAAATGGAGTGT R-AAGTCGATAATTGATGCCCATGCCAA
Mouse PAT1	F-GAAATGGAGCTGCAGAGGA R-GCTGGAGCAGAAGAGAATGG

Immunostaining

Frozen sections of 5 µm from mice distal colon were fixed with 4% paraformaldehyde for 20 min. These sections were then permeabilized with 0.3% NP-40 (Fisher scientific) for 5 min and blocked with 5% normal goat serum (NGS) in PBS for 2 h at RT. These were then incubated with primary antibodies for DRA (rabbit polyclonal antibody raised against the same sequence as above at Pocono Rabbit Farm, Canadensis, PA) at 1:100 ratio with mouse villin antibody (Invitrogen, Carlsbad, CA) in 1% NGS in PBS. After three washes of PBS, the sections were incubated with fluorescently tagged secondary antibodies (Invitrogen). The slides were once again washed in PBS and mounted with DAPI (Invitrogen) using cover slips. The slides were sealed with clear nail polish and stored at -20 °C until imaged. The images were captured using the BX-100 fluorescent microscope.

Hematoxylin and Eosin Staining

Formalin-fixed paraffin-embedded ileal and distal colonic tissues were sectioned at 5 µm thickness using a microtome and stained with hematoxylin and eosin according to the manufacturer's protocol (Sytek Laboratories, Upsala, MN). These sections were imaged at 10X and 20X magnifications using Olympus BX light microscope.

Results

Adoptive Transfer of Naïve CD4$^+$ T Cell Demonstrated Severe Inflammation in the Colon with Almost No Sign of Inflammation in the Ileum

Four weeks post-transfer of CD4$^+$CD45RB^{high} T cells, the ileum had no apparent histopathological damage (Fig. 1a, b). However, colon developed severe colitis characterized by histological changes including inflammatory infiltrate, disrupted mucosal, and crypt architecture with transmural

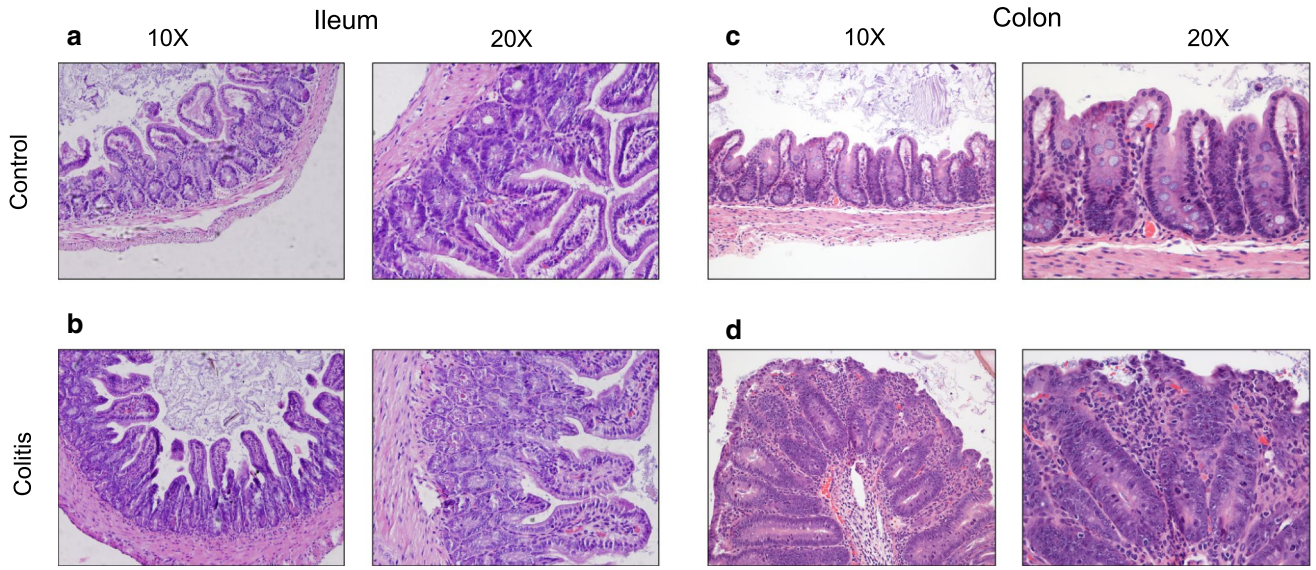

Fig. 1 Histology of ileum and colon. Representative image of ileum of **a** control mice, **b** T cell transfer colitis mice, **c** colon of control mice, **d** colon histology of T cell transfer colitis mice. Histology of the ileum appeared similar between control vs colitis mice. Colonic histology of colitic mice appeared inflamed with aberrant architecture compared to control mice. Paraffin-embedded 5 μM sections from mouse ileum and colon were stained with hematoxylin and eosin and imaged at × 10 and × 20 magnifications

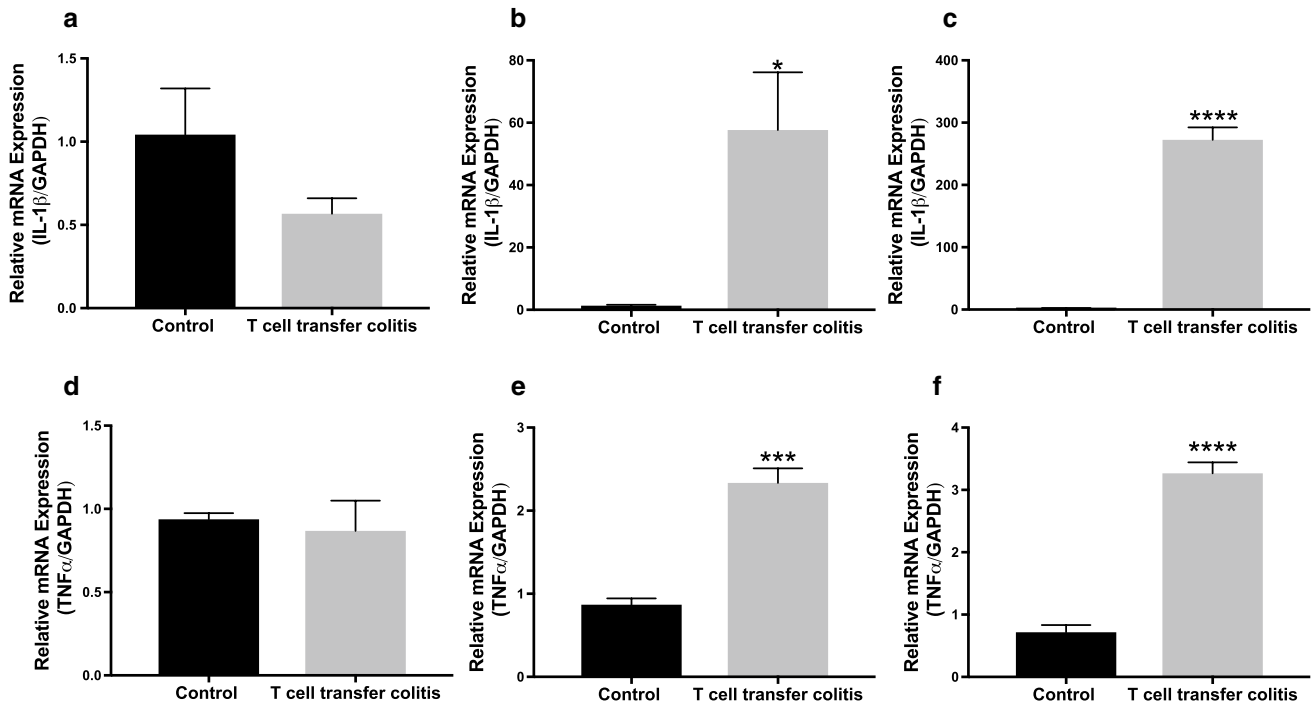

Fig. 2 mRNA expression of pro-inflammatory cytokines IL-1β and TNF-α in mice ileal and colonic mucosa. IL-1β mRNA expression in the **a** ileum, **b** proximal colon, and **c** distal colon. TNF-α mRNA expression in the **d** ileum, **e** proximal colon, and **f** distal colon. Gene expression normalized to internal control GAPDH. mRNA isolated from mouse intestinal mucosa from ileum, and proximal and distal colons was subjected to qPCR with specific primers for respective cytokines. Data represented as average ± SEM, $N = 4$, $^{*}p < 0.05$; $^{***}p < 0.0005$; $^{****}p < 0.0001$ versus control

inflammation (Fig. 1c, d). In parallel with the changes in histology, there was a significant increase in pro-inflammatory cytokine mRNA levels (IL-1β and TNF-α) in the distal and proximal colonic mucosa (Fig. 2b, c, e, f). Once more, these changes were only evident in the colon and not ileum, and the cytokine mRNA expression in the ileum remained unchanged, comparable to that of control animals (Fig. 2a, d). The mRNA levels of anti-inflammatory cytokines such as interleukin-10 and interleukin-22 remained unchanged in all regions of the intestine (data not shown).

Naïve CD4$^+$ T Cell Transfer Colitis Demonstrated Diarrheal Phenotype and Reduced Expression of DRA with No Change in NHE3

One of the key features of intestinal inflammation is the manifestation of diarrhea. In this regard, the excised whole colon of mice with colitis was observed for its gross morphology. The colons of mice with colitis appeared shortened with loose stools, indicative of diarrheal phenotype (Fig. 3).

To examine the basis of diarrheal phenotype observed in these mice, we next determined the mRNA and protein expression of DRA, the major chloride bicarbonate exchanger in the mammalian intestine. As with other parameters, ileum showed no variation in DRA mRNA expression (Fig. 4a). However, similar to other models of colitis, including DSS, TNBS, IL-10 knockout, and infectious models, the expression of DRA mRNA level was markedly reduced in the proximal and distal colons of mice with naïve CD4$^+$ T cell transfer colitis (Fig. 4b, c). To further confirm these findings, protein expression of DRA was also determined in the distal colonic tissues (Fig. 4d). Distal colonic tissues had a significantly lower amount of DRA protein as compared to control mice. In parallel, the Immunostaining of the colonic tissues with specific DRA antibody (green) also demonstrated a significant loss of signal for DRA in mice with colitis compared to the control in the distal colon (Fig. 4e). The latter result further corroborates with previous findings to the reduction in DRA expression during colonic inflammation.

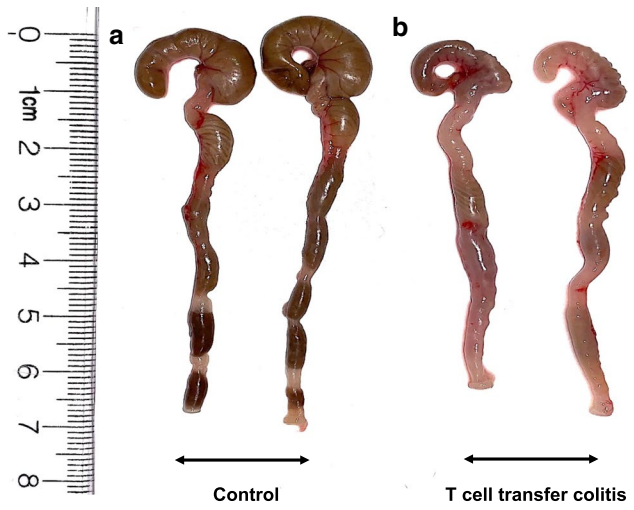

Fig. 3 Morphology of whole excised colon. Representative images of colon of **a** control mice and **b** mice with T cell transfer colitis

The electroneutral sodium chloride absorption of the intestine is mainly mediated by the key ion transporters DRA and NHE3. NHE3 is the key sodium hydrogen exchanger isoform, crucial for sodium absorption in the intestine, and the reduced function of this transporter has also been implicated in the pathogenesis of diarrhea in intestinal inflammation. Therefore, we pursued to determine the expression on NHE3 in the proximal, distal and ileal mucosa in control vs mice with colitis. In contrast to DRA, the mRNA and protein levels of NHE3 in the colon were not affected by T cell transfer colitis. This was apparent both in the colon, where inflammation is predominant, and in the ileum (Fig. 5).

In addition to DRA, PAT-1, another important anion transporter mRNA level, was also significantly decreased in the distal colonic mucosa in mice with colitis (Fig. 6). Since inflammation was not present in the ileum, the mRNA level of PAT-1 was also unaltered (Fig. 6a–c).

In addition, another important epithelial sodium transporter ENACα mRNA was significantly lower in mice with colitis in both the proximal and distal colonic mucosa with no change in the ileum (Fig. 6d–f). Thus, although the sodium hydrogen exchanger, NHE3, levels were unaltered as with other models of colitis and IBD, the levels of ENACα were downregulated.

Differential Expression of Other Important Intestinal ion Transporters in Naïve CD4$^+$ T Cell Transfer Colitis Mouse Model

The intestine houses many other important ion transporters which regulate water absorption and secretion. In this regard, several other ion transporters were also considered. These included cystic fibrosis transmembrane conductance regulator (CFTR) and the monocarboxylate transporters MCT1 and SMCT1. CFTR levels remained unchanged after colitis insult in the colon as well as the ileum (Fig. 6g–i). However, the important monocarboxylate transporters, both MCT1 and SMCT1 mRNA expressions, were significantly reduced in the distal colon of mice with colitis (Fig. 7).

Discussion

The naïve CD4$^+$ T cell transfer model of colitis has gained widespread usage in the field of IBD research, due to its close resemblance to the human Crohn's disease [14]. The

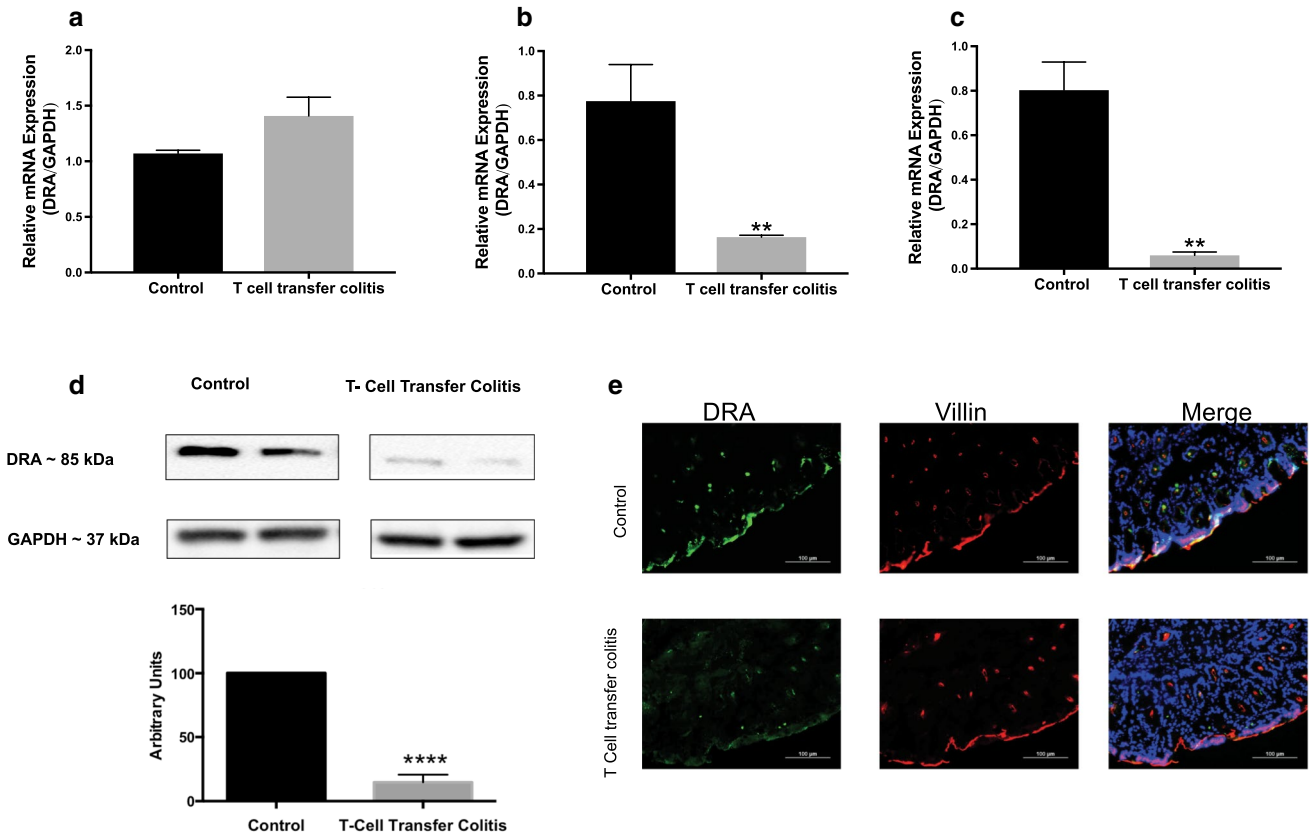

Fig. 4 Effect of T cell transfer colitis on DRA expression. Graphical representation of DRA mRNA in the **a** ileum, **b** proximal colon, and **c** distal colon. Gene expression normalized to internal control GAPDH. mRNA isolated from mouse intestinal mucosa from ileum, and proximal and distal colons was subjected to qPCR with specific primers for DRA. **d** Representative image of western blot for DRA in distal colonic mucosa and graphical representation of densitometric analysis conducted using ImageJ software normalized to internal control GAPDH. Data represented as average ± SEM, $N = 4$, $^{**}p < 0.005$; $^{****}p < 0.0001$ versus control. **e** Immunofluorescence staining of DRA (green) and Villin (red) in 5 µM distal colonic cryosections imaged at × 20

basis of colitis development in this model involves disruption on T cell homeostasis, which has been suggested to be a key initial factor in the pathogenesis of IBD [15]. Although mice with T cell transfer colitis unfailingly develop diarrhea, the ion transporter basis of this phenotype has not yet been investigated. In this regard, we sought to determine the levels of important intestinal ion transporters which have been implicated in the pathogenesis of diarrhea under inflammatory conditions [16].

Our observations in this study clearly demonstrate the significant downregulation of important sodium and chloride transporters including DRA, PAT1, and ENACα in the colonic mucosa, with no effect on CFTR and NHE3. These observations are reasonably parallel to investigations conducted in other models of colitis including the chemically induced DSS and TNBS as well as in patients with IBD [7, 9, 17]. In addition, the monocarboxylate transporters, MCT1 and SMCT1, were also significantly reduced in the inflamed colonic mucosa compared to control mice. However, inflammation as well as downregulation of ion transporters was not observed in the ileum in mice with T cell transfer. Therefore, this study provides further evidence for the important role intestinal NaCl and monocarboxylate transporters play, in IBD-associated pathophysiological basis of diarrhea.

Coupled electroneutral NaCl absorption is the predominant mechanism of water absorption in the distal small intestine and the colon [18]. This process involves coupling of NHE3 and DRA. DRA or downregulated in adenoma is the major chloride transporter involved in the electroneutral NaCl absorption in the mammalian intestine [19]. This ion transporter is highly expressed in the colon, and the loss of its function and expression is implicated in many intestinal disorders [7, 20]. The sodium counterpart transporter which works together with DRA is the key sodium hydrogen exchanger isoform, NHE-3. The knockdown of both transporters in mice leads to the manifestation of severe diarrhea [21, 22]. Although there was a significant downregulation of the mRNA and protein expression of DRA compared to control animals in T cell transfer colitis as anticipated, that

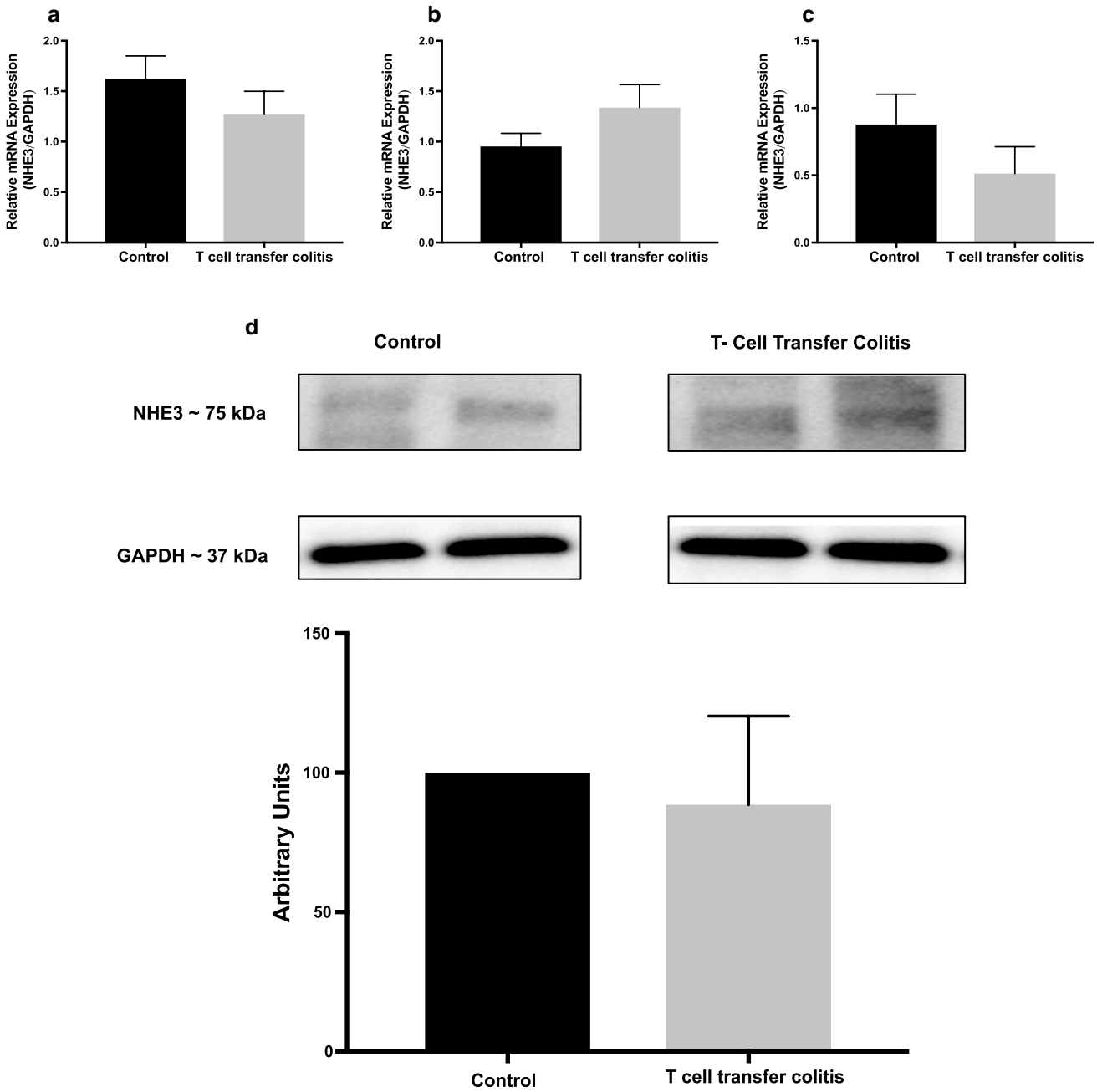

Fig. 5 Effect of T cell transfer colitis on NHE3 expression. Graphical representation of NHE3 mRNA in the **a** ileum, **b** proximal colon, and **c** distal colon. Gene expression normalized to internal control GAPDH. mRNA isolated from mouse intestinal mucosa from ileum, and proximal and distal colons was subjected to qPCR with specific primers for NHE3. **d** Representative image of western blot for NHE3 in proximal colonic mucosa and graphical representation of densitometric analysis conducted using ImageJ software normalized to internal control GAPDH. Data represented as average ± SEM, $N = 4$

of NHE3 remained unaltered showing only a trend toward a decline.

This observation could be due to the following reasons: In human and mouse models of ulcerative colitis, it has been perceived that the expression of NHE3 was preserved in the mucosa and only its function was affected [9, 23]. On the contrary, there are several studies where the expression of NHE3 level has also been shown to be downregulated by inflammation [17, 24]. The expression of NHE3 is highest in the ileum, followed by the proximal colon, and is least expressed in the distal colon. Therefore, since the key transporter involved in electrolyte reabsorption in the distal colon is DRA, the levels of NHE3 expression may not be altered by colonic inflammation. Additionally, there are several reports

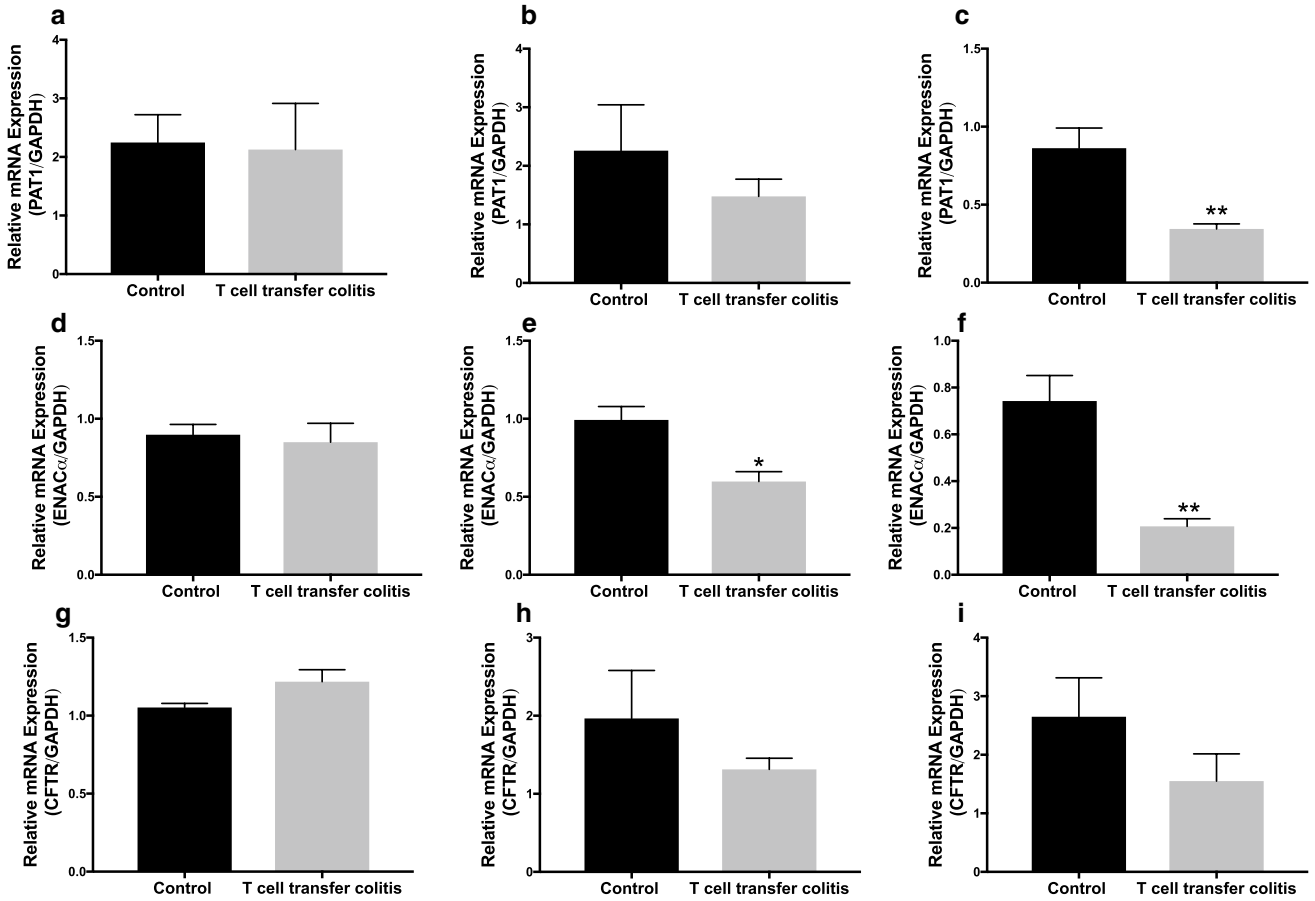

Fig. 6 Effect of T cell transfer colitis on PAT1, ENACα, and CFTR mRNA expression. Graphical representation of PAT1 mRNA in the **a** ileum, **b** proximal colon, and **c** distal colon. ENACα mRNA in the **d** ileum, **e** proximal colon, and **f** distal colon. CFTR mRNA in the **g** ileum, **h** proximal colon, and **i** distal colon. Gene expression normalized to internal control GAPDH. mRNA isolated from mouse intestinal mucosa from ileum, and proximal and distal colons was subjected to qPCR with specific primers for PAT1, ENACα, and CFTR. Data represented as average ± SEM, $N=4$, $*p<0.05$; $**p<0.005$ versus control

of the reciprocal regulation of NHE3 and DRA, which may also contribute to this pattern of expression [25, 26]. The other important anion transporter PAT-1 was also significantly downregulated in the colon of mice with adoptive T cell transfer colitis. However, the expression of PAT1 in the colon in physiological conditions is much less as compared to DRA and therefore may not contribute as much as DRA in chloride absorption [27]. The PAT-1 transporter is expressed at higher levels in the small intestine and thus maybe more important in the ion absorption in the small intestine.

However, the epithelial sodium channel or ENACα in our studies was markedly reduced in parallel with DRA. ENACs also participate in the apical transport of sodium ions into enterocytes and are highly expressed in the colon. Furthermore, in IBD-associated diarrhea and in mouse models of colitis, ENAC has been shown to be downregulated [11]. Overall, since diarrhea observed in colitis is mainly caused due to the repression of the expression and function of the key transporters in the colon, DRA itself can be the key driver responsible for this phenotype. In addition, the relatively lower levels of cytokines in the proximal colon and ileum (in response to colitis, where NHE3 is highly expressed) may not be enough to downregulate mRNA or protein levels for this transporter.

Another important ion transporter, which was not affected by T cell transfer colitis, was CFTR. This chloride channel is important in secreting chloride ions into the lumen and therefore, typically works opposite to the other transporters [20]. However, in previous studies also CFTR has not been shown to play a role in IBD-associated diarrhea [20]. Our current studies agree with the previous reports. In our studies, the mRNA levels were intact, showing a trend toward a decrease. Additionally, compared to the ileum, the expression of CFTR in the colon is known to be localized in enterocytes at the base of the crypts as opposed to the villus; this may also have a contribution to the differential expression of this transporter in this model of colitis.

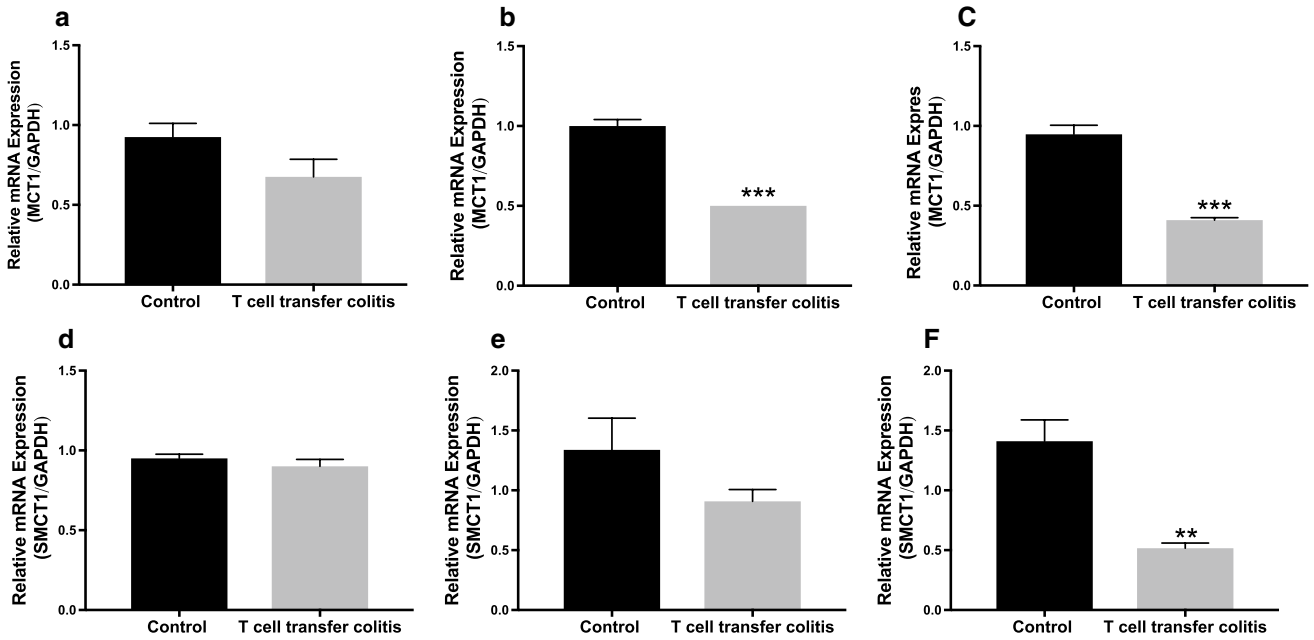

Fig. 7 Effect of T cell transfer colitis on monocarboxylate transporter mRNA expression. Graphical representation of MCT1 mRNA in the **a** ileum, **b** proximal colon, and **c** distal colon. SMCT1 mRNA in the **d** ileum, **e** proximal colon, and **f** distal colon. Gene expression was normalized to internal control GAPDH. mRNA isolated from mouse intestinal mucosa from ileum, and proximal and distal colons was subjected to qPCR with specific primers for MCT1 and SMCT1. Data represented as average ± SEM, $N = 4$, $^{**}p < 0.005$; $^{***}p < 0.0005$ versus control

Apart from electrolyte transporters, an interesting finding in this study was the marked reduction in the mRNA expression of the monocarboxylate transporters MCT1 and SMCT1 in the distal colonic mucosa. The colon is a reservoir for a multitude of bacteria out of which important probiotics are also coexistent. These bacteria produce by-products such as lactate, acetate, pyruvate, propionate, butyrate, and other short-chain fatty acids which all serve as substrates for MCT1 and SMCT1. Several studies from the recent past have highlighted the biological functions and protective effects of these molecules in health and disease [28]. In line with that, the expression of MCT1 has been demonstrated to be downregulated in human IBD, further highlighting the similarity of this model with human disease [12, 29]. Downregulation of nutrient uptake is also known to reduce osmotic water absorption resulting in fluid accumulation in the lumen. In addition, secondary to the reduction in these transporters, the uptake of butyrate reduces, which in turn may contribute to the pathogenesis of diarrhea, due to its beneficial effect on the expression of ion transporters. Another important factor which has been shown to be involved in the pathogenesis of IBD and associated diarrhea is the compromised barrier function [30]. Previously, it has been demonstrated that adoptive T cell transfer leads to increased permeability in the gut barrier [31]. In addition, very recent studies have shown a close link between the downregulations of ion transporters such as DRA in altering the expression of tight junction proteins, ultimately causing defects in barrier integrity [32]. Therefore, it is quite likely that the compromised barrier function observed in these mice may also partake in contributing to the overall diarrhea apart from reduced ion transporter expression.

In conclusion, our studies validate that mice with adoptive naïve $CD4^+$ T transfer inflammatory colitis model demonstrate inflammation in the colon, characterized by diarrheal phenotype. The pathophysiological basis of diarrhea may be in part explained by the significant downregulation of important NaCl and monocarboxylate transporters.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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