Upregulation of CFTR expression but not SLC26A3 and SLC9A3 in ulcerative colitis

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Lohi, H., S. Mäkelä, K. Pulkkinen, P. Höglund, M.-L. Karjalainen-Lindsberg, P. Puolakkainen, and J. Kere. Upregulation of CFTR expression but not SLC26A3 and SLC9A3 in ulcerative colitis. Am J Physiol Gastrointest Liver Physiol 283: G567–G575, 2002. First published April 10, 2002; 10.1152/ajpgi.00356.2001.—In inflamed colonic mucosa, the absorption of salt is disturbed. We compared the expression of three major mediators of the intestinal salt transport between healthy and inflamed colonic mucosa to understand the pathophysiology of diarrhea in inflammatory bowel disease. Expression levels of the cystic fibrosis transmembrane regulator (CFTR) (Cl− channel), SLC26A3 (Cl−/HCO3− exchanger) and SLC9A3 (Na+/H+ exchanger) mRNAs were measured by real-time quantitative RT-PCR in peroperative colonic samples from controls (n = 4) and patients with ulcerative colitis (n = 10). Several samples were obtained from each individual. Tissue samples were divided into three subgroups according to their histological degree of inflammation. Expression of CFTR and SLC26A3 proteins were determined by immunohistochemistry and Western blotting from the same samples, respectively. Increased expression of CFTR mRNA was observed in all three groups of affected tissue samples, most pronounced in mildly inflamed colonic mucosa (5-fold increase in expression; P < 0.001). The expression of the CFTR protein was detected from health and inflamed colon tissue. Although the expression of the SLC26A3 mRNA was significantly decreased in severe ulcerative colitis (P < 0.05), the SLC26A3 protein levels remained unchanged in all groups. The expression of SLC9A3 mRNA was significantly changed between the mild and severe groups. Intestinal inflammation modulates the expression of three major mediators of intestinal salt transport and may contribute to diarrhea in ulcerative colitis both by increasing transepithelial Cl− secretion and by inhibiting the epithelial NaCl absorption.

IN NORMAL COLONIC MUCOSA, the absorption of salt is driven by active transporters followed by passive movements of water. Much of the absorption of NaCl is mediated by electroneutral pH-coupled Na+/H+ and Cl−/HCO3− exchangers (27). Cystic fibrosis (CF) transmembrane regulator (CFTR), the gene for one of the most common inherited diseases worldwide, CF (OMIM #219700), acts as the major mediator of cAMP- and Ca2+-activated Cl− secretion in the colonic epithelium (33). The intestinal phenotype of CF results from luminal obstruction by thick mucoid secretions (15). In contrast, overstimulation of Cl− secretion, e.g., by bacterial enterotoxins, is associated with secretory diarrhea (16, 28, 48).

A recent advance in the understanding of the colonic Cl−/HCO3− transport resulted from the identification of the SLC26A3 gene mutated in congenital chloride diarrhea (CLD; OMIM #217400) (23). CLD is a recessively inherited intestinal disorder that manifests with massive losses of Cl− in stools with respective defects in intestinal HCO3− secretion. As a result, the intestinal content is acidic, with corresponding systemic metabolic alkalosis. Low pH secondarily impairs the intestinal absorption of Na+ through the Na+/H+ exchanger, leading to both high fecal chloride and sodium (24). Because water is transported only passively along the gradient in the impermeable colonic mucosa, these patients have massive diarrhea. The function of the SLC26A3 protein in the mature surface epithelium of ileum and colon has been shown to mediate Na+-independent Cl−/OH− and Cl−/HCO3− exchange (9, 20, 23, 34, 35, 42). Evolutionarily, the SLC26A3 protein belongs to the SLC26 family of tissue-specific anion transporters (31).

A distinct phenotype results from the malfunction of intestinal Na+/H+ exchangers: mice lacking SLC9A3 develop sodium diarrhea with acidosis (OMIM #600530) (40). Several compensatory mechanisms have been shown to modify the mouse phenotype (34, 40), and no human cases of sodium diarrhea have been associated with mutations in the six Na+/H+ exchangers cloned to date (36).

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Little is known about the possible role of electrolyte-transport abnormalities in the pathogenesis of diarrhea associated with chronic intestinal inflammation (3, 4, 7, 44). In animal models, colitis has been associated with a reduced expression of SLC26A3 and unchanged SLC9A3 (52). Human inflammatory colon tissues may show no change in the expression of SLC26A3 (20). Although CFTR has multiple effects on cellular electrolyte and fluid homeostasis, not much is known about its expression in inflamed colonic mucosa.

The aim of this study was to examine the effect of inflammatory bowel disease (IBD) on the expression of the SLC26A3, SLC9A3, and CFTR genes. The activity of inflammation was determined by histological evaluation of the preoperative colonic specimens graded in three subgroups (mild, moderate, and severe). The expression levels were measured by real-time quantitative RT-PCR, immunohistochemistry, and Western blotting.

**MATERIALS AND METHODS**

**Tissue samples.** Altogether, 30 preoperative tissue samples from 10 patients undergoing surgery for ulcerative colitis (UC) and 12 samples from 4 control individuals with noninflamed colon mucosa were obtained from the Department of Surgery, Helsinki University Central Hospital by permission of the respective ethical review board. Inflamed colonic tissue samples were taken from two areas: those that macroscopically appeared normal and those demonstrating the highest degree of inflammation. Control tissue samples were taken from morphologically unaltered, uninflamed colonic mucosa of benign (tubular adenoma) or malignant (two adenocarcinomas) tumors at distant nonneoplastic area, as well as of diverticulosis. Tissue samples used for mRNA and protein analysis were taken from the same area as those samples used for histopathological analysis. Tissue samples were handled immediately after surgery on ice or snap frozen in liquid nitrogen before homogenization.

**Histological grading.** The samples for histological analyses were fixed in 10% neutral buffered formalin immediately after surgery. Formalin-fixed paraffin-embedded samples were prepared for histology and stained by hematoxylin-eosin for histological grading. The degree of inflammation was graded as suggested by Truelove and Richards (46) on a four-point scale: normal (no significant inflammation, n = 12); mild (elevated number of mucosal leukocytes but intact epithelium, n = 11); moderate (aggregates of leukocytes with intact epithelium, n = 7); and severe (significant ulceration of the epithelium by mononuclear cell infiltrate, n = 12). Histological grading was performed by a pathologist (M.-L. Karjalainen-Lindsberg) without knowledge of surgery or the endoscopic reports and experimental data.

**Quantitative RT-PCR.** Adjacent colonic tissue samples were used for histological grading and RNA extraction. Total RNA was extracted from fresh tissues using RNeasy Mini Kit (Qiagen), and 400 ng total RNA were further transcribed to cDNA with random hexamers (Taqman Reverse Transcription Reagents, Perkin-Elmer). Dilutions of the cDNA were used for real-time quantitative PCR (5’ fluorogenic nuclease assay) using Perkin-Elmer’s ABI Prism 7700 Sequence Detector System (30). The probes used were CCA ATC GAA TTC ATT ATG ACC GTG ATT GC for SLC26A3, ATG CAG TCT CTG GAG CAG CGA CGG for SLC9A3, and CAG AAG CGT CAT CAA AGC ATG CCA AC for CFTR. The following primers were used for amplification: SLC26A3 (100 nM), AGA CAA ACT TCC ACT GCC CAT and ACA GCC GGA TAC ACC TGC; SLC9A3 (900 nM), TCC CCC AGC ACC GAC A and TCC CGG ATG CTC CGG; CFTR (300 nM), TCT TGT GTG TTT CTT CTT ATG ATG AAT ATA GAT and GCA AAC TTG GAG ATG TCT CCT TCT. Primers were designed into exon-intron junctions to avoid amplification of genomic DNA. An 18s rRNA probe and primers were used as an endogenous control gene (S18 control kit, Perkin-Elmer). PCR conditions were: 2 min 50°C, 10 min 95°C, and 40 cycles of 15 s 95°C and 1 min 60°C. The PCR assays were performed in separate tubes, and relative quantitation of the SLC26A3, SLC9A3, and CFTR mRNAs was performed using the standard curve method according to the manufacturer’s instructions (PE Applied Biosystems, User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

**Immunohistochemistry.** Serial sections to those used for histological grading from the same blocks of tissue were used for immunohistochemistry. The peroxidase-antiperoxidase technique was performed using Vectastain Elite ABC Avidin Biotin Complex Kit (Vector Laboratories, Burlingame, CA). For pretreatment, the deparaffinized slides were boiled in a microwave oven for 20 min in 10 mM citrate buffer (pH 6.0) or 0.01 M EDTA buffer (pH 8.0). Monoclonal CFTR antibody [Ab-2 (MM13–4), Neomarkers, Fremont, CA] was used at 3 μg/ml. Diaminobenzidine was used as the chromogenic substrate, and the slides were counterstained with hematoxylin. Normal mouse IgG (Dako, Glostrup, Denmark) at 3 μg/ml was used as a negative control on parallel sections.

**Production of antisera.** The COOH-terminal antigen was raised in rabbits against the synthetic peptide corresponding to nucleotides 2375–2416 of the published SLC26A3 sequence (GenBank L02785; Research Genetics, Huntsville, AL). Nucleotides 205–407, corresponding to the first 68 amino acids of the NH2-terminal region of SLC26A3 gene, were amplified with the following primers: 5’-CGT GGA TCC ATG ATT GAA CCC TTT GGG AAT C-3’ and 5’-CGA GAA TAT GCT GCC AAC CAA GAT-3’ by PCR, and the subsequent PCR product was cloned into the BamH I-EcoR I sites of the pGEX-2T expression plasmid (Amersham Pharmacia Biotech, Uppsala, Sweden). BamH I-EcoR I recognition sequences were added to the 5’-end of the primers. The cloned region was produced and purified as glutathione S-transferase (GST) fusion protein according to the manufacturer’s instructions (Amersham Pharmacia Biotech). The purified fusion protein was used as antigen in the immunisation of two rabbits (Animal Core Facility, Viikki Biocenter, University of Helsinki). Presera were collected before the first immunisation. A total of five immunisations were performed at 3-wk intervals using 400 μg of the GST fusion protein mix 1:1 with Freund’s adjuvant. Rabbits were bled after the immunization, and the serum was divided to negative control on parallel sections.

**Western analysis.** Surface epithelial cells of human colon tissues were scraped and transferred to ice-cold lysis buffer [50 mM core buffer (Roche, Basel, Switzerland), 150 mM NaCl, 0.1 mg/ml phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1% Nonidet P-40, and 0.5% sodium deoxycholate]. The lysed cells were homogenized by a syringe and needle followed by centrifugation at 12,000 g for 10 min at 4°C. The supernatant was preserved for protein analyses. Protein concentration was measured using the colorimetric Bradford protein assay (Bio-Rad Laboratories, Hercules, CA). Total proteins were separated by SDS-PAGE using 9% gels and electrophoresed onto Hybond C-extra membranes (Amersham Pharmacia Biotech). Nonspecific binding
sites were blocked by incubating the membranes in a solution of 5% nonfat dry milk in PBS containing 0.1% Tween-20. The primary antibody sera were diluted 1:500–1:1,000, and biotin-conjugated goat anti-rabbit IgG (Roche) was used as a secondary antibody 1:2,000 in PBS. To visualize the protein bands by enhanced chemiluminescent reaction, membranes were incubated in 1:2,000 dilution of horseradish peroxidase-conjugated streptavidin (Roche). Computer-assisted scanning densitometry (Biometra, BioDocAnalyze, Göttingen, Germany) was used to analyze the intensity of the immunoreactive bands in the autographs. The optic densities were normalized to the amount of protein in lane. Statistical significance between groups was analyzed by Kruskal-Wallis nonparametric test.

**Statistical analysis.** Statistical analyses were performed using the PRISM Statistic Package, version 3.0 (GraphPad Software, San Diego, CA). The degree of statistical significance between two groups was calculated using the nonparametric Mann-Whitney U test. Comparisons between three or more groups were performed using the Kruskal-Wallis test. The groups were considered different at a P value <0.05. In these cases, P values for comparisons between groups were calculated using Dunn's multiple comparison as a nonparametric posttest.

**RESULTS**

To study the effect of intestinal inflammation on the expression of the genes responsible for major NaCl and fluid movements, we examined the relative mRNA expression of CFTR, SLC26A3, and SLC9A3 genes in preoperative samples from normal (n = 12) and UC patients' colons (n = 30) by quantitative RT-PCR. Expression levels were normalized to S18 transcripts in the same cDNA sample. In normal human colonic mucosa, all three genes had different basal expression levels (Fig. 1). However, a direct comparison of mRNA levels for the three genes is not possible, because we did not normalize the PCR assays for mRNA copy numbers. For each gene separately, comparison of mRNA levels between the samples gives information about their relative levels.

**Expression of the CFTR gene was increased in inflamed compared with normal colonic mucosa.** In inflamed colonic mucosa, the CFTR expression was increased 2.5-fold compared with noninflamed samples (Fig. 1A; P = 0.0002, Mann-Whitney U test). Detailed characterization of the three subgroups of inflammation (mild, moderate, and severe) demonstrated upregulated expression of the CFTR mRNA in all stages of inflamed tissues: fivefold in mildly inflamed samples (P < 0.001), 2.4-fold in moderate, and 1.5-fold in severely inflamed colon mucosa compared with normal (Fig. 2A). Most pronounced upregulation was in mildly inflamed mucosa at 3.4-fold higher than in severely inflamed sets of samples (Fig. 2A).

**The expression of the SLC26A3 and SLC9A3 genes varies in inflamed colonic mucosa.** In contrast to CFTR, the expression levels of the SLC26A3 and SLC9A3 mRNA were not different between normal and inflamed mucosa: the median expression of the SLC26A3 decreased 0.6-fold (P = 0.1095), and the SLC9A3 expression elevated 1.3-fold in the inflamed samples (P = 0.5130; Fig. 1, B and C). Increased expression of
SLC26A3 in mild inflammation (1.4-fold) was followed by sharp reduction in moderate (1.9-fold) and severe inflammation (2.6-fold; Fig. 2B). Expression of SLC26A3 was significantly changed in the severely inflamed tissue and between the mild and severe groups \((P < 0.05)\). In case of SLC9A3, the initial up-regulation in both mild (3.3-fold) and moderate (1.5-fold) was followed by return to the same level as in noninflamed samples (1.1-fold; Fig. 2C). A statistically significant difference was seen between the mild and severe groups.

The CFTR protein is expressed in the healthy and inflamed colon mucosa. To assess putative alterations in the CFTR protein expression in response to inflammation, a set of inflammatory samples was studied using immunohistochemistry with a monoclonal CFTR antibody and compared with the normal control. As expected, normal colon epithelium showed CFTR expression at the bottom of the crypts and in the lower two-thirds of the crypt epithelium, whereas the immunostaining was absent in the upper one-third of the crypt epithelium and the lumenal surface epithelium. The CFTR-specific immunoreactivity was detected only at the apical edge of the cryptal epithelial cells, corresponding well with the functional CFTR protein at the apical membrane. The immunostaining in colon was, however, faint and sporadic compared with pancreas that was used as a positive control (Fig. 3).

All UC samples demonstrated the expression of the CFTR protein similar to the normal colon epithelium. Interestingly, some inflammatory samples demonstrated also cytoplasmic expression of the CFTR protein that was not detected in normal control samples.

The SLC26A3 protein expression remains unchanged even in severely inflamed colon mucosa. To study whether the levels of the SLC26A3 mRNA correlate with the protein expression level, Western blotting using similar amounts of differently inflamed colon tissues was performed (Fig. 4A). Antibody against the NH₂-terminal part of the SLC26A3 protein detected a specific band of 85 kDa in all samples, and antibody against the COOH-terminal tail of the SLC26A3 peptide detected two protein bands at \(\approx 85\) and 75 kDa. The intensities of the bands in mildly and severely inflamed samples appeared identical, suggesting that the expression of SLC26A3 remains unchanged even in severely inflamed colon tissue. These results were statistically verified by measuring altogether 30 immunoreactive bands from 10 patients by densitometric scanning of the autographs (Fig. 4B). There was no significant difference \((P = 0.7841)\) in mean protein expression in normal vs. inflamed tissues. The characterization of the subgroups demonstrated also that SLC26A3 expression remains unchanged in all stages of inflamed tissues (Fig. 4B).

The aminoterminal part of the SLC26A3 protein might undergo posttranslational cleaving, because the NH₂-terminal antibody detected only the larger \(\approx 85\)-kDa band corresponding with the predicted molecular

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**Fig. 2.** Relationship between histological degree of acute inflammation in colon samples from normal colon and inflamed areas of patients with UC and mRNA expression of the CFTR (A), SLC26A3 (B), and SLC9A3 (C) measured by quantitative RT-PCR. Scattergrams of all measurements in a logarithmic scale are shown; bars represent the medians of the groups. Degree of statistical significance calculated by the Kruskal-Wallis test is given top left. Statistically significant differences between 4 groups \((^*P < 0.05; ^{**}P < 0.01; ^{***}P < 0.001)\) were calculated using Dunn’s post hoc test.
mass of the SLC26A3 protein. This observation is supported by the previous studies showing that the SLC26A3 protein expressed in COS-1 and Sf9 cells and in normal colonic mucosa had an apparent size of 73–75 kDa, ~10 kDa less than predicted from the deduced amino acid sequence and in vitro translated full-length SLC26A3 cRNA (8, 9, 41). The shorter 75-kDa protein, expressed more abundantly than the 85-kDa protein in all samples, may represent the functional protein as suggested by previous studies (8).

DISCUSSION

Malabsorption of NaCl and water is a well-characterized feature of an inflamed intestinal mucosa and leads to diarrhea (4, 7, 10, 38). The underlying mechanisms may include activation of secretion, reduction of absorption, and a defect in epithelial barrier function (39). We examined the effect of UC on the expression of three intestinal electrolyte transporter genes suggested to account for the major colonic NaCl and fluid movements: SLC26A3, SLC9A3, and CFTR. Functional defects of these genes have been previously associated with diarrheal diseases such as CLD and sodium diarrhea; and a distinct phenotype with lack of secretory response and meconium ileus is seen in CF (19, 25, 40).

Although CFTR has a crucial role in the regulation of the intestinal electrolyte homeostasis, not much is known about its expression or function in inflamed colon mucosa. In general, inflammatory stimulation results both in an increased epithelial permeability and altered patterns of proliferation and differentiation of intestinal epithelial cells (32, 53). Elevated CFTR expression and enhanced cAMP-dependent Cl− secretion have been demonstrated in hyperproliferated mouse intestine (47). As a transepithelial protein on the luminal surface along the intestine, it has a major task in mediating Cl− secretion into the intestinal lumen. Other CFTR-regulated transport functions include inhibition of Na+ reabsorption in intestine and activation of Cl−/HCO3− exchange in at least pancreas and trachea (2, 13, 29, 33, 43, 51). Thus the upregulation of CFTR, as shown in our series of inflamed tissues, might result in both increased secretion of Cl− and decreased absorption of sodium of the mucosa and ultimately contribute to diarrhea.

Fig. 3. Expression of the CFTR protein in normal colon and pancreas and in inflammatory colon. A: immunostaining for the CFTR protein was detected in the apical surface of epithelial cells of lower 2/3 of the normal crypt. The expression was absent in the epithelial cells of the upper 1/3 of the crypt and in the luminal surface epithelium. B: in pancreas, CFTR antibody demonstrated strong immunostaining especially in intercalated ducts as expected. C: higher magnification of B. D: no staining was detected in a serial section of B stained with normal mouse IgG at same concentration. E-G: inflammatory colon showed apical expression of the CFTR protein similar to the normal epithelium, here shown in histologically mild (E), moderate (F), and severe (G) inflammation. G: cytoplasmic immunoreactivity was seen in some inflamed samples, here shown in sample with severe inflammation. A, C, E-G: ×200; B, D: ×100.
Previous studies with intestinal epithelial cells have produced contradictory results, because one group reported a decrease in CFTR expression, and others have observed that CFTR expression is unchanged (6, 11, 32). In our study, the expression of the CFTR protein was detected both in healthy and inflamed colon mucosa by immunohistochemistry, although the overall expression level of CFTR in colon is not abundant. Interestingly, some inflammatory samples demonstrated cytoplasmic expression of CFTR that could not be detected in normal control. It likely corresponds to a newly synthesized or retarded form of the CFTR protein in an intracellular compartment, such as endoplasmic reticulum or Golgi apparatus. Although no significant changes in the levels of CFTR expression were detected in the apical surface of the colonic epithelial cells by immunohistochemistry, the cytoplasmic expression of CFTR might suggest that the translation of the CFTR protein is enhanced in the inflamed colon mucosa.

The CFTR-induced upregulation of the SLC26A3 and SLC9A3 mRNA expression in heterologous systems was reported recently (1, 17, 51). Speculatively, the slight but insignificant increase in the expression of SLC26A3 and SLC9A3 in mildly inflamed intestine could also reflect an increased activation of CFTR. Thus CFTR may play an important role in the pathogenesis of inflammation-associated diarrhea by modifying the function and properties of Cl⁻, Na⁺, and K⁺ channels, the Cl⁻/HCO₃⁻ exchanger, Na⁺/H⁺ exchanger, mucous secretion, and, secondarily, water permeability (28).

The inflamed colonic mucosa contains increased levels of different inflammatory mediators, which are capable of triggering Cl⁻ secretion. Thus the secretion of chloride and water may contribute to the pathogenesis of diarrhea in UC (49, 50). However, there are also several human studies as well as animal models of colitis supporting the idea that diarrhea in IBD is likely due to antiabsorptive rather than prosecretory effects (18). The high-lumen, negative transmucosal electrical potential difference present in normal colon has been shown to be decreased in UC patients, suggesting impaired electrogenic Na⁺ absorption rather than enhanced Cl⁻ secretion (37, 38). In addition, malabsorption of Na⁺ and Cl⁻ has also been associated

Table 1. Clinical data of the patients with UC

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Sex/Age</th>
<th>Duration of UC, y</th>
<th>Recent Treatment</th>
<th>Clinical Severity of UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/46</td>
<td>13</td>
<td>none</td>
<td>mild</td>
</tr>
<tr>
<td>2</td>
<td>M/24</td>
<td>2</td>
<td>corticosteroid, 5-aminosalicylate</td>
<td>severe</td>
</tr>
<tr>
<td>3</td>
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<td>corticosteroid, 5-aminosalicylate</td>
<td>distal moderate</td>
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<tr>
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<td>13</td>
<td>5-aminosalicylate</td>
<td>severe</td>
</tr>
<tr>
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<td>moderate</td>
</tr>
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<tr>
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<td>proximal aggressive, distal</td>
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<tr>
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<td>10</td>
<td>corticosteroid</td>
<td>mild</td>
</tr>
<tr>
<td>9</td>
<td>M/56</td>
<td>6</td>
<td>corticosteroid, 5-aminosalicylate</td>
<td>severe</td>
</tr>
<tr>
<td>10</td>
<td>M/46</td>
<td>13</td>
<td>corticosteroid, 5-aminosalicylate</td>
<td>moderate</td>
</tr>
</tbody>
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UC, ulcerative colitis; M, male; F, female.
with inflamed colonic mucosa (5, 14, 21, 22, 45) as well as a notably diminished activity of basolateral membrane Na\(^+\)-K\(^+\)-ATPase, which is a prerequisite for apical Cl\(^-\) secretion (38). These studies indicate that impaired water absorption secondary to impaired Na\(^+\) and Cl\(^-\) absorption rather than Cl\(^-\) secretion may be a major pathogenic factor in the diarrhea of acute colitis. Therefore, further studies are still required to correlate the putative functional changes in inflamed human colon with the elevated level of CFTR and its putative intracellular redistribution found in this study.

This study supports our earlier findings demonstrating similar expression of the SLC26A3 mRNA in IBD and ischemic colitis when compared with normal colon epithelium (20). In this study, there were no significant changes in the expression of the SLC26A3 and SLC9A3 genes in UC compared with noninflamed tissues when all subgroups were analyzed together. However, there is variation in the individual levels of expression of the genes, especially with SLC9A3, which may reflect alterations in region-specific expression of the genes, because the tissue samples were collected at different locations along the colon. Furthermore, all the patients were treated with glucocorticosteroids known to alleviate intestinal inflammation and modulate the expression of some genes involved in transport processes, e.g., SLC9A3 gene (2, 26).

Characterization of the effect of disease activity on epithelial gene expression revealed slight changes for SLC26A3 and SLC9A3. An initial increase in mild disease was followed by a decrease in moderate and severe UC in case of SLC26A3. SLC9A3 expression increased more than that of SLC26A3 in mild UC but remained unchanged in severe UC. There are several reasons that may be responsible for the reduction of the SLC26A3 mRNA seen in severe UC. It may be secondary, due to a loss of epithelial cells by erosions and ulceration, or due to an expansion of undifferentiated crypt cell compartment. However, no changes in the expression of the SLC26A3 protein was noted even in severe UC colitis. In our earlier study, we showed the expansion of the SLC26A3 expression deeper in the crypts to the cells of the proliferative cell compartment in a set of inflammatory colon samples (20). Similarly, SLC9A3 staining has been suggested to remain unchanged in a colon tissue specimen of an UC patient (52). It is likely that the protein translation machinery in inflamed intestinal cells can compensate small fluctuation in the mRNA level.

A rabbit model of chronic ileal inflammation has demonstrated that the inhibition of coupled NaCl absorption by the villus cells occurs as a result of diminished Cl\(^-\)/HCO\(_3\) but not Na\(^+\)/H\(^+\) exchange activity (44). The mechanism has been shown to be a decrease in affinity for Cl\(^-\) rather than an altered number of transporters (12). Because there was no significant change in the levels of SLC26A3 and SLC9A3 transporters even in severe colitis, it is tempting to speculate that a similar model of inhibition of NaCl absorption might exist in human colon too. However, several different studies using different conditions or models have produced ambiguous results regarding the regulation of the expression of different apical and basolateral transporters (6, 11, 32, 38, 52). Significant differences may occur in different models, and there may be differences even between UC and other types of intestinal inflammation.

In summary, we have determined the relationship between the activity of intestinal inflammation and the degree of the expression of the human CFTR, SLC26A3, and SLC9A3 genes in normal colon and in UC. Intestinal inflammation modulates the expression of these major mediators of intestinal salt transport and may contribute to diarrhea in UC both by increasing transepithelial Cl\(^-\) secretion and by inhibiting the epithelial NaCl absorption.

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