Distinct epithelial responses in experimental colitis: implications for ion uptake and mucosal protection

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The colonic epithelium consists of two major cell types, enterocytes and goblet cells, which play a key role in the maintenance of colonic functions. The enterocytes express specific proteins like carbonic anhydrases (CAs) and sodium/hydrogen exchangers (NHEs) that are involved in colonic CO₂ excretion, intracellular pH regulation, Na⁺ and Cl⁻ absorption and, indirectly, in water transport (5). CA I, an isoform of the CAs, is localized in the cytoplasm of the surface enterocytes (32). CA I catalyzes the reversible hydration of CO₂, providing H⁺ and HCO₃⁻ ions to the apical Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchangers (5). In contrast to CA I, the exact function of CA isoenzyme IV still awaits characterization. CA IV protein is strategically positioned along the apical membrane of the surface enterocytes (14). Therefore, it seems likely that CA IV, similar to CA I, participates in the colonic ion and fluid transport.

The apical NHEs that are responsible for electroneutral Na⁺ absorption in the colon are NHE2 and -3 (6, 22). Both NHE isoforms are expressed by colonic surface enterocytes (3, 4). Together with Cl⁻/HCO₃⁻ exchangers the two NHE isoforms regulate Na⁺ and Cl⁻ absorption. Because water passively follows ion movements, this process is an important factor in colonic water absorption as well. Supportive to the role of NHE3 in colonic water absorption is the fact that NHE3-deficient mice suffer from diarrhea (29).

The colonic enterocytes also express gene products that are assumed to be involved in fatty acid uptake and cellular transport of fatty acids, the fatty acid-binding proteins (FABPs) (1). In the colon, two isoforms of FABPs were identified, intestinal (i-) and liver iFABP. Both types of FABP are expressed by colonic surface enterocytes (30, 31).

Interestingly, colonic enterocytes also express alkaline phosphatase (AP), which is known to detoxify endotoxin and thus plays a significant role in the innate defense of the colonic mucosa (25).

Goblet cells, the second major cell type in the colonic epithelium, express the secretory mucin Muc2 (34), which is the structural component of the mucus layer. Muc2 protein is expressed by crypt and surface goblet cells in the proximal as well as distal colon. After synthesis, Muc2 is secreted into the lumen and forms a

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gel-like mucus layer. This mucus layer serves as a barrier to protect the epithelium from mechanical stress, noxious agents, viruses, and other pathogens (16, 37). Goblet cells are also known to synthesize and secrete trefoil factor family peptide-3 (TFF3), a bioactive peptide that is involved in epithelial repair (21). In the proximal colon, TFF3 protein is expressed by goblet cells in the upper one-third of the crypts and in surface epithelium, whereas in the distal colon, TFF3 protein is observed in goblet cells located in the upper two-thirds of the crypts and in the surface epithelium. Because TFF3 acts as a motogen, i.e., promotes cell migration without promoting cell division, it stimulates epithelial restitution and thus epithelial repair (39).

In healthy colon, the above-described enterocyte- and goblet cell-specific functions are tightly regulated. Yet, during inflammatory diseases like ulcerative colitis (UC) and in experimental colitis, colonic enterocyte and goblet cell functions are altered. For example, in humans with active UC, CA I protein levels and total CA activity were significantly reduced (15). Furthermore, in UC, aberrations in Na+ and Cl− absorption and secretion were observed, suggesting alterations in the expression levels or activity of electrolyte exchangers (12, 18, 26). In experimental colitis, a disruption in colonic electrolyte transport was reported (2). Also, goblet cell-specific Muc2 expression was significantly reduced in humans with active UC (36). In these studies, the colonic epithelium was investigated during chronic inflammation; nevertheless, information on enterocyte and goblet cell functioning during acute inflammation is limited.

In the present study, we investigated cell-type-specific gene expression, as a measure of enterocyte and goblet cell function, in the proximal and distal colon during different phases of acute colitis induced with dextran sulfate sodium (DSS). Enterocyte-specific functions were studied by the analysis of CA I and IV, NHE2 and -3, iFABP, and AP expression. Muc2 and TFF3 expression was analyzed to study goblet cell-specific functions. In conjunction, these data were used to determine the functioning of enterocytes and goblet cells during DSS-induced acute colitis.

MATERIALS AND METHODS

Animals. Eight-week-old, specified pathogen-free, male Wistar rats (Broekman, Utrecht, The Netherlands) were housed at constant temperature and humidity on a 12:12-h light-dark cycle. One week before and during the experiment, the rats had free access to a standard pelleted diet (Hope Farms, Woerden, The Netherlands) and sterilized tap water (controls) or sterilized tap water supplemented with DSS. All of the experiments were performed with the approval of the Animal Studies Ethics Committee of our institution.

Experimental design. Rats were given 7% DSS (37–40 kDa, TdB Consultancy, Uppsala, Sweden) in their drinking water for 7 days, followed by a 7-day recovery period during which DSS was omitted from the drinking water. Fresh DSS solutions were prepared daily. On day 0 (control), day 2 (onset of disease), day 7 (active colitis), and day 14 (regenerative phase), five animals per time point were killed. Segments of the proximal and distal colon were dissected and prepared for light microscopy or were snap frozen in liquid nitrogen and stored at −70°C until RNA and protein isolation. Additionally, to study Muc2 and TFF3 secretion, two tissue explants (10 mm3) of the proximal colon and three explants of the distal colon were cultured in RPMI medium (GIBCO-BRL, Gaithersburg MD) for 4.5 h. Thereafter, the tissue, as well as the culture medium, was collected and homogenized in, or culture medium was mixed with, a Tris buffer containing 1% (wt/vol) SDS and protease inhibitors, as described previously (10, 36).

Immunohistochemistry. Five-micrometer-thick sections were cut and prepared for immunohistochemistry as described previously (38). Briefly, sections were incubated overnight with one of the following enterocyte-specific antibodies: anti-mouse CA I (1:16,000), anti-rat CA IV (1:16,000) (14), anti-rat NHE2 (1:1,500) (4), anti-rat NHE3 (1:1,500) (3), anti-rat iFABP (1:4,000) (8); and the goblet cell-specific antibody WE9 (1:300) (55) to detect Muc2 and anti-TFF3 (1:6,000). Immunoreaction was detected using the Vectastain ABC Elite Kit (Vector Laboratories, Burlingame, UK), and staining was developed using 3,3′-diaminobenzidine.

Histochemistry. Enterocyte-specific AP activity was assayed on colonic tissue sections by use of a one-step assay. Deparaffinized and rehydrated tissue sections were incubated with a Tris buffer (pH 9.5) containing 50 µl of 4-nitroblue tetrazolium chloride (NBT; Vector Laboratories) and 37.5 µl of 5-bromo-4-chole-3-indolyl phosphate (BCIP; Vector Laboratories) according to the manufacturer’s protocol. The color reaction was performed for 1 h in the dark and was stopped with distilled water and mounted with Aquamount Improved (Gurr, Brunschwig, Amsterdam, The Netherlands).

In situ hybridization. Nonradioactive in situ hybridizations were performed according to the method described previously, with slight modifications (19). Briefly, sections were deparaffinized, hydrated, and incubated in the following solutions: 0.2 M HCl, distilled water, 0.1% (wt/vol) pepsin (Sigma, St. Louis, MO) in 0.01 M HCl, 0.2% (wt/vol) glycine in phosphate-buffered saline (PBS), 4% (wt/vol) paraformaldehyde in PBS, and finally in 2× SSC. Until hybridization, sections were stored in a solution of 50% (vol/vol) formamide, 2× SSC at 37°C. For hybridization, cell type-specific probes were diluted in hybridization solution [50% (vol/vol) deionized formamide, 10% (wt/vol) dextran sulfate, 2× SSC, 1× Denhardt’s solution, 1 µg/ml tRNA, 250 µg/ml herring sperm DNA] to a concentration of 100 ng/ml, incubated at 68°C for 15 min and layered onto the sections. Sections were hybridized overnight at 55°C in a humid chamber. Posthybridization washes were performed at 45°C using the following steps: 50% (vol/vol) formamide in 2× SSC, 50% (vol/vol) formamide in 1× SSC and 0.1× SSC. A 15-min incubation with RNase T1 (2 U/ml in 1 mM EDTA in 2× SSC) at 37°C was followed by washes of 0.1× SSC at 45°C and 2× SSC at room temperature. The digoxigenin-labeled hybrids were detected by incubation with antidigoxigenin (Fab, 1:2,000) conjugated to AP for 2.5 h at room temperature. Thereafter, sections were washed in 0.025% (vol/vol) Tween 20 (Merck, Darmstadt, Germany) in Tris-buffered saline, pH 7.5. For staining, sections were layered with detection buffer, pH 9.5 (0.1 M Tris, 0.1 M NaCl, 0.05 M MgCl2), containing 0.33 mg/ml NBT, 0.16 mg/ml BCIP, 8% polyvinyl alcohol (MW 31,000–50,000, Aldrich Chemical, Milwaukee, WI) and 1 mM levamisol (Sigma). The color reaction was performed overnight in the dark and was stopped when the desired intensity of the resulting blue precipitate was reached. Finally, sec-
ions were washed in 10 mM Tris containing 1 mM EDTA, pH 9.5, and distilled water and mounted with Aquamount Im-proved. As control for aspecific binding of probes or for aspe-
cific signal (i.e., endogenous AP activity), the cell type-specific probes were replaced by sense-strand RNA or omitted from the hybridization solution, respectively. No color reaction was seen on sections incubated with these types of control hybridization solution.

Probe preparation for in situ hybridization. Digoxigenin-
11-UTP-labeled RNA probes were prepared according to the manufacturer’s protocol (Boehringer-Mannheim, Mannheim, Germany) using T3, T7, or SP6 RNA polymerase. The follow-
ing enterocyte-specific probes were used: an 890-bp XhoI/
BamHI fragment of mouse CA I cDNA cloned in pBlue-
script KS (17) and a 690-bp XbaI/EcoRI fragment of rat CA
IV cDNA cloned in pGEM4 (13). As goblet cell-specific probes, a 200-bp EcoRI/NotI fragment based on the 1.1-kb
fragment of rat Muc2, as described previously (38), and a
438-bp EcoRI fragment of rat TFF3 ligated in pBlueScript KS
were used (33). Probes longer than 700 bp were hydrolyzed.
Probe detection. The expression of enterocyte and goblet
specific marker was corrected for GAPDH mRNA to correct
for the amount of loading. In addition to the enterocyte- and
oblet cell-specific probes that were used for the in situ
hybridization studies, a 736-bp XbaI/EcoRI fragment of rat
NHE2 cDNA clone ligated in pGEM-7Z, a 359-bp EcoRI/
HindIII fragment of rat NHE3 cDNA clone ligated in
pGEM-4Z, and a 564-bp fragment of rat iFABP cDNA clone
ligated in pBR322 (20) were also used as enterocyte-specific
markers. Average mRNA levels of the cell-type-specific
markers were calculated per segment per rat followed by
calculation of the mean mRNA expression levels of the mark-
ers (± SE) for each of the disease phases studied. When the
above-described probes were used under the same conditions
by Northern blot, a specific band for each of the probes was
obtained.

Statistical analysis. To compare two groups, an unpaired
t-test was used, and to compare three or more groups, anal-
ysis of variance was performed followed by an unpaired
t-test. Differences were considered significant when p 0.05.
Data are represented as means ± SE.

RESULTS

Localization of enterocyte-specific markers. Cell-type-
specific markers were detected in situ at the mRNA
and/or at the protein level by means of in situ hybrid-
ization and immunohistochemistry, respectively. CA I
and iFABP expression levels strongly decrease from mid-
to distal colon (14, 31); thus slight differences in sampling
position of the distal colonic segments might influence
the expression levels of CAI and iFABP. Therefore, CA I
and iFABP were used only as markers for enterocyte function in the proximal colon. CA IV, NHE2, and NHE3 were used as enterocyte markers in both proximal and distal colon.

The mRNA of the enterocyte-specific marker CA I, which is normally expressed in the upper half of the crypts in the proximal colon (Fig. 1A), remained ex-
pressed during the onset of disease (not shown). Dur-
ing active disease, CA I mRNA expression was still
detected at low levels in areas with a normal-appearing
morphology (Fig. 1B) but not in areas with crypt dam-
age and a flattened-surface epithelium (not shown).
During the regenerative phase, CA I mRNA expression
pattern was comparable to that of controls (Fig. 1C).
Similarly, CA I protein, which is normally expressed by
the surface epithelial cells (32), was absent in
the flattened epithelial enterocytes during the active
phase of the disease but reappeared during the regenerative
phase (not shown).

The mRNA and protein of CA IV is expressed by
surface enterocytes in the proximal and distal colon
(Fig. 1, D and G). During and after DSS treatment, CA
IV mRNA expression was seen in the normal-appearing
surface epithelium as well as in the flattened-
surface epithelium of the proximal and distal colon
(e.g., Fig. 1, E and F). Focusing on CA IV protein, we
observed that the flattened-surface enterocytes were
CA IV negative during active disease in the proximal
(not shown) as well as distal colon (Fig. 1H). During the
regenerative phase, CA IV protein expression was seen
in surface epithelium with a normal-appearing mor-
phology and also in some of the flattened-surface cells (Fig. 1).

The expression pattern of NHE2 and -3 was analyzed by immunohistochemistry. Both NHE2 and -3 were expressed in the apical membrane of the surface enterocytes in the proximal and distal colon of controls (Fig. 1I, NHE2). During DSS treatment, NHE2 and -3 proteins were decreased or absent in many surface enterocytes, with a normal-appearing morphology in the proximal and distal colon (not shown). Additionally, most of the flattened-surface enterocytes were also NHE2 and -3 protein negative (Fig. 1K, NHE2). During the regenerative phase, NHE2 and -3 proteins were expressed by all of the enterocytes with a normal-appearing morphology (not shown) and by some of the flattened-surface enterocytes (Fig. 1L, NHE2).

iFABP was expressed in the surface epithelium of the proximal colon (Fig. 1M). During the onset of disease and during active disease, the colonic surface epithelium became iFABP negative (Fig. 1N). During the regenerative phase, most surface enterocytes were iFABP positive again (Fig. 1O).

The in situ AP activity was observed in the brush border of the surface enterocytes in both colonic segments (Fig. 1, P–R; proximal colon). During and after DSS treatment, AP activity remained present in the surface epithelium (Fig. 1, Q and R), even in the flattened-surface enterocytes during active disease and during the regenerative phase. Moreover, during active disease, AP activity seemed to be increased (Fig. 1Q).

Quantitation of enterocyte-specific mRNA and protein expression. The mRNA and/or protein expression of the enterocyte-specific markers CA I, CA IV, NHE2, NHE3, and iFABP was detected and quantified by means of mRNA and/or protein dot blotting. We observed a significant decrease in CA I mRNA levels in the proximal colon during active disease (Fig. 2A). Subsequently, during the regenerative phase, CA I mRNA levels normalized again. Similarly, at the protein level, CA I expression in the proximal colon was significantly decreased during active disease and increased again during the regenerative phase (Fig. 3A). Unlike the mRNA levels, CA I protein levels remained significantly lower than the control levels during the latter phase. The mRNA levels of the other CA isoform, CA IV, were only slightly and not significantly decreased in the proximal colon and were unaltered in the distal colon during DSS-induced disease (Fig. 2). However, CA IV protein levels were decreased during active disease in both proximal and distal colon (Fig. 3). During the regenerative phase, CA IV protein levels increased again in both colonic segments. Despite the increase in CA IV protein levels in the proximal and distal colon during the regenerative phase, the protein levels remained lower than control levels.

The mRNA levels of NHE2 were strongly decreased in the proximal and distal colon during each disease phase (Fig. 2, A and B). Similar to NHE2 mRNA levels, NHE3 mRNA levels significantly decreased in the
proximal colon during each phase of disease. Furthermore, in the distal colon, NHE3 mRNA levels were decreased during active disease and the regenerative phase. The amount of protein in the excised colonic tissue was limited; therefore, NHE2 and -3 levels in the tissue samples were not determined.

Focusing on iFABP, we observed a significant down-regulation of iFABP mRNA and protein levels in the proximal colon during each phase of disease (Figs. 2A and 3A).

**Localization of goblet cell-specific markers.** Recently, we demonstrated that DSS induced crypt loss, ulcerations, and concomitant goblet cell loss in the proximal and distal colon (27). In the present study, we focused on the areas in which the goblet cells remained. In rat controls, the mRNA and protein of the mucin Muc2 are expressed by all goblet cells in the proximal and distal colon (Fig. 4, A and D). During each of the DSS-induced disease phases, MUC2 mRNA and protein expression by goblet cells was observed in areas with elongated crypts as well as in areas with flattened crypt and surface cells. Especially in the distal colon, the elongated crypts mainly contained Muc2 mRNA and protein-positive goblet cells during active disease and the regenerative phase (Fig. 4, B, C, and H).

The mRNA and protein of TFF3 are expressed by goblet cells in the upper half and upper two-thirds of the crypts and surface epithelium in the proximal colon and distal colon, respectively (Fig. 4E, distal colon). During disease, in both the proximal and distal colon, TFF3 mRNA and protein expression extended from surface epithelium toward the lower crypt region, in areas with elongated crypts, and in areas with flattened crypts. In the distal colon, TFF3 mRNA and protein expression were even observed at the crypt bottom during these disease phases, especially in elongated crypts (Fig. 4, F and G).

It is noteworthy that goblet cells positive for Muc2 and TFF3 mRNA and protein accumulated in the surface epithelium in the proximal and distal colon during each of the DSS-induced disease phases (Fig. 4, D and H, Muc2 protein expression in the distal colon).

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Fig. 4. Localization of goblet cell-specific gene expression during DSS-induced colitis. Expression of the mucin Muc2 mRNA (A-C) and protein (D and H) and trefoil factor family peptide-3 (TFF3) mRNA (E-G) in the distal colon of controls (A, D, and E) on day 7 of the DSS treatment (B and F), and on day 14 (C, G, and H). Sections used for immunohistochemistry were counterstained with hematoxylin (D and H). Note the accumulation of Muc2-positive goblet cells in the elongated crypts and surface epithelium on day 14 (H). Bar in A, 110 μm; magnifications of A-C and E-G are similar. Bar in D = 215 μm; magnifications of D and H are similar.
Quantitation of goblet cell-specific mRNA and protein expression. The expression of the goblet cell-specific markers Muc2 and TFF3 was determined and quantified at the mRNA and protein levels by protein and RNA dot blots, respectively. The Muc2 mRNA expression appeared largely unaltered in the proximal and distal colon during the onset of disease and active disease compared with controls (Fig. 5, A and B). In contrast, during the regenerative phase, Muc2 mRNA levels significantly decreased in both colonic segments. Muc2 protein levels showed a slight but not significant increase in the proximal colon during onset of disease and during active disease (Fig. 6A). During the regenerative phase, Muc2 protein levels normalized in the latter segment. In contrast, in the distal colon, Muc2 protein levels were unaltered during onset of disease and active disease but significantly increased during the regenerative phase (Fig. 6B).

Remarkable alterations in TFF3 expression levels were observed at the mRNA as well as protein levels (Figs. 5 and 6). In the proximal colon, TFF3 mRNA was significantly increased during the onset of disease and active disease (Fig. 5A). During the regenerative phase, TFF3 mRNA levels in the proximal colon decreased and were comparable to control levels. TFF3 mRNA expression in the distal colon seemed to be slightly, but not significantly, increased during the onset of disease and active disease (Fig. 5B). Moreover, within the latter colonic segment, a strong and significant increase in TFF3 mRNA expression was observed during the regenerative phase. TFF3 protein levels in the proximal colon were maintained during each disease phase (Fig. 6A). In the distal colon, no alteration in TFF3 protein levels were seen during onset of disease and active disease (Fig. 6B). During the regenerative phase, however, TFF3 protein levels were significantly increased in the latter segment.

Secretion of Muc2 and TFF3. The percentage of Muc2 secretion was calculated as the amount of Muc2...
in the medium divided by the sum of the amount of Muc2 in tissue and in the medium. TFF3 secretion levels were calculated in a similar way as the Muc2 secretion levels. In both proximal and distal colon, Muc2 secretion levels were maintained during each phase of disease (Fig. 7A). In contrast to the unaltered Muc2 secretion levels, TFF3 secretion levels progressively increased during disease in both colonic segments (Fig. 7B). In the proximal colon, a threefold increase in TFF3 secretion level was seen during active disease and the regenerative phase. Moreover, in the distal colon the upregulation of TFF3 secretion was fourfold during active disease and the regenerative phase.

**DISCUSSION**

In the present study, we investigated enterocyte- and goblet cell-specific functions during DSS-induced colitis by measuring cell-type-specific gene expression. The in situ detection of the enterocyte-specific gene products revealed a downregulation of CA I mRNA and protein, CA IV mRNA protein, NHE2 and -3 protein, and iFABP protein in some of the normal-appearing enterocytes and in most of the flattened-surface enterocytes during DSS treatment. In contrast, the enterocyte-specific AP activity is maintained or even upregulated during DSS-colitis in both normal-appearing and flattened enterocytes. These data demonstrate that distinct enterocyte-specific genes are downregulated during the process of epithelial restitution, whereas others are maintained or even upregulated. The downregulation of the CAs, NHEs, and iFABP may indicate loss of enterocyte function, and it may contribute to the pathology seen in DSS-induced colitis. Additionally, because AP activity is known to play a critical role in the innate defense of the intestinal mucosa (24, 25), these data suggest that epithelial defense is maintained or even increased during DSS-induced colitis.

Quantitative analysis revealed remarkable alterations in the enterocyte-specific CA I and CA IV expression during DSS-colitis. Specifically, CA I mRNA and protein levels were reduced during active disease in the proximal colon. During the regenerative phase, both CA I mRNA and protein were normalized again. In contrast, CA IV expression was downregulated only at the protein level, but not at the mRNA level, during active disease in the proximal and distal colon. In both colonic segments, CA IV protein levels increased again during the regenerative phase. These findings suggest that DSS effects on CA I expression were mediated via effects on transcript abundance, whereas effects on CA IV were mediated at the posttranscriptional level. Furthermore, the alterations in CA I mRNA and protein levels during DSS-induced acute colitis are in line with alterations observed in humans with UC. Namely, in patients with active UC, both CA I mRNA and protein were significantly downregulated, whereas in UC in remission, these levels increased again (15). Thus, although DSS-induced colitis is a relatively acute model, whereas UC in patients is chronic, similar alterations in CA I expression levels seem to occur. Unfortunately, data on CA IV expression levels in humans or other experimental colitis models are currently lacking. Nevertheless, what are the consequences of the downregulation of CAs during inflammation? We speculate that, as CA I catalyzes the formation of $H^+$ and $HCO_3^-$ ions (5, 14), downregulation of CA I would lead to reduced levels of $H^+$ and $HCO_3^-$ levels and thus indirectly to a reduction in the activity of the apical Na$^+$/H$^+$ and Cl$^-$/$HCO_3^-$ exchangers. This in turn may lead to reduced NaCl and water absorption. In other words, downregulation of CA I protein levels might contribute to the diarrhea that is observed during DSS-induced colitis (9, 23). Furthermore, because CA IV is likely to participate in the colonic ion and fluid transport as well, the downregulation of CA IV during DSS treatment might also contribute to the induction and/or perpetuation of the DSS-induced diarrhea.

NHE2 and NHE3 were downregulated at the mRNA and protein level during DSS-induced colitis in the proximal and distal colon, suggesting that DSS affects these genes at the mRNA level. Previous studies demonstrate that aldosterone, glucocorticoids, and inter-
feron (IFN-γ) downregulate NHE2 and NHE3 at mRNA, protein, and activity levels in rat colon (7, 28, 40). It is very likely that the DSS-induced downregulation in NHE2 and -3 mRNA and protein leads to decreased activity levels of both exchangers. This, in turn, suggests that DSS-induced diarrhea, i.e., reduced water absorption, might be partly caused by a downregulation of the sodium exchangers. Presently, it remains unclear whether the damage induced by DSS is responsible for the decrease in these CAs and/or NHEs or whether the epithelium actively downregulates the expression of these specific genes to initiate watery diarrhea to expel pathogens and noxious agents like DSS from the intestinal lumen.

Focusing on iFABP, we observed a downregulation of iFABP mRNA and protein levels in the proximal colon during DSS-induced disease, suggesting that, similar to NHE2 and -3, DSS affected iFABP expression at the mRNA level. More generally, these results suggest that, in addition to electrolyte and water absorption, uptake and/or cellular transport of fatty acids were also diminished during DSS-colitis.

In situ hybridization and immunohistochemical studies demonstrated that, despite the DSS-induced changes in epithelial morphology, i.e., crypt and surface cell flattening and crypt elongation, goblet cells continued to express MUC2 and TFF3 mRNA and protein. These findings demonstrate that goblet cells maintain their capacity to express Muc2 and TFF3 during the process of restitution, i.e., flattening of epithelial cells. During active disease and the regenerative phase, TFF3 mRNA and protein expression was extended toward the crypt bottom. Additionally, elongated crypts contained mainly goblet cells positive for Muc2 and TFF3 mRNA and protein. Similarly, goblet cells positive for Muc2 and TFF3 mRNA and protein accumulated in the surface epithelium during each disease phase. Recently, we demonstrated that DSS treatment induced loss of crypt and surface epithelium and concomitant goblet cell loss (27). These data suggest that the DSS-induced loss of crypts and surface epithelium and the ensuing loss of goblet cells in some areas are at least partly compensated by an increase in the number of goblet cells in elongated crypts and surface epithelium in other areas. Taken together, these data emphasize that goblet cells, and particularly Muc2 and TFF3, are of critical importance to maintain epithelial protection and to stimulate epithelial repair during acute inflammation and regeneration, respectively.

Quantitative analysis of goblet cell-specific Muc2 expression revealed pronounced changes in mRNA and protein levels in the proximal and distal colon during DSS-induced colitis. Specifically, both Muc2 mRNA and protein levels were maintained or even upregulated during onset and active disease in both colonic segments. During the regenerative phase, Muc2 mRNA levels decreased in both colonic segments, whereas Muc2 protein levels were comparable to control levels or even upregulated. Additionally, Muc2 secretion levels appeared to be maintained during each of the DSS-induced disease phases in both colonic segments. The downregulation of Muc2 mRNA in conjunction with the retained or even increased Muc2 protein levels during the regenerative phase suggest an increased translation efficacy and/or an altered mRNA/protein stability. More importantly, the maintained or increased Muc2 protein levels in conjunction with the retained Muc2 secretion levels suggest that the thickness of the mucus layer is at least maintained or even increased, offering optimal protection to the colonic epithelium during DSS-induced colitis.

TFF3 mRNA and protein levels were maintained or even increased during each DSS-induced disease phase in the proximal as well as distal colon. Because alterations in TFF3 protein levels were similar to alterations in TFF3 mRNA levels, we conclude that DSS effects on TFF3 expression levels are mediated primarily via transcript abundance. Besides the maintained or upregulated TFF3 protein levels during DSS-colitis, TFF3 secretion levels appeared to increase progressively, indicating that the luminal TFF3 content is increased during each phase of DSS-induced colitis. Presently, information on TFF3 protein expression and secretion in other colitis models is lacking, yet TFF3-deficient mice had impaired mucosal healing and manifested poor epithelial regeneration after DSS treatment (21). Rectal instillation of TFF3 was able to prevent the marked ulceration that occurred after DSS treatment in these TFF3-deficient mice. Furthermore, in an in vitro model of epithelial restitution, the addition of TFF3 to wounded monolayers of confluent IEC-6 cells stimulated epithelial migration (11). In concert, these findings suggest that TFF3 plays a pivotal role in epithelial repair during acute inflammation and that the epithelial repair capacity is enhanced in this acute model of colitis.

In summary, DSS induced a downregulation of CA I, CA IV, NHE2 and NHE3, and iFABP gene expression during active colitis. Downregulation of these genes may account for some of the pathology seen during DSS-induced colitis. Furthermore, enterocyte-specific AP activity was maintained or even upregulated in normal-appearing and flattened-surface cells during active disease, supporting an important role for enterocytes in the innate defense of the mucosa during acute colitis. DSS-induced diarrhea may be largely attributed to downregulation of the CAs and NHEs. In contrast to enterocyte-specific gene expression, goblet cells continued to express Muc2 and TFF3 during DSS-colitis. Moreover, Muc2 and TFF3-positive goblet cells accumulated in the surface epithelium, and TFF3 expression extended from surface epithelium to crypt bottom. Collectively, these data imply that goblet cells play a pivotal role in epithelial defense against luminal substances and pathogens via Muc2 synthesis and secretion and in epithelial repair via TFF3 synthesis and secretion.

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