Effect of Ectopic Expression of Rat Trefoil Factor Family 3 (Intestinal Trefoil Factor) in the Jejunum of Transgenic Mice*

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To further examine the function of the trefoil factor family (TFF), the expression of which is up-regulated at sites of injury, we have produced transgenic mice that chronically express rat TFF3 within the jejunum (using a rat fatty acid-binding protein promoter). The expression of rat TFF3 was limited to the villi of the jejunum and had no effect on base-line morphology. Rat TFF3 expression did result, however, in a reduced sensitivity to indomethacin (85 mg/kg subcutaneously), which only caused a 29% reduction in villus height in transgenics versus 51% reduction in controls (p < 0.01). Indomethacin increased initial intestinal epithelial cell proliferation and migration, but the presence of rat TFF3 caused no additional change in proliferation (bromodeoxyuridine), cell migration (1H)thymidine and bromodeoxyuridine), apoptosis (terminal deoxyuridine nucleotidyl nick end labeling), or E-cadherin immunostaining. In vitro studies following changes in resistance of intestinal strips in Ussing chambers (voltage-clamp technique) showed increased base-line resistance in the rat TFF3-expressing region (326 ± 60 versus 195 ± 48 ohm-cm2 in controls, p < 0.05) and reduced the fall in resistance following HCl exposure by about 40% (p < 0.01). Overexpression of TFF3 stabilizes the mucosa against noxious agents, supporting its role in mucosal protection/repair. It may therefore provide a novel approach to the prevention and/or treatment of intestinal ulceration.

Gastrointestinal mucosal integrity depends on the dynamic equilibrium between aggressive factors such as luminal acid, enzymes, and bacteria and host defense mechanisms such as mucus secretion, rapid cell turnover, and efficient blood supply. When an injury does occur, it is usually rapidly repaired by an initial process involving cell migration (restitution), increased proliferation, and subsequent remodelling.

There is increasing evidence that a group of molecules, termed the trefoil factor family (TFF),1 are involved in gastrointestinal defense and repair, possibly acting by more than one mechanism. Three TFF members have been identified in mammals: TFF1 (previously termed pS2), TFF2 (previously spasmytic polypeptide), and TFF3 (previously intestinal trefoil factor). All three TFF members contain one or two highly conserved trefoil domains, are located clustered within a 50-kilobase sequence on chromosome 21q22.3 (1), and are remarkably resistant to proteolytic digestion (2), probably in part because of their extensive intrachain disulfide cysteine bridging. Under nondamaged circumstances, expression of the three TFF mammalian homologues are geographically distinct. TFF1 and TFF2 are both primarily located in the stomach, whereas TFF3 is predominantly present in the mucous cells of the small and large intestine (3–5). Up-regulation of expression of all three TFF members occurs at sites of damage in conditions such as peptic ulcer and Crohn’s disease (6, 7). The temporal relationship to acute injury varies with each peptide, however, suggesting they may have different pathophysiological roles (7, 8). This idea is also supported by the finding that mice that have had the TFF1 gene functionally deleted (“knock-out”) have a markedly different phenotype (gastric adenomas and carcinomas; Ref. 9) compared with mice who have had TFF2 and TFF3 deleted (giving an essentially normal phenotype under nonstressed situations; Refs. 10 and 11).

To gain further insight into the function of the TFF family and TFF3 in particular, we have now established a model that chronically overexpresses rat TFF3 in the proximal small intestine of mice. We use this model to determine the effect of TFF3 overexpression under basal circumstances and its ability to influence sensitivity to damage using both in vitro and in vivo models of injury.

MATERIALS AND METHODS

Materials were obtained from Sigma Chemicals (Poole, Dorset, UK) unless otherwise stated.

Ethical Approval of Procedures Involving Animals

Genetic modification of animals and all animal procedures were approved by the appropriate local and national authorities. All of the animals were kept on standard chow diet ad libitum and were killed by cervical dislocation.

TFF: FABP, intestinal fatty acid-binding protein; rFABP, rat FABP; PCR, polymerase chain reaction; bp, base pair(s); BrdUrd, bromodeoxyuridine; ANOVA, analysis of variance; KH, Krebs-Henseleit; EGF, epidermal growth factor.
confirmed by immunohistochemical staining using methods described from positions 28 to 586 (kindly donated by A. Gandarillas, Imperial labeled DNA sequence corresponding to the coding region of 18 S RNA. The probe was labeled with $^{32}$P using a blot analyses were performed using a specific 110-bp probe to the radiation (UV Stratalinker 2400, Stratagene, Cambridge, UK). Northern Pharmacia Biotech) by capillary transfer and cross-linked by UV irra-

150 live-born mice, PCR analysis identified one founder mouse that had water, boiled for 20 min, and stored at

Fatty Acid-binding Protein (rFABPi) Promoter FABPi

Expression of rTFF3 peptide was

Parameters and Sensitivity to Indomethacin-induced Small Intestinal Damage

This study was performed to examine the effect of rTFF3 expression on base-line and post-indomethacin morphometry (microdissected villi), proliferation (BrdUrd staining), apoptosis (terminal deoxyuridine nucleotidyl nick end labeling), cell migration up the crypt and villus (using double labeling with BrdUrd and $[^3H]$thymidine), and cell adhesion molecule distribution (E-cadherin immunohistochemistry).

Six groups (n = 6/group) of control and rTFF3 transgenic animals were injected with $[^3H]$thymidine (0.5 mCi/kg, intraperitonealy; Amersham Pharmacia Biotech) 17 h before killing and BrdUrd (50 mg/kg, intraperitoneally) 1 h before killing, i.e. the “double labeling technique,” to determine the differences in proliferation and the cell migration between groups. All animals also received a single dose of indomethacin (85 mg/kg, subcutaneously) at various time points either before or after the $[^3H]$thymidine and BrdUrd, so that at the time of killing, changes in morphology, morphometry, and cell migration could be assessed 0, 6, 12, 18, 24, and 30 h after injection of indomethacin.

Collection of Samples—Following killing, the various sections of the intestine were dissected free, and the weights and lengths of the small and large intestine of transgenic and negative littermates were re-

Immunohistochemistry for rTFF2—Expression of rTFF3 peptide was confirmed by immunohistochemical staining using methods described previously (14). The antibody used for these immunostaining studies, rTFF1#7, is a rabbit polyclonal antibody raised against the C-terminal decapeptide of TFF3 and detects the presence of both the rat and mouse forms of TFF3. The samples were fixed in neutral buffered formalin and embedded in paraffin wax. 4-μm sections were cut onto poly-l-ornithine-coated slides, and then labeled. The slides were incubated with normal goat serum (1:20) for 30 min, primary antibody (1:1000) for 1 h, and biotinylated goat anti-rabbit IgG (1:200) for 30 min. The location of rTFF3 antibody binding was visualized using the avidin-biotin method (Vector), and a brown reaction product was obtained with a peroxidase substrate (diaminobenzidine and phosphate-buffered saline in addition to 0.3% hydrogen peroxide).

Study 2: Influence of Rat TFF3 Expression on Base-line Parameters and Sensitivity to Indomethacin-induced Small Intestinal Damage

Ectopic Rat TFF3 Expression and Intestinal Injury

FIG. 1. Map of rFABPi-rTFF3 construct containing relevant restriction sites. The construct cassette includes nucleotides $^{−1178}$ to $^{+28}$ of the rFABPi promoter. This promoter sequence includes the first 28 bases of the nontranslated region of the FABP. This was linked to a 413-bp sequence of TFF3 derived by reverse transcriptase-PCR from RNA from rat distal small intestine. This component comprises the entire 246-bp coding region of TFF3, which includes the AUG initiation site at position 1 and the TGA stop codon at 244. The sequence also includes the putative polyadenylation signal AAUAAA at position 374. The FABP DNA sequence, inserted at the EcoRI-SmaI site of pUC13, was restricted at the BamHI and HindIII sites to allow the addition of the 413-bp rTFF3 cDNA. The final construct was retrieved by restriction with PviII and Drdl and consists of a 1607-bp sequence comprising 92 bp of pUC13 (between PviII and EcoRI sites), $^{−1178}$ to $^{+28}$ of rFABPi, 7 bp of pUC13 (between SmaI and BamHI sites), and 1–394 of the rTFF3 cDNA.
were hydrated, hydrolyzed, stained with the Feulgen reaction, and transferred to 45% (v/v) acetic acid, and the crypts and villi were teased apart under a stereo dissecting microscope. The tissues were then placed in between the two halves of a modified Ussing chamber (0.2 cm² of exposed area) and bathed on each side with 5 ml of Krebs-Henseleit (KH) solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, and 11.1 mM glucose). Full thickness tissue sections were cut from 20 individual crypts and villi. The highest labeled position was determined for both the [³H]thymidine- and BrdUrd-labeled cells. The number of positively staining cells/crypt and villus. The number of positively stained cells/100 cells was expressed as the apoptotic index (%).

Study 3: Influence of Rat TFF3 Expression on Mucosal Integrity

Determined Using the In Vitro Voltage Clamp Technique

This in vitro method measures the short circuit current (Isc) across mucosal preparations under voltage-clamp (0 mV) conditions. The changes in resistance following challenge with luminal thus provides an indirect method of assessing tissue sensitivity to injurious agents (19).

Transgenic mice and negative littermates (n = 6/group/site) were killed, and four adjacent segments of either jejunum and ileum intestine were placed immediately into Krebs-Henseleit (KH) solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, and 11.1 mM glucose). Full thickness tissue sections were then placed in between the two halves of a modified Ussing chamber (0.2 cm² of exposed area) and bathed on each side with 5 ml of oxygenated (85% O₂, 5% CO₂) KH solution at 37 °C, pH 7.4. Tissues were voltage-clamped at 0 mV, and the resultant basal Isc was recorded.
continuously, on top of which 1-mV voltage pulses were applied at 100-s intervals throughout the period of experimentation. The resultant deflections in the current were recorded, and the resistance (in ohms-cm²) was determined using Ohm’s Law. The mean resistance calculated from six consecutive voltage pulses, 10 min prior to washes with either KH (both sides for 10 min) or an acid wash (50 mM HCl apical and KH basolateral for 10 min) was denoted as 100% for each preparation. Following 10 min of wash (as described above), tissues were bathed once again with fresh KH. The individual resistance measurements were calculated as a percentage of the mean pre-wash values for a further 60 min.

For statistical analyses, the data from all experiments are expressed as the means ± S.E. For the study on base-line parameters and sensitivity to indomethacin, the data were analyzed by two-way ANOVA using the presence of transgene and the time since administration of indomethacin as factors. Where a significant effect (p < 0.05) was found, individuals t tests were performed, based on the group means and residual obtained from the ANOVA, a method equivalent to repeated measures analyses. For the voltage-clamp study, basal I_sc and resistance were compared between the transgenic and control groups using unpaired two-tailed t testing. The changes in resistance following HCl exposure were analyzed by two-way ANOVA using the presence of transgene and the time since acid exposure as factors (zero time point not included as zero variance). Subsequent analyses were performed as for the indomethacin study.

RESULTS

Study 1: Production of Transgenic Mice Using the rFABPi Promoter FABPi-1178 to +28-rTFF3 Fusion Gene

Positive heterozygote offspring were detected using PCR (Fig. 2A). As expected when using this promoter (20), Northern blot analyses (Fig. 2B) and immunohistochemical staining showed that expression of rTFF3 was restricted to the jejunum of transgenic animals. Expression of rTFF3 was limited to enterocytes on the surface of the villi, with the crypts being negative (Fig. 3).

Study 2: Influence of Rat TFF3 Expression on Base-line Parameters and Sensitivity to Indomethacin-induced Small Intestinal Damage

Morphology and Morphometry—The morphology of microdissected villi in the jejunum of control and transgenic animals under base-line conditions was identical, with long, round ended villi (Fig. 4, top panel). The villi obtained from control animals that had received indomethacin were progressively shorter with distal bulbous expansion (Fig. 4, middle panel). This effect of indomethacin was much less marked in the jejunum of transgenic animals (i.e. the rTFF3-expressing region; Fig. 4, bottom panel). Morphometric analyses showed a progressive fall in the villus height of both the jejunum and ileum, reaching a minimum size after 12 h in the jejunum (Fig. 5, upper panel) and 24 h in the ileum (Fig. 5, lower panel). ANOVA for jejunal villus height gave a significant effect for the presence of transgenic DNA (p < 0.001) and administration of indomethacin (p < 0.001), with a significant interaction between transgenic status, administration of indomethacin, and timing (p < 0.001). This showed that the effect of the presence of transgenic DNA varied depending on the presence of indomethacin and its time since administration.

In contrast to the finding in the jejunum, there was no difference in the degree of shortening of the ileal villi between control and transgenic animals caused by indomethacin (Fig. 5, presence of (mouse) TFF3 in the goblet cells of the jejunal villi (initial magnification, ×100). B, in the transgenic animals, additional positivity (because of the presence of rat TFF3) was seen in the enterocytes of the jejunal villi but, as expected using the FABP promoter, did not result in expression in the crypt region (initial magnification, ×37). C, immunostaining of villi of a transgenic animal that have been cut in cross-section showing the enterocytes stained positive for TFF3, whereas the cells and mesenchyme of the villi core were negative (initial magnification, ×100).
ANOVA gave a significant effect caused by indomethacin administration \((p < 0.001)\) but none caused by the presence of transgenic DNA \((p = 0.747)\), with no interaction between transgene and indomethacin \((p = 0.768; \text{Fig. 5, lower panel})\).

For both the jejunal and ileal regions, assessment using villus surface area as the parameter of damage gave similar results to using villus height (data not shown). There was no significant effect on the crypt depths caused by the presence of transgenic DNA or indomethacin administration (data not shown).

**Proliferation, Cell Migration, and Apoptosis**—There was no significant difference in proliferation, as assessed by the number of BrdUrd-labeled mitotic cells/crypt, between transgenic mice and their negative littermates at any time point in either the jejunum or the ileum (effect of presence of transgenic DNA on ANOVA, \(p = 0.514\) for jejunum and \(p = 0.388\) for ileum). Administration of indomethacin caused a significant increase in the number of mitoses compared with base line at 6 and 12 h post-indomethacin administration and was followed by a decrease at 30 h post-indomethacin administration (Fig. 6, upper panel).

Administration of indomethacin also caused a significant increase in the rate of migration compared with base line at 6, 12, and 18 h in transgenic and control animals (Fig. 6, lower panel). However, there was no significant difference in the rate of migration, as assessed by the change in the cell position over 16 h, between transgenic mice and their negative littermates at any time point in either jejunum or ileum (effect of presence of transgenic DNA on ANOVA, \(p = 0.122\) for jejunum and \(p = 0.231\) for ileum).

Base-line apoptotic index of combined crypt and villus values was 1.54% in the jejunum of controls and 2.72% in transgenic animals (group SEM 1.03). ANOVA showed no significant effect of the presence of transgenic DNA \((p = 0.133)\) and no effect...
of indomethacin ($p = 0.299$), with no interaction ($p = 0.314$). Subanalyses of the crypt and villi apoptotic indices also showed no significant differences between groups (data not shown).

E-cadherin Staining—Both the anti-human and anti-mouse monoclonal antibodies used gave identical results; the enterocytes on the villi and within the crypts showed the presence of an adhesion molecule, E-cadherin. Strong basolateral positivity was found along the entire length of the crypt and villi and was not influenced by indomethacin administration or presence of rTFF3 transgene. The section shown is villus tips from a jejunal sample of a transgenic animal not given indomethacin and reacted with a monoclonal antibody against E-cadherin. Initial magnification, ×60; bar, 0.1 mm.

**DISCUSSION**

Ectopic expression of the rat homologue of TFF3 in the jejunum of mice resulted in a normal phenotype under non-damaged circumstances but reduced the amount of initial injury sustained using in vivo and in vitro models. This protective effect of rTFF3 in the transgenic animals was limited to regions that overexpressed the peptide and, based on results from the in vivo experiment, did not appear to be mediated by alteration in proliferation, migration, or apoptosis.

The trefoil peptides form a family of molecules that share a motif comprising six-cysteine residues linked by three intrachain disulfide bonds. This configuration, termed a trefoil or P domain, is distinct from those found in other peptide families such as epidermal growth factor. Three members of this family, TFF1, TFF2, and TFF3, are found in mammals, and the amino acid sequence is highly conserved across species (21). In the normal gastrointestinal tract, TFF1 and TFF2 are predominantly expressed in the stomach, whereas TFF3 is mainly found in the mucus-producing cells of the small and large intestine. In contrast to the region-specific expression of TFF peptides under normal circumstances, up-regulation of all three peptides occurs around sites of injury in conditions such as peptic ulcer and inflammatory bowel disease, leading to speculation that they may be involved in the repair process.

Several lines of evidence now support this idea of a protective/repair role for TFF peptides; the addition of recombinant trefoil peptides has been shown to stimulate migration of intestinal cell lines (2,22), suggesting that they might be involved in the restitution process (23). Furthermore, systemic
administration of trefoil peptides, when given at about 75 μg/ 
rat, reduced indomethacin-induced gastric injury (2). Much 
higher concentrations (1–15 mg/rat) of TFF peptides adminis-
tered intraluminally have also been shown to reduce ethanol-
induced gastric injury (24) and the amount of damage sus-
tained in dinitrobenzene sulfonic acid-induced colitis (25). This 
major difference in dosage requirements between systemic ver-
sus luminal administration has led to the idea that the TFF 
receptor(s) may have a basolateral distribution, similar to that 
found for EGF (26), although an alternative mechanism of action 
for luminal TFF may be through stabilization of the overlying 
mucus layer (27).

Although there is a general consensus regarding the pro-
motogenic activity of the TFF, the literature regarding their 
other actions provides confusing and sometimes apparently 
contradictory results; TFF2 was initially reported to have 
smooth muscle relaxant (“spasmolytic”; Ref. 28) and gastric 
antisecretory effects (28, 29), although more recent publica-
tions refute these reports (2, 30). The TFF peptides have been 
reported to have pro-proliferative activity (31, 32), no effect 
(2, 22, 33), or antiproliferative activity (34) on various cell 
lines in vitro. Similarly, TFF3 has been reported to have 
pro-apoptotic (35) or anti-apoptotic (36) activity when added 
to cell culture of various gastrointestinal cell lines. Loss of 
function (knock-out) mice for the three mammalian TFF have 
now been reported. Gene disruption of TFF2 and TFF3 
causes minimal effects on the phenotype under base-line 
circumstances (10, 11), although loss of TFF3 increases sen-
sitivity to colonic injury, suggesting a role in mucosal de-
fense. Interestingly, loss of TFF1 causes marked gastric hyper-
proliferation, achlorhydria, and the development of gastric 
adenomas and carcinomas (9). This has led to the 
suggestion that TFF1 acts as a tumor suppressor gene or 
anti-proliferative factor, findings that have subsequently 
been supported using a human gastric cancer cell line (34). 
The different phenotypical results from the knock-out stud-
ies, in combination with the marked geographical and tem-
poral differences of TFF expression under normal and dam-
aged conditions (6, 7, 37), suggest that the three TFF 
members may play different if somewhat overlapping roles.

To gain further insight into the function of TFF3, which is 
the major TFF peptide present in the small intestine and 
colon, we have now produced site-specific up-regulation of 
rTFF3 within the jejunum of transgenic mice. The use of the 
rat FABP1/1178 to -29 promoter has the major advantage of 
directing expression only to the proximal small intestine, 
allowing the distal intestine to act as an internal “intra-
animal” control, and removes concerns regarding nonspecific 
effects of genetic disruption. In addition, the use of rTFF3-
positive heterozygote animals for the studies allows rTFF3-
negative littermates to act as additional controls. Two com-
plementary damaging models were used to test these 
animals; indomethacin-induced small intestinal injury in 
vivo and HCl exposure of intestinal strips in vitro. Indo-
methacin was chosen because it is a robust, reproducible 
model that we have used previously to test healing effects of 
other peptide factors (20, 38). In addition, non-steroidal anti-
flammatory drug-induced gut injury is a major source of 
morbidity in humans, and this model therefore has some 
clinical relevance. The complementary use of in vitro studies 
measuring Isc and resistance across intestinal strips in 
Ussing chambers removes some of the potentially confound-
ing variables such as differences in blood flow and systemic 
factors that may influence results in whole animal in vivo 
studies.

Indomethacin causes intestinal injury via multiple mech-
anisms including reduction in mucosal blood flow, reduction 
in prostaglandin levels, and actions on neutrophil function 
(39). The amount of injury sustained in rTFF3-expressing 
regions was markedly reduced compared with controls, with 
the major effect being reduced initial damage rather than 
 quicker repair. This suggests that the presence of the rTFF3 
is able to “stabilize” the mucosa against damaging insults in a 
“cytoprotective” fashion similar to that reported for EGF 
(40). Our previous study examining the effect of indometha-
cin administration on transgenic mice overexpressing human 
TFF1 gave a similar result to that found in the present series 
of experiments (20), suggesting that this is a TFF class effect. 
Detailed analyses of potential mechanisms of action of TFF1 
against indomethacin injury (proliferation, migration, etc.) 
were not, however, performed in these previous experiments. 
In the present studies, we also extended our experiments to 
exclude isolated strips of intestine to gain further insight into 
the potential protective function of TFF members. The 
results from the Ussing chamber assay gave broadly similar 
results to those found in the indomethacin model. The sensi-

![Graph](image_url)

**FIG. 8.** Effect of 50 mM HCl on the jejunal (upper panel) and ileal (lower panel) resistance in transgenic and control mice. Full thickness preparations of either jejunum or ileum from control (○) or transgenic (●) mice were placed in Ussing chambers and voltage-clamped (0 mV), and resistance values were calculated using Ohm’s law from the I, deflections obtained consequent to 1-mV pulses applied at 100-s intervals. For each preparation, six base-line resistance measure-
ments were taken over a 10-min period, averaged, and standardized to 100%. The serial measurement of changes in electrical resistance fol-
lowing a 10-min apical exposure to HCl (50 mM) was then performed as a 
marker of tissue sensitivity to injury. The values are expressed as the 
means ± S.E. from six tissue samples/site. Upper panel, the fall in 
resistance caused by HCl exposure was significantly less (p < 0.01) in the 
jejunum (rTFF3-expressing region) of transgenic animals compared 
with controls at all time points after t = 0. Lower panel, there was no 
difference in the fall of resistance caused by exposure to HCl in the 
ileum (non-rTFF3-expressing region) of transgenic and control animals.
tivity of the jejunal (rTFF3-expressing) region to the damaging effects of HCl was significantly reduced compared with controls, showing that the protective effect of rTFF3 expression was not limited to indomethacin exposure alone. These results indicate that these effects of rTFF3 were likely to be mediated via local rather than systemic mechanisms. In addition, the finding that the base-line electrical resistance in TFF3-expressing samples was higher than controls further supports the concept that the “integrity” of the mucosa is greater in the presence of TFF3.

Analyses of the tissue from indomethacin-treated animals showed that TFF3 expression did not affect proliferation (either before or after indomethacin exposure) or migration up the villi. Most in vitro studies have reported little if any pro-mitogenic activity for TFFs. Both human and rodent TFFs have, however, been shown to be pro-mitogenic in vitro (2, 22), and TFF3 knock-out animals have been reported to have decreased migration of enterocytes up the crypt (10). A possible explanation for the apparent discrepancy between the present findings and previous studies is that because of the promoter used, the expression of rTFF3 was restricted to enterocytes of the jejunal villi once they had left the crypt. If the normal major stimulus for migration of enterocytes in vivo is in the crypts, then any pro-mitogenic activity of rTFF3 would not be able to exert its effects. Cell culture studies examining the effect of the TFF peptides have also reported that they cause a down-regulation of membranous E-cadherin (16). This was not seen in our studies and may reflect species differences (human cell line versus murine tissue) or differences in responses to TFF peptides when tested in vitro as opposed to using whole animal studies. Alternatively, immunohistochemical staining may be too insensitive to detect subtle changes of membranous E-cadherin expression in response to the TFF3 because the in vitro study showing down-regulation of E-cadherin in HT29 cells (colonic cancer line) was only seen when analyzed by fluorescence-activated cell sorting analysis (16). The influence of TFF peptides on apoptosis in vivo is currently unclear; our present studies found no statistically significant difference in the apoptotic index between transgenic and control animals. Previous reports examining the influence of exogenous TFF3 on apoptosis in vitro have reported pro-apoptotic (35) or anti-apoptotic (36) activity.

The exact mechanism by which the strengthening of the mucosa in response to rat TFF3 expression is mediated is unclear, although one possibility is that TFF3 increases tight junction function. This mechanism could explain the increased base-line resistance in the Ussing chamber studies but is difficult to reconcile with the finding of decreased cell-cell interaction (membranous E-cadherin expression) caused when TFF3 is added to HT29 cells in vitro (16). Other mechanisms that have been suggested to explain the cytoprotective effect of EGF, such as alteration in mucosal blood flow and motility, may also be relevant in explaining the effect of TFF3 in vivo. However, they would not explain the increased resistance to HCl shown in the Ussing chamber studies. Further studies are therefore required, including additional efforts to identify TFF receptor(s). Evidence for their presence includes immunoprecipitation and cross-linking experiments that have identified a 45-kDa protein complex from rat intestinal membranes that undergoes tyrosine phosphorylation in response to TFF3 (41) and binding of radiolabeled TFF2 to rat enterocytes after intravenous administration (42). We have previously shown synergistic activity between EGF and the trefoil peptides in vitro and in vivo (43, 44). This interaction between the EGF receptor pathway and TFF peptides is incompletely understood but is probably at the post-receptor binding stage (16). Recombinant rTFF3 causes rapid tyrosine phosphorylation of β-catenin and the EGF receptor in HT29 cells (16). TFF3 has also been shown to inhibit extracellular signal-related protein kinase activity, which is part of the mitogen-activated protein kinase pathway (45).

The use of recombinant peptides to treat gastrointestinal conditions is currently of much clinical interest (46). Advances in gene technology (particularly promoter identification) now allow the potential to direct therapeutic genes to specific areas of interest. Our current findings suggest that mucosal up-regulation of TFF3 can stabilize the mucosa from damaging agents by actions independent of proliferation, migration, and apoptosis. Chronic or inducible expression of additional TFF peptides in the gastrointestinal mucosa might therefore prove to be a novel approach to the treatment of relapsing ulcerative conditions of the bowel. Further work appears to be warranted.

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