Tumour necrosis factor α converting enzyme (TACE) activity in the colonic mucosa of patients with inflammatory bowel disease

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Background: Anti-tumour necrosis factor α [TNF-α] antibodies are effective in Crohn’s disease and perhaps ulcerative colitis but antigenicity and the high cost have raised interest in other strategies to block TNF-α. These include the TNF-α converting enzyme (TACE) which releases soluble TNF-α from transmembrane pro-TNF-α.

Aim: To investigate whether TACE activity is present in human colonic mucosa.

Materials and methods: Detergent extracts of cell membranes from colonic biopsies were obtained from 12 controls and 28 patients with inflammatory bowel disease. Enzyme activity was measured by hydrolysis assays using pro-TNF-α or oligopeptide substrates spanning the known pro-TNF-α cleavage site at Ala76-Val77. Cleavage products were identified by western blotting, high pressure liquid chromatography, or mass spectrometry. TACE protein was localised by immunohistochemistry and identified by western blotting of detergent extracts from purified lamina propria mononuclear cells (LPMNC) or epithelial cells.

Results: Detergent extracts released TNF-α from pro-TNF-α and cleaved a model oligopeptide as predicted. Substrate hydrolysis was sensitive to TACE/matrix metalloproteinase (MMP) inhibitors, but not trocadi which has low activity against TACE. The median TACE level was increased in active ulcerative colitis (1.47 arbitrary units [AU]/mg; p<0.01) but not in Crohn’s disease (81 AU/mg) compared with controls (79 AU/mg). Both the full length proform and the active form of TACE protein were expressed in LPMNC cells and epithelial cells.

Conclusions: Functional TACE activity is ubiquitously expressed in the human colon and increased in ulcerative colitis, raising interest in MMP inhibitors targeting TACE.

Tumour necrosis factor α converting enzyme (TACE) is translated as a 26 kDa type II transmembrane precursor protein which requires specific proteolytic cleavage in the extracellular domain at the Ala76-Val77 bond to release the soluble and presumably biologically active 17 kDa chain. The protease responsible for this cleavage has recently been identified as a membrane anchored multidomain metalloproteinase called TNF-α converting enzyme (TACE). TACE (ADAM 17) belongs to the ADAM (a disintegrin and metalloproteinase) family of cell surface proteins which are involved in diverse functions such as fertilisation, myogenesis, neurogenesis, neutrophil migration, and ectodomain shedding of cell surface proteins like TNF-α. Northern blot analysis has shown strong expression of mRNA for TACE in several organs such as the heart, placenta, testes, ovaries, and small bowel, whereas weaker expression was observed in libraries of a variety of other human organs, including the colon.

Using the more sensitive reverse transcription-polymerase chain reaction technique, we found that TACE mRNA was ubiquitously expressed in human colonic mucosa and that transcript levels were upregulated in IBD. Preliminary data also suggested the presence of TACE activity; however, other matrix metalloproteinases (MMP) present in colonic mucosa can also cleave pro-TNF-α in vitro, and the enzyme responsible for TNF-α release in the colon has not yet been identified. Here, we provide evidence that TACE activity is present in human colonic mucosa and increased in ulcerative colitis.

METHODS

Materials

Oligopeptides with the sequence ac-SPLAQAVRSSSR-NH2 or dinitrophenol (dnp)-SPLAQAVRSSSRSTPS-NH2, corresponding to the known pro-TNF-α cleavage site by TACE at Ala76-Val77 were synthesised by commercial solid phase synthesis (KE Jørgensen, Copenhagen, Denmark) and purified in our laboratory using reverse phase high pressure liquid chromatography (HPLC) on a preparative nucleosil 5 µ C18 column (Pharmacia Biotech, Denmark). Purity was at least 95%, as determined by high pressure liquid chromatography.

Abbreviations: ADAM, a disintegrin and metalloproteinase; AU, arbitrary units; BSA, bovine serum albumin; DMSO, dimethylsulphoxide; dnp, dinitrophenol; EDTA, ethylene-diamoinediacetic acid; EGTA, ethylene glycol-bis-[β-aminoethyl ether]-N,N,N′,N′-tetraacetic acid; GST, glutathione S-transferase; FITC, fluorescein isothiocyanate; HPLC, high pressure liquid chromatography; IBD, inflammatory bowel disease; LPMNC, lamina propria mononuclear cells; MMP, matrix metalloproteinase; MT, membrane type; PBS, phosphate buffered saline; PE, phycoerythrin; SDS, sodium dodecyl sulphate; TACE, TNF-α converting enzyme; TLCK, Nα-p-tosyl-L-hydrolysis chloromethyl ketone; TNF-α, tumour necrosis factor α.
judged by HPLC analysis. Peptides for identification of break-
down products in HPLC analysis were prepared in the same
way. Recombinant human TACE, recombinant glutathione-S-
transferase (GST) pro-TNF-α substrate, and the MMP inhibi-
tors CH4474, BB 94, and trocace, were obtained from Celltech
Chiroscience (Cambridge, UK). A goat polyclonal antibody
against a peptide mapping the carboxy terminus of human
TACE (designated C-15 by the manufacturer) and an epitope
specific blocking peptide were obtained from Santa Cruz Bio-
technology (UK).

Patients
Colonicoscopic biopsies were obtained from 18 patients with
ulcerative colitis and 10 patients with Crohn’s disease accord-
ing to standardised diagnostic criteria.20 21 Fifteen men and
13 females with a median age of 46 years (range 19–72) were
included. Three patients were receiving oral prednisolone
(12.5–30 mg/day) at the time of the study and only one was
receiving azathioprine (150 mg/day). Six patients were receiv-
ing topical treatment with a prednisolone enema (25 mg/day)
or a 5-aminosalicylic acid suppository. Twenty four patients
including 20 21 were receiving azathioprine (150 mg/day). Six patients were receiv-
ing treatment with a prednisolone enema (25 mg/day)
or a 5-aminosalicylic acid containing drug (1–3 g/day). Disease activity was graded as described previously.20 21 The control group consisted of three healthy
controls and nine patients with no signs of neoplastic or inflam-
mmatory disease undergoing routine colonoscopy. Nine
patients were females with a median age of 57 years (range
25–78) were studied. In patients with IBD, biopsies were col-
lected from endoscopically inflamed or non-inflamed colonic
mucosa, or both, using standard biopsy forceps (Olympus,
Japan). Biopsies were immediately washed in isotonic saline
and stored in 200 µl of Tris buffer at –80°C for later detergent
extraction of cell membrane enzyme activity. For western
blotting and immunohistochemical studies, biopsies were col-
llected from a further nine control patients, three males and six
females, with a median age of 54 years (range 25–73). These
biopsies were processed as described below. Permission for
collection of biopsies was obtained from the regional ethics
committee and all participants gave informed and written
consent.

Detergent extraction of TACE activity from cell
membranes
Five biopsies (average 5 mg each) were homogenised by hand
in a glass to glass homogeniser (clearance 0.1 mm) in 250 µl
of Tris buffer (10 mM) at pH 7.5. Crude membranes were
separated by precipitation at 15 000 g, for 20 minutes after a
15 minute incubation period on ice after addition of CaCl2 (10
mM) to a volume of 1 ml. The resulting pellet was dissolved in
50 µl of 1% Nonidet P-40 (Sigma, St Louis, Missouri, USA) at
0–5°C for one hour followed by centrifugation at 15 000 g, for
five minutes. The supernatant used for enzyme analysis is
referred to in the following as detergent extract. For initial
HPLC experiments, a pool of detergent extract was established
from normal colonic tissue obtained in a patient undergoing
surgical resection.

Pro-TNF-α hydrolisys assay
Recombinant human TACE or detergent extract (0.1 ng) was
incubated for 60 minutes at 37°C with a recombinant GST
pro-TNF-α substrate (6 ng) in a total incubation volume of 20
µl. The incubation volume contained the following buffers: Tris
(10 mM, pH 7.5), MgCl2 (1 mM), CaCl2 (0.2 mM), and protease
inhibitors (pepsstatin A (10 µM), leupeptin (10 µM), soybean
trypsin inhibitor (1 µg/ml), trasyol (100 units/ml), and
a-1-antitrypsin (0.2 mg/ml)) (all from Sigma). The inhibitory
effects of ethylene-diamine-tetraacetic acid (EDTA) (5 mM), a
zinc (and metal) chelating agent, and CH4474 (1 µg/ml), a
MMP/TACE inhibitor, were studied in separate experiments.20 21 The reaction was stopped by addition of sam-
ple loading electrophoresis buffer (5 µl) with sodium dodecyl
sulphate (SDS) (2.3%) followed by immediate heating to 95°C.
Electrophoresis was performed by application of 4 µl samples
on a 20% continuous acrylamide gel using the Phast system
(Pharmacia Biotech, Uppsala, Sweden). Western blotting was
performed using electrophoretic transfer onto nitrocellulose
membranes (Biorad, Sundbyberg, Sweden) followed by
inactivation of the nitrocellulose membrane by incubation for
one hour in 2% bovine serum albumin (BSA) (RIA grade; Sigma).
Cleavage products of pro-TNF-α were detected by rabbit-anti-GST
(AH Diagnostic, Aarhus, Denmark) and rabbit-anti-human
TNF-α (Sigma), both diluted 1:4000 in BSA (1%). The secondary
goat-antirabbit antibody (Dako, Copenhagen, Denmark) was
diluted 1:4000 in 1% BSA. Detection was performed with ECL western blotting reagents
(Amersham Pharmacia Biotech, Denmark) following the
manufacturer’s instructions. Gels were photographed using a
Polaroid camera designed for the purpose (Amersham, UK).

A modification of the assay was used in separate
experiments to study the effect of Nα-p-tosyl-l-lysine-
chloromethyl ketone (TLCK), a serine protease inhibitor, on
recombinant TACE activity. This assay was based on direct
detection of substrate breakdown using silver staining to
demonstrate substrate and products. The only other modifica-
tion was an increase in the amount of GST-pro-TNF-α to 0.5
µg. Electrophoresis and silver staining were performed
according to the manual for the Phast system.

Oligopeptide hydrolsisy assays
Substrate peptide with either the sequence ac-
SPLAQAVRSSSR-NH₂ or dnp-SPLAQAVRSSSTTPS-NH₂ (0.5
µg/ml) was added to the incubation medium to start the reac-
tion and incubated at 37°C for 60 minutes. The incubation
buffer and protease inhibitors were the same as described
above. The total incubation volume was 20 µl and the sample
protein concentration ranged from 3 to 9 mg/ml. Freshly dis-
solved TLCK (10 µg/ml), the zink chelating agents, phenantro-
lin (5 mM), and EDTA (5 mM) (without MgCl₂ and CaCl₂), or
one of the MMP inhibitors, CH4474 (1 µg/ml), BB94 (10
µg/ml), or trocace (5 µl), were added to the buffer in some
experiments. CH4474 and BB94 are broad spectrum MMP
inhibitors that also inhibit TACE. In contrast, trocace has on-
ly low activity against TACE but inhibits most MMPs.21 22 CH4474
or BB94 was reconstituted in dimethylsulphoxide (DMSO) at
a final concentration of 0.05%. The same DMSO concentration
was used in parallel control experiments. The reaction was
stopped by addition of 900 µl of HCl (0.1 M). Peptide
degradation was determined by reverse phase HPLC using a
215x4 mm Machery-Nagel MN-C18 5 µ silica column (Mach-
ery Nagel, Germany). Separation was monitored at 215 nm for
unlabelled peptide substrate, at 340 nm for dnp labelled pep-
tide substrate, and expressed as arbitrary units (AU) corre-
sponding to the peak area in the chromatogram.

The position of the breakdown products was identified by
synthetic standards with the predicted sequences for cleavage
in the Ala-Val position. The breakdown products from
the unlabelled peptide substrate were also identified by mass
spectrometry (M-Scan, Oxford, UK). Fractions for this analy-
sis were obtained by fraction collection from the HPLC analy-
sis using peak detection and pooling of samples from 20
analyses. Unpaired and paired data were compared using
non-parametric statistical methods.

Immunohistochemistry
Formalin fixed paraffin embedded tissue sections were
dehydrated in xylene for 10 minutes and rehydrated in ethanol.
After inhibition of endogenous peroxidase by a solution of
hydrogen peroxide in ethanol (30 minutes), sections were
heated at 60°C overnight in 10 mM Tris 0.25 mM EDTA, pH
9.0. Non-specific reactions with endogenous biotin were
bloked using a biotin blocking system (Dako), and other non-specific reactions were blocked using Tris buffered saline (0.05 M Tris/HCl, pH 7.6, 0.15 M NaCl) and cascin 0.5%. The primary goat anti-TACE antibody (1:1000) was applied for 12–20 hours at 4°C. Biotinylated antigen rabbit immunoglobulin was used as a secondary antibody. Finally, avidin DH and biotinylated horseradish peroxidase H reagents (Vector Laboratories, Burlingame, California, USA) were applied and visualised using diaminobenzidine as a chromogen. The reaction was enhanced using biotinylated thymidine followed by further avidin-peroxidase treatment. The nuclei were counterstained with haematoxylin. The specificity of the immunohistochemical analysis was confirmed by substituting the primary antibody with unspecific goat immunoglobulins (Dako) on parallel sections.

**Isolation of epithelial cells and lamina propria mononuclear cells**

Colonic epithelial cells were isolated from 5–7 biopsies obtained from endoscopically normal colonic mucosa using short term (10 minutes) EDTA/EGTA (ethylene glycol-bis (β-aminoethyl ether)- N.N.N’,N’-tetraacetic acid) treatment, as described in detail recently. This method has been shown to release crypts from crypt fragments with less than 5% contaminating cells. The isolated cells were washed with phosphate buffered saline (PBS) and stored until analysis at −80°C in a buffer containing Tris (10 mM, pH 7.5), MgCl2 (1 mM), CaCl2 (0.2 mM), and protease inhibitors (pepsatin A (10 µM), leupeptin (10 µM), soybean trypsin inhibitor (1 µg/ml), trysylol (100 units/ml), and α–1-antitrypsin (0.2 mg/ml)). All reagents were obtained from Sigma.

Lamina propria mononuclear cells (LPMNC) were isolated from 7–10 biopsies, essentially as described previously. Epithelial cells were removed using 60 minute EDTA/EGTA treatment followed by overnight incubation in the presence of 48 U/ml type I collagenase (Sigma) in RPMI 1640 medium (Gibco-BRL, UK). LPMNC were separated by passage through a cell strainer (100 µ nylon mesh; Falcon, UK), and purified by centrifugation on a Lymphoprep gradient (Nycomed, Norway). Cells were either suspended in 100 µl PBS and subjected to fluorescent flow cytometry or suspended in 10 µl protease inhibitor buffer (as above) and kept at −80°C for later western blot analysis. Flow cytometry analysis (FAC-Scan; Becton-Dickinson, Massachusetts, USA) of LPMNC was carried out using fluorescein isothiocyanate (FITC) conjugated or phycoerythrin (PE) conjugated antibodies against CD45 (PE) or CD68 (FITC) (Dako). In a representative experiment, >95% of 50 000 cells counted were CD45 positive. Further analysis showed a total of 42% lymphocytes estimated by forward scatter and side scatter (cell size and granulation) and 32% monocytes/macrophages.

**Western blotting of TACE protein**

Colonic epithelial cells or LPMNC were homogenised in 1% Nonidet P40 (Sigma) in a total volume of 50 µl, which included the same protease inhibitor cocktail as described in the cell isolation procedure. 1 mM EDTA and phenyl methyl sulphonyl fluoride (Sigma). After incubation for 60 minutes at 0°C, undissolved membranes were removed by centrifugation and the supernatant was used for SDS electrophoresis. In order to ensure sufficient amounts of protein, cells were pooled from 2–5 individual patients. Total protein (50 µg) was separated by electrophoresis on a 4–12% SDS gel and transferred onto ethanol activated polyvinylidene difluoride membranes (Amersham Pharmacia Biotech). The membranes were blocked for one hour in Tris 10 mM, NaCl 0.15 M, and 0.05% Tween-20 supplemented with 2% BSA and incubated overnight at 4°C with anti-TACE antibody diluted 1:5000. After additional washes in buffer, the membrane was incubated for one hour at room temperature with horseradish peroxidase conjugated goat rabbit antibody (Dako) diluted 1:20 000. Detection was performed with ECL western blotting reagents (Amersham Pharmacia Biotech) following the manufacturer's instructions. Gels were photographed using a Polaroid camera designed for the purpose (Amersham, UK). In control experiments the TACE antibody was incubated for two hours at room temperature with an epitope mimicking blocking peptide prior to incubation with membrane protein.

**RESULTS**

**Demonstration of TACE activity in colonic biopsies using a recombinant pro-TNF-α assay and an unlabelled oligopeptide hydrolysis assay**

Figure 1 (A, B) shows that detergent extract of cell membranes isolated from normal colonic mucosa cleaved a recombinant GST-pro-TNF-α substrate into a GST fragment and a TNF-α fragment, as identified by antibodies and standards. The GST fragment was sometimes seen as a double band, probably due to the action of other proteases. Similar results were obtained when recombinant TACE was used to cleave the substrate, and both processes were sensitive to inhibition with EDTA (fig 1A, B) and CH4474, a TACE/MMP inhibitor (fig 1C). To determine the precise cleavage site of pro-TNF-α, a TACE-like activity in detergent extracts, an assay based on cleavage of an unlabelled synthetic peptide mimicking the known pro-TNF-α cleavage site was devised. As shown in fig 2 (A, B), the oligopeptide substrate was cleaved by detergent extracts yielding two products identified by HPLC. Also, this process was inhibited by EDTA and CH4474 (fig 2C, D). The molecular weight of the resulting peptides produced by cleavage of the model substrate deviated <2% from predicted. This indicates that cleavage occurred between Ala(76) and Val(77) in the corresponding pro-TNF-α sequence. Deviation should be >10% if cleavage shifts one amino acid.

**Characterisation of TACE activity in human colonic mucosa using a dnp labelled oligopeptide hydrolysis assay**

A modified assay allowing detection at 340 nm was required to study the effect of additional protease inhibitors as some of these interfered with HPLC detection of unlabelled peptide breakdown due to high absorbance in the relevant concentration ranges (data not shown). The reaction was linear for both
MMPs, but not TACE (fig 3). At least two bands, which may be due to differences in glycosylation, were identified by synthetic standards (A), (B) Magnification of product formation. (C, D) Inhibition by EDTA (5 mM) and CH4474 (1 µg/ml). Similar results were obtained in five further independent experiments.

The amount of detergent extract added and for time up to one hour (data not shown). As summarised in fig 3, EDTA and CH4474 also inhibited cleavage of the dnp labelled oligopeptide substrate mimicking the pro-TNF-α cleavage site. TACE activity was quantitated by high pressure liquid chromatography and expressed relative to protein concentration. Mean values of three individual experiments of material pooled from individual patients are shown. Samples contained dimethylsulphoxide (DMSO) 0.05% (*), which was necessary for solubilisation of the inhibitors. Controls with (*) and without DMSO are shown. TLECK, Nα-p-tosyl-L-hydroxylamine chloromethyl ketone.

Localisation and expression of TACE protein in human colonic mucosa

Using immunohistochemistry, we found that TACE appeared to be predominantly expressed in mononuclear cells of the lamina propria and in the epithelium (fig 6A, C). No significant staining was seen in control sections stained with a goat immunoglobulin (fig 6B, D). To corroborate this finding, western blotting was performed on detergent extracts of cell membranes from epithelial cells and LPMNC isolated from normal human colonic biopsies. The full length proform as well as the active form of TACE with molecular weights of 94 AU/mg, range 67–123, n=6) (NS). In contrast, the median TACE level in patients with ulcerative colitis (147 AU/mg, range 73–337, n=12) compared with controls (94 AU/mg, range 67–123, n=12) (p<0.01). Breakdown according to the grade of clinical activity showed that the median TACE level was higher in those with clinically active ulcerative colitis (147 AU/mg, range 73–337, n=12) compared with controls (p<0.01) but not in those with inactive disease (94 AU/mg, range 67–123, n=6) (NS). The amount of the primary antibody was preincubated with an epitope specific blocking peptide.

Measurement of TACE activity in colonic mucosa from patients with IBD

The dnp labelled oligopeptide assay was used to measure TACE activity in IBD patients because sample interference was avoided, which simplified analyses. The median TACE activity level was significantly increased in patients with ulcerative colitis (118 AU/mg, range 67–337, n=18) compared with controls (79 AU/mg, range 60–130, n=12) (p<0.01). Breakdown according to the grade of clinical activity showed that the median TACE level was higher in those with clinically active ulcerative colitis (147 AU/mg, range 73–337, n=12) compared with controls (p<0.01) but not in those with inactive disease (94 AU/mg, range 67–123, n=6) (NS).
or ulcerative colitis disease (90 AU/mg, range 70–136, n=10) (NS). However, separate analysis of paired biopsies from five patients with ulcerative colitis and one with Crohn’s disease showed that median TACE levels were slightly higher in endoscopically inflamed areas (77 AU/mg, range 66–144) compared with normal mucosa (65 AU/mg, range 27–106) (p<0.05).

**DISCUSSION**

In this study, we found that detergent extracts of cell membranes from normal human colonic mucosa cleaved a full length pro-TNF-α substrate and released TNF-α. As release of TNF-α was sensitive to both EDTA and a TACE/MMP inhibitor, we concluded that biopsies contained TACE-like activity (fig 1). Next, we found that detergent extracts also cleaved a synthetic peptide mimicking the hydrolysis site for TACE in the pro-TNF-α sequence at amino acids 76–77. From these data we concluded that biopsies contained TACE-like activity that cleaves a model peptide for the human TNF-α precursor at the correct site (fig 2). Finally, we used a dnp labelled oligopeptide assay to study the effect of inhibitors. The effect of these was as predicted (fig 3) with the

**Figure 5** Sodium dodecyl sulphate (SDS) electrophoresis showing hydrolysis of glutathione-S-transferase-pro-tumour necrosis factor α (GST-pro-TNF-α) by recombinant TNF-α converting enzyme (TACE). Samples were analysed on continuous SDS polyacrylamide gels (20%) using silver staining. Lane 1: GST-pro-TNF-α substrate; lane 2: recombinant TACE, t=0 minutes; lane 3: recombinant TACE, t=60 minutes; lane 4: recombinant TACE, t=60 minutes + Nα-p-tosyl-l-lysine chloromethyl ketone (TLCK 10 µg/ml). Similar results were obtained in two further independent experiments.

**Figure 6** Immunohistochemistry of tumour necrosis factor α (TNF-α) converting enzyme (TACE) protein expression in normal human colonic mucosa. (A) (×20) and (C) (×80) show that TACE was widely expressed in lamina propria mononuclear cells and in crypt epithelial cells. (B) (×20) and (D) (×80) show the corresponding background staining when the primary antibody was substituted with unspecific goat immunoglobulins. Examples of cells regarded as positive for TACE are indicated by arrows (C). Similar results were obtained in two independent experiments.

**Figure 7** Western blotting of tumour necrosis factor α (TNF-α) converting enzyme (TACE) protein immunoreactivity in detergent extract of membranes from intact biopsies (lane A), isolated epithelial cells (lane B), or purified lamina propria mononuclear cells (lane C). The arrows indicate the proform (p) and active (a) forms of TACE. Lanes D and E show the absence of immunoreactivity when an epitope mimicking blocking peptide was present during incubation. The TACE antibody was the same as that used in fig 6. Similar results were obtained in three independent experiments.
widely expressed in LPMNC protein in human colonic mucosa. TACE protein was, as expected, of TACE cell fractions expressed the proform as well as the active form human colonic epithelial cells or LPMNC showing that both by western blotting of cell membrane protein from purified human colonic epithelium. The function of TACE protein expressed in human colonic epithelial cells.

The presence of TACE protein in human colonic epithelial cells has been demonstrated in arthritis affected cartilage as well as in synovial tissue of patients with rheumatoid arthritis. TACE activity was also present in Crohn’s disease mucosa but not increased compared with controls (fig 8). This contrasts our previous finding of a correlation between TACE mRNA expression and Crohn’s disease activity and one possibility is that inactivation of TACE activity has been catalysed in Crohn’s disease mucosa by other MMPs as part of a self-regulatory mechanism similar to that described for MT1-MMP–MMP-2 interactions. Increased mRNA expression, but not increased enzyme activity, would be detected in such a setting. Recently, phorbol 12-myristate-13-acetate, a potent inducer of shedding, has shown to downregulate TACE expression in vitro, and since the effect was inhibited by a MMP inhibitor, shedding processes appear to be under feedback control. Whether a similar downregulatory mechanism is operative in vivo in Crohn’s disease mucosa, but not in ulcerative colitis, remains purely speculative at this stage.

Inhibition of TACE activity strongly downregulates T cell release of soluble TNF-α in vitro, which may lead to accumulation of the membrane bound precursor thought to be biologically active. Shedding of regulatory soluble TNF-α receptors is also compromised and TACE/MMP inhibition may in theory carry a proinflammatory potential. However, a recent study showed that oral administration of a potent MMP/TACE inhibitor to healthy human subjects strongly reduced lipopolysaccharide induced TNF-α release without influencing the level of monocyte bound TNF-α. Synthetic MMP inhibitors have been shown to downregulate inflammation in rat models of arthritis and IBD and to protect against endotoxic shock in mice by preventing TNF-α processing. Although the MMP inhibitors tested have a variable ability to block sheddase enzyme activity, the data make it less likely that this class of drugs has a general proinflammatory effect in vivo. Shedding of -selectin and transforming growth factor α is also compromised in TACE deficient mice and MMP inhibitors reduce the release of the soluble Fas ligand by T cells in vitro, but the exact clinical significance of these effects remains unclear.

In conclusion, we have shown that functional TACE activity is ubiquitously expressed in normal or Crohn’s disease colonic mucosa and upregulated in ulcerative colitis. Secretion of TNF-α is definitively increased in IBD and drugs targeting the final stage of TNF-α processing may therefore be of benefit. As TACE is structurally related to the family of MMPs, which have been strongly implicated as final mediators of TNF-α-induced proteolytic gut mucosal damage, our data may be helpful in creating a rationale for therapeutic intervention in ulcerative colitis using specifically tailored synthetic MMP inhibitors that also target TACE.

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