

Increased expression of transforming growth factor α precursors in acute experimental colitis in rats

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Abstract

Background and aim—Epidermal growth factor (EGF) and transforming growth factor α (TGF- α), members of the EGF family of growth factors, protect rat gastric and colonic mucosa against injury. Having shown previously that exogenously applied EGF protects rat colonic mucosa against injury, the aim of the present study was to evaluate the endogenously expressed ligand mediating the protective effect of EGF/TGF- α in vivo.

Methods—In an experimental model of trinitrobenzene sulphonic acid (TNBS)/ethanol induced colitis in rats EGF and TGF- α expression was evaluated using a ribonuclease protection assay, northern blot analysis, western blot analysis, and immunohistochemistry.

Results—TGF- α mRNA increased 3–4 times at 4–8 hours after induction of colitis and returned to control levels within 24 hours. TGF- α immunoreactive protein with a molecular size of about 28 kDa representing TGF- α precursors increased markedly after induction of colitis with a peak at 8–12 hours. No fully processed 5.6 kDa TGF- α protein was detected in normal or inflamed colon tissue. Only a weak signal for EGF mRNA expression was detected in the rat colon and no EGF protein was observed by immunohistochemistry or western blot analysis.

Conclusions—TGF- α precursors are the main ligands for the EGF receptor in acute colitis. It is hypothesised that TGF- α precursors convey the biological activity of endogenous TGF- α peptides during mucosal defence and repair.

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Keywords: transforming growth factor alpha (TGF- α); epidermal growth factor (EGF); precursor molecules; colitis; rat

The epidermal growth factor (EGF) family consists of several peptide hormones which share the ability to bind to a common receptor.^{1–4} Among the members of the EGF family, EGF and transforming growth factor alpha (TGF- α) have been studied extensively and both were identified previously in the human^{5–6} and rat gastrointestinal tract.^{6–8} EGF was first isolated from mouse submandibular glands as a 53 amino acid peptide¹ and TGF- α

was first isolated from the medium of retrovirus-transformed fibroblasts⁴ as a 50 amino acid peptide. TGF- α shares a 30% homology with EGF.⁹ Both EGF and TGF- α are synthesised as large membrane anchored precursors. The EGF precursor has a molecular weight of 150–180 kDa¹⁰ while TGF- α precursors are smaller with molecular weights between 18 and 68 kDa.^{11–12} Size heterogeneity of the TGF- α species results from differential proteolytic cleavage and N- and O-glycosylation.^{11–13–14} Although enzymes responsible for proteolytic processing of TGF- α precursors in vivo are unknown, the mature 50 amino acid TGF- α peptide can be enzymatically cleaved in vitro from the precursor molecule by elastase.¹⁴ Both EGF and TGF- α membrane anchored and soluble precursor molecules are able to bind and activate the EGF receptor.^{10–15} The membrane anchored TGF- α precursor has been shown to interact with EGF receptors on adjacent cells in vitro.¹⁵

EGF and TGF- α are potent mitogens for certain types of cells of the gastrointestinal tract in vitro¹ and in vivo.¹⁶ Besides their mitogenic effects, members of the EGF family of growth factors are able to modulate cell migration,¹⁷ mucosal blood flow,¹⁸ gastrointestinal motility,¹⁹ mucus production and secretion,²⁰ and gastric acid secretion.²¹

TGF- α mRNA and immunoreactive peptide have been identified in human and rat colonic mucosa,^{5–6–8} and EGF immunoreactivity has been detected in human and rat colon.^{5–7} In patients with Crohn's disease expression of EGF immunoreactivity has been described around colonic ulcers.²²

The role of the EGF family of peptides in mucosal defence and repair after injury of the colon is currently under investigation. Using an experimental model of colitis in the rat, we have shown that EGF given exogenously prior to the induction of colitis protects the colonic mucosa.²³ These results indicate that the EGF family of peptides plays an important part in mucosal protection in the early phase of colitis.

The aim of our present study was to determine the endogenously expressed ligand for the EGF receptor in the early phase after colonic injury using the same animal model of colitis.

Methods

EXPERIMENTAL DESIGN

Male Sprague-Dawley rats with body weights of 250–300 g were fasted overnight with free

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access to drinking water. Animals were given 50 mg/kg trinitrobenzene sulphonic acid (TNBS) in 50% ethanol as enemas to induce colitis as described previously.²³ Three animals were sacrificed before and at 2, 4, 8, 12, and 24 hours after induction of colitis. Representative colonic tissues were processed for evaluations of histological tissue damage, myeloperoxidase activity, and TGF- α and EGF expression.

Evaluation of histological tissue damage

Specimens were fixed in zinc formalin, embedded in paraffin wax blocks, cut in 6 μ m thick sections, and consecutively stained with haematoxylin and eosin. The extent of tissue damage was evaluated microscopically and macroscopically as percentage oedema, erosions, and ulcers as described previously.²³

Myeloperoxidase assay

Tissues were homogenised in 0.5% hexadecyltrimethylammonium bromide (Sigma, St Louis, Missouri, USA). Myeloperoxidase activity was measured photometrically as changes at 470 nm after adding the substrate peroxide and compared with a standard dilution with horseradish peroxidase. Protein contents were estimated by the Biuret method and myeloperoxidase activity was expressed as U/mg protein.²⁴

Preparation of rat TGF- α , EGF, β -actin, and 18S probes

Rat EGF and TGF- α cDNA fragments were obtained by reverse transcription and polymerase chain reaction (RT-PCR). Sense and antisense primers were designed and synthesised based on the published sequences.^{25, 26} Rat submandibular gland and liver total RNA were prepared and used as templates to generate a 430 bp EGF and a 156 bp TGF- α cDNA by RT-PCR, respectively. Both cDNA fragments were subcloned into plasmid pBlueScript II and multiple positive clones were sequenced from both directions to ensure no PCR infidelity.

EGF primers: sense 5'TGGAAAAGATGGCTGCCACTGGGTC and antisense 5'GTGTTTCTCTAGGACCACAAACCA.

TGF- α primers: sense 5'GCAGTGGTGTCTCACTTCAA and antisense 5'CACTGCCAGGAGATCTGCATGCTC.

Rat β -actin was donated by Dr D Cooper, Harbor UCLA Medical Center, Division of Pulmonology, and human 18S cDNA probe was donated by Dr T M Gress, University of Ulm, Germany.

Plasmids containing TGF- α , EGF or β -actin inserts were linearised by appropriate restriction enzymes and incubated with appropriate RNA polymerase in transcription buffer containing ³²P-UTP (ICN, Biomedicals Inc, Costa Mesa, California, USA) using a standard in vitro transcription procedure (Promega, Madison, Wisconsin, USA). The human 18S cDNA probe was excised with EcoRI and random labelled using ³²P-CTP (Pharmacia, Piscataway, New Jersey, USA). Final cRNA and cDNA products were extracted by phenol-chloroform and precipitated with alcohol to remove unincorporated radioactive UTP.

Preparation of total cellular RNA and poly-A RNA

Total cellular RNA was extracted by the acidic guanidium thiocyanate method as described previously.²⁷ In order to purify poly-A RNA biotinylated oligo(dT) was annealed in solution to mRNA and the hybrids were captured with streptavidin-coated paramagnetic particles in a magnetic separation stand (PolyA-Tract, Promega, Madison, Wisconsin, USA).

Ribonuclease protection assay

Nuclease protection assays were performed as described previously²⁸ and were used to quantitate the TGF- α and EGF mRNA before and after induction of colitis. Samples containing 15 μ g total RNA were annealed with 1–2 \times 10⁶ disintegrations per minute (dpm) of labelled probe for 16 hours at 45°C in buffer containing 80% formamide. After sequential digestion of hybrids with ribonucleases A and T1 (Boehringer Mannheim, Indianapolis, Indiana, USA) and proteinase K (Boehringer Mannheim) the RNA was extracted with phenol-chloroform, precipitated with 2.5 volumes of ethanol, washed with 70% ethanol, and dissolved in 99% formamide and sequencing dye. After heating for three minutes at 90°C the samples were briefly cooled on ice and applied to a 8% acrylamide in 8.3 M urea DNA sequencing gel. After electrophoresis, the gel was dried and exposed to a Kodak XAR 5 film at –70°C with a DuPont Lightning Plus intensifying screen (DuPont, Boston, Massachusetts, USA) for 3–6 days. The size of protected fragments was determined by including a DNA sequencing ladder in adjacent lanes. RNA abundance was calculated using scanning laser densitometry (BioRad, Hercules, California, USA) with the integrated area expressed as the average of five individual determinations.

Northern blot analysis

Poly A-RNA (2–4 μ g) was electrophoresed in a 1% formaldehyde agarose gel and transferred to a nitrocellulose membrane (ICN, Biomedicals Inc, Costa Mesa, California, USA). Membranes were prehybridised in 7% sodium dodecyl sulphate (SDS), 1 mM EDTA pH 8, 1% bovine serum albumin (BSA) in 0.5 M sodium phosphate, pH 7.2, containing 100 μ g/ml denatured salmon sperm DNA for one hour. After prehybridisation either denatured EGF or TGF- α cRNA or 18S cDNA probes were added (10⁶–10⁷ cpm/ml hybridisation solution) and membranes were hybridised overnight. After hybridisation membranes were washed in 0.5 \times sodium chloride, sodium citrate (SSC) and 0.1% SDS at room temperature and x-ray films were then exposed for two to four days using intensifying screens at –70°C.

Antibodies used for western blotting and immunohistochemistry

Both a monoclonal antibody raised against the amino acid sequence 34–50 of the fully processed 5.6 kDa human TGF- α peptide (Oncogene, New York, USA) that cross reacts with rat TGF- α and a polyclonal antibody

(Pro-Hormone Science, Los Angeles, California, USA) raised against rat TGF- α were used for immunohistochemical analysis. The polyclonal antibody was used in western blot analysis. A polyclonal antibody raised against rat EGF (Pro-Hormone Science) was used to perform immunohistochemical analysis. The polyclonal TGF- α antibody did not cross react with EGF on western blot analysis. No cross reaction of TGF- α antibodies with EGF was observed in immunostaining of rat submandibular glands. The rat EGF antibody stained the submandibular gland intensively (data not shown).

Immunohistochemistry

Intact colonic rings were fixed overnight in Zamboni's solution,²⁹ washed in 0.1 M phosphate buffered saline (PBS), pH 7.5, containing 25% sucrose for cryoprotection, and then frozen in OCT compound. Sections 12 μ m thick were cut using a cryomicrotome. Immunohistochemical analysis was performed using the ABC technique as described previously.³⁰ In brief, on the day of the assay sections were allowed to thaw and were washed in 0.1 M PBS containing 0.3% Triton-X 100. Tissues were incubated with normal goat serum (30% in 0.1 M PBS v/v) and incubated either with the polyclonal (1:750) or with the monoclonal (1:50) TGF- α antibody or with the polyclonal rat EGF antibody (1:750) for 12–15 hours at 4°C. After washing the sections in 0.1 M PBS/0.3% Triton-X 100, tissue sections were incubated with secondary antibody (goat anti-rabbit IgG or goat anti-mouse) for two hours at room temperature. Endogenous peroxidase was quenched in ice cold methanol with 1% hydrogen peroxide. Binding was visualised after incubation with avidin biotin complex with hydrogen peroxide in the presence of diaminobenzidine (DAB). Sections were counterstained with haematoxylin and mounted with Permount medium. All pictures were taken with a Zeiss photomicroscope using a Kodak colour print film.

Western blot analysis

Colonic tissues were homogenised (250 mg/ml w/v) in 20 mM Tris-HCl buffer, pH 8.8, containing EDTA (10 mM), NP-40 (0.5%), and sodium deoxycholate (0.5%) using a tissuemiser (Tekmar, Ohio). The homogenates were diluted 1:5 with Laemmli's electrophoretic sample buffer³¹ containing β -mercaptoethanol and heated in a boiling water bath for 10 minutes. Samples were cooled, centrifuged, and aliquots equivalent to 3 mg wet tissue were subjected to electrophoresis on a 15% SDS separating gel. The proteins were electrophoretically transferred to Immobilon-P membranes (Millipore, Milford, Massachusetts, USA) and immunoblotted after incubation with the polyclonal antiserum to rat TGF- α (1:1000) or with the polyclonal antibody to rat EGF (1:1000). Immunoreactive bands were detected by autoradiography after labelling with ¹²⁵I-goat anti-rabbit IgG. For reference synthetic rat TGF- α (Bachem, Philadelphia, Pennsylvania, USA) of known

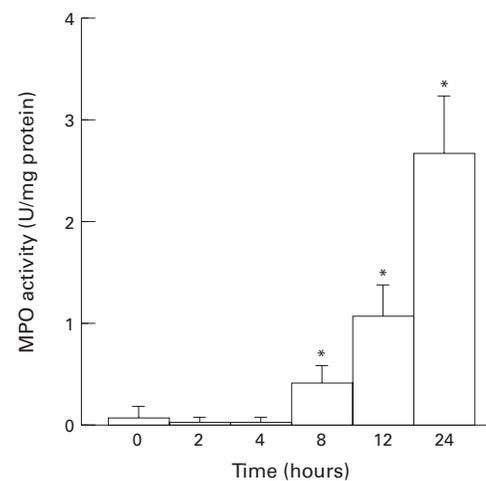


Figure 1: Myeloperoxidase (MPO) activity in U/mg protein before and up to 24 hours after induction of colitis ($n=6$).

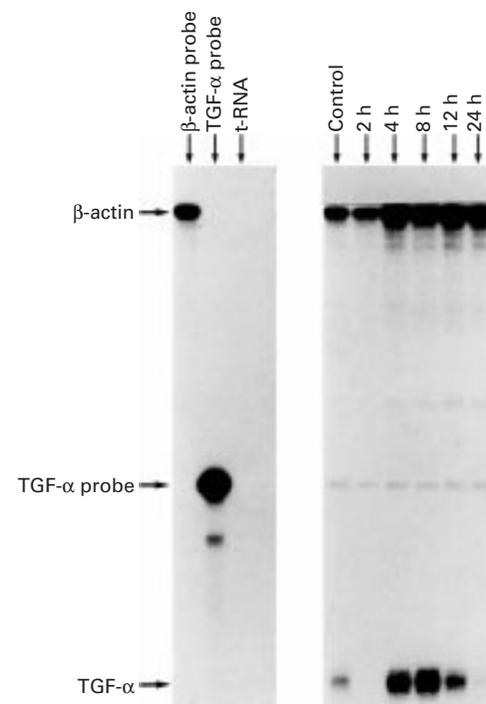


Figure 2: Nuclease protection assays analysing expression of TGF- α mRNA before (control) and at 2, 4, 8, 12 and 24 hours after induction of colitis. 15 μ g of total RNA per sample was loaded in each lane. In lanes 1 and 2 the synthesised TGF- α and β -actin probes were loaded which were slightly longer due to extension of the probe by parts of the copied polycloning sites of the host plasmids. Note the marked increase in TGF- α mRNA expression at 4 and 8 hours after induction of colitis and the increase in β -actin expression after induction of colitis.

quantity was loaded in a separate lane on each gel. Non-specific binding was determined by incubating corresponding membranes either with secondary antibody alone or with different non-immune rabbit sera. Membranes were exposed to x-ray film for four to seven days.

Statistical analysis

The quantities of EGF, TGF- α , and β -actin RNA from ribonuclease protection assays and TGF- α immunoreactivity on western blots were calculated using scanning laser densitom-

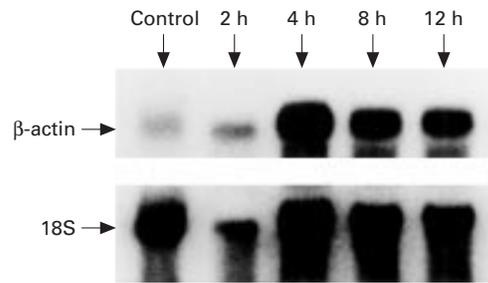


Figure 3: Comparison of β -actin and 18 rRNA expression before and at 2–12 hours after induction of colitis on northern blot analysis. A marked increase in β -actin mRNA is seen during colitis.

etry (BioRad, Hercules, California, USA) with the integrated area expressed as the average of five individual determinations. The unpaired Student's *t* test was used for comparison of the data and a *p* value of <0.05 was considered significant.

Results

EXPERIMENTAL COLITIS

Irritation of the colonic mucosa with 50% ethanol in TNBS resulted in a severe inflammation that involved all layers of the colon. At 24 hours 80% of the exposed mucosa showed erosions but no ulcers were detected. Numerous inflammatory cells and severe tissue oedema were present in the colon. Myeloperoxidase activity expressed by neutrophil granulocytes and macrophages increased markedly within the first 24 hours (fig 1).

TGF- α and EGF mRNA expression

Within the first two hours after mucosal injury TGF- α mRNA expression decreased significantly compared with uninflamed colon. At four and eight hours after induction of colitis TGF- α mRNA increased 3–4 times over the levels of uninflamed colon and returned to basal levels of TGF- α mRNA expression after 12–24 hours (fig 2). Northern blot analysis performed with poly-A RNA extracted from uninflamed and inflamed colon tissue revealed a 4.5 kb transcript known to encode TGF- α (data not shown).

β -actin mRNA expression in inflamed colonic tissue was also increased compared with uninflamed colonic tissue and was therefore

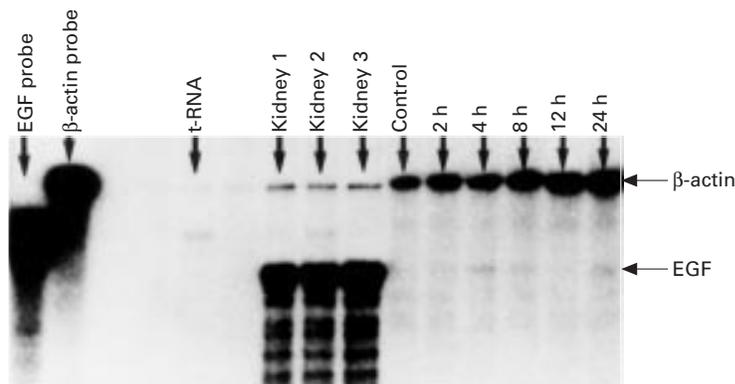


Figure 4: Nuclease protection assay analysing expression of EGF mRNA in normal and inflamed colonic tissue. Weak protected bands were detected in both normal and inflamed tissue. Abundant EGF mRNA was present in kidney extracts.

not suitable as a housekeeping gene (fig 2). 18S ribosomal RNA expression was comparable to uninflamed control colons at 4–12 hours, the time points when a marked increase in expression of β -actin mRNA was seen (fig 3). The pattern of 18S rRNA and β -actin mRNA expression was observed consistently in our experiments, making it unlikely that differences in the amounts of total RNA used in the ribonuclease protection assays were responsible for the observed increase in the expression of TGF- α . Increased expression of housekeeping genes in different experimental settings has also been described by others.³²

Figure 4 shows the expression of EGF mRNA in normal and inflamed colonic tissue up to 24 hours after induction of the colitis. After prolonged exposure of the *x*-ray film weak protected bands of EGF mRNA became visible. No changes were detected before and after induction of colitis. Marked expression of EGF mRNA was detected in kidney extracts. Note that about 10 times less RNA isolated from kidney was used as a positive control to perform nuclease protection assays compared with RNA extracted from colonic tissues due to the expected high expression of EGF mRNA in the kidney. No EGF mRNA was detected on northern blots (data not shown).

Immunohistochemistry for TGF- α and EGF

Figure 5 shows TGF- α -like immunoreactive staining in colonic tissue before and at different time points after mucosal injury. In uninflamed colon TGF- α -like immunoreactivity was predominantly found in the upper half of the crypts and in the luminal surface epithelium (fig 5A). Within the first two hours after mucosal injury the upper half of the mucosa became necrotic and TGF- α -like immunoreactive staining disappeared (fig 5B). Whereas some of the damaged areas remained necrotic and presumably will turn into ulcers, TGF- α -like immunoreactivity reappeared in remaining epithelial cells at the bottom of the crypts and in epithelial cells of areas adjacent to necrotic areas (fig 5C). Between 8 and 12 hours TGF- α -like immunoreactivity was detected in epithelial cells along the entire crypts (fig 5C) and in migrating epithelial cells (fig 5D). At 24 hours after induction of colitis TGF- α -like immunoreactivity was again more pronounced in the upper half of the crypts (fig 5E).

No differences between staining with the monoclonal anti-TGF- α antibody and polyclonal anti-TGF- α antibody were detected. The staining with both antibodies was completely abolished after overnight preabsorption of either antibody with excess rat TGF- α (figs 5F and G).

EGF-like immunoreactivity was not detected in either normal or inflamed colonic tissue.

Western blot analysis for TGF- α

Figure 6A shows a representative western blot of homogenates of control and inflamed colon. In every experiment standard rat TGF- α was loaded in a separate lane (fig 6B). Figure 6C demonstrates the efficiency of the method used

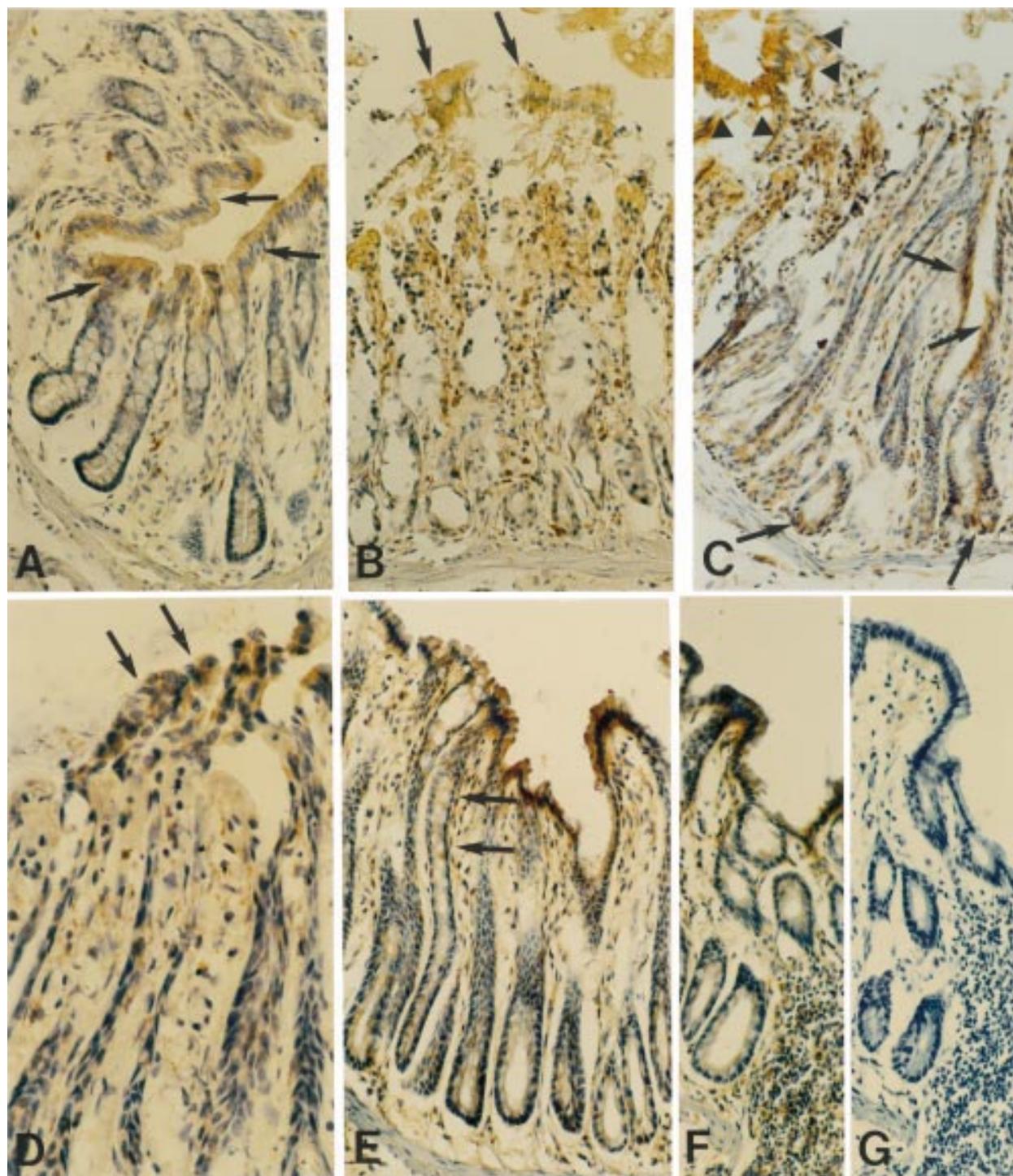


Figure 5: TGF- α -like immunoreactive staining in colonic tissue before and at different time points after mucosal injury. (A) In uninflamed colon TGF- α -like immunoreactivity was predominantly found in the upper half of the crypts and in the luminal surface epithelium. (B) Within the first two hours after mucosal injury the upper half of the mucosa became necrotic and TGF- α -like immunoreactive staining disappeared. (C) While some of the damaged areas remained necrotic and presumably will turn into ulcers, TGF- α -like immunoreactivity reappeared in remaining epithelial cells at the bottom of the crypts and in epithelial cells of areas adjacent to necrotic areas. Between 8 and 12 hours TGF- α -like immunoreactivity was detected in epithelial cells along the entire crypts (C) and in migrating epithelial cells (D). (E) Twenty four hours after induction of colitis TGF- α -like immunoreactivity was again more pronounced in the upper half of the crypts. (F, G) The staining with both antibodies was completely abolished after overnight preabsorption of either antibody with excess rat TGF- α .

to detect 250 ng synthesised rat TGF- α . The majority of TGF- α -like immunoreactivity was found in bands of about 28 kDa. A 5.6 kDa band corresponding to mature TGF- α was not detected in either control or inflamed colon. Compared with uninflamed colon, TGF- α -like immunoreactivity disappeared within two

hours after induction of colitis but was increasingly expressed at 8–12 hours of inflammation. TGF- α levels returned to basal expression at 24 hours after induction of colitis. Figure 7 shows a comparison of the expression of TGF- α mRNA and TGF- α -like immunoreactivity. As can be seen, expression of TGF- α

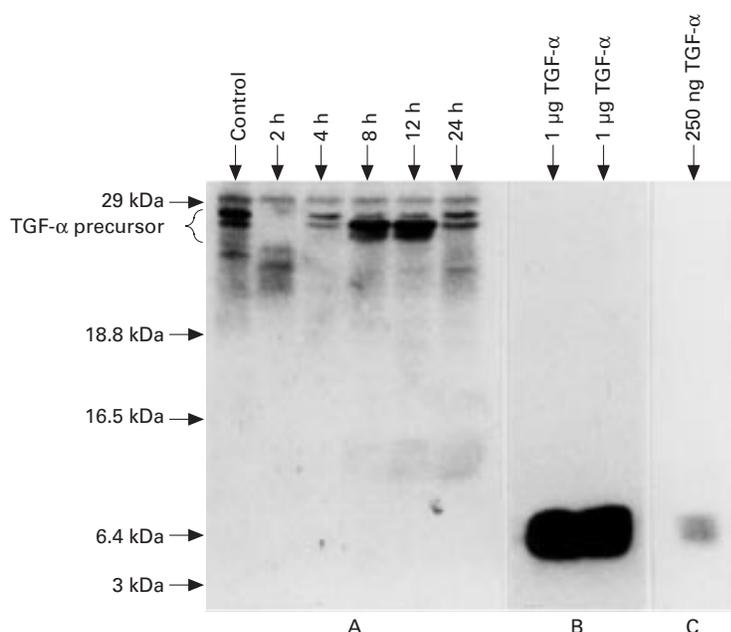


Figure 6: TGF- α protein expression on western blot analysis before and after induction of colitis. The same polyclonal antibody was used as for immunohistochemical analysis. No immunoreactive band was found in the molecular size of mature processed 5.6 kDa TGF- α . Immunoreactive bands were found with a higher molecular weight of about 28 kDa. Additional weak non-specific bands were seen which also appeared after incubation of corresponding blots with different non-immune rabbit sera.

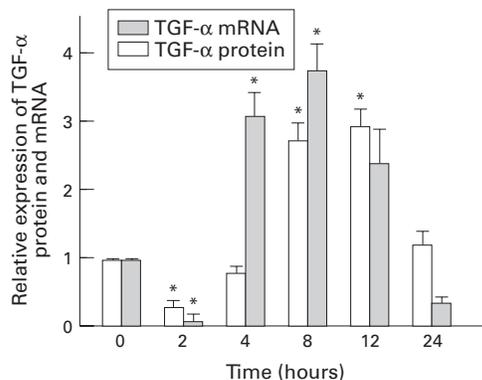


Figure 7: Comparison of TGF- α protein ($n = 6$) and mRNA ($n = 3$) expression during the first 24 hours of colitis by western blot analysis and nuclease protection assays, respectively.

protein increased slightly later than TGF- α mRNA during colitis.

No immunoreactive EGF was found by western blot analysis in tissue extracts of normal or inflamed colon.

Discussion

In response to acute mucosal injury expression of TGF- α mRNA and protein levels increased significantly. Although small amounts of EGF mRNA were detected on nuclease protection assays, no increase was seen during the course of colitis. Furthermore, no EGF protein was found by immunohistochemical or western blot analysis in the rat colon, suggesting that locally produced EGF is not a key component of mucosal repair after acute injury. Having shown previously that activation of the EGF receptors by exogenously administered EGF protected colonic mucosa against ethanol and TNBS induced injury,²³ we now hypothesise

that TGF- α is the locally expressed member of the EGF family that mediates the capability of EGF to maintain mucosal integrity and to accelerate restitution after injury. The importance of TGF- α in mucosal protection is supported by recent studies with mice deficient in TGF- α (waved 1 mice) where mucosal damage after application of oral dextran sulphate was significantly more pronounced in TGF- α deficient mice than in controls.³³ Increased expression of TGF- α , but not EGF, was also reported by Polk *et al* using experimental ulcer models in the rat stomach.³⁴ Of interest, in the experimental stomach ulcer model as well as in our experimental model of colitis TGF- α expression increased early within six hours after injury, underlining the importance of endogenous TGF- α for mucosal protection and restitution. In contrast, Alison *et al*³⁵ found an abundant expression of both EGF and TGF- α in rat stomach tissue and an increased and prolonged expression of both EGF and TGF- α after cryoprobe induced injury. In this experimental setting EGF expression was increased from days 1–10 after induction of injury and TGF- α expression was increased from day 6 after injury. It is therefore possible that EGF and TGF- α are expressed simultaneously in the rat stomach and that the pattern of TGF- α expression is dependent on the way mucosal injury is induced and the additional expression of EGF. We cannot exclude the possibility that, in the colon, other members of the EGF family such as amphiregulin³ and heparin-binding epidermal growth factor² participate in the mucosal response to injury. It is also possible that luminal EGF may play an additional role in protecting or healing colonic mucosa after injury.

Although we do not have direct evidence from the present study for a TGF- α induced biological effect, the mechanisms of action of TGF- α could involve mucus production and release in response to injury,²⁰ induction of TGF- β expression leading to cell migration and epithelial cell restitution,¹⁷ increase in prostaglandin production,³⁶ inhibition of inducible nitric oxide synthase leading to diminished NO production,³⁷ increased mucosal blood flow,¹⁸ and cell proliferation and differentiation.¹ This is a matter for further study.

Wright *et al*²² reported an increase in EGF immunoreactivity in humans that was associated with a cell lineage involved in the repair of chronic ulcerations in the stomach and intestine of patients with peptic ulcer and inflammatory bowel disease. These findings support the hypothesis that EGF family peptides are important in mucosal repair. It is possible that in humans EGF, and not TGF- α , is the key mediator of mucosal repair in response to injury. However, it will be interesting to study concomitantly the expression of different EGF family members' mRNA and protein levels in patients with gastrointestinal ulcerations to characterise further the interactions of the peptides of the EGF family. To our knowledge our study is the first to combine these

techniques to characterise EGF/TGF- α expression in response to mucosal injury.

Of interest was our observation that the TGF- α precursors are not metabolised into the mature soluble 5.6 kDa TGF- α protein after injury. This suggests that in rat colonic mucosa the biological action of TGF- α peptides is mediated by large TGF- α molecules produced by epithelial cells. These molecular forms could still be membrane anchored and act locally on the same or adjacent cells, favouring the assumption of an autocrine or juxtacrine pathway. In fact, it has been shown that soluble and membrane bound TGF- α precursors are biologically active by binding and activating the EGF receptor on adjacent cells *in vitro*.¹⁵ Another explanation for the fact that no mature processed 5.6 kDa TGF- α was detected in our experiments could be that the 5.6 kDa TGF- α metabolite was rapidly removed into the lumen after cleavage from the precursor. However, when the luminal content of inflamed colon at 8 or 12 hours was homogenised and subjected to western blot analysis only the larger molecular forms, but no 5.6 kDa TGF- α , were present (data not shown). Our finding that TGF- α precursors are the predominant molecular forms of TGF- α in the inflamed intestine is supported by a recent report in human fetal intestine where only TGF- α precursors with molecular weights of 30 and 68 kDa—and not the 5.6 kDa TGF- α —were found on western blot analysis in intestinal extracts.³⁸ Our report is the first to show that, after acute mucosal injury, TGF- α precursors, and no mature 5.6 kDa TGF- α protein, were found. Our data strongly support the hypothesis that the biological activity of TGF- α is mediated by TGF- α precursors.

In uninfamed colonic mucosa TGF- α is expressed predominantly in epithelial cells in the upper half of the crypts and in epithelial cells of the luminal surface. In response to injury these surface cells became necrotic and were shed into the lumen. At this time TGF- α protein and mRNA expression fell markedly as assessed by nuclease protection assay and western blot analysis. At 8 and 12 hours of inflammation, when peak expression of TGF- α mRNA and protein was obtained, TGF- α was localised in the remaining intact epithelial cells of the crypts and in entire crypts in areas adjacent to the damaged mucosa. With restoration of the mucosal integrity TGF- α expression was again predominantly found in cells in the upper half of the crypts and the surface epithelium and TGF- α expression returned to levels of uninfamed colon.

The signal for the upregulation of TGF- α in response to mucosal injury is unknown. Increased TGF- α gene expression occurred within 4 and 6 hours and prior to the maximum influx of inflammatory cells, suggesting that mucosal injury by itself—and not mediators released from inflammatory cells, especially neutrophils—are responsible for this effect.

In summary, the marked expression of TGF- α on the levels of mRNA and protein early after mucosal injury suggests a pivotal

role for TGF- α within the kindred of the EGF family responsible for mucosal protection and restitution. Detection of TGF- α precursors and not of the fully processed 5.6 kDa TGF- α protein in response to injury indicates that soluble and/or membrane bound TGF- α precursors mediate the actions of TGF- α and thus favour the assumption of an autocrine or juxtacrine pathway.

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