Immunoreactivity of transforming growth factor alpha in the normal adult gastrointestinal tract

D M Thomas, M M Nasim, W J Gullick, M R Alison

Abstract

The immunolocalisation of transforming growth factor alpha (TGF α) in the normal human oesophagus and the gastrointestinal tract was elucidated using two different antibodies – Ab2, a monoclonal antibody reacting with part of the human TGFα molecule, and 26T a rabbit affinity purified polyclonal antibody raised against part of the rat TGFα peptide. There have been conflicting reports on the distribution of this growth factor in the gut. The results clearly showed that this peptide regulatory factor is confined to the luminal surface and foveolae in the stomach, restricted to the villous epithelium in the small intestine, and in the colon was seen in the upper one third of the crypt. This pattern indicates that the distribution of this peptide regulatory factor is within the differentiated compartment and indicates a role in differentiation besides its well known proliferative effects.

Transforming growth factor α (TGF α) is an acid heat stable, 50 amino acid peptide of 5600 Daltons first isolated from an extract produced by cells in culture transformed by retroviruses. It was subsequently found to possess the ability to transform normal rat kidney cells and to stimulate tumber formation in the mouse. It is structurally related to epidermal growth factor (EGF) and shares the same cell surface receptor (EGFR), a 170 kDa glycoprotein that has sequence homology with the v-erb B oncogene. Having been identified in a number of neoplastic tissues as well as in mouse embryos and the human placenta, TGF α was once thought to be absent from normal adult tissue. However, it has recently been shown to be expressed in skin and in normal breast tissue. Using radioimmunoassay, Cartilage and Elder have shown its presence in normal human gastrointestinal mucosa. Bennett et al found low levels of TGF α mRNA in most of a series of normal human stomach mucosal samples and differential expression has been shown in rat intestinal epithelial cells.

We have used two antibodies to characterise immunohistochemically the expression of TGF α in the normal adult human oesophagus and gastrointestinal tract.

Methods

Formalin fixed, paraffin embedded blocks of normal adult oesophagus (n=5), stomach (n=25), small intestine (n=10), and colon (n=15) were selected from the files of Hammersmith Hospital. Two antibodies reacting with TGF α were used. The first was commercially available monoclonal antibody (TGF α; Ab2; GF10; Oncogene Science) directed against the COOH terminus (residues 34–50) of the human TGF α peptide. This antibody has been fully characterised elsewhere. The second antibody was an affinity purified rabbit polyclonal antibody (26T) raised against the C-terminal 17 amino acids of mature rat TGF α peptide. This antibody has also been fully characterised elsewhere. The primary antibody to EGF was a rabbit polyclonal antibody against recombinant human EGF/urogastrone (URO), and the staining protocol has been described elsewhere. Controls omitting the primary antibody and controls absorbing the primary antibody with recombinant EGF/URO were also performed.

immunohistochemistry

Four µm sections were mounted on poly-L-lysine coated slides, air dried overnight at room temperature, dewaxed, and rehydrated by passage through xylene and graded alcohols. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in distilled water applied for 30 minutes. Sections were postfixed in either Zamboni’s (for Ab2) or Bouin’s (for 26T) fixative for 25 minutes and then incubated with 1:20 dilutions of normal swine serum and normal goat serum respectively for 15 minutes before the application of the primary antibodies at dilutions of 1:180 (that is, 5-5 µg/ml) for Ab2 and 1:20 (36 µg/ml) for 26T. Sections were incubated overnight at 4°C. Biotinylated swine anti-rabbit immunoglobulin (Dakopatts, UK) and biotinylated goat antimouse immunoglobulin (Dakopatts, UK) were then added at dilutions of 1:500 and 1:200 respectively for half an hour followed, after washing three times with phosphate-buffered saline (PBS), by a streptavidin complex (Dakopatts, UK) for 30 minutes. The slides were then developed with diaminobenzidine, counterstained with Mayer’s haematoxylin and mounted in DPX. PBS and 1:1000 normal swine serum were used as negative controls for the monoclonal and polyclonal antibodies respectively and, in addition, the specificity of staining of the primary antibodies was verified by preincubation of primary antibodies with their cognate peptides. Normal human placenta was used as a positive control.

Results

In all cases, suprabasal oesophageal squamous stratified epithelium showed weak cytoplasmic staining while basal cells remained unstained. In the stomach, all cases showed strong, diffuse ---

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Results

In all cases, suprabasal oesophageal squamous stratified epithelium showed weak cytoplasmic staining while basal cells remained unstained. In the stomach, all cases showed strong, diffuse

**Department of Histopathology and ICRF Oncology Group, Royal Postgraduate Medical School, Hammersmith Hospital, London**

D M Thomas
M M Nasim
W J Gullick
M R Alison

Correspondence to:
Dr M R Alison, Department of Histopathology, Royal Postgraduate Medical School, University of London, Du Cane Road, London W12 ONN.

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cytoplasmic and membrane immunoreactivity within epithelial cells at the luminal surface and in cells lining the gastric pits, with some weaker immunoreactivity in cells at the base of the gastric gland: cells within the isthmic, proliferative zone did not stain (Figs 1 and 2). There was an apparent gradient of increasing expression from the neck region to the luminal surface. In specialised mucosa, parietal cells consistently showed stronger immunoreactivity than chief cells. In the small intestine, villous enterocytes showed abundant immunoreactivity with a negative gradient towards cells lining the crypts, which were completely unstained (Fig 3). Paneth cells showed no immunoreactivity for TGF α, nor did Brunner's glands in the duodenum. There was no ileal Peyer's patch or lymphocyte staining. Similarly, in the colon there was a gradient from stronger positivity at the surface to a total lack of staining at the bases of crypts (Fig 4). Immunolocalisation of both TGF α antibodies was similar, though the polyclonal antibody produced more background staining. All negative controls (either replacing the primary antibodies or absorbing the primary antibodies with the immunising peptides) showed abolition of staining. Staining with the antibody against EGF/URO was found at only two sites within the tissues studied, namely in Brunner's glands of the duodenum where there was an abundant immunoreactive EGF/URO (Fig 5) and also in the mucous neck cells of the gastric glands (Fig 6). Staining was abolished by the appropriate negative controls.

Discussion

This study shows that TGF α is expressed ubiquitously throughout the normal adult oesophagus and gastrointestinal tract. Furthermore, it was apparently localised to those areas which, in the normal adult, do not undergo cell division - that is, immunoreactivity was seen almost exclusively within the differentiated, non-proliferative zones of oesophageal, gastric, small intestinal, or colonic mucosa.

The mucosa of the gastrointestinal tract is an example of labile tissue - that is, tissue that in the normal adult constantly and rapidly undergoes cell division, differentiation, and cell loss. This rapid turnover demands a precise mechanism of control to maintain the equilibrium between proliferation and differentiation. The selective localisation of expression of TGF α to the differentiated compartments of the gastrointestinal tract, together with its known mitogenic and morphogenic properties, strongly suggest that this peptide is involved in such a control mechanism. TGF α is known to potentiate the cellular proliferation effects of gastrin and to limit ethanol induced gastric injury in rats. In addition, TGF α has been implicated in the upward migration of keratinocytes in another labile tissue, the skin, and it could fulfill a similar role in the gut.

Our finding of consistently abundant TGF α immunoreactivity in parietal cells is consistent with the work of Beauchamp who showed by northern blot analysis higher levels of TGF α in guinea pig parietal cell enriched fractions than in chief cell fractions. TGF α is known to inhibit gastric acid secretion and its acid stable nature makes it a suitable candidate for a role in the physiological control mechanisms of gastric acid secretion.
Figure 3: Transforming growth factor α expression in the small intestine showing immunoreactivity confined to the villous epithelium with the crypts being totally negative. (Original magnification ×100.)

Figure 4: Transforming growth factor α expression in the colon showing strong immunoreactivity in the upper two thirds of the crypts and on the luminal surface; the bottom one third of the crypts are totally unstained. (Original magnification ×100.)

Figure 5: Brunner's glands showing abundant immunoreactive epidermal growth factor/urogastrone. (Original magnification ×200.)

Figure 6: Epidermal growth factor/urogastrone expression confined to mucous neck cells in the gastric gland with no staining in the foveolar epithelium (cf transforming growth factor α in Figures 1 and 2). (Original magnification ×200.)

Reports on the distribution of EGF in the normal human gastrointestinal mucosa have been somewhat conflicting. Poulsen et al.13 reported its presence in Paneth cell granules besides Brunner's glands, while Elder et al.16 found EGF immunoreactivity in antral glands and some surface goblet cells in the duodenum and jejunum. Together with the data presented in this study, it is clear that the distribution of EGF is very limited within the gastrointestinal tract, and that TGF α is more likely the natural ligand for the epidermal growth factor receptor, which is confined to the laterobasal membranes of villous enterocytes.11 One intriguing possibility is that there is a gradient of TGFs towards the proliferative compartment in the gastric gland and intestinal mucosa, thus limiting the size of the differentiated compartment (for example, foveolus or villus). Indeed, in normal rat kidney fibroblasts, retinoic acid, a well known inducer of differentiation, stimulates EGF receptor gene expression.17 Further support for the notion that TGFs/EGF is involved in gut differentiation comes from the fact that EGF promotes nutrient absorption in the rabbit jejunum.18

We are grateful to Nick Wright for the EGF data.

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