Immunoelectron microscopic localisation of transforming growth factor alpha in rat colon

R Pérez-Tomás, X Cullerè, M Asbert, C Díaz-Ruiz

Abstract
Transforming growth factor alpha (TGF α) is a polypeptide, which binds to the epidermal growth factor receptor to carry out its function related to cell proliferation and differentiation. The ultrastructural localisation of TGF α was studied in both the proximal and the distal colon. The columnar cells, lining the surface epithelium of the proximal colon, showed a strong immunoreactivity in the polyribosomes and in the interdigitations of the lateral membrane. The columnar cells of the crypts and the goblet cells in both the proximal and the distal colon showed the immunostaining in the cis and trans cisternae of the Golgi apparatus. TGF α seems to be processed differently in the surface columnar cells and in the crypt columnar cells and goblet cells. Moreover, it probably has different roles in proliferation and differentiation.

Methods
TISSUES
Adult Sprague Dawley rats weighing 150–320 g were killed by cervical dislocation. The abdominal wall was opened and the proximal and distal colon were dissected out intact.

IMMUNOELECTRON MICROSCOPY
Small pieces of proximal and distal colon were fixed in a mixture of 2% paraformaldehyde and 1% glutaraldehyde in phosphate buffered saline (PBS) for two hours at 4°C. After washing in PBS the specimens were incubated in 50 mM NH₄Cl in PBS for 60 minutes to block aldehyde residues. The specimens were dehydrated in a graded ethanol series and embedded in Lowicryl K4M. Ultrathin sections were cut on a Reichert-Imy ultracut ultramicrotome using a diamond knife, and mounted on a nickel grid coated with formvar and carbon.

Labelling of the immunoreactive sites was achieved by indirect grid immunogold staining as previously described. The sections were incubated on a drop of normal goat serum (diluted 1:30 in PBS pH 7-2, 0-1% bovine albumin, 0-01% sodium azide (NaAz)) for 30 minutes to block the background and for two hours at room temperature in mouse monoclonal anti-TGF α (diluted 2 μg/ml) in PBS-bovine serum albumin-NaAz; Oncogene Science, ref GF10). The anti-TGF α antibody recognises a human TGF α epitope (residues 34–50) and it does not crossreact with epidermal growth factor. The sections were washed on five drops of PBS and the primary antibodies were localised using colloidal gold (10 nm diameter) labelled goat antimouse IgG (diluted 1:60 in PBS-bovine serum albumin-0-05% Tween 20; Sigma, ref G2272) for one hour at room temperature. The sections were washed on drops of PBS and distilled water and stained with 2% uranyl acetate and lead citrate. Electron micrographs were taken using a Philips 301 electron microscope.

NEGATIVE CONTROL
The following were included as negative controls: (a) primary incubation with PBS-bovine serum albumin-NaAz, (b) primary...
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Results

The proximal and the distal colon, lined by columnar cells, showed abundant microvilli projecting from the apical plasma membrane. Membrane bound vesicles were commonly seen in the web region. A Golgi complex, small clear vesicles, and abundant mitochondria were seen in the supranuclear region. Both smooth and rough endoplasmic reticulum could also be seen through the cytoplasm. The nucleus was located in the basal third of the cell.

Positive staining with the anti-TGF α antibody was seen in the columnar cells of the proximal colon and the strongest reaction confined to the cytoplasm (Fig 1), was regularly associated with polyribosomes (Fig 2). Nuclei, mitochondria, smooth and rough endoplasmic reticulum, and small clear vesicles did not show any immunoreactivity. Positive immunoreactivity was also identified in the interdigitations of the lateral membranes (Fig 3). Furthermore, hardly any immunoreactivity was seen on the microvilli of the columnar cells.

In the proximal colon, the crypts are longer than in other parts of the colon. The upper two thirds are lined by columnar and goblet cells. The lower part is covered by goblet cells. The distal colon has shorter crypts. The upper part is lined by columnar and goblet cells. The lower part is mainly covered by goblet cells and a smaller number of columnar cells. The columnar cells of the crypts in both the proximal and the distal colon showed positive staining with the anti-TGF α antibody in the cisternae of the Golgi apparatus (Fig 4). We could not find any immunoreactivity in the cytoplasm.

In both the proximal and the distal colon, the goblet cells are characterised by mucous granules occupying the apical two thirds of the cell. The nucleus is located in the basal...

Figure 1: Electron micrograph of surface columnar cell in the proximal colon. An intense labelling with 10 nm gold particles is seen throughout the cytoplasm. Mitochondria (M), microvilli (arrowhead), and clear vesicle (asterisk) are shown. (Immunogold test; bar represents 0.35 μm.)

Figure 2: Electron micrograph of surface columnar cell in the proximal colon. TGF α immunoreactivity is associated with the polyribosomes. Note that the rough endoplasmic reticulum (rER) did not show any staining. (Immunogold test; bar represents 0.35 mm.)

Figure 3: Electron micrograph of surface columnar cell (GC) in the proximal colon. TGF α immunoreactivity is localised with 10 nm gold particles in the interdigitations of the lateral membranes and in the polyribosomes. No immunoreactivity was seen in the neighbouring goblet cell (GC). Mitochondria (M), interdigitations (arrowhead), and rough endoplasmic reticulum (rER) are shown. (Immunogold test; bar represents 0.35 μm.)

Figure 4: Electron micrograph of crypt columnar cell in the distal colon. TGF α antibody shows an intense labelling in the cisternae membranes of the Golgi apparatus (G). (Immunogold test; bar represents 0.35 μm.)

incubation with anti-TGF α (2 μg/ml) pre-absorbed with rat TGF α (30 μg/ml, fragment 1–50, Bachem, ref H-5545) for 24 hours at 4°C.
region of the cell and a Golgi complex is usually seen in the supranuclear region. The immunoreactivity was mainly located in the Golgi apparatus of both cis and trans cisternae. The gold particles were confined to the cisterna membranes (Figs 5 and 6). The mucous granules were also heavily labelled with gold particles (Fig 7).

**Discussion**

The intestinal epithelium is a very dynamic tissue, in which cells migrate distally along the crypt-villus axis and the capacity for proliferation is lost. Sophisticated, differentiated, absorptive, and digestive functions are progressively acquired. TGF-α may have a role in colonic mucosal proliferation and differentiation. We report here, for the first time, the ultrastructural identification of TGF-α in the proximal and distal rat colon.

TGF-α mRNA in CHO cells (Chinese hamster ovary fibroblast) is processed in the rough endoplasmic reticulum and Golgi apparatus, and anchored to the plasmatic membrane. We found, however, the specific immunostaining in the polyribosomes of the surface columnar cells of the proximal colon. This localisation could suggest that the coding sequence of the TGF-α mRNA has been processed by alternate splicing of the gene in the surface columnar cells. The alternate splicing of the TGF-α mRNA has been reported before in the brain. Some cell lines such as A375, FeSV/FRE and SW620 (colon adenocarcinoma) showed a high synthesis of TGF-α and displayed a cytoplasmic staining, as we described in surface columnar cells. On the other hand, columnar cells in both proximal and distal crypts presented TGF-α staining in the cisternae of the Golgi apparatus as described by Teixido et al in CHO cells. Furthermore, the apical membrane of both surface and crypt columnar cells did not show gold particles label suggesting that the TGF-α precursor is not anchored at the apical membrane in those cell types. This finding may be supported by the experiments of Choudry et al and Barnes et al. They identified an endopeptidase-2 in the intestinal brush border, which hydrolysed the TGF-α (this enzyme degraded about 30 nmol of h-TGF-α/h per mg of protein), and this may explain the absence of staining in the luminal surface. We also found, however, some label associated with the interdigitations of the lateral membrane in the surface and crypt columnar cells. In this case, TGF-α could be functioning as a type of receptor or interacting with the epidermal growth factor/TGF-α receptor on a neighbouring cell, having a role that requires discrete cell-cell interactions, not compatible with release of the soluble factor. Many studies have been performed on the presence of epidermal growth factor/TGF-α receptor in colon epithelium in the adult rodent. Thus, the influence of TGF-α in the colonic mucosa may regulate the balance between proliferation and differentiation by means of binding to epidermal growth factor/TGF-α receptor.

Goblet cells in the proximal and distal colon showed the immunoreactivity located in the Golgi apparatus and in the mucous granules. This suggests TGF-α must be secreted by goblet cells and also bind to epidermal growth factor/TGF-α receptor.
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