

## Roles for Transforming Growth Factor- $\alpha$ in Gastric Physiology and Pathophysiology

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Transforming growth factor  $\alpha$  (TGF $\alpha$ ) is a 5.6 kd single-chain polypeptide that acts through binding to the epidermal growth factor receptor (EGFR). TGF $\alpha$  is produced in a wide range of normal as well as embryonic and neoplastic cells and tissues. TGF $\alpha$  and EGFR, but not EGF, are expressed in normal gastric mucosa. We have identified the following biological roles for TGF $\alpha$  in the stomach, using a variety of primate and rodent models: inhibition of acid secretion; stimulation of mucous cell growth; protection against ethanol- and aspirin-induced injury. This last effect is associated with a time- and dose-dependent increase in levels of insoluble gastric mucin.

Based on these known biological actions of TGF $\alpha$ , we have examined TGF $\alpha$  production in Ménétrier's disease, a disorder characterized by foveolar hyperplasia, hypochlorhydria, and increased gastric mucin content. In four patients with Ménétrier's disease, there was enhanced TGF $\alpha$  immunostaining throughout the gastric mucosa. Furthermore, metallothionein (MT)-TGF $\alpha$  transgenic mice which overproduce TGF $\alpha$  in the stomach exhibit histopathological and biochemical features characteristic of and consistent with the diagnosis of Ménétrier's disease. Thus locally produced TGF $\alpha$  may mediate a number of biological processes in the stomach, and its altered production may participate in the pathogenesis of selected pathological states.

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### INTRODUCTION

The purpose of this article is to review work, in large part from this laboratory, on the role of TGF $\alpha$  in selected aspects of gastric physiology, and its apparent dysregulation in Ménétrier's disease. Before doing so, we will summarize recent advances in the study of TGF $\alpha$  and attempt to place this peptide into the context of EGF and other EGF-like molecules, with special emphasis on the gastrointestinal tract. This review is not intended to be comprehensive and in part will be highly speculative.

### BACKGROUND

#### *Discovery of Transforming Growth Factors*

Transforming growth factors (TGFs) were first identified in 1978 in medium conditioned by fibroblasts transformed by the Rous sarcoma virus [1]. Addition of this partially purified material to normal fibroblasts caused the reversible appear-

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*Abbreviations:* AR: amphiregulin EGF: epidermal growth factor EGFR: epidermal growth factor receptor ELISA: enzyme-linked immunosorbent assay HB-EGF: heparin binding-epidermal growth factor MT: metallothionein NDF: neu differentiation factor PAS: periodic acid-Schiff TGF: transforming growth factor

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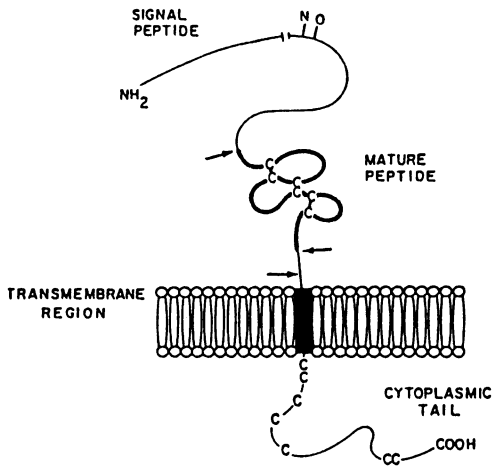


FIG. 1. Schema of TGF $\alpha$  peptide. Arrows indicate sites of proteolytic cleavage. N- and O-linked glycosylation sites are indicated in the NH<sub>2</sub> terminus.

ance of a malignant (transformed) phenotype. Consequently, the protein was named transforming growth factor. Later it was shown that this transforming activity was composed of two distinct proteins, now designated TGF $\alpha$  and TGF $\beta$  [2]. It was postulated that TGF $\alpha$  functioned in an autocrine manner and that overexpression of this protein might contribute to malignant transformation [3]. Since TGF $\alpha$  was also observed in embryonic cells and tissues [4,5], it was suggested that TGF $\alpha$  was an embryonic growth factor inappropriately expressed in neoplasia.

#### *Structure of the TGF $\alpha$ Gene and Protein*

The human TGF $\alpha$  gene spans 70–100 kb on chromosome 2 and contains six exons [6,7]. The 4.5–4.8 kb TGF $\alpha$  mRNA transcript encodes a 160 amino acid peptide, which is schematically depicted in Fig. 1. A signal peptide in the amino terminus is presumably cleaved prior to exit from the cell. N- and O-linked glycosylation sites are indicated by the asterisks. The 50 amino acid polypeptide is produced by proteolytic cleavage of the ALA VAL VAL residues that flank either end of the mature molecule. Six cysteine residues in the mature peptide form three disulfide bridges. There is a hydrophobic transmembrane region followed by an intracellular cytoplasmic tail with seven cysteine residues, some of which are covalently linked to palmitate [8].

The reported sizes of the TGF $\alpha$  protein range from 5 to 20 kd. This variation may reflect differential glycosylation and proteolytic cleavage, as well as dimerization and the presence of binding proteins. Possible distinct biological roles of TGF $\alpha$  forms of higher molecular weight have not been explored. In a number of TGF $\alpha$  expressing cell lines, the protein is detected in cell extracts but not in the conditioned medium [9]. The biological significance of this observation has been explored in parallel by two independent groups [10,11]. The arrows in Fig. 1 indicate sites of mutation engineered by these investigators that result in membrane fixation of TGF $\alpha$ . These mutated forms of TGF $\alpha$  are able to activate epidermal growth factor (EGF) receptors on neighboring cells. This observation has important implications for the actions of TGF $\alpha$ ; e.g., this cell-cell stimulation (“juxtacrine” stimulation) might play a role in developmental processes that depend on discrete cell-cell interactions, or it might target proTGF $\alpha$  expressing cells to tissue sites rich in EGFR [12,13]. There is

increasing evidence that local production/processing of TGF $\alpha$  may confer biological consequences distinct from systemic delivery/exogenous administration of the growth factor [14].

#### *The EGF/TGF $\alpha$ Receptor*

There is 35 percent structural identity between TGF $\alpha$  and EGF; however, all six cysteine residues are conserved, and formation of three disulfide bridges imparts sufficient structural identity for both peptides to bind to the epidermal growth factor receptor (EGFR) [15]. The EGFR is a 170 kd protein that consists of a cell surface ligand receptor domain, a single hydrophobic transmembrane segment, and a highly conserved cytoplasmic tyrosine kinase domain. Binding of EGF or TGF $\alpha$  to the receptor initiates a complex program of activation of intrinsic kinase activity, increases in cytosolic calcium, and ultimately DNA synthesis and cellular growth. In addition, clustering and dimerization of receptors occurs with binding of ligand to cell surface receptor, followed by internalization and degradation of the ligand/receptor complexes within lysosomes. A 65 amino acid cytoplasmic stretch of the EGFR has been identified, one that mediates the increase in cytoplasmic calcium and ligand/receptor internalization (the CAIN domain) [16]. Activation of the EGFR tyrosine kinase appears to be necessary for subsequent biological activity. An active area of research is identification of substrates for this tyrosine kinase; activation of phospholipase C- $\gamma$  1 appears to be a promising candidate [17,18].

#### *Cellular Distribution of TGF $\alpha$*

TGF $\alpha$  expression is clearly not restricted to the embryonic and neoplastic state. We were the first to demonstrate that TGF $\alpha$  is produced *in vitro* and *in vivo* by a non-transformed epithelial cell, human keratinocytes [19]. Subsequently, production of TGF $\alpha$  has been detected in a wide range of normal cells and tissues, including activated macrophages [20], mammary epithelium [21,22], and gastrointestinal tissues [23–28]. In the rat small intestine, epithelial cells eluted from the jejunal crypt-villus axis expressed TGF $\alpha$  mRNA at twofold higher levels in the villus tip than in the crypt, and immunostaining for TGF $\alpha$  showed uniform immunoreactivity in the villus cells, whereas crypt cells did not stain [27]. Localization of TGF $\alpha$  in this post-mitotic, differentiated compartment may have important implications for its mode of action (see below). Production of TGF $\alpha$  in the stomach also will be discussed in more detail below.

It should be noted that the examples cited above represent cells and tissues in which TGF $\alpha$  mRNA, as well as protein, have been detected, thus reflecting true local synthesis rather than delivery from a remote site. Since EGF is expressed by a selective population of normal cells and tissues (e.g., salivary gland, Brunner's gland, kidney), the widespread production of TGF $\alpha$  has led us to suggest that *in vivo* TGF $\alpha$ , and not EGF, is the major ligand for the EGFR. This statement must be qualified by certain caveats. First, Wright's group has demonstrated that a novel cell lineage produces EGF in the chronically injured gastrointestinal tract [29]. Second, Snedeker and co-workers have shown that both TGF $\alpha$  and EGF are expressed in the developing mouse mammary gland but are restricted to different compartments; TGF $\alpha$  co-localizes with EGFR to the proliferative terminal end bud compartment, whereas EGF resides at the luminal surface, where it might play a role in fluid transport and/or milk secretion [30]. Third, additional members of the EGF/TGF $\alpha$

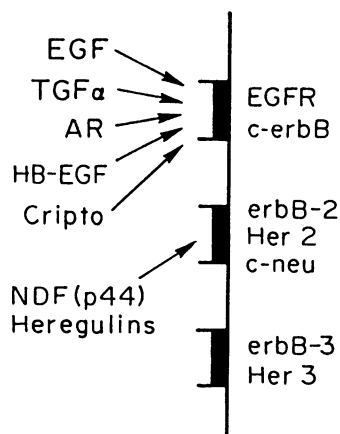


FIG. 2. Family of TGF $\alpha$  ligands and receptors.

family have been identified (see below), and certain of these ligands may be as widely expressed as TGF $\alpha$ .

#### *Family of TGF $\alpha$ /EGF Ligands and Receptors*

Figure 2 lists the family of TGF $\alpha$  ligands and receptors. EGF and EGFR were the first to be characterized [15]. Two additional receptors with homology to EGFR have been identified, erbB-2 and erbB-3 [31,32]. Of interest is the fact that a 44 kd glycoprotein designated neu differentiation factor (NDF) has been purified to homogeneity from ras-transformed rat cells and appears to be a ligand for erbB-2 [33]; this glycoprotein may be the rat homologue of a 45 kd protein heregulin- $\alpha$  that has been purified from the conditioned medium of a human breast cancer cell line (MDA-MB-231), cloned, and sequenced [34]. Addition of NDF to mammary epithelial cells results in a differentiated phenotype [35]. No ligand for erbB-3 has been identified thus far.

There is an expanding number of members of the TGF $\alpha$  family of ligands. These share structural similarities, including the conservation of six cysteines of the EGF motif which, in EGF, are involved in the three disulfide bonds defining the tertiary structure and conferring the ability to bind the EGFR. With the exception of cripto (for which recombinant peptide is not yet available), these family members have been shown to bind the EGFR. The best characterized of these ligands are EGF, TGF $\alpha$ , and amphiregulin (AR), whose structures are shown in Fig. 3. The disulfide bonds formed between cysteines 1 and 3, 2 and 4, and 5 and 6 result in formation of three loops that provide the backbone structure to these molecules. AR was initially cloned from TPA-induced MCF-7 cells [36,37]; more recently, it has been isolated from conditioned medium of human keratinocytes [38], in which, like TGF $\alpha$ , it appears to act as an autocrine growth factor. Newer members of the EGF/TGF $\alpha$  family which have not been as well characterized include heparin binding (HB)-EGF and cripto. HB-EGF was cloned from human macrophages [39], and cripto was identified initially in a human teratocarcinoma cell line [40].

Production of TGF $\alpha$ , AR, and cripto has been examined in gastrointestinal neoplasia. In contrast to TGF $\alpha$ , production of AR and cripto appears to be more consistently elevated in gastrointestinal neoplasms relative to normal gastrointestinal epithelium. Salomon and co-workers have observed AR expression in normal

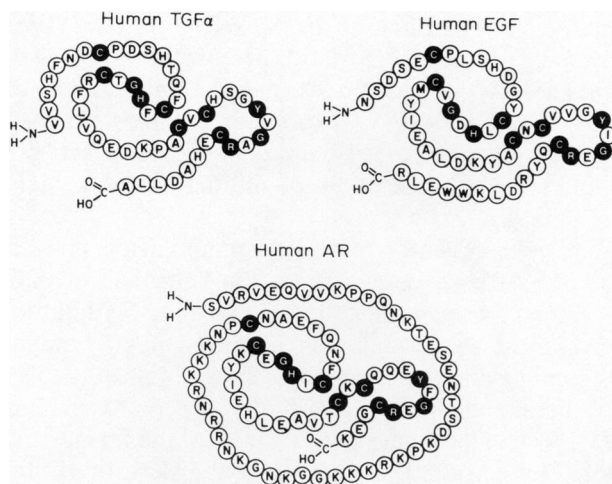


FIG. 3. Amino acid structure of human TGF $\alpha$ , EGF, and amphiregulin (AR).

colonic epithelium, but cripto expression was restricted to neoplastic tissue [41]. We have observed AR expression to be consistently enhanced in gastric and colonic carcinoma relative to adjacent normal epithelium; the expression is confined to the epithelial versus stromal elements by in situ hybridization [42]. TGF $\alpha$  expression was variable in intensity between normal epithelium and carcinoma. It should be noted, however, that enhanced expression of AR is not restricted to neoplasia, as it was also increased in the involved skin of patients with psoriasis [42].

#### *Biological Actions of TGF $\alpha$*

The biological actions of TGF $\alpha$  and EGF have been reviewed recently in detail [15]. These peptides share a similar spectrum of activity, since both peptides activate the same receptor. Shared properties that are of potential importance to the gastrointestinal tract include stimulation of cellular proliferation [15], cell migration [43,44], angiogenesis [45], and arterial blood flow [46], as well as inhibition of gastric acid secretion [47]. Quantitative differences in activity have been reported. For example, TGF $\alpha$  is more potent in stimulation of calcium release from fetal long bones [48]. Also, TGF $\alpha$  is more potent in stimulating regional arterial blood flow in dogs; pre-treatment with EGF or TGF $\alpha$  desensitizes the vascular response to EGF but not to TGF $\alpha$  [46]. No explanation for these differences in biological activity has been reported; however, differential processing or degradation of ligand-receptor complexes exist as possible mechanisms [49–51]. A final point to emphasize is that these polypeptides clearly subserve functions other than growth.

### TGF $\alpha$ IN THE STOMACH

#### *Localization Studies and Role in Acid Regulation*

Identification of TGF $\alpha$  expression in human keratinocytes spurred us to examine a battery of normal cells and tissues for TGF $\alpha$  production. TGF $\alpha$  mRNA expression was detected in the scraped gastric mucosa of a number of species, including human, dog, guinea pig [26], and rat [Coffey RJ: personal observation]. To further localize TGF $\alpha$  production in the gastric mucosa, guinea pig gastric mucosa was separated into a 65 percent pure parietal cell fraction and 95 percent pure chief cell fraction by

the differential centrifugation method of Raufman et al. [52]. The 4.8 kb TGF $\alpha$  transcript was greatest in the parietal cell fraction (5.8-fold increase), but was also enhanced in the chief cell fraction (1.9-fold increase) relative to the unfractionated gastric mucosa. Like TGF $\alpha$  expression, EGFR mRNA expression was most intense in the parietal cell-enriched fraction (7.8-fold increase), but was also increased in the chief cell-enriched fraction (2.7-fold increase) relative to the unfractionated guinea pig gastric mucosa [52].

These studies have been extended by examination of TGF $\alpha$  immunohistochemical staining in the normal human adult gastric mucosa [53]. At the light microscopic level, TGF $\alpha$  immunoreactivity is greater in the fundus than the antrum. Within the fundus, TGF $\alpha$  immunoreactivity is most concentrated in parietal cells, although other cell populations exhibit immunostaining, including surface mucous cells. Furthermore, both TGF $\alpha$  (by radioimmunoassay) and EGFR (by Western blot analysis) have been detected in H<sup>+</sup>, K<sup>+</sup>-ATPase-enriched fractions of human gastric mucosal membranes [Coffey RJ, Goldenring JR: unpublished observation]. In addition, a 2.7-fold increase in immunoreactive TGF $\alpha$  released into gastric secretions was observed over basal levels following pentagastrin administration to three normal volunteers (142  $\pm$  16 pg/minute basal versus 382  $\pm$  168 pg/minute post-pentagastrin). In a separate study, extremely low basal immunoreactive TGF $\alpha$  levels (13  $\pm$  9 pg/minute) were detected in human saliva, which further decreased after pentagastrin administration. Furthermore, we have previously shown that pretreatment with TGF $\alpha$  in isolated rabbit parietal cells results in a dose-dependent reduction of histamine-stimulated, but not acetylcholine-stimulated [<sup>14</sup>C-aminopyrine uptake] [54]. These studies have led us to suggest an autocrine/intracrine role for TGF $\alpha$  in the modulation of gastric acid secretion. A tentative model is as follows: TGF $\alpha$  functionally binds to its receptor in tubulovesicles of quiescent parietal cells to suppress basal acid production; parietal cell activation by secretagogues results in fusion of tubulovesicles to the canaliculus, activation of H<sup>+</sup>, K<sup>+</sup>-ATPase and acid production, a process which would dissociate TGF $\alpha$  from its receptor and lead to its release into the gastric lumen. The net effect would be to augment acid production by removal of this acid inhibitory factor. Additional studies are under way to test the validity of this highly speculative model.

#### *Mitogenic Effect of TGF $\alpha$ in the Stomach*

TGF $\alpha$  has been shown to be mitogenic for cultured canine fundic epithelial cells [55]. In collaborative studies with Michael Rutten, we have shown that TGF $\alpha$  is a potent mitogen for cultured guinea pig gastric mucous cells [56]. In fact, recombinant human TGF $\alpha$  is at least tenfold more potent than recombinant human EGF in stimulating the growth of these cells under serum-free conditions. Since these cultured cells express a 4.8 kb TGF $\alpha$  transcript and have detectable TGF $\alpha$  binding sites [Rutten MJ, Coffey RJ: unpublished observation], the elements are present for a TGF $\alpha$  autocrine loop in the growth of these cells.

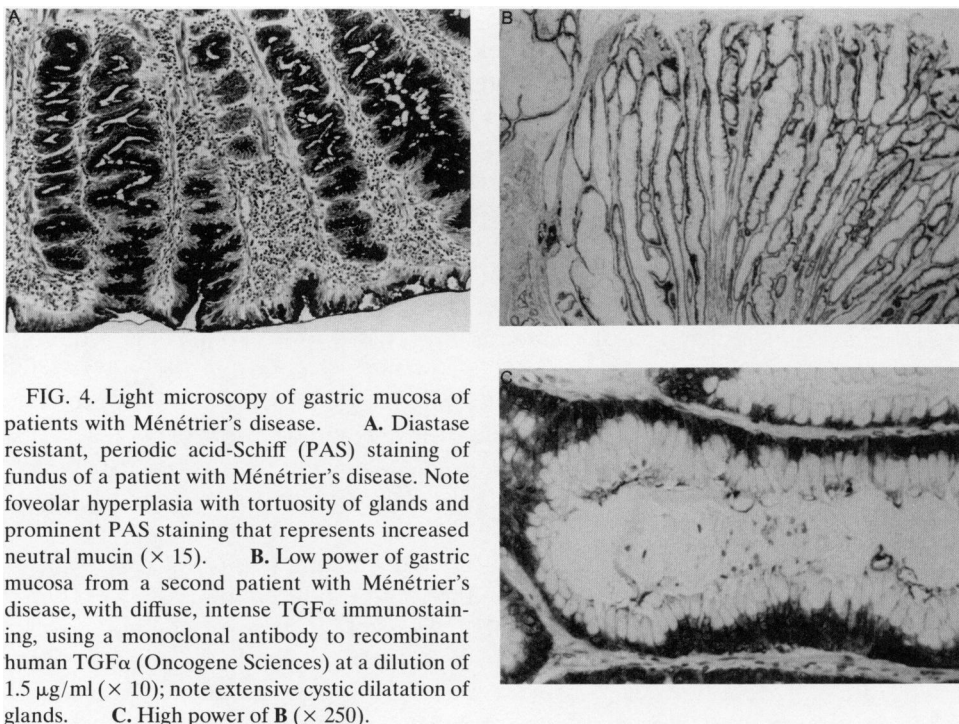
#### *Role of TGF $\alpha$ in Gastric Injury*

There is an orderly temporal sequence of reparative events following acute gastric injury. Repair of superficial epithelial cell loss, a process that is dependent on cell migration, begins within five minutes of acute injury and is nearly complete within one hour. Deeper mucosal erosions may persist for five days after acute injury and

require DNA synthesis for repair. Since TGF $\alpha$  mediates cell migration and is a mitogen for a number of epithelial cells (including gastric mucous cells), we postulated that TGF $\alpha$  might be upregulated following acute gastric injury so as to participate in the subsequent reparative events. After orogastric administration of both acidified sodium taurocholate and hydrochloric acid to induce acute gastric injury in rats, enhanced TGF $\alpha$  expression was observed by Northern blot analysis of mRNA isolated from scraped gastric mucosa [57]. In the taurocholate model, there was a dose-dependent increase in TGF $\alpha$  mRNA expression four hours after orogastric administration of 5, 15, and 30 mM taurocholate. At the 30 mM dose, a 1.3-fold increase in TGF $\alpha$  mRNA expression was observed at one hour, increasing to 2.6-fold at six hours, and returning to baseline at 24 hours. More striking was a tenfold increase in levels of immunoreactive TGF $\alpha$  in the gastric juice 30 minutes after administration of hydrochloric acid. This rapid appearance of TGF $\alpha$  probably represents release of a biologically active transmembrane form of TGF $\alpha$ . Thus production of TGF $\alpha$  is enhanced in a time frame consistent with its participation in subsequent reparative events, although these observations certainly do not prove that TGF $\alpha$  acts in this manner.

In an additional set of experiments, we studied whether TGF $\alpha$  was protective to the gastric mucosa against acute ethanol- and aspirin-induced injury [58]. Systemic administration of TGF $\alpha$  dose-dependently decreased 100 percent ethanol-induced gastric mucosal injury; an intraperitoneal dose of 50  $\mu$ g/kg delivered 15 minutes prior to ethanol decreased macroscopic mucosal injury by greater than 90 percent. At the microscopic level, TGF $\alpha$  significantly prevented deep gastric necrotic lesions and reduced disruption of surface epithelium. Pre-treatment with orogastric TGF $\alpha$  (200  $\mu$ g/kg) only partially (40 percent) decreased macroscopic ethanol damage. Intraperitoneal administration of TGF $\alpha$  at a dose of 10  $\mu$ g/kg, which does not significantly inhibit gastric acid secretion, decreased aspirin (200 mg/kg)-induced macroscopic damage by greater than 80 percent. Thus TGF $\alpha$  is truly cytoprotective, as it protects against acid-independent and acid-dependent forms of acute gastric injury. TGF $\alpha$  protection did not seem to be mediated by prostaglandin, glutathione, or ornithine decarboxylase-related events, as evidenced by lack of influence of the inhibition of their production. Pre-treatment with the sulfhydryl blocking agent N-ethylmaleimide partially abolished (40 percent) the protective effect of TGF $\alpha$ .

In addition, systemic administration of TGF $\alpha$  resulted in a time- and dose-dependent increase in levels of immunoreactive gastric mucin. Gastric mucin was measured in lightly scraped gastric mucosa by a reverse enzyme-linked immunosorbent assay (ELISA) with an antibody that recognizes biologically active, insoluble gastric mucin [59]. Fifteen and 30 minutes following intraperitoneal administration of 100  $\mu$ g/kg of TGF $\alpha$ , there was, respectively, a 7.3- and 14.6-fold increase in levels of gastric mucin, which corresponds to the timing of TGF $\alpha$ -induced mucosal protection. The role of gastric mucus as a protective barrier for the gastric mucosa is, however, controversial [60–63]. Adherent mucus is reported to be permeable to damaging agents such as ethanol and aspirin [63], which gain access through the gel to the superficial epithelial cells. On the other hand, removal of the gelatinous layer of mucus in cellular debris which formed after exposure of the gastric mucosa to 70 percent ethanol inhibited the protection against a rechallenge with the same necrotizing agent [64]. We postulate that the TGF $\alpha$ -induced increase in the adherent mucous gel layer covering the epithelial surface may act as a dilutional barrier to



damaging agents, may delay and/or restrict further damage induced by acid pepsin, and may accelerate early reparative events. An alternative mechanism by which mucin might protect the gastric mucosa is through its ability to scavenge toxic oxygen metabolites [65], which are generated by ethanol and aspirin [66]. The rapid increase in mucin levels is probably due to release of pre-formed mucin. Studies are under way to examine the effect of TGF $\alpha$  on rat gastric mucin mRNA expression and protein production.

#### *Upregulation of TGF $\alpha$ in Ménétrier's Disease*

Ménétrier's disease is an uncommon disorder characterized by enlarged gastric folds with foveolar hyperplasia and glandular cystic dilatation [67–72]. Biochemical features that are seen frequently include hypoproteinemia, hypochlorhydria, and increased gastric mucin content. From the cumulative results of several small series, it has been reported that there is a 10–15 percent incidence of gastric cancer in patients with Ménétrier's disease [70], but its exact incidence is uncertain, since the disease is rare, and few patients have been followed prospectively. The etiology of this disorder is unknown. Since TGF $\alpha$  stimulates the growth of gastric mucous cells, inhibits gastric acid secretion, and increases gastric mucin content, we speculated that its overproduction might be involved in a pathogenesis of this disorder. Therefore, we characterized TGF $\alpha$  immunostaining in the gastric mucosa of four patients with Ménétrier's disease [53]. In contrast to the normal pattern of TGF $\alpha$  immunostaining, in which TGF $\alpha$  appears most concentrated in parietal cells, there was intense staining in the majority of mucous cells (Fig. 4). In one patient, from



whom sufficient fresh tissue was obtained to isolate RNA, expression of TGF $\alpha$  and EGFR was increased in the gastric mucosa relative to a normal volunteer.

In addition, metallothionein (MT)-TGF $\alpha$  transgenic mice, which overexpress TGF $\alpha$  in the gastric mucosa, exhibit a number of features characteristic of and consistent with the diagnosis of Ménétrier's disease, including foveolar hyperplasia and glandular cystic dilatation, increased gastric neutral mucin staining, and reduced basal and histamine-stimulated rates of acid production [53]. Our findings of enhanced TGF $\alpha$  immunostaining in the gastric mucosa of four patients with Ménétrier's disease (with increased TGF $\alpha$  in mRNA expression in one patient), coupled with the histological lesions and altered functional characteristics in the stomachs of MT-TGF $\alpha$  transgenic mice, provide compelling evidence for a role for TGF $\alpha$  (or, more generally, an EGFR-linked signal transduction pathway) in the pathogenesis of Ménétrier's disease.

### SUMMARY

In this brief manuscript, we have attempted to provide the reader with a historical perspective of TGF $\alpha$ , with particular emphasis on its possible roles in normal gastric physiology and how its overproduction in Ménétrier's disease might contribute to the pathogenesis of this disorder.

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