# Hypertrophic Gastropathy Resembling Menetrier's Disease in Transgenic Mice Overexpressing Transforming Growth Factor α in the Stomach

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## Abstract

**Transforming growth factor α (TGFα) is thought to participate in the normal and pathologic processes of numerous tissues, including the gastric mucosa. To explore its role in vivo, transgenic mice were generated overexpressing TGFα in the stomach. TGFα induced dramatic structural and functional lesions of the glandular stomach that were similar to Menetrier's disease in humans. Transgenic mice developed severe adenomatous hyperplasia that resulted in a striking nodular thickening or hypertrophy of the gastric mucosa. Secretions obtained from affected stomachs contained no detectable gastric acid, suggesting that parietal cell function had been greatly impaired. These findings demonstrate that overproduction of TGFα can stimulate cellular proliferation, suppress acid secretion, and perturb organogenesis of the stomach of transgenic mice. Moreover, TGFα may contribute to the pathogenesis of related human hypertrophic gastropathies, such as Menetrier's disease. (*J. Clin. Invest.* 1992. 90:1161–1167.) Key words: Menetrier's disease • transforming growth factor α • transgenic mice • stomach • parietal cells**

## Introduction

Transforming growth factor α (TGFα)[1] is a potent mitogen and a member of the epidermal growth factor (EGF) family of peptides (1–5). TGFα, which induces biological responses (5) by binding to and activating the tyrosine kinase of the EGF receptor (6), has been implicated in malignant transformation. Human primary and metastatic tumors often overexpress TGFα (7), and highly active TGFα expression vectors can be transforming when introduced into cultured cells (8–10). TGFα and EGF have also been shown to participate in the physiologic function of a multiplicity of tissues (5), including the mucosa of the stomach, where TGFα and its receptor have been detected in rodents and humans (11). TGFα is a potent inhibitor of gastric acid secretion (12), and EGF can stimulate gastric mucosal growth in rodents (13, 14).

Transgenic mice have proven to be extremely useful as models for human diseases (15). We and others have shown that transgenic mice overexpressing TGFα experience a number of phenotypic abnormalities, including neoplasia of the liver and mammary gland (16–19). Here, we report that TGFα induces dramatic structural and functional lesions of the transgenic stomach that are reminiscent of Menetrier's disease, a human syndrome that is characterized by a diffuse thickening of the gastric wall caused by extensive hyperplasia of the surface epithelium, and is associated with carcinoma of the stomach (20, 21).

## Methods

*Animals.* Transgenic mice bearing the metallothionein–TGFα fusion gene were created and identified as described previously (16). Briefly, one-cell mouse embryos from the inbred line FVB/N (Harlan Sprague Dawley, Inc., Indianapolis, IN) were microinjected with an active expression construct containing the mouse metallothionein I gene promoter, the human TGFα cDNA, and the human growth hormone gene polyadenylation signal. Microinjected embryos were transferred into pseudopregnant CD1 females (Charles River Breeding Laboratories, Inc., Wilmington, MA) under Avertine anesthesia (0.38 g/kg body wt 2,2,2-tribromoethyl alcohol; Aldrich Chemical Company, Milwaukee, WI). Where indicated, the metallothionein promoter of the transgene was induced to maximum activity by maintaining mice on drinking water containing 50 mM zinc sulfate. All mice used in this study were cared for and maintained in accordance with guidelines set forth by the National Institutes of Health. Animals that became moribund or appeared in acute distress were euthanized.

*Analysis of gastric juice.* Routinely, gastric juice was obtained from mice that were starved for 24 h. The pylorus was ligated under methoxyflurane (Metofane; Pitman-Moore, Inc., Mundelein, IL) anesthesia. After ~ 2.5 h without food or water, adult mice were again anesthetized and all fluid was removed from the stomachs. Because the stomachs of juvenile mice contained smaller amounts of juice, 0.25 ml saline was injected into the juvenile stomach and removed after 5 min. The pH of gastric juice was determined using a semi-micro combination pH electrode (Orion Research, Inc., Cambridge, MA). In some experiments, 500 μg/kg body wt of pentagastrin (Sigma Chemical Co., St. Louis, MO) was injected subcutaneously 3 h after gastric ligation to stimulate acid secretion. 1 h after pentagastrin injection, mice were killed and the acid content of their stomachs was determined by titration with sodium hydroxide.

*RNA analysis.* Total tissue RNA was isolated and analyzed using Northern blot hybridization, as previously described (16, 22). 15 μg of total tissue RNA was loaded per lane, and hybridization was conducted with a $^{32}$P-radiolabeled 925-bp human TGFα cDNA probe (16). Mice were injected with 5 mg/kg body wt zinc chloride 4–5 h before death to maximize transgene expression.

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1. *Abbreviations used in this paper:* EGF, epidermal growth factor; H&E, hematoxylin and eosin; TGFα, transforming growth factor α.

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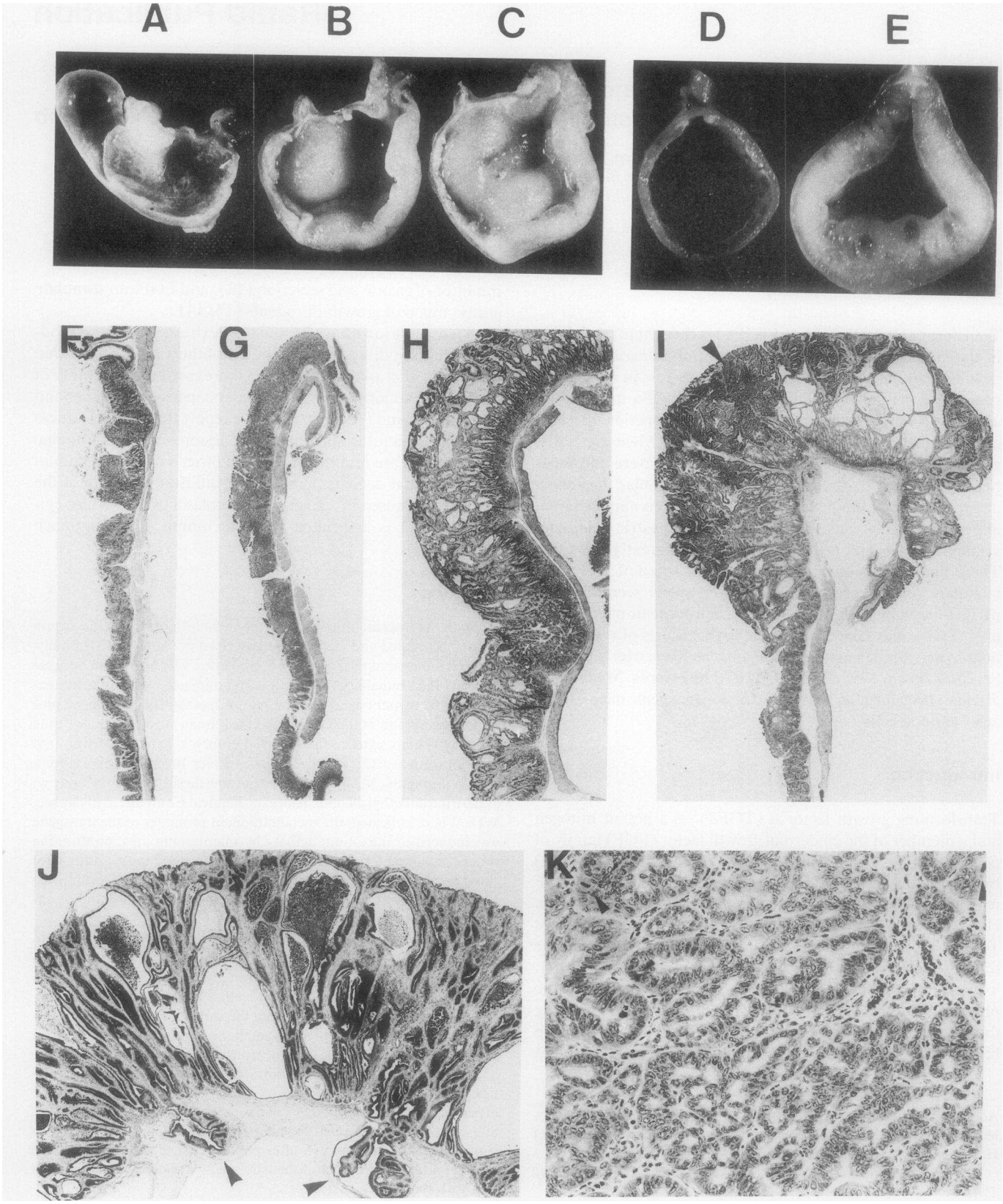



*Figure 1.* Hyperplastic lesions of the glandular stomach of MT100 TGFα transgenic mice. (*A–E*) Gross analysis of stomachs from nontransgenic FVB (*A, D*) and transgenic MT100 (*B, C* and *E*) mice in sagittal (*A–C*) or cross (*D* and *E*) section. Note thickening (hypertrophy) of the transgenic mucosa (*E*), the appearance of nodules (*B* and C) and cysts (*E*). (*F–K*) Histologic analysis of stomachs from nontransgenic FVB (*F*) and transgenic MT100 (*G–K*) mice after thin sectioning along greater curvature, staining with H&E (*F–I, K*) or PAS (*J*). Note progressive hyperplastic and cystic transgene-specific alterations (*F–I*). The stomach in *I* contains regions of moderate dysplasia; one (*I, arrowhead*) is

*Table I. Elevation in the pH of Gastric Juice from TGFα Transgenic Mice**

| | Juvenile | | Adult | |
|---|---|---|---|---|
| | FVB | MT100 | FVB | MT100 |
| pH | 2.7±0.2 | 3.1±0.5 | 3.9±1.0 | 7.6±0.4 |
| Age (mo) | 1.3±0.2 | 1.4±0.2 | 5.4±2.3 | 5.7±2.3 |
| *n* | 5 | 6 | 15 | 13 |

* Study includes male and female mice. All animals were starved overnight. In adult mice (≥3 mo old), gastric juice was removed directly from the stomach; in juvenile mice (≤1.5 mo old), juice was recovered after injection of saline into the stomach (see Methods). *n*, number of mice in which pH of stomach juice was examined. Average age of mice is given in months±SD. pH units are given±SD.

*Immunohistochemistry.* Mouse tissues were fixed in Bouin's solution, paraffin embedded, and sectioned. Immunohistochemistry was performed using standard techniques, except that deparaffinized sections were treated with 0.05% saponin for 2 h and 0.1% trypsin for 10–15 min at room temperature. TGFα was localized using rabbit polyclonal antiserum against a rat pro-TGFα intracellular peptide (residues 137–159) (23). Antibody was diluted up to 1:20,000 and incubated for 1 h at room temperature. Horseradish peroxidase staining was achieved using the ABC mouse IgG elite kit (Vector Laboratories, Burlingame, CA). Tissues to be processed only for routine histologic analysis were fixed in 10% buffered formalin and stained with hematoxylin and eosin (H&E).

## Results

Adult TGFα transgenic mice from line MT100, where stomach lesions were most severe, all developed age-dependent symptoms of malnutrition. The body wt of transgenic animals > 5 mo old was 25–33% less than controls, although food intake by TGFα and control mice was determined to be virtually identical. Heterozygous and homozygous MT100 mice died or were humanely killed by 10 and 6 mo of age, respectively. Gross morphologic analysis revealed that the glandular stomach was always firm and enlarged; the weight of the stomachs from the oldest MT100 mice was three to six times greater than nontransgenic controls. The gastric mucosa was extraordinarily thick (hypertrophic) with large nodular projections on the inside wall (Fig. 1, *B* and *C*), while the lumen was severely constricted (Fig. 1 *E*) and contained viscous, mucous-laden secretions.

Histologic analysis revealed that hyperplastic changes were obvious in 31 of 31 MT100 mice between 1.5 and 10 mo of age, compared to minimal or no changes in 15 of 15 FVB control mice (Fig. 1, *G–I*, and *F*, respectively). 6-wk-old MT100 mice exhibited small cysts and diffuse epithelial hyperplasia within the glandular portion of the gastric mucosa (Fig. 1 *G*). By 6 mo, cystic hyperplasia was dramatic (Fig. 1 *H*), but less severe in the antrum. The gastric mucosa was much enlarged, mainly because of hyperplasia of mucin-secreting columnar epithelia, as evidenced by the appearance of periodic acid Schiff (PAS)

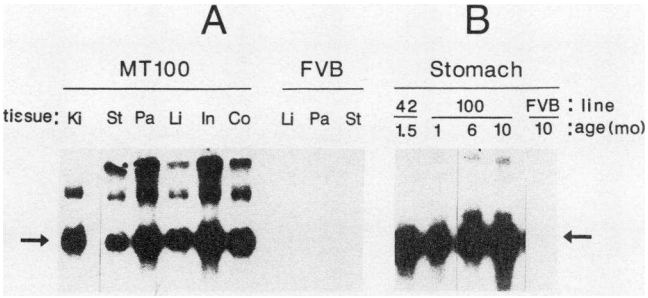



*Figure 2.* Detection of MT-TGFα fusion transgene expression by Northern blot hybridization. (*A*) Tissue RNA isolated from zinc-induced 10-mo-old MT100 TGFα transgenic and nontransgenic FVB male littermates. *Ki,* kidney; *St,* stomach; *Pa,* pancreas; *Li,* liver; *In,* small intestine; and *Co,* colon. (*B*) Levels of TGFα RNA found in the stomachs of MT42, MT100, and control FVB adult and juvenile male mice. 15 μg of total RNA were loaded per lane, and hybridization was with a human TGFα cDNA probe. Arrows indicate the expected 1-kb mature human TGFα RNA. Higher molecular weight bands probably represent transgenic TGFα RNAs generated by read-through transcription into adjacent transgenes and/or flanking mouse genomic sequences. Blots were rehybridized with a β-actin probe to confirm the relative amounts of total RNA loaded and transferred (data not shown).

positive secretory material in cytoplasmic droplets of ductules and in many cysts (Fig. 1 *J*). Microscopic analysis of thin sections of the MT100 gastric mucosa stained with Azure Eosin-Nocht suggested that parietal cells were frequently compressed and decreased in number, particularly around or under cysts (data not shown). In addition, inflammatory cell infiltration and diffuse edema of the submucosa were evident. The gastric mucosa of mice surviving 8–10 mo of age grew increasingly thick and cystic, and contained foci of dysplastic cells possessing enlarged basophilic nuclei with abnormal polarity, and pseudostratification (Fig. 1, *I* and *K*). Hyperplastic secretory epithelium extended deep into the mucosa, and occasionally penetrated the submucosal layer to form diverticuli (Fig. 1 *J*).

To determine if the transgenic parietal cell population was functional and effectively secreting acid, gastric juice was collected from the stomachs of MT100 and control mice. The pH of gastric juice from MT100 transgenic mice > 3 mo old was neutral or even slightly basic, strongly suggesting that parietal cell function was impaired in MT100 mice (Table I, Adult). This hypothesis was strengthened by measuring titratable gastric acid in untreated and pentagastrin-stimulated mice; the gastric juice in MT100 transgenic mice contained no detectable titratable acid, regardless of pentagastrin administration. Interestingly, the pH of gastric juice obtained from MT100 mice ≤ 6 wk of age was not significantly different from nontransgenic controls (Table I, Juvenile).

TGFα transgene expression in the stomach of MT100 mice was demonstrated using Northern blot analysis. Fig. 2 *A* shows that the stomach, pancreas, liver, small intestine, colon, and kidney of adult MT100 mice all contained abundant human

magnified in *K* showing cellular atypia and multiple mitotic figures (*K, arrowheads*). (*J*) Note hyperplasia of PAS-positive mucin-secreting cells, cystic dilatation, calcium deposition, and formation of diverticuli (*arrowheads*). Age: 2 (*G*), 5 (*B* and C), 6 (*E* and *H*), 8 (*A* and *D*), or 10 (*F* and *I–K*) mo. Mag: ×2.9 (*A–C*), ×3.6 (*D* and *E*), ×9 (*F–I*), ×21.5 (*J*), or ×200 (*K*).

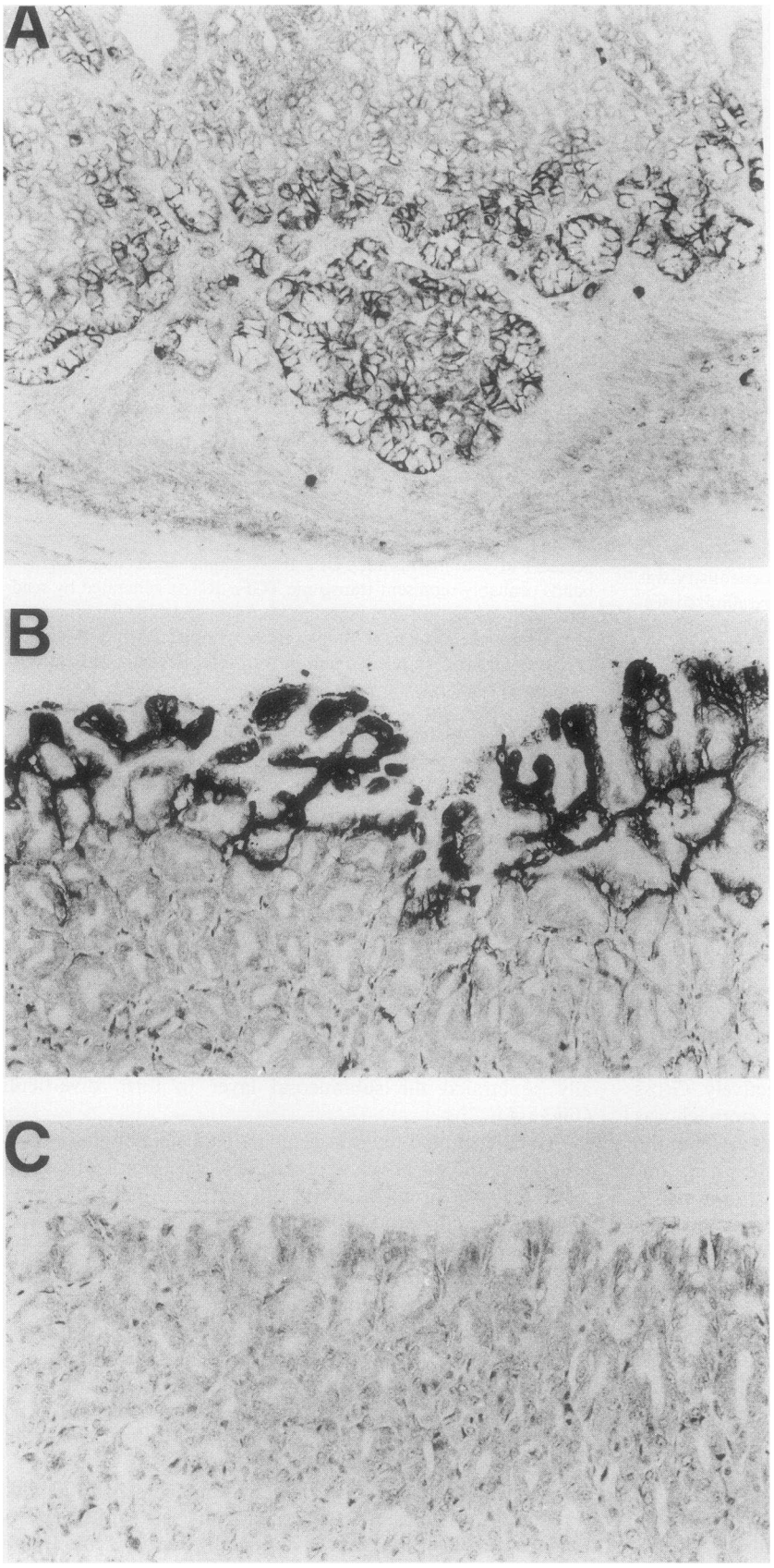
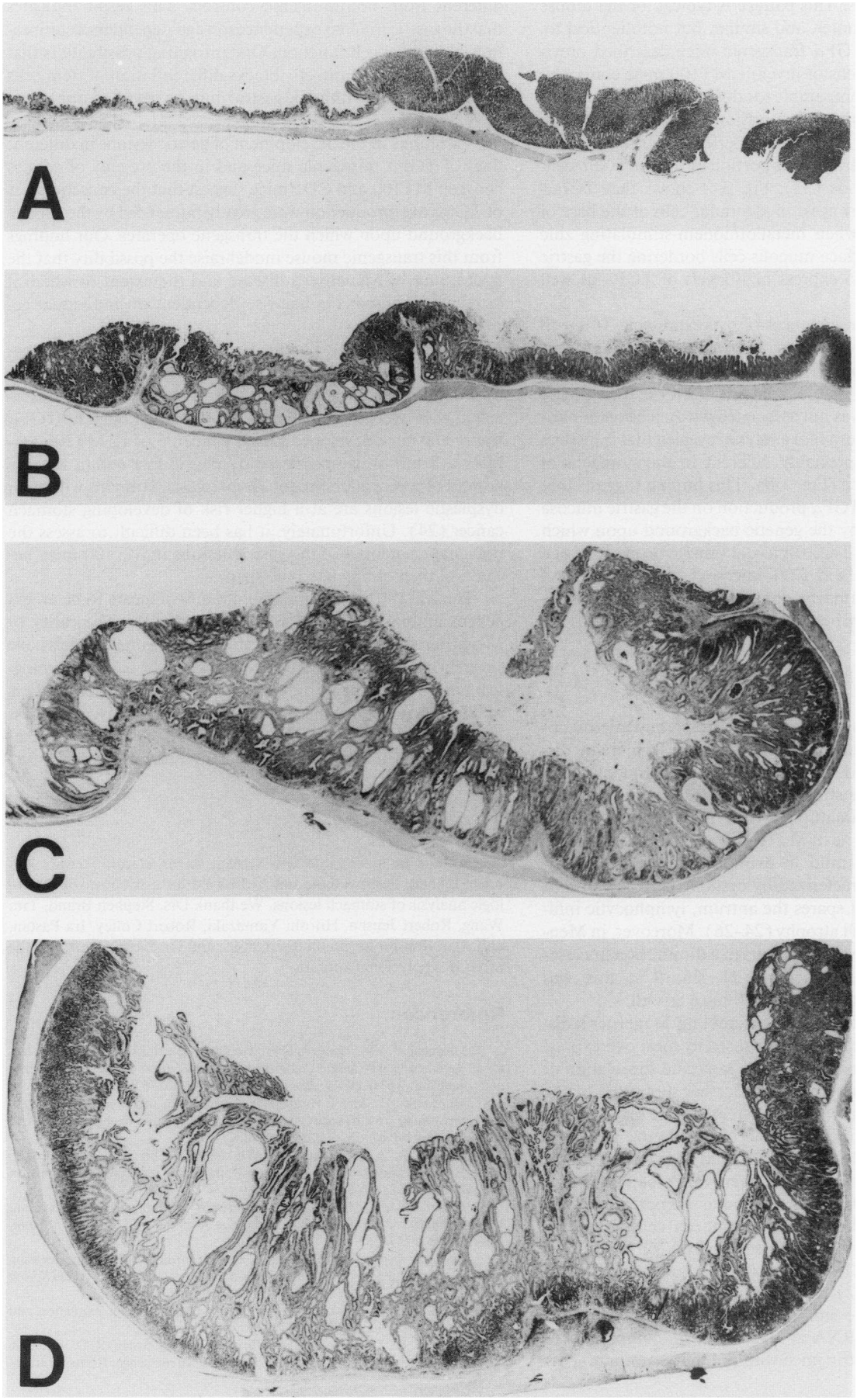



*Figure 3.* Immunohistochemical localization of TGFα in the gastric mucosa of transgenic MT100 (*A* and *B*) and nontransgenic FVB (*C*) mice. Mice were injected with zinc chloride at 5 mg/kg body wt 4 h before (*A*), or given drinking water containing 50 mM zinc sulfate 44 h before (*B* and *C*) death. Note membranous peroxidase staining of glandular cells at the base of the foveolar crypts in mouse with advanced hyperplastic disease (*A*), and intense staining in surface mucous cells exposed to zinc water in the lumen (*B*). Age: 10 mo (*A*), or 6 wk (*B* and *C*). ×200 (*A–C*).

*Figure 4.* Effect of genetic background on TGFα-induced stomach lesions. Histologic analysis of stomachs of 10-mo-old male mice. (*A*) Nontransgenic control progeny of a cross between heterozygous MT100 and CD1 mice. (*B* and *C*) TGFα transgenic progeny of a cross between heterozygous MT100 and CD1 mice. (*D*) heterozygous MT100. Mice used for (*A–C*) were littermates. Note amelioration of TGFα-induced hypertrophic lesions in FVB-derived MT100 transgenic mice when crossed with the outbred strain CD1 (*B* and *C*). All sections were stained with H & E. ×7.5 (*A–D*).


A
B
C
D

TGFα RNA transcripts. This pattern is typical for the mouse metallothionein I promoter, and similar, but not identical to, expression in MT42 TGFα transgenic mice described previously (16). The stomachs of juvenile MT100 mice contained levels of TGFα RNA comparable to adult transgenic mice (Fig. 2 *B*). To localize transgene expression within the stomach, immunohistochemical staining was performed using an antibody specific for the intracellular portion of the proTGFα cell-surface precursor peptide (23). Fig. 3 *A* shows that TGFα-specific staining was strongest in glandular cells at the base of the crypts. However, when metallothionein-stimulating zinc water was ingested, surface mucous cells bordering the gastric lumen were induced to express high levels of TGFα as well (Fig. 3 *B*).

The phenotypic consequences of overexpressing TGFα in the stomach varied in different transgenic lines. MT42 transgenic mice, derived from the outbred strain CD1, also exhibited cystic hyperplasia of the stomach; however, the disease developed slowly and was not associated with cachexia or parietal cell atrophy. Northern blot analysis revealed that transgene expression was not appreciably different in the stomachs of MT100 and MT42 mice (Fig. 2 *B*). This finding suggests that the effect of enhanced TGFα production on the gastric mucosa is strongly influenced by the genetic background upon which the transgene operates. The 10-mo-old transgenic progeny of a cross between MT100 and CD1 mice exhibited no signs of cachexia, and their stomachs contained variable but consistently less severe hypertrophic lesions (Figure 4, *B* and *C*).

## Discussion

Gastric lesions in TGFα transgenic mice grossly and microscopically resemble those seen in patients suffering from a rare disease originally described by Menetrier (20). The nodular projections observed in the stomachs of MT100 mice are overtly analogous to the prominent giant rugal folds found in Menetrier's disease (20, 21). As in MT100 mice, patients with Menetrier's disease exhibit mild to extraordinary cachexia, and their stomachs are characterized by cystic hyperplasia of mucous-secreting cells that spares the antrum, lymphocytic infiltrations, and parietal cell atrophy (24–26). Moreover, in Menetrier's disease, the latter often results in a diminution or cessation of gastric acid production (21, 26, 27); this was consistently observed in adult MT100 mice as well.

Our data suggest that lesions resembling Menetrier's disease in these transgenic mice were caused by local overexpression of TGFα, and autocrine and/or paracrine stimulation of the EGF receptor. This hypothesis is further supported by the finding that gastric disease was consistently more severe in homozygous transgenic mice, capable of generating twice the amount of TGFα. Furthermore, TGFα has been reported to be elevated in the gastric mucosa of Menetrier's patients (28). Although it is likely that overproduction of this potent mitogen directly induces hyperplasia of gastric epithelial cells, the mechanism by which TGFα compromises parietal cell function is not obvious. TGFα has been shown to be a potent inhibitor of gastric acid secretion (12), and may directly affect mature parietal cells. However, the stomachs of juvenile MT100 mice, which produce TGFα RNA at levels that are comparable to adult mice, contain gastric juice with pH that is not markedly different from nontransgenic controls. This result indicates that the gastric mucosa experiences an age-dependent deterioration of parietal cell function. One intriguing possibility is that TGFα directly or indirectly blocks differentiation of stem cells into parietal cells within the gastric mucosa of young transgenic mice.

Variations in the development of gastric lesions in different lines of TGFα transgenic mice and in the progeny of crosses between MT100 and CD1 mice suggest that the consequences of TGFα overproduction were greatly influenced by the genetic background upon which the transgene operates. Our findings from this transgenic mouse model raise the possibility that the appearance of Menetrier's disease and the extent to which it develops in humans is likewise dependent on individual genetic makeup.

Menetrier's disease is considered by many to be a premalignant condition because 15% of reported cases included gastric carcinoma (26). Furthermore, gastric cancer has been associated with overexpression of TGFα (29). Although no TGFα transgenic mice developed gastric cancer, 5 of 12 MT100 animals ≥ 8 mo of age possessed dysplastic foci within a background of severe adenomatous hyperplasia. Humans with such dysplastic lesions are at a higher risk of developing stomach cancer (24). Unfortunately, it has been difficult to assess the malignant potential of the gastric lesions in MT100 mice because of their abbreviated life span.

The MT100 TGFα transgenic mouse appears to be an excellent animal model for Menetrier's disease. The rarity of Menetrier's disease and the dearth of good animal models have severely handicapped attempts to elucidate the pathophysiological nature of this syndrome. By permitting a detailed analysis of the pathogenesis of this hypertrophic gastropathy, our TGFα transgenic mouse model may shed light on the cause and treatment of Menetrier's disease and related human stomach disorders.

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