

## Brief Report

## TREATMENT OF MÉNÉTRIER'S DISEASE WITH A MONOCLONAL ANTIBODY AGAINST THE EPIDERMAL GROWTH FACTOR RECEPTOR

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**M**ÉNÉTRIER'S disease (hypoproteinemic hypertrophic gastropathy) is a rare, acquired, premalignant disorder of the stomach.<sup>1-4</sup> It is characterized by giant hypertrophic folds that most often involve the fundus, excess mucus secretion, decreased acid secretion (hypochlorhydria), and hypoproteinemia due to selective loss of serum proteins across the gastric mucosa.<sup>5,6</sup> The cause of Ménétrier's disease is unknown, although infection with cytomegalovirus (CMV) in children and infection with *Helicobacter pylori* have been implicated.<sup>7,8</sup> Symptoms include epigastric pain, vomiting, edema, anorexia, and weight loss. Gastric cancer has been reported at diagnosis or during follow-up in patients with hypertrophic gastropathy. Evidence of the benefits of anticholinergic drugs, acid suppression, octreotide, and eradication of *H. pylori* is inconsistent.<sup>2,9,10</sup> Except in cases of CMV-associated Ménétrier's disease in children,<sup>11</sup> spontaneous remissions are rare. Partial or total gastrectomy is generally reserved for patients with debilitating disease and for cases in which there is concern over the development of gastric cancer.<sup>12</sup>

Increased signaling of the epidermal growth factor receptor has been implicated in the pathogenesis of Ménétrier's disease.<sup>13</sup> We recently cared for a patient

with this disorder who had not had a response to drug therapy. We hypothesized that the administration of a neutralizing monoclonal antibody against the epidermal growth factor receptor might be of clinical benefit. After one day of treatment with this antibody, the patient's nausea and vomiting decreased, and after one month there was clinical and biochemical improvement, including an increase in the serum albumin concentration and a decrease in the loss of protein in the stool.

### CASE REPORT

A 48-year-old man presented with nausea, vomiting, edema up to the waist, and hypoalbuminemia in July 1998. A gastric analysis revealed the secretion of a large volume of viscous gastric juice with a pH of 7.0. An endoscopic evaluation showed enlarged gastric folds in the fundus, and snare biopsies of the fundus showed foveolar hyperplasia (an expansion of surface mucous cells) and the absence of parietal cells. The results of a carbon-13 urea breath test were normal, and the rate of basal secretion of gastric acid was 0 mmol of hydrogen in 30 minutes (as compared with the normal rate of 4.0 mmol of hydrogen per hour). Trials of prochlorperazine, promethazine, ondansetron, lansoprazole, cisapride, octreotide, and glycopyrrolate did not alleviate the patient's symptoms. He was not considered for gastrectomy, because of the risk associated with anesthesia and surgery as a result of his primary pulmonary hypertension. This condition had been diagnosed in 1993, at which time a continuous infusion of epoprostenol (prostacyclin) had been initiated. Reduction in the dose of epoprostenol did not ameliorate his symptoms.

Approval for compassionate use of a monoclonal antibody against the epidermal growth factor receptor (C225, ImClone Systems) was granted by the Food and Drug Administration and the institutional review boards of the University of Texas Southwestern Medical Center and the Vanderbilt University Medical Center, and the patient gave informed written consent for a month-long course of treatment with this antibody. Intravenous infusion was initiated at a loading dose of 490 mg per square meter of body-surface area

**TABLE 1.** CLINICAL AND BIOCHEMICAL FINDINGS IN A PATIENT WITH MÉNÉTRIER'S DISEASE BEFORE AND DURING TREATMENT WITH A MONOCLONAL ANTIBODY AGAINST THE EPIDERMAL GROWTH FACTOR RECEPTOR.

| CHARACTERISTIC                                 | BASE-LINE VALUE | VALUE AFTER 1 MO OF TREATMENT |
|--|-----------------|-------------------------------|
| Episodes of vomiting per week                  | 70              | 1                             |
| Quality-of-life score*                         | 8               | 15                            |
| Serum albumin (g/dl)†                          | 2.0             | 3.1                           |
| Serum gastrin (pg/ml)‡                         | 82              | 591                           |
| Stool alpha <sub>1</sub> -antitrypsin (mg/dl)§ | 296             | 108                           |
| Parietal cells on gastric biopsy               | Absent          | Present                       |

\*Ferrans and Powers' quality-of-life index measures four domains: health and functioning, psychological and spiritual state, family dynamics, and socioeconomic status.<sup>15</sup> Higher scores indicate better quality of life. The quality-of-life index has been validated in patients with cancer and chronic medical conditions as well as in healthy subjects. The maximal possible score is 30; the mean ( $\pm$ SD) score in normal subjects is 20 $\pm$ 6.

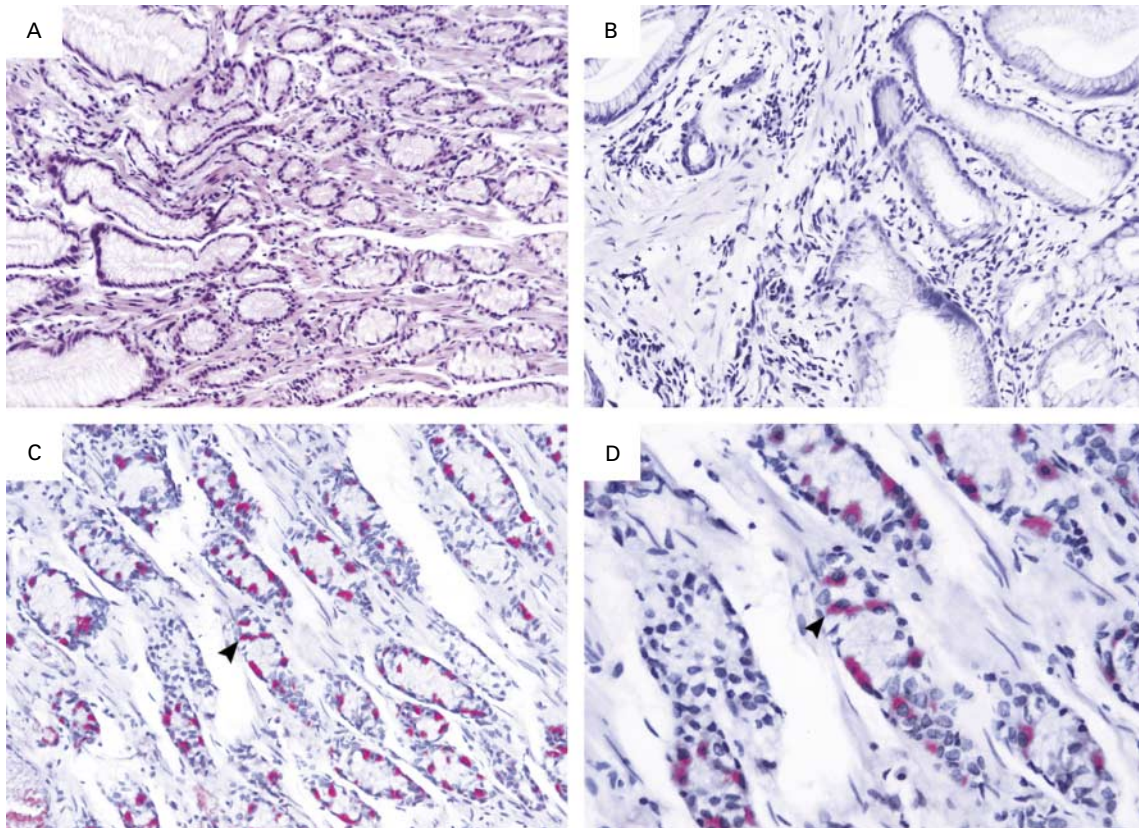
†The normal value is 3.2 to 5.0 g per deciliter.

‡The normal value is <100 pg per milliliter.

§The normal value is <54 mg per deciliter. Stool alpha<sub>1</sub>-antitrypsin concentrations provide quantitative measurements of loss of protein through the stool.<sup>16</sup>

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**Figure 1.** Staining of Parietal Cells in the Fundic Mucosa of a Patient with Ménétrier's Disease before and after One Month of Treatment with a Monoclonal Antibody against the Epidermal Growth Factor Receptor.

In Panel A ( $\times 200$ ), hematoxylin-and-eosin staining of a  $10\text{-}\mu\text{m}$  section from a gastric fundic specimen obtained before treatment shows marked foveolar hyperplasia. In Panels B, C, and D, immunostaining for  $\text{H}^+/\text{K}^+ \text{-ATPase}$  was performed to identify parietal cells in the gastric mucosa. Biopsy specimens were stained with monoclonal antibody against the  $\alpha$  subunit of  $\text{H}^+/\text{K}^+ \text{-ATPase}$  (clone 12.18, at 1:5000)<sup>17</sup> and then alkaline phosphatase-conjugated goat antimouse IgG (with Vector Red substrate detection). The sections were counterstained with Mayer's hematoxylin. In Panel B ( $\times 200$ ), a biopsy specimen obtained before treatment shows a complete absence of parietal cells. In Panels C ( $\times 200$ ) and D ( $\times 400$ ), a fundic-biopsy specimen shows numerous parietal cells near the base of the gastric fundic glands after one month of treatment, although foveolar hyperplasia is still present. The arrowheads in Panels C and D indicate the same immunostained parietal cell.

given over a two-hour period, followed by three weekly treatments of 250 mg per square meter, each given over a two-hour period.

### RESULTS

After the initiation of treatment with antibody against the epidermal growth factor receptor, the patient's nausea and vomiting decreased immediately. An actiniform eruption with pustular lesions and erythematous papules developed across his face and chest during the first week of treatment. These lesions responded to topical clindamycin therapy.

One day after the initiation of treatment, the concentrations of transforming growth factor  $\alpha$  increased by a factor of approximately three in serum and gastric juice (from 16 to 44 pg per milliliter and from 64 to 201 pg per milliliter, respectively), as determined by radioimmunoassay. This increase provided evidence

of effective blockade of the epidermal growth factor receptor.<sup>14</sup>

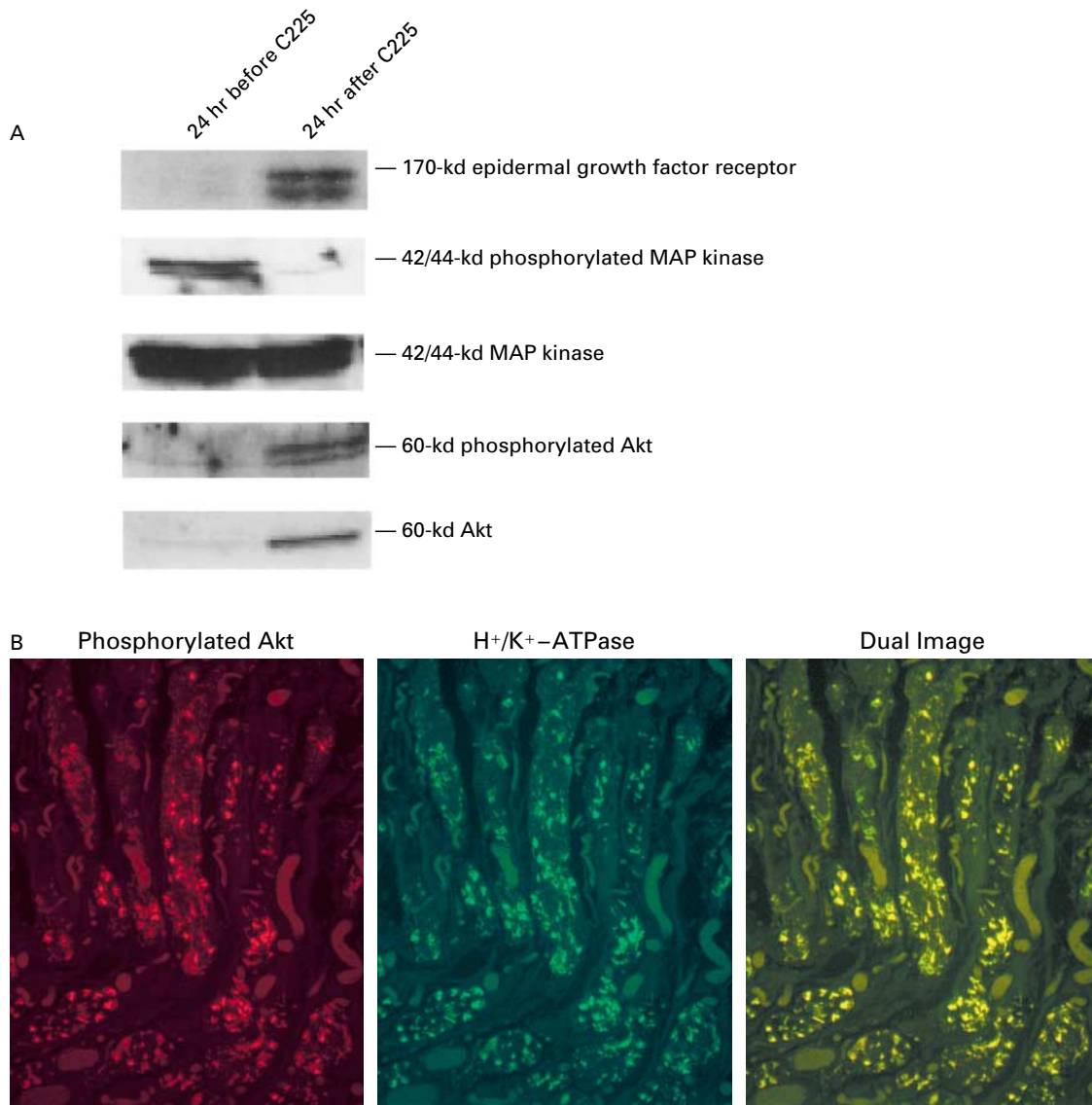
After one month of treatment, the patient's vomiting had decreased from an average of 70 episodes to just over 1 episode per week (Table 1), and there was a marked improvement in the quality-of-life score. The serum albumin concentration had increased, and loss of protein through the stool had decreased. Repeated endoscopy showed enlarged gastric folds, and biopsy revealed numerous parietal cells despite persistent foveolar hyperplasia (Fig. 1). The pH of the gastric juice remained at 7.0.

Protein extracts prepared from fundic-biopsy specimens obtained by gastroscopy before and after one day and one month of treatment were analyzed by Western blotting with antibodies against the epidermal growth factor receptor, mitogen-associated protein

kinase, and Akt, a protein apparently involved in cell survival. There was a marked increase in the tissue content of the epidermal growth factor receptor after treatment (Fig. 2A) — a finding consistent with the reversal of receptor down-regulation after receptor blockade. In addition, there was a marked decrease in the con-

centration of active phosphorylated mitogen-associated protein kinase but no change in the total kinase concentration. Activation of the mitogen-associated protein kinase pathway has been linked to increased proliferation of cells.

To examine this relation further, we determined the



**Figure 2.** The Effect of a Monoclonal Antibody against the Epidermal Growth Factor Receptor (C225) on Selected Signaling Events in the Gastric Mucosa of a Patient with Ménétrier's Disease.

Gastric-biopsy specimens were obtained during upper endoscopy, and protein lysates were prepared. In Panel A, 40  $\mu$ g of total protein was electrophoresed on a 12.5 percent denaturing polyacrylamide gel. After transfer to a nitrocellulose filter, the blots were probed sequentially with antibodies against the ectodomain of the epidermal growth factor receptor (mouse monoclonal IgG 31 G7 at 1:1000, Zymed Laboratories, South San Francisco), active phosphorylated and total mitogen-associated protein (MAP) kinase (both rabbit polyclonal at 1:1000, New England Biolabs, Beverly, Mass.), and active phosphorylated and total Akt (rabbit polyclonal anti-Ser<sup>473</sup> Akt and anti-Akt antibodies at 1:1000, New England Biolabs). In Panel B ( $\times$ 400), sections of the gastric mucosa obtained after one month of treatment were dual-stained with antibodies against the  $\alpha$  subunit of H<sup>+</sup>/K<sup>+</sup>-ATPase (clone 12.18, at 1:2000)<sup>17</sup> and phosphorylated Akt (sheep polyclonal anti-Ser<sup>473</sup> Akt at 1:500, Upstate Biotechnology, Lake Placid, N.Y.) and developed with Cy2- and Cy3-conjugated secondary antibodies, respectively (Jackson ImmunoResearch, West Grove, Pa.). Dual staining overlap is shown at right.

proliferative index in the fundic mucosa by immunostaining for Ki-67, a marker of actively dividing cells. The proliferative index, representing the mean number of Ki-67–positive surface mucous cells per gastric glandular unit and obtained by averaging the counts in 10 glandular units, decreased from an average of 45 (range, 20 to 65) before treatment to 5 (range, 0 to 15) in the biopsy specimen obtained one day after the initiation of treatment. At one month, the proliferative index was 18 (range, 7 to 34).

A striking finding one month after the initiation of treatment was the emergence of parietal cells. Since gastrin is a trophic factor for parietal cells, we measured serum gastrin concentrations, which increased from 82 to 425 pg per milliliter one day after the start of treatment and remained high (591 pg per milliliter) one month later. To examine the underlying molecular events, we measured the levels of fundic Akt. Total and active phosphorylated Akt increased significantly one day after the initiation of treatment, as determined by Western blot analysis (Fig. 2A). This increase was correlated with increased Akt activity in the fundus (data not shown). There was no detectable immunohistochemical staining for active phosphorylated Akt in the gastric-biopsy specimens obtained one day before and one day after the initiation of treatment, but intense immunoreactivity was seen in newly emergent parietal cells after one month of treatment (Fig. 2B). Akt and H<sup>+</sup>/K<sup>+</sup>–ATPase immunoreactivity colocalized within parietal cells.

After one month of treatment, the patient was evaluated for heart–lung transplantation. Unfortunately, he had cardiac arrest resulting from an anaphylactic reaction to the iodinated radiographic contrast material given during a transjugular liver biopsy, and he died suddenly. An autopsy was not performed.

## DISCUSSION

Transforming growth factor  $\alpha$  and the epidermal growth factor receptor are produced in the stomach.<sup>18</sup> Overproduction of transforming growth factor  $\alpha$  in the stomach could account for several of the features of Ménétrier's disease, including decreased acid production, increased hyperplasia of surface mucous cells, oxyntic atrophy, and increased mucin production.<sup>19–22</sup> Transgenic mice that overproduce transforming growth factor  $\alpha$  in the stomach have many of the features of Ménétrier's disease, including foveolar hyperplasia, increased mucin content, a reduced number of parietal cells, and decreased acid production.<sup>23,24</sup> Transforming growth factor  $\alpha$ , however, is one of six mammalian ligands that bind to the epidermal growth factor receptor,<sup>25</sup> and increased production of any of these ligands may contribute to Ménétrier's disease. Our previous results and the clinical and biochemical response to antibody against the epidermal growth factor receptor in this patient are

evidence that increased signaling of epidermal growth factor receptor has a role in the pathogenesis of Ménétrier's disease.

Our current understanding of the differentiation of fundic glandular elements suggests that the cells of the gastric gland arise from a progenitor zone located in the upper neck region of the gland. This population of progenitor cells then gives rise to two groups of cells with different life spans. Surface mucous cells with a life span of four to six days differentiate and migrate toward the lumen. In contrast, the other glandular cells (parietal cells, chief cells, and enterochromaffin-like cells) migrate basally; their predicted life spans are in excess of 60 to 100 days.<sup>26,27</sup> In transgenic mice that overexpress transforming growth factor  $\alpha$  in the stomach, there is a selective expansion of surface mucous cells at the expense of other cell lineages.<sup>28</sup> Serum gastrin concentrations are inappropriately low in these mice<sup>28</sup> and in patients with Ménétrier's disease (unpublished data), despite an alkaline gastric pH, which is a potent stimulus for gastrin secretion.<sup>29</sup> We speculate that increased signaling of the epidermal growth factor receptor in the presence of inappropriately low serum gastrin concentrations contributes to the histologic features of Ménétrier's disease. The prompt and sustained increase in serum gastrin concentrations after the administration of antibody against epidermal growth factor receptor probably stimulates the production of parietal cells.

There is no known association between primary pulmonary hypertension and Ménétrier's disease.<sup>30,31</sup> The long-term side effects of epoprostenol, which include nausea and vomiting, are dose-dependent and resolve with dose reduction. Our patient's gastrointestinal symptoms did not resolve after the dose of epoprostenol was decreased. There is no evidence at this time to implicate the patient's long-term epoprostenol therapy in the causation of Ménétrier's disease.

In this patient with Ménétrier's disease, systemic blockade of the epidermal growth factor receptor improved the clinical and biochemical features of the disease. Whether these results are reproducible and whether long-term treatment will be effective are not known. In the future, this treatment could be combined with inhibition of tumor necrosis factor  $\alpha$ –converting enzyme, the enzyme that cleaves transforming growth factor  $\alpha$  at the cell surface,<sup>32</sup> since this combined regimen results in cooperative inhibition of the growth of mammary and colorectal cancer cells *in vitro*.<sup>33</sup>

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