Impairment of gastric secretion modulation in duodenal ulcer and long-term PPI treatment

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Impairment of Gastric Secretion Modulation in Duodenal Ulcer and in Long-Term PPI Treatment

Quantitative Morphologic Findings and Pathophysiologic Implications

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Helicobacter pylori affects gastric secretion. This functional effect might have a morphometric counterpart. Therefore, the gastric cell secretory compartment was morphometrically assessed in different pathophysiologic conditions related to *Helicobacter pylori* infection. Nineteen *Helicobacter pylori*-positive nonduodenal ulcer subjects, 15 omeprazole chronically treated subjects, and 19 duodenal ulcer patients were studied against 19 controls. Somatostatin, gastrin, enterochromaffin-like, and parietal cell density was assessed in gastric biopsies. No differences in any cell type density were found between *Helicobacter pylori*-positive nonduodenal ulcer subjects and controls. On the contrary, differences were significant when comparing omeprazole and duodenal ulcer patients to controls (higher density of gastrin, enterochromaffin-like, and parietal cells, lower density of somatostatin cells). In duodenal ulcer a reversion to control values followed *Helicobacter pylori* eradication and ulcer healing. A direct linear correlation between enterochromaffin-like, gastrin, and parietal cell density was demonstrated. An almost complete map of mucosal cells involved in gastric secretion is provided by this study. The cell density pattern, identical to the omeprazole group, points to an impaired feedback control of secretion in duodenal ulcer. The reversion to control values after *Helicobacter pylori* eradication and ulcer healing demonstrates the pathogenetic role of *Helicobacter pylori*–host interaction in these changes.

KEY WORDS: duodenal ulcer; *Helicobacter pylori*, immunohistochemistry; PPI.

A strong association between *Helicobacter pylori* (*H. pylori*) infection and duodenal ulcer (DU) is well established (1, 2). Convincing evidence that *H. pylori* affects gastric secretion has been provided, and this may represent a major contributive factor to DU. The first step into this regard has been the demonstration of increased gastrin release in *H. pylori*-positive subjects, which has been theorized as the "gastrin link" between the infection and DU (3). More recently, it has been reported that, although all *H. pylori*-positive subjects have increased gastrin response to meal and to gastrin-releasing peptide (4), those who develop DU have an acid response to gastrin six times greater than controls

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and two times greater than *H. pylori*-positive non-DU patients (5).

A decrease in somatostatin release from antral D cells has also been shown in *H. pylori*-positive subjects (6–8), and this finding fits into the whole scenario perfectly, since somatostatin inhibits both gastrin/histamine release and chlorhydropeptic secretion in humans (9). Furthermore, complex interactions between *H. pylori* and gastric secretion regulation are suggested by the finding that enterochromaffin-like (ECL) cell hyperplasia after long-term treatment with PPIs is further enhanced in the presence of *H. pylori* infection (10).

We hypothesized that these functional effects of *H. pylori* infection could have histologic counterparts, such as quantitative alteration in the number of gastric endocrine and acid secreting cells. To address this issue, we morphometrically evaluated the density of mucosal cells involved in acid secretion and its regulation in DU and other *H. pylori*-related pathophysiologic conditions.

MATERIALS AND METHODS

Subjects. Seventy-two subjects entered the study. They were part of a series of outpatients consecutively referred to our endoscopy unit with dyspeptic symptoms. Thirty-eight patients were considered to be affected with functional dyspepsia according to Heading's definition (11), since no macroscopic abnormalities were detected at endoscopy. Of these dyspeptic patients, 19 (10 women and 9 men; mean age 44.8 years, range 21–66 years) were *H. pylori* negative, with neither macroscopic nor histologic abnormalities, and were consequently considered as controls; 5 were smokers (10 or more cigarettes a day). The remaining 19 dyspeptic patients (12 women and 7 men; mean age 50.3 years; range 26–72 years) were *H. pylori* positive as demonstrated by histology and culture; 5 were smokers.

Fifteen patients (5 women and 10 men; mean age 60.8 years, range 38–72 years) had been taking 20 mg daily omeprazole for at least 6 months (mean 32.0 months, range 6–120), due to grade 1–5 reflux esophagitis (12). One of them was affected with Barrett's esophagus; 5 were *H. pylori* positive and 10 *H. pylori* negative; 3 were smokers.

In 19 patients (7 women and 12 men; mean age 50.9 years, range 27–75 years) a DU was endoscopically demonstrated, and all 19 were shown to be *H. pylori* positive in the study protocol evaluation; 11 of them were smokers.

Age and sex distribution did not significantly differ between the various groups. None of the 72 subjects had taken PPIs, H_2-receptor antagonists, nonsteroidal antiinflammatory drugs, or other relevant drugs (except for omeprazole in the omeprazole group) in the two months before the study. None was suffering from any metabolic or systemic disorder. Alcohol intake was negligible in all subjects. All gave informed consent to undergo the study protocol, which consisted of upper endoscopy with multiple gastric biopsies and basal gastrin assessment. Gastrin assessment and endoscopy with biopsies were repeated in 8 patients of the DU group 30 days after completion of DU treatment. Treatment consisted of 8 days therapy with omeprazole 20 mg twice daily, clarithromycin 500 mg twice daily, metronidazole 400 mg three times daily, followed by omeprazole 20 mg once daily for three weeks. Ulcer healing was achieved in all the 8 patients. However, bacterial eradication was attained only in 6 of them and these only were evaluated for morphometry.

Fasting Serum Gastrin Concentration Assessment. Fasting serum gastrin concentrations were determined by a specific radioimmunoassay (GASK-PR, CIS Bio International, Gif-Sur-Yvette, France) in 10 ml of blood, which was quickly centrifuged and frozen at −80°C until measurement.

Endoscopy. All the esophagogastroscopies were performed by one of the authors (P.B.), with only local pharyngeal anesthesia using 2% lidocain (Xylocaine, Astra, Kings Langley, England). Sites of biopsy are schematically represented in Figure 1. Two grasp biopsies for histochemical assessment (forceps 413 26 180, open diameter 7.7 mm; Endo-Technik, Hilden, Germany) were taken from the antrum (greater curvature ~2 cm proximal to the pylorus), body (middle third of the vertical part of the greater curvature), and fundus (greater curvature in proximity of the body). One additional biopsy was also taken from the antrum, body, and fundus (as close as possible to the biopsies for histochemistry) and from the angulus for histologic evaluation. One more biopsy for *H. pylori* culture was taken from the antrum.

Histology. Gastric biopsy specimens were fixed in formalin, routinely processed, and embedded in paraffin. Sequential 5-μm-thick sections were stained with hematoxylin–eosin and Alcian blue/periodic acid Schiff (pH 2.5) for histopathologic evaluation. A modified Giemsa technique for the detection of *H. pylori* was used. Antrum and body biopsy specimens were classified for the gastritis pattern according to the updated Sydney System (13). Chronic inflammation, neutrophil infiltration (i.e., activity), glandular atrophy, intestinal metaplasia, and *H. pylori* density were graded on a 0–3 scale for each biopsy site.

For parietal cell morphometry, specimens were fixed with 4% formaldehyde (obtained from paraformaldehyde) in 0.1 mol/liter phosphate buffer, pH 7.4, dehydrated, and embedded in paraplast. Specimens were used for analysis regardless of orientation. Sections of the gastric body and fundus were stained with hematoxylin and Congo red for parietal cells (14) and photographed at ×400. The volume density of parietal cells was estimated by the ratio between the surface area of Congo red-stained cells and the overall surface area of sections of the glandular part of the mucosa (15). The glandular part of the mucosa corresponds to the area of the mucosa including the neck, body, and bottom of the gastric glands, while excluding the surface epithelium and the foveolar region. For each biopsy, a surface area of at least 1 mm^2 was scanned for these measurements. The cell surface area and the glandular part of the mucosa were measured with a suitable software program (Image 1.49, National Institutes of Health, Bethesda, Maryland, USA) applied to photomicrographs captured into a Macintosh PowerPC by a Color OneScanner 600/27 (Apple, Cupertino, California, USA).

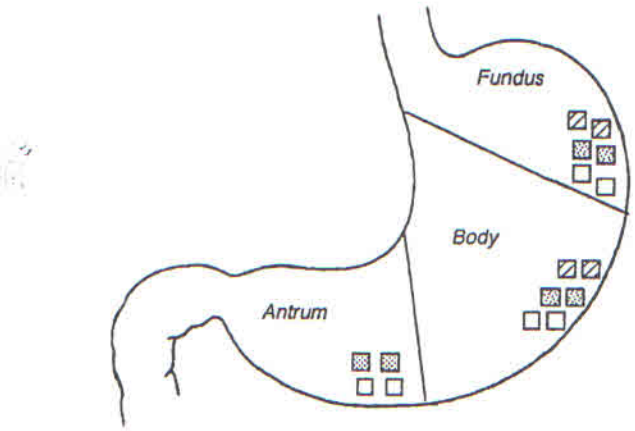

Fig 1. Sites of biopsies for morphometric assessment of each cell type.

Histochemistry. After fixation for 8 h with 8% ethyldimethylaminopropyl-carbodiimide (EDCDI; Sigma, Milan, Italy) in 0.1 mol/liter phosphate buffer, pH 6.9 (16), some specimens were dehydrated and embedded in paraplast. Sections of formaldehyde-fixed specimens were used to analyze gastrin D and G immunoreactive cells, whereas sections of EDCDI fixed specimens were used to analyze histamine immunoreactive cells (16). Polyclonal antibodies against gastrin (Sigma; diluted 1:200), somatostatin (Dako, Milan, Italy; undiluted) and histamine (Chemicon, Rome, Italy; diluted 1:500) and a monoclonal antibody against human chromogranin A (clone PHE5, Ortho Diagnostic System, Milan, Italy; undiluted) were used as primary antibodies and were revealed, with fluorescein isothiocyanate-labeled goat anti-rabbit antibodies and with rhodamine isothiocyanate-labeled goat anti-mouse antibodies (both from Sigma, diluted 1:50), as appropriate. The sections were examined with an Axioskop microscope (Zeiss, Oberkochen, Germany) equipped for epifluorescence. The specificity of the immunostaining was tested by omitting the first antibodies or by substituting them with nonspecific ones. In some experiments, anti-histamine antiserum was preadsorbed with histamine succinylated ovalbumine (1–10 mg/ml) for 24hr before applying to sections. No reactivity was observed in any control section. In order to keep approximation and assumptions to a minimum, the density of immunolabeled cells was expressed as the number of stained cells per unit section surface area. All G cells. D cells, and double immunoreactive cells for histamine and chromogranin A (ECL cells) (17) were counted, and the surface area was measured in one section per biopsy, comprising the full thickness of the mucosa. The number of cells per square millimeter of section surface area was eventually computed. The values obtained in this study were exactly four times those obtained from previous investigations (18, 19) as a consequence of readjustment of the microscopic system used for morphometry. This does not however, diminish previous conclusions based on the comparative analysis of results, because the relative experimental differences were independent of the absolute values.

Statistical Analysis. Statistical evaluation was performed by means of Kruskal-Wallis and Dunn tests for nonparametric multiple comparisons and the Spearman rank correlation test for independent samples. As for cell morphometry, analysis of variance as well as Student's t test for paired values, with two tails for comparisons between pre- and posttreatment DU patients, were carried out. $P < 0.05$ was considered significant. Results are given as mean ± SE.

RESULTS

Histologic Findings. Absence of histologic abnormalities in biopsy specimens was a prerequisite for inclusion in the control group. Only mild inflammation was present in the six patients of the DU group after eradication and healing. Therefore, data concerning these two groups do not appear in Table 1, which gives the scores for each of the main histopathologic parameters in the remaining three groups.

Fasting Serum Gastrin Concentration. In the *H. pylori*-positive non-DU, omeprazole, DU, and DU after eradication and healing groups mean serum gastrin concentrations were respectively 44.1 ± 3.5, 160.9 ± 39.6, 78.2 ± 4.1 and 70.8 ± 10.5. Differences from controls (45.1 ± 2.4) were significant for the omeprazole and DU groups ($P < 0.001$). Within the omeprazole group, *H. pylori*-positive subjects (212.4 ±

TABLE 1. SCORES FOR HISTOLOGICAL FEATURES IN *H. pylori*-POSITIVE NON-DU PATIENTS, OMEPRAZOLE SUBJECTS, AND DU PATIENTS*

	Antrum			*Fundus*		
	H. pylori *pos*^{I} *non-DU* (N = 19)	*Omeprazole*^{II} (N = 15)	*DU*^{III} (N = 19)	H. pylori *pos*^{I} *non-DU* (N = 19)	*Omeprazole*^{II} (N = 15)	*DU*^{III} (N = 19)
Inflammation	2.79 ± 0.10	0.60 ± 0.19	2.90 ± 0.07	2.21 ± 0.16	0.33 ± 0.16	2.26 ± 0.17
Activity	2.37 ± 0.16	0.13 ± 0.13	2.79 ± 0.09	1.53 ± 0.19	0.13 ± 0.13	1.47 ± 0.22
Atrophy	0.42 ± 0.18	0.07 ± 0.07	0.37 ± 0.19	0	0	0
Intestinal Metaplasia	0.26 ± 0.13	0.07 ± 0.07	0.26 ± 0.17	0	0	0
H. pylori density	2.47 ± 0.16	0.13 ± 0.13	2.84 ± 0.09	1.79 ± 0.22	0.13 ± 0.13	1.68 ± 0.26

*Values are mean ± SEM. Statistical analyses were performed with the Kruskall-Wallis and Dunn tests. Both in the antrum and fundus: Inflammation I vs II and II vs III: $P < 0.001$, activity I vs II and II vs III: $P < 0.001$, *H. pylori* density I vs II and II vs III: $P < 0.001$.

69.8) did not differ from *H. pylori*-negative ones (138.0 ± 48.7). Values decreased significantly ($P < 0.05$) in the DU group after eradication and healing.

G-Cell Density. Antral G-cell density was twice as high in the omeprazole and DU groups as in controls and the *H. pylori*-positive group. Moreover, density significantly decreased in the DU group after eradication and healing with reversion to values similar to those of controls (Figure 2).

D-Cell Density. In controls, D-cell density was eight times greater in the antrum than in the body and the fundus ($P < 0.001$) (Figures 2–4). Density did not differ significantly between the two latter sites. While D-cell density in the antrum was significantly lower in the omeprazole and pretreatment DU patients than in controls, there was no difference between controls, *H. pylori*-positive non-DU subjects, and DU patients after eradication and healing (Figure 2). In the body, values were nearly the same in all the groups (Figure 3). In the fundus, significant differences were found in the DU and omeprazole groups when compared to controls, but with much lower absolute values overall than in the antrum (Figure 4).

ECL-Cell Density. Body ECL-cell density was approximately three times greater in the omeprazole and DU groups than in the other groups. A significant decrease was noted in the DU group after eradication and healing, but this did not reach control values (Figure 3). Fundus ECL-cell density behaved in almost exactly the same way (Figure 4).

Parietal Cell Volume Density. Parietal cell volume density in the body did not differ from the fundus either in controls or in any of the study groups (Figure 5). Values were more than twice as high in the omeprazole and DU groups as in controls and the *H. pylori*-positive non-DU group. A

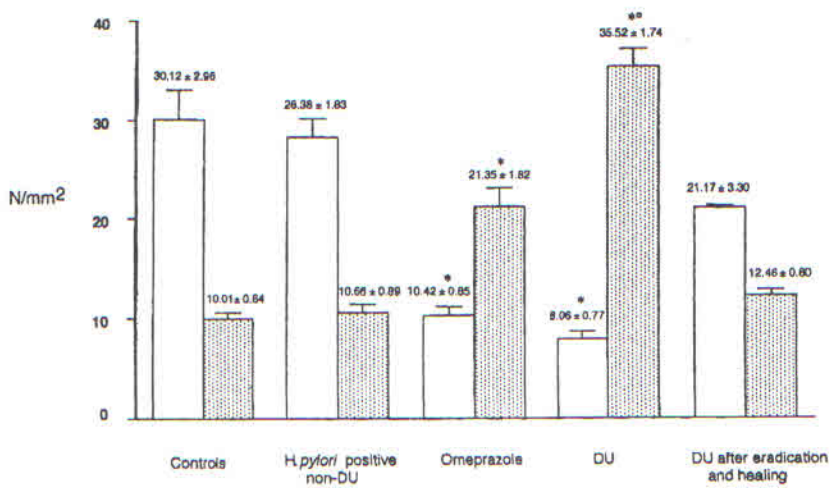

Fig 2. D (□) and G (▨) cell density (mean ± SEM) in the antrum. *$P < 0.001$ vs controls. *H. pylori* positive, and DU after eradication healing, °$P < 0.001$ vs omeprazole. Only comparisons yielding significant differences are mentioned.

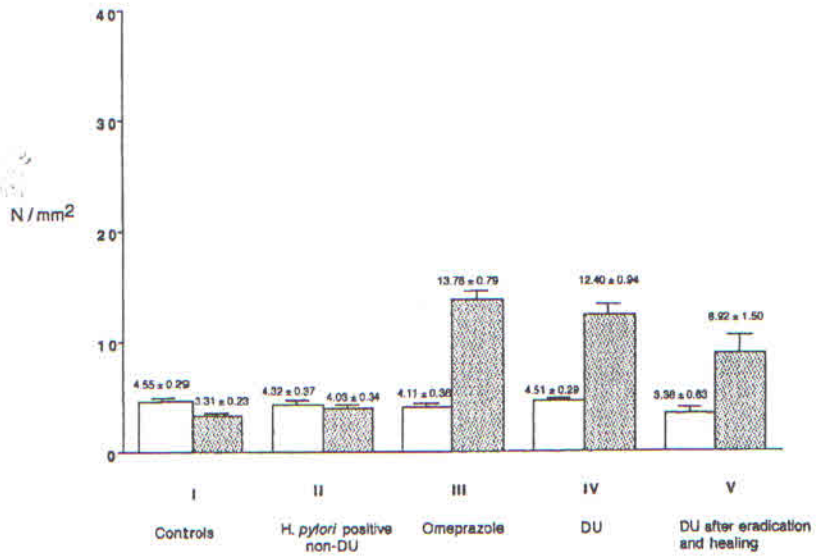

Fig 3. D (□) and ECL (▨) cell density (mean ± SEM) in the body. I vs III, IV, and V: $P < 0.001$; II vs III, IV, and V: $P < 0.001$; III vs V; $P < 0.001$; IV vs V; $P < 0.005$. Only comparisons yielding significant differences are mentioned.

significant decrease took place in the DU group after eradication and healing, reverting completely to control values in the body as well as in the fundus (Figure 5).

Relationships Between Different Cell Type Densities in the Whole Population. A direct linear correlation was shown between gastrin concentration and parietal cell density both in the body and the fundus ($P < 0.05$). Antrum G-cell density, antrum and body ECL-cell density, and body and fundus parietal cell volume density were reciprocally correlated ($P < 0.005$). An inverse linear correlation was shown between antrum D cell density and the density of all other cell types ($P < 0.005$).

Comparison of the Effects of Chronic PPI Therapy Within Omeprazole Group, Depending on *H. pylori* Status. In this group of subjects no significant difference was shown in the density of any cell type be-

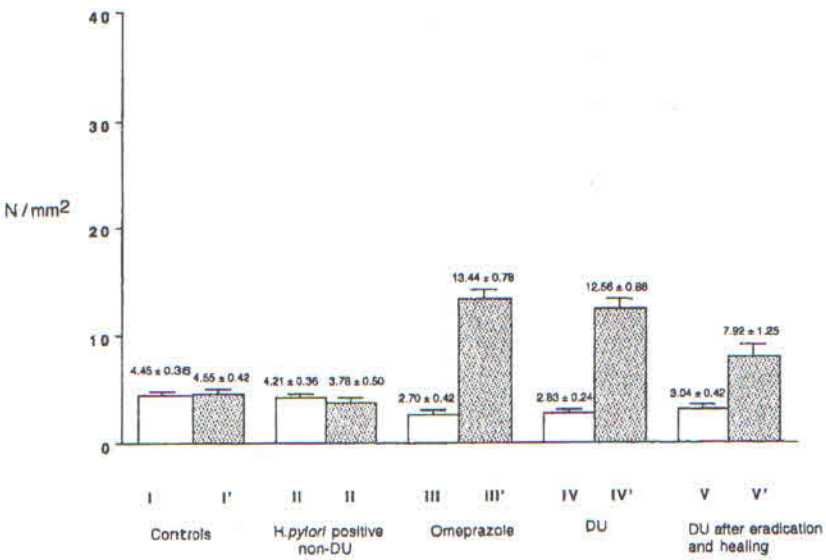

Fig 4. D (□) and ECL (▨) cell density (mean ± SEM) in the fundus. I vs III, and IV: $P < 0.01$; I' vs III', and IV'; $P < 0.001$; I' vs V' $P < 0.005$; II' vs III', and IV': $P < 0.001$; II' vs V'; $P < 0.001$. Only comparisons yielding significant differences are mentioned.

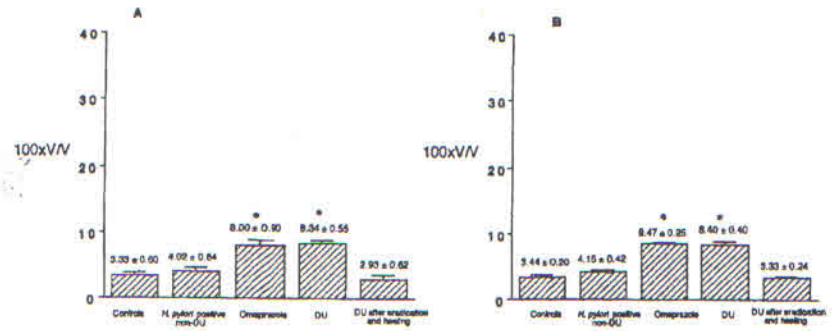

Fig 5. Parietal cell (▨) volume density in the body (A) and in the fundus (B). $^*P < 0.001$ vs controls, *H. pylori* positive, and DU after eradication and healing. Only comparisons yielding significant differences are mentioned.

tween *H. pylori*-positive and *H. pylori*-negative subjects.

DISCUSSION

This study has demonstrated that *H. pylori* is associated with significant changes in the number of D-, G-, and ECL-cells and in the volume of parietal cells in DU subjects, but not in those without DU, and that these changes revert towards control values after *H. pylori* eradication and ulcer healing.

H. pylori is known to affect gastric secretion. This effect seems to be exerted through the somatostatin–gastrin axis and has been considered relevant in DU development (3–9, 20, 21). However, only a minority of subjects with this widespread infection develop DU. The different clinical outcome might be determined by bacterial characteristics and/or host factors, which could result in different effects on gastric secretion (22).

Although the present study is mainly morphometric and does not directly explore functional aspects, our results seem capable of providing an insight into gastric secretion modifications in DU and other *H. pylori*-related pathophysiologic conditions, since morphology is generally affected by long-lasting functional changes.

These findings are interesting even from a solely physiologic point of view. Cell distribution in the control group now allows us to compile an almost complete map of the gastric mucosal cells involved in gastric secretion. Although predictable, the direct correlation between G-, ECL-, and parietal-cell density, as well as the inverse correlation between the density of D and all other cells, is remarkable.

However, the main finding of the study is the greater density of all types of secretion-regulating cells (with the exception of D cells, having an opposite behavior) in both DU and omeprazole subjects when compared with controls. The density distribution of each cell type is the same in the DU and omeprazole groups, with exactly the same differences from controls. PPI treatment inhibits the last step of gastric secretion: acid release into the glandular lumen. Therefore, in the omeprazole group, the secretion feedback mechanism, activated by luminal acidity and exerted through the somatostatin–gastrin–histamine pathway, is also inhibited. This determines the well-known increase in serum gastrin concentration, confirmed here, and provides an explanation of the differences in cell density between controls and the omeprazole group. Considering that morphometric findings are identical in the DU and omeprazole groups, an impairment of the feedback mechanism, rendering secretion partially independent of antral physiologic inhibitory pathways, may be hypothesized in DU. These data support previous functional findings, which point to a defect in the autoregulation of acid secretion in DU (23). Different mechanisms by which *H. pylori* could interfere with various steps of the secretion feedback loop can be hypothesized: (1) *H. pylori* is capable of $N\alpha$-methyl histamine synthesis, and this could inhibit antral somatostatin release by binding H_3 receptors on D cells (24); (2) the infection induces antral cytokine production, which in turn is capable of increasing gastrin release and inhibiting that of somatostatin (25, 26); (3) in spite of high luminal acid concentrations, bacterial ammonia production may be responsible for an alkaline mucosal environment surrounding the cells and modulating gastric secretion, thus preventing an effective feedback mechanism (27).

As previously demonstrated in drug discontinuation after long-term PPI treatment (28), *H. pylori* eradication and ulcer healing in DU determine rear-

rangement in cell populations involved in gastric secretion, with reversion of cell density to values comparable to those of controls. This indicates a causative role of *H. pylori* and omeprazole in cell density changes, found, respectively, in DU and patients chronically treated with PPI. The decrease in G-, ECL-, and parietal-cell density in DU patients after *H. pylori* eradication and ulcer healing is even more relevant if we consider that the inherent action of PPIs (administered to these patients for one month) has an opposite effect on these cells (18, 29). Complete reversion of the parietal cell volume density to control values is of special interest in challenging the hypothesis that a genetically expanded parietal cell mass (that could preexist in *H. pylori* infection) is important in the pathogenesis of DU (30). Differences in the preexisting cell mass could explain why only some of the *H. pylori*-positive subjects develop DU (30). The volume density of parietal cells expresses the percentage of mucosal volume occupied by functioning parietal cells. Its reversion to control values, after *H. pylori* eradication, suggests that the greater density of these cells in DU is not a preexisting congenital feature but is due to parasite–host interactions.

These data represent an expansion of our previous results concerning ECL cell density and mucosal histamine concentration in DU (19). The previously shown three times greater ECL density in DU patients, when compared to *H. pylori*-positive non-DU subjects, could certainly explain the exaggerated acid response to gastrin inherent to DU patients and could be the reason why only these subjects develop DU. The presently shown changes in density of all the cell types involved in gastric secretion and their reversion to control values after bacterial eradication and ulcer healing provide a more complete picture of secretory rearrangement and suggest its relevance in DU pathogenesis. Moreover, the very close similarity of cell distribution in DU and omeprazole-treated patients seems to point to a partial lack of efficacy of the physiologic acid-control feedback mechanism as the possible cause of cell rearrangement shown in DU.

Two previous studies have analyzed the number of G and D cells in the antrum of DU patients. Arnold et al (31) did not find any significant differences from controls. Graham et al (32) found a lower G-cell density in the antrum of DU patients when compared to controls and *H. pylori*-positive non-DU patients. Moreover, the number of cells per area differs greatly from our findings and there is also a discrepancy between these two previous reports. We are unable to thoroughly explain this, although differences in the selection of controls (*H. pylori* status was not assessed in Arnold's study), and in morphometric methods can partly explain the discrepancies.

From the histopathologic standpoint, two facts are noteworthy: the dramatic regression after treatment of gastric mucosal inflammation in the six cured DU patients, and the absence of linear and/or micronodular ECL-cell hyperplasia in the omeprazole group. After five years of PPI treatment, a prevalence of 29.2% has been reported for the latter finding, which is considered a precursor of ECL carcinoids (10). Methodological reasons (a lower number of biopsies for histopathology in our study, due to its different aim) and differences in the population under study (only reflux subjects undergoing shorter-term PPI treatment in our study) may account for this discrepancy.

The significant differences in gastrin serum concentration between controls and both the omeprazole and DU groups are coherent with present morphometric findings and in keeping with previous reports (5, 10, 19). The lack of significant differences between *H. pylori*-positive and -negative subjects within the omeprazole group is also consistent with previous data (10). Gastrin decrease after treatment in the DU group indicates both a causative role of the bacterium–host interaction in gastrin modifications and a pathogenetic role of increased gastrin levels in DU.

Morphometric findings in the course of omeprazole treatment are consistent with the functional ones recently reported (33) and provide the anatomic substratum for the transitory rebound hypersecretion after PPI discontinuation (33).

In conclusion, the mucosal density of the cells involved in gastric secretion is significantly different between DU patients and controls. This finding is specific to *H. pylori*-positive DU patients and is not generalized to all *H. pylori*-positive subjects. The close similarity in secretory compartment morphometry of the DU and the chronically treated omeprazole subjects points to an impaired feedback control of acid secretion, which could be instrumental in ulcer formation. The complete readjustment of the secretory compartment after *H. pylori* eradication and ulcer healing suggests a pathogenetic role of *H. pylori*–host interaction in these changes.

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