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Hypergastrinemia and Enterochromaffin-like Cell Hyperplasia

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The enterochromaffin-like (ECL) cells, the most frequent endocrine cells of the oxyntic mucosa of the stomach, are under the trophic stimulus of gastrin. These cells undergo a hyperplastic increase in variety of hypergastrinemic diseases. The most widely accepted nomenclature for the description of hyperplastic proliferation has been retrospectively arranged in a sequence presumed to reflect a temporal evolution of the proliferative process. A comparative, prospective study aimed to verify, in human hypergastrinemic diseases such as atrophic body gastritis (ABG), Zollinger-Ellison syndrome (ZES) and antral gastrin cell hyperfunction (AGCH), the effect of exposure of ECL cells to different pattern of gastrin hypersecretion, is lacking. To this purpose, we studied a series of consecutive patients with ABG, ZES and AGCH at the time of first diagnosis. Material and Methods: The patients included in this study (124 ABG, 18 ZES and 10 AGCH) were selected on the basis of two previously performed screening studies aimed to diagnose these diseases. All patients at the time of diagnosis underwent gastroscopy, with multiple biopsies of the gastric body mucosa for the evaluation of qualitative pattern of ECL cells hyperplasia, and basal fasting gastrin determination. A sample of hypergastrinemic patients from each group was further investigated by meal-stimulation of gastrin secretion and quantitative morphometry for CgA positive gastric body endocrine cells. Results: AGCH patients showed only the normal or simple hyperplasia pattern. In the ZES group, simple and linear grades accounted for 38.4 percent and 46.1 percent, respectively. MEN-I patients showed only these two patterns. The majority of ABG patients showed the presence of micronodular pattern (59.7 percent). A correlation analysis between fasting gastrin levels and grade of hyperplasia ($r = 0.5580$, $p < 0.0001$), indicates that the greater the gastrin levels, the higher is the degree of severity of ECL hyperplasia pattern. In conclusion, our data support the role of gastrin as the selective contributor to the progression of ECL cell hyperplasia in humans.

INTRODUCTION

The enterochromaffin-like (ECL)[b] cells, the most frequent endocrine cells of the oxyntic mucosa of the stomach, are under the trophic stimulus of gastrin. In fact, they have been shown to proliferate in experimental [1] and pathologic [2-3] states associated with hypergastrinemia. The hyperplastic response of the ECL cells to hypergastrinemia, either endogenous or exogenous, has been observed in rodents in elegant studies performed by Håkanson and co-workers [4]. If the gastrin stimulus was sustained for days, the ECL cells responded with hypertrophy [4]. If the stimulus was further sustained for weeks and months, a five-fold increase in ECL cell number (hyperplasia) developed [1, 5-6].

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[b]*Abbreviations:* ECL, enterochromaffin-like (cells); ABG, atrophic body gastritis; ZES, Zollinger-Ellison syndrome; MEN I, multiple endocrine neoplasia type I; AGCH, antral gastrin cell hyperfunction; TVD, total volume density; CgA, chromogranin A; SH, simple (or diffuse) hyperplasia; LH, Linear hyperplasia; MH, micronodular hyperplasia; AH, adenomatoid hyperplasia.

Although many experimental studies have been done on ECL cells with different models of induced hypergastrinemia, studies involving humans are usually restricted to patients with rare hypergastrinemic conditions such as atrophic body gastritis (ABG), Zollinger-Ellison syndrome (ZES) and antral gastrin cell hyperfunction (AGCH).

Various nomenclature have been used to describe the qualitative histological patterns of gastric body mucosa endocrine cell in humans. The most widely accepted, formulated by a panel of pathologists with specific interest in gastrointestinal endocrine pathology, has been retrospectively arranged in a sequence presumed to reflect a temporal evolution of the proliferative process [7]. This supposed successive development of increasingly more advanced grades of ECL cell growth has been indirectly verified in the aforementioned experimental animal models, in which the successive steps of the qualitative patterns of hyperplasia observed in humans have been produced by sustained levels of hypergastrinemia [8]. In addition, in these experimental conditions, the ECL cells also evolved to dysplastic lesions and carcinoid tumors [8]. Thus, the experimental observations and the occurrence of such qualitative morphologic changes may suggest, as also in humans, gastrin acts as a promoter of ECL cell growth. In a recent study in patients with carcinoid arising on ABG and ZES, it has been demonstrated by ultrastructural morphometry as gastrin exerts a selective role on ECL cell component of the endocrine cell population of the gastric body mucosa, thus strengthening the specific trophic action of gastrin on human ECL cells [9].

Qualitatively recognizable ECL cell hyperplasia in the human gastric mucosa has been mainly investigated in two pathological conditions: atrophic body gastritis [10] and Zollinger-Ellison syndrome [11]. ABG is characterized by chronic inflammation of oxyntic mucosa resulting in a progressive atrophy of the oxyntic glands and, subsequently, achlorhydria [12]. The unopposed feedback due to achlorhydria determines a sustained hypersecretion of gastrin by the G cells located in the antrum. ABG is a consistent finding in pernicious anemia, but can be found independently from it [13]. In ZES the hypergastrinemia is determined by a gastrin-secreting tumor, mostly of pancreatic or duodenal origin [14]. The hypergastrinemia induces a state of unremitting gastric acid hypersecretion and recurrent ulcer peptic disease. In 25 percent of the cases [14], ZES can also be a manifestation of the multiple endocrine neoplasia type 1 (MEN I), a disease characterized by an inherited predisposition to develop endocrine tumors.

Another human disease associated with increased gastrin levels is the antral gastrin cell hyperfunction (AGCH) syndrome, also known as pseudo-ZES. This syndrome is a debated clinical entity described to occur with or without antral gastrin cell hyperplasia in adults and in infants [15-16], probably reflecting one side of the spectrum of *H. pylori*-related duodenal ulcer disease [17]. Few data concerning the ECL cell hyperplasia in this rare condition are available, but a qualitative description of the pattern of ECL proliferation is lacking.

In these three diseases, a significant correlation has been demonstrated between circulating gastrin concentrations and ECL cell number [10, 18-20]. In addition, the entire spectrum of qualitative ECL cell proliferation, from hyperplasia to dysplasia and to neoplasia, has been observed in patients with ABG and ZES [11]. However, most of the studies regarding the prevalence of ECL cell pattern of growth in these hypergastrinemic conditions are retrospective in nature and based on filed histological specimens for which complete functional data do not exist [7, 21]. In addition, a prospective comparative analysis of the ECL cell changes present in these three human diseases has never been presented.

Another item that deserves attention is the possible role of stimulated gastrin levels in contributing to ECL cells proliferation. The main stimulus for gastrin secretion is constituted by the daily meals, which induce different gastrin response in the human hypergastrinemic conditions. In fact, even though in ZES patients tumoral hypergastrinemia is independent from any physiological stimuli, both ABG and AGCH, characterized

by G cells hyperplasia/hyperfunction, are greatly affected by stimulants such as meals [22]. Thus, the role of different pattern of gastrin response to the meals and the consequent different time and levels of exposure of human ECL cell to the specific trophic hormone should also be taken in account in evaluating the relationship between gastrin and its cellular target.

Aim of the present study was to verify, in human hypergastrinemic diseases, the effect of exposure of ECL cells to different pattern of gastrin hypersecretion. To this purpose, we studied a series of consecutive patients with ABG, ZES and AGCH at the time of first diagnosis.

MATERIAL AND METHODS

Patients

The patients included in this study were selected on the basis of two screening studies. The first concerned a screening program for early detection of ABG in patients with unexplained anemia or long-standing history of dyspepsia, as described in detail elsewhere [13]. In the second study, ZES and AGCH patients were identified among a group of patients with resistant duodenal ulcer, which represents one of the main clinical characteristic of these diseases, as previously published [23].

Atrophic body gastritis. One-hundred and twenty-four patients (35 males and 89 females; age range: 22-83 [median: 56.5]) in whom ABG was newly diagnosed from June 1991 to December 1996. The patients who were found to have gastrin values exceeding the normal range at our laboratory were further investigated with test for gastric acid secretion (basal and pentagastrin-stimulated) and underwent gastroscopy with multiple biopsies of the gastric body mucosa for conventional histopathological examination and for the evaluation endocrine cells. Only patients with histologically proven ABG, irrespective of the type [24], were included. Pernicious anemia was present in 48 patients (38.7 percent) (Table 1).

Zollinger-Ellison syndrome. Eighteen patients (12 males and six females; age range: 11-67 [median: 53.5]) in whom the diagnosis of ZES was established by demonstration of repeated basal plasma hypergastrinemia, basal acid hypersecretion and positive secretin test (plasma gastrin increase more than 200 pg/ml over basal value after IV bolus of 2 U/kg secretin) [25]. MEN-1 was present in five patients (four males and one female; 27.7 percent) (Table 1).

Antral gastrin cell hyperfunction. Ten patients (eight males and two females; age range: 19-54 [median: 35.5]) defined by co-existence of basal and meal-stimulated gastrin levels exceeding the normal range, a negative secretin test, an increased basal and/or peak acid output (BAO and PAO, respectively), as previously described [15-16, 23] (Table 1).

Control group. Seventeen individuals (two males and 15 females; age range: 21-67 [mean: 44.2]) with dyspeptic complaints but no histological findings of body mucosal atrophy and normal fasting and stimulated gastrin values.

Biopsies

All patients investigated underwent gastroscopy with multiple biopsies of the gastric body mucosa for the same morphological investigations performed in ABG patients. In each patients three to four biopsies were obtained from the mid-part of the gastric body mucosa approximately 4 to 5 cm above the corpus/antrum border along the great curve and from the antral mucosa with the use of a fiberoptic gastroscope. Immediately after recovery, the biopsy specimens were fixed in Bouin's fluid for three to four hours at room temperature. After rinsing in 70 percent ethanol, they were alcohol dehydrated and embedded in paraffin.

Table 1. Clinical and biochemical features of Zollinger-Ellison (ZES), Antral G cell hyperplasia (AGCH) and atrophic body gastritis (ABG) patients. Results are expressed as median (range).

	ZES n = 18[a]	AGCH n = 10	ABG n = 124[b]
Age (yrs)	53.5 (11-67)	35.5 (19-54)	56.5 (22-83)
Sex (male/female)	12/6	8/2	35/89
Basal serum gastrin (pg/ml[c])	950 (235-8000)	56.1 (45-77)	375 (49-2700)
Basal acid output (mEq/h)	48.8 (12-96)	15 (11-22.4)	0 (0-1.3)
Pentagastrin acid output (mEq/h)	57 (24-105)	46.5 (24.7-96.1)	0 (0-10)

[a]Including 5 with MEN-1 (four males, one female).
[b]48 patients had pernicious anemia.
[c]Normal value = 10-40 pg/ml.

Morphological investigations of gastric body mucosa

Histology and Immunocytochemistry. Serial 5 μm-thick sections of gastric body mucosa perpendicular to the mucosal surface were stained with hematoxylin-eosin for conventional histopathological examination and for immunostaining of endocrine cells using monoclonal antibody against chromogranin A (CgA; clone LK2H10, Biogenex Laboratory, San Ramon, CA) followed by avidin-biotine complex procedure (Dako, LSAB2 kit, Dakopatts, Glostrup, Denmark). Biotinylated goat antirabbit Igs (code BA1000, Vector Laboratories, Burlingame, CA; dilution, 1:200) were then used as a secondary antibody, followed by the avidin-biotin-complex peroxidase technique (Vectastain ABC Kit, Vector Laboratories, Burlingame, CA). For the immunoreaction, diaminobenzidine tetrahydrochloride was used as a chromogen substrate and nuclear counterstaining with hematoxylin was performed. Glandular atrophy of the body mucosa was defined as replacement of oxyntic glands by metaplastic pyloric and/or intestinal glands [19].

The endocrine cell changes were evaluated in non-intestinalized areas of gastric body mucosa. The following patterns of endocrine cell growth were identified according to the classification proposed by Solcia et al. [7] and independently evaluated by two examiners who were unaware of the patient's clinical data: 1) normal pattern; 2) simple (or diffuse) hyperplasia (SH); 3) linear hyperplasia (LH); 4) micronodular hyperplasia (MH); and 5) adenomatoid hyperplasia (AH). The patients were subdivided into different groups according to the highest grade of endocrine cell hyperplasia [23]. Consequently, patients with Normal and SH were assigned to the group of SH, those with both SH and LH were assigned to the subgroup of LH, those with both LH and MH were assigned to the group of MH and those with both MH and AH were assigned to the group of AH.

Morphometry. The methodological procedure employed in the study of the volume density of all endocrine cells (identified by CgA immunostaining) has been described in detail elsewhere [26]. On the basis of the stereological principle dictating that the area fraction of any tissue component is equal to its volume fraction [27], the volume density of endocrine cells was calculated as the fraction of the area of the epithelial mucosal component (glandular lumina excluded) occupied by immunostained endocrine cell profiles and the results were expressed as a percentage of the endocrine cell mass over the total volume of the mucosal epithelial structures.

Gastrin Assay and test meal

Plasma gastrin levels were determined by radioimmunoassay using antiserum 4562, kindly supplied by Prof. J.F. Rehfeld, Copenhagen, Denmark, as previously described [28]. This antiserum measures gastrin components I, II and III with almost equimolar potency [29]. Plasma values are expressed as pg/ml equivalent of SHG 17 I. Plasma gastrin was measured in all patients. Previous evaluation of normal subjects in our laboratory using this antibody showed that the normal range for basal gastrin was 10-40 pg/ml [24]. A protein-rich meal stimulation was performed, as described [15], in a representative sample of patients from each group.

Statistics

Data were expressed as median (range) or number/total (percentage, percent). Analyses were performed using the Mann-Whitney test for unpaired non parametric data. The Spearman rank test was applied for correlation analysis. To evaluate the relationship between gastrin values and qualitative pattern of ECL cell growth, each pattern was graded as follows: normal pattern = 1; simple = 2; linear = 3; micronodular = 4; adenomatoid = 5. Two-tailed P values less than 0.05 were considered statistically significant.

RESULTS

The respective percentage of ECL pattern hyperplasia in each group of the investigated hypergastrinemic patients at the time of diagnosis is shown in Figure 1. Both controls and the AGCH patients showed only the normal or SH pattern. However, in the controls, the normal pattern predominated, whereas the AGCH group showed a predominance of the SH pattern. In the ZES group Normal pattern of ECL cells was not observed, hyperplastic changes were mainly composed by the Simple and Linear grades accounting for 38.4 percent and 46.1 percent respectively, and in the MEN-I group the same patterns were found. Micronodular and adenomatoid grades of hyperplasia were minimally represented only in

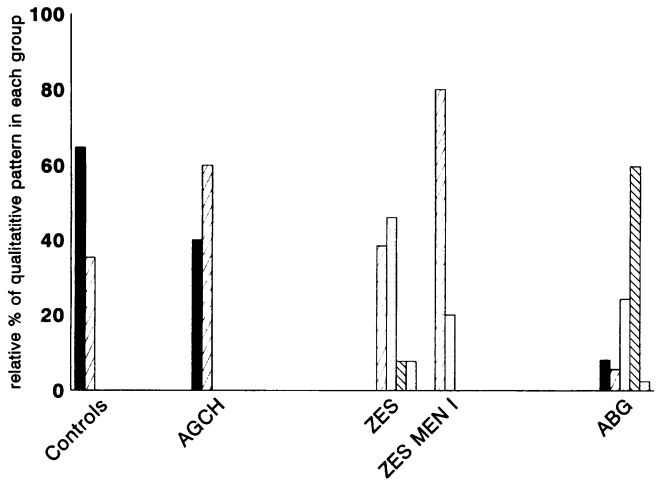

Figure 1. Relative percentage of qualitative pattern of ECL cell in each group of hypergastrinemic patients and controls investigated. ■ = Normal pattern; ▨ = Simple hyperplasia pattern; □ = Linear hyperplasia pattern; ▧ = Micronodular hyperplasia pattern; ▥ = Adenomatoid hyperplasia pattern.

Table 2. Median (range) of fasting gastrin levels in hypergastrinemic patients at the time of diagnosis subdivided according to the qualitative pattern of endocrine (ECL) cells[a].

	Normal	SH	LH	MH	AH
ZES	—	440 (235-2205)	1050 (315-2625)	350	8000
n = 18	n = 0	n = 9	n = 7	n = 1	n = 1
AGCH	51.5 (45-58.6)	59 (45-77.3)	—	—	—
n = 10	n = 4	n = 6	n = 0	n = 0	n = 0
ABG	76.8[a] (49-315)	140[b] (65-450)	235[c] (99-1800)	500[d] (70-2700)	500 (419-750)
n = 124	n = 10	n =7	n = 30	n = 74	n = 3

SH, Simple hyperplasia; LH, linear hyperplasia; MH, micronodular hyperplasia; AH, adnomatoid hyperplasia
[a]$p < .05$ vs. simple.
[b]$p < .005$ vs. micronodular.
[c]$p < .001$ vs. micronodular.
[d]$p < .0001$ vs. simple

the sporadic ZES subgroup (Fig. 1). On the contrary, in the ABG group, all ECL cell hyperplasia pattern were present. However, the majority of patients showed the presence of the micronodular pattern (59.7 percent). In addition, even if the adenomatoid grade was observed in three patients, it represented only the 2.4 percent of total observations.

Table 2 shows the median (range) fasting gastrin levels of the hypergastrinemic patients subdivided according to the qualitative pattern of endocrine (ECL) cell. In AGCH patients no apparent relationship exists between the slight fasting hypergastrinemia and the qualitative pattern of endocrine cell. On the contrary, in both ZES and ABG patients, a relationship between fasting gastrin values and grade of ECL cell hyperplasia seemed to occur. In fact, ZES patients (including the five MEN I patients) with simple pattern had a median fasting gastrinemia, which was half of that of patients with the linear one (440 pg/ml vs. 1050 pg/ml), and adenomatoid changes were observed only in the single patient with the highest levels of gastrin. In the ABG group, no significant difference in median gastrin levels was achieved between the normal and SH groups. However, the increasing severity of the pattern of endocrine cell hyperplasia, from simple to micronodular was associated with a stepwise statistical increase of median fasting gastrin values (Table 2).

In order to verify if the fasting gastrin levels were related to the qualitative pattern of ECL hyperplasia, a correlation analysis between hormonal levels and grade of hyperplasia for all the four patient's group investigated (n = 169), was performed (see material and methods). Spearman rank test was highly significative ($r = 0.5580$, $p < 0.0001$), indicating that the greater were the gastrin levels, the higher was the degree of severity of ECL hyperplasia pattern.

To evaluate the pattern of gastrin response to physiologic stimulus, a protein-rich meal was performed in a sample of patients from each group. Figure 2 shows, as expected, that tumoral gastrin levels were substantially not affected by the meal. On the contrary, in ABG the meal test was able to significantly increase, for all the time of observation, the already elevated basal gastrin levels ($p < .05$). In addition, a more prolonged stimulatory effect of the meal was observed during all the two-hour test in ABG patients, compared to the levels observed in AGCH and controls, indicating a pronounced and protracted gastrin response

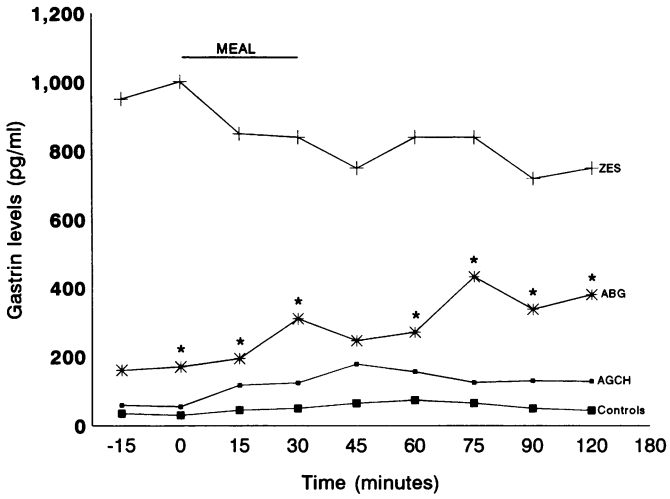

Figure 2. Median values for gastrin plasma concentration before, during and after proteic meal in controls (n = 10) (■); AGCH patients (n = 10) (▪); ZES patients (n = 10) (+); and Atrophic body gastritis (n = 25) (✳). Full line indicates the length of the meal.* Mann Whitney rank test two-sided p <0.05 of ABG compared with AGCH and controls.

in the atrophic patients. AGCH patients had a significantly higher gastrin increase in response to meal than controls ($p < .01$), with a tendency to remain elevated at the end of test.

The different percentage in qualitative pattern observed in body endocrine cells in the hypergastrinemic conditions were analyzed by morphometric analysis in a representative number for each condition reflecting the relative percentage pattern of hyperplasia (see Figure 1). Figure 3 shows the total volume density (TVD) of CgA-positive endocrine cell of the gastric body mucosa observed at diagnosis. Control and AGCH patients had a overlapping median TVD. AGCH patients had a statistical lesser number of ECL cells than all other hypergastrinemic patients evaluated. ZES patients had a five-fold TVD than that of control group (2.77 percent [1.53-5.8] vs. 0.73 percent [0.24-1.50]; $p < .005$), being not different if analyzed separately for the presence of MEN I. Median total volume density of ECL cells of ABG patients was about seven-fold the control values and more than two times that of ZES (6.04 percent [3.4-7.58] vs. 2.77 percent [1.53-5.8]; $p < .05$).

DISCUSSION

In this study, we have investigated the role of hypergastrinemia in the determination of ECL cell qualitative pattern of proliferation in the human gastric body mucosa. To this purpose, a series of consecutive patients with ABG, ZES and AGCH were selected on the basis of screening programs and studied at the time of diagnosis.

In respect to the other group of patients, the initial picture of patients with ABG showed a more severe qualitative histopathological pattern of endocrine cell, since the micronodular pattern was present in the 59.7 percent of total atrophic patients. In contrast, most ZES and all the AGCH patients, at the moment of diagnosis, showed less severe patterns of endocrine cell hyperplasia. In ZES, the normal pattern, however, was never observed, the SH and LH pattern predominated, while the micronodular was infrequent as previously observed [11, 20]. These distinct patterns found at diagnosis are due to different gastrin levels as shown by the highly significant correlation between hormonal values and qualitative

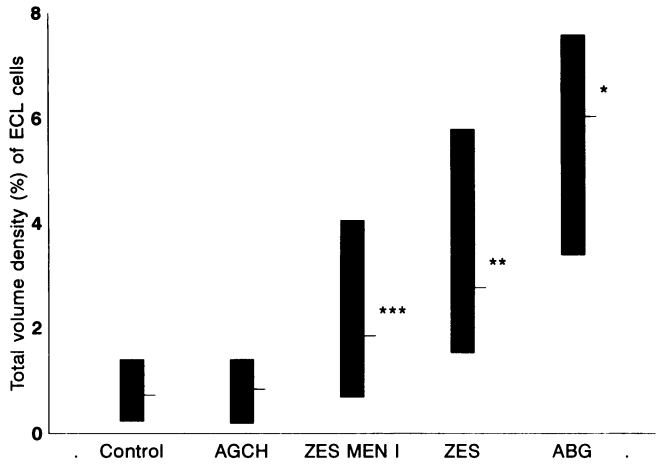

Figure 3. Median and range values for total volume density of ECL express as percentage in the controls (n = 6), AGCH (n = 6), ZES patients (n = 10), ZES MEN I (n = 5) and ABG patients (n = 14). Full line indicates median values.* vs. ZES $p< 0.05$; vs. ZES MEN I $p< 0.01$; vs. AGCH $p< 0.01$; vs. control $p< 0.01$. ** vs. AGCH $p < 0.05$; vs. controls $p< 0.05$. *** vs. AGCH $p < 0.05$; vs. controls $p< 0.05$.

grade of hyperplasia. Moreover, in these diseases, different time of exposure to the trophic stimulus of gastrin before the diagnosis should also be considered. In fact, ABG is a barely symptomless disease whose course prior to diagnosis may be estimated in terms of decades of life [30-31], resulting in a long-term exposure to elevated gastrin levels. In contrast, given the clinically more active and manifest nature of ZES, development of symptoms to the time of diagnosis is relatively short, three to six years [14]. In the AGCH group, characterized by a severe duodenal ulcer disease, the time before diagnosis is similar to that of ZES patients, but the mildly elevated levels of fasting gastrin well reflect the milder qualitative pattern observed (i.e., normal and simple hyperplasia).

Another aspect that must be evaluated analyzing the differences in the qualitative pattern of ECL in our hypergastrinemic patients is the origin of hypergastrinemia, which is different for each human diseases considered. ZES is a tumoral disease where gastrin secretion proceeds unrelated to the acid feed-back or to physiologic stimuli as meals, determining a continuous exposure of ECL cells to severely and constantly elevated gastrin levels, where the role of stimulated gastrin levels is minimal if not irrelevant. Atrophic body gastritis is characterized by a secondary G cell hyperplasia due to the lack of physiological negative feed-back between acid and antral gastrin secretion. Thus, ABG fasting gastrin levels well reflects the extent of the oxyntic damage and express the wide range of mucosal alteration present in this disease [13]. Moreover, due to occurrence of G cells hyperplasia, these patients had a more prolonged and sustained gastrin response to the meal, which adds, for several hours of the day, a supplemental amount of gastrin to the already elevated hormonal values, thus suggesting that during the day ABG patients' ECL cells are exposed to a dual gastrin modulation: the fasting hypergastrinemic background and the additional amount induced by meals.

AGCH syndrome arises from an unbalanced regulation between antral G and D cell, related to the *H. pylori* infection [17]. These patients display slightly elevated fasting gastrin levels and a moderate meal-induced hypergastrinemia, well different from the gastrin values observed in ABG and ZES patients. Thus, although the behavior of daily gastrin

secretion is similar to that of atrophic patient, the magnitude of gastrin release is significantly lesser, as also reflected by the predominance of milder pattern of hyperplasia in AGCH patients.

However, if one compares the median fasting gastrin values in the different groups, it can be noted that at the same pattern of hyperplasia, overlapping median levels of gastrinemia do not correspond. For example, the simple pattern is found with quite different median gastrin values: ZES, 440 pg/ml; AGCH, 59 pg/ml; and ABG, 140 pg/ml. This finding suggests that gastric mucosa status (atrophic or hypertrophic changes) is another possible key factor influencing the supposed evolutive sequence of ECL cell pattern. In particular, it has been suggested that the MH pattern is not exclusively due to the gastrin trophic effect exerted on the ECL cells but may also represent an epiphenomenon of mucosal atrophy due to the persistence of endocrine cells after disappearance of the other epithelial components [32-33]. On the other hand, in the non-atrophic gastric mucosa of ZES patients, the high gastrin levels were only associated with minimal prevalence of micronodules, thus the role of mucosal height deserves a careful investigation in order to exclude apparent differences only due to epithelial alteration.

It has been observed as in ZES the most advanced hyperplasia pattern (micronodular and adenomatoid) occur almost exclusively in patients with MEN I [21]. Unexpectedly, we observed that this did not occur in our population, in fact one sporadic ZES patient showed the presence of MH, while another one had the adenomatoid. We have no data able to explain this occurrence, but we think that one possible explanation can be found in the effect of prolonged pharmacological treatment. In fact, the marked inhibition of gastric acid secretion produced by the use of antisecretory drugs has been suggested as a possible cause for the increase in gastrin level and for the consequent increase of ECL cell in ZES patients [20].

The different percentage in prevalence of qualitative ECL cells pattern observed in the hypergastrinemic conditions, were further supported by morphometric analysis. The total volume density of ECL cells in controls and AGCH patients overlapped, in keeping with the observed presence of the normal, or at maximum, of the SH pattern. TVD of sporadic ZES was not different from that of ZES MEN I patients, supporting the lack of differences in qualitative findings and in fasting gastrin levels between these two subgroups found in this study. Interestingly, ABG patients showed the most elevated TVD values, more than two times those of ZES, suggesting again that more than the absolute gastrin levels, the time (decades) of exposure to trophic hormone could be crucial.

In conclusion, our data further support the concept of gastrin as the selective contributor to the development and progression of ECL cell hyperplasia in humans. However, the mucosal status of the hypergastrinemic patients remains to be clarified in detailed and comparative studies.

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REFERENCES

1. Håkanson, R., Ekelund, M., Sundler, F. Activation and proliferation of gastric endocrine cells. In: Falkmer, S. and Håkanson, R. and Sundler, F., eds. Evolution and tumor pathology of the neuroendocrine system. Amsterdam: Elsevier; 1984, pp. 371-398.
2. Bordi, C., Costa, A., and Missale, G. ECL cell proliferation and gastrin levels. Gastroenterology 68:205-206, 1975.
3. Creutzfeldt, W. The achlorhydria-carcinoid sequence: role of gastrin. Digestion 39:61-79, 1988.
4. Håkanson, R., Chen, D., and Sundler, F. The ECL cells. In: Johnson, L.R., ed. Physiology of the gastrointestinal tract. New York: Raven Press; 1994, pp. 1171-1183.
5. Brenna, E. and Waldum, H.L. The trophic effect of gastrin on the enterochromaffin-like cells of the rat stomach. Establishment of a full-range concentration-response relationship. Gut 33:1303-1306, 1992.
6. Ryberg, B., Tielemans, Y., Axelson, J., Carlsson, E., Håkanson, R., Mattsson, H., Sundler, F., and Willems G. Gastrin stimulates the self-replication rate of enterochromaffin-like cells in the rat stomach. Effect of omeprazole, ranitidine, and gastrin-17 in intact and antrectomized rats. Gastroenterology 99:935-942, 1990.
7. Solcia, E., Bordi, C., Creutzfeldt, W., Dayal, Y., Dayan, A.D., Falkmer, S., Grimelius, L., and Havu, N. Histopathological classification of nonantral gastric endocrine growths in man. Digestion 41:185-200, 1988.
8. Modlin, I.M., and Tang, L.H. The enterochromaffin-like cell: an enigmatic cellular link. Gastroenterology 111:783-810, 1996;.
9. D'Adda, T., Annibale, B., Delle Fave, G., and Bordi, C. Oxyntic endocrine cells of hypergastrinaemic patients: differential response to antrectomy or octreotide. Gut 38:668-674, 1996.
10. Borch, K., Renvall, H., Liedberg, G., and Andersen, N. Relations between circulating gastrin and endocrine cell proliferation in the atrophic gastric fundic mucosa. Scand. J. Gastroenterol. 21:357-363, 1986.
11. Bordi, C., D'Adda, T., Azzoni, C., Pilato, F.P., Baggi, M.T.,and Yu, J-Y. Hyperplasia of endocrine cells in the human oxyntic mucosa. In: Håkanson, R and Sundler F, eds. The stomach as an endocrine organ. Elsevier: Amsterdam; 1991, pp. 403-424.
12. Strickland, R.G. and Mackay, E.R. A reappraisal of the nature and significance of chronic atrophic gastritis. Am. J. Dig. Dis. 18:426-444, 1973.
13. Annibale, B., Marignani, M., Aprile, M.R., D'Ambra, G., Caruana, P., D'Adda, T., Delle Fave G., and Bordi C. Atrophic body gastritis: distinct features associated with *Helicobacter pylori* infection. *Helicobacter*. 2:57-69, 1997.
14. Jensen, R.T. and Gardner, J.D. Gastrinoma. In: Go, V.L.W., DiMagno, E.P., Gardner, J.D., Lebenthal, E., Reber, H.A., Scheele, G.A., eds. The pancreas: biology, pathobiology, and disease. Second Edition. New York: Raven Press; 1993, pp. 931-978.
15. Delle Fave, G., Annibale, B., Puoti, M., Giordano, V., Corleto, V., De Magistris, L., and Torsoli, A. Medical treatment of antral gastrin cell hyperfunction: role of non-antisecretory therapy. Digestion 46:65-71, 1990.
16. Annibale, B., Bonamico, M., Rindi, G., Villani, L., Ferrante, E., Vania, A., Solcia, E., and Delle Fave, G. Antral gastrin hyperfunction in children. Gastroenterology 101:1547-1551, 1991.
17. Annibale, B., Rindi, G., D'Ambra, G., Marignani, M., Solcia, E., Bordi, C., and Delle Fave, G. Antral gastrin cell hyperfunction and *Helicobacter pylori* infection. Aliment. Pharmacol. Ther. 10:607-615, 1996.
18. Bordi, C., Azzoni, C., Pilato, F.P., Robutti, F., D'Ambra, G., Caruana, P., Rindi, G., Corleto, V., Annibale, B., and Delle Fave, G. Morphometry of gastric endocrine cells in hypergastrinemic patients treated with somatostatin analogue octreotide. Regul. Pep. 47:307-318, 1993.
19. Maton, P.N., Lack, E.E., Collen, M.J., Cornelius, M.J., David, E., Gardner, J.D., Jensen, R.T. The effect of Zollinger-Ellison syndrome and omeprazole therapy on gastric oxyntic endocrine cells. Gastroenterology 99:943-950, 1990,.
20. Cadiot, G., Lehy, T., Ruszniewski, P., Bonfils, S., and Mignon, M. Gastric endocrine cell evolution in patients with Zollinger-Ellison syndrome: influence of gastrinoma growth and long-term omeprazole treatment. Dig. Dis. Sci. 38:1307-1317, 1993.
21. Solcia, E., Fiocca, R., Villani, L., Luinetti, O., and Capella, C. Hyperplastic, dysplastic, and neoplastic Enterochromaffin-like cell proliferations of the gastric mucosa. Am. J. Surg. Pathol. 19 (suppl 1):S1-S7, 1995.
22. Jensen, R.T. Gastrinoma as a model for prolonged hypergastrinemia in the human. In Gastrin. Walsh, J.M., ed. New York: Raven Press; 1993, pp. 373-393.

23. Annibale, B., de Magistris, L., Corleto, V., D'Ambra, G., Marignani, M., Iannoni, C., Delle Fave, G. Zollinger Ellison syndrome and Antral G-cell hyperfunction in patients with resistant duodenal ulcer disease. Aliment. Pharmacol. Ther. 8:87-93, 1994.
24. Correa, P. and Yardley, J.H. Grading and classification of chronic gastritis: one american response to the Sydney system. Gastroenterology 102:355-359, 1992.
25. Wolfe, M,M. and Jensen, R.T. Zollinger-Ellison syndrome. N. Engl. J. Med. 317:1200-1209, 1987;
26. Ferraro, G., Annibale, B., Marignani, M., Azzoni, C., D'Adda, T., D'Ambra, G., Bordi, C., and Delle Fave, G. Effectiveness of octreotide in controlling fasting hypergastrinemia and related enterochromaffin-like cell growth. J. Clin. Endocrinol. Metab. 81:677-683, 1996.
27. Loud, A.V. and Anversa, P. Morphometric analysis of biologic processes. Lab. Invest. 50:250-261, 1984.
28. Delle Fave, G., Khon, A., de Magistris, L., Annibale, B., Bruzzone, R., Sparvoli, C., Severi, C., and Torsoli, A. Effect of bombesin in gastric acid secretion in patients with duodenal ulcer. Gut 24:231-235, 1983.
29. de Magistris, L. and Rhefeld, J.F. A simple enzymatic procedure for radioimmunochemical quantitation of the large molecule forms of gastrin and cholecystokinin. Ann. Biochem. 102:126-133, 1980.
30. Borch, K., Renvall, H., and Liedberg, G. Gastric endocrine cell hyperplasia and carcinoid tumors in pernicious anemia. Gastroenterology 88:638-48, 1985;.
31. Sjoblom, S.M., Sipponen, P., Karonen, S.L., and Jarvinen, H.J. Argyrophil cell hyperplasia and carcinoid tumors of the stomach dependence on duration of pernicious anemia. Eur. J. Gastroenterol. Hepat. 3:153-157, 1991.
32. Creutzfeldt, W. and Lamberts, R. Inter-relationship between serum gastrin levels, gastric mucosal histology and gastric endocrine cell growth. Digestion 51(suppl 1):76-81, 1992.
33. Lamberts, R., Creutzfeldt, W., Strüber, H.G., Brunner, G., and Solcia, E. Long-term omeprazole therapy in peptic ulcer disease: gastrin, endocrine cell growth, and gastritis. Gastroenterology 104:1356-1370, 1993.