Altered Distribution of Metaplastic Paneth, Gastrin and Pancreatic Acinar Cells in Atrophic Gastritic Mucosa with Endocrine Cell Lesions

M. Salih Deveci and Guzin Deveci

Department of Pathology, Gülhane Military Medical Academy, School of Medicine, Ankara, Turkey

Deveci, M.S. and Deveci, G. Altered Distribution of Metaplastic Paneth, Gastrin and Pancreatic Acinar Cells in Atrophic Gastritic Mucosa with Endocrine Cell Lesions. Tohoku J. Exp. Med., 2004, 202 (1), 13-22 —— The mechanism of progression from gastric endocrine cell hyperplasias (ECHs) to carcinoid tumor (GCT) is still unknown. In these lesions, the distribution of metaplastic Paneth, gastrin and pancreatic acinar cells developing due to consequences of corporal mucosal atrophy has not been investigated in detail. In this study, 33 gastric endoscopic biopsies with endocrine cell lesions were examined. In all cases except 6 with solitary GCT, complete-type (small intestine) intestinal metaplasia (IM) with Paneth cells was observed. The density of lysozyme-positive Paneth cells in IMs in cases with GCTs was less than those in ECH alone. The density of gastrin-positive cells in IMs and average number of micronodules of ECHs were similar. Pancreatic acinar metaplasia (PAM) was observed in 6 cases of GCTs with ECH. The size of GCTs with ECH was smaller than those without ECH. By image analysis, the percentage of Ki67 (MIB-1, proliferation marker) expressing cells of GCTs with ECH was 5.1 ±0.6%, and GCT without ECH 7.8±1%. Our results indicate that few Paneth cells and many PAMs in atrophic corporal mucosa are seen more frequently in cases of GCTs with ECH, compared to those in ECH alone. Gastrin-positive cells in the corporal IM may stimulate enterochromaffin-like (ECL) cells, which may induce hyperplasia, dysplasia or neoplasia by augmenting the effects of hypergastrinemia through a paracrine mechanism on local gastrin-sensitive cells. ——— endocrine cell lesions; paneth cells; gastrin cells; pancreatic acinar cells

© 2004 Tohoku University Medical Press
The gastric endocrine cell hyperplasia (ECH) is often seen in cases of chronic atrophic gastritis and pernicious anemia with hypo- or achlorhydria (Black and Haffner 1968; Mendelsohn et al. 1987; Berendt et al. 1989; Borch 1989; Bordi et al. 1991; Rindi et al. 1993; D’Adda et al. 1994; Bordi et al. 1995; Rindi 1995; Solcia et al. 1995; Lewin and Appelman 1996). There are only a few cases that were accompanied by primary G-cell hyperplasia, multiple endocrine neoplasia (MEN type-1) or Zollinger-Ellison syndrome (Doumith 1982). Gastric acid in normal situations suppresses gastrin secretion. Hypo- or achlorhydria interrupts the gastrin feedback mechanism inducing hyperplasia of gastrin-producing cells in the antral mucosa. The resulting hypergastrinemia is regarded as the trophic stimulus responsible for argyrophil cell hyperplasia. In ECHs, a variety endocrine cells like gastrin (G), pancreatic polypeptide (PP), somatostatin, enterochromaffin like (ECL), enterochromaffin (EC), and D1 cells have been identified. Most of these are seen microscopically as foci of ECL cell proliferation (Borch et al. 1985; Berendt et al. 1989; Borch 1989; Bordi et al. 1991; D’Adda et al. 1994).

Gastrointestinal endocrine cell proliferations have been classified into hyperplasia, adenomatoid hyperplasia, endocrine cell dysplasia (ECD) and neoplasia (Solcia et al. 1995; Lewin and Appelman 1996). ECH may be seen as linear (five or more adjacent endocrine cells within a pre-existing gastric gland) or nodular type (clusters of 5 or more endocrine cells outside of a gland). The latter does not exceed the average diameter of a gastric mucous gland. Adenomatoid hyperplasia is defined as five or more adjacent nodular clusters in the mucosa. ECD consists of enlargement and fusion of nodules of adenomatoid hyperplasia with discontinuity and irregularity of intervening basement membrane. The mechanism of progression from ECH to ECD or GCT is still unknown.

The aim of this study is to investigate the distribution of several gastric metaplasias such as complete-type IM with Paneth cells (accepted as mucosal defense cells) and gastrin cells, and pancreatic acinar metaplasia in endoscopic biopsy material of cases with endocrine cell lesions. In addition, the degree of Ki67 (MIB-1) and p53 protein expression in these lesions was analyzed quantitatively to compare their proliferative status by image analysis method.

**MATERIALS AND METHODS**

The gastric endocrine cell lesions in 33 consecutive patients with atrophic gastritis diagnosed between 1995-2003 in Department of Pathology, Gülhane Military Medical Academy were examined. For the study procedures involving human materials, the approval from the local ethic committee has been obtained. These patients underwent gastric endoscopy clinically to evaluate the cause of anemia, or to evaluate *Helicobacter pylori* infection, or to investigate other gastric disorders. High serum gastrin levels and/or the presence of antral G-cell hyperplasia were accepted as significant markers of hypergastrinemic process of ECHs with or without GCT. Routinely, five gastric biopsy specimens from patients were taken, which of two were from the antrum, two from body, and one from the incisura angularis. Additionally, three or five endoscopic biopsies from every patient with endocrine cell lesion, if present, were taken. The tissues were embedded in paraffin without specific orientation. The 3-5 µm sections of all tissue samples were prepared and stained with hematoxylin-eosin, toluidine blue, alcian blue and periodic acid-Schiff (PAS).

Gastritis, atrophy and intestinal metaplasia (IM) in gastric endoscopic biopsies were histologically graded as mild, moderate and marked in accordance with Updated Sydney System (Dixon et al. 1996). For patients with ECH, endoscopy was reperformed at a six-month to one-year period. The size of multifocal GCTs or ECDs was measured on the slides. Endocrine cell nodules smaller than 0.5 millimeter in diameter were diagnosed as ECD. When an ECD focus at the beginning biopsy of the patient was present, the biopsy was repeated within a shorter period to evaluate other possible lesions and to eliminate
GCT. About 15 endoscopic biopsy samples were examined from each case.

For immunohistochemistry, standard avidin biotin-peroxidase complex method was employed on formalin fixed and paraffin embedded tissue sections. Immunohistochemical staining was performed using antibodies against chromogranin-A (LK2H10+PHE5, 1:200, NeoMarkers, Fremont, CA, USA), gastrin (Rabbit, 1:200, NeoMarkers), lysozyme/muramidase (Rabbit, 1:100, NeoMarkers), Ki67 (MIB-1, 100, NeoMarkers), p53 protein (DO-7+BP53-12, 1:100, NeoMarkers). AEC (3 amino-9 ethyl carbazole) was used as chromogen.

Average number of chromogranin-A positive micronodules (per corporal mucosa with ECH) was recorded in gastric endoscopic biopsy of every one of the patients with ECH. The density of gastrin-positive G-cells and lysozyme-positive Paneth cells in at least 10 metaplastic intestinal glands or crypts (per gland/crypt with IM) were determined under light microscope. Ki67 (MIB-1) expressions in GCTs were analyzed by using KS-400 image analysis software.

Image analysis

A computer system composed of following components was used in image analysis: An IBM compatible computer running Microsoft Windows NT 4.0 Service Pack 6a operating system, a light microscope with monitored stage (Zeiss Axioskop, Göttingen, Germany), frame grabber card (Matrox Meteor, Matrox Imaging, Dorval, Quebec, Canada), a digital camera attached to the microscope (Sony AVT Horn 3 CCD, Tokyo), a 21 inch video monitor (Philips, Chungli, Taiwan) and a special image analysis software (Karl Zeiss KS 400 version 3.0 for Windows, Zeiss, Göttingen, Germany).

With the aid of KS 400 version for Windows software, semiautomatic, interactive ‘macro’ (a batch file containing all of the instructions to accomplish a predetermined set of measurements) was prepared. First area of the study was selected with plan 2.5x objective. Then in the selected area (with 20x objective) a grid having lines and dots have been generated by the system. At the end of the process (after 50 cells measurement on each case), this macro allowed determination of percentage of positive nuclei as square micron within the tumor cell population.

Statistical analysis

The data were expressed as the ± s.e. in all cases. A non-parametric method of statistical analysis was used. Statistical analysis of more than two groups was performed using the Kruskal Wallis test followed by Mann Whitney’s U-test. To compare two groups, Mann Whitney’s U-test was used. Comparisons between groups of discrete variables were performed with the chi-square test. A $p$-value of $<0.05$ was considered significant.

RESULTS

The clinical, histopathological and immunohistochemical findings of the cases are summarized in Table 1. Endoscopically grayish-yellow polyps or flat lesions have been detected in many cases. Gastric endocrine cell lesions in 33 cases were diagnosed histologically and immunohistochemically.

Patients’ age ranged from 21 to 83 years. The mean age of patients with GCT accompanying ECH was lower than that of others (41.4±4 vs. 54±5.3 and 58.3±4.1). ECH was comparatively more frequent in females, and GCT with ECH in males ($p=0.06$).

None of the cases with endocrine cell lesions had clinical features of MEN-1 and Zollinger-Ellison Syndrome or carcinoid syndrome. Two of the solitary GCTs invaded the serosal fatty tissues. One of these also had hepatic metastases at the time of biopsy.

In all cases with atrophic gastritis accompanied by ECH, moderate-to-marked degree complete type IM was noted in the corporal mucosa (Fig. 1). A mild inflammation without significant activity or metaplasia was seen in the antral biopsies. In standard alcian blue-periodic acid-Schiff (PAS) stained sections of biopsies of 33 cases
with endocrine cell lesions, any incomplete-type metaplasia was not detected. There were mild atrophy and complete type of IM without Paneth cells in the gastric biopsies of three cases with solitary GCTs.

In thirteen patients with GCTs accompanied by ECH, 34 foci of GCT were detected. Of these patients, ten also had ECD (Fig. 2). The diameter of tumors ranged from 0.1 cm to 1.0 cm (mean 0.3 cm ± 0.3). Of The 34 GCTs, 29 were intramucosal, others submucosal. In the remaining 6 cases, GCTs were not accompanied by ECH. The mean diameter of 6 solitary GCTs was 2.6±0.3cm (range: 0.8 cm-to-8 cm). The difference between the two groups (with and without ECH) was statistically significant ($p<0.01$).

Chromogranin-A expression was present in all nodular-type ECH in the corporal mucosa (Fig. 3). Focal expressions involving a small number of cells were also selected in areas of coexistent pseudo-pyloric metaplasia, complete-type IM and PAM. Average number of hyperplastic micronodules

| Table 1. Clinico-pathological features of the cases with gastric endocrine cell lesions and the results of immunohistochemical analyses |
|---------------------------------|-----------------|-----------------|
|                                 | ECH only        | GCT with ECH    | GCT without ECH |
| Total number of patients        | 14              | 13              | 6               |
| Gender (Female/Male)            | 10F/4M          | 5F/8M           | 3F/3M           |
| Mean Age (Years)               | 54±5.3          | 41.4±4          | 58.3±4          |
| Lesion site (Corpus/Antrum)    | 14/0            | 13/0            | 2/4             |
| Grading of Atrophic Gastritis:  | Mild/Moderate/Marked | 0/10/4        | 0/8/5           | 3/0/0           |
| Complete IM (±Paneth cells):    | Mild/Moderate/Marked | 5/3/6         | 2/8/3           | 3/0/0           |
| Anemia ‡                       | 5               | 5               | 0               |
| The density of Lysozyme antibody (+) Paneth cells (per gland or crypt) | 5.6±0.5         | 2.9±0.5*        | -               |
| The density of Gastrin antibody (+) G-cells (per gland or crypt)         | 2.2±0.3         | 2.5±0.1         | -               |
| Average number of Chromogranin-A (+) micronodules (per corporal mucosa with ECH) | 19.1±2.4        | 19.2±1.7        | -               |
| Pseudo-pyloric metaplasia       | 8               | 9               | 2               |
| Pancreatic acinar metaplasia (PAM) | 0            | 6               | 0               |
| Helicobacter pylori infection   | 0               | 2               | 1               |
| Ulceration                      | 0               | 0               | 3               |
| The number of gastric carcinoid tumors (GCTs)                              | -               | 34              | 6               |
| Mean diameter of GCTs (cm)      | -               | 0.3±0.3         | 2.6±1.3*        |
| Diameter of GCTs: (<0.5 cm / 0.5-1 cm / >1 cm)                           | -               | 29/5/0          | 0/2/4           |
| Gastric stromal tumors/other tumors or disorders \( ^\)                  | 3\( ^\)         | 3\( ^\)         | 0               |
| Proliferative activity: Ki67 (MIB-1) expression (%)                        | -               | 5.1±0.6         | 7.8±1           |
| p53 protein expression (%)                                               | -               | 0               | 0               |
| Treatment:                     | LEE             | -               | 10              |
|                                | STG & TG        | -               | 3               |

ECH, Endocrine cell hyperplasia; ECD, Endocrine cell dysplasia; GCT, Gastric carcinoid tumor; LEE, local endoscopic excision; STG & TG, Subtotal and total gastrectomy.

\( ^\)Pernicious anemia (7 cases), Multivitamin (D, A, E, B\(_{12}\) deficiency (one case), hypersplenism and anemia with unknown etiology (2 cases).

\( ^\)Parathyroid adenoma, ovarian endometrioid carcinoma, duodenal total villous atrophy, hypercalcemia due to renal disorder.

\( ^\)Gastric leiomyoma, schwannoma, colonic adenoma and carcinoma.

\( ^\)Updated Sydney System (Dixon et al.1996). \( ^p<0.05\), Significantly different from other group(s).
Fig. 1. Nodular-type endocrine cell hyperplasia (arrows) in base of the complete-type intestinal metaplasias with numerous Paneth cells (Hematoxylin-Eosin, ×200)

Fig. 2. Endocrine cell dysplasia (in the center) surrounded by hyperplastic micronodules, metaplastic glands and lymphoplasmacytic inflammatory infiltrate (Hematoxylin-Eosin, ×200)

Fig. 3. Chromogranin-A positive clusters of nodular-type endocrine cell hyperplasias (×200)

Fig. 4. G-cells (arrows) in complete (small intestine) type intestinal metaplasias (Gastrin, ×400)

Fig. 5. Numerous Paneth cells showing immunoreactivity with lysozyme antibody in metaplastic intestinal glands of the case with only ECH (×400)

Fig. 6. A few Paneth cells (arrows) in metaplastic intestinal glands of the case with gastric carcinoid tumor and ECH (not seen in photograph) (Lysozyme, ×400)
ules in the two groups (with ECH only and GCT accompanying ECH) was almost equal (19.1±2.4 vs. 19.2±1.7/per corporal mucosa).

Gastrin-positive G-cells in the antral mucosa, supporting G-cell proliferation due to hypergastrinemia, were shown with gastrin antibody. In addition, these cells were detected in the complete-type IMs with Paneth cells of the corporal mucosa (Fig. 4). In these cases, the number of G-cells in each metaplastic gland ranged from 1 to 5. There was no statistical difference between groups with ECH only or ECH with GCT (2.2±0.3 vs. 2.5±0.1/per metaplastic gland). No gastrin-positive GCT was detected immunohistochemically.

In the twenty-seven cases with ECH (with or without GCT), complete (small intestine) type metaplasias contained Paneth cells. Paneth cells were counted in immunostained sections with lysozyme-antibody (Figs. 5 and 6). The density of Paneth cells ranged from 1 to 10 cells per metaplastic gland. The density of Paneth cells in the cases with both ECH and GCT was lower (2.9±0.5 vs. 5.6±0.5/per metaplastic gland) than that in the cases with ECH only (statistically significant, p<0.01).

In the 6 cases of GCT with ECH, after the diagnosis of GCT by endoscopic biopsy, subtotal gastrectomy or local excision revealed the presence of scattered PAM lobules. The lobules of PAM were also immunohistochemically strong positive with lysozyme-antibody (Fig. 7).

Nuclear Ki67 (MIB-1, proliferation marker) expressions (Fig. 8) were only seen in larger tumors than 0.5 cm. These tumors include 6 GCTs without ECH in 6 patients and 6 GCTs (of the 34 GCTs) with ECH in 13 patients. Mean Ki67 (MIB-1) expressions determined by image analysis in GCTs with or without ECH were 5.1±0.6% and 7.8±1%, respectively. No expression of p53 tumor-suppressor gene protein was observed in any GCT.

The patients were followed up from 1995-2003. Follow-up ranged from 1 to 8 years (mean 2.5 years). No progression to dysplasia or neoplasia has been detected in our patients with ECH. In three cases with ECD, GCTs were detected during the follow-up period of 6, 12 and 18th months. Metastatic disease was not seen in the cases, except in a case with solitary GCT who died after 8 months.

**DISCUSSION**

The sequence of hyperplasia-dysplasia-neoplasia has also been reported in the pathogenesis of gastrointestinal endocrine neoplasms (Borch et al. 1987). ECHs may sometimes progress to ECD and finally GCTs (Goldman et al. 1981; Mendelsohn et al. 1987; Berendt et al. 1989; Rindi et al. 1993). Some glycoprotein hormones have been reported to be risk in tumor development (Bordi et al. 1988). However, there is no
certain evidence explaining the etiopathogenesis (Modlin and Sandor 1997). Almost half of the cases in our series had GCTs with ECH at the time of their first diagnosis. Also, most ECDs were associated with GCTs (10/13). ECD was frequently observed in neighboring areas of intramucosal GCT or in those of ECH.

The G-cells have been previously reported in the epithelium of pyloric region and in small intestinal mucosa (Larsson et al. 1975; Yamada et al. 1979; Satoh 2000). The distribution of immunoreactive G-cells has been studied in 38 partial gastrectomy specimens to determine the effect of varying degrees of gastritis (Sloan et al. 1979). They reported that the G-cells were almost totally absent in the IMs, and only a few G-cells were found within areas of pseudo-pyloric metaplasia in the corporal mucosa.

However, in our study, G-cells have been found in both the complete-type IM and corporal pseudo-pyloric metaplasia accompanying ECH (with or without GCT). The G-cells in areas of pseudo-pyloric metaplasia were fewer than in those of IM. The presence of G-cells in IM of ECH or GCT cases suggests that gastrin secretion may locally affect the activity of ECL cells in the corporal mucosa through a paracrine mechanism. We suggest that G-cell metaplasia may augment the well-known trophic effect of hypergastrinemia over ECL cells in areas of IM, leading to hyperplasia, dysplasia or neoplasia.

In patients with atrophic gastritis, pseudo-pyloric glands sometimes may differentiate into chief and parietal cells (Tang et al. 1995; Chew et al. 2000). However, IMs replacing total corporal glands hardly regress. Therefore, IMs with Paneth cells in atrophic gastritis are irreversible. In IM lesions, the stem cells show abnormal cell differentiation but preserve their normal direction of migration (Dayal et al. 1987). Paneth cells migrate inwards and their turnover time is much slower than that of other crypt cells. They are renewed in every 15 days (Karam 1999; Inada et al. 2001). The Paneth cells may contribute to mucosal integrity (Lee et al. 1999).

In our series, compared to the cases with ECD/GCT, the number of Paneth cells in cases with ECH only was significantly higher. Gassler et al. (2002) reported Paneth cells of IMs in the gastric mucosa and Paneth cell-rich adenoma express the proliferation marker Ki67, a phenomenon that was never observed in Paneth cells of normal small intestine. Moreover, Wong et al. (2000) emphasized that numerous Paneth cells and goblet cells in areas of intestinal metaplasia were in cell cycle; however, the endocrine cells were non-proliferative. Regulatory or apoptotic effect of Paneth cells has also been proposed (Lopez-Lewellyn and Erlandsen 1980; Watson 1995; Moller et al. 1996).

A possible relationship between endocrine cells and Paneth cells has first been reported by Sakaki et al. (2000) in a case of gallbladder adenocarcinoma composed of a mixture of neuroendocrine cell nests, scattered metaplastic goblet cells and Paneth-like cells. They postulated that Paneth cell-type carcinoma cells might be intimately related to the promotion of neuroendocrine cells to proliferate, releasing undefined trophic substance(s). In addition, Bae et al. (2002) documented a case of intestinal type of bile duct cholangiocarcinoma with prominent Paneth cell differentiation of carcinoma cells. Goblet cells and neuroendocrine cells were also present in this case. These reports documented a possible relationship of Paneth cells in the proliferation of endocrine cells in various neoplastic lesions.

Paneth cells may also play some roles in the pathogenesis of gastric endocrine cell lesions. In an experimental study, Seno et al. (2002) reported the proliferation of other cells due to destruction of Paneth cells. They detected TNF-alpha released from degenerated Paneth cells through activation of NF-kappaB, suggesting its proliferative effect on other cells.

In advanced forms of atrophic gastritis, altered distribution of Paneth cells, including destruction, due to severity of inflammation, antibody-mediated mechanisms, individual secreting hormones, proliferating factors, deficiency of
minerals such as zinc (Elmes and Jones 1980), genetic factors or other unknown factors may take place. This may lead to disregulation of adjacent cells in areas of IM, which contribute progression to ECD or GCT. In some of our cases, anemia of unknown etiology, multivitamin deficiency, hypersplenism, hyperparathyroidism and hypercalcemia were present. It is, at present, not clear whether these factors participated in the endocrine cell lesions seen in our cases.

PAM was present in up to 1.2% of gastric biopsy samples and 13% of gastrectomy specimens (Doglioni et al. 1993). In our series, there were multifocal PAMs in 6 cases of GCTs with ECH. PAM may be regarded as metaplasia of gastric chief cells to acinar cells because of similarity of secretory substance(s) (Doglioni et al. 1993; Tang et al 1995; Chew et al. 2000). However its association with ECHs or GCTs is still unclear.

In this series, most GCTs with ECH were smaller than in those without ECH (p < 0.01). GCTs with ECH were detected at rather early stage, limited to mucosa or submucosa. The low proliferative activity shown by Ki67 staining, and absent p53 protein expression in GCTs are consistent with their relatively benign biological behavior.

In conclusion, our results indicate that few Paneth cells and many PAMs in atrophic corporal mucosa are seen more frequently in cases of GCTs with ECH, compared to those in ECH alone. Gastrin-positive cells in the corporal IMs may stimulate enterochromaffin-like (ECL) cells, which may induce hyperplasia, dysplasia or neoplasia by augmenting the effects of hypergastrinemia through a paracrine mechanism on local gastrin-sensitive cells.

References


