Distribution of GEP Endocrine Cells in the Gastric Mucosa of Mice with Experimental Gastritis[*]

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(Received September 13, 1984)

ABSTRACT

The aim of this study was to ascertain the morphological changes in the endocrine cell profile in the atrophic mucosa of mice with autoimmune gastritis induced by neonatal thymectomy and administration of carcinogenic chemical, N–methyl–N'–nitro–N–nitrosoguanidine (MNNG).

Proliferation of argyrophil cells, gastrin (G17, G34) cells in areas of pseudopyloric metaplasia, enteric hormone (GIP, secretin, substance P, enteroglucagon) containing cells and enterochromaffin cells in areas of intestinal metaplasia of the atrophic mucosa was observed in mice with autoimmune gastritis together with proliferation of enterochromaffin-like cells and undifferentiated endocrine cells in their atrophic mucosa. These findings suggest that atrophic mucosae, especially epithelia of pseudopyloric and intestinal metaplasia as well, have the potential of producing endocrine cells which are normally present in the gastric antrum and intestine.

INTRODUCTION

The endocrine profile is colorful in the gastric mucosa of patients with pernicious anemia and body confined atrophic gastritis (A type gastritis)[1,5,14,16,18),21]. Rubin[1] has observed proliferation of enterochromaffin-like cells in the atrophic mucosa of the gastric body in patients with pernicious anemia. These changes in the endocrine profile are mainly due to atrophic changes of the gastric mucosa. Proliferation of enterochromaffin cells has been found only in islets of the intestinal metaplasia (ectopic intestinal epithelium)[3,18]. The changes do not occur in the atrophic mucosa without intestinal metaplasia. These suggest that argyrophil cells as well as argentaffin cells and endocrine cells containing enteric hormones appear only in the enteric epithelium of the atrophic mucosa in man.

The present study was made to determine whether these endocrine cells appear in the enteric epithelium of the atrophic mucosa in mice with experimentally induced atrophic gastritis which closely resembles the gastric mucosa in patients with pernicious anemia.

MATERIALS AND METHODS

1. **Production of atrophic gastritis and perparation of tissue sections**

Neonatal mice (BALB/C (+/?)) of both sexes 3 days after birth were thymectomized according to the method of Kojima et al.[8] The animals were kept in a conventional manner for two months and for more 6 months thereafter[23] were given N–methyl–N'–nitro–N–nitrosoguanidine (MNNG) at a concentration of 50 microgram/ml in deionized water containing 0.04% of Tween 60. The drinking bottles were shielded with foil

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to prevent deterioration of MNNG by exposure to light and water was changed every 3 days. The treatment of this chemical was made with the expectation that atrophic changes would increase in the gastric mucosa induced after thymectomy.

The animals were sacrificed 8 months after the operation. The abdomen was opened and the stomach was resected and opened along the greater curve. The gastric mucosa was carefully examined for tumours which might have developed after MNNG, but no tumours were found in all the treated mice. However, a few tissue samples (0.5 × 0.5 cm) were taken from the body (glandular) and antral mucosa. The tissue samples were fixed in Bouin's solution for 3 hr at 4°C. After fixation the tissue specimens were embedded in paraffin wax and sectioned 3 microns in thickness.

2. Conventional histopathology and histochemistry

Sections were stained with hematoxylin and eosin for histopathologic examination and with PAS– alcian blue at pH 2.5[13] for evaluation of intestinalization of the gastric mucosa. Fibrous changes in the stomach were evaluated by Azan stain.

The number of parietal cells was counted under a microscope and the mean number was shown per unit area (cells per mm^2, ×400, 3μ).

Grimelius silver stain[2] and Masson-Fontana silver impregnation[11] were utilized in this study for identification of argyrophil and argentaffin cells, respectively.

3. Immunocytochemistry for identification of endocrine cells

Unlabeled antibody enzyme (peroxidase antiperoxidase) method[20] was utilized in the present study for identification of endocrine cells. Prior to application of primary antisera, the sections were first treated with hydrogen peroxide to exhaust the endogenous peroxidase activity and then they were incubated with normal goat serum to block nonspecific background staining. The specific antisera were applied for 12–18 hr at 4°C. The conjugated antiglobulins were used at room temperature. The following peroxidase antiperoxidase complexes were then applied: horse radish peroxidase and rabbit anti-horse radish peroxidase (Dakopatt A/S Kyowa)at

Table 1. Antisera and dilution for PAP

Gastrin	2000
Somatostatin	4000
GIP	4000
Glucagon	4000
Enteroglucagon *	4000
Substance P	800
Secretin	2000
5–hydroxytryptamine	4000
Neurotensin	2000
VIP	2000
M–enkephalin	1000
Motilin	4000
ACTH	3000

* As total glucagon

1:300 at room temperature. The peroxidase activity was demonstrated by incubation for 2-3 min using a freshly prepared solution of 0.05% of 3, 3–diaminobenzidine tetrahydrochloride in phosphate buffered saline, pH 7.2, containing 0.01% hydrogen peroxide. The used antisera and their dilution are shown in Table 1.

4. Control for immunocytochemistry

In order to demonstrate that the immunocytochemical reactions were specific, the following tests were performed. 1) Prior to immunostaining, the diluted antisera were absorbed with samples of synthetic peptides (gastrin 17, pancreatic glucagon, somatostatin, substance P, secretin and 5–hydroxytryptamine)[15], 2) Normal rabbit serum was used instead of the primary antibody as the first layer, 3) PAP complex was applied alone and developed by the unlabeled antibody enzyme technique.

5. Electron microscopic study

The specimens from the body and antrum were fixed for 2 hr at 4°C in 3% glutaraldehyde and in the same buffer at 4°C for 1 hr, which were dehydrated in graded alcohol and then embedded in Epon. Ultrathin section one micron in thickness was doublestained with uranyl acetate and lead citrate, and individual cells were examined uneder a JEM 100S electron microscope and photographed.

6. Definition of atrophic gastritis

Atrophic gastritis was defined in the present study as reduction of parietal cells in the body mucosa of the stomach associated with

inflammatory cell infiltration and interstitial fibrosis as well as intestinal metaplasia.

RESULTS

Atrophic gastritis confined to the body (glandular) mucosa of the stomach developed 2 months after thymectomy in neonatal mice. These atrophic changes increased by admistration of carcinogenic dosage of MNNG as shown in Fig. 1, which were associated with a marked reduction of parietal cells (parietal cell; 12. 3± 3. 6 cells/mm², control non-thymectomy; 121. 1 ±24. 5) and chief cells together with loose glandular structure and proliferation of prematured cells resembling mucous neck cells which grew from the glandular neck to the base (Fig. 2). Inflammatory cells, which were composed of lymphocytes, plasma cells and eosinocytes, infiltrated markedly and diffusedly in the lamina propria mucosae and cysts of various sizes as well as lymph follicles existed, which were very similar to the findings of chronic atrophic cystic gastritis, and intestinal metaplasia was also observed (Fig. 3). Antral mucosa was nearly normal, though glandular structure was slightly loose and irregular (Fig. 4). These changes, however, were less severe than those in the body (glandular) mucosa.

Proliferation of argyrophil cells was observed in the body mucosa (Fig. 5). G cells (G17 and G34) were increased in the antrum and these were hyerplasia (Fig. 6), while D cells did not show any remarkable change in number in the antrum of this type of gastritis when compared to those in the antrum of normal mice. D cells in the body mucosa, however, were proliferated (Fig. 7).

Endocrine cells containing enteropancreatic hormones were found in the region of intestinal metaplasia in the body mucosa. These were gastric inhibitory polypeptide-like immunoreactive cells (Fig. 8), pancreatic glucagon-like immunoreactive cells (Fig. 9), enteroglucagon-like immunoreactive cells (Fig. 10), substance P–like immunoreactive cells, secretin-like immunoreactive cells and 5 hydroxytryptamine-like immunoreactive cells. Gastrin (G17 and G34)-like immunoreactivities were found in enteric or pseudopyloric cells of the atrophic body mucosa (Flg. 11).

Enterochromaffin–like cells were increased in the atrophic body mucosa and undifferentiated

endocrine cells were identified in the atrophic body mucosa at the ultrastructural level (Fig. 12).

Changes in endocrine cells such as enterochromaffin cells except G and D cells were not observed in the antrum. Neurotensin, motilin, m–enkephalin, ACTH, and VIP containing cells were not identified in both the atrophic body mucosa and antral mucosa.

DISCUSSION

Thymectomy of 3-day old neonatal mice resulted in development of atrophic gastritis which satisfied the definition described earlier[7]. The definition was that atrophic gastritis has a reduction of parietal and chief cells in the body mucosa essentially[12].

The histopathology in the gastric mucosa of this type of gastritis is similar to that in the gastric mucosa of patients with pernicious anemia and A type atrophic gastritis. In addition to these morphological findings, as a high incidence of autoantibodies and seropositive parietal cell antibodies[17] was observed in these mice with gastritis, this type of gastritis may be autoimmune atrophic gastritis[4,9]. Atrophic gastritis developed when MNNG was administered to rats[24]. As it was, therefore, expected that the atrophic change would increase in the gastric mucosa where atrophic gastritis had already developed after thymectomy, the authors attempted to administer MNNG to thymectomized mice. The results revealed that more severe atrophic gastritis developed in the gastric body mucosa of mice with thymectomy, associated with pseudopyloric metaplasia and intestinal metaplasia in the gastric body mucosa than that which developed in mice with thymectomy alone[7,17]. Development of gastric cancer, however, did not occur although an amount of MNNG sufficient to develop gastric cancer in the case of rat[22] was administered to the thymectomized mice.

According to Solcia et al.[19], marked changes of endocrine cells are usually associated with relevant modifications of gastric glands, which occur in the area of intestinal metaplasia and are often observed in chronic antritis and gastritis. Namely, pyloric or fundic type endocrine cells disappear in such areas, being replaced by endocrine cells known to be present normally in the mucosa of the upper small
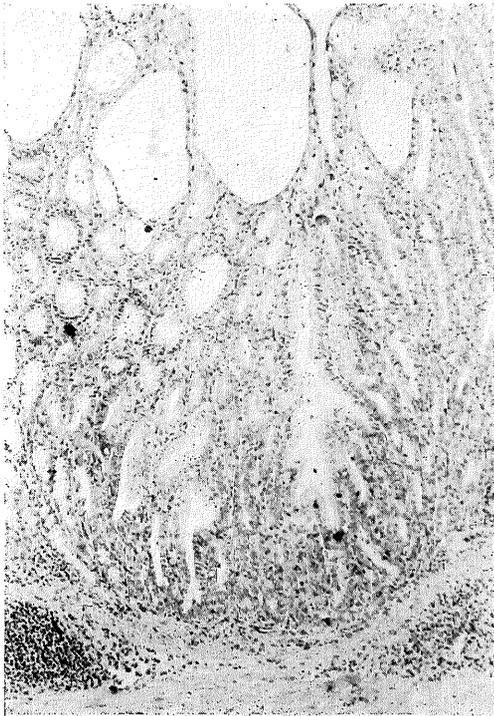
Fig. 1. Markedly atrophic gastritis in the body mucosa of a mouse after thymectomy and MNNG (Hematoxylin and eosin stain, ×80 original magnification).
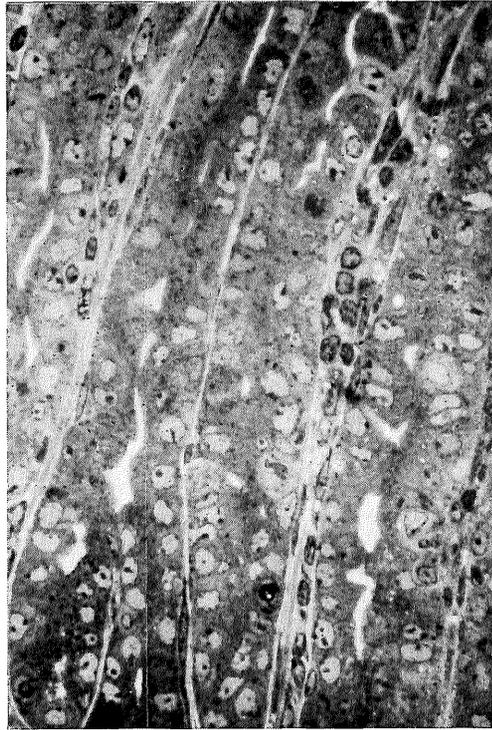
Fig. 2. Proliferation of prematured cells resembled closely mucous neck cells grew from the glandular neck to base (Toluidine blue stain, ×1000 original magnification).

Fig. 3. Atrophic changes of the body mucosa in which inflammatory cell infiltration, cystes and intestinal metaplasia were present (Alcian blue at pH 2.5-PAS stain, ×80 original magnification).
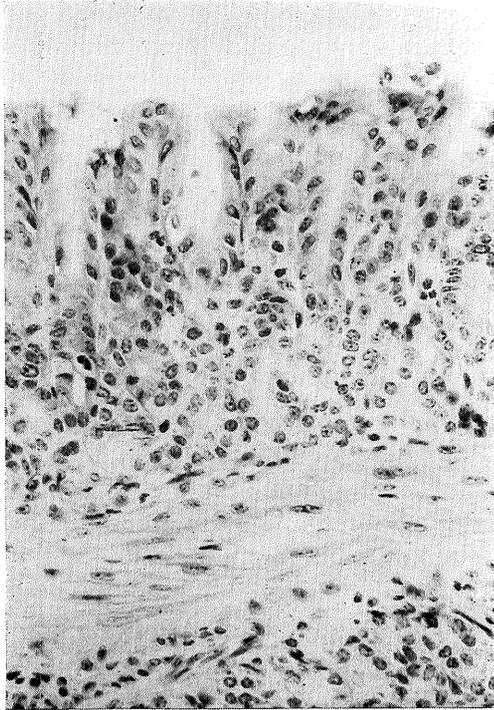
Fig. 4. Almost normal mucosa of the antrum in a mouse after thymectomy and MNNG (Hematoxylin and eosin stain, ×80 original magnification).
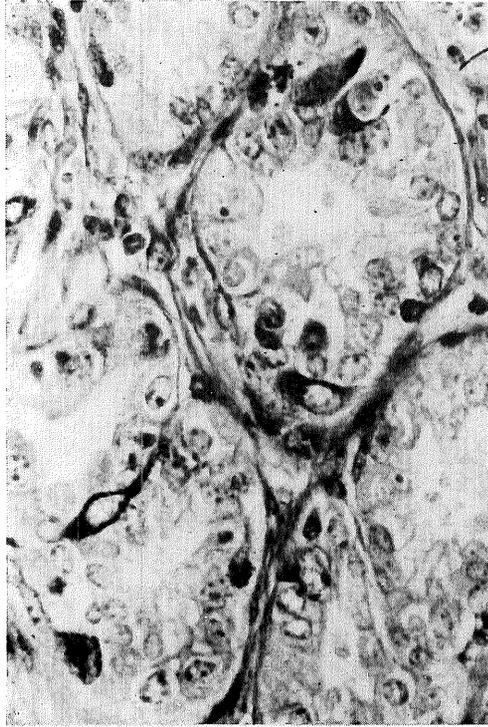
Fig. 5. Proliferation of argyrophil cells in the atrophic body mucosa (Grimelius stain, ×400 original magnification).

Fig. 6. Hyperplasia of antral G cells (G17) in a mouse with body atrophic gastritis (PAP, ×400 original magnification).

Fig. 7. Poliferation of D cells in the atrophic body mucosa (PAP ×100 original magnification).

Fig. 8. GIP-like immunoreactive cells in the atrophic body mucosa (PAP ×400 original magnification).
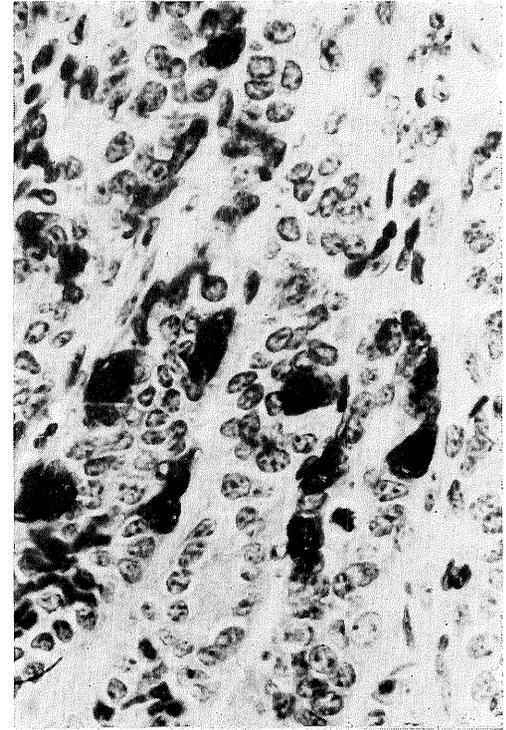
Fig. 9. Pancreatic glucagon-like immunoreactive cells in the atrophic body mucosa (PAP ×400 original magnification).
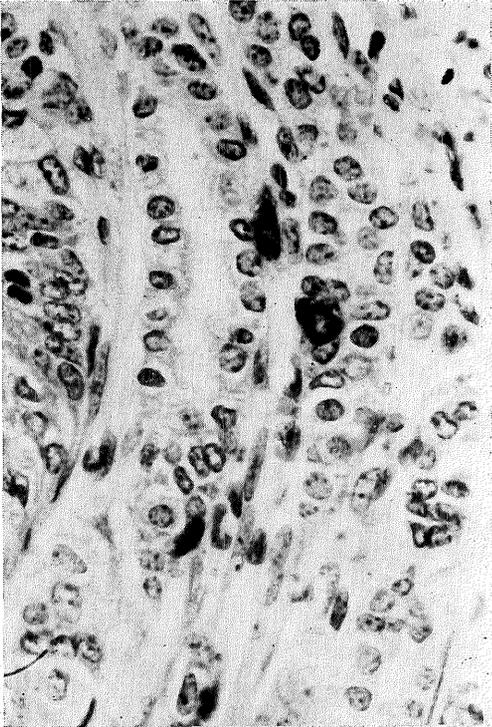
Fig. 10. Enteroglucagon-like immunoreactive cells in the atrophic body mucosa (PAP ×400 original magnification).
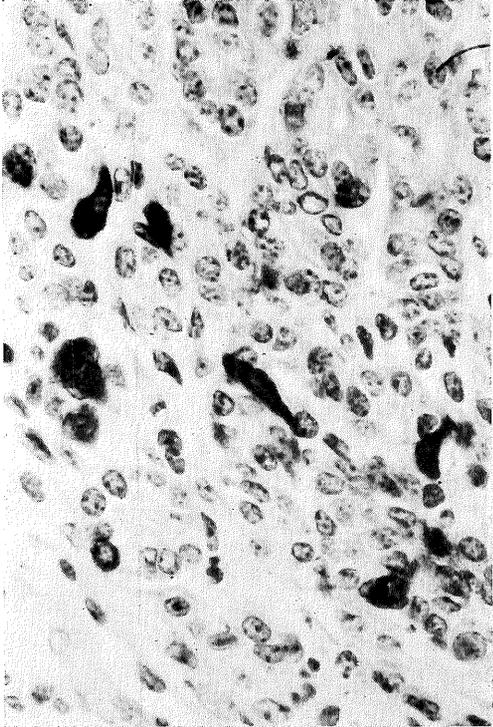
Fig. 11. Gastrin (G34)-like immunoreactive cells in the area of pseudopyloric metaplasia of the atrophic body mucosa (PAP ×400 original magnification).
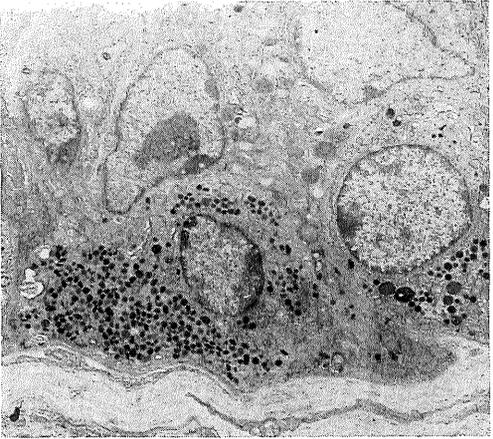
Fig. 12. Enterochromaffin-like cells and undifferentiated endocrine cells were proliferated in the atrophic body mucosa (EM ×7000 original magnification).

intestine, though with a relative increase of enterochromaffin cells and decrease of the remaining cell types. These cells normally in the upper small intestine are I (CCK), S (secretin), K (GIP) and gastrin cells.

Pseudopyloric metaplasia occurring in the body and fundus of chronic atrophic gastritis shows pyloric type endocrine cells such as G cells, D cells, and enterochromaffin cells, although fewer in number than in normal pyloric mucosa[19].

In the present study, the authors confirmed that these findings observed in human cases of atrophic gastritis were also seen in mice with fundobody confined atrophic gastritis which developed after thymectomy and administration of MNNG. Actually, a few G cells containing G17-like and G34-like immunoreactivities and relatively many enterochromaffin cells appeared in the areas of pseudopyloric metaplasia and epithelia which closely resembled intestinal cells in the fundobdy mucosa of mice with atrophic gastritis. A relative increase of D cells was also observed in occasionally surviving areas of fundobody mucosa of the stomach in such mice. This shows normal endocrine profile in orthotopic fundobody mucosa.

A relative increase of argyrophil cells was found in the atrophic mucosa. Endocrine cells which contained GIP-like-, secretin-like, substance P-like and enteroglucagon-like immunoreactivities replaced the fundobody type endocrine cells in the atrophic mucosa with intestinal metaplasia and epithelia which resembled intestinal epithelia. These endocrine cells are present normally in the mucosa of the small intestine. Furthermore, many endocrine cells which reacted positive to the serum against pancreatic glucagon were observed in the atrophic mucosa.

Proliferation of enterochromaffin-like cells and undifferentiated and prematured mucous cells was observed in the atrophic mucosa at the ultrastructural level.

Marked changes in endocrine profile of G, D and enterochromaffin cells, however, were not found in the orthotopic antral mucosa of mice with atrophic gastritis, because the antral mucosa was kept almost normal in this type of atrophic gastritis.

These findings observed in the present study showed that ectopic intestinal epithelium (intestinal metaplasia) had the potential of producing endocrine cells, which are normally present in intestinal mucosa, in mice with experimental autoimmune atrophic gastritis confined in the fundobody mucosa by thymectomy and MNNG, and in addition to be associated with hypergastrinemia, antral G cell hyperplasia, and seropositive parietal cell antibodies. Endocrine profile, thus, was markedly changed in the gastric mucosa of this type of atrophic gastritis in mice. Further these findings strongly support that the profile of endocrine cells was different in the gastric mucosa of the human case of atrophic gastritis, especially associated with pernicious anemia, from that in normal gastric mucosa.

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