

## Histochemical and Ultrastructural Studies on Experimental Gastritis in Mice<sup>\*</sup>

Takaji TAMARU, Kazuma OKAMOTO, Akihiko KAMBARA,  
Satoru SHIMIZU, Hassei KOH, Masahiro YAMAMOTO,  
Kohichi SANO, Kunihiko DAITOKU, Shinya KISHIMOTO  
and Goro KAJIYAMA

*The 1st Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3, Kasumi,  
Minami-ku, Hiroshima 734, Japan*

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### ABSTRACT

Experimental gastritis was produced in BALB/c (?/+) mice by the method of neonatal thymectomy. This atrophic gastritis was confined to the gastric fundobody mucosa and spared antrum. The morphological changes in the gastric mucosa of this type of gastritis have been described in detail during the growth process after thymectomy (1, 2, 3, 6, 10, and 15 months). The mucosal changes developed from the second months after thymectomy, which were characterized by destruction and degeneration of both the parietal and chief cells in the deep glands of gastric fundobody mucosa associated with cellular infiltration in the lamina propriae. From 3 to 6 months after the operation, however, the structure of the whole fundobody glands became composed of mostly immature cells and mucus-containing cells. These cells were similar in appearance to mucous neck cells of the normal stomach and stained positive with PAS-alcian blue at pH 2.5. At the ultrastructural level, however, the size, electron density and number of secretory granules of mucus-containing cells were different from those of cells of the normal stomach. During this period, both parietal and chief cells were markedly decreased in number and both cells were very immature. With growth (10 to 15 months after the operation), the mucosal thickness increased, although the cells composing the glands were essentially the same as those at the early to middle stages after the operation. Thus, this type of gastritis was characteristic of fundobody-confined atrophic gastritis associated with immature and undifferentiated cells as well as mucus-containing cells and hypertrophic mucosae. These peculiar changes in the gastric mucosae increased with growth after thymectomy.

The precise mechanism of induction of this type of gastritis still remains to be defined. However, first, the mucosal destruction and increased cellular proliferation are one of the possible causes, and secondly, unknown inhibitory mechanism for maturing immature cells can participate in the development of this type of gastritis. This is the other possible cause.

### INTRODUCTION

Experimental autoimmune gastritis in mice was first reported by Kojima and his coworkers<sup>1,2)</sup>. This gastritis was induced in nude mice

by injecting suspension of spleen cells obtained from neonatally thymectomized mice. Morphologically, the atrophic changes and thickened rugae which were confined to the mucosae of the gastric body were characteristic, and very

<sup>\*</sup>田丸隆二, 岡本一馬, 神原明彦, 清水 哲, 黄 八成, 山本昌弘, 佐野宏一, 大徳邦彦, 岸本真也, 梶山梧朗: 実験胃炎の組織化学的電子顕微鏡的研究

similar to findings observed in human type A gastritis<sup>3)</sup> and in Menetrier's disease.

A similar gastritis was also produced by the same authors and Sano in athymic mice (BALB/c (?/+)) after neonatal thymectomy<sup>4)</sup>. The aim of this study was to investigate the histochemical and ultrastructural characteristics of this type of gastritis which developed in athymic mice after neonatal thymectomy.

### MATERIALS AND METHODS

Production of gastritis and preparation of tissue samples

Ninety neonatal mice (BALB/c (?/+)) of both sexes 3 days after birth were thymectomized. The mice were fed in a usual manner for 1, 2, 3, 6, 10, and 15 months thereafter. The mice were divided into the following two groups: the first group for histopathological study of the stomach (light microscopic study), and the second group for electron microscopic study.

#### 1) Light microscopic study

The abdomen of athymic mice was removed under ether anesthesia, fixed in Bouin's solution, dehydrated in graded ethanol and embedded in paraffin wax. Three micron-wax tissue sections were stained with hematoxylin and eosin for histopathological study and PAS-alcian blue for mucin staining.

#### 2) Electron microscopic study

The abdomen and thorax of the athymic mice were opened under ether-anesthesia and the stomach of each mouse was fixed by perfusion with saline followed by 3% glutaraldehyde solution in 0.1 m Millonig's phosphate buffer at pH 7.4 into the left ventricle for 5 min. After the tissue samples taken from the stomach were refixed in the same fixative for 2 hr at 4°C, they were postfixed in 2% OsO<sub>4</sub> solution for 1 hr at 4°C.

The tissue samples treated as mentioned above were dehydrated in graded ethanol and embedded in epon. Semi-thin tissue sections cut with a Porter-Bloom ultramicrotome were each stained with Richardson's toluidine blue solution and then with PAS after blockade of aldehyde residues<sup>5)</sup>. Ultrathin tissue sections were stained with uranyl acetate and Millonig's lead<sup>6)</sup>. Each stained sample was photographed by a JEM-100S electron microscope.

### RESULTS

#### (1) One month after thymectomy

The gastric mucosae of thymectomized mice 3 days after birth were almost normal and not different from the gastric mucosae of sham operated mice. The gastric glands were usually composed of surface epithelial cells, mucous neck cells, parietal cells, chief cells, and endocrine cells. Cell infiltration was not observed in the intercellular space at this time.

#### (2) Two months after thymectomy

Gastric mucosal changes were observed in 30% of the thymectomized mice at this stage. The most characteristic changes were cell infil-

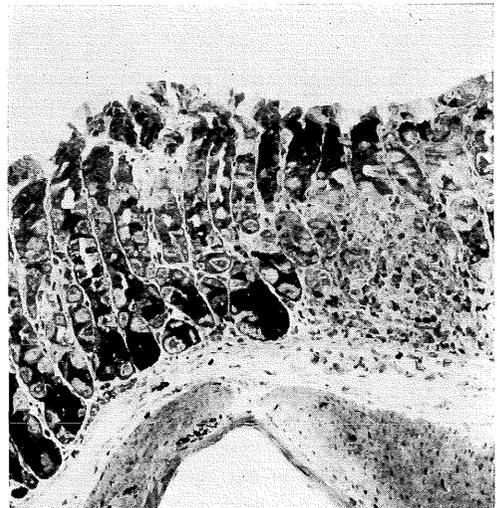


Fig. 1. Focal cell infiltrations in the base of the glands, 2 months after thymectomy. Toluidine blue  $\times 290$

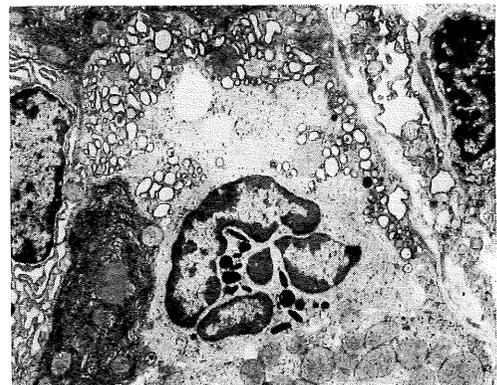
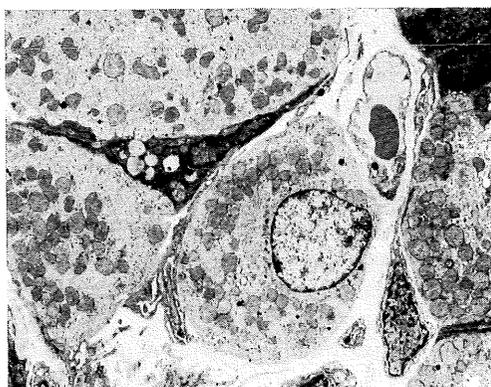


Fig. 2. An intraepithelial eosinophilic leucocyte surrounded by degenerated chief cells and a parietal cell in the epithelium, 2 months after thymectomy. EM  $\times 3700$



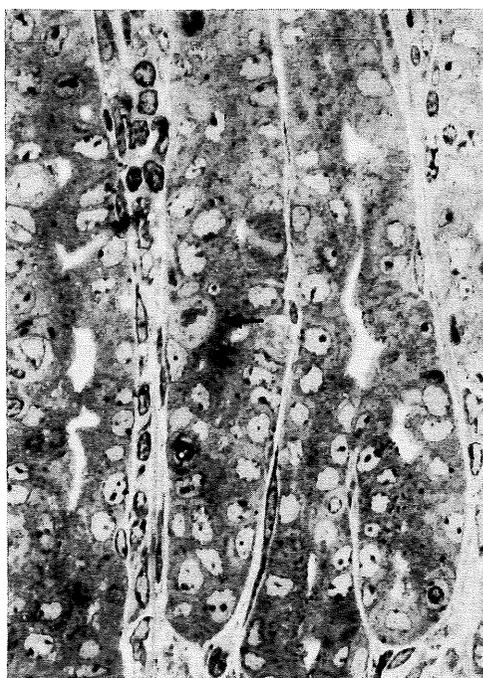
**Fig. 3.** A fibroblast surrounded by parietal cells, 2 months after thymectomy. EM  $\times 2100$

tration and lymph follicle appearing in the deep parts of the fundic mucosae, which destroyed the normal glandular structures (Fig. 1). These infiltrated cells were composed mainly of mononuclear cells, plasma cells, and lymphocytes. Eosinophilic leucocytes, however, were usually observed in the connective tissues and also in glands of the stomach (Fig. 2).

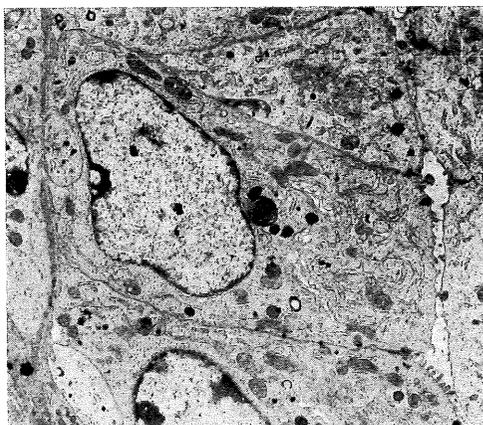
Although at the light microscopic level glandular structures and their composing cells appeared to be normal, many glandular cells were degenerated at the electron microscopic level. The secretory granules of the degenerated chief cells were not altered, although RER in the cells were remarkably enlarged and vacuolated. Their cell membranes were found to be destroyed and disappeared, while basement membranes were infolded. Fibroblasts were often seen in the intercellular space of the epithelial cells and formed frameworks with some fibroblasts projecting into the intracellular space (Fig. 3).

### (3) 3-6 months after thymectomy

The mucosal thickness was increased when compared to that of sham-operated mice and mice without gastritis after thymectomy. The increased mucosal thickness was caused by elongation of the glands which were mostly composed of undifferentiated mucus-containing cells or immature cells (Fig. 4). Parietal cells and chief cells were scarcely seen. The mitotic cells were observed in all glands at the electron microscopic level. Some mucous granules, RER, Golgi apparatus, mitochondria, and many free ribosomes as well were seen in the mucus-containing cells, which resembled mucous neck cells (Fig. 5).

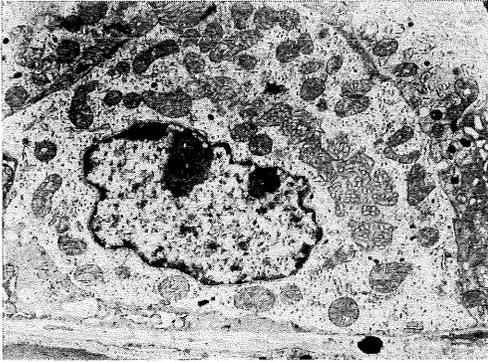


**Fig. 4.** Chief and parietal cells are scarcely seen and a mitotic cell is observed in the lower part of the elongated gland. (Arrow shows a mitotic cell) 3 months after thymectomy. Toluidine blue  $\times 1180$



**Fig. 5.** The glands are mostly composed of undifferentiated cells which are abundant in free ribosomes which lack special organelles. 3 months after thymectomy. EM  $\times 2960$

Immature cells possessed RER, Golgi apparatus, mitochondria, and also many free ribosomes, but they did not have secretory granules. Most of the parietal cells, which were small in number, were also immature (Fig. 6). They contained mitochondria, intracellular canaliculi,



**Fig. 6.** A poorly developed parietal cell containing many free ribosomes is occasionally seen. 3 months after thymectomy. EM  $\times 4000$

few SER, and many free ribosomes. Chief cells were more scarce than parietal cells and were also immature. Poorly developed RER, Golgi apparatus, and many free ribosomes were present in these chief cells. Small and electron dense granules which were  $0.5\text{--}1\ \mu\text{m}$  in length were observed in the apical region of chief cells.

Parietal cells were located in the middle to the lower part of the glands. On the other hand, endocrine cells were well kept in number and structure. Endocrine cells of normal gastric mucosae of mice were differentiated into five types, that is, A, D, EC, ECL, and A-like (X) cells. In the case of gastritis, all endocrine cells were well matured and contained typical secretory granules which did not differ from those in the control mice. Some of the endocrine cells showed lamellar structure or enlarged



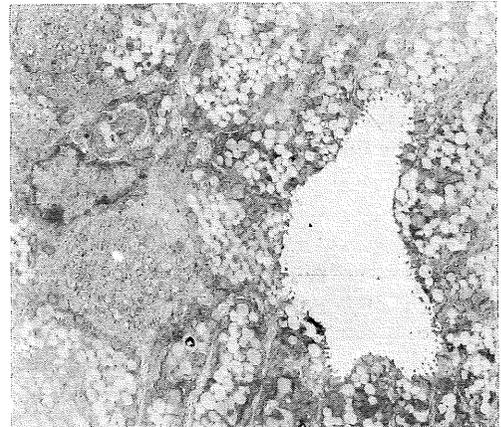
**Fig. 7.** A peculiar form of glands is surrounded by many infiltrated cells, and cystic formation is observed. 10 months after thymectomy. Toluidine blue  $\times 125$

SER. Infiltrated cells in the connective tissues were composed of plasma cells, lymphocytes, and eosinophils.

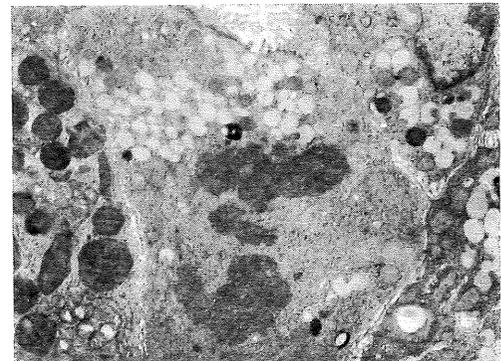
(4) 10–15 months after thymectomy

The gastric mucosae of the mice with gastritis 10–15 months after thymectomy were thick with giant rugae and the surface epithelia were rough (Fig. 7). Elongated glands were tortuous and tubulo-alveolar as well as dense with proliferated epithelial cells. Mitotic cells were frequently observed in these glands. Cysts were often observed in the deep regions of the glands.

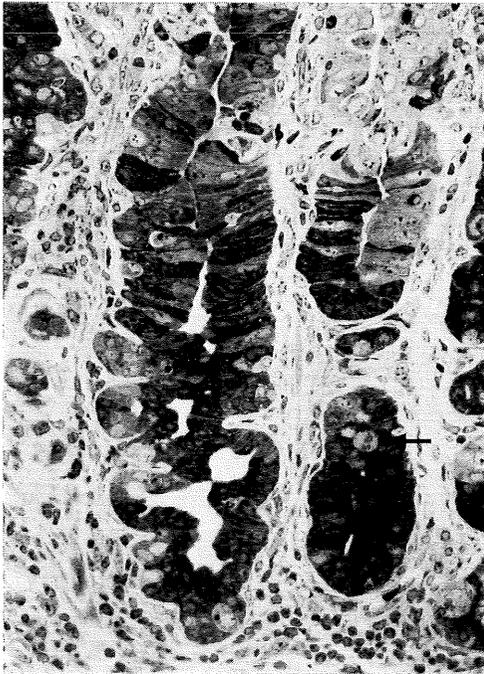
The cells which composed these glands were almost the same as those of mice with gastritis 3–6 months after thymectomy. Mucus-containing cells were most frequently observed among the cells of mice with gastritis in all parts of the gland and even in the base of the glands (Fig. 8). Some of these cells showed mitotic changes. These mucus-containing cells varied



**Fig. 8.** Glands are mostly composed of mucus-containing cells. Many types of granules are seen. 10 months after thymectomy. EM  $\times 2480$



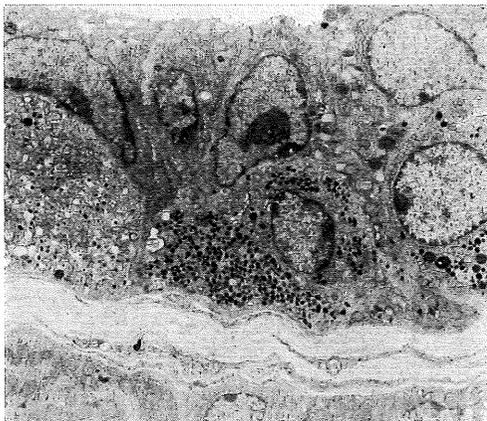
**Fig. 9.** A mitotic cell containing mucus. 10 months after thymectomy. EM  $\times 5000$



**Fig. 10.** Tortuous glands are mainly composed of mucus-containing cells and immature cells (Arrow shows a mitotic cell), 10 months after thymectomy. Toluidine blue  $\times 1180$

in shape (Fig. 9) and mostly faced the glandular tubulus. Mucous granules were located in the apical cytoplasm of these cells, which were  $0.4\text{--}1\ \mu\text{m}$  in size. The granules had various electron density and were composed of electron dense central cores and surrounding halos.

Immature cells were also frequently observed in the mucosae of mice with gastritis. The cells were proliferated in clusters, by which the



**Fig. 11.** Three types of endocrine cells are seen, 10 months after thymectomy. EM  $\times 2550$

glands were enlarged (Fig. 10). These immature cells were similar to those observed 3–6 months after thymectomy, although the cells after 10–15 months did not face the lumen. Many of the immature cells were in mitosis and proliferation.

A few fibrillovesicular cells and a few parietal cells in various stages of development were scatteringly observed among these cells. Most of the chief cells, however, were immature and very few in number when compared to parietal cells, and located in the lower parts of the gland. Secretory granules of these chief cells were few in number,  $0.7\text{--}1.5\ \mu\text{m}$  in size, and resembled those of chief cells in the upper parts of the glands of normal mice.

Most of the endocrine cells were well developed as those of mice 3–6 months after thymectomy and were found sporadically among mucus-containing cells, but these endocrine cells were not seen in the cluster of immature cells (Fig. 11).

Markedly cellular infiltrations were present in the lamina propria mucosae. The infiltrated cells were the same as those of mice 3–6 months after thymectomy. A few degenerated epithelial cells were left insular among these infiltrated cells.

#### (5) Histochemistry

In the gastric mucosae of normal mice, both surface epithelial and mucous neck cells were stained positive with PAS and showed neutral mucin, but most of these cells were stained negative with alcian blue at pH 2.5. Mucin present in the cells of deep glands and surface epithelia reacted faintly to alcian blue at pH 2.5 between the fore-stomach and glandular stomach in adult mice 3 months after birth. Stainability with PAS–alcian blue at pH 2.5 of the gastric mucosae of mice 2 months after thymectomy was not different from that in normal gastric mucosae. The surface and foveolar mucus were stained positive with PAS, although they were not stained at all with alcian blue at pH 2.5.

The glands of mice with gastritis 3 months after thymectomy were mostly composed of immature cells and mucus-containing cells, which were strongly stained with alcian blue at pH 2.5 and partly with PAS (Fig. 12). The surface mucus in the upper corpus was weakly stained with alcian blue at pH 2.5.



**Fig. 12.** Mucus-containing cells in the glands are stained with alcian-blue at pH 2.5, 6 months after thymectomy. PAS-AB  $\times 290$



**Fig. 14.** The glands are mostly composed of alcian-blue positive and PAS positive cells, 15 months after thymectomy. PAS-AB  $\times 280$



**Fig. 13.** Mucus-containing cells in the glands are stained with PAS, 6 months after thymectomy. Semi-thin PAS  $\times 1180$

As the mice aged, the glands became elongated, tortuous, and disarranged. The secretory granules of mucus-containing cells also increased in number and demonstrated strong stainability to PAS-alcian blue at pH 2.5 with growth (Fig. 13). Epithelial cells composing gastric glands were stained variably with PAS-alcian blue at pH 2.5 (Fig. 14). The surface mucus of gastric mucosae of mice with gastritis was more weakly stained with PAS than that of gastric mucosae of normal mice. Some surface mucous cells were not covered with PAS-positive mucus.

Ultrastructural observations showed that mucus-containing cells resembled mucous neck cells and all of these cells seemed to belong to one type of cells. Mucus-stainabilities of these cells varied and were different in character from those of the gastric mucosae of normal mice.

## DISCUSSION

In this study, the gastric mucosae of mice 2 months after thymectomy showed atrophic changes, characteristic degeneration of parietal and chief cells, cellular infiltration, and destruc-

tion of glandular structure. Sano<sup>4)</sup> reported that parietal cell antibodies (PCA) were seen in 65% of mice with gastritis, but these were observed only in 10% of mice without gastritis. He concluded that PCA was one of the causes of this type of atrophic gastritis.

Lymphocytic and plasma cells mostly infiltrated into the lamina propriae. Many eosinophilic leucocytes were also seen not only in the interepithelial tissue but also in the spaces among degenerated epithelial cells. These suggest that allergic factors might be involved as one of the causes for the development of this type of gastritis. Furthermore, according to Archer<sup>7)</sup> eosinophilic leucocytes were concentrated at the site of antigen-antibody reaction and phagocytosed antigen-antibody complexes. The findings observed here supported his hypothesis. During the course of destruction of glandular structure, cytoplasmic processes and the bodies of fibroblasts, which were the frameworks of epithelium, were observed in the spaces between epithelial cells and played an important role in the development of gastric mucosal lesions.

Mainly immature cells and mucus-containing cells appeared in the gastritic mucosa 3 months after thymectomy. With aging, the gastric glands became thick, elongated and tortuous. The glands were composed of immature and mucus-containing cells. However, at all stages of development, cell infiltration into the lamina propriae, degeneration and destruction of the epithelia were also observed. In addition, mitotic and immature cells as well as mucus-containing cells were usually observed in the deep glands during the period of the experiment. These suggest that mitotic and proliferative activities of the cells increased in the mucosae of mice with gastritis and these findings indicate that this type of gastritis was induced by repeated destruction and regeneration of the gastric mucosae.

It has been commonly considered that immature and mucus-containing cells appeared first in case of regeneration of gastric mucosae. Wattel<sup>8)</sup> observed that restoration commenced with the appearance of differentiating mucous cells and undifferentiated cells in mouse gastric mucosae exposed to fast neutron. He concluded that proliferative activity was confined to both of these cells. Yeoman<sup>9)</sup> also described that

undifferentiated cells appeared first and mucoid cells and young parietal cells were seen secondarily during the restoration of aspirin-induced erosions. Furthermore, the authors reported here that proliferated activity was confined to immature cells and mucus-containing cells. In the present study, as described before, mitotic and proliferative cells were largely composed of both undifferentiated and mucus-containing cells in the mucosa of mice with gastritis. This supports Yeoman's hypothesis.

With regard to inhibition of maturation of these cells, Lopes et al.<sup>10)</sup> have suggested that positive parietal cell antibodies may participate in this mechanism. Finally, mucosal destruction and increased cellular proliferation are the most important causes of induction to this type of gastritis. Moreover, repeated mucosal destruction may be a possible factor to inhibit the maturation of these cells.

Mucous neck cells do not show alcianophilia at all in the normal mucosae of mice<sup>11)</sup>. In the present study, however, mucus-containing cells in the gastric mucosae of mice with gastritis showed positive reactivities to alcian blue and PAS staining, although these cells were similar in appearance to mucous neck cells of normal mice. Furthermore, at the ultrastructural level, the size, electron densities, and number of secretory granules in these cells were quite different from those of mucous neck cells of normal mice. These suggest that the mucus-containing cells in the mucosae of mice with gastritis were not always the same in character as mucous neck cells in normal mice. Therefore, some inhibitory factors on maturation of the undifferentiated and immature cells may play an important role in development of gastritis in this study.

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