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Morphological Observations of Rabbit Gastric Fundus Cells in Primary Culture

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ABSTRACT

The morphological properties of the cells isolated from rabbit gastric fundus and the cells grown on a collagen gel sheet were investigated, using both light and scanning electron microscopes. The cells used for this study were obtained in the form of lumps from the isthmus region. The lumps consisted of 4 different types of cells (mucous and immatured cells, and a small number of chief and parietal cells). These isolated cells were then grow on collagen gel in a defined medium. However, two of those cells (chief and parietal cells) gradually disappeared as the culture time progressed. The majority of the cells grown on the gel were found to be mucous and assorted unidentified cells. The mucous cells were covered with many short microvilli, and many PAS positive granules in their cytoplasm. Some of those cells were covered with a mucous like substance. Judging from the staining properties of each cell, the unidentified cells appeared to indicate that they were immature cells. It was found that the cell's morphology and population retained constant regardless of whether Bh-EGF was administered or not.

The cells located within gastric fundus gland, such as chief, parietal and mucous cells etc. seem to proliferate and differentiate from the isthmus region cells which consisted of multipotent immature cells[3,4,6].

It is well known that the *in vitro* culture system is a useful model for studying cell development and cellular function as well as observing the effects of agents such as hormones, growth factors and other artificial drugs[1,2,5,7,9,10]. We recently found that the gastric fundus mucous cells isolated from rabbit's fundus gland was able to proliferate even in a serum free medium when the cells were cultured on a collagen gel sheet. Further, those cells could respond to hormones and maintain their functions[8,12].

In this study, we investigated the morphological properties of those cells cultured in a defined medium, using both light and scanning electron microscope. Furthermore, whether or not Bh-EGF was able to influence the cell differentiation of isthmus cells was investigated, morphologically.

MATERIALS AND METHODS

Materials

Medium 199 (M199, Earle's base), proteinase and collagenase were purchased from GIBCO (New York, USA) and Wako (Osaka, Japan). Biosynthetic human EGF (Bh-EGF) was obtained from Wakunaga Pharm. Co., Ltd. (Hiroshima, Japan). Antibiotics (penicillin and streptomycin) were supplied by Meiji (Tokyo, Japan) and Banyu (Tokyo, Japan). Culture petri dishes (ϕ 35mm) were procured from Nunc (Roskilde, Denmark). Hematoxylin, alcian blue and May-

Grünward solution were purchased from Merch (Rahway, USA) while percoll was obtained from Sigma (St. Louis, USA).

Cell isolation

The method has been described in detail elsewhere[8,12]. Briefly, Japanese white rabbit fundic cells were dispersed with 0.1% proteinase for 30 min and then with 0.05% collagenase for 40 min. Furthermore, the percoll density gradient (1.13 to 0.15g/ml) centrifugation was performed in order to eliminate matured chief, parietal and mucous cells. Over 95% of cell viability was secured through this procedure. The aliquot of the dispersed cells were mounted on glass slides and stained with May-Grünwald or periodic acid Schiff (PAS)-hematoxylin. The cells were obtained as lumps. These lumps chiefly consisted of deep foveolar mucous cells and undifferentiated cells (so-called the isthmus cells), and a small amount of matured chief, parietal and mucous neck cells. The majority of the isthmus cells had basophilic cytoplasm and PAS positive granules in it.

Isolated cells were washed thoroughly with Hank's balanced salt solution, suspended in M199 without serum, and with 100 U/ml penicillin and 100 μg/ml streptomycin, seeded on the plastic dishes coated with rat tail collagen gel[11] at the density of approximately 5×10^5 cells, and incubated in a humidified atmosphere of 95% air / 5% carbon dioxide at 37°C.

After being attached to this substrate, the cells were washed and cultured in the medium with 1.0 – 100 ng/ml of Bh-EGF. Each medium was changed daily.

Morphological study

To examine the same cell with both light and electron microscope, the specimens were prepared using the following procedures.

The cells cultured on the collagen gel were washed twice with phosphate buffered saline at 37°C and fixed with 4% paraformaldehyde or 2% glutaraldehyde buffered with 0.1M phosphate buffer pH 7.4 (PB) for 2 hr at 4°C. After fixation, the specimens were washed overnight with PB, and then stained with periodic acid Schiff (PAS). Hematoxylin or May-Grünwald reagent was used as a counter staining. First, the cells were observed with a light microscope (LM) and photographed. After the observation, the specimens were treated with 2% tannic acid, and postfixed with 2% osmium tetroxide buffered with PB for 1 hr at 4°C. These cells were then dehydrated using graded ethanol and to ensure that absolute dehydration was achieved, the use of the critical point drying method was applied. Following this step the dehydrated cells were coated with gold and examined under the JEOL-T200 scanning electron microscope (SEM).

RESULTS

To investigate the histological properties of the fundic region in rabbit stomach, the specimens stained with hematoxylin-eosin, alcian blue (pH 2.5)-PAS (AB-PAS) and alcian blue (pH 1.0) were observed. Rabbit fundus mucosa consisted of gastric foveolae, isthmus and fundus gland. Histochemically almost all the cells distributed in the upper part of isthmus region contained only neutral mucin glycoproteins. In the mucous neck region, the cells had many PAS positive granules but the cells containing acidic mucosubstances were hardly observed in this region (data not shown).

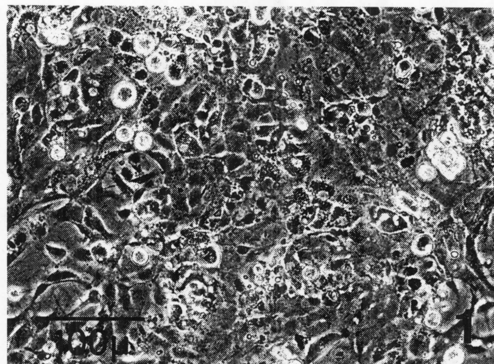

Fig. 1. Phase contrast microphotograph of primary monolayer culture. After forty-eight hours in culture, cells were attached on the substrate, and well spreaded and confluented.

LM observation

Isolated cells were grown on a collagen gel in a serum free defined medium (Fig. 1). Using light microscope, chief, parietal, mucous and unidentified cells were distinguished (Figs. 2 and 6), when the cells were stained using the previously mentioned method. Chief cells could be

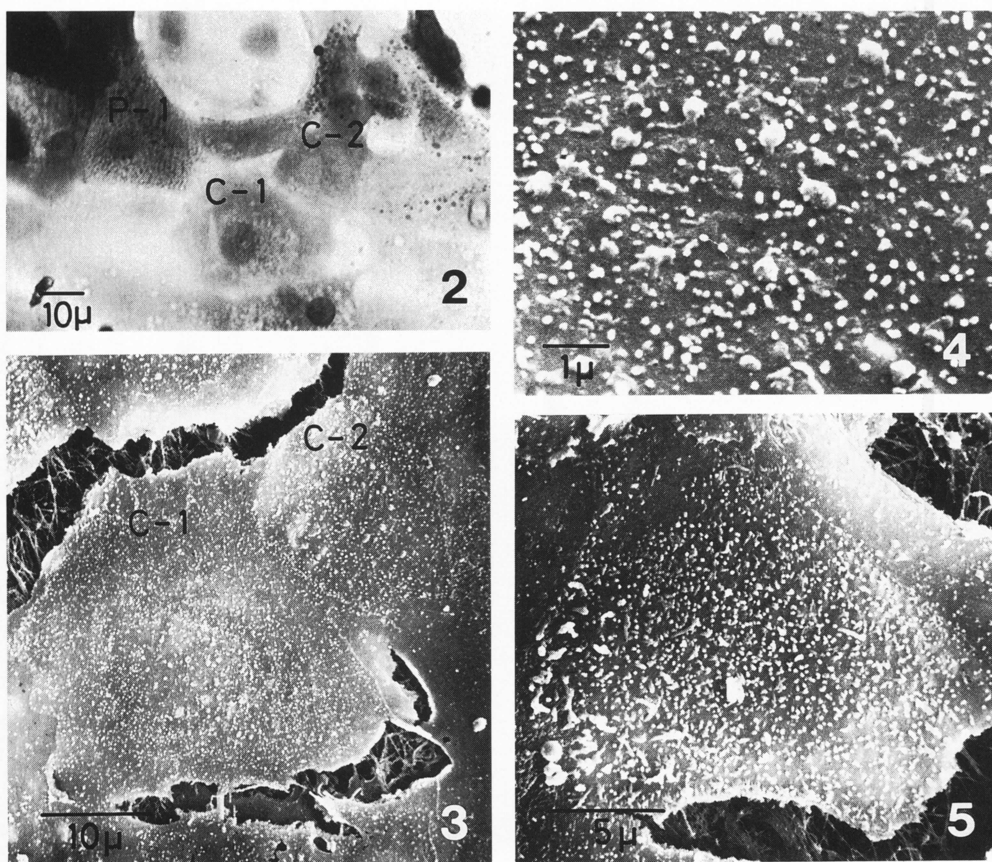

Fig. 2. Micrograph of the fundic cells cultured for 24 hr stained with PAS-May-Grünwald technique. By staining pattern, chief and parietal cells were distinguishable. Chief cells show basophilic cytoplasm and contain many large lucent granules (C-1 and C-2). Parietal cells show eosinophilic and a large number of mitochondria were observed (P-1).

Fig. 3-5. Scanning electron micrograph corresponding to C-1, C-2 and P-1 in Fig. 3, respectively.
Figs. 3 and 4. Chief cells possessed very short microvilli and spherical projection on the cell surface.
Fig. 5. Parietal cell was covered with many long microvilli.

identified as the cell containing basophilic cytoplasm, while at the same time containing many lucent granules in their cytoplasm (Fig. 2). Parietal cells were stained at eosinophilic, and their cytoplasm reacted weakly to PAS (Fig. 6). The mucous cells comprising the vast majority of cells grown on the gel, contained various number of PAS positive granules in their cytoplasm (Fig. 6). As the culture time progressed, the ratio in these cells gradually changed and the chief and parietal cells finally disappeared. Unidentified cells had none of the characteristics except for a slight staining in their cytoplasm basophilically.

SEM observation

In order to observe the surface property, the same cells observed with LM (Figs. 2 and 6) were chosen for the observation of SEM, ultrastructually. The chief cells, which adhered to the gel at the early stages of the culture, possessed many grain-like projections measuring 0.15 μm in diameter and in length. In addition, several knob-like vesicles projected from the cell surface. These spherical projections were 0.5 μm in diameter and were scattered on the whole cell surface (Figs. 3 and 4). These vesicles, however, did not correspond to the lucent granules observed with LM. The nuclear regions seemed to be either flattened or somewhat raised. The surface

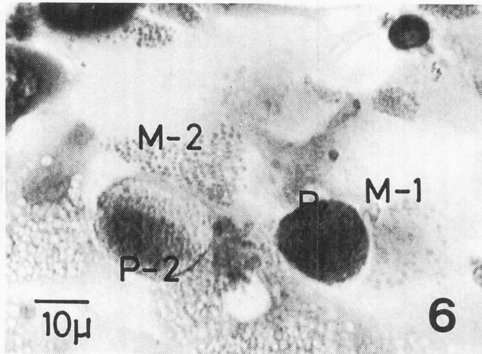

Fig. 6. This micrograph shows parietal cells (P and P-2) and mucous cells (M-1 and M-2). Mucous cells contain many PAS positive granules in their cytoplasm.

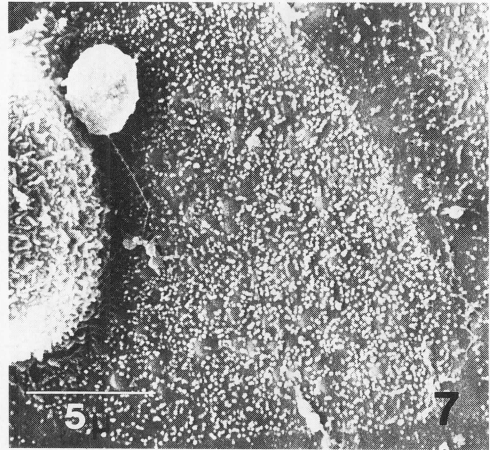

Fig. 7. Mucous cells are covered with various amounts of short microvilli and mucous granules are distinguished as hemispherical swellings (M-1 in Fig. 6).

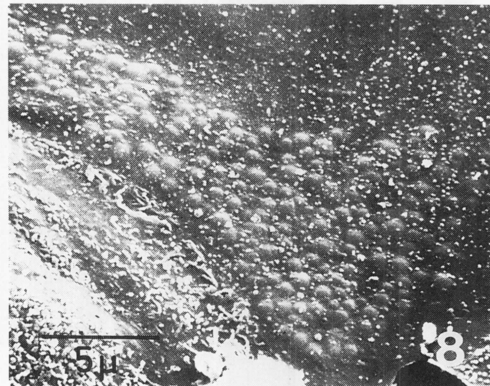

Fig. 8. Higher magnification of the mucous cell (M-2 in Fig. 6). Hemispherical swellings of mucous granules are apparently seen. At lower left of the figure, hemispherically attached parietal cell (P-2) which shows many long and curved microvilli is seen.

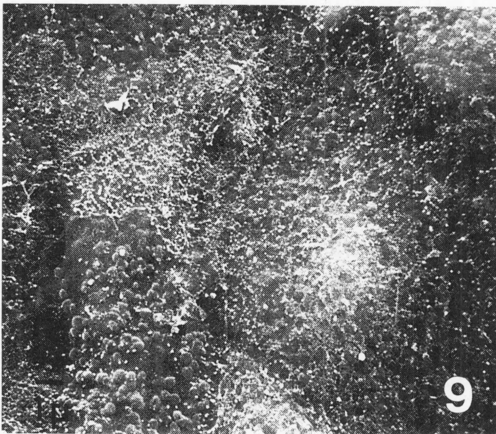

Fig. 9. Electron micrograph of a crowded region of mucous cells. Mucous granules are distinct and two mucous cells are covered with mucus-like substances. Forty-eight hours into be culture time.

of parietal cells were covered with numerous microvilli. The length of microvilli was dependent upon the degree of cell spread on the collagen gel. In the flattened cells, the microvilli at 0.15 to 0.3 μm in length and a small number of long microvilli (1.0 μm in length) were observed (Fig. 5). Hemispherically attached parietal cells had long (0.5 μm) and curved microvilli (Fig. 8). Mucous cells which contained PAS positive granules in their cytoplasm were covered with various number of short microvilli (80-130nm in length). Mucous granules were distinctly confirmed as hemispherical swelling sites of the cell membrane (Figs. 7 and 8). The size of the mucous granules were between 0.5 and 0.8 μm in diameter. In the cells having many granules, the surface was covered with a mucus-like substance (Fig. 9). Unidentified cells were very flat and covered with a moderate number of short microvilli.

DISCUSSION

In *in vitro* study, it is important to identify the cells grown as well as to investigate what functions are maintained in the cultured cells.

In this study we investigated the morphological properties of the rabbit gastric cells grown on a collagen gel, using LM and SEM. To collerate the information obtained from both microscope varieties, the same cells observed with LM were chosen for the investigation by SEM.

The viable cells of rabbit stomach were able to be isolated by the methods reported by Berglindhn et al[1] and Bouhours et al[2] with some modification[8,12]. And only the isthmus region cells which were isolated as lumps were well proliferated in serum free defined medium. In the histological observations, those lumps consisted of four cell types. The majority of the lumps, consisted of mucous cells, and unidentified cells which possessed a small amount of PAS positive granules. At the early stages of the culture, four types of cells grown or attached on the collagen gel were recognized using light microscopically. From the observation of SEM, chief and parietal cells which have basophilic and eosinophilic cytoplasm respectively were covered with various lengths of microvilli. However, as the culture progressed two of those cells gradually disappeared. From the above observations, our culture condition seems to be unsuitable for the maintenance and growth in those cells.

Under the *milieu*, almost all cells that grew from the isthmus cells were found to be either mucous or unidentified cells. Mucous cells have a lot of short microvilli on the cell surface, and have many PAS positive granules in their cytoplasm. Previously, we had demonstrated that those mucous cells grew and maintained their function, at the cells were able to synthesize and secrete mucin glycoprotein for a long time[12]. The fact that some of the mucous cells were covered with mucin like substance indicated the secretion of mucin from the cell, supported our previous report. The fate of the unidentified cells which seemed to be the immature cells, because of their staining properties, is not clear yet. Generally, most of the isthmus region cells were found to be immature, and developed into various types of cells associated with the gastric gland, such as mucous, chief and parietal cells. Under proper culture conditions, the unidentified cells observed here may well develop to be actually a stomach cell.

In this culture system, biosynthetic human EGF which was one of the growth factors, didn't induce the morphological changes nor the differentiation of isthmus cell as far as examined.

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