Histopathology and Physiopathology of Gastric Mucous Hyperplasia in Rats Heavily Infected with *Taenia taeniaeformis*

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**ABSTRACT.** Rats heavily infected with larval *Taenia taeniaeformis* show hyperplasia of the gastric mucosa accompanied by mucous cell proliferation, increase in the level of intragastric pH and hypergastrinemia. Sixty one rats were divided into 2 groups designed as infected (36 rats) and control (25 rats) group. These rats were examined with time course of the infection histopathologically and physiopathologically, during 14–112 days postinfection (DPI). In the infected rats, gastric mucosal hyperplasia began to be observed at 56 DPI, and the structural disturbance of zymogenic units in the corpus and mucous units in the antrum had increased with time. However, the degree of these changes in the antrum was weaker than those in the corpus. Alcianblue and/or PAS-positive cells increased in their numbers with time, and 4 types of cells other than typical surface mucous cell and mucous neck cell were observed by electron-microscopy. However, zymogenic and parietal cells decreased in their number after 56 DPI. Further, the infected rats showed changes in the serum concentration of alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, glucose and total protein. Some similarities with Menetrier’s disease were discussed.—**KEY WORDS**: gastric hyperplasia, histopathology, physiopathology, rat, *Taenia taeniaeformis*.

In general, when parasites infect host, histopathological changes are observed in the organ or the tissue where they are located. For example, nematode parasites of the abomasum, such as *Trichostrongylus axei*, *Ostertagia circumcincta* and *Haemonchus contortus*, cause histopathological changes and an increase in pH in the host’s abomasum where they infect [5, 14, 19, 20]. However, when rats were heavily and chronically infected with *Taenia taeniaeformis* larvae in the liver, a remarkable increase in their stomach weight accompanied by mucous cell proliferation occurs [1–4, 17]. Increase in level of intragastric pH and serum gastrin, and decrease in number of zymogenic and parietal cells were reported in the infected rats [1, 2, 17]. Since Bullock and Curtis [3] first reported the hyperplasia of the stomach in rats heavily infected with *T. taeniaeformis* larvae, some works have followed. Rikihsa and Lin [17] reported that gross hyperplasia of the gastric mucosa and excessive mucus production in the stomach occurred in rats heavily parasitized with larvae of *T. taeniaeformis*, and that PAS-positive mucous cells were the major cell types in the hyperplastic stomach. Although the cause and the mechanism were suggested, through *in vivo* and *in vitro* experiment, in some works [1, 2, 4, 18], they seemed to be very complex and were still unsolved. Similarly, many gastrointestinal diseases have been reported and classified, and their cause and mechanism are also various, very complex and still not well understood.

Menetrier’s disease [12, 13], Zollinger-Ellison syndrome [12] and hypertrophic hypersecretory gastropathy [12] are all uniquely characterized with hyperplastic gastropathy, and classified according to morphological and clinical differences. Many causes, such as the infection of cytomegalovirus [7, 8], *Helicobacter pylori* [15, 21], and *Giardia lamblia* [16], and the abnormal proliferation caused by peptide growth factors [6] etc., have been proposed. Especially, since Menetrier’s disease was first reported in 1888, many works have followed. However, the exact cause and the mechanism of this gastropathy are still unsolved. The patients with this disease have enlarged gastric folds with foveolar hyperplasia, and were frequently accompanied by hypoproteinemia, hypochlorhydia, and enhanced mucous production [6]. These characteristics are very similar to those of the rats heavily infected with *T. taeniaeformis* larvae in the liver. So the gastric mucosae of the infected rats were histopathologically examined at the different post infection dates, and concentrations of their selected serum components were also measured.

**MATERIALS AND METHODS**

*T. taeniaeformis* eggs used in this study were collected from proglottids expelled by cats infected with rat strain originally isolated from Norway rat (*Rattus norvegicus*) in Sapporo, Japan, and maintained in our laboratory using cats and rats.

Sixty one, 4-week-old male Wistar rats were purchased from a commercial source (CLEA, Japan) and divided into 2 groups designed as infected (36 rats) and control (25 rats) groups. When the animals were 5 weeks old, rats of infected...
Fig. 1. Body weight of infected and control rats.
Fig. 2. Stomach weight of infected and control rats.
Fig. 3. Liver weight of infected and control rats.
Fig. 4. *Taenia taeniaeformis* larval weight (per 100 larvae) in the liver of infected rats.

Figs. 1-4. Differences between infected and control group were observed on body and stomach weight after 56 days postinfection (DPI). Increase of liver weight was agreed with that of larval weight.

Fig. 5. Mucosa in the corpus of an infected rat. The mucosal changes in the corpus of the infected rats were observed after 56 DPI. The mucosa increased in thickness at 56 DPI, which was continued until 112 DPI. AB and/or PAS-positive mucous cells such as SMC or MNC increased in their numbers and the gastric glands dilated to cystic cavities with time until 84 DPI.

Fig. 6. Gastric mucosa in the corpus of an infected rat at 56 DPI. Gastric glands elongated near luminal surface of the mucosa, where AB and/or PAS-positive mucous cells increased in their number, and most of them in dilated gastric glands were columnar in shape. Scale bar=25 μm.

Fig. 7. Type I cell observed at the cystic glands of the corpus in an infected rats after 56 DPI. They were rectangle in shape, and their cytoplasm had a few big secretory granules which were almost homogeneous substances, and a few small granules which had a dense core. Nucleus was located at the base, and organelles seemed to be pressed against the nucleus. Scale bar=2 μm.

Fig. 8. Type II cell observed at the cystic glands of the corpus in an infected rats after 56 DPI. They were square in shape, and their cytoplasm contained many secretory granules, some of which had a dense core. The granules occupied most of the cytoplasm along with several mitochondria and some rough endoplasmic reticula, which were seen near nucleus and seemed to be pressed. Scale bar=2 μm.

Fig. 9. Gastric mucosa in the corpus of an infected rat at 70 DPI. Gastric glands further expanded along with the thickness of the mucosa, where AB and/or PAS-positive mucous cells were smaller than those seen at 56 DPI, while they increased in their number. Scale bar=25 μm.

Fig. 10. Type III cell observed at the large cystic glands of the corpus in an infected rats after 70 DPI. They were square in shape, and their cytoplasm was few and contained large spherical nuclei, some mitochondria and rough endoplasmic reticula. Some secretory granules were located in apical cytoplasm and had a dense core. Scale bar=2 μm.

Fig. 11. Type IV cell observed at the thin edge of the much larger cystic cavities of the corpus in an infected rats after 84 DPI. They were ovoid in shape, and had slender nucleus at the base. Many granules, some of which had a core, were observed in the cytoplasm. Scale bar=2 μm.

Fig. 12. Mucosa in the antrum of an infected rat. The mucosal changes in the antrum of the infected rats were observed after 56 DPI. The mucosa increased in thickness at 56 DPI, which was continued until 112 DPI. AB and/or PAS-positive mucous cells increased in their number and cystic cavities were observed. However, degree of the changes was less than that of the corpus.
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group were orally dosed with approximately 4,000 *T. taeniaeformis* eggs. Two to four rats from both groups were sacrificed every 2 weeks after the infection until 112 days postinfection (DPI). After the stomach was removed from the abdomen, the greater curvature was opened and placed into 2 types of fixative solution. Tissues for light microscopic observation were fixed in 20% formalin. Sections were stained either with hematoxylin and eosin (HE), with pH 2.5 alcianblue (AB), with Periodic acid Sciff (PAS), or with both AB and PAS, and then observed under light microscope. Tissues for electron microscopic observation were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, and postfixed for 1.5 hr in 1% OsO₄ in 0.1 M cacodylate buffer. After dehydration, tissues were embedded in Quetol 812 (NISSIN EM Co., Ltd., Tokyo, Japan). Thin sections were stained with 10% uranyl acetate in 50% ethanol and lead citrate, and observed using a JEM-1210 electron microscope (JEOL, Tokyo, Japan).

Blood samples were collected every 2 weeks from the external jugular vein of 6 rats (3 rats each in infected and control groups) planned to be sacrificed at 112 DPI, immediately placed on ice, and allowed to clot. Concentration of the serum components was measured on an automated analyzer (COBAS MIRA (Roche Nippon, Tokyo, Japan)). Concentration of the serum components was measured on an automated analyzer (COBAS MIRA (Roche Nippon, Tokyo, Japan)). Blood samples were collected every 2 weeks after a 24-hr fast from the external jugular vein of 6 rats (3 rats each in infected and control groups) planned to be sacrificed at 112 DPI, immediately placed on ice, and allowed to clot. Concentration of the serum components was measured on an automated analyzer (COBAS MIRA (Roche Nippon, Tokyo, Japan)). Concentration of the serum components was measured on an automated analyzer (COBAS MIRA (Roche Nippon, Tokyo, Japan)). Concentration of the serum components was measured on an automated analyzer (COBAS MIRA (Roche Nippon, Tokyo, Japan)). Concentration of the serum components was measured on an automated analyzer (COBAS MIRA (Roche Nippon, Tokyo, Japan)). Concentration of the serum components was measured on an automated analyzer (COBAS MIRA (Roche Nippon, Tokyo, Japan)).

RESULTS

**Weight of body, stomach, liver of infected and control rats, and mean number and weight of hepatic larvae in infected rats:** The mean number of the hepatic larvae in the infected rats was 604 ± 41. The changes of body, stomach and liver weights in the infected and control rats are shown in Figs. 1, 2 and 3, respectively. However, data of 11 dead rats before the sacrifice were excluded. Significant difference on body weight between the infected and the control rats was seen after 56 DPI. Moreover, drastic change on the increase of the stomach weight was seen in the infected rats after 56 DPI, though the infected rats did not show such increase until 42 DPI. The liver of the infected rats increased in their weights with time after the infection, and this increase matched with the larval growth (Fig. 4).

**Histopathological and physiopathological observation in the corpus:** PAS-positive cells in the surface and the pit, known as surface mucous cells (SMC), were observed in the corpus of infected and control rats until 42 DPI. In the isthmus, AB and/or PAS-positive cells were located, and they were immature SMC. Faint PAS-positive cells, known as mucous neck cells (MNC), were observed in the neck. Parietal cells, whose cytoplasm were not stained with any dyes used, were located from the pit to the base, and zymogenic cells, whose cytoplasm were stained with hematoxylin, were located at the base (Fig. 5). Degenerated cell with dispersing nucleus or apoptotic body was not observed.

At 56 DPI, the mucosa of the infected rats increased in thickness in the corpus. The gastric glands elongated, and cystic cavities were seen near the luminal surface of the mucosa, where cell proliferation was very active. Normal structure of the zymogenic units composed of the pit, the isthmus, the neck and the base was disturbed and could not be differentiated from each other. Mucous cells markedly increased in number, and most of them in dilated gastric glands were columnar in shape and their cytoplasm were filled with mucigen granules stained with AB and/or PAS. The characteristics of these cells were similar to those of immature SMC (Figs. 5, 6), and these cells were further observed using transmission electron microscope. Two types of cells were recognized, and their forms and granules were different from each other. One (type I) was rectangle in shape, whose cytoplasm was occupied by a few large secretory granules which were almost homogeneous substances and a few small granules containing a dense core (Fig. 7). Organelles were located around the nucleus, and also seemed to be pressed. The other (type II) was square in shape, its cytoplasm contained many secretory granules, some of which had a dense core (Fig. 8). The granules occupied nearly all of the cytoplasm, where several mitochondria and some rough endoplasmic reticula (rER) were observed near nucleus which was located at the base and seemed to be pressed. Both zymogenic and parietal cells decreased in their numbers. Apoptotic bodies were observed in the dilated glands, but few.

At 70 DPI, the mucosa increased in thickness, gastric glands further expanded along with the thickness of the mucosa, and the zymogenic units were disturbed. AB and/or PAS-positive cells were distributed throughout the mucosa (Fig. 5). AB-positive and faint PAS-positive cells were observed at the middle and the lower part of the mucosa, respectively. Though type I and II cells were observed, the mucous cells showing AB and/or PAS-positive around the large cystic cavities were smaller than those (Fig. 9), and further observed using transmission electron microscope. They were square in shape, and their cytoplasm was few and contained large spherical nuclei, mitochondria, rER, and secretory granules which had a dense core (type III) (Fig. 10). Apoptotic bodies were sometimes observed in the dilated glands and increased in number slightly.

At 84 DPI, though the degree of hyperplasia was the same as that at 70 DPI, the cystic cavity increased in number and size (Fig. 5). Around the larger cystic cavities in the corpus, type III cells increased in number. Furthermore, at the thin edge of the very large cystic cavity, PAS-positive pressed ovoid cells were observed. They had slender nucleus and many granules, some of which had a core, were observed using transmission electron microscope (type IV) (Fig. 11). Apoptotic bodies were observed more in the dilated glands, and this condition continued until 112 DPI.
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At 98 and 112 DPI, the conditions of the mucosa in the corpus were very similar to those at 84 DPI. Inflammatory cells, predominantly lymphocytes and some eosinophils, were found at the base of the mucosa in the corpus until 42 DPI. After 56 DPI, however, most of them were observed among the edematous hyperplastic area of the upper lamina propria in the corpus. They also increased in their number. Though this trend continued until 70 DPI, it became weaker after 84 DPI.

Histopathological and physiopathological observation in the antrum: Until 42 DPI, in the antrum, PAS-positive SMC spread into upper half of the mucosa. AB-positive mucoid cells of pylorum were observed at the base. Further, some AB and PAS-positive cells were also observed at the upper half of the mucosa (Fig. 12).

At 56 DPI, the mucosa in the antrum increased in thickness. AB and PAS-positive mucous cells were observed near the luminal surface and at the lower half of the mucosa, and typical PAS-positive SMC were observed.

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Fig. 13. Concentration of serum alkaline phosphatase.
Fig. 14. Concentration of serum alanine aminotransferase.
Fig. 15. Concentration of serum aspartate aminotransferase.
Fig. 16. Concentration of serum blood urea nitrogen.
Fig. 17. Concentration of serum cholesterol.
Fig. 18. Concentration of serum creatinine.
Fig. 19. Concentration of serum glucose.

Fig. 20. Concentration of serum neutral fat.

Fig. 21. Concentration of serum total bilirubin.

Fig. 22. Concentration of serum triglyceride.

Fig. 23. Concentration of serum total protein.

Figs. 13–23. Concentrations of the serum components measured in the infected and control rats was observed on alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), glucose (Glu) and total protein (TP). However, the concentrations of alkaline phosphatase (ALP), cholesterol (CHO), creatinine (Crea), neutral fat (NEFA), total bilirubin (T-Bil) and triglyceride (TG) did not show significant differences between the infected and the control group.

at the upper half of the mucosa. AB-positive mucoid cells were observed at the base of the mucosa and slightly increased in number of its granules. Some cystic cavities were observed near the luminal surface of the mucosa (Fig. 12). Apoptotic body was not observed.

At 70 DPI, the degree of hyperplasia was almost the same as that of 56 DPI, and some cystic cavities were observed near the luminal surface of the mucosa. AB and PAS-positive cells decreased in number and were observed at the middle of the mucosa. PAS-positive cells were distributed throughout the mucosa. At the base, weak PAS-
positive cells lining along the glands, and AB-positive cells decreased in number (Fig. 12). Apoptotic body was not observed.

At 84 DPI, though the mucosa did not increase in thickness, mucous units were disturbed and many large cystic cavities were observed throughout the mucosa. AB and/or PAS-positive cells were seen, and further observation was carried out using electron microscope. These cells were found to be similar to type I, II, and III cell (Figs. 7, 8, 10). The degree of the changes was less than that of the corpus, though these mucosal changes in the antrum were similar to those in the corpus (Fig. 12). Apoptotic bodies were sometimes observed in the cystic cavities, and this continued until 112 DPI.

At 98 and 112 DPI, the conditions of the mucosa in the antrum were very similar to those at 84 DPI. Inflammatory cells, predominantly lymphocytes and some eosinophils, were found at the base of the lamina propria in the antrum until 42 DPI. Although they increased in their number at the base of the lamina propria, the degree was weaker than those in the corpus after 56 DPI.

Changes of serum component levels: Concentrations of the 11 serum components measured are shown in Figs. 13–23. Significant differences were observed between the infected and control groups with regard to ALP, ALT, BUN, Glu and TP. The concentrations of BUN, ALP and ALT in infected group were higher than those of the control after 56–70 DPI, while the concentrations of TP and Glu were lower at 70 DPI. However, the rest of the components measured did not show any significant difference between the infected and the control groups.

DISCUSSION

In the corpus, after 56 DPI, the AB and/or PAS-positive mucous cells such as type I and II cells similar to immature SMC increased in their numbers and the gastric glands dilated with time until 70 DPI. Contrary to these increases, zymogenic and parietal cells decreased in their numbers, which continued to 112 DPI. It was previously reported that parietal and zymogenic cells were rare in hyperplastic gastric mucosa of the rats dosed orally with *T. taeniaeformis* eggs [17]. However, it was reported that the mean number of days spent for the turnover of parietal cell were 35 days in the pit, 31 days in the isthmus, 54 days in the neck and 189 days in the base [9], and that for zymogenic cell were 138 days [11]. The degenerated cells with nuclei dispersing were also observed in the corpus after 56 DPI and became stronger with time. At 70 DPI, the type I, II and III cells, classified according to their morphological differences, were observed in the corpus. This observation was similar to the report by Rikihisa and Lin [17]. At 84 DPI, though type IV cells were often observed at the thin edge of the large cystic cavities in the corpus. These observations may suggest that apoptosis occurred, and that death or removal of zymogenic and parietal cells as well as the disorder of cell proliferation and differentiation occurred in the corpus.

Although most researchers have reported on the gastric corpus of the patients with Menetrier’s disease, Dempsey et al. [6] reported that only 1 of 4 patients with Menetrier’s disease had enlarged folds in the antrum as well as the corpus. In our study, similarly, degree of the changes in the antrum was less than that in the corpus, though the mucosal changes were observed to begin at 56 DPI in the antrum of the infected rats. It was also suggested that apoptosis occurred after 84 DPI in the antrum. These observations may suggest that the disorder of cell proliferation and differentiation occurred in the antrum, though the degree of them was less than that in corpus and the appearance of apoptotic body was delayed.

Karam and Leblond [10] reported that about 67% of stem cells differentiate into SMC, about 24% into MNC which further differentiate into zymogenic cells, and about 9% into parietal cells in mice. These observations suggested that the cell proliferation and the differentiation were out of order both in the corpus and the antrum of the infected rats after 56 DPI.

The infiltration of the inflammatory cells into the hyperplastic gastric mucosa was observed in the infected rats. This observation was similar to the report of the patients with Menetrier’s disease with gastritis [12].

Although the number of serum samples collected were not enough to evaluate the change of the component levels in serum, some suggestions could be made. The peak levels of ALT and AST occurred at 14 or 28 DPI and 70 or 84 DPI, respectively. The higher levels suggested some hepatic damages. The decrease of TP level was observed after 70 DPI, when the gastric hyperplasia became prominent. Edematous changes were also observed in the corpus after 56 DPI. Chronical manifestation of hypoproteinemia was also a frequent finding in patients with Menetrier’s disease [6], and Oderda et al. [15] reported that the increase of the tight junction width among the mucosal cells of the corpus was observed in the patients with Menetrier’s disease.

Several suggestions as the cause of gastric hyperplasia in *T. taeniaeformis* larvae infected rats were made in some works [1, 2, 4, 18]. One of the strongest was that the hyperplasia in the infected rats was caused by factor/s in blood by means of parabiosis [4], and that protein components of larval excretory-secretory products affected the differentiation and proliferation of the gastric cell through an *in vitro* experiment [18]. In line with these, we observed gastric mucosal hyperplasia in rats surgically implanted with the larvae into the abdominal cavity (unpublished). Dempsey et al. [6] further reported that the majority of mucous cells in the gastric mucosa of patient with Menetrier’s disease was stained by the immunostaining of gastrin with Menetrier’s disease [12].

In several experiments, we observed gastric mucosal hyperplasia in rats with *T. taeniaeformis* larvae infected in liver and those hyperplastic gastraphathies such as Menetrier’s disease, further study on rats infected with *T. taeniaeformis* larvae may prove to be a useful model in solving the mechanism of such disease process.
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