Comparative Studies on Distribution and Fine Morphology of the Intestinal Peyer's Patches in Mongolian Gerbils (*Meriones unguiculatus*) and Mice

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ABSTRACT. The intestinal Peyer's patches (PP) were significantly different in distribution and fine morphology between Mongolian gerbils and mice. The PP of gerbils were evenly distributed from the duodenum to ileum, whereas the PP of mice were more densely distributed in the lower ileum than other parts of the intestine. In gerbils, each PP had a much greater number of lymphoid follicles than in mice, although the total number of PP was smaller. Electron microscopy revealed that the PP dome of gerbils was covered with two types of epithelial cells, one with shorter microvilli and the other with longer ones, whereas the epithelial cells of the murine PP dome was uniform-shaped with numerous medium-sized microvilli. Some dome absorptive cells of gerbils were morphologically similar to poorly differentiated crypt cells of mice, suggesting to be immature M cells.—**KEY WORDS:** follicle-associated epithelium (FAE), M cell, Mongolian gerbil, mouse, Peyer's patch.

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The epithelial surface of Peyer's patches (PP) of the intestine has been extensively studied by electron microscopy in domestic [5, 11, 13] and laboratory [4, 12, 14] animals as well as in man [23, 24]. The distribution of PP and the morphology of follicle-associated epithelial cells (FAE), that is M cells [4], were described to vary among animal species [8, 9, 11]. Distribution, structure and development of the PP were shown to be similar in rodents, rabbit and man [8].

We recently observed that the Mongolian gerbils, being different from mice, were highly susceptible to peroral inoculation with the Tyzzer's organism showing severe enterohepatitis, suggesting that the FAE and PP might play important role in bacterial invasion [7, 25]. The present study is to see the comparative distribution and electron microscopy of the intestinal PP and FAE of Mongolian gerbils and mice.

MATERIALS AND METHODS

Animals: Eight-week-old female ICR mice weighing 25 to 29 g came from a commercial breeder (Charles River Japan, Atsugi). Mongolian gerbils of TUM stock were bred at this laboratory and they were used at 8 to 12 weeks of age weighing 60 to 70 g. All the animals were kept in a conventional (CV) environment and given commercial pellets (MF; Oriental Yeast, Tokyo) and tap water *ad libitum*.

Electron microscopy: Tissue samples were taken

from the small intestine with PP domes and washed repeatedly with chilled phosphate buffered saline, pH 7.2 (PBS). For scanning electron microscopy (SEM) tissues were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, respectively, for 2 hr. Then, they were dehydrated in a graded ethanol series, transferred to isoamylacetate, dried at a critical point and coated with gold-palladium by evaporation. Observation was made using a Hitachi S-450 electron microscope. For transmission electron microscopy (TEM) tissues were cut into pieces under a dissecting microscope and fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, respectively, for 2 hr. Fixed specimens were dehydrated in a graded ethanol series and then transferred to acetone and embedded in epoxy resin. Serial ultrathin sections about 100 nm in thickness were made and stained with uranyl acetate and lead citrate. They were observed with a JEOL JEM-1200EX electron microscope.

Gross and ultrastructural measuring: The number of follicles within each PP was counted under a magnifying glass. On the TEM figures of the longitudinal ultrathin sections of the epithelium, the density of microvilli on the central 2 μm -brush border of each cell was measured according to Sicinski *et al.* [20]. The length of microvilli was determined on the central brush border of each cell.

RESULTS

In Mongolian gerbils, the PP were rather evenly distributed from the duodenum to ileum and char-

Table 1. Numerical comparison of intestinal PP^{a)} between gerbils and mice

Animal	Total number of PP	Number of LF ^{b)} /PP	Total number of LF/Animal
Gerbil ^{c)}	5-9 ^{e)} 6.6±1.3 ^{f)}	3-26 11.2±0.5	60-110 74±4.2
Mouse ^{d)}	9-14 10.8±0.6	3-9 5.5±0.2	45-70 55±5.3

a) Peyer's patch.

b) Lymphoid follicles.

c) Thirty female TUM Mongolian gerbils aged 10 to 12 weeks.

d) Fifteen female ICR mice aged 10 to 12 weeks.

e) Range.

f) Mean±SE.

Fig. 1. Distribution of PP at each level of the small intestine (vertical scale) and the number of lymphoid follicles within each PP (horizontal scale) in gerbils and mice. 0 to 60=duodenum; 61 to 240=jejunum and ileum length (mm). The length of the murine small intestine was expressed with a reduction to 58% for comparison to the gerbil one.

acterized by prominent bulges on the serosal surface (Table 1, Fig. 1). Each gerbil had 5 to 9 PP in the whole intestine. Each PP contained 3 to 26 lymphoid follicles, and 60 to 110 lymphoid follicles were counted in total. The larger PP mostly appeared in the duodenum and were equipped with a larger number of follicles.

In mice, the distribution of PP in the jejunum was much less dense than in the duodenum and ileum. The murine small intestine had 9 to 14 PP containing 3 to 9 lymphoid follicles, and 45 to 70 follicles were counted in total. While the murine PP domes were less in number than those in gerbils, the PP of both species were very similar in size and shape.

On light microscopy no significant difference in morphology was detected between the PP of gerbils and those of mice.

SEM revealed that each PP of gerbils and mice formed a hemispherical dome 200 to 600 μ m in diameter (Fig. 2). By TEM, while the villous surface was covered with epithelial cells with denser and longer microvilli, the dome surface was covered with polygonal absorptive cells with longer microvilli as well as diamond-shaped M cells with shorter microvilli (Fig. 3). The micro-villi of both types of dome cells had poor glycocalyx. The dome absorptive cells

Fig. 2. Hemispherical PP domes (D) of a gerbil. SEM.

Table 2. Numerical comparison of the intestinal epithelium with microvilli between gerbils and mice

Animal	Cell type	Density ^{a)}	Length (μm)
Gerbil	Dome absorptive cell	11.3 $\pm$ 0.2 (5–16) ^{b)}	2.05 $\pm$ 0.05(1.25–3.5)
	M cell; mature	7.7 $\pm$ 0.3(3–11)	0.63 $\pm$ 0.03(0.2 –1.0)
	immature	9.2 $\pm$ 0.7(5–12)	0.82 $\pm$ 0.06(0.55–1.3)
	Villous absorptive cell	18.5 $\pm$ 0.2(11–17)	1.80 $\pm$ 0.11(1.0 $\pm$ 3.5)
Mouse	Dome absorptive cell	14.5 $\pm$ 0.4(11–19)	1.14 $\pm$ 0.03(0.85–1.6)
	M cell ^{c)}	7.9 $\pm$ 0.5(3–10)	0.50 $\pm$ 0.05(0.25–1.0)

a) Number of microvilli per 2 μm -length of the brush border.b) Mean $\pm$ SE (Range).

c) Not including immature-M cells.

Fig. 3. Number of microvilli per 2 μm -length of the brush border (horizontal scale) and the length (μm) of microvilli of different cell types (vertical scale) in the PP domes of gerbil and mice. $\blacktriangle$: Murine absorptive cell (MA), $\blacksquare$: Murine M cell (MM), $+$: Gerbil absorptive cell (GA), $\diamond$: Gerbil M cell (GM). Fig. 4. Number of microvilli per 2 μm -length of the brush border (horizontal scale), and the length (μm) of microvilli (vertical scale) of M cells and dome or villous absorptive cells in gerbils. $\blacklozenge$: Mature M cell (mM), $\times$: Immature M cell (iM), $+$: Dome absorptive cell (DA), $\blacksquare$: Villous absorptive cell (VA).

were shown to have a fewer and comparatively longer microvilli showing well-developed terminal webs (Table 2, Figs. 3–5). They had a moderate number of endocytic vesicles in the apical cytoplasm.

The gerbil M cells had shorter microvilli with poorly developed terminal webs, and a few mitochondria and numerous vesicles were seen within the apical cytoplasm (Table 2, Fig. 6). They were shown to contact with the dome absorptive

Fig. 5. Gerbil dome absorptive cells (D) with rather sparse microvilli, facing on a villous absorptive cell (V) with denser and longer microvilli. TEM.

Fig. 6. Gerbil M cell (M) with irregular-shaped shorter microvilli and numerous small apical cytoplasmic vesicles. Adjacent dome absorptive cells (A) have sparse and longer microvilli and no distinguished Golgi complexes and mitochondria. A lymphoid cell (L) is contacting with the both types of cells. TEM.

Fig. 7. Gerbil M cell (M) with irregular-shaped, shorter and thicker microvilli and a dome absorptive cell (A) with fine microvilli, contacting each other through desmosomes or junctional complexes. TEM.

cells by desmosomal complexes (Fig. 7). There was another type of dome cells showing an intermediate morphology between M cells and dome absorptive cells, which were suggestive of immature M cells (Table 2, Figs. 4, 6 (A)). Lymphoid cells were frequently present beside or within M cells (Fig. 6).

In mice, FAE were mostly composed of hexagonal absorptive cells with fine microvilli, which were much shorter than those of gerbils (Fig. 3). Around the domes there were a small number of M cells with a few short microvilli without glycocalyx cover. The murine M cells were almost similar in morphology to those of the gerbil (Fig. 8). The apical surface of M cells was clearly demarcated and characterized by poorly developed terminal webs. There were a few branched microvilli, which were shorter and thicker than those of adjacent absorptive cells.

DISCUSSION

There have been many studies on murine FAE [1,

Fig. 8. Murine lymphoid cells (L) above or beneath an M cell (M) having branched and fused microvilli and mitochondria mostly distributed at the apical cytoplasm. TEM.

2, 20–22], but no information on the FAE of gerbils has been available. The present study revealed significant difference in distribution of the PP and fine morphology of dome absorptive cells between Mongolian gerbils and mice, probably being related with the difference in length of the intestine between both species of animals. In the gerbil having a shorter gut the PP were rather evenly distributed through the whole small intestine, while the PP of murine species less numerous in the jejunum.

In the gerbil the dome absorptive cells showed a lower density of microvilli than those of villous absorptive cells. They showed characteristic morphology being very similar to that of immature M cells or poorly differentiated crypt cells, lacking developed cytoskeletons and regular polygonal arrangement. Immature murine M cells were shown to have a regularly arranged hexagonal structure on the luminal surface [10]. These findings suggested that the dome absorptive cells of gerbils might be poorly differentiated cells or intermediate forms between the villous absorptive cells and M cells. On the other hand, the transformation of differentiated

absorptive cells into M cells was not evidenced.

In this study lymphoid cells were seen to pass through a gap of the basal lamina underlying FAE and to migrate to the intestinal lumen, suggesting that they might play important roles in the differentiation of M cells. The precursors of all FAE cells including M cells, might be present within the follicle-associated crypt, suggesting that the interaction between enterocytes and lymphoid cells might trigger the differentiation of M cells at the follicle epithelium [15, 19, 20–22].

M cells are known to be active for taking macromolecules or microorganisms [3, 6, 17, 18] and to transfer antigenic information to adjacent macrophages and lymphoid cells through an intracellular tubulovesicular system [3, 14, 16, 23]. In cases of enteric infection with Tyzzer's organisms [7, 25] and other bacteria [26, 27], to which the gerbil was shown to be highly susceptible, M cells might be of importance in interaction between microbes and the host. On the other hand, the immune system of the gerbil was described to be less active than that of the guinea pig and hamster [26, 27]. The difference in susceptibility to enteric infections between gerbils and mice might be possibly related with difference in morphology and function of FAE within the PP.

Smith and Peacock [22] stated that M cells within the murine PP were increased in number when specific pathogen-free (SPF) animals were transferred to CV environment. Sicinski *et al.* [20] reported that the microvilli of PP dome absorptive cells were longer in SPF mice than in conventional ones. In the present study gerbils as well as mice were kept in a CV environment, and they were found to have dome absorptive cells with a few microvilli being shorter than those of villous absorptive cells. The control of microbial environment is needed for further comparative studies between two species of animals. Although the present observations were of a very limited range, there seemed to be difference in the number and length of microvilli between the absorptive cells of gerbils and those of mice. Such interspecies difference will be studied further in details.

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