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A Morphological Study of "Microfold" Cells of Lymphoid Aggregative Tissue in the Small Intestine of the Chicken

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ABSTRACT. The morphology of lymphoid aggregative tissue of the small intestine was studied in the Japanese bantam chicken. Lymphoid aggregative tissue were composed of leaf-like villi unlike those in mammals. In the present study, these lymphoid aggregative tissues histologically were identified as Peyer's patches. Light and electron microscopy of the covering epithelium of the Peyer's patches showed that the cells had morphologically the same features of "Microfold" ("M") cells previously described in mammalian species. The authors demonstrated that the "M" cells of birds were not limited to the bursa of Fabricius, but also existed in the covering epithelium of the Peyer's patches in the small intestine. The proportion of "M" cells enfolding lymphoid cells much in mature chickens (18%) than in mature rats (100%).

Key words : Microfold cell (M cell) — Peyer's patch — Chicken

In 1971, Bockman and Cooper[1] gave the term "Follicle-associated epithelium (FAE)" to the epithelium covering follicles of rabbit appendix and chick bursa. In 1974, Owen and Jones[2] found that specialized cells exist in the FAE of human Peyer's patches. These epithelial cells, which cover lymphoid follicles in the Peyer's patches, have been variously termed "Microfold cells",[2] due to their characteristic luminal surface microfolds, "Membranous cells",[3] due to the formation of a membrane separating lymphoid cells from the lumen and, more recently, simply "M" cells. These M cells have since been also identified in mammalian tonsil, appendix and solitary lymph nodes.[4] It has been found that they have the function of transporting various macromolecules[3–9] and microorganisms[10–14] from intestinal lumen into lymphoid follicles. Recently, these cells, which are important in the "first line of mucosal defense",[15] have attracted much attention.

Most of the research of M cells has centered around mammals, and there are almost no detailed reports concerning birds. Therefore, chicken ileum was investigated morphologically in this study.

MATERIALS AND METHODS

Twelve 4 to 12-month-old Japanese bantam chickens employed in these experiments were fasted, but were allowed water for 48 hours prior to sacrifice

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under ether anesthesia. Tissues of the small intestine were removed and washed with a jet of cold saline from a injector to remove surface mucus. After washing, tissues were immediately fixed 2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.4. Observing the mucosa under a dissecting microscope (WILD M-400), we divided the area of leaf-like villi that differed from surrounding mucosa into 1-mm cube specimens. The specimens were fixed for 2 hours in 1% osmium tetroxide in 0.1M phosphate buffer, pH 7.3, after which they were dehydrated with graded ethanol series. Samples for scanning electron microscopy were placed in iso-amyl acetate, critical point dried, coated with gold-palladium by evaporation, and observed under a HITACHI HHS-2R scanning electron microscope. For transmission electron microscopy, samples were placed in propylene oxide, and embedded in epoxy resin. Ultrathin sections were prepared with a glass knife using a Porter-Blum MT2-B type microtome. After double staining with uranium acetate and lead citrate, they were observed with a HITACHI H-500 transmission electron microscope. Thin sections for light microscopic examination were stained with hematoxylin and eosin.

RESULTS

1) Dissecting microscopy and light microscopy

Careful observation with the dissecting microscope revealed 1 to 3 areas with abundant capillaries and leaf-like villi distinctly different from the finger-like villi of surrounding mucosa at 5 to 10 cm from the ileocecal junction on

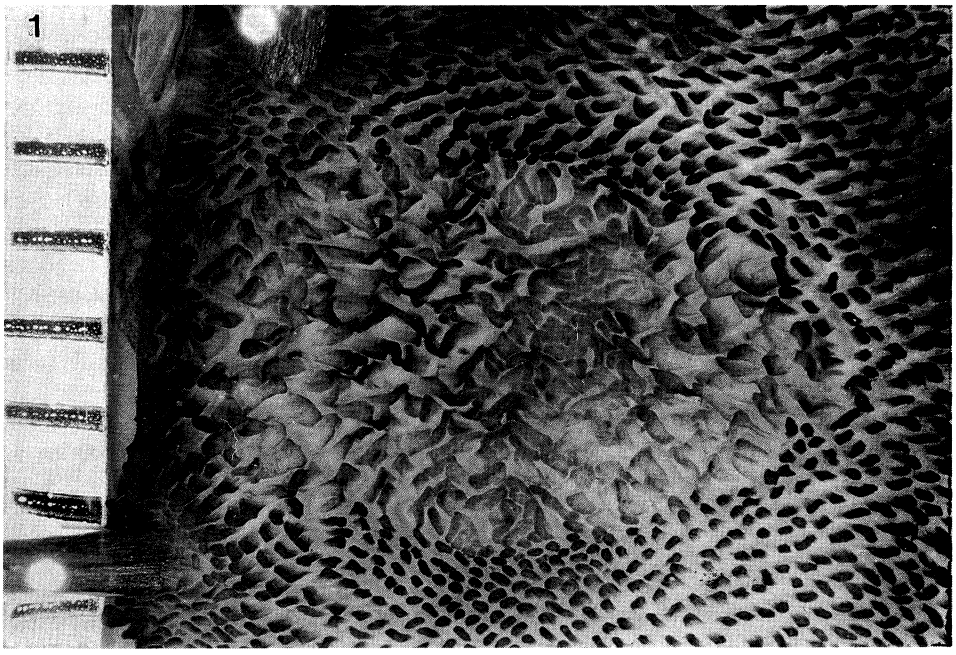

Fig. 1. A dissecting micrograph showing a Peyer's patch in the chicken. Note the broad, leaf-like villi of the patch, as compared with the smaller, finger-like villi laterally.

the antimesenteric wall of the small intestine (Fig. 1). Light microscopy revealed these areas to be composed of many lymphoid follicles in lamina propria and submucosal tissue (Fig. 2a) and it was noted that they displayed the histological feature of Peyer's patches that had previously been reported in mammals.[16,17] Parts of the epithelium covering the leaf-like villi had characteristics of a lymphoepithelium, particularly at the base of the villi where goblet cells were few. On enlarged observation of the lymphoepithelium, it was noted that the cells had short brush borders and were associated with many mononuclear cells (Fig. 2b).

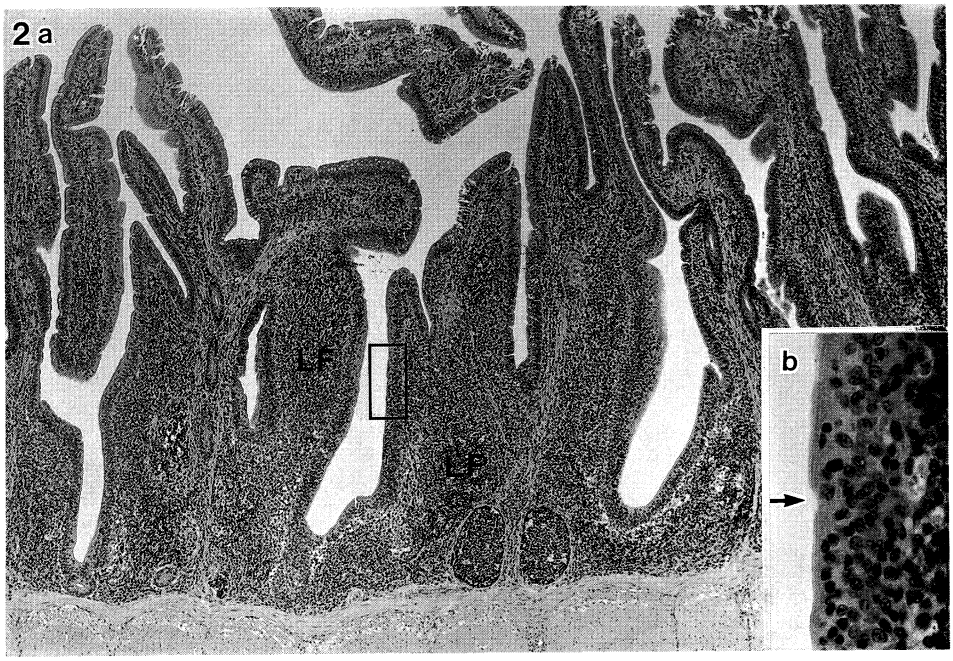

Fig. 2 a. A light micrograph of a section of a Peyer's patch containing lymphoid follicles. (Hematoxylin and eosin, original ×75) Inset: b. At a higher magnification of the lymphoepithelial area, the cell with a shortened brush border and associated lymphoid cells is suspected to be an "M" cell. (Hematoxylin and eosin, original ×470) LF; lymphoid follicle

2) Electron microscopy

Scanning electron microscopy of the surface of the leaf-like villi of the so-called Peyer's patches revealed on the surface to be indented and with coarse microvilli (Fig. 3). These were clearly different from surrounding cells ultrastructurally. The villi of the small intestine outside of the Peyer's patches had no such cells, on transmission electron microscopy of the Peyer's patch, cells with short, coarse microvilli associating with lymphoid cells were observed (Fig. 4). The proportion of these cells associating with lymphoid cells was 18% (6/34). The surface coat of the microvilli of these cells was thinner than that of adjacent columnar cells (Fig. 5). In their cytoplasm were numerous small vesicles. The microvilli had central actin filaments which entered the cytoplasm at the apex, but the terminal web was not as well developed as

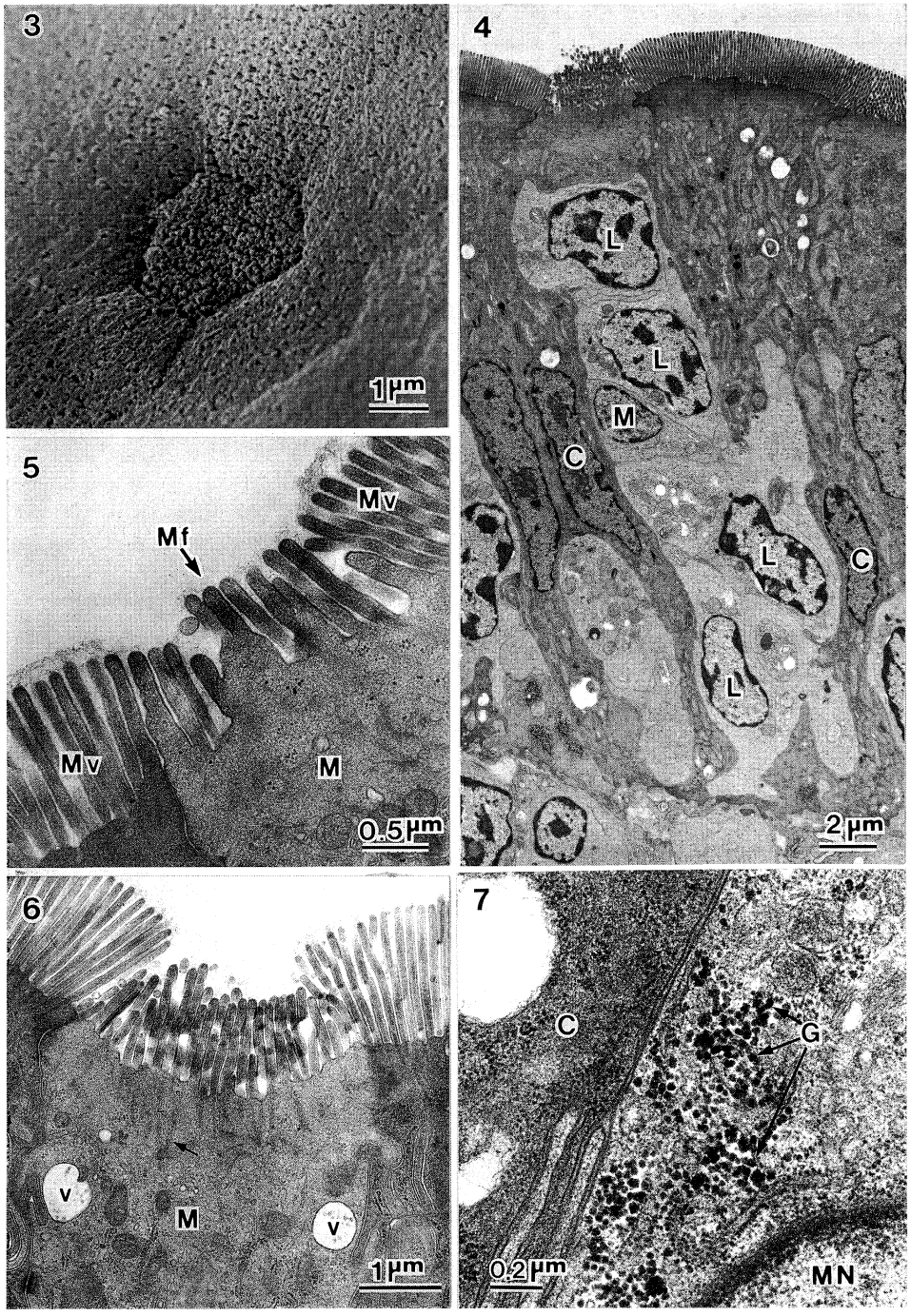

Fig. 3. A scanning electron micrograph of the surface of the leaf-like villi. Note that the surface of the leaf-like villi. Note that the surface of the M cell is indented and that its microvilli are coarse. ×8,000

Fig. 4. A low magnification transmission electron micrograph showing an "M" cell associated with lymphoid cells. ×2,500, M; M cell, C; columnar cell, L; lymphoid cell

Fig. 5. The surface coat of an "M" cell is thinner than that of adjacent columnar cells. ×20,000, Mf; microfolds, Mv; microvilli
Fig. 6. The apical cytoplasm of an "M" cell. ×35,000, G; glycogen particles, MN; M cell nucleus

that of neighboring columnar cells (Fig. 6), and many glycogen particles were present (Fig. 7).

The present study has confirmed that in the chicken these cells have the same characteristics of mammalian M cells[2,14,18,22] ultrastructurally.

DISCUSSION

Once Peyer[19] stated that he had found the intestinal glands in animals of lower orders, in door mice, moles, hedge-hogs and birds with the naked eye. Then, Bruecke[19] supposed, with the aid of injections of colored liquids, that "the patches of Peyer" were related to the intestinal lymphatic vessels and might be themselves lymphatic glands. At present, the Peyer's patch is morphologically defined as an aggregation of more than five lymphoid follicles[16,17] and it has been recognized that they play important roles with regards to gut immunity.

In 1968, Fichterius *et al.*[20] reported that Peyer's patches did not exist in animals of a lower order than mammals. In 1980 McGarry *et al.*[21] observed annular bands of lymphoid tissue in the intestine of the Mallard Duck Anas platyrhychos. The results of the present study, however, showed that Peyer's patches also exit in the chicken. Electron microscopic observation showed that M cells also exist in the covering epithelium of Peyer's patches of the chicken ileum and other mammals.[2,14,18,22]

An ontogenetic study[22] of rat M cells has shown the proportion of M cells associating with lymphoid cells to be 0% (0/17) ten days after birth 31% (5/16) 20 days after birth and 100% (17/17) one year after birth, indicating that the proportion increases as the rat matures. Among 34 M cells of mature chickens viewed in the present study, only 6 (18%) were associated with lymphoid cells. In a study of the relation between M cells and intraepithelial lymphocytes in mammalian Peyer's patches, Shimazui[23] demonstrated two types ; an intracellular type, the so-called enclosing type and an intercellular type. In the M cells of chicken Peyer's patches, they may be of the intercellular type. Cells similar to mammalian M cells have been recognized in the avin bursa of Fabricius, but there have been no detailed reports of such cells enclosing lymphoid cells. However, specialized epithelial cells in the follicle-associated epithelium of the bursa[24] are known to transport antigens via the intercellular spaces of the epithelium to lymphoid cells.

The authors demonstrated morphologically that the "M" cells of birds were not limited to the bursa of Fabricius, but also existed in the covering epithelium of the Peyer's patches in the small intestine.

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