

Ultrastructural Study on the Differentiation and the Fate of M cells in Follicle-Associated Epithelium of Rat Peyer's Patch

Sachiko ONISHI¹⁾, Toshifumi YOKOYAMA²⁾, Keigi CHIN¹⁾, Midori YUJI¹⁾, Tetsuro INAMOTO¹⁾, Wang-Mei QI¹⁾, Katsuhiko WARITA¹⁾, Nobuhiko HOSHI¹⁾ and Hiroshi KITAGAWA^{1)*}

¹⁾Department of Bioresource and Agrobiosciences, Graduate School of Science and Technology, Kobe University, Kobe 657-8501 and

²⁾Department of Anatomy and Neurobiology, Faculty of Medicine, Kagawa University, Kagawa 761-0793, Japan

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ABSTRACT. The differentiation process of immature microvillous epithelial cells to M cells and the fate of M cells in the follicle-associated epithelium (FAE) of the mucosa-associated lymphoid tissues are still unclear. In this study, the differentiation process and the fate of M cells were clarified in rat Peyer's patches under a transmission electron microscope. Almost all immature epithelial cells were found to possess long, slender microvilli, which gradually shortened, thickened and dispersed as the immature epithelial cells migrated away from the crypt orifices. These morphological changes started in the centers and moved to the peripheries of the apical surfaces of epithelial cells, accompanied by the protrusion of apical cytoplasm out of the terminal web. During these changes, the bundles of microfilaments of microvilli never shortened, and both small vesicles in the apical cytoplasm and tiny invaginations of the apical membranes were found. The intraepithelial migrating cells gradually accumulated to form typical intraepithelial pockets. In all FAE, there was no morphological sign of cell death in M cells. The rearrangement of microfilament bundles, the reconstruction of microvilli and the disappearance of pockets resulted in the transformation of M cells into microvillous epithelial cells. These serial ultrastructural changes suggest that M cells are a temporal and transitional cell type caused by the active engulfment of luminal substances and that when the engulfment ceases, the M cells transform into mature microvillous epithelial cells.

KEY WORDS: cellular differentiation, M cell, Peyer's patch, rat, ultrastructure.

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Owen and Jones [21] first designated as "M cells" the special epithelial cells that enfold intraepithelial migrating cells in the follicle-associated epithelium (FAE) of human Peyer's patches. A peculiar function of M cells is the transportation of antigenic substances from the lumen to the subepithelial immunocompetent cells, thereby initiating mucosal immune responses or oral immunological tolerance [10]. Detailed ultrastructural characteristics of M cells have been determined in various mammalian and avian species such as mice, humans, rabbits and chickens [10, 14, 16, 29]. However, the ultrastructural characteristics of typical M cells have not been established in the mucosa-associated lymphatic tissues of rats.

The epithelial cells in FAE are generally derived from the neighboring intestinal crypts [1, 2, 8]. In the epithelial migration, the epithelial cells are induced apoptosis and are finally exfoliated from the most luminal portion of the FAE in chicken cecal tonsils [15, 27] and mouse Peyer's patches [24]. Most M cells are constantly distributed in the basal to middle regions rather than apical regions of the FAE in the Peyer's patches of rats [3], rabbits [13], mice [4, 24], and humans [5], the cecal patches of rabbits [6] and the cecal tonsils of chickens [15]. In addition, no cell death is detected by light and scanning electron microscopy at the demarcation around the clusters of M cells in chicken cecal tonsils [15, 28]. Detailed three-dimensional observation demonstrates the redifferentiation of M cells into

microvillous columnar epithelial cells in chicken cecal tonsils [15]. In mice and pigs, the redifferentiation of M cells to microvillous epithelial cells has been also proposed [18, 24]. However, the fate of M cells has not yet been clarified ultrastructurally.

There are two hypotheses on the cellular origin of M cells. One is that M cells differentiate from mature epithelial cells [1, 11, 22, 25, 26], and the other is that M cells directly differentiate from immature epithelial cells [2, 4, 5, 8, 12, 13, 27]. The latter hypothesis has thus far predominated in the literature and is clearly demonstrated by detailed three-dimensional observation in chicken cecal tonsils [15]. In all animal species, however, details of the differentiation process of epithelial cells to M cells in the FAE of the mucosa-associated lymphoid tissues remain unclear.

In this study, the FAEs in rat Peyer's patches were observed under transmission electron microscope, and the typical morphological features of M cells were clarified. Because of one-way migration of epithelial cells in the FAE, the predominant existence of transitional epithelial cells to M cells was expected at the lower region from the accumulation of M cells, whereas the predominant one from M cells to microvillous epithelial cells was expected to occur at the upper region from the accumulation of M cells in the FAE. In consideration of this assumption, we observed the detailed ultrastructural changes of epithelial cells to clarify the differentiation process from immature microvillous epithelial cells to M cells and the fate of M cells.

* CORRESPONDENCE TO: KITAGAWA, H., Graduate School of Science and Technology, Kobe University, Kobe 657-8501, Japan.

MATERIALS AND METHODS

Animals: Six male Wistar rats aged 7 weeks (Japan SLC Inc., Japan) were used according to the guidelines for the care and use of experimental animals at Rokkodai Campus of Kobe University. The animals were maintained under conventional laboratory housing conditions. The animals had free access to food (Lab MR Stock; Nosan Corp., Japan) and water. The animal facility was maintained under conditions of a 12 hr light/dark cycle at $21 \pm 1^\circ\text{C}$ and 50–60% humidity. Clinical and pathological examinations of all animals revealed no sign of disorder.

Tissue preparation: Animals were intravascularly perfused with 2.5% glutaraldehyde –2.0% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) after euthanasia of diethyl ether inhalation. Small tissue blocks of the Peyer's patches were extracted and immersion-fixed in the same fixative for 24 hr at 4°C . After postfixation with 1.0% OsO_4 in 0.1 M PB for 2.0 hr at room temperature, the small specimens were dehydrated and embedded in a Quetol 812-mixture.

Transmission electron microscopy: Ultrathin sections were cut using an ultramicrotome (Sorvall MT-1, U.S.A.). Sections stained with both uranyl acetate and lead citrate were observed under a transmission electron microscope (Hitachi H-7100, Japan) at an accelerating voltage of 75 kV.

Quantitative histology: Epithelial cells except for goblet cells and endocrine cells were classified into 5 types as described in results. The diameters of apical surfaces in each cell type were divided into 100% on the electron micrographs. In the central 50% areas and the peripheral 15% areas, the longitudinal diameter and the transversal diameter were measured on electron micrographs of 10 genuinely transversely or longitudinally sectioned microvilli that had been randomly selected. The distribution density of microvilli was measured in $1 \mu\text{m}$ of each area on 5 epithelial cells of each cell type. Data were presented as mean number \pm standard deviation. One way ANOVA was employed with Tukey-Kramer multiple comparison test in statistical analysis. *P* values of less than 0.05 were considered statistically significant.

RESULTS

Distribution of Epithelial cells in FAE: The FAE were predominantly composed of microvillous columnar epithelial cells, a few M cells and goblet cells, and rare gastrointestinal endocrine cells. M cells were preferentially accumulated at the lower half of the FAE, and never located at the top. Accumulating at the top of the FAE were epithelial cells with typical characteristics of late apoptotic stage, characterized by the shrinkage of both cytoplasm and nucleus, the latter of which had chromatin condensed beneath the nucleolemma. The epithelial cells in the FAE except for goblet cells and endocrine cells were classified into 5 types: typical M cells, immature microvillous epithelial cells, transitional epithelial cells from immature

microvillous epithelial cells to M cells, transitional epithelial cells from M cells to mature microvillous epithelial cells, and mature microvillous epithelial cells (Fig. 1).

Typical M cells: Typical M cells were characterized by the lack of almost all microvilli and marked intraepithelial pockets occupied by migrating cells such as lymphocytes and macrophage-like cells (Fig. 1c). When a few microvilli remained, the microvilli were dispersed on the apical surfaces and were slightly shorter in the central surface areas than in the peripheral surface areas (Fig. 2a, c). Terminal webs were lacking in the central surface areas rather than in the periphery. In the microvilli-lacking areas, however, bundles of microvillous microfilaments occasionally remained in the apical cytoplasm (Fig. 3d).

The flattened apical cytoplasm of M cells were supported by slender pillars which reached the basal lamina. The oval nucleus located at the mid-basal cytoplasm and possessing one or two nucleoli were slightly bigger than those of the microvillous epithelial cell. The nuclei and cytoplasm of typical M cells also tended to be slightly clearer than those of the ordinary microvillous epithelial cells in FAE.

The cytoplasm between the apical membranes and the terminal webs in almost all M cells protruded slightly beyond those of the neighboring microvillous epithelial cells (Fig. 3d). A few vesicles or occasional tubules of various diameters were dispersed in the apical cytoplasm beneath the apical membranes of M cells (Fig. 3d). Thick cytoplasmic processes containing numerous small vesicles and tubules were rarely found at the apical surfaces of M cells. Distribution of other cytoplasmic organelles in M cells was similar to those in the microvillous epithelial cells.

Immature microvillous epithelial cells: Morphological characteristics of immature microvillous columnar epithelial cells around the crypt orifices were very similar to those of mature ones at the apical region of the FAE (Fig. 1a). Namely, the epithelial cells possessed elliptical nuclei that were located in the mid-basal cytoplasm and contained less heterochromatin. The microvilli on the apical surface were uniformly slender and long (Fig. 2a, b). The bundles of microvillous microfilaments elongated to the terminal web. The length between the base of microvilli and the terminal web (LBT) was short.

Transitional epithelial cells from immature microvillous epithelial cells to M cells: In the base of the FAE, microvillous epithelial cells with shorter microvilli than those of immature microvillous epithelial cells were predominant. In the transitional epithelial cells with slightly shorter microvilli, the LBTs were slightly longer. The lengths of microvilli were generally in inverse proportion to the LBTs, whereas the lengths between the top of microvilli and the terminal web were almost constant (Fig. 4). The apical cytoplasm outside the terminal web was slightly low in electron density and protruded into the lumen (Fig. 1b). In general, the distribution densities of microvilli were low and their diameters were large in the epithelial cells with

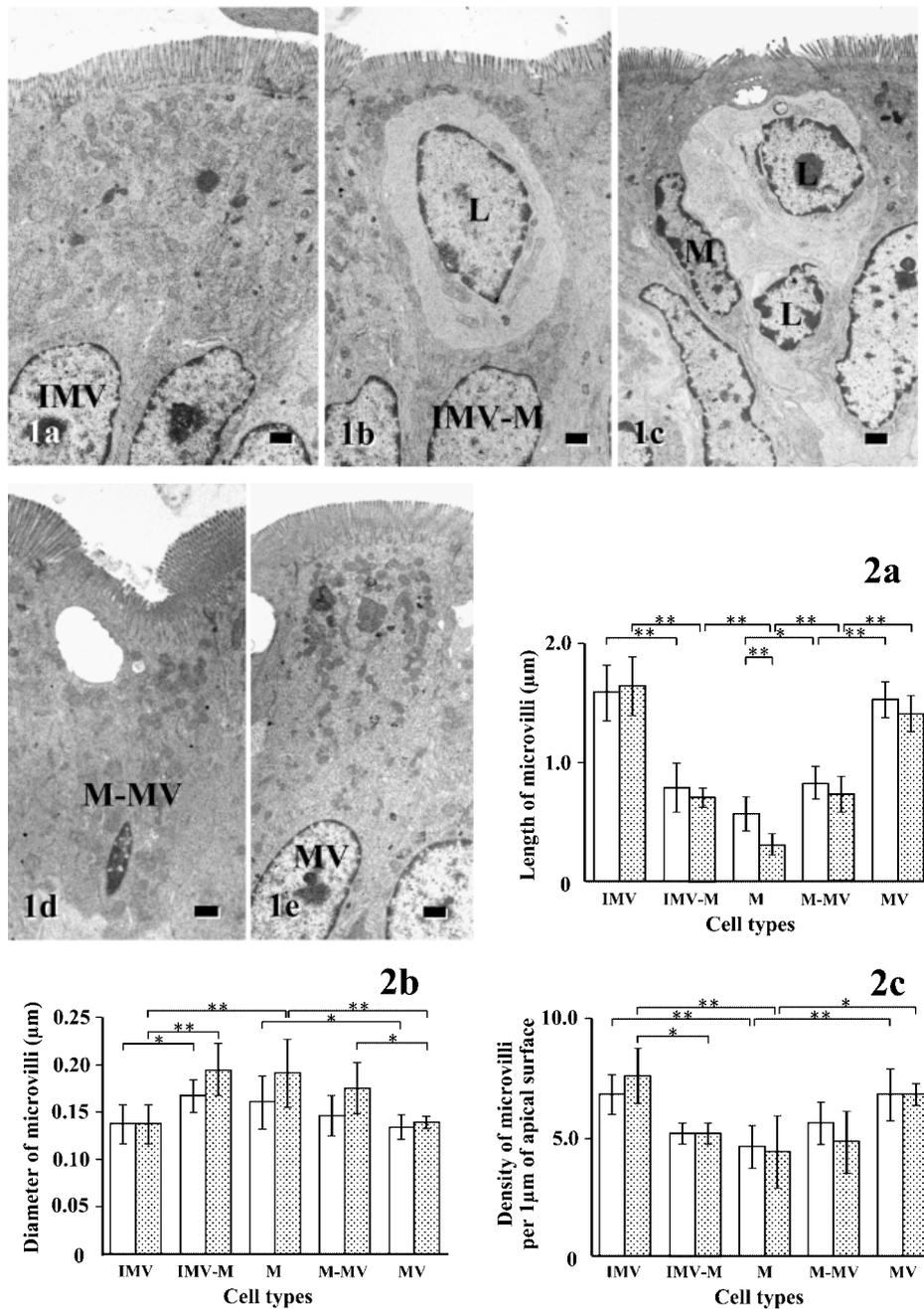


Fig. 1. Low-magnification electron micrographs of various types of epithelial cells in the FAE of a rat Peyer's patch. a) Immature microvillous epithelial cell (IMV) with long, slender microvilli. b) Transitional epithelial cell from immature microvillous epithelial cell to M cell (IMV-M). The microvilli are shorter than those of the immature microvillous epithelial cell in a). A lymphocyte (L) is visible in the apical cytoplasm of IMV-M. c) A typical M cell (M) with few microvilli, which are short and sparse on the apical surface. The apical cytoplasm protrudes slightly into the lumen and forms an intraepithelial pocket enfolding lymphocytes (L). d) Transitional epithelial cell from M cell to mature microvillous epithelial cell (M-MV). The microvilli are irregularly formed on the apical surface, which is slightly invaginated compared with those of neighboring epithelial cells. e) Mature microvillous epithelial cell (MV). The MV possesses regular and closely-packed microvilli. Bar = 1 μ m.

Fig. 2. The length (a), diameter (b) and density (c) of the microvilli in various types of epithelial cells in the FAEs of rat Peyer's patches. Open columns represent microvilli at the peripheral 15% areas of apical surfaces and dotted columns the central 50% areas. *, $P < 0.05$; **, $P < 0.01$; IMV, immature microvillous epithelial cell; IMV-M, transitional epithelial cell from immature microvillous epithelial cell to M cell; M, typical M cell; M-MV, transitional epithelial cell from M cell to mature microvillous epithelial cell; MV, mature microvillous epithelial cell.

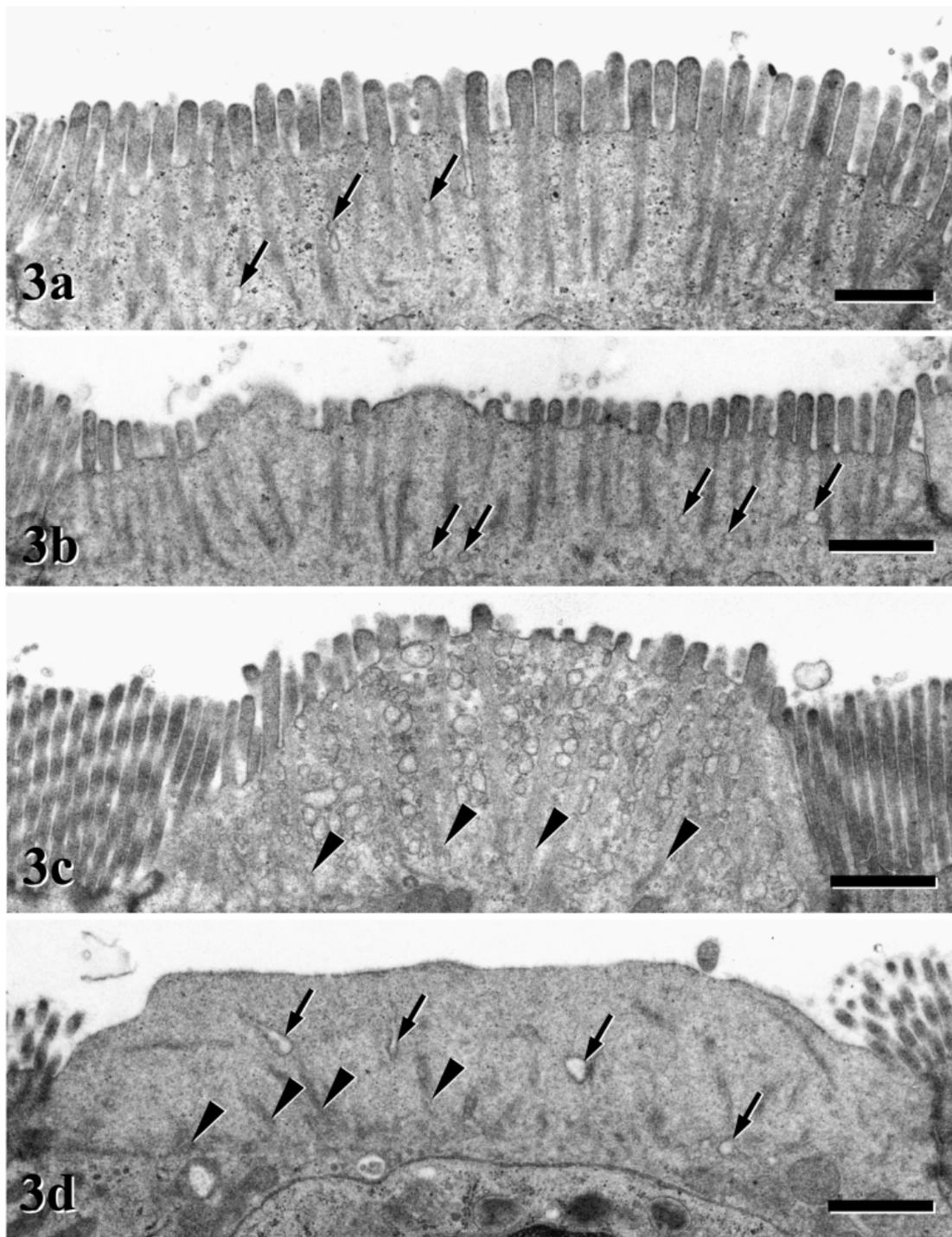


Fig. 3. High-magnification electron micrographs of changes in the apical cytoplasm of transitional epithelial cells (a-c) from an immature microvillous epithelial cell to an M cell (d) in the FAE of rat Peyer's patch. In c, microvilli are short and thick compared with those of neighboring ordinary microvillous epithelial cells. The microvilli shorten and disperse gradually in the process from a) to c). The microvilli are shorter in the central apical surfaces than in the peripheral ones (a-c). In b), the microfilament bundle lengths are almost constant in spite of different lengths of microvilli. A few small vesicles (arrows) are visible in a, b), whereas numerous small vesicles are seen in c). Slightly large vesicles or tubules (arrows in d) and the remnants of microfilament bundles (arrowheads in c and d) are seen in the apical cytoplasm. Bar = 1 μ m.

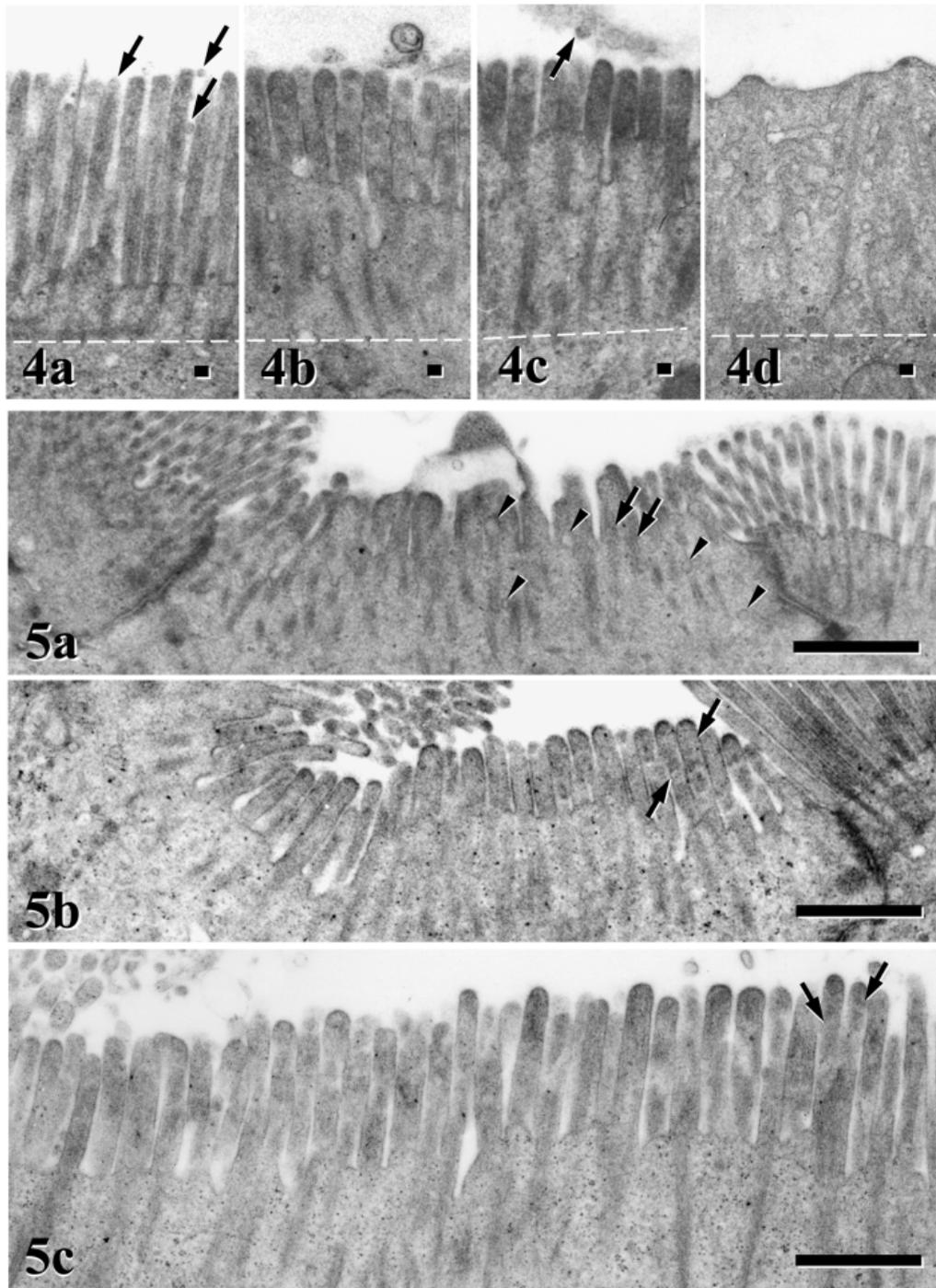


Fig. 4. High-magnification electron micrographs of changes in the microvilli of transitional epithelial cells (a-c) from an immature microvillous epithelial cell to an M cell (d) in the FAE of rat Peyer's patch. The lengths of microvilli are in inverse proportion to the depth from the base of microvilli to the terminal web (broken lines), whereas the lengths between the top of microvilli and the terminal web are almost constant. Small fragments of microvilli (arrows) are visible in a, c). Bar = 0.1 μm .

Fig. 5. High-magnification electron micrographs of changes in the apical cytoplasm in transitional epithelial cells from M cells to mature microvillous epithelial cells. a) In the early transitional cell out of the M cell, the apical surface is caved in irregularly into various-sized clumps, which contain two bundles of microvillous microfilaments (arrows). Some small vesicles (arrowheads) are visible in the apical cytoplasm. b) Thick microvilli are seen in the central portion of the apical surface. The apical surface level of this epithelial cell is apparently low compared with those of neighboring epithelial cells. c) Short microvilli are irregularly formed in the apical surface. Clumps that are further divided into two microvilli are visible (arrows) in b, c). Bar = 1 μm .

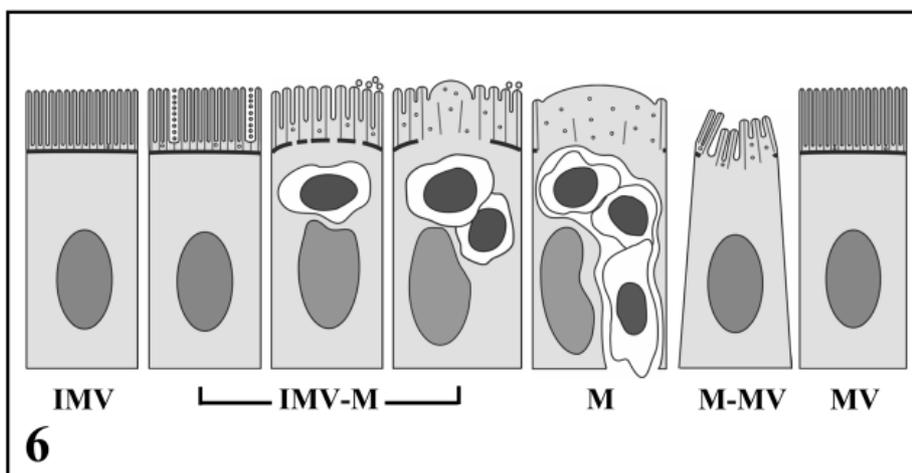


Fig. 6. A schematic diagram of transition processes both from an immature microvillous epithelial cell to an M cell and from an M cell to a mature microvillous epithelial cell. IMV, immature microvillous epithelial cell; IMV-M, transitional epithelial cell from immature microvillous epithelial cell to M cell; M, typical M cell; M-MV, transitional epithelial cell from M cell to mature microvillous epithelial cell; MV, mature microvillous epithelial cell.

short microvilli (Fig. 3a-c). These morphological changes of microvilli appeared markedly in the central portions of the apical surfaces, whereas the changes were minor in the peripheral portions (Fig. 3a-c). The terminal webs were more markedly diffused and disappeared in the central portions of the luminal surfaces than in the peripheral portions. The microvillous fragmentation was occasionally found (Fig. 4a-c). As the shortening of microvilli progressed, the number of intraepithelial migrating cells increased.

Small vesicles were found around the root of microvilli in the epithelial cells with morphological changes in microvilli (Fig. 3a-c). As the epithelial cells changed into M cells, the numbers of small vesicles in the apical cytoplasm increased. Invaginations of apical membranes were also found around the root of the microvilli. No other organelles were found in the apical cytoplasm outside the terminal webs.

Transitional epithelial cells from M cells to mature microvillous epithelial cells: The columnar epithelial cells possessing short microvilli of various lengths were located in the middle to apical FAE exclusively. In these epithelial cells, bundles of microvillous microfilaments with uniform length were constantly visible in the apical cytoplasm. Many small vesicles were occasionally and locally accumulated in the space between the bundles of microfilaments. Invaginations of the apical membranes were also found. The superficial cytoplasm was irregularly divided into various-sized clumps by various depths of invagination. The successive invaginations finally created irregular microvilli, such as thick or forked microvilli of various lengths (Fig. 5). In the epithelial cells whose microvilli were reorganized, terminal webs also appeared in the apical cytoplasm, although the LBTs were greater than those in mature microvillous epithelial cells. At the initial

stage of the above described morphological changes of microvilli, the intraepithelial migrating cells decreased suddenly, and consequently the intraepithelial pockets disappeared. These epithelial cells were slightly invaginated from the apical surface levels of neighboring epithelial cells because of the short cell bodies (Fig. 1d).

Mature microvillous epithelial cells: Typical microvillous epithelial cells similar to those in the intestinal villi were predominantly distributed in the apical FAE. The long, slender microvilli were densely packed in the apical surfaces (Fig. 1e). The bundles of microfilaments from the microvilli connected with the marked terminal web in the apical cytoplasm, where small vesicles were rarely found.

DISCUSSION

The epithelial cells time-dependently migrate from the crypts to the apices along the dome in chicken cecal tonsils [27]. In Peyer's patches from mice at 12 hr after ^3H -thymidine administration, the labeled epithelial cells appear at the base of the FAE and by 48 hr reach the apex of the FAE [1]. Epithelial cells with typical morphological characteristics of apoptosis are exclusively located at the apical FAE in chicken cecal tonsil and murine Peyer's patch [15, 24, 28]. Thus, the migration of epithelial cells is one-way from the crypt to the apex of the FAE.

M cells are most abundantly distributed at the upper portions of the FAE in mouse Peyer's patches [25]. No labeled M-cells appear in the FAE until 3 days after administration of ^3H -thymidine in mouse Peyer's patches [1]. From these findings, the differentiation of M cells from mature epithelial cells in the FAE has been proposed [1, 11, 22, 25, 26]. In rat Peyer's patches, however, M cells are predominantly located in the lower half of the FAE and

decrease in number toward its apex [3]. In human Peyer's patch, M cells also distribute near the intestinal crypts encircling the dome rather than on the upper part of the FAE [5]. Immature-appearing M cells are labeled at 24 hr after injection of ^3H -thymidine just as the labeled absorptive cells appear in the FAE [2]. From these observations, the hypothesis of the direct derivation of M cells from undifferentiated crypt epithelial cells has predominated [2, 4, 5, 9, 12, 13, 15, 27]. In the present study, M cells with short microvilli were predominantly distributed in the lower region of the FAE. Moreover, the serial differentiation process from immature epithelial cells to M cells was clarified under a transmission electron microscope. Therefore, our observations strongly support the hypothesis that M cells differentiate from immature epithelial cells.

During the active pinocytosis and phagocytosis of luminal contents in M cells, the area of apical membranes is considered to be depleted by vesicle formation, resulting in attenuation of cytoplasmic projections in M cells [20]. In the present study, during the differentiation process from immature epithelial cells to M cells, the microvilli gradually reduced their lengths, but the microfilament bundles did not. During this process, invaginations of cell membranes and small vesicles were found around the roots of the microvilli. On the other hand, sporadic fragmentation of the microvilli was found in immature epithelial cells at differentiation to M cells. Therefore, we consider that the active pinocytosis induces the reduction of length of the microvilli, and that the fragmentation of microvilli results in the reduction of density of the microvilli in the immature epithelial cells in the FAE of rat Peyer's patches.

The transition processes of epithelial cells in the FAE that we observed are summarized in Fig. 6. During the transition from immature microvillous epithelial cells to M cells, the reduction of length of the microvilli proceeded almost evenly over the apical surfaces of immature microvillous epithelial cells, although the microvilli in the central portions were somewhat more rapidly shortened than those in the peripheral portions of the apical surfaces. In contrast, during the transition from M cells, the formation of microvilli proceeded irregularly over the apical cell membranes of M cells. This morphological difference in the apical surfaces might be a reliable identification datum of immature M cells or redifferentiating M cells. In the immature and the redifferentiating M cells, small vesicles were visible in the cytoplasm beneath the apical cell membranes. However, the uptake of luminal substances might be scarcely carried out during the redifferentiation process of M cells, because the epithelial pockets acutely disappeared at the initial stage of the redifferentiation process.

In rat Peyer's patches, apoptotic epithelial cells with chromatin condensation and cytoplasmic shrinkage are also frequently observed in the apical portions of the FAE [3]. Moreover, it has been proposed that M cells never die and redifferentiate into microvillous epithelial cells [15, 24, 28]. Therefore, the present successive morphological changes

from M cells to microvillous epithelial cells might demonstrate the hypothesis of the redifferentiation of M cells.

M cells have been characterized by the presence of microfolds, fewer microvilli, thin rims of cytoplasm, numerous small cytoplasmic vesicles, and intraepithelial pockets harboring lymphoid cells [10, 21]. However, M cells with several different morphological characteristics have been reported in various animal species. That is, in rat, mouse, and Guinea pig Peyer's patches, the M cells have short, stub-like microvilli [2, 17, 19, 23]. In rabbit cecal lymphoid patches, the M cells have longer and thicker microvilli than those of microvillous epithelial cells [6, 7]. In the present study, small vesicles were located in the cytoplasm beneath the apical cell membranes during the transition process from immature epithelial cells to M cells, suggesting the possibility of active transportation of luminal substances, as occurs in typical M cells. Further, the different morphologies of microvilli in M cells might be reflected by the functional differences in uptake of luminal antigens between immature M cells and typical M cells.

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