The Development of M Cells in Peyer's Patches Is Restricted to Specialized Dome-Associated Crypts

Andreas Gebert, Susanne Fassbender,
Kerstin Werner, and Annikka Weissferdt

From the Center of Anatomy, Medical School of Hannover,
Hannover, Germany

It is controversial whether the membranous (M) cells of the Peyer's patches represent a separate cell line or develop from enterocytes under the influence of lymphocytes on the domes. To answer this question, the crypts that produce the dome epithelial cells were studied and the distribution of M cells over the domes was determined in mice. The *Ulex europaeus* agglutinin was used to detect M cells in mouse Peyer's patches. Confocal microscopy with lectin-gold labeling on ultrathin sections, scanning electron microscopy, and laminin immuno-histochemistry were combined to characterize the cellular composition and the structure of the dome-associated crypts and the dome epithelium. In addition, the sites of lymphocyte invasion into the dome epithelium were studied after removal of the epithelium using scanning electron microscopy. The domes of Peyer's patches were supplied with epithelial cells that derived from two types of crypt: specialized dome-associated crypts and ordinary crypts differing not only in shape, size, and cellular composition but also in the presence of M cell precursors. When epithelial cells derived from ordinary crypts entered the domes, they formed converging radial strips devoid of M cells. In contrast to the M cells, the sites where lymphocytes invaded the dome epithelium were not arranged in radial strips, but randomly distributed over the domes. M cell development is restricted to specialized dome-associated crypts. Only dome epithelial cells that derive from these specialized crypts differentiate into M cells. It is concluded that M cells represent a separate cell line that is induced in the dome-associated crypts by still unknown, probably diffusible lymphoid factors. (Am J Pathol 1999, 154:1573-1582)

The epithelium that covers the domes of Peyer's patches differs from that of the villi in the paucity of goblet cells and in the presence of the membranous (M) cells. These highly specialized epithelial cells have a unique, important function: to transport antigenic and potentially harmful substances from the gut lumen across the epithelial barrier to cells of the lymphoid tissue and thereby to

initiate mucosal and systemic immune reactions.¹⁻³ The epithelial cells that cover the villi and likewise those of the domes derive from stem cells in the middle third of the crypts.⁴⁻⁷ However, it is controversial whether the M cells represent a separate cell line or develop from enterocytes on the dome under the influence of lymphocytes that lie in the dome epithelium. The latter hypothesis was initially postulated by Smith and Peacock^{8,9} and Bhalla and Owen⁵ but has repeatedly been questioned.¹⁰⁻¹² It has also recently been supported by findings of Kernéis et al¹³ in cell culture experiments, where direct cell-cell interaction of an epithelial cell line with Peyer's patch lymphocytes led to the formation of M-cell-like cells that resembled intestinal M cells in morphology and transport function. To clarify the developmental pathway of M cells *in vivo*, we investigated in detail the sites where the initial steps of M cell differentiation most likely take place: the crypts that produce epithelial cells for the domes. To study the origin, the formation, and the migration of M cells, a new approach combining different histochemical and ultrastructural techniques was used to follow the developing cells from the crypts to the dome.

This study was performed in the Peyer's patches of mice, because this animal model is well established and has previously been used in a multitude of studies concentrating on the ultrastructure,^{6,8} the histochemistry,¹⁴⁻¹⁷ the transport function,^{1,6,18} and the interaction of murine M cells with microorganisms.¹⁹⁻²² In addition, it is a great advantage that M cells of the murine Peyer's patches possess large amounts of α 1-2-linked fucose in the glycocalyx of their apical membrane¹⁵⁻¹⁷ and thereby can easily be identified by light microscopy using the *Ulex europaeus* agglutinin (UEA-I). Lectins are histochemical markers, which not only reliably discriminate between the cell types of the gut epithelium²³ but also represent remarkably sensitive tools to describe the differentiation pathway of the intestinal cells.²⁴ To follow the developmental pathway of the dome epithelial cells, we characterized, both ultrastructurally and histochemically, the crypts that produce them, investigated the distribution of M cells over the domes, and determined the sites of entry of lymphocytes into the dome epithelium.

The crypts at the base of murine Peyer's patch domes were investigated using a combination of optical sections

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Address reprint requests to Dr. A. Gebert, Abteilung Anatomie 2, Medizinische Hochschule Hannover, 30623 Hannover, Germany.

acquired by confocal laser scanning microscopy (CLSM) with histochemically labeled cryosections, semi-thin and ultrathin sections. The association of individual crypts with epithelial regions at the flanks of the domes was investigated by scanning electron microscopy (SEM) combined with low-power CLSM, a method that allows fluorescence microscopic views of whole domes to be generated with an extended depth of focus. To ascertain that the M cell marker UEA-I used in these fluorescence techniques detected all M cells of the Peyer's patches, lectin-gold labelings were performed on ultrathin sections that allow murine M cells to be identified by their typical ultrastructure and the extent of lectin binding to be correlated with the ultrastructural characteristics. In addition, the basement membrane component laminin was detected immunohistochemically to outline the crypts and to define their spatial positions. To determine the sites where lymphocytes enter the dome epithelium, we chemically removed the dome epithelium and revealed the distribution of holes in the basal membrane associated with the trespassing cells using SEM. The combination of the various techniques makes it possible to follow the differentiation pathway of intestinal M cells in the *in vivo* situation, to decide whether M cells develop as a separate cell line, and to locate the compartments of the gut epithelium where this differentiation takes place.

Materials and Methods

Animal Experiments

Female BALB/c mice kept under specific-pathogen-free conditions, 4 to 8 weeks old and weighing ~20 g, were anesthetized intraperitoneally with a mixture of Ketanest (WDT, Garbsen, Germany) and Rompun (Bayer, Leverkusen, Germany). The Peyer's patches of the small intestine were removed and rinsed in Ringer's solution at 4°C for a few seconds to remove feces and mucus. The specimens were cut into small pieces using a razor blade and fixed as described for the different methods (see below). The mice were killed by cervical dislocation.

Thin Section Electron Microscopy

Some of the Peyer's patches were fixed in a solution of 2% formaldehyde and 0.05% glutaraldehyde in phosphate buffer, pH 7.3, for 16 hours. After rinsing in phosphate-buffered saline (PBS) containing 1% L-lysine (Sigma, Deisenhofen, Germany) for 16 hours to block free aldehyde groups, single domes were microdissected, partially dehydrated in ethanol dilutions (30%, 50%, 70%) and transferred to LR White resin, medium grade (Plano, Marburg, Germany). The tissue blocks were enclosed in gelatin capsules and allowed to polymerize at 50°C for 48 hours. Semi-thin sections, 0.1 to 1.0 μm in thickness, were cut with glass knives and stained with toluidine blue. Ultrathin sections, 60 to 80 nm thick, were cut with a diamond knife and mounted on nickel grids.

The lectin-gold labeling procedure was performed with PBS, containing 0.15% bovine serum albumin-c (BSA-c,

Biotrend, Cologne, Germany) and 0.1% sodium azide. Free aldehyde groups were blocked in a drop of PBS containing 0.7% L-lysine for 15 minutes. After rinsing in PBS containing 5% bovine serum albumin (Serva, Heidelberg, Germany), 0.1% cold water fish skin gelatin (Biotrend), and 1% normal goat serum (Sigma), the sections were incubated with a solution containing UEA-I-biotin (12.5 $\mu\text{g}/\text{ml}$; Sigma) and 0.05% Tween 20 (Serva) at 4°C overnight. After rinsing, the grids were incubated for 4 hours with a goat anti-biotin antibody, conjugated to 20-nm colloidal gold (BioCell, Cardiff, UK). Finally, the grids were treated with 2% glutaraldehyde in PBS, washed in distilled water, and stained with uranium acetate and lead citrate. The sections were observed in a Zeiss EM10 electron microscope (Zeiss, Oberkochen, Germany). Controls were carried out either by omitting the lectin or by preincubating the lectin with 0.03 mol/L L-fucose overnight. In both controls, only a negligible uniform background staining was found over all tissue components and over the pure resin, probably resulting from some nonspecific binding of the gold-labeled secondary antibody.

Immunohistochemistry for Laminin

Eight freshly excised Peyer's patches taken from four mice were frozen and stored in liquid nitrogen. Cryosections running parallel to the mucosal surface, 4 μm in thickness, were fixed in a mixture of methanol and acetone for 10 minutes at -20°C and transferred to PBS. The sections were incubated with a polyclonal anti-laminin antiserum (Sigma) derived from rabbit for 1 hour. After rinsing in PBS, the binding sites were detected using the avidin-biotin complex method and 4-chloro-1-naphthol as substrate or an anti-rabbit antibody conjugated to FITC. Controls were performed by replacing the primary antibody with buffer and resulted in unstained sections, except for some granulated cells in the lamina propria.

Scanning Electron Microscopy

Twenty-two domes taken from the Peyer's patches of four mice were microdissected as described for confocal microscopy (see below) and fixed as described for thin-section electron microscopy. The tissue samples were rinsed in PBS and dehydrated in a series of acetone dilutions. Critical-point drying was performed using a Balzers CPD 030 (Balzers, Liechtenstein) and sputter-coating using a Polaron E 5400 (Polaron, Watford, UK) and a gold-palladium target. The specimens were examined in a PSEM 500 scanning electron microscope (Philips, Eindhoven, The Netherlands) equipped with a PC-based device for recording and processing digital images at high resolution.²⁵

To investigate the basal membrane of the dome epithelium and the distribution of trespassing lymphocytes, the dome epithelium was removed according to the method described by Low and McClugage²⁶ and McClugage et al.²⁷ Twenty-two unfixed Peyer's patches taken from nine mice were incubated with 1% boric acid for 24

hours, thoroughly rinsed in PBS, dehydrated in acetone, and prepared and examined as described for conventional SEM. The positions of holes in the basal membrane were marked with colored dots on digital images of complete domes using Photoshop software (version 4.0, Adobe, Edinburgh, UK). Longer incubation with boric acid (eg, for 48 hours) enlarged neither the number nor the diameter of holes.

Confocal Microscopy

For CLSM, the Peyer's patches of eight mice were fixed in pure ethanol for 16 hours, rehydrated in an ethanol series, and transferred to PBS. Single domes were microdissected under a binocular dissecting microscope so that the complete dome surface could be examined without overlying villi. Lectin labeling was performed with *Ulex europaeus* agglutinin conjugated with tetramethylrhodamine (UEA-I-TRITC, Sigma) at concentrations of 50 or 12.5 $\mu\text{g/ml}$ for 2 hours. After rinsing in PBS, the samples were stored and examined in a Tris-buffered solution containing 30% glycerol and 0.1% NaN_3 . CLSM was performed with a BioRad 600 confocal laser scanner (Biorad, Munich, Germany) attached to an Axiovert 35M inverted microscope (Zeiss) and equipped with a krypton-argon mixed gas laser. Serial optical sections of the lectin-labeled domes were recorded using COMOS software (version 6.0, Biorad) and the 568-nm line for excitation of TRITC. Maximal projections providing an extended depth of focus were generated with Lasersnap software (version 1.02, Biorad) and Photoshop software (Adobe). Controls, performed by preincubation of the lectin with 0.03 mol/L L-fucose overnight, resulted in a drastic decrease of the lectin binding.

Results

Dome-Associated Crypts Were Distinct from Ordinary Crypts

Semi-thin sections across the crypt region running parallel to the mucosal surface revealed that the lymphoid tissue and the underlying follicle of each Peyer's patch dome was surrounded by ~ 28 crypts (range, 21 to 34) that showed two morphological types (Figure 1). Although some of these crypts closely resembled ordinary crypts, the majority differed considerably from ordinary crypts in size, symmetry, epithelial height, and cellular composition (Figure 1b). These dome-associated crypts were oval or kidney-shaped in cross section with maximal diameters of $70.6 \pm 10 \mu\text{m}$ (range, 52 to 90 μm ; $n = 22$) as compared with $51 \pm 8.7 \mu\text{m}$ (range, 33 to 66 μm ; $n = 27$) measured for ordinary crypts. Although the crypt epithelium on the (outer) villous side did not differ from that of ordinary crypts, the epithelium facing the (inner) dome side was less intensely stained, its height was considerably increased, and it only rarely contained goblet cells (Figure 1b). The dome-associated crypts were in close contact with the lymphoid tissue of the dome but contained only relatively few lymphocytes as compared

with the epithelium at the flanks of the dome. In addition to these peculiarities in cellular composition and structure, laminin immunohistochemistry showed that the dome-associated crypts were separated from the ordinary crypts by a layer of smooth muscle cells (Figure 1c). Serial cross and longitudinal sections of the patches revealed that such layers surrounded each of the lymphoid follicles and were continuous with the lamina muscularis mucosae.

M Cell Precursors Were Detected in the Dome-Associated Crypts

Ultrathin sections of the dome-associated crypts showed that a subpopulation of epithelial cells possessed thicker and more irregular microvilli than the remaining cells (Figure 2, a and b). Labeling with UEA-I as a marker for murine M cells, visualized on ultrathin sections by colloidal gold, showed that the brush border of these cells, but not that of the other epithelial cells, was rich in $\alpha 1$ -2-linked fucose residues (Figure 2, a and b). Most of these M cell precursors were present at the side of the crypt that was in contact with the lymphoid tissue and continuous with the dome epithelium, whereas only very few of these cells were found at the villous side of the dome-associated crypts. Although some lectin binding was seen in ordinary crypts, mostly associated with the mucus of some of the goblet cells or the granules of Paneth cells, no epithelial cells with the morphological and lectin histochemical characteristics of M cell precursors were found (Figure 2c).

Ordinary Crypts Contributed to the Dome Epithelium

Conventional SEM was performed to investigate the transition from the crypt epithelium to the epithelium at the base of the domes. In the cleft between dome and villi, crypt mouths were recognized that were associated with regions at the lower flanks of the domes showing the typical patchwork of enterocytes and interspersed M cells. The M cells were identified by their brush border, which was shorter and darker than that of surrounding enterocytes. Whereas most of the crypt mouths were associated with such regions rich in M cells (Figure 3, c and e), a minority of crypt mouths was observed that obviously produced epithelial cells for the dome (ie, enterocytes, goblet cells, and brush cells) but did not produce M cells (Figure 3d).

The Two Types of Crypts Were Associated with Radial Strips in the Domes

To determine how the regions rich or poor in M cells were associated with the two types of crypts seen in the sectioning techniques, low-power CLSM of whole domes labeled with UEA-I-TRITC was used. Beside M cells, the goblet cells and the brush cells bind UEA-I.^{15,17} Because these two cell types are rare in the dome epithelium and

Figure 1. Crypt region neighboring the lymphoid follicles of Peyer's patches. Semi-thin sections (a and b) show that the inner crypts that directly contact the lymphoid tissue (L) possess some morphological peculiarities. These dome-associated crypts (DAC) have a larger diameter than ordinary crypts (OC) and an oval or longish shape. The epithelium at the dome side of the dome-associated crypts is higher and more lightly stained than the epithelium opposite and contains only very few goblet cells. Immunohistochemistry for laminin (c), a regular component of basal membranes, not only outlines the two types of crypts but also detects a layer of smooth muscle cells (SM) separating the dome-associated from the ordinary crypts. Serial sections and longitudinal sections revealed that this layer is part of the lamina muscularis mucosae. Magnification, $\times 50$ (a), $\times 500$ (b), and $\times 175$ (c).

can be distinguished from the M cells by their size and binding intensity, they could be excluded from further evaluation. Small bright dots, each representing an individual M cell, were most numerous at the flanks of the domes (Figure 4). In the majority of the domes investigated by low-power CLSM (12 of 20 domes), the M cells were not randomly distributed over the flanks of the domes but arranged in radial strips (Figures 4 and 5). Approximately 22 radial strips per dome (range, 15 to 28) rich in M cells alternated with regions that were poor in or even devoid of M cells. The M-cell-rich strips varied in

width between 24 and 88 μm and were $\sim 250 \mu\text{m}$ long. These strips were associated with groups of intensely labeled cells at the periphery of the domes (Figure 5). The analysis of single confocal sections running through the base of the domes revealed that each of the strips was associated with an individual crypt. The M-cell-rich strips were associated with crypts of $\sim 70 \mu\text{m}$ in diameter, representing the specialized dome-associated crypts described above (Figure 5c). In contrast, radial strips poor in or devoid of M cells were associated with crypts of $\sim 50 \mu\text{m}$ in diameter, typically lying more peripheral to the

Figure 2. Cross sections of a dome-associated crypt (**a** and **b**) and an ordinary crypt (**c**). The micrographs were taken from the same ultrathin section lectin-gold labeled for $\alpha 1$ -2-linked fucose residues. In the upper third of the dome-associated crypts (**a**), a few epithelial cells, mostly located at the dome side of these flattened crypts, possessed thick irregular microvilli (**arrows**). Whereas the brush border of these M cell precursors was labeled by the lectin (**b**), that of the remaining epithelial cells lacked gold particles. Some mucus in the crypt lumen and in some of the goblet cells was also lectin positive. In contrast, the brush border of epithelial cells in ordinary crypts (**c**) remained unlabeled. UEA-I colloidal gold 20 nm; magnification, $\times 8600$ (**a**) and $\times 17,500$ (**b** and **c**).

domes and representing ordinary crypts (Figure 5d). On a minority of the domes investigated (the remaining 8 of 20), a relatively uniform distribution of M cells was seen.

The Lectin-Negative Strips Lacked M Cells

The M cell population of individual domes may be heterogeneous with respect to its lectin-binding properties (see Refs. 7 and 17). To exclude that, in CLSM, the M cell marker UEA-I detected only a minor subpopulation of all M cells, the ultrastructural morphology was used as an additional criterion for the identification of M cells. In 54 ultrathin sections, taken from 10 domes, 98 different M cells were identified by their typical morphology, ie, short stub-like microvilli, numerous cytoplasmic vesicles, and/or a pocket-like invagination of the basolateral membrane (Figure 6). Intense labeling by gold particles was found on the brush border and the apical cytoplasm of 95 M cells (97%) identified by morphology but not on ordi-

nary enterocytes of the domes (Figure 6). Thus, it was excluded that a major population of M cells existed that was not detected by the *Ulex* lectin, and it must be assumed that the lectin-negative strips seen in CLSM did indeed lack M cells.

The Sites of Lymphocyte Invasion Were Not Arranged in Strips

After removal of the epithelium with boric acid, the sites where lymphocytes had entered or left the dome epithelium appeared in SEM as holes, $\sim 5.6 \mu\text{m}$ (range, 3.6 to $9.2 \mu\text{m}$, $5.6 \pm 1.1 \mu\text{m}$; $n = 46$) in diameter (Figure 7). Trespassing lymphocytes were found lying on the basal membrane near the holes or in the holes in most of the domes examined (Figure 7c). The holes in the basal membrane of the dome epithelium were evenly distributed over the domes; no regular arrangements were detected in the 110 domes studied by this technique, and in

Figure 3. Scanning electron microscopy of a single microdissected dome (a) of a murine Peyer's patch. The M cells are identified by their relatively short, dark brush border; they are restricted to the dome epithelium (upper half in b, asterisks in c and e). Crypts (arrows) are opening to the cleft between the dome and the neighboring villi. Whereas the crypts shown in c and e do produce M cells and form M-cell-rich strips on the flank of the dome, no such cells are produced by the crypt shown in d. Note that the mouths of the M-cell-producing dome-associated crypts are mostly flattened (e), whereas those of ordinary crypts are typically round (d). Magnification, $\times 120$ (a), $\times 1350$ (b), and $\times 2100$ (c–e).

particular no radial strips rich or poor in holes and/or lymphocytes were found. This impression was confirmed by image analysis of 15 randomly selected domes taken from eight mice, which showed no preferential distribution, eg, arrangements in strips (Figure 7d).

Discussion

The present study, investigating the development of the Peyer's patch dome epithelium *in vivo*, detects a sub-population of immature epithelial cells in the crypts pre-

Figure 4. Low-power confocal laser scanning microscopy of a microdissected Peyer's patch dome (D) and adjacent villi (V). The *Ulex europaeus* agglutinin (UEA-I-TRITC) binds to the M cells which are most numerous at the flanks of the domes. In addition, brush cells and the mucus of some immature goblet cells in the crypts are labeled by the lectin. The M cells are not randomly distributed over the domes, but arranged in radial strips, rich or poor in M cells. Magnification, $\times 70$ (a), maximal projection of 34 optical sections, and $\times 170$ (b), maximal projection of 38 optical sections.

Figure 5. Confocal view of a microdissected UEA-I-TRITC-labeled dome (**a**) and higher magnifications (**b–d**, frame in **a**) of the top (T), the flanks, and the base with associated crypts. Maximal projections (**a** and **b**), which provide an extended depth of focus, reveal radial strips rich in M cells (**white arrows**) and others poor in M cells (**black arrow**). Single optical sections from the same image stack running across the crypt region (**c** and **d**) show that each strip is associated with an individual crypt. Most of the inner crypts are relatively large in diameter (**c**) and associated with M-cell-rich strips (**white arrows**). The M-cell-poor strips, however, are associated with smaller crypts (**black arrow**) that resemble ordinary crypts in size and shape (**d**). It must be noted that the three crypts were at different positions along the *z* axis and therefore are seen in different optical sections. Magnification, $\times 90$ (**a**), maximal projection of 33 optical sections; $\times 280$ (**b**), maximal projection of 101 optical sections; $\times 280$ (**c**), section 74 of the image stack shown in **b**; and $\times 280$ (**d**), section 86 of the image stack shown in **b**.

determined as M cells, shows that this differentiation pathway is restricted to specialized dome-associated crypts and thus challenges the hypothesis of a lymphocyte-induced formation of M cells. It is shown that those crypts that produce M cells differ fundamentally from ordinary crypts in size, shape, cellular composition, and location. M cell precursors were detected at the (inner) dome side of these crypts by ultrastructural characteristics and the binding of UEA-I, the M cell marker in mice. At the lower flanks of the dome, they develop into cells with relatively short stub-like microvilli representing immature M cells, previously identified by ultrastructure, transport function, and histochemistry.^{6,28} Mature M cells are present at the upper flanks of the domes, where they acquire their full transport capacity and mostly possess the typical pocket-like invagination of the basolateral membrane filled with lymphocytes.⁶

Lectin histochemical studies revealed that the epithelial cells, which derive from an individual crypt of the small intestine, are of monoclonal origin^{29,30} and that M cells that derive from a common crypt are also monoclo-

nal.^{7,31} The present study shows that the clone of epithelial cells, which derives from an individual dome-associated crypt, forms a radial strip on the flank of the dome rich in M cells. In addition, the combination of sectioning techniques with SEM and CLSM revealed not only that the specialized dome-associated crypts contribute to the dome epithelium but also that ordinary crypts produce epithelial cells that enter the dome. However, we were unable to detect M cell precursors in such ordinary crypts, and the radial strips associated with these were devoid of M cells, as demonstrated by scanning and confocal microscopy. The sensitivity of 97% determined for UEA-I as a marker for murine M cells further documents the absence of M cells in epithelial regions that derive from ordinary crypts. We therefore conclude that the differentiation of M cells is induced in and restricted to the dome side of the specialized dome-associated crypts and takes place neither in other crypts nor later on at the flanks of the domes.

This finding implies that lymphocytes of the dome epithelium do not induce the formation of M cells, as has

Figure 6. Ultrathin sections lectin-gold labeled for $\alpha 1$ -2-linked fucose residues showing the Peyer's patch dome epithelium of four mice. The M cells (M) are characterized by short stub-like microvilli and numerous small vesicles in their apical cytoplasm. Of 98 M cells identified as such by these morphological criteria, 95 were intensely labeled at the apical membrane and in the apical cytoplasm. In contrast, cells morphologically identified as enterocytes (E) by their typical dense brush border lacked gold particles. The **arrows** point to the junctional complexes between M cells and enterocytes. UEA-I colloidal gold 20 nm; magnification, $\times 22,000$ (a-d).

previously been suggested.^{5,8,9,13,32} Our conclusion is further supported by the observation of a random distribution of holes in the basal membrane of the dome epithelium and the trespassing lymphocytes. If M cell development were induced by dome-epithelial lymphocytes via direct cell-cell interaction, the distribution of M cells over the domes would correspond with that of lymphocytes. As no such correlation was observed, it can be ruled out that M cells develop from enterocytes in the specific environment of the dome epithelium. The results of this study indicate that M cells represent a separate cell line distinct from enterocytes, goblet cells, Paneth cells, or other cell types of the intestinal epithelium. The different stages of M cell development are related to specific compartments, starting with M cell precursors in the upper crypt epithelium and ending with mature M cells at the tips of radial M-cell-rich strips. Although epithelial cells labeled by M cell markers have previously been described in the upper crypts using light microscopy,^{7,17} for the first time we identify and characterize M cell precursors at an ultrastructural level. The presence of such cells at the position where the initial differentiation into the main epithelial cell types of the intestine takes

place⁴ indicates that M cells also derive directly from undifferentiated crypt stem cells.

The radial strips rich in M cells were associated with specialized M-cell-producing crypts, and the number of M-cell-rich strips per dome was similar to that of the dome-associated crypts. However, radial strips rich or poor in M cells were detected in most, but not in all, domes examined. The presence or absence of strips varied between the domes of individual patches and was not related to individual animals. As a Peyer's patch is formed by 5 to 10 follicles in mice, most of the follicles lie at the periphery of their patch and are in contact with ordinary crypts. They should consequently possess domes with M-cell-negative strips. Some follicles, however, lie in the center of the patch, are completely surrounded by other follicles, and are not in contact with ordinary crypts. They are thus homogeneously supplied with M cells and enterocytes, as was observed for some domes.

The dome-associated crypts possess a specialized epithelium at the dome side, in which the development of M cells from stem cells is induced and the generation of goblet cells is suppressed. These specialized epithelial

Figure 7. Scanning electron microscopy of a murine Peyer's patch (a) after removal of the epithelium. Whereas the basement membrane of ordinary villi and crypts shows only a few small pores, that of the domes (b) possesses numerous holes. Small roundish cells, probably representing trespassing lymphocytes, were found in or near some of these holes (c). In the corresponding images b and d, the holes of the dome epithelial basement membrane are labeled by dots. Because the periphery of the domes was slightly out of focus and/or covered with some detritus, a region of interest (ROI) was defined and analyzed. All twelve domes examined by this method showed an irregular random distribution of holes over the dome surface and in particular no radial strips rich or poor in holes. The arrows in a point to the openings of the dome-associated crypts. Magnification, $\times 45$ (a), $\times 200$ (b), $\times 560$ (c), and $\times 200$ (d).

regions are in direct contact with the lymphoid tissue of the follicle but only rarely contain lymphocytes. Based on the present observations, we postulate that the differentiation pathway of M cells is triggered by a still unknown humoral factor in the crypts rather than by direct cell-cell interaction, as suggested by findings in cell cultures.¹³ The percentage of M cells of all dome epithelial cells is relatively constant in and characteristic for each species and location (eg, 8% to 10% in mouse Peyer's patches¹⁵), but both increases and decreases of the relative number of M cells have been described under the influence of antigens, inflammation, drugs, or microbes.³³⁻³⁷ It is therefore speculated that the humoral factors that induce and maintain the development of M cells are regulated by the cells of the lymphoid tissue. In conclusion, the *in vivo* situation described in the present study enables us to follow the differentiation pathway of intes-

tinal M cells and to state that their formation is restricted to specialized epithelial regions in the dome-associated crypts and probably not induced by direct interaction with intraepithelial lymphocytes in these crypts or on the dome. Additional studies are needed to identify the factors and the molecular mechanisms involved in the induction of M cells in the dome-associated crypts.

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