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ORIGINAL ARTICLE

The identification of intestinal M cells in the sacculus rotundus and appendix of the Angora rabbit

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Abstract The present study was aimed at the immunohistochemical demonstration of M cells, found in the follicle-associated epithelium (FAE) of the sacculus rotundus (SR) and appendix of the Angora rabbit, using anti-vimentin primary antibodies, and at the determination of certain fine structural characteristics. Ten adult Angora rabbits constituted the material of the study. Immunohistochemical staining revealed that many cells composing the FAE, which covered the dome regions of the SR and appendix, reacted positively with vimentin. FAE contained two different types of vimentin-positive cells. The first type surrounded intraepithelial lymphocytes (IEL) with a basolateral invagination in the apex and periphery of the dome epithelium, whilst the second type consisted of columnar cells found in the FAE near crypts. The immunoreactivity of the cells found in the FAE covering the apex and periphery of the domes was observed particularly in the perinuclear cytoplasm and the cytoplasm surrounding the IEL. Electron microscopic examination demonstrated that the M cells found in the FAE covering the apex and periphery of the dome regions of the SR and appendix did not exhibit any microvilli on their apical surface. The FAE near crypts contained columnar cells, which resembled enterocytes. The apical membrane of these cells exhibited shorter and irregular microvilli, in contrast to neighbouring enterocytes. It was determined that M cells, found in the FAE of the SR and appendix in the Angora rabbit, displayed similarities in terms of localization and fine structure. This situation may be indicative of the two lymphoid structures with different localization having similar functional properties.

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Introduction

The gastrointestinal duct is exposed to numerous antigens and pathogenic agents throughout the life period of the organism (Brandtzaeg et al. 2008; Cesta 2006; Fortuna–Lamothe and Boullier 2007; Liebler–Tenorio and Pabst 2006). Gut-associated lymphoid tissue (GALT) is involved in the development of an immune response or immune tolerance to the antigens and pathogens found in the intestinal tract (Magalhaes et al. 2007; Mowat 2003). GALT is composed of solitary and aggregated lymphoid structures (Cesta 2006; Newberry and Lorenz 2005; Newberry 2008). Compared to other mammalian species, rabbits have a very well-developed GALT structure (Cesta 2006; Haley 2003). The GALT of the rabbit consists of lymphoid structures, including Peyer's patches, the sacculus rotundus, caecal patches and the appendix (Gebert and Bartels 1991; Gebert et al. 1992; Haley 2003).

Lymphoid structures composing the GALT are lined by a specialized epithelium known as follicle-associated epithelium (FAE) or the dome epithelium (Beyaz 2004; Magalhaes et al. 2007; Neutra 1998; Shaykhiev and Bals 2007). FAE consists of M cells and enterocytes. However, sometimes FAE also contains goblet cells and enteroendocrine cells (Cesta 2006; Newberry 2008). M cells are epithelial cells specialized in the uptake and transport of antigens, and are characterized both with their membranous thin cytoplasm and their surrounding IEL with a basolateral invagination (Kraehenbuhl and Neutra 2000; Man et al. 2004; Miller at al. Corr et al., 2008). M cells transfer macromolecules, bacteria and viruses through their thin cytoplasm to the lymphoid cells beneath them (Hathaway and Kraehenbuhl 2000; Kucharzik et al. 2000; Neutra 1998). These cells are easily distinguished from neighbouring enterocytes in that they exhibit short, irregular microvilli (Neutra et al., 2001). Although M cells are best observed electron microscopically, they are also observed at light microscopic level by means of UEA-1 (Ulex europaeus agglutinin-1) lectin in mice (Clark et al. 1993), anti-cytokeratin 8 in rats (Rautenberg et al. 1996), anti-cytokeratin 18 in pigs (Gebert et al. 1994) and anti-vimentin primary antibodies in rabbits (Gebert et al. 1992; Jepson et al. 1992).

The SR is a lymphoid organ unique to rabbits, which is characterized with an ampoule-like enlargement of the terminal ileum, and is composed solely of aggregated lymphoid follicles (Beşoluk et al. 2006; Snipes 1978). This organ is also named as the tonsilla iliaca or circular Peyer's patches (Haley 2003; Mage 1998). It has been demonstrated that the appendix, which is located medially to the caecum, similar to the bursa of Fabricius of birds, is a primary lymphoid organ in the development of B lymphocytes, particularly in young rabbits (Pospisil and Mage 1998; Weinstein et al. 1994). A limited number of researches have been carried out on the structural properties of M cells in the SR (Gebert and Bartels 1991) and appendix (Gebert and Bartels 1991; Kraehenbuhl and Neutra 2000; Marchetti et al. 2004) of rabbits. The present study was aimed at the immunohistochemical demonstration of M cells found in the FAE of the SR and appendix of the Angora rabbit, using anti-vimentin primary antibodies, and at the determination of the fine structural properties of these cells.

Material and methods

Ten healthy adult Angora rabbits, obtained from the Research Farm of Erciyes University Faculty of Veterinary Medicine, constituted the material of the study. The animals were

provided with standard laboratory feed and fresh water and were housed at a temperature of 23±2 ^{0}C. The rabbits were euthanized by an intravenous injection of 0.1 ml/kg sodium pentobarbital. The taken tissue samples were first rinsed in physiological saline to eliminate intestinal contents, and were later fixed in Bouin's solution for 2 hours. The tissues were passed through graded alcohols, methyl benzoate and benzole series, respectively, and were embedded in paraffin. The 5 μm–thick sections cut from the paraffin blocks were applied Mallory's triple stain modified by Crossmon (1937) in order to perform an examination of the general structure.

For the purpose of determining vimentin immunoreactivity, sections were placed on poly-L-lysine slides and were applied immunohistochemical staining with the avidin-biotin-peroxidase complex (ABC) method. The sections were kept in 3% hydrogen peroxide (H_20_2) in methanol for 20 minutes to eliminate endogen peroxide. Subsequently, they were boiled in 96 ^{0}C citrate buffer (pH 6.0) for 20 minutes with an aim to restore antigenic properties. The sections, which were left to cool at room temperature for 20 minutes, after being washed in phosphate-buffered saline (PBS, pH; 7.4) solution, were applied 10% goat serum (Thermo Scientific, USA) for 5 minutes in order to prevent non-specific binding. Subsequently, the sections were incubated with 1/200 diluted monoclonal mouse anti-vimentin (Clone; V9, DAKO) primary antibodies at room temperature for 1 hour. Following incubation, the sections were treated with anti-mouse secondary antibodies with biotin (Thermo Scientific, USA) for 20 minutes and with avidin-peroxidase solutions (Thermo Scientific, USA) for 20 minutes. In order to display the antigen-antibody reaction, the chromogen 3-amino 9-ethyl carbazole (AEC) (Thermo Scientific, USA) was applied for 5-10 minutes, and later Gill's hematoxylin was applied for background staining. While all washing steps were performed with PBS, staining procedures were performed in a humid chamber (Shandon, UK). The assessment of immunohistochemical stainings was performed using positive and negative controls. Rabbit kidney sections were used as positive controls for anti-vimentin primary antibodies. Goat serum replaced primary antibodies as negative control, and staining procedures were applied as usual.

For electron microscopic examination, the taken tissue samples were first prefixed in glutaraldehyde-paraformaldehyde (pH 7.4) in compliance with the method described by Karnovsky (1965), and then fixed in 1% osmic acid solution for 2 hours. Following the second fixation, the tissues were treated with 1% uranyl acetate solution for two hours, passed through graded alcohols and propylene oxide, and finally embedded in Araldite M. 1-micron-thick semi-thin sections cut from these blocks were applied the toluidine blue staining method, and following the marking of determined areas in these sections, thin sections of a thickness of 30–40 nm were cut. These sections were applied contrast staining as described by Veneable and Coggeshall (1965), and were examined under a Carl Zeiss EM 9S2 model electron microscope.

The animals used in the study were euthanized with the approval of the Ethics Board of Erciyes University Faculty of Veterinary Medicine (Date of meeting; 09.04.2003, Decision No: 25).

Results

Light microscopic findings

Light microscopic examinations revealed that both the SR (Fig. 1A) and appendix (Fig. 1B) were composed solely of aggregated lymphoid follicles. Lymphoid follicles protruded

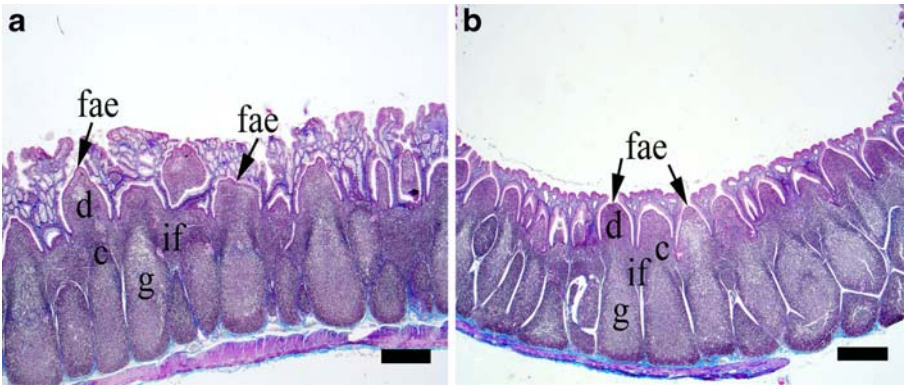


Fig. 1 General histological appearance of the SR and appendix, composed of aggregated lymphoid follicles. A) Histological appearance of the SR, FAE; follicle associated epithelium, d; dome region, c; corona, g; germinal centre; if; interfollicular area, bar; 500 μm. B) Histological appearance of the appendix, FAE; follicle associated epithelium, d; dome region, c; corona, g; germinal centre; if; interfollicular area, bar; 500 μm

towards the lumen, thereby, forming the dome villi. The surface of aggregated lymphoid follicles, which faced the lumen, was lined by FAE. In both organs, each follicle was composed of a subepithelial dome region, a light coloured germinal centre in the middle, a corona region separating the indicated two regions from each other, and interfollicular areas in-between neighbouring follicles (Fig. 1A and B).

Immunohistochemical staining demonstrated that many vimentin-positive cells existed in the FAE covering the dome regions of the SR and appendix (Fig. 2A–F). While some FAE in the apex of the dome regions were composed of only enterocytes (Fig. 2A and D), in some FAE, many vimentin-positive cells were observed to exist among enterocytes (Fig. 2B and C). The basolateral invagination formed by the vimentin-positive cells in the apex and periphery of the dome epithelium contained abundant IEL (Fig. 2A–D). Vimentin immunoreactivity was observed particularly in the perinuclear cytoplasm of cells and in the cytoplasm surrounding IEL (Fig. 2A–D). No staining was observed apically or basally to these cells. These cells, due to the features indicated above, displayed the typical appearance of mature M cells. In the FAE near the crypts, columnar vimentin-positive cells, lacking a basolateral invagination surrounding lymphocytes, were observed (Fig. 2E and F).

In semi-thin sections, the M cells in the FAE of the SR and appendix were characterized with the presence of a thin apical cytoplasm between neighbouring enterocytes and with their surrounding IEL (Fig. 3A and B). These cells were easily distinguished, based on their staining a lighter colour compared to neighbouring enterocytes, and their possessing a basally located large euchromatic nucleus (Fig. 3A and B). The nuclei of M cells were located below the IEL, which they surrounded.

Electron microscopic findings

Electron microscopic examinations revealed that the apical membrane of mature M cells in the SR and appendix lacked microvilli (Fig. 4A–C). These cells surrounded the IEL beneath them with a distinct invagination (Fig. 4A–C). Their apical cytoplasm contained abundant vesicles, vacuoles and mitochondria (Fig. 4B–C), and their nuclei were situated beneath the invagination surrounding the IEL, near the basal membrane (Fig. 4A). In the FAE near the crypts, the presence of columnar cells, which resembled enterocytes, was observed. These

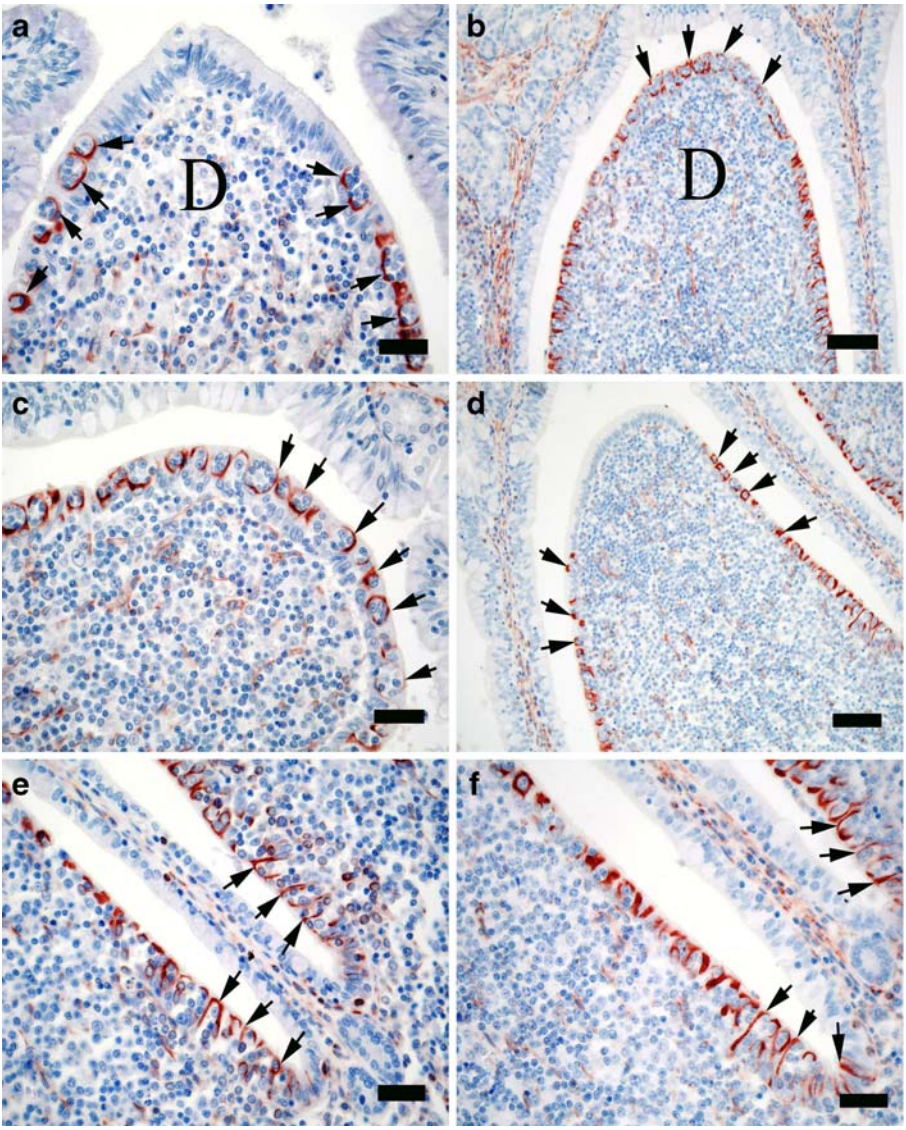


Fig. 2 Vimentin-positive cells in the SR and appendix, Immunohistochemical staining, AEC. A) Mature M cells found in the FAE in the periphery of the dome region of the SR (arrows), bar; 25 µm. B) Mature M cells found in the FAE in the periphery and apex of the dome region of the SR (arrows), bar; 50 µm. C) Mature M cells found in the FAE in the periphery and apex of the dome region of the appendix (arrows), bar; 25 µm. D) Mature M cells found in the FAE in the periphery of the dome region of the appendix (arrows), bar; 50 µm. E) Columnar vimentin-positive immature M cells found in the FAE near the crypts in the SR (arrows), bar; 25 µm. F) Columnar vimentin-positive immature M cells found in the FAE near the crypts in the appendix (arrows), bar; 25 µm

cells, which were electron lighter compared to neighbouring enterocytes, exhibited shorter and irregular microvilli on their apical membrane, compared to the microvilli of enterocytes. Furthermore, these cells possessed a more euchromatic nucleus (Fig. 4D). The organelles and vacuoles of these cells were located nearer the apical membrane.

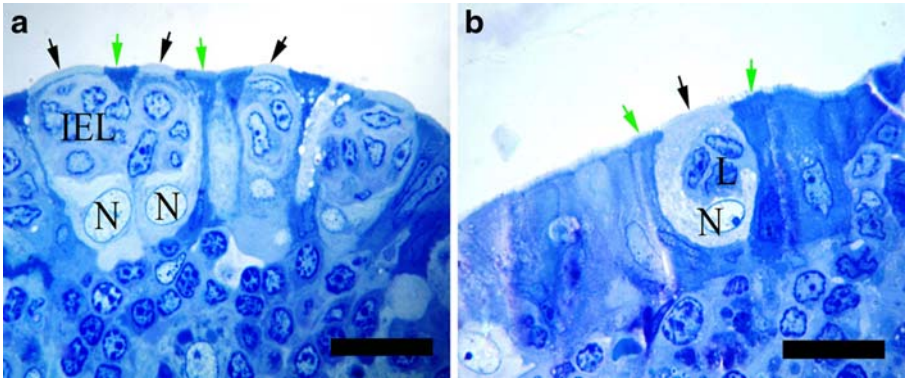


Fig. 3 General appearance of the FAE of the SR and appendix, semi thin section, toluidine blue. A) M cells characterized with a lighter staining and their surrounding IEL (black arrows) compared to neighbouring enterocytes (green arrows) in the SR, N; Euchromatic nuclei of the M cells, bar; 8 μm. B) An M cell characterized with a lighter staining and its surrounding IEL (black arrow) compared to neighbouring enterocytes (green arrows) in the appendix, N; Euchromatic nucleus of the M cell, bar; 8 μm

Discussion

GALT is composed of aggregated and solitary lymphoid follicles located along the gastrointestinal tract (Cesta 2006; Liebler–Tenorio and Pabst 2006; Newberry 2008). In comparison to other mammals, rabbits have a well developed GALT structure (Cesta 2006; Haley 2003). In general, the GALT of the rabbit is considered to be composed of the isolated lymphoid follicles, Peyer's patches, SR, appendix, and ceacal patches (Gebert and Bartels 1991; Haley 2003). In agreement with classical literature reports available on the SR and appendix (Cesta 2006; Gebert and Bartels 1991), it was determined in the present study that these lymphoid structures were composed of many aggregated lymphoid follicles, and that each follicle is comprised of four sections, namely, the dome, corona, germinal centre and interfollicular area, with the dome regions being lined by FAE, a specialized type of epithelium.

The M cells in the FAE are specialized epithelial cells, which are involved in the development of an immune response or immune tolerance to antigens by transferring intraluminal macromolecules and pathogenic agents to the antigen-presenting cells beneath them by transepithelial transport route (Beyaz 2004; Man et al. 2004; Neutra 1998). The observation of abundant mitochondria and vesicles in the apical cytoplasm of the M cells in the FAE of the SR and appendix in electron microscopic examinations in the present study is in agreement with the reports of Magalhaes et al. (2007) and Hathaway and Kraehenbuhl (2000). Furthermore, the determination of the M cells in the FAE of the SR and appendix having a thin apical cytoplasm and their surrounding IEL with a basolateral invagination, was in agreement with the findings reported by Kraehenbuhl and Neutra (2000), Miller et al. (2007) and Neutra et al. (2001). In addition, the mature M cells of the Angora rabbit lacking microvilli and having a smooth apical membrane was in contrast with the report of Gebert and Bartels (1991), which indicated that the M cells of the Peyer's patches in the chinchilla rabbit exhibited irregular microvilli and the M cells of the caecal patches had longer microvilli, in comparison to neighbouring enterocytes. However, the same finding was in agreement with the reports of Gebert and Bartels (1991) and Marchetti et al. (2004) for the appendix. Thereby, it was observed that mature M cells, excluding their having a smooth apical membrane, displayed a similarity to typical M cells for all their structural

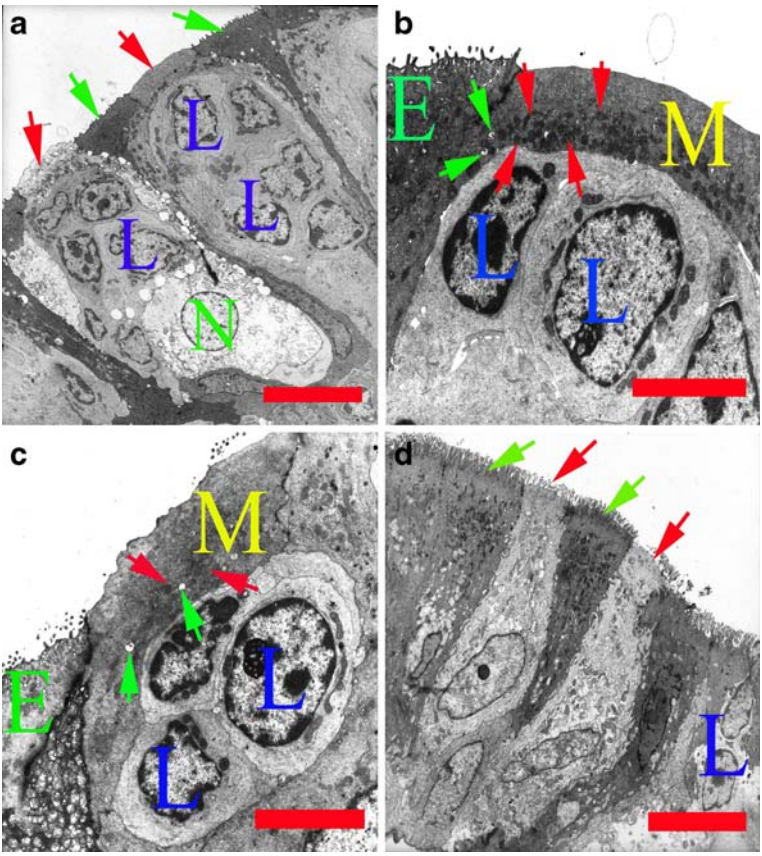


Fig. 4 Electron microscopic appearance of M cells in the FAE of the SR and appendix. A) M cells characterized with their membranous apical cytoplasm (red arrows) among neighbouring enterocytes (green arrows) in the FAE of the SR, L: IEL surrounded by M cells, N; basally located nucleus of M cell, bar: 6 μm B) M cell in the FAE of the SR with a larger appearance and smooth apical membrane lacking microvilli (M), green arrows; abundant small vacuoles in the apical cytoplasm of an M cell, red arrows; mitochondria, L: lymphocytes surrounded by an M cell, E; enterocyte, bar: 2 μm. C) M cell in the FAE of the appendix with a smooth apical membrane lacking microvilli (M), green arrows; abundant small vacuoles in the apical cytoplasm of an M cell, red arrows; mitochondria, L: lymphocytes surrounded by an M cell, E; enterocyte, bar: 2 μm. D) Immature M cells observed to be more electron light (red arrows) compared to neighbouring enterocytes (green arrows) in the FAE near the crypts in the SR, L; lymphocyte, bar: 6 μm

features. In the present study, enterocyte-like columnar cells were observed in the FAE near the crypts. These cells, which were electron lighter and exhibited shorter and irregular microvilli, in comparison to neighbouring enterocytes, had organelles and vacuoles that were located nearer the apical membrane. Based on these morphological features, these cells were considered to be immature M cells, similar to those described by Bye et al. (1984) in mice.

It has been reported that the M cells in the FAE of the GALT (Gebert et al. 1992; Jepson et al. 1992; Jepson et al. 1993), BALT (Gebert and Hach 1992) and tonsils (Gebert et al. 1995) of the rabbit contain vimentin intermediate filaments in their cytoplasm. Similar to the report of Gebert et al. (1992), in the present study, it was found that the FAE of the SR and appendix contained two different types of vimentin-positive cells. The first type of

these cells was mature M cells, which surrounded the IEL with a basolateral invagination, and the second type was columnar immature vimentin-positive cells. It has been reported that in all of the dome follicles of the appendix (Gebert and Bartels 1991; Jepson et al. 1993) and SR (Gebert and Bartels 1991), M cells are located in the peripheral FAE, whilst only enterocytes exist in the apex of the dome region. In the present study, whilst some FAE in the apex of the dome regions of the SR and appendix were composed of only enterocytes, as also reported by these researchers, contradictorily, in many dome apexes, a great number of vimentin-positive cells existed among enterocytes.

Vimentin, which is an intermediate filament, is generally found in cells of mesenchymal origin (Goldman et al. 1996; Helfand et al. 2005; Ivaska et al. 2007). Vimentin filaments function in the elasticity of cells and the fixation of organelles in the cytosol, and are also a part of the cytoskeleton (Goldman et al. 1996). Furthermore, it has been determined that these filaments establish a structural connection between the cell membrane and nuclear laminae, thereby, acting in the intracellular transfer of signals (Helfand et al. 2005; Ivaska et al. 2007). In addition, there are literature reports indicating that in the intestinal M cells of the rabbit, vimentin intermediate filaments extend from the periphery of the nucleus to the membrane of the intraepithelial packet surrounding the lymphocytes (Gebert et al. 1992; Jepson et al. 1992). In the present study, vimentin immunoreactivity was determined in both the perinuclear cytoplasm and cytoplasmic parts surrounding the IEL in mature M cells, and in only the perinuclear cytoplasm in immature M cells. Immunoreaction was not observed in the apical cytoplasm of the cells. Vimentin, which was dense in the perinuclear cytoplasm and in the cytoplasmic parts surrounding the IEL in mature M cells, can be considered to function in the elasticity of the cell and to preserve cellular morphology by preventing IEL from making pressure on the basally located nucleus of M cells and enabling lymphocytes to continuously enter and leave the packet. Furthermore, the interaction of immature M cells with B lymphocytes is thought to be the particular reason for immature M cells displaying the typical morphology of M cells (Golovkina et al. 1999; Shaykhiev and Bals 2007). Another function of vimentin filaments is to communicate signals between IEL, which have close contact with M cell membranes.

The origin of M cells, their mechanism of differentiation and cell death have not been fully explained (Miyazawa et al. 2006). Nonetheless, there are two opinions regarding the formation of M cells (Mach et al. 2005). The first opinion suggests that M cells develop from stem cells found in crypts, different from those which later form the enterocytes (Gebert et al. 1999; Gebert et al. 2004; Lelouard et al. 2001), whilst the other opinion suggests that enterocytes differentiate between M cells as a result of interaction with B lymphocytes (Kerneis et al. 1997) and stimulation of pathogenic bacteria (Borghesic et al. 1999). Miyazawa et al. (2007) reported that in pigs, immature M cells, as a result of close contact with lymphocytes in the periphery of FAE, first differentiate into mature M cells, and later into enterocytes near the apex of the dome. These researchers also reported that the M cells of the Peyer's patches of the pig, similar to M cells of the mouse (Sierro et al. 2000) and rabbit (Takeuchi and Gonda 2004), after differentiating between enterocytes, go through apoptosis in the apex, and are later excreted into the lumen. In the present study, based on the presence of mature M cells with a thin, narrow cytoplasm, which surround IEL with a basolateral packet in the FAE in the periphery of the dome region, and the identification of columnar immature M cells lacking IEL packets in the FAE near the crypts by vimentin immunohistochemical staining, the opinion suggesting that M cells originate from crypts has been favoured. Furthermore, due to the observation of many vimentin-positive M cells in the FAE in the apex of some dome regions in both the SR and the appendix in the present study, we are not in support of the view that M cells

differentiate between enterocytes and are excreted into the lumen from the apex (Miyazawa et al. 2006).

Similar to the bursa of Fabricius of birds, it is reported that the appendix, particularly in young rabbits, is a primary lymphoid organ in the development of B lymphocytes (Pospisil and Mage 1998; Weinstein et al. 1994). Furthermore, the SR is considered to have functions similar to those of the appendix (Shi–Jin and Nan–Hui 2007). In the present study, the M cells in the FAE of the SR and the appendix of the Angora rabbit displayed close similarity to each other in terms of localization and fine structure. This may be indicative of the two lymphoid structures with different localization having the same functional properties.

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References

Beşoluk K, Eken E, Sur E (2006) A morphological and morphometric study on the sacculus rotundus and ileum of the Angora rabbit. Veterinarni Medicina 51: 60–65.

Beyaz F (2004) M cells: membranous epithelial cells. Erciyes Univ Vet Derg, 1: 133–138.

Borghesic C, Taussig MJ, Nicoletti C (1999) Rapid appearence of M cells after microbial challenge is restricted at the periphery of the follicle-associated epithelium of Peyer's patch. Lab Invest 79: 1393–1401.

Brandtzaeg P, Kiyono V, Pabst R, Russel MW (2008) Terminology: nomenclature of mucosa-associated lymphoid tissue. Mucosal Immunol 1: 31–37. doi:10.1038/mi.2007.9

Bye WA, Allan CH, Trier JS (1984) Structure, distribution, and origin of M cells in Peyer's patches of mouse ileum. Gastroenterology 86: 789–801.

Cesta MF (2006) Normal structure, function, and histology of mucosa-associated lymphoid tissue. Toxicol Pathol 34: 599–608. doi:10.1080/01926230600865531

Clark MA, Jepson MA, Simmons NL, Booth TA, Hirst BH (1993) Differential expression of lectin-binding sites defines mouse intestinal M-cells. J Histochem Cytochem 41: 1679–1687.

Corr SC, Gahan CGM, Hill C (2008) M-cells: Origin, morphology and role in mucosal immunity and microbial pathogenesis. FEMS Immunol Med Microbiol 52: 2–12. doi:10.1111/j.1574-695X.2007.00359.x

Crossmon G (1937) A modification of Mallory's connective tissue stain with a discussion of the principles involved. Anat Rec 69: 33–38.

Fortuna–Lamothe L, Boullier S (2007) A review on the interactions between gut microflora and digestive mucosal immunity. Posssible ways to improve the health of rabbits. Livestock Sci 107: 1–18. doi:10.1016/j.livsci.2006.09.005

Gebert A, Bartels H (1991) Occluding junctions in the epithelia of the gut-associated lymphoid tissue (GALT) of the rabbit ileum and caecum. Cell Tissue Res 266: 301–314. doi:10.1007/BF00318186

Gebert A, Hach G (1992) Vimentin antibodies stain membranous epithelial cells in the rabbit bronchus-associated lymphoid tissue (BALT). Histochemistry 98: 271–273. doi:10.1007/BF00271042

Gebert A, Hach G, Bartels H (1992) Co-localization of vimentin and cytokeratins in M-cells of rabbit gut-associated lymphoid tissue (GALT). Cell Tissue Res 269: 331–340. doi:10.1007/BF00319625

Gebert A, Rothkotter HJ, Pabst R (1994) Cytokeratin 18 is an M-cell marker in porcine Peyer's patches. Cell Tissue Res 276: 213–221. doi:10.1007/BF00306106

Gebert A, Willfuhr B, Pabst R (1995) The rabbit M-cell marker vimentin is present in epithelial cells of the tonsil crypt. Acta Otolaryngol 115: 697–700.

Gebert A, Fassbender S, Werner K, Weissferdt A (1999) The development of M cells in Peyer's patches is restricted to specialized dome-associated crypts. Am J Pathol 154: 1573–1582.

Gebert A, Steinmetz I, Fassbender S, Wendlandt KH (2004) Antigen transport into Peyer's patches: Increased uptake by constant numbers of M cells. Am J Pathol 164: 65–72.

Goldman RD, Khuon S, Chou YH, Opal V, Steinert PM (1996) The function of intermediate filaments in cell shape and cytoskeletal integrity. J Cell Biol 134: 971–983.

Golovkina TV, Shlomchik M, Hannum L, Chervonsky A (1999) Organogenetic role of B lymphocytes in mucosal immunity. Science 286: 1965–1968. doi:10.1126/science.286.5446.1965

Haley PJ (2003) Species differences in the structure and function of the immune system. Toxicology 188: 49–71. doi:10.1016/S0300-483X(03)00043-X
Hathaway LJ, Kraehenbuhl JP (2000) The role of M cells in mucosal immunity. Cell Mol Life Sci 57: 323–332.
Helfand BT, Chou YH, Shumaker DK, Goldman RD (2005) Intermediate filament proteins participate in signal transduction. Trends Cell Biol 15: 568–570. doi:10.1016/j.tcb.2005.09.009
Ivaska J, Pallari HM, Nevo J, Eriksson JE (2007) Novel functions of vimentin in cell adhesion, migration, and signaling. Experimental Cell Res 313: 2050–2062. doi:10.1016/j.yexcr.2007.03.040
Jepson MA, Mason CM, Bennett MK, Simmons NL, Hirst BH (1992) Co-expression of vimentin and cytokeratins in M-cells of rabbit intestinal lymphoid follicle associated eptihelium. Histochem J 24: 33–39.
Jepson MA, Simmons NL, Hirst GL, Hirst BH (1993) Identification of M cells and their distribution in rabbit intestinal Peyer patches and appendix. Cell Tissue Res 273: 127–136.
Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J Cell Biol 27: 137–138.
Kerneis S, Bogdanova A, Kraehenbuhl JP. Pringault V (1997) Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. Science 277: 949–952. doi:10.1126/science.277.5328.949
Kraehenbuhl J-P, Neutra MR (2000) Epithelial M cells: Differentiation and function. Annu Rev Cell Dev Biol 16: 301-332. doi:10.1146/annurev.cellbio.16.1.301
Kucharzik T, Lugering N, Rautenberg K, Lugering A, Schmidt MA, Stoll R, Domschke W (2000) Role of M cells in intestinal barrier function. Ann NY Acad Sci 915: 171–183 doi:10.1111/j.1749-6632.2000.tb05240.x
Lelouard H, Sahuquet A, Reggio H, Montcourrier P (2001) Rabbit M cells and dome enterocytes are distinct cell lineages. J Cell Sci 114: 2077–2083.
Liebler-Tenorio EM, Pabst R (2006) Malt structure and function in farm animals. Vet Res 37**:** 257–280. doi:10.1051/vetres:2006001
Mach J, Hshieh T, Hsieh D, Grubbs N, Chervonsky A (2005) Development of intestinal M cells. Immunol Rev 206: 177–189. doi:10.1111/j.0105-2896.2005.00281.x
Magalhaes JG, Tattoli I, Girardin SE (2007) The intestinal epithelial barrier: How to distinguish between the microbial flora and pathogens. Semin Immunol 19: 106–115.
Mage R (1998) Immunology lagomorph. In: Griebel P, Pastoret PP, Bazin H, Govaerts A (ed.) Handbook of Vertebrate Immunology, Academic Press Limited, pp 673.
Man AL, Prieto-Garcia ME, Nicoletti C (2004) Improving M cell mediated transport across mucosal barriers: do certain bacteria hold the keys? Immunology 113: 15–22. doi:10.1111/j.1365-2567.2004.01964.x
Marchetti M., Sirard JC, Sansonetti P, Pringault E, Kerneis S (2004) Interaction of pathogenic bacteria with rabbit appendix M cells: bacterial motility is a key feature in vivo. Microbes and Infections 6: 521–528. doi:10.1016/j.micinf.2004.02.009
Miller H, Zhang J, Kuolee R, Patel GB, Chen W (2007) Intestinal M cells: The fallible sentinels? World J Gastroenterol 13: 1477–1486.
Miyazawa K, Aso V, Kanaya T, Kido T, Minashima T, Watanabe K, Ohwada V, Kitazawa H, Rose MT, Tahara K, Yamasaki T, Yamaguchi T (2006) Apoptotic process of porcine intestinal M cells. Cell Tissue Res 323: 425–432. doi:10.1007/s00441-005-0086-z
Mowat AM (2003) Anatomical basis of tolerance and immunity to intestinal antigens. Nature Rev Immunol 3: 331–341. doi:10.1038/nri1057
Neutra MR (1998) Current concepts in mucosal immunity V. Role of M-cells in transepithelial transport of antigens and pathogens to the mucosal immune system. Am J Physiol 274: 785–791.
Neutra MR, Mantis NJ, Kraehenbuhl JP (2001) Collaboration of epithelial cells with organized mucosal lymphoid tissues. Immunol Nature 2: 1004–1009. doi:10.1038/ni1101-1004
Newberry RD, Lorenz RG (2005) Organizing a mucosal defense. Immunol Rev 206: 6–21. doi:10.1111/j.0105-2896.2005.00282.x
Newberry RD (2008) Intestinal lymphoid tissues: is variety an asset or a liability. Curr Opin Gastroenterol 24: 121–128. doi:10.1097/MOG.0b013e3282f4906d
Pospisil R, Mage RG (1998) Rabbit appendix: a site of development and selection of the B cell repertoire. Curr Top Microbiol Immunol 229: 59–70.
Rautenberg K., Cichon C., Heyer G, Demel M, Schmidt MA (1996) Immunohistochemical characterization of the follicle-associated epithelium of Peyer's patches: anti-cytokeratin 8 antibody (clone 4.1.18) as a molecular marker for rat M cells. Eur J Cell Biol 71: 363–370.
Shaykhiev R, Bals R (2007) Interactions between epithelial cells and leukocytes in immunity and tissue homeostasis. J Leukoc Biol 82: 1–15.
Shi-Jin W, Nan-Hui C (2007) Characteristics of histological location of bursin in sacculus rotundus and gut-associated lumphoid tissues of rabbits. J Shanghai Univ 11: 189–192. doi:10.1007/s11741-007-0219-6

Sierro F, Pringault E, Assman PS, Kraehenbuhl JP, Debard N (2000) Transient expression of M-cell phenotype by enterocyte-like cells of the follicle-associated epithelium of mouse Peyer's patches. Gastroenterology 119: 734–743. doi:10.1053/gast.2000.16481

Snipes RL (1978) Anatomy of rabbit cecum. Anat Embryol 155: 57–80.

Takeuchi T, Gonda T (2004) Cellular kinetics of villous epithelial cells and M cells in rabbit small intestine. J Vet Med Sci 66: 689–693. doi:10.1292/jvms.66.689

Veneable JH, Coggeshall R (1965) A simplified lead citrate stain for use in electron microscopy. J Cell Biol 25: 407–408.

Weinstein PD, Mage RG, Anderson AO (1994) The appendix functions as a mammalian bursal equivalent in the developing rabbit. Adv Exp Med Biol 355: 249–253.