Ultrastructural study on the follicle-associated epithelium of nasal-associated lymphoid tissue in specific pathogen-free (SPF) and conventional environment-adapted (SPF-CV) rats

KWANG IL JEONG, HODAKA SUZUKI, HIROYUKI NAKAYAMA AND KUNIO DOI

Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan

(*Accepted 11 January 2000*)

ABSTRACT

Membranous (M) cells in follicle-associated epithelium (FAE) play an important role in the mucosal immunity through transport of a variety of foreign antigens to the underlying mucosa-associated lymphoid tissue (MALT). We aimed to investigate the ultrastructure of M cells in the FAE covering nasal-associated lymphoid tissue (NALT) both in specific pathogen-free (SPF) rats and in conventional environment-adapted (SPF-CV) rats aged 8–38 wk. In NALT of both SPF and SPF-CV rats, FAE included the nonciliated microvillous cell, which appears to be an analogue of M cell previously described in other MALT. In SPF rats, M cells increased in number only slightly with age, and they maintained morphological uniformity irrespective of age. In SPF-CV rats, M cells selectively increased in number resulting in prominent expansion of FAE surface area in parallel with the duration of maintenance in a conventional environment. In addition, M cells in SPF-CV rats showed heterogeneity in their surface morphology such as the length and number of microvilli and cell surface area and outline. In addition, the FAE was stratified by various subtypes of M cells, which were characterised by several subcellular alterations including the presence of many keratin filaments, homogeneous dark bodies and extensive cytoplasmic interfoliation with wide intercellular spaces filled with amorphous proteinaceous material. These characteristics of M cells in SPF-CV rat were intimately related with a preferential influx of immunocompetent cells into the FAE, which was not seen or was very rare in SPF rats irrespective of age. The results suggest the possibility that NALT may effectively carry out the mucosal immune response against antigenic stimuli of different magnitude through the unique dynamics of M cells which seem to be influenced by the infiltration of immunocompetent cells.

Key words: NALT; M cells; structural heterogeneity; immune surveillance.

INTRODUCTION

The upper respiratory mucosa is the first site that is in contact with airborne infectious agents and it acts as a line of defence against these pathogens. Recently the nasal-associated lymphoid tissue (NALT), which is regarded as an equivalent of Waldeyer's rings in man, has been recognised in the upper respiratory tract of rodents as the only well-organised mucosa-associated lymphoid tissue (MALT) (Spit et al. 1989). The morphology of bronchus-associated lymphoid tissue (BALT) and gut-associated lymphoid tissue (GALT) has been extensively described (Bienenstock et al. 1973; Bockman & Cooper, 1973; Owen & Jones, 1974; Mair et al. 1987), while a detailed morphological description of NALT, especially of the epithelium overlying this lymphoid organ, is lacking.

The follicle-associated epithelium (FAE) contains the membranous (M) cells (Owen & Jones, 1974) which take up and transport macromolecules and pathogenic antigens into the MALT (Owen, 1977; Wolf et al. 1983; Siciński et al. 1990). The passage of antigens through M cells is an essential step for the induction of mucosal immune responses and is involved in the development of many infectious diseases (Amerongen et al. 1991; Morin et al. 1994). Because M cells are a minor population in the FAE in germ-free and specific pathogen-free (SPF) animals, it

Correspondence to Dr Kwang Il Jeong, Department of Veterinary Pathology, Graduate School of Agricultural and Life Science, University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan.

is difficult to characterise their morphology. Several investigators (e.g. Smith et al. 1987) showed that the number of M cells increased when SPF animals were transferred to a conventional (CV) environment. The purpose of this study was to provide detailed information on the ultrastructure of FAE containing M cells in NALT not only of SPF rats but also those transferred to a conventional environment from SPF condition.

MATERIALS AND METHODS

Specific pathogen-free (SPF) male Wistar rats aged 8 wk were obtained from Japan SLC, Shizuoka, Japan. After arrival at our animal facility, the rats were divided into 2 groups. One group of 40 rats was kept under SPF conditions (SPF rats) and the other (64 rats) was maintained under conventional laboratory housing conditions (SPF-CV rats) throughout the experimental period. Eight SPF-CV rats were killed at intervals of 4 wk from 10 to 38 wk. In addition, 8 SPF rats were killed at 8, 10, 14, 18 and 22 wk. During the experiment, SPF rats were routinely checked for common murine pathogens and were confirmed to be free. All rats were killed by decapitation under anaesthesia with diethyl ether.

The nasal tissue was fixed by infusing 2.5% glutaraldehyde in 0.1 M phosphate buffer into the nasal cavity through the posterior opening of the pharyngeal duct. Thereafter, the mucosa was split longitudinally along the median line of the palate to obtain 2 equal halves. After prolonged fixation in the fresh fixative for 4 h at 4 °C, NALT was extirpated from the bony fossa as described previously (Jeong et al. 1999). Fixed tissue was washed in fresh buffer, postfixed in buffer containing 1% OsO_4, and then dehydrated through graded ethanol solutions to absolute ethanol at 4 °C.

For transmission electron microscopy (TEM), small pieces of NALT were then embedded in epoxy resin. Semithin sections were stained with toluidine blue for light microscopic examination. Ultrathin sections perpendicular to the epithelial layer were contrasted with uranyl acetate and lead citrate and observed under a 1200EX electron microscope (JEOL, Tokyo). For scanning electron microscopy (SEM), the whole intact NALT was transferred to absolute amyl acetate, critical-point dried with CO_2 in an HCP-2 critical-point drier (Hitachi, Tokyo), mounted on stubs with the epithelium upwards and then coated using E-1030 ion sputter (Hitachi, Tokyo) for subsequent viewing in S-4000 scanning electron microscope (Hitachi, Tokyo).

RESULTS

Light microscopically, the FAE was simple columnar and usually had direct contact with the basal lamina in SPF rats (Fig. 1*a*), while it was stratified into 2–3 layers of nonciliated microvillous cells in SPF-CV rats (Fig. 1*b*, *c*). The microvillous cells enfolding lymphocytes in the cytoplasmic pocket were sparse in young

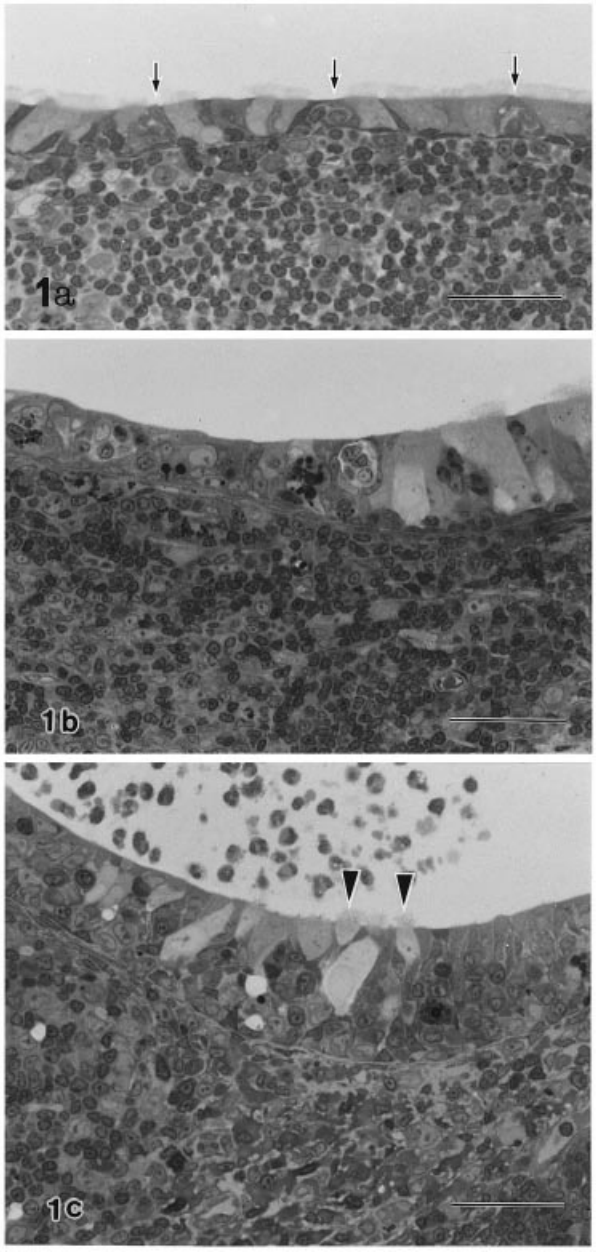

Fig. 1. Light micrographs of FAE in 10 wk SPF rats (*a*), 22 wk (*b*) and 38-wk-old (*c*) SPF-CV rats. Several M cells (arrows), which have no cilia on the luminal surface and enfold lymphocytes in the cytoplasmic pocket, are scattered in the simple columnar FAE (*a*). M cells are prominently increased and stratified into 2–3 layers in the FAE apex where most of the ciliated respiratory epithelial cells have disappeared and the remaining cells (arrowheads) are frequently pushed up toward the nasal lumen in SPF-CV rats (*b*, *c*). Toluidine blue. Bars, 50 μm.

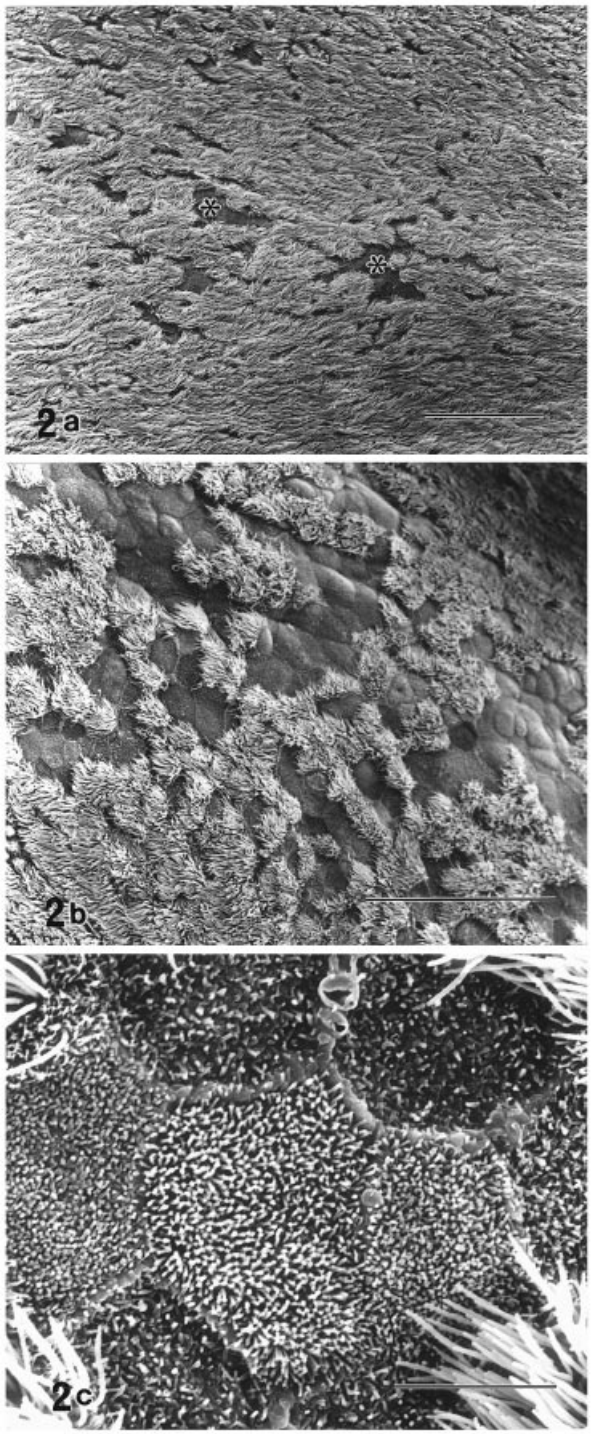

Fig. 2. Scanning electron micrographs of FAE in 10 wk (*a*) and 22-wk-old (*b*, *c*) SPF rats. M cells (asterisks) have numerous short microvilli, and are readily distinguishable from the adjacent respiratory epithelial cells with long cilia. They increase slightly in number with age (*a*, *b*) and sometimes have cytoplasmic protrusions along cell boundaries (*c*). They show a comparatively uniform contour irrespective of age. Bars: *a*, *b*, 50 μm; *c*, 5 μm.

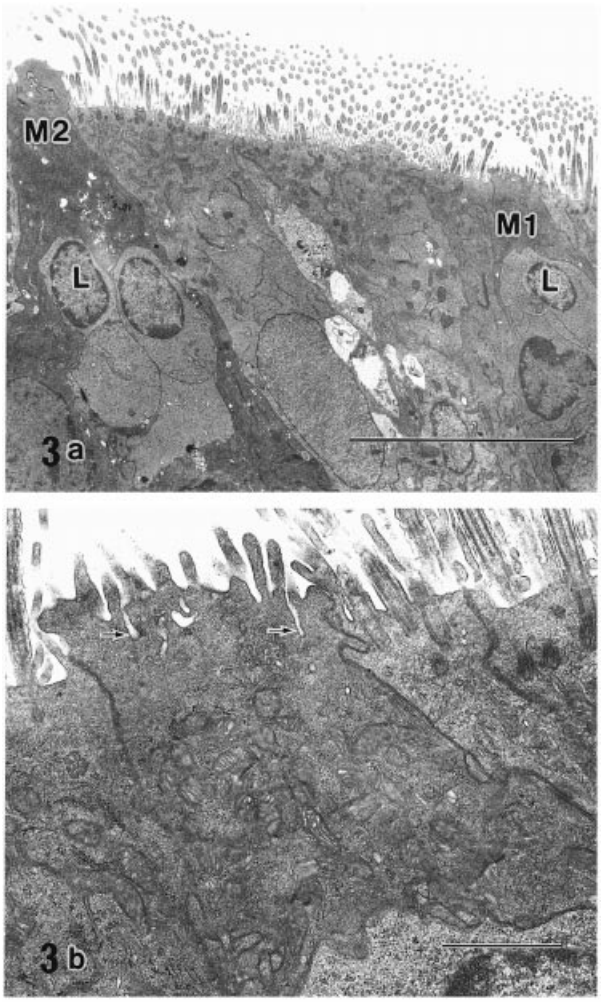

Fig. 3. Transmission electron micrographs (TEM) of FAE in 10-wk-old SPF rats. M cells (M1, M2) enfold 2 to 3 lymphocytes (L) in cytoplasmic pockets (*a*). One M cell (M2) possesses a blunt fold in the luminal surface and several variable-sized vesicles in the apical cytoplasm which is stained darker than that of adjacent ciliated cells. The other M cell (M1), shown at higher magnification in *b*, has short microvilli on its irregular luminal surface, many mitochondria and several invaginating pits (arrows) in the apical cytoplasm. Bars: *a*, 10 μm; *b*, 2 μm.

SPF rats and only slightly increased in number even in older SPF rats. On the other hand, a conspicuous increase in number of microvillous cells occurred with age in SPF-CV rats, although they were restricted almost entirely to the apex of FAE which became heavily infiltrated with lymphocytes. In addition, most of the ciliated respiratory cells at that site gradually disappeared and those remaining were frequently pushed up towards the nasal lumen (Fig. 1*c*).

In SPF rats, FAE included a characteristic population of nonciliated microvillous cells with a polygonal cell surface in SEM observations (Fig. 2). Those cells were easily distinguished from the adjacent respiratory cells which had numerous long cilia on their surface. They were often scattered in small groups and increased only slightly in number with the age in SPF rats, resulting in moderate expansion of the FAE surface area (Fig. 2*a*, *b*). They had numerous

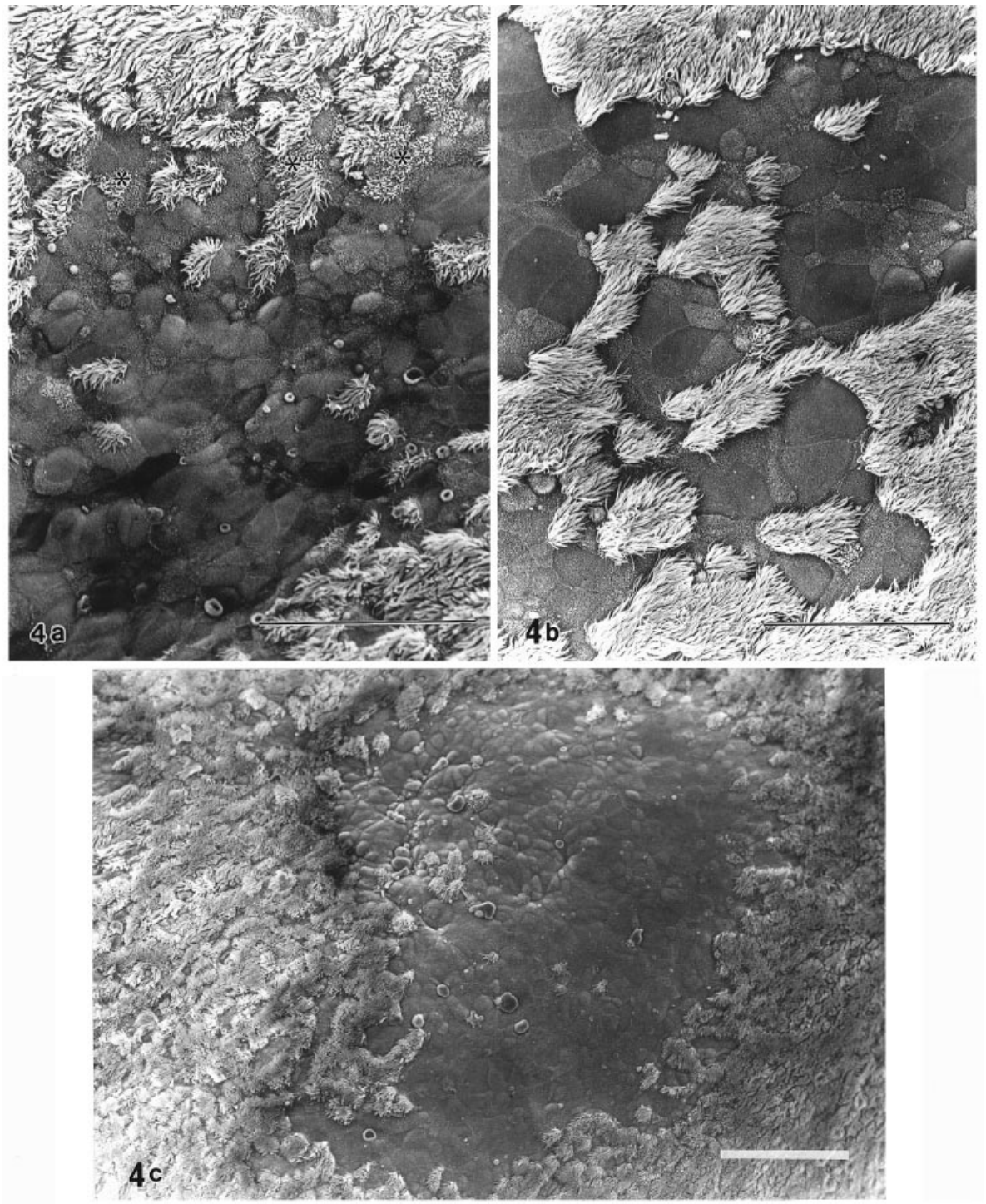

Fig. 4. For legend see opposite.

short microvilli showing only slight variations in number, density and length, but sometimes displayed slight cytoplasmic protrusions along the cell margin (Fig. 2*c*). TEM also showed small groups of nonciliated microvillous cells. They possessed irregular microvilli on the luminal surface and contained variable-sized vacuoles, many mitochondria and several invaginating pits in the cytoplasm, which was stained darker than that of the adjacent ciliated respiratory cells (Fig. 3*a*, *b*). These cells were found immediately above the dome area of each small lymphoid follicle and were usually columnar in shape. They were infiltrated by slightly increased numbers of lymphocytes in older rats.

In SPF-CV rats, the FAE surface area expanded in proportion to the duration of maintenance in a conventional environment. This was mainly due to an increase in number of nonciliated microvillous cells

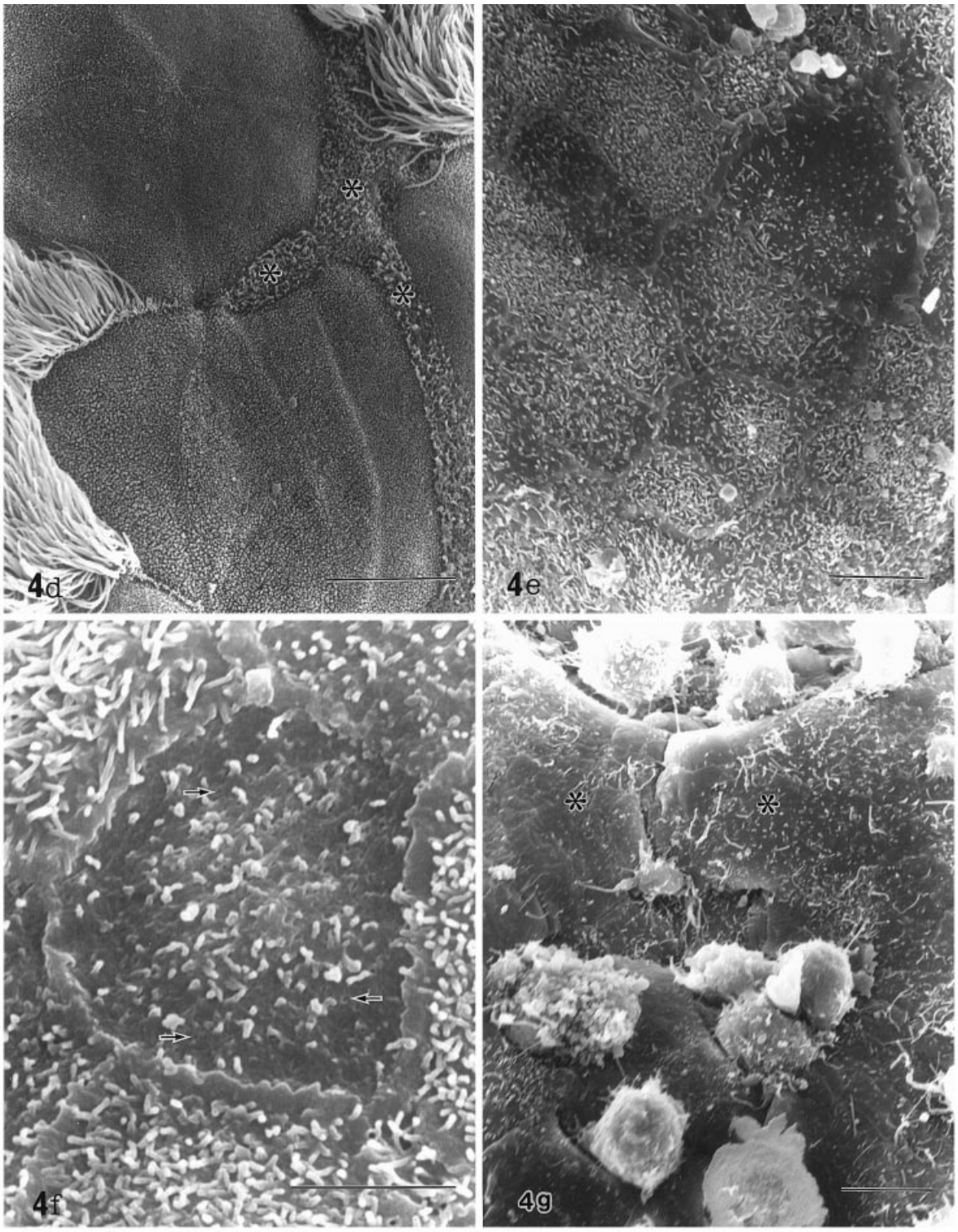

Fig. 4. SEM of FAE in 14 wk (*a*), 22 wk (*b*), 30 wk (*c*, *d*) and 38-wk-old (*e–g*) SPF-CV rats. After transfer to conventional environment, M cells have increased in number and cover most of the expanded FAE area (*a*, *c*) where the ciliated respiratory cells (asterisks in *a*, *d*) which have gradually disappeared possess sparse cilia and slender microvilli. M cells show the heterogeneities in the cell surface area (*b*), and in the length and density of microvilli (*d*, *e*, *g*). Some are characterised by a cell boundary with prominent protrusions (*e*, *f*), numerous pinpoint pits (arrows in *f*), or an almost smooth luminal surface (asterisks in *g*). Bars: *a–c*, 50 μm; *d–g*, 5 μm.

(Fig. 4*a*, *c*). The nonciliated microvillous cells had various contours of luminal surface (Fig. 4*b*) and covered most of the FAE apex area (Fig. 4*c*), where few ciliated cells remained. These nonciliated cells showed prominent heterogeneities in the length and number of microvilli and frequently displayed cytoplasmic protrusions along cell to cell borders (Fig. 4*d–g*) compared with those in SPF rats. Such a

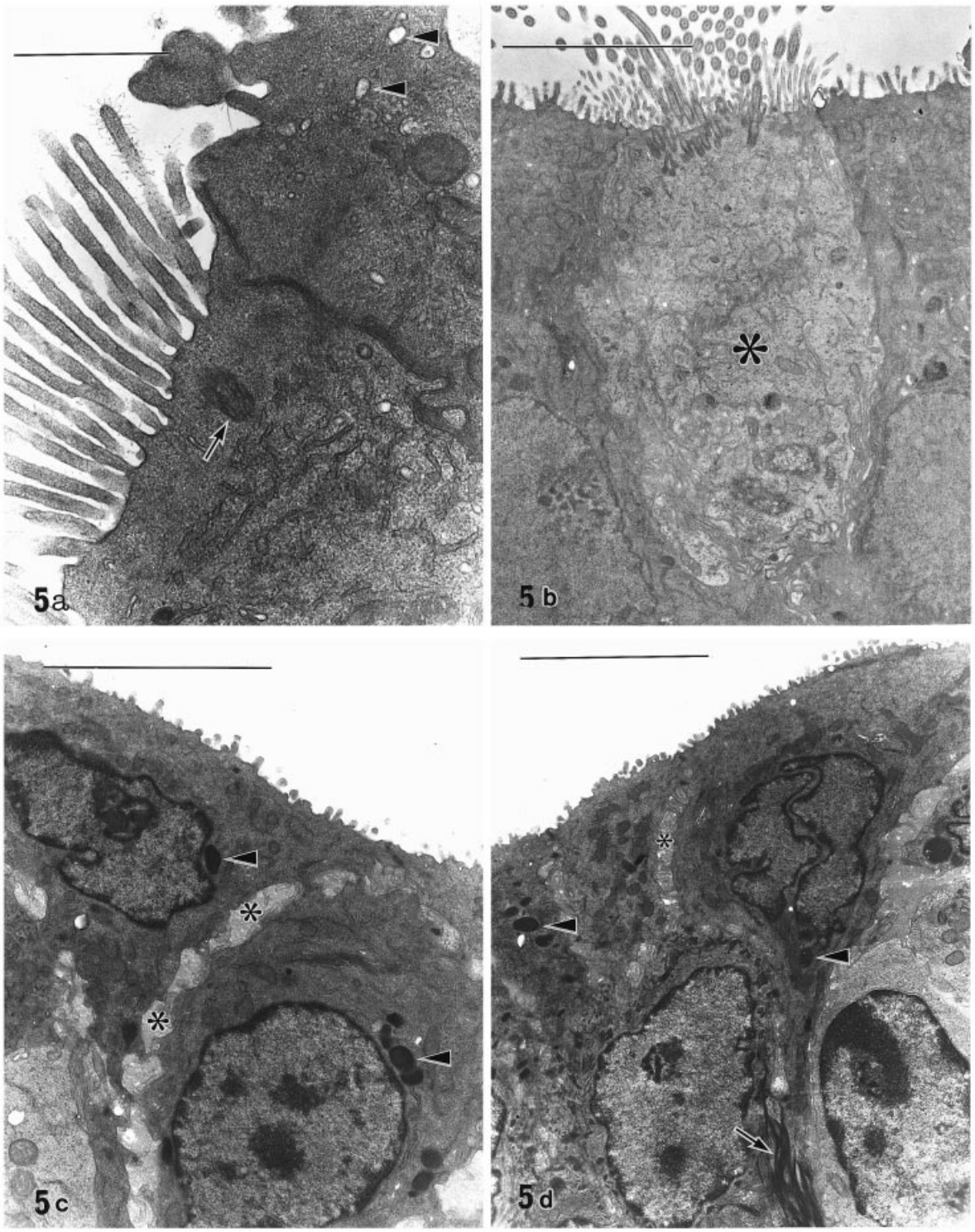

Fig. 5. For legend see opposite.

variation in the surface structure of nonciliated cells became more prominent in parallel with the duration of maintenance under conventional conditions. In the older SPF-CV rats, some of the microvillous cells were marked by numerous pinpoint pits in the surface (Fig. 4*f*) or revealed an almost smooth apical surface due to absence of the unique microvilli (Fig. 4*g*).

The ciliated epithelial cells were scattered among nonciliated microvillous cells or in the periphery of FAE of NALT. They possessed sparser and shorter cilia and more slender microvilli in the FAE than those in the surrounding respiratory epithelium, and frequently had a narrow or elongated cell surface compressed by the adjacent nonciliated microvillous cells (Fig. 4*a*, *d*). Such a loss of cilia was confined almost entirely to the ciliated cells in the FAE region,

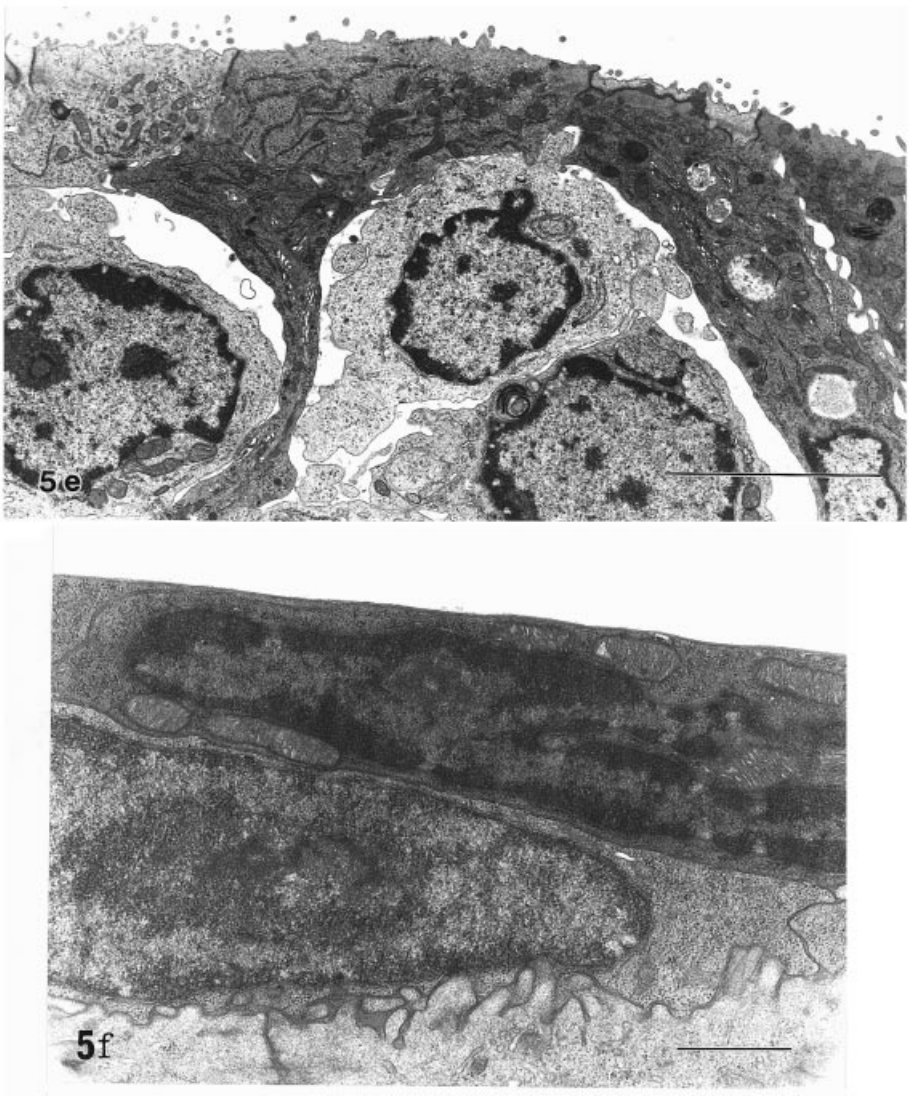

Fig. 5. TEM of FAE in 10 wk (*a*), 22 wk (*b*), 30 wk (*c*, *d*) and 38-wk-old (*e*, *f*) SPF-CV rats. The ciliated respiratory cells residing in the FAE have lost most of their cilia but have numerous slender microvilli, and still possess a basal body (arrow in *a*) in the apical cytoplasm. M cells have several vesicles in their cytoplasm (arrowhead in *a*) and an irregular luminal contour. In parallel with the duration of maintenance under conventional conditions. The ciliated respiratory cells (asterisk in *b*) frequently contain necrotic organelles in their cytoplasm and are pushed towards the nasal lumen. The FAE is stratified stepwise into 2–3 layers of various types of M cells (*c–f*). They show extensive cytoplasmic interfoliation with the intercellular space being filled with proteinaceous material (asterisks in *c*, *d*), and have electron dense bodies (arrowheads in *c*, *d*) and many keratin filaments (arrow in *d*) in their cytoplasm. M cells enfold several lymphocytes or macrophages in their attenuated cytoplasm (*e*) and have sparse (*c–e*) or limited numbers of microvilli (*f*) on the luminal surface. Bars: *a*, *f*, 1 μm; *b–e*, 5 μm.

and progressed moderately according to the duration of maintenance under conventional conditions.

TEM revealed that the respiratory cells in the FAE maintained the characteristics of ciliated cells such as ciliary rootlets or basal bodies in their apical cytoplasm (Fig. 5*a*), although some had sparse short cilia and showed similar luminal surface appearances to those of the nonciliated microvillous cells as described above (Fig. 4*a*, *d*). Moreover, the ciliated cells in the FAE region were frequently pushed upwards by the neighbouring microvillous cells, and contained autophagosomes and necrotic organelles in their cytoplasm (Fig. 5*b*), especially in older SPF-CV rats.

On the other hand, the nonciliated microvillous cells in young SPF-CV rats had similar features to those in SPF rats in that they enfolded lymphocytes in cytoplasmic pockets and possessed irregular microvilli on their luminal surfaces and several vesicles in the apical cytoplasm (Fig. 5*a*). The FAE became stratified stepwise into 2–3 layers of cuboidal to columnar or flat cells, which usually showed a heterogeneous morphology compared with the microvillous cells in SPF rats, as the duration for which they were kept under conventional conditions became longer (Fig. 5*c–f*). The suprabasal cells in the FAE showed extensive interfoliation with some wide intercellular

spaces filled with an amorphous proteinaceous material, and frequently had homogeneous dark spherical bodies in their cytoplasm and many keratin filaments around their nuclei (Fig. 5*c*, *d*). In addition, the superficial cells possessed sparse stubby microvilli on their surface, and had several vesicles and many mitochondria in their cytoplasm (Fig. 5*c–e*). Some of the flattened cells in the most superficial layer of stratified FAE had almost no microvilli on the luminal surface and contained few organelles in the cytoplasm (Fig. 5*f*). In general, the FAE showed more intimate contact with lymphocytes or macrophages, which were found frequently in the apex of FAE rather than in the periphery, in SPF-CV rats (Fig. 5*e*) than in SPF rats. The epithelial cells in FAE, most of which lost direct contact with basal lamina, came to be heavily infiltrated with lymphocytes or macrophages in SPF-CV rats depending on the length of time that they were kept under conventional conditions.

DISCUSSION

Detailed ultrastructural observations were made to clarify the morphological characteristics of M cells in the FAE of both SPF and SPF-CV rats at various ages. Previous reports have shown that immunocompetent cells in the nasal mucosa are fewer in number and immunologically less mature in germ-free (GF) and SPF animals than in CV animals (Ichimiya et al. 1991), and the numbers of T and B cells in NALT are 2–3 times greater after an experimental infection (Asanuma et al. 1997). In the present study, NALT had more numerous and well-developed follicles in SPF-CV rats than in age-matched SPF rats. Furthermore, the FAE surface area was considerably expanded in SPF-CV rats, while it only increased slightly in SPF rats according to age. These results support the hypothesis that NALT plays an important role in mucosal immunity against foreign antigens in the upper respiratory tract. In addition, the expansion of FAE surface area in the present SPF-CV rats was mainly due to an increase in number of the nonciliated microvillous cells, which are thought to be the homologues of the M cells previously described in GALT or BALT (Bienenstock et al. 1973; Bockman & Cooper, 1973; Spit et al. 1989). Accordingly, they possess an irregular outline of the apical membrane, which is devoid of cilia and carries microvilli. They also contain variable-sized vacuoles and invaginated pits in their apical cytoplasm, and enfold lymphocytes in cytoplasmic pockets. The present result is completely compatible with the previous finding that exposure of GF mice to a selected pathogen causes a prominent increase in number of M cells in Peyer's patches (Savidge et al. 1991).

The expansion of FAE surface area in SPF-CV rats was mainly due to an increase in number of M cells, and was accompanied by morphological alterations in M cells. SEM observation showed that M cells maintained a uniformity in surface structure in SPF rats irrespective of age, while they revealed marked heterogeneities in the length and number of microvilli as well as in the individual cell surface area and outline in SPF-CV rats. The surface heterogeneity in NALT of SPF-CV rats seems to be intimately related to the presence of the various subtypes of M cells interfacing the luminal surface. In the present TEM examination, M cells facing the nasal lumen had a relatively uniform columnar appearance in SPF rats, whereas they extended into the lumen with a variety of shapes, flat to cuboidal or columnar, in SPF-CV rats.

M cells, especially in SPF-CV rats, present a heterogeneous structure which may be necessary for expansion of the 3-dimensional space for the influx of lymphocytes and macrophages into FAE (Regoli et al. 1995). Furthermore, such a spatial expansion could be acquired by a stratification of the FAE consisting of various subtypes of M cells with columnar to cuboidal or flat appearances as shown in the present SPF-CV rats. In fact, the infiltration of immunocompetent cells into the FAE containing M cells was increased when the duration of maintenance under conventional conditions was protracted. Lymphocyte migration into M cells therefore seems to be an antigen-dependent event (Regoli et al. 1994), and the immune cells in the FAE appear to be able to induce an increase in the number of M cells (Förster et al. 1996) and alterations in the FAE (Borghesi et al. 1996). This structural heterogeneity, however, cannot be explained simply by the difference in the stage of M cell maturation (Spit et al. 1989). In turn, there is a possibility that the excessive influx of immunocompetent cells into FAE might subsequently induce some subcellular changes in M cells (Regoli et al. 1994). This is also suggested by the present observation that M cells in SPF-CV rats showed a relative increase in keratin filaments and electron-dense spherical bodies, and extensive cytoplasmic interfoliation. These subcellular structures were rarely found in SPF rats, and might be a characteristic of M cells in SPF-CV rats undergoing metaplasia (Boysen, 1984) as well as differing or altered stages of cell maturation (Bye et al. 1984; Spit et al. 1989). Accordingly, these subcellular differences of M cells between SPF and SPF-CV rats perhaps imply that the

maturation and differentiation of M cells in NALT can be influenced by a close association with immunocompetent cells (Smith & Peacock, 1980; Savidge et al. 1991). The infiltration of these cells into FAE is subject to modifications in environmental conditions such as SPF on conventional conditions in which the magnitude of antigenic stimuli differs. However, there is also the possibility that the subcellular changes in M cells can be caused directly by antigenic stimulation under conventional conditions (Borghesi et al. 1996).

In NALT of SPF-CV rats, M cells were increased to become the main subpopulation within the FAE where most of the respiratory ciliated cells and goblet cells disappeared. Many of the ciliated and goblet cells that remained had autophagosomes and other necrotic organelles in their electron-lucent cytoplasm which was shrunken or elongated under the pressure of adjacent microvillous cells. The selective increase of M cells in the FAE could be influenced not only by direct contact with immune cells but also by some humoral mediators (Kernéis et al. 1997), which originate from those immune cells activated during antigenic stimuli and afford the pluripotent cells a priority of differentiation into M cells as suggested previously (Bye et al. 1984; Savidge et al. 1991).

In conclusion, the present results suggest (1) that the number of M cells is selectively increased within the FAE of NALT, (2) that the structural heterogeneity of M cells may be closely related to the presence of intraepithelial immune cells, and (3) that they contribute to control the overall NALT response to respiratory infection. However, further investigation is needed to clarify the genesis of M cells in NALT and their exact function under various environmental conditions.

REFERENCES

AMERONGEN HM, WELTZIN RA, FARNET CM, MICHETTI P, HASELTINE WA, NEUTRA MR (1991) Transepithelial transport of HIV-1 by intestinal M cells: a mechanism for transmission of AIDS. *Journal of Acquired Immune Deficiency Syndromes* **4**, 760–765.

ASANUMA H, THOMPSON AH, IWASAKI T, SATO Y, INABA Y, AIZAWA C et al. (1997) Isolation and characterization of mouse nasal-associated lymphoid tissue. *Journal of Immunological Methods* **202**, 123–131.

BIENENSTOCK J, JOHNSTON N, PEREY DYE (1973) Bronchial lymphoid tissue. I. Morphologic characteristics. *Laboratory Investigation* **28**, 686–692.

BOCKMAN DE, COOPER MD (1973) Pinocytosis by epithelium associated with lymphoid follicles in the bursa of Fabricius, appendix, and Peyer's patches: an electron microscopic study. *American Journal of Anatomy* **136**, 455–478.

BORGHESI C, REGOLI M, BERTELLI E, NICOLETTI C (1996) Modifications of the follicle-associated epithelium by short-term exposure to a non-intestinal bacterium. *Journal of Pathology* **180**, 326–332.

BOYSEN M (1984) The surface structure of the human nasal mucosa: I. Ciliated and metaplastic epithelium in normal individuals. *Virchows Archiv B: Cell Pathology* **40**, 279–294.

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.

FÖRSTER R, MATTIS AE, KREMMER E, WOLF E, BREM G, LIPP M (1996) A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. *Cell* **87**, 1037–1047.

ICHIMIYA I, KAWAUCHI H, FUJIYOSHI T, TANAKA T, MOGI G (1991) Distribution of immunocompetent cells in normal nasal mucosa: comparisons among germ-free, specific pathogen-free, and conventional mice. *Annals of Otology, Rhinology and Laryngology* **100**, 638–642.

JEONG KI, UETSUKA K, NAKAYAMA H, DOI K (1999) Glycoconjugate expression in follicle-association epithelium (FAE) covering the nasal-associated lymphoid tissue (NALT) in specific pathogen-free and conventional rats. *Experimental Animal* **48**, 23–29.

KERNÉIS S, BOGDANOVA A, KRAEHENBUHL J-P, PRINGAULT E (1997) Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. *Science* **277**, 949–952.

MAIR TS, BATTEN EH, STOKES CR, BOURNE FJ (1987) The histological features of the immune system of the equine respiratory tract. *Journal of Comparative Pathology* **97**, 575–586.

MORIN MJ, WARNER A, FIELDS BN (1994) A pathway for entry of reoviruses into the host through M cells of the respiratory tract. *Journal of Experimental Medicine* **180**, 1523–1527.

OWEN RL (1977) Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultrastructural study. *Gastroenterology* **72**, 440–451.

OWEN RL, JONES AL (1974) Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. *Gastroenterology* **66**, 189–203.

REGOLI M, BORGHESI C, BERTELLI E, NICOLETTI C (1994) A morphological study of the lymphocyte traffic in Peyer's patches after an in vivo antigenic stimulation. *Anatomical Record* **239**, 47–54.

REGOLI M, BERTELLI E, BORGHESI C, NICOLETTI C (1995) Three-dimensional (3D) reconstruction of M cells in rabbit Peyer's patches: definition of the intraepithelial compartment of the follicle-associated epithelium. *Anatomical Record* **243**, 19–26.

SAVIDGE TC, SMITH MW, JAMES PS, ALDRED P (1991) *Salmonella*-induced M-cell formation in germ-free mouse Peyer's patch tissue. *American Journal of Pathology* **139**, 177–184.

SICIŃSKI P, ROWIŃSKI J, WARCHOŁ JB, JARZABEK Z, GUT W, SZCZYGIEŁ B et al. (1990) Poliovirus type 1 enters the human host through intestinal M cells. *Gastroenterology* **98**, 56–58.

SMITH MW, PEACOCK MA (1980) "M" cell distribution in follicle-associated epithelium of mouse Peyer's patch. *American Journal of Anatomy* **159**, 167–175.

SMITH MW, JAMES PS, TIVEY DR (1987) M cell numbers increase after transfer of SPF mice to a normal animal house environment. *American Journal of Pathology* **128**, 385–389.

SPIT BJ, HENDRIKSEN EGJ, BRUIJINTJES JP, KUPER CF (1989) Nasal lymphoid tissue in the rat. *Cell and Tissue Research* **255**, 193–198.

WOLF JL, KAUFFMAN RS, FINBERG R, DAMBRAUSKAS R, FIELDS BN, TRIER JS (1983) Determinants of reoviruses interaction with the intestinal M cells and absorptive cells of murine intestine. *Gastroenterology* **85**, 291–300.