

Morphological changes of myoepithelial cells of mouse lacrimal glands during postnatal development

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Summary. To reveal the correlation between myoepithelial cell configuration and size/shape of glandular endpieces (acini), we observed postnatal developmental changes of myoepithelial cells in the lacrimal glands of mice. Glandular and myoepithelial cells were examined in paraffin sections and in isolated acini. In newborns, rudiments of acini showed no clear lumina and glandular cells had few secretory granules. There were no myoepithelial cells with actin. At 3 days after birth, some rudiments showed lumina; however, secretory granules were not salient. Round cells in the periphery of the acini showed immunoreactivity for actin. In 1-week-old mice, glandular cells were polarized: luminal cytoplasm contained some secretory granules, and nuclei were located basally. Most myoepithelial cells were flattened and sometimes projected thin processes in various directions. At 2 weeks, the glandular cells increased their size and contained numerous secretory granules, and the myoepithelial cells were almost stellate. In 4-8 week-old mice, acini increased their size, and myoepithelial cells were very thin and processes were prolonged in length and increased in number. During postnatal development, their distribution of myoepithelial cells was more scarce, while the size of acini increased. This reciprocal relation of myoepithelial cell distribution and acinar size may indicate that the changes of myoepithelial cell configurations depend on the change of acinus size.

Key words: Myoepithelial cell, Lacrimal gland, Postnatal development, Morphometry, Mouse

Introduction

Myoepithelial cells (*myoepitheliocytus*) are flat contractile cells with processes that embrace the glandular endpieces and small ducts of many mammalian exocrine glands (e.g., mammary, salivary,

sweat, lacrimal, and Harderian glands). In mammary glands, the myoepithelial-cell contraction can reduce the luminal volume in glandular endpieces, and these cells play a role in expelling secretory products from glandular endpieces to the excretory duct system (Pitelka et al., 1973; Moore et al., 1987). The expelling role of myoepithelial cells in mammary glands has often been generalised to any gland (e.g. Fawcett, 1986).

The configuration of myoepithelial cells was quite different between various exocrine glands, and the morphological variety suggested that a generalised assignment of a myoepithelial-cell role was very difficult (Satoh et al., 1994). If myoepithelial cell contraction plays a role in expelling secretory products from glandular endpieces to the excretory duct system, the configuration and arrangement of myoepithelial cells can be correlated with the nature of the secretory products (Nagato et al., 1980; López and Alvarez-Uría, 1993) and/or the luminal width of the glandular endpieces. However the configuration of myoepithelial cells can also depend on the outer contour of the glandular endpieces (Satoh et al., 1994).

Emerman and Vogl (1986) reported that the myoepithelial cells covering large glandular endpieces in lactating mammary gland were stellate, whereas myoepithelial cells in virgin glands on the ducts and small and nipple-like gland buds were spindle-shaped. Myoepithelial cell configuration can be changeable at different maturation stages of a gland; however, it has not been ascertained whether the myoepithelial cell change is accompanied with the presence of secretory materials in glandular cells and/or with size/shape of glandular endpieces. To clarify this, in the present study, we observed the postnatal development of the lacrimal glands of mice. In lacrimal glands, glandular endpieces (acinus) were covered with a myoepithelial cell envelope (Leeson, 1960; Leeson and Leeson, 1971). In newborn mice with the eyelid closed the lacrimal apparatus cannot function well, but gradually develops. The observation of postnatal development of lacrimal apparatus in rodents is suitable for evaluation of the correlation between the functional stages of glandular

cells and the changes of myoepithelial cell configuration.

Materials and methods

Animals

Albino mice (BALB/C; both sexes, aged 0 day, 3 days, 1 week, 2 weeks, 4 weeks, and 8 weeks) were used in the present study. They were bred and housed in plastic cages, in the Animal Laboratory for Medical Research of Asahikawa Medical College under constant conditions (temperature 22 °C, Light: Dark= 14:10 hours with light from 5 a.m. to 7 p.m.). They were given commercial food pellets and water ad libitum.

Paraffin sections

The animals (number of animals: aged 0 day, n= 6; 3 days, n= 5; 1 week, n= 5; 2 weeks, n= 6; 4 weeks, n= 6; and 8 weeks, n= 6) were anaesthetised by ether, thoracotomized and then perfused via the left cardiac ventricle with a fixative containing 4% paraformaldehyde (about 4 °C; phosphate buffered, pH 7.4) for several minutes. Exorbital lacrimal glands were taken out and then additionally immersed in the same fixative overnight in a refrigerator. The fixed tissues were dehydrated and embedded in paraffin. Sections (ca. 5 µm thick) were cut, deparaffinized, and some of them were stained with haematoxylin-eosin.

Other deparaffinized sections were incubated in anti-smooth muscle actin antibody (A-2547, Sigma, St. Louis, MO, USA), diluted 1:6000, for 24 hours in a refrigerator, and then incubated with biotinylated antibody diluted 1:200 (BA-2000, Vector Laboratories, Burlingame, CA, USA) and with the ABC reagent (Vectastain Elite ABC kit, Vector) for 120 minutes, each at room temperature (Hsu et al., 1981). Subsequently, the specimens were developed for 5-10 minutes in 0.02% 3,3'-diaminobenzidine containing 0.01% hydrogen peroxide in 0.025M TRIS-HCl buffer (pH 7.6). After dehydrating them in ethyl alcohol, we mounted the sections in Entellan (Merck, Darmstadt, FRG) and observed them under a light microscope. The specificity of the immunohistochemical staining was confirmed by replacing the primary antiserum with nonimmune swine serum.

Myoepithelial cells of isolated acini

To get whole images of myoepithelial cell configuration, we observed those in isolated glandular endpieces (acini) from mice (n= 6 in each group). Details have been previously described (Sato et al., 1994). Briefly, after decapitation, exorbital lacrimal glands were taken out and immersed in oxygenated HEPES-buffered Ringer solution (HR) (37 °C, pH 7.4, adjusted with NaOH) containing collagenase (Type I, Sigma) for 45 minutes with constant agitation (120 c/min) to digest the connective tissue. Finally, slight

pipetting yielded the isolated acini. After being attached onto the glass slide by Cell-Tak (Collaborative Res., Bedford, Mass., USA), isolated acini were fixed with 4% paraformaldehyde in a refrigerator. Myoepithelial cells were stained by Bodipyl FL-phalloidin (1 U/ 5 µl, B-607, Molecular Probe, Eugene, OR, USA) for 20-30 minutes. Cells containing huge amounts of filamentous actin (F-actin) can be easily stained by phalloidin (Wulf et al., 1979). The specimens were mounted in Permafluor (Shandon/Lipshaw/Immunon, Pittsburgh, PA, USA), and observed under a fluorescent microscope or a laser scanning confocal microscope (MRC-600, Bio-Rad Microscience Division, Hemphstead, UK).

Morphometrical analysis

Sections immunohistochemically stained for actin were photographed. Two photographs were taken per animal (magnification at x350, and showing about 400,000 µm² of tissue area).

The following parameters were measured by a digitizer (Nikon Cosmозome II S, Tokyo, Japan) with a personal computer (NEC PC-9801 VX, Tokyo, Japan): 1) total area of acinus profile (including glandular and myoepithelial cells); and 2) areas of myoepithelial cell profiles showing actin immunoreactivity in acini. From these values, the ratios of myoepithelial cell areas to acinus areas were obtained. Mean values and standard deviation were calculated, and measured values were statistically analysed by ANOVA. The level of significance was set at $p \leq 0.05$.

Results

In newborn (aged 0 day) mice, epithelial cells clustered in the subcutaneous connective tissue at the temporal region. The clusters were oval and ellipsoid (ca. 100 µm in diameter), and were rudiments of acini of the lacrimal gland. Glandular cells and myoepithelial cells were not differentiated under a light microscope (Fig. 1a). No rudiment showed a clear lumen. There were no cells containing secretory granules. Each rudiment was separated by interstitial connective tissue. There were no cells immunohistochemically stained by anti-actin antibody in the rudiments (Fig. 2a). In this stage, the rudiments were very fragile to the collagenase digestion procedure, so we could not isolate the intact rudiments, but epithelial cells dispersed easily.

At 3 days after birth, the number of rudiment profiles in each section increased, while their size decreased (about 50-80 µm in diameter). Clear lumina were recognised in some rudiments; however, no glandular cells contained secretory granules (Fig. 1b). Several rudiments were grouped, although connective tissue intervening between rudiments was clearly observed. Many cells in the peripheral region of the rudiments showed faint immunoreactivity for actin (Fig. 2b). These immature myoepithelial cells were almost round, and few processes were observed. About 16% of

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the epithelial cells showed immunoreactivity for actin (Fig. 4). In the isolated specimens, each isolated rudiment was round or oval in shape, and a few rudiments clustered. However, at this stage, the myoepithelial cells were not clearly stained by Bodipy FL-phalloidin (data is not shown). It is likely that the immature myoepithelial cells contained little F-actin,

and we could not visualise the myoepithelial cell configuration.

At 1 week after birth, the glandular cells accumulated secretory granules in their supranuclear regions, and the nuclei located basally: epithelial cells differentiated to polarised secretory cells. Glandular endpieces (acini) and ducts were clearly distinguished

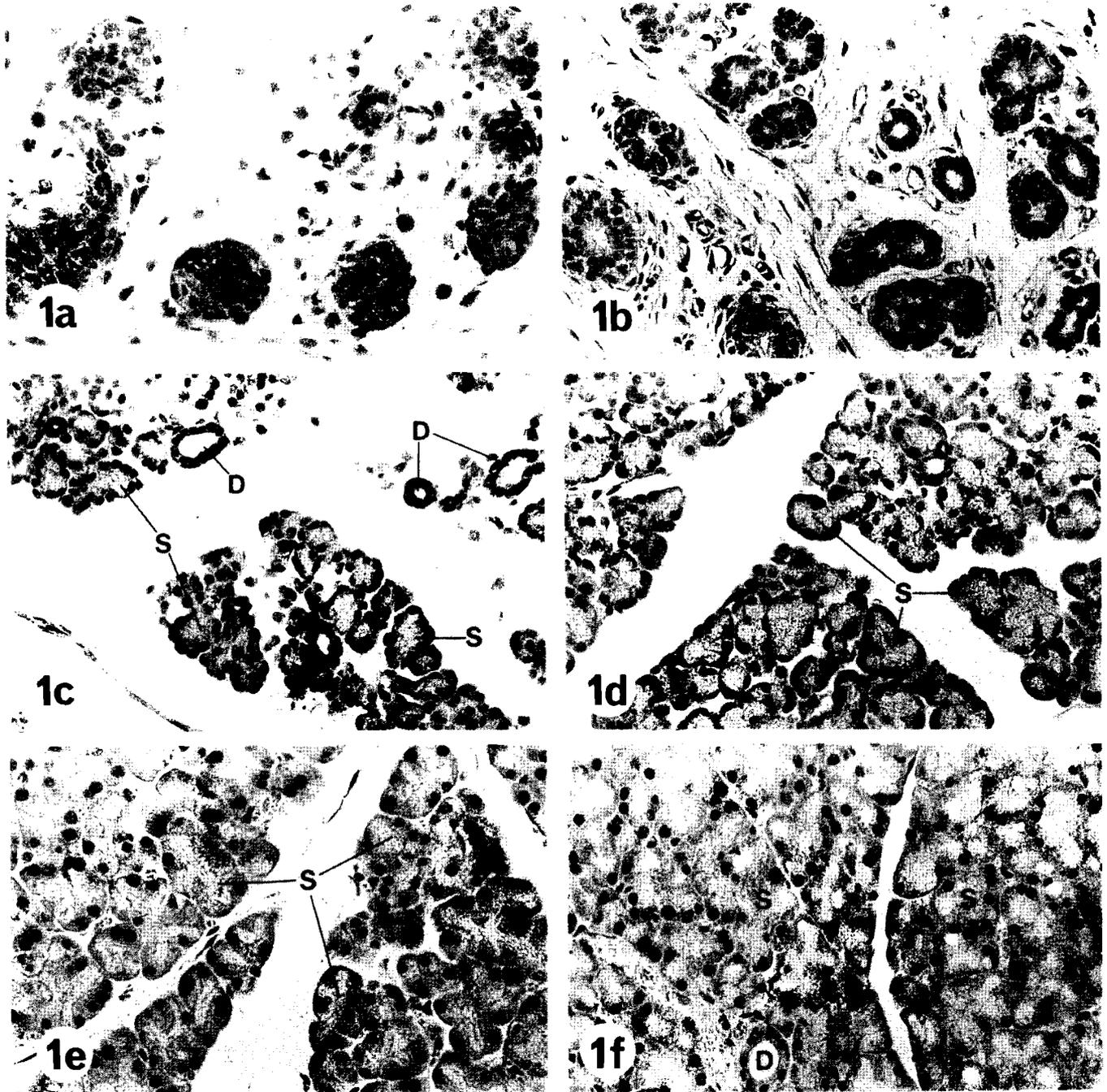


Fig. 1. Paraffin sections stained by haematoxylin-eosin. Lacrimal glands at birth (**a**), at 3 days after birth (**b**), at 1 week after birth (**c**), at 2 weeks after birth (**d**), at 4 weeks after birth (**e**), and at 8 weeks after birth (**f**). Glandular cells containing secretory granules (S), and ducts (D) are recognized at 1 week after birth. a-f, x 150

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(Fig. 1c). Many acini (about 35-40 μm in diameter) were grouped and glandular lobules were formed. After the isolation procedure, it was hard to isolate each acinus, and many acini clustered. In the periphery of the acini, many myoepithelial cells stained for actin. They were flat and prolonged with a few short processes around the

acini. Extremities of the processes were blunt or tapered. Most myoepithelial cells at this stage were starfish-like or maple leaf-like in shape, and some were fusiform (Figs. 2c, 3a). No contact among myoepithelial cells was observed. The central cell bodies were stained diffusely by Bodipy FL-phalloidin, and actin bundles were not

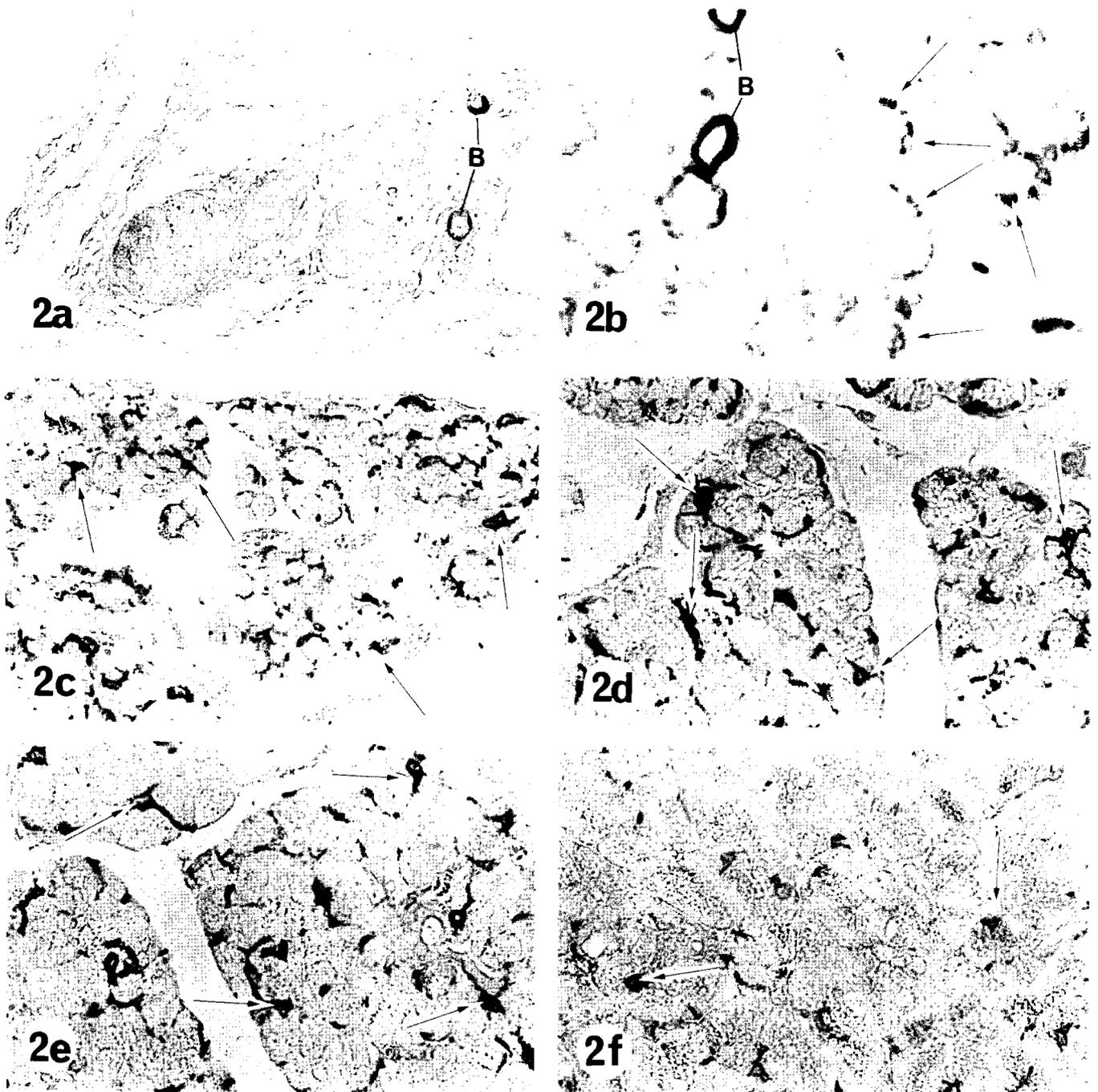


Fig. 2. Paraffin sections stained by anti-actin antibody. Lacrimal glands in newborn (a), at 3 days after birth (b), at 1 week after birth (c), at 2 weeks after birth (d), at 4 weeks after birth (e), and at 8 weeks after birth (f). In newborn, only muscles of blood vessel (B) are stained, and after 3 days myoepithelial cells (arrows) are seen. a-f, x 150

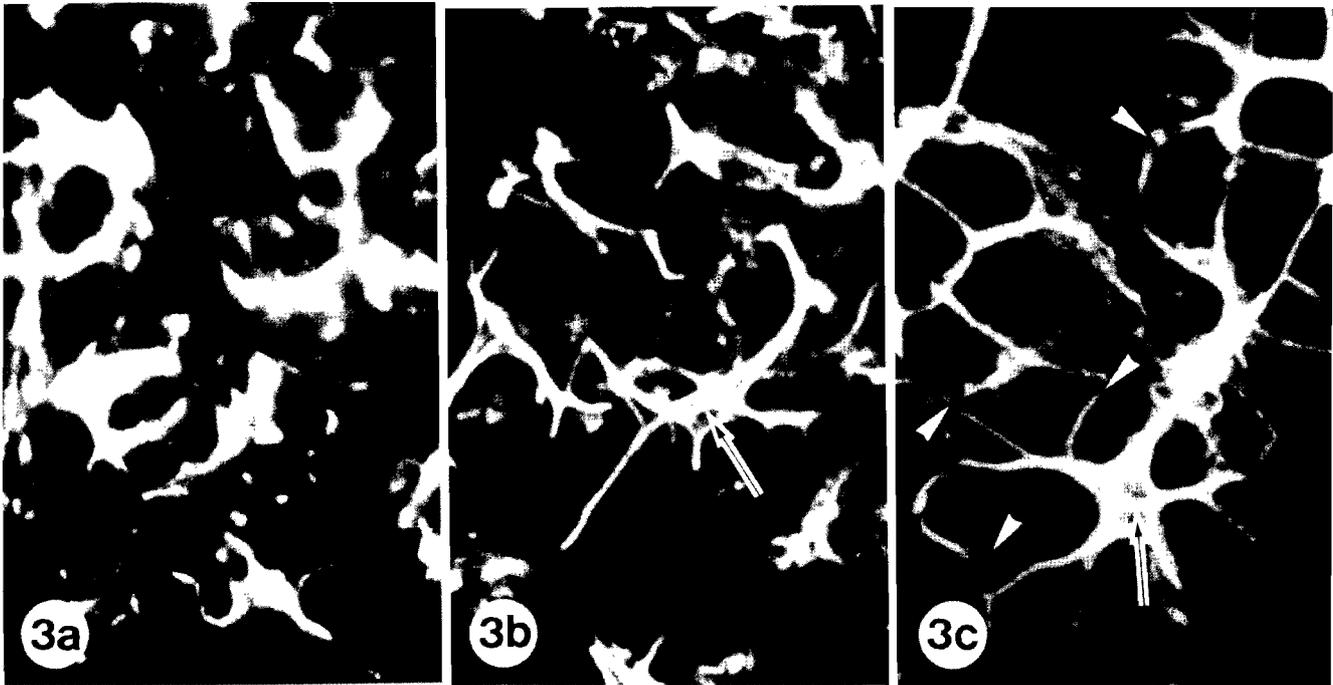


Fig. 3. Myoepithelial cells in isolated acini. Laser scanning micrographs show myoepithelial cells, stained by phalloidin, at 1 week after birth (a), at 2 weeks after birth (b), and 4 weeks after birth (c). Actin bundles are recognized (arrows), and the processes of myoepithelial cells at 4 weeks after birth often contact each other (arrowheads). a and b, x 700; and c, x 1,100

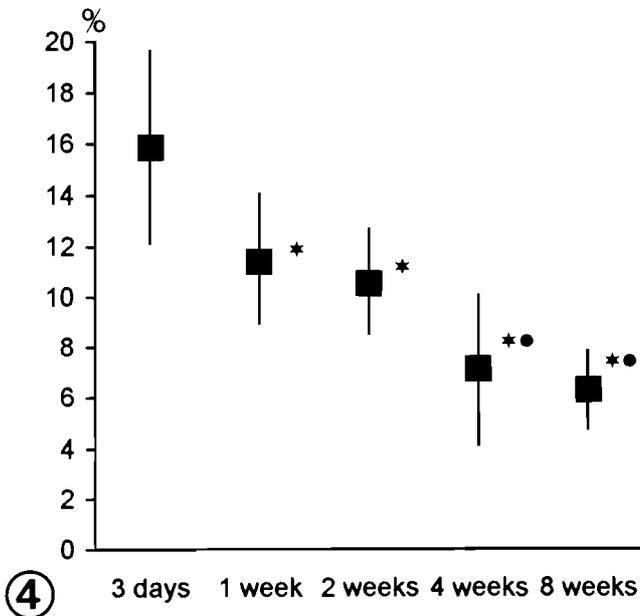


Fig. 4. Graph showing the ratio (%) of myoepithelial cell area to acinus area. Mean \pm SD are depicted. asterisk: significant ($p < 0.01$) between the values of 1, 2, 4, and 8 weeks after birth and that of 3 days after birth; black circle: significant ($p < 0.05-0.01$) between the values of 4 or 8 weeks after birth and those of 1 or 2 weeks after birth.

observed. About 12% of the acinus profile was occupied by myoepithelial cells (Fig. 4).

At 2 weeks after birth, the number of acini conforming a lobule increased. The secretory granule area in glandular cells was prominent, and the size of the glandular acini increased (about 50 μ m in diameter) (Fig. 1d). Some processes of myoepithelial cells prolonged. However, there were few contacts of myoepithelial cells to each other at the ends of the processes. Actin bundles in the myoepithelial cells were recognisable (Fig. 2d, 3b). The ratio of myoepithelial cells to acinus area decreased further, to about 10% (Fig. 4).

Between 4- and 8-week-old mice, there was no significant morphological difference in the glandular acini and myoepithelial cells. At these stages, lobules increased in size and contained many acini (Fig. 1e,f). The size of each acini increased (about 60-80 μ m in diameter). Flat myoepithelial cells with their processes intervening between the acini were observed (Fig. 2e,f). The processes of the myoepithelial cells increased in number, length and width. From the cell body, 4-6 processes extended in all directions and embraced the acinus (Fig. 3c). Arborization was frequently observed. Each acini was attached by a few myoepithelial cells, but no entire basal surface of the acini was covered. The terminal ends of the processes often contacted each other. However, the contact areas were too narrow to bind strongly to each other. Actin bundles in the cell body were thick. The processes of myoepithelial cells

increased, whereas the area of glandular cells increased further, therefore the ratio of myoepithelial cells to acinus area decreased to 6-7% (Fig. 4).

Discussion

It has been considered that myoepithelial cell contraction plays a role in expelling secretory products from glandular endpieces to the excretory duct system in various glands, and that the myoepithelial cell configuration is closely related to the secretory products. In the present study on the postnatal development of myoepithelial cells, we discuss this view.

While lacrimal glands were only rudiments within 1 week after birth, the differentiation of myoepithelial cells had already started. There was no differentiation between glandular and myoepithelial cells in the rudiments in newborns, and then 3 days after birth, faint immunoreactivity for actin was observed in the peripheral cells in acinus rudiments. However, the cells showing actin-immunoreactivity did not contain enough F-actin to visualize the whole cell configuration by phalloidin-staining. About 1 week after birth, the lacrimal gland of mice would function, because at this stage, secretory granules had accumulated in the glandular cells and acinus structures were established. Simultaneously, myoepithelial cells were clearly distinguished by immunohistochemistry and phalloidin-staining. In conclusion, within 1 week after birth, there is a close positive correlation between glandular cell maturation and myoepithelial cell differentiation. This corresponds to the development of myoepithelial cells in the hamster Harderian gland, another ophthalmic exocrine gland (López et al., 1992). Within 10 days after birth, the myoepithelial cells of the Harderian gland contain few myofilaments, and the secretory granules in the glandular cells are few in number.

After 1 week, during postnatal development, the glandular cells contained more secretory granules, and the size of the acini increased gradually. Additionally, the number of acini in the lobule increased conspicuously. However, the myoepithelial cell profile did not increase as much, although prolonged processes in various directions were observed. Acini in the immature stage (3 days - 1 week old) were more often surrounded by myoepithelial cells than those in mature mice (4-8 weeks old). The ratio of myoepithelial cells to acinus area decreased from 16% (at 3 days) to 6% (at 8 weeks); after 1 week, the distribution of myoepithelial cells was more scarce, while the size of glandular acini increased. The reciprocal correlation of myoepithelial cell distribution and acinus size/number suggests that the expelling function may be only one of the roles of myoepithelial cells. Should the expelling function indeed be the main function, it would be likely that the area of the myoepithelial cells would increase in parallel to an increase in the glandular acini. However, this was not the case.

Additionally, the processes were thin, and most basal

surfaces of the acini were not covered by the myoepithelial cells in mature lacrimal glands (4-8 weeks). The contact of the process extremities seemed not to be robust. The configurations of the myoepithelial cells are not suitable for co-ordinating a contraction to cause a glandular volume reduction. Another physiological role for the myoepithelial cell has to be considered.

A supporting role for the myoepithelial cells has been proposed: myoepithelial-cell contraction may also play a role in maintaining the contour of glandular endpieces against increasing luminal pressure during secretion (Garrett and Emmelin, 1979). Exocrine glands (including the exorbital lacrimal gland) in the subcutaneous tissues can be exposed by physical force from the outside environment. The salivary glands in the oral region are mechanically moved during mastication. Myoepithelial cells in these glands may act as an exoskeleton, to inhibit the straggling of the glandular endpieces (Satoh et al., 1994).

Finally, how do the myoepithelial cells change their configuration? Myoepithelial cell configuration at 1 week was starfish-like, and then the processes branched and elongated. The elongation of the processes was accompanied by the growth in size of the acinus. Recently, from a comparative study on myoepithelial cell configuration in various exocrine glands, it was suggested that the shape of the myoepithelial cells depended on the outer contour of the glandular endpieces rather than on the nature of the secretory material or luminal width (Satoh et al., 1994). The present result in postnatal development can support this view. However, we could not ascertain whether the myoepithelial cells prolonged their processes actively and/or stretched passively during the change of the outer contour of the glandular endpieces.

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