Mucous Cysts in the Thymus of the New Born Mouse

By

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Summary. Mucous cysts are observed in the medulla of the thymus of new born mice. Three types of cells, mucous cell, ciliated cell and cell with microvilli, can be seen in the wall of cysts. In addition, degenerating cells and large phagosomes are occasionally found in the wall. The mucous cells contain lamellar arrays of the rough endoplasmic reticulum, prominent Golgi apparatus, and mucous granules which can be subdivided into three groups according to their sizes and components. Epithelium lining the cysts are not surrounded by the basal lamina. Cytochemical studies indicate that the Golgi apparatus, mucous granules, and intracystal material contain glycoprotein. Acid phosphatase activity is negative in the mucous granules. The possible origin of these actively mucus-secreting structures will be discussed.

Introduction

The presence of colloidal cysts and mucous cells in the thymus has been reported in various animals (for review see Hammer, '05, '09; Bargmann, '43; Yamada and Hosaka, '54; Arnesen, '58; Kostowiecki, '67). Electron microscopic studies on such structures have been reported in the mice (Clark, '63), rat (Van Haelst, '67), frog (Curtis et al., '72), and nude (nu nu) mice which is deficient in most thymic function (Cordier, '74). However, ultrastructural descriptions on such cells in normal mammalian thymus were rather brief, and cytochemical studies seem to be deficient.

This paper will describe cysts lined with mucous cells in the thymus of the normal new born mice. In addition to their cytochemical characterization, possible nature of such structures will be discussed.

Material and Methods

Preparation for electron microscopy

Five ICR-GCL-strain-mice of both sexes, one day old, were fixed by perfusion with an aldehyde mixture according to Palay et al. ('68). Small blocks of thymus were post-fixed for 2 hr with cold, buffered osmium tetroxide (Palay et al., '68). Some of them were stained in block with 0.5% uranyl acetate in acetate-Veronal buffer (Farquhar and Palade, '65). They were dehydrated and embedded in Epon 812 (Luft, '61). Ultrathin sections were stained with saturated aqueous uranyl acetate followed by lead tartrate (Millonig, '61), and examined in a Hitachi HS-7 or a JEOL JEM-100B electron microscope.
Histochemical procedure

Four ICR-GCL-strain-mice of both sexes, one or two days old, were fixed with an aldehyde mixture followed by post-fixation with osmium tetroxide, and embedded in Epon 812. Three entire lobes of thymus were cut serially into one or two micrometer thick sections, They were stained with periodic acid-Schiff (PAS) technique according to Chang and Leblond (71), and examined with a light microscope.

Ultrathin sections from the other Epon-embedded blocks were treated with periodic acid-thiosemicarbazide-silver proteinate method (Thiery, '67). They were mounted on gold grids, and floated onto 1% aqueous periodic acid for 25 min followed by washing in distilled water, and refloated on 1% thiosemicarbazide in 10% acetic acid for 70 hr at room temperature. Subsequently, they were washed in a graded series of acetic acid and distilled water, and refloated on 1% aqueous silver proteinate in darkness for 30 min. Control sections were processed without periodic acid. In addition, some specimens from six animals were fixed for 1 hr with 2.5% glutaraldehyde in 0.1 M sod. cacodylate buffer at pH 7.4, and used for the detection of acid phosphatase-activity. After washing overnight in cold cacodylate buffer containing 7.5% sucrose, 50 μm thick sections were cut with a Sorval TC 2 sectioner, and incubated for 60 min at 37°C in a modified Gomori's incubating medium (Barka and Anderson, '62). They were post-fixed in 1% osmium tetroxide and embedded in Epon 812. Control sections were incubated in a medium without substrate or in a medium containing 0.01 M sodium fluoride.

Results

Light microscopy

In the PAS-stained sections, mucous cells, which contain bright red granules in the cytoplasm, usually can be seen in the medulla of the thymus (Fig. 1). Most of them are observed in the wall of round or irregularly elongated cysts, while a few solitary cells can be seen among the epithelial reticulum of the medulla. Cysts contain negligibly or strongly PAS-positive material. Three different types of cells can be found in the wall of cysts. One is the cubo-cylindrical mucous cell which has bright red granules and a pale nucleus in the basal part of the cell body. The second type is a cubo-squamous cell with or without a distinct cuticular border (Fig. 2). The third type is a cubo-squamous cell with cilia and a cuticular border. Mucous cells are conspicuous in the wall.

Fig. 1. Longitudinal section of the thymus of the new born mouse, illustrating the distribution of mucous cells (dots) and cyst (encircled dots). C, cortex of the thymus; M, medulla of the thymus. Arrow indicates the cranial direction.
of small cysts (70–200\(\mu m\) in diameter), and only a few in those of large cysts (300–600\(\mu m\) in diameter). On the contrary, ciliated cells are numerous in the large cysts, and only a few in small cysts.

Electron microscopy

The mucous cell contain an oval or elongated nucleus near the cell base, well-developed lamellar arrays of the rough endoplasmic reticulum in the basal and perinuclear region, prominent Golgi complex in the supranuclear area, numerous mucous granules in the upper half of the cell body, and tonofilaments (Fig. 3). It has also microvilli at the free surface, tight junction between adjacent cells, immediately subjacent to the free surfaces, and desmosomes and cell folds at the lateral and basal borders (Fig. 8). It is noticed that intracellular membranes, especially the membranes of mucous granules, could hardly be visible, if the uranyl acetate-block-staining was not carried out. The mucous granules can be divided into three types according to their sizes and components. The first type of granules (0.2–1.5\(\mu m\) in diameter) contains 3–6 nm thick fibrillar matrix (Fig. 4). The second type of granules (0.2–1.5\(\mu m\) in diameter) contains not only fibrillar matrix but also a centrally-located amorphous dense core (0.2–0.7\(\mu m\) in diameter) (Fig. 5). The third type of granules is large (1–4\(\mu m\) in diameter) and often coalesces. This granule also contains somewhat dispersed fibrillar matrix and several clear spaces (Fig. 6). Mucous cells usually contain either of three types (Figs. 1, 6, 11). The large granules occasionally protrude from free surfaces of the cells into the lumen (Fig. 6). In such cases, the membranes of granules fuse with the plasma membrane, resulting in a five-layered structure, which consists of three dense laminae altering with two electron-transparent ones. Furthermore, some protrusions are bounded by a single membrane (Fig. 7). Material similar to fibrillar matrix in the granules can be seen in the intracystal spaces (Fig. 6). Discharge of the mucus seems to be accomplished by rupture of a single membrane surrounding the protrusion followed by the liberation of mucous content.

Cubo-squamous cells with microvilli are also found in the walls of cysts. They contain free ribosomes, rough endoplasmic reticulum, Golgi apparatus, mitochondria, and tonofilaments (Fig. 3). The third cell-type in the wall is the ciliated cells. They contain cilia and microvilli at their luminal surface, many free ribosomes, relatively small number of rough endoplasmic reticulum, mitochondria, and tonofilaments (Fig. 9). In addition, apparently degenerating mucous cells are found among the epithelial linings. Large vacuoles containing a cellular debris, which are similar to the large phagosomes in the intestine (Cheng and Leblond, '74), are occasionally found in the cytoplasms of epithelial cells (Fig. 10). These epithelial cells lining the cyst are not surrounded by basal lamina, and are continuous with epithelial reticulum of the medulla.

Cytochemistry

In the periodic acid-thiosemicarbazide-silver proteinate-treated sections, strong silver deposits characterizing positive reaction are observed in the fibrillar matrix of the mucous granules, while an amorphous core of the second type-granule shows negative reaction (Figs. 10, 11). Golgi complex, including the saccules and small vesicles, is weakly positive. Immature mucous granules in the vicinity of the Golgi complex react weakly (Fig. 12). Intracystal materials react negligibly to strongly (Figs. 10, 11). Fuzzy coats of microvilli and glycogen are positive (Fig.
Control sections are negative. These results indicate that fibrillar matrix of the mucous granules, Golgi complex and intracystal material contain polysaccharides. Acid phosphatase activity is negative in the mucous granules, although it is positive in the Golgi apparatus and lysosomes in the same section. Control sections show negative activity. It seems reasonable to conclude that these granules are non-lysosomal.

**Discussion**

The mucous cells in the thymus are very similar to those in the intestine and the salivary glands. The granules with dense core, which may be proteinaceous, have been reported in the intestinal mucous cells (Cheng, '74) as well as in the salivary gland (Alvares and Sesso, '75). Present cytochemical studies reveal glycoprotein in the Golgi complex as well as in the mucous granules. This is in agreement with the current concept of secretory process in the mucous cell which indicates that the synthesis of carbohydrate moiety of the mucus and its linkage with protein occur in the Golgi complex (Neutra and Leblond, '66a, b). The mode of discharge of the mucus in the thymus is probably similar to the merocrine process in the goblet cell of the human intestine reported by Trier ('63). He has described that the membranes of the mucous granules fuse with the apical plasma membrane, and that only the contents of granules are liberated into the lumen. In the thymic mucous cells, the discharging process of the mucus can be divided into the following three steps. The first step is the fusion of granule membrane with the plasma membrane. Such fused membranes have also been reported in the rat sublingual gland (Kim et al., '72). The second step is the conversion of such fused membranes into the single membrane surrounding the protrusion. This single membrane may possibly be explained as being produced by such "complete fusion" of membranes of mucous granule and cell surface as the model advocated by Robertson ('61). The final step may probably be the rupture of this single membrane followed by the liberation of the mucous content. Further fate of the mucus in the intracystal space remains unsolved in this study. The presence of degenerating mucous cells seems to indicate that the turn over of mucous cells occur in the vicinity of cysts. They, at least some of them, probably phagocytosed by an adjacent epithelial cell lining the cyst. Such case has been reported in the small intestine (Cheng and Leblond, '74).

The epithelial components of the mouse thymus are derived from the endoderm of the 3rd pharyngeal pouch, and in some parts even from the ectoderm of the cervical sinus (Crisan, '35). Hammer ('09) as well as Bargmann ('43) have considered that thymic cysts originate not only from a remnant of embryonal pharyngeal pouch but also from epithelial reticular cells. The number of cysts increases with the period of so-called age-involution (Hammer, '09; Bargmann, '43) as well as with that of accidental involution (Selye '36; Cowan and Sorenson, '63). Mucification of Hassall corpuscles has been reported in the guinea pig after the injection of estrogen (Izard, '65). In addition, the multipotential cell, which is capable of differentiation into several cell-types, has been reported in the intestinal epithelium (Cheng and Leblond, '74). Furthermore, the present electron micrographs of the epithelial cells lining the cysts show that they are continuous, without the basal lamina, with the reticulum of the medulla. These facts seem to suggest that the pharyngeal pouch derivatives, probably
some of the epithelial reticular cells in the thymus, may have the ability to differentiate into mucous or ciliated cells, and that the errors of differentiation may be accelerated at the period of involution.

Since mucous cysts are numerous in the thymus of nude (nu nu) mice which are immunologically incompetent (Cordier, '74), it seems reasonable to conclude that these structures do not have the function of inducing immunological competence.

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**References**


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PLATES
Explanations of Figures

Plate I

Fig. 2. Photomicrograph of a section of a cyst surrounded by mucous cells containing strong PAS-positive granules in their cytoplasms. Intracystal material is weakly PAS-positive. ×400.

Fig. 3. Electron micrograph of a mucous cyst containing moderately electron-dense material. Its lower wall consists of mucous cells containing an elongated nucleus, rough endoplasmic reticulum, prominent Golgi complex, numerous mucous granules, and microvilli at their free surfaces. Upper wall consists of a squamous cell lacking the secretory granules. A mucous cell containing several small granules is in the wall on the right. ×12,000.
Plate I

K. Kishi
Plate II

Fig. 4. Golgi complex of a mucous cell in the mice thymus. Mucous granules of the first type containing fibrillar matrix are visible in its vicinity. ×60,000.

Fig. 5. Mucous granule of the second type containing an amorphous dense core surrounded by the fibrillar matrix. ×90,000.
Plate III

Fig. 6. Large coalescing granules and their protrusions into the lumen (L). ×30,000.

Fig. 7. A higher magnification of two protruding mucous masses. The protrusion on the left (a) is surrounded by five layered structure consisting of three dense laminae and two electron-transparent ones (double arrows), indicating the fusion of membranes of mucous granules and the cell surface. The protrusion on the right (b) is, for the most part, surrounded by the single membrane (Arrow). ×144,000.

Fig. 8. Tight junction is visible between adjacent epithelial cells, immediately subjacent to the free surfaces. ×144,000.

Fig. 9. A ciliated cell containing many cilia and microvilli at the free surface, many free ribosomes, and mitochondria. ×17,500.
Plate IV

Fig. 10. Electron micrograph of a periodic acid-thiosemicarbazide-silver proteinate-treated section. Peripheral rims of mucous granules show strongly positive reaction, while central cores are negative. Glycogens react strongly. Intracystal material reacts negligibly. Fuzzy coats of microvilli react positively. V, a large vacuole containing a cellular debris. ×18,000.
Plate V

Fig. 11. Electron micrograph of a periodic acid-thiosemicarbazide-silver proteinate-treated section. The mucous cell on the lower right contains numerous granules without core (the first type-granules), while the cell on the left is filled with granules with an electron-transparent core (the second type-granules). Intracystal material reacts strongly. \( \times 9,000 \).

Fig. 12. Electron micrograph of a periodic acid-thiosemicarbazide-silver proteinate-treated section. Weakly positive reaction can be seen in the Golgi complex, including saccules and small vesicles (arrow), as well as in the immature mucous granules (doubly headed arrow). Mature mucous granules and glycogen react strongly. \( \times 26,300 \).