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Primary Cultures of Human Myasthenia Gravis Thymus and Normal Thymus

Studies of Cell Morphology, Cell Proliferative Pattern and Localization of α-Bungarotoxin Binding Sites on Cultured Thymic Cells

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SUMMARY

We have established primary cultures of human myasthenia gravis (MG) thymuses and normal thymuses. In cultures of 19 thymuses with hyperplasia among 23 MG thymuses and 12 thymuses among 13 normal thymuses, epithelial cells migrated in a mosaic-like arrangement and were maintained for more than 5–10 weeks. There were, among mononuclear epithelial cells, some multinucleated cells, some of which were considered to be derived from epithelial cells because they had a desmosome-like structure and contained tonofilaments in their cytoplasm. There was no significant difference in the morphology of epithelial cells between MG thymuses and normal thymuses.

The growth rate of thymic epithelial cells was identified by [^{3}H]thymidine autoradiography(ARG), labeling indices rising to a peak around a week and falling to low levels gradually. There was no significant difference in the growth rate of epithelial cells between MG thymuses and normal thymuses.

An autoradiographic method with ^{125}I-labeled α-bungarotoxin was used to detect the presence of acetylcholine receptor (AChR) on the cultured cells. ARG of human MG thymuses and normal thymuses, which were cultured for 4 weeks, revealed

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diffusely distributed silver grains on the epithelial cells. Toxin binding sites (AChRs) were considered to be present on the epithelial cells. There was no significant difference in the distribution of AChR on the epithelial cells between MG thymuses and normal thymuses.

Key words: *Acetylcholine receptor – Epithelial cell – Myasthenia gravis – Tissue culture*

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease characterized by circulating autoantibodies against the acetylcholine receptor (AChR) present on the neuromuscular endplate. The role of the thymus in the pathogenesis of MG has been controversial. Since the myasthenic thymus contains AChR-bearing cells (Engel et al. 1977; Kao et al. 1977), it has been suggested that thymic AChR is important in the pathogenesis of MG. Though animal studies indicate that AChR-bearing cells are found in normal thymus (Lindstrom et al. 1976; Kao and Drachman 1977; Fuchs et al. 1980), there have been few reports studying whether AChR-bearing cells occur only in myasthenic thymus.

There have been several reports on the cultures of human thymus (Matagne-Dhoossche 1972; Pyke and Gelfand 1974; Papiernick et al. 1975; Oosterom et al. 1979), but there have been few reports on the cultures of both human MG thymus and normal thymus.

In this study we have established primary cultures of human MG thymuses and normal thymuses, observed the morphology of cultured thymic cells, identified the growth rate of cultured thymic cells by the autoradiographic method, and studied localization of AChR on cultured thymic cells of MG thymuses and normal thymuses by the autoradiographic method in vitro.

MATERIALS AND METHODS

(1) Tissue culture of human MG thymus and normal thymus

Specimens of 23 MG thymuses (18 years-old ~ 58 years-old, hyperplasia in 15 cases and thymoma in 8 cases) and 13 normal thymuses (3 years-old ~ 62 years-old, all had heart diseases without immunological abnormalities) were used. After the removal of the capsule, thymic tissue was minced with scissors in minimal essential medium (MEM) until fragments of 1 mm^3 or less were obtained. The fragments were explanted on collagen coated cover slips in culture dishes containing MEM supplemented with 15% fetal bovine serum (FBS) and antibiotics (penicillin 100 IU/ml). Thymic epithelial cells started to grow from the explant by day 3–5 and expanded eccentrically thereafter. We observed the morphology of thymic cells by phase-contrast microscope, transmission electron microscope (TEM) and scanning electron microscope (SEM).

For TEM, specimens were fixed for 30 min in 2.5% glutaraldehyde, fixed for 15 min in 2% osmium tetroxide, dehydrated through graded alcohols, and embedded in resin. These sections were stained with uranyl acetate and lead citrate and viewed in a Hitachi H-800 electron microscope. Specimens for SEM were dehydrated through graded alcohols, critical point dried, mounted on specimen stubs, and coated with gold. They were examined in a Hitachi S-430 scanning electron microscope with an acceleration of 20 or 25 kV.

(2) Cell proliferative pattern of cultured human thymic cells

Specimens of 1 MG thymus with hyperplasia and 3 normal thymuses were used. Explants cultured for 1–4 weeks were incubated in the medium containing 5 μCi/ml [^{3}H]thymidine for an hour. They were rinsed with phosphate buffer saline (PBS), fixed in 2.5% glutaraldehyde for 30 min, dehydrated through graded alcohols, and dried in air. They were covered with Kodak NTB2 emulsion, exposed for a week, and developed with Dektol for 4 min at 16 °C. They were stained with toluidine blue. Labeling indices (epithelial cells with a labeled nucleus/total epithelial cells) were calculated at each period.

(3) Localization of α-bungarotoxin binding sites on cultured thymic cells

Specimens of 4 human MG thymuses and 2 normal thymuses were used. Explants cultured for 4–5 weeks were fixed in 2% paraformaldehyde for 15 min after they were rinsed with PBS, preincubated in PBS/BSA (bovine serum albumin) for 30 min, and incubated in a solution containing 5 μCi/ml[^{125}I]α-bungarotoxin for 1 h. They were rinsed with PBS, fixed in 2.5% glutaraldehyde with 1% tannic acid for 30 min, dehydrated through graded alcohols, and dried in air, covered with Sakura NR-M2 emulsion, exposed for 4–5 weeks, and developed with Konidol-X for 5–6 min at 20 °C. They were stained with toluidine blue.

In order to check non-specific binding capacity, control tissues were incubated in a solution containing both labeled α-bungarotoxin and 10^{-2} M *d*-tubocurarine. Also chick muscle cultures were used to confirm the reliability of ARG with [^{125}I]α-bungarotoxin.

RESULTS

(1) Culture morphology

In cultures of 19 thymuses with hyperplasia among 23 MG thymuses and 12 thymuses among 13 normal thymuses, epithelial cells migrated in a mosaic-like arrangement and were maintained for more than 5–10 weeks.

Many mononuclear cells, which were chiefly composed of lymphocytes, migrated shortly after the start of the culture and epithelial cells migrated 3–5 days after the explant (Fig. 1a). They expanded eccentrically around the explant thereafter. Some multinucleated cells were found among mononuclear epithelial cells. Some of them had some nuclei which were arranged in trapezoid form and contained abundant cytoplasmic vacuoles. Others of them had some nuclei which were arranged longitudinally

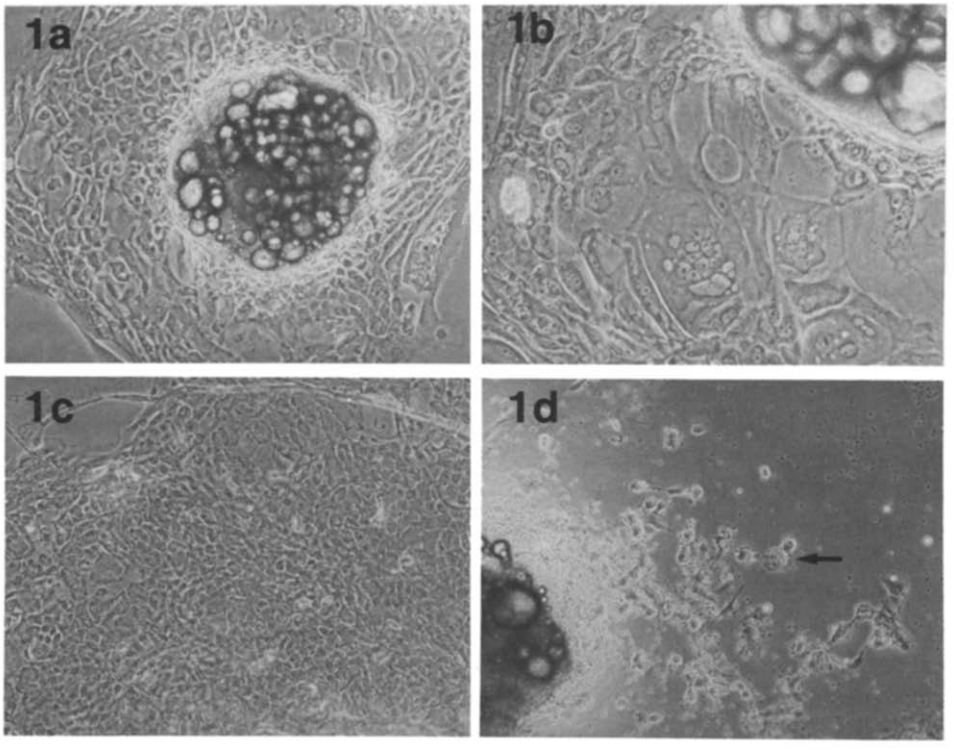

Fig. 1. Phase-contrast photomicrographs of culture of human normal thymus.
a: Culture for 7 days. Epithelial cells migrate from the explant. × 100.
b: Culture for 7 days. Multinucleated cells are observed among mononuclear epithelial cells. × 200.
c: Culture for 30 days. Epithelial cells proliferate well around the explant. × 50.
d: Culture for 7 days. Some viable lymphocytes are surrounding some epithelial cells (arrow). This phenomenon may be autosensitization of lymphocytes against self-epithelial cells. × 100.

in their myotube-like cytoplasm (Fig. 1b). Lymphocytes were eliminated after 7–10 days. Multinucleated cells did not increase after 2 weeks, but cytoplasmic vacuoles increased gradually. Epithelial cells proliferated well around the explant at 4 weeks (Fig. 1c). Fibroblasts started to proliferate after a week and replaced epithelial cells gradually in some explant cultures.

Morphologically at least two types of mononuclear epithelial cells could be distinguished by light-microscopic examination, one being cells which had a large nucleus in their scarce cytoplasm, the other one being round cells which had a small nucleus in their abundant cytoplasm. Except for epithelial cells and multinucleated cells, some giant macrophage-like cells were observed at the sides of cultured cells.

In cultures for 7–10 days we recognized the phenomenon of some viable lymphocytes surrounding some epithelial cells (Fig. 1d). Since these epithelial cells stopped to proliferate and were destroyed by degeneration thereafter, we considered that this phenomenon might be autosensitization of lymphocytes against self-epithelial cells. Therefore, since the persistence of viable lymphocytes in such cultures would lead to

their autosensitization with destruction of the epithelium, successful growth of epithelial cells was dependent on the extensive removal of thymocytes both at the beginning of the culture and at days 2–3, when the explants were attached to the culture dish.

Although in cultures of the human MG thymuses from 4 of the 23 cases, no epithelial cells migrated, these cases were the cultures in which parenchyma of thymoma itself was explanted. It was necessary to explant hyperplastic thymus, which existed around thymoma, rather than thymoma itself for success of epithelial cell growth. In cultures of the human normal thymuses of one case among 13 cases, no epithelial cells migrated because we could not detect the parenchyma of the thymus. It was easy to detect thymic parenchyma in young-aged thymuses, but it was difficult to detect it in old-aged thymuses because they were involuted in adipose tissue. However, we succeeded in the culture of old-aged thymuses if we detected thymic parenchyma, which looked like white clear tissue under the simple microscope, and explated it certainly. In cultures of normal old-aged thymuses lymphocytes, which migrated shortly after the start of the culture, were fewer than in cultures of young-aged thymuses and MG thymuses. But epithelial cells migrated as well as in cultures of young-aged thymuses. There was no significant difference in the growth rate of epithelial cells between young-aged thymuses and old-aged thymuses.

There was no significant difference in the morphology of epithelial cells between MG thymuses and normal thymuses by light-microscopic examination.

Transmission electron-microscopic examination was done in tissue cultures for 2 and 4 weeks. In cultures for 2 weeks some epithelial cells with desmosome-like structure contained some bundles of microfilaments with a diameter of 4–6 nm under their cell membrane and some intermediate tonofilaments with a diameter of 8–10 nm in their cytoplasm (Fig. 2a). These epithelial cells frequently had a pale nucleus with marginal heterochromatin and contained a well-developed Golgi complex, long cisternae of the rough endoplasmic reticulum (RER), lipid droplets and mitochondria in the electron-lucent cytoplasm (Fig. 2a). Other epithelial cells contained abundant cytoplasmic vacuoles. There were a few epithelial cells with a dark nucleus which scarcely contained a Golgi complex and RER in the electron-dense cytoplasm at 2 weeks. In cultures for 4 weeks, some epithelial cells with a dark nucleus contained abundant intermediate filaments, and scarcely contained a Golgi complex and RER in the cytoplasm (Fig. 2b). Other epithelial cells contained scarce intermediate filaments in the cytoplasm. A large interpatient variation was observed in the quantity of these intermediate filaments. There were a few epithelial cells with a pale nucleus which had electron-lucent cytoplasm at 4 weeks. Some of the multinucleated cells were considered to be derived from epithelial cells because they had a desmosome-like structure and contained tonofilaments in their cytoplasm (Fig. 2c). These cells had intermediate nuclei between a pale and dark nucleus, long cisternae of RER and mitochondria in the cytoplasm.

Morphologically at least three types of epithelial cells could be distinguished by transmission electron-microscopic examination. Type-1 cells had a pale nucleus with marginal heterochromatin, a well-developed Golgi complex and abundant RER in the electron-lucent cytoplasm (Fig. 2a). Type-2 cells had an intermediate nucleus between

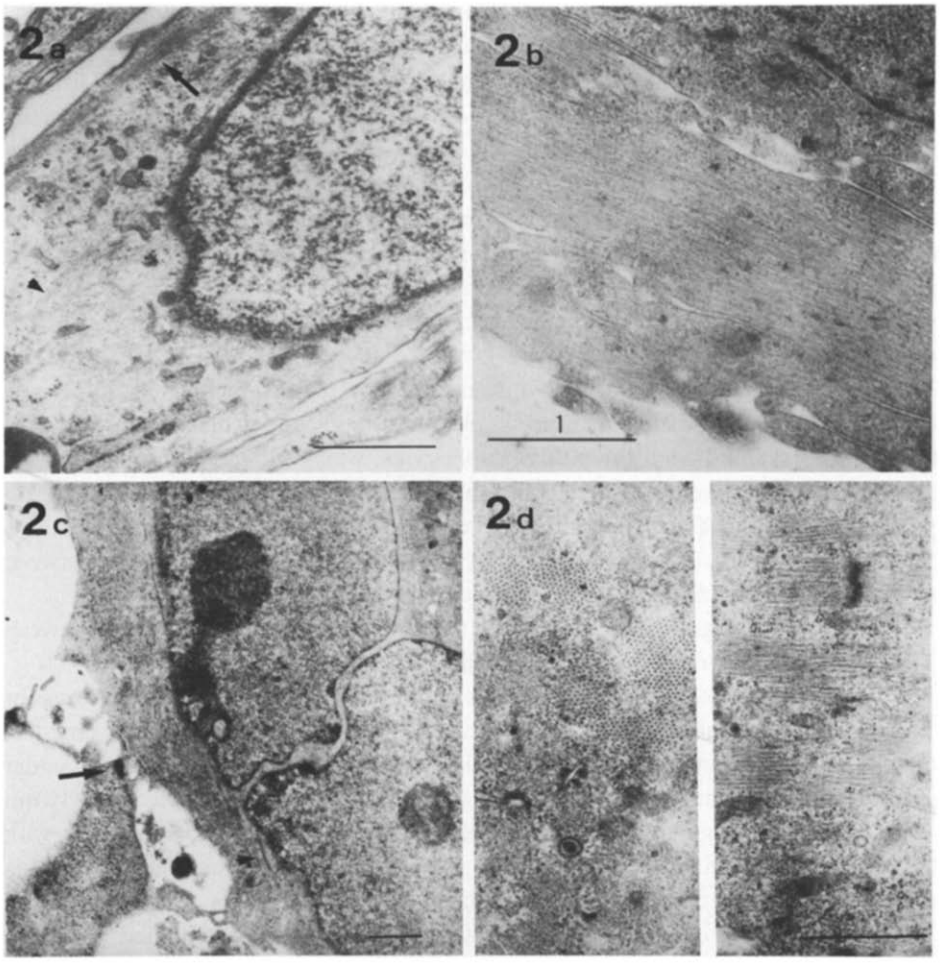

Fig. 2. Transmission electron-micrographs of human MG thymus and normal thymus.

a: Culture of MG thymus for 2 weeks. An epithelial cell (type-1 cell) has a pale nucleus with marginal heterochromatin, a bundle of microfilaments (arrow) under the cell membrane, some intermediate tonofilaments (arrow head) and short profiles of RER in the electron-lucent cytoplasm.

b: Culture of MG thymus for 4 weeks. An epithelial cell (type-3 cell) with a dark nucleus contains abundant intermediate filaments without a Golgi complex and RER in the electron-dense cytoplasm.

c: Culture of normal thymus for 2 weeks. A multinucleated cell (type-2 cell) has intermediate nuclei with pronounced nucleoli, desmosome-like structure (arrow) and tonofilaments (arrow head) in the cytoplasm.

d: Culture of MG thymus for 4 weeks. A thymic cell contains myofilaments (left: longitudinal section, right: transverse section).

a pale and dark nucleus and contained RER, mitochondria, lipid droplets and a Golgi complex in the electron-dense cytoplasm (Fig. 2c). Type-3 cells had a dark nucleus, and frequently contained abundant intermediate filaments but scarcely contained a Golgi complex and RER (Fig. 2b). In cultures of 2 weeks most of the epithelial cells were type-1 or type-2 cells, and type-3 cells, on the other hand, were few. In cultures of

4 weeks most of the epithelial cells were type-3 or type-2 cells and type-1 cells, on the other hand, were scarcely observed.

Scanning electron-microscopic examination was done in tissue cultures for 3 weeks. There were some flat epithelial cells and other epithelial cells with a swollen nucleus (Fig. 3a). Numerous microvilli with a diameter of 100–170 nm and a length of 170–850 nm were observed on the cell surface (Fig. 3b). These microvilli frequently stood up erectly from the cell surface. There were some epithelial cells with numerous microvilli and other epithelial cells with scarce microvilli (Fig. 3c). A large variation was observed in the number of these microvilli on the epithelial cells. There was, however,

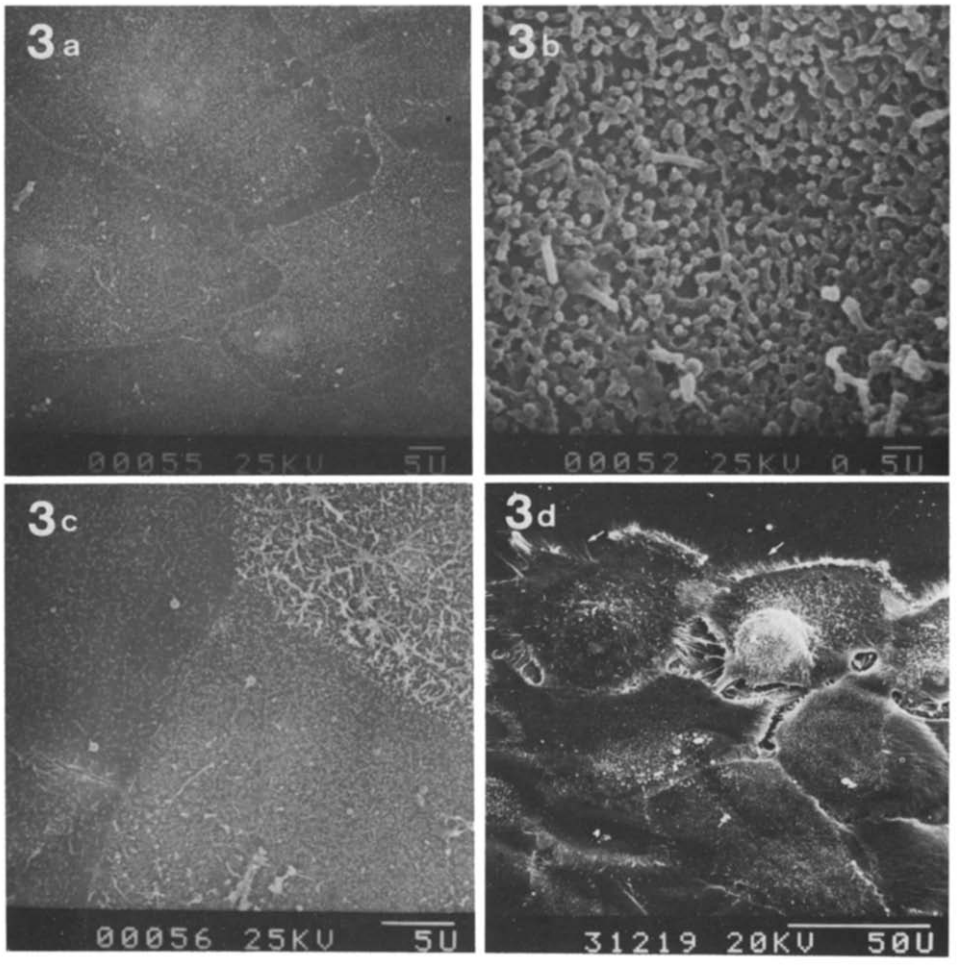

Fig. 3. Scanning electron-micrographs of culture of human normal thymus of 3 weeks.
a: Flat epithelial cells have achieved confluence.
b: Numerous microvilli are observed on the cell surface of epithelial cells.
c: The number of microvilli varies among epithelial cells.
d: Long and slender filopodia (arrow) extend from the edges of the epithelial cells. They appear to link cell with cell or cell with substrate, and frequently arborize at their tip ends.

another type of surface extension, a very long and slender filopodium which seemed frequently to extend from the edges of the epithelial cells (Fig. 3d). These filopodia appeared usually to link cell with cell or cell with substrate and frequently arborized at their tip ends. Epithelial cells with filopodia tended to be observed chiefly at the sides of the cultures.

There was no significant difference in the morphology of epithelial cells between MG thymuses and normal thymuses by electron-microscopic examination.

(2) The study of cell proliferative pattern of cultured thymic cells

Tritiated thymidine labeling indices were measured from 1 to 4 weeks. A large interpatient variation was observed in the number of labeled cells per culture, but the overall pattern of labeling was similar in all cases. Labeled cells were observed diffusely from the base of the cultures to the sides of the cultures at 1 week and they tended to be observed at the sides of the cultures at 2 weeks. Labeling indices rose to a peak around 1 week and fell to low levels gradually and few labeled cells were observed at 4 weeks. There was no significant difference in the growth rate of thymic epithelial cells between MG thymuses and normal thymuses (Table 1).

(3) The study of localization of AChR on cultured thymic cells

ARG of human MG thymuses and normal thymuses, which were cultured for 4 weeks, revealed diffusely distributed silver grains on the epithelial cells (Figs. 4a and 4b). Few silver grains were observed on the fibroblasts. The number of silver grains on more than a hundred epithelial cells was measured and average grains on a unit area (6.25×10^{-4} mm^2) were calculated. Average grains on the epithelial cells of MG thymus were 21.15 ± 2.15 and those of normal thymus were 20.87 ± 3.24. There was no significant difference in the distribution and number of silver grains on the epithelial cells between MG thymus and normal thymus. In control tissue incubated in a solution containing both labeled toxin and 10^{-2} M *d*-tubocurarine, few silver grains were observed on the epithelial cells (Fig. 4c). The average number of silver grains on a unit area, calculated in a similar manner, was 5.61 ± 0.32. There was a statistically significant difference between the former and the latter. Therefore these α-bungarotoxin binding sites were considered to be blocked by *d*-tubocurarine and this binding was considered to be specific binding.

TABLE 1

LABELING INDICES OF CULTURED THYMIC CELLS AT EACH PERIOD

	1 week	2 weeks	4 weeks
The cultures of MG thymus	$17.8 \pm 9.9\%$ (n = 8)	$6.5 \pm 5.3\%$ (n = 7)	$0.6 \pm 0.3\%$ (n = 3)
The cultures of normal thymus	$17.0 \pm 9.1\%$ (n = 10)	$6.7 \pm 6.3\%$ (n = 2)	$0.5 \pm 0.2\%$ (n = 4)

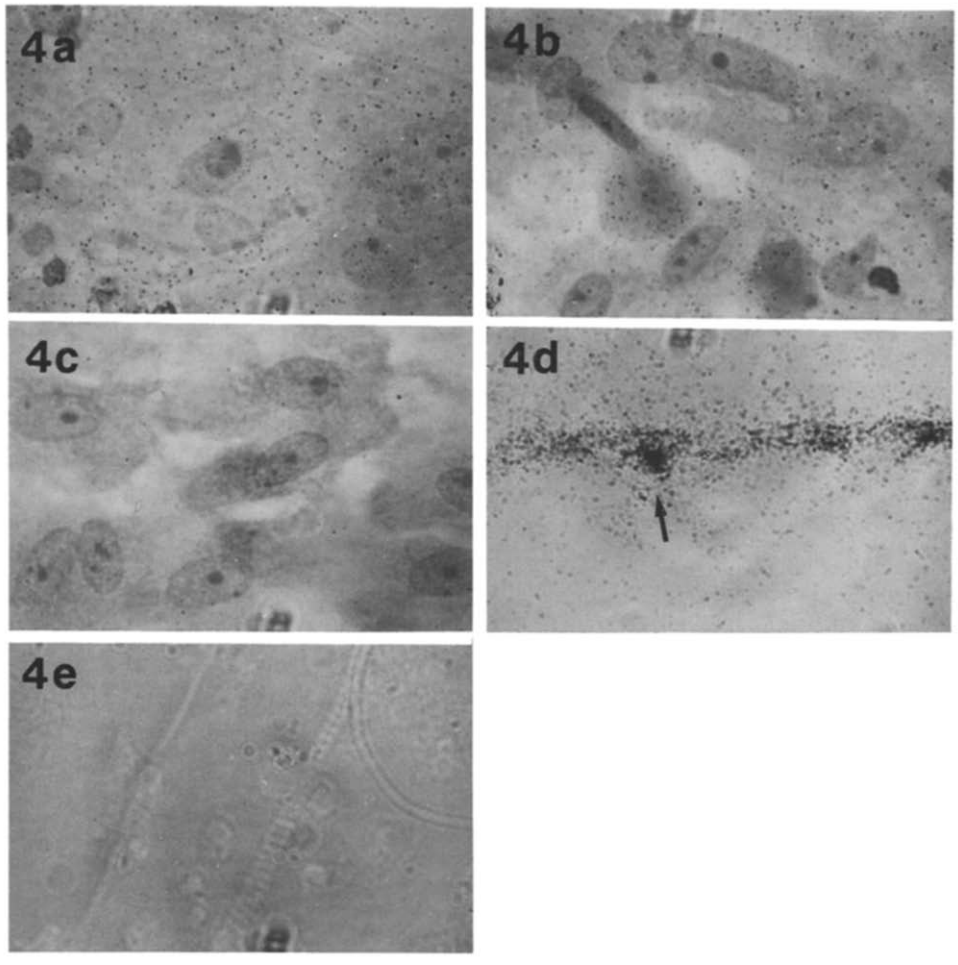

Fig. 4. Autoradiography of human MG thymus and normal thymus cultured for 4 weeks (*a–c*) and chick muscle cultured for 17 days (*d–e*) with [^{125}I]α-bungarotoxin.

a: MG thymus; *b*: normal thymus. Diffuse distribution of silver grains is observed on the epithelial cells. Toluidine blue, × 400.

c: Control tissue incubated in a solution containing both labeled toxin and 10^{-2} M *d*-tubocurarine. Few silver grains are observed on the epithelial cells. Toluidine blue, × 400.

d: Silver grains are observed diffusely on the myotube and the aggregation of silver grains is scattered (arrow). No staining, × 400.

e: Control chick myotube incubated in a solution containing both labeled toxin and 10^{-2} M *d*-tubocurarine. Few silver grains are observed on the myotube. No staining, × 200.

In ARG of chick muscle cultures silver grains were observed diffusely on myotube and aggregation of silver grains was scattered (Fig. 4d). In control tissue incubated in a solution containing both labeled toxin and 10^{-2} M *d*-tubocurarine, few silver grains were observed on myotube (Fig. 4e).

DISCUSSION

In our study the growth pattern of thymic epithelium explants was similar to that described by other investigators (Matagne-Dhoossche 1972; Pyke and Gelfand 1974; Papiernick et al. 1975; Oosterom et al. 1979).

When parenchyma of thymoma itself was explanted, no epithelial cells migrated. We considered that it was partly because of the culture conditions and that thymoma tissue might grow in suitable culture media.

In the cultures of old-aged thymuses fewer lymphocytes migrated after the start of the culture than in the cultures of MG thymuses and normal young-aged thymuses. We considered that it was partly because the thymic cortex, which was rich in lymphocytes, decreased in old-aged thymuses (Hammar 1926). However, there was no significant difference in the growth rate of epithelial cells between young-aged thymuses and old-aged thymuses. Our data suggest that the growth rate of epithelial cells is not dependent on age. Previous reports described that adult thymus was transformed into a mass of adipose tissue, containing scattered islands of lymphatic tissue composed of thymic epithelial regions, and that the epithelial regions of the thymus did not disappear completely even in old age (Hammar 1926; von Gaudecker 1978). In recent years increasing functional evidence suggested that the human thymus served an immunological function throughout life (von Gaudecker 1978). Our data suggest that the remaining thymus epithelial cells may serve as an immunological function in a rather old person.

In the cultures of MG thymuses and normal thymuses multinucleated cells were observed among mononuclear epithelial cells. The origin and function of these multinucleated cells are obscure. There have been only a few reports on these multinucleated cells in human thymus. Von Gaudecker (1978) first reported that multinucleated giant cells were found rarely in several tissue blocks of human thymuses. He suggested that he defined them as histiocytic giant cells with phagocytic capacity. In an animal study Scheiff (1976) reported that multinucleate epithelial cells occurred in the thymus of C3H mice. In the cultures of human thymus most other investigators did not mention multinucleated cells. Only Matagne-Dhoossche (1972) reported that multinucleated cells regularly formed in cell cultures of human thymic tissue from different ages and suggested that they were formed by a confluence of macrophages. In our study some of them were considered to be derived from epithelial cells because they had a desmosome-like structure and tonofilaments in the cytoplasm (Fig. 2c). In the cultures of human skin epidermal cells few multinucleated cells were observed. These data suggest that human thymic epithelial cells may have the ability to differentiate into multinucleated cells in vitro.

By transmission electron-microscopic examination 3 subtypes of epithelial cells were found. These various types of epithelial cells may represent stages in a differentiation process. Type-1 cells show characteristics of a high activity, such as a large round euchromatin nucleus with a well developed Golgi complex and several profiles of RER (Fig. 1a). In contrast, the morphological characteristics of type-3 dark cells indicate inactivity, possibly degeneration, of the cell, as illustrated by the increase

in nuclear heterochromatin and virtual absence of a Golgi complex and RER (Fig. 2b). Concerning the morphology of type-2 intermediate cells, transitional forms between cells of type-1 and type-3 were also observed. In our study type-1 cells were frequently observed and type-3 cells were scarcely observed at 2 weeks, but type-1 cells decreased in number and type-3 cells increased in number at 4 weeks. These data may suggest a differentiation process progressing from type-1 to type-3 cells.

In the culture of only one case of MG, myoid cells with striated muscle structure were observed in transmission electron-microscopic examination. The origin of myoid cells has been controversial and there are two main theories about it. One theory is that they are derived from the mesenchymal cells surrounding the thymus and secondarily incorporated into the parenchyma (Van De Velde 1966; Drenckhahn et al. 1979). The other theory is that they are derived from epithelial cells (Töro et al. 1969; Frazier 1973). In our study epithelial cells contained intermediate tonofilaments with a diameter of 8–10 nm and thin actin microfilaments with a diameter of 4–6 nm, and the distribution of both types of filaments was conspicuously not related (Fig. 2a). Myoid cells, on the other hand, contained thick (18 nm) myosin filaments and thin (4–6 nm) actin microfilaments arranged in a correlated pattern comparable to the arrangement in striated muscle (Fig. 2d). Therefore thymic epithelial cells and myoid cells showed different ultrastructural features, particularly concerning their filaments. In confirmation of the former theory, the statement can be made that they are two entirely different entities. We prefer the former to the latter theory.

By scanning electron-microscopic examination a large variation was observed in the number of microvilli and filopodia on the epithelial cells. The functional significance of the microvilli and filopodia is not entirely understood. Concerning the filopodia, Porter et al. (1973) reported that they first appeared on the dividing cell and they increased greatly in number as the cell entered mitosis. In our study epithelial cells with abundant filopodia tended to be observed at the sides of the cultures at 3 weeks. Also in our study of the growth rate of epithelial cells by ARG labeled cells tended to be observed at the sides of the cultures at 2 weeks. These data support the notion that filopodia are present on dividing cells.

In our study of the proliferative pattern of epithelial cells they ceased to proliferate after 4–5 weeks in culture. Perhaps they are, as other epithelial cells of mammals, programmed for a given number of generations, then stop dividing and degenerate (Rafferty 1975).

There was no significant difference in the morphology and growth rate of the thymic epithelial cells between MG thymuses and normal thymuses. This finding suggests that there is no qualitative difference of epithelial cells between MG thymuses and normal thymuses.

In the cultures of MG thymuses and normal thymuses for 7–10 days some viable lymphocytes were surrounding some epithelial cells (Fig. 1d). These epithelial cells ceased to proliferate and were destroyed by degeneration thereafter. Therefore, we considered that this phenomenon might be autosensitization of lymphocytes against self-epithelial cells. In an animal study Cohen and Wekerle (1973) reported autosensitization of lymphocytes against self-epithelial cells in the cultures of mouse thymus. They

suggested that some regulatory mechanism was involved which inhibited autosensitization in vivo. In our study a similar phenomenon was observed and it is likely that this phenomenon occurs in vivo. This finding may be consistent with the notion that sensitization to AChR occurs in the thymus.

The role of the thymus in the pathogenesis of MG has been controversial. There are three main roles to be considered at present: (1) AChR antigens exist in the thymus (Engel et al. 1977; Kao and Drachman 1977; Fuchs et al. 1980). (2) Anti-AChR antibodies are produced in the thymus (Vincent et al. 1978; Fujii et al. 1984). (3) AChR-specific helper T-cells are exported from the thymus to other antibody-producing sites (Newsom-Davis et al. 1981, 1983). Concerning AChR antigens in the thymus, Kao and Drachman (1977) reported that thymic myoid cells bore AChRs in the cultures of MG thymus by ARG. Engel et al. (1977) reported in an immunoenzyme study that thymic epithelial cells contained AChRs. Fuchs et al. (1980), in an immunofluorescence study, reported that thymocytes of the mouse bore AChR-like antigen. Raul et al. (1983) reported in an immunofluorescence, study that AChR-bearing cells were present in human normal thymus as well as human MG thymus. There have been few reports in which the localization and number of AChR on human MG thymus and normal thymus cells were compared. In our study by ARG, silver grains were diffusely distributed on thymic epithelial cells (Figs. 4a and b). Since this binding was obviously blocked by *d*-tubocurarine (Fig. 4c), this binding was considered to be a specific binding and toxin binding sites (AChRs) were considered to be present on thymic epithelial cells. The pattern of staining was different from that in chick muscle cultures (Fig. 4d). However, Engel et al. (1977) reported that they demonstrated a diffuse AChR of thymic epithelial cells, resembling that of uninnervated skeletal muscle fibers in tissue culture (Askanas et al. 1976). In human muscle cultures AChRs were diffusely distributed without foci of increased staining on the myotube (Askanas et al. 1977). Therefore, the distribution of AChR on thymic epithelial cells was not quite different from that on uninnervated human skeletal muscle fibers in tissue culture, but the number of AChR on thymic epithelial cells was much less than that on the myotube. There was no significant difference in the distribution and number of AChRs on the epithelial cells between MG thymuses and normal thymuses. This finding suggests that epithelial cells containing AChR are not a pathologic feature of the human MG thymus. We consider that the development of MG is not dependent on the difference of quantity of AChR antigens but dependent on autosensitization against self-AChR by an unknown mechanism. It is likely that the break-down of self-tolerance against AChR occurs in the thymus by an unknown mechanism.

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