FINE STRUCTURE PECULIARITIES OF MEDULLARY EPITHELIAL CELLS AND THEIR RELATION TO THYMUS LYMPHOPOIESIS

Tz. Marinova, E. Boshnakova

The medullary epithelial cells of the thymus synthesize and secrete humoral factors (4, 6, 15, 19, 23), which presumably secure the intrathymus production of immunocompetent lymphoid cells (1, 3, 5, 16, 17, 21). We tried to find out cytological evidence of the possible participation of medullary epithelial cells in the build up of immunocompetent thymocytes.

Material and methods

Material from the medulla of thymus of 7 four-month-old mice (Swiss line), killed through decapitation, was prepared after the standard method for electron microscope investigation: prefixation in 5 per cent glutaraldehyde (0.1 mol phosphate buffer with pH 7.4, for two hours at 4° C), postfixation in 1 per cent osmium tetroxide (0.1 mol phosphate buffer with pH 7.4, for 1.5 hours at 4° C), dehydration in ascending alcohol grades, and Durcupan ACM embedding. The semi-thin sections were stained, whilst the ultrathin ones were subjected to double contrast staining after Reynolds (1963). Photographs were taken in electron microscope JEM 7A.

Results and discussion

In the medullary part of the thymus lobulus, the epithelial cells form a specific microenvironment for the thymocytes, and considerably exceed them in number. The study of their fine structure allows for differentiation of two basic types of cells — dark and clear. All medullary epithelial cells possess common characteristic features — a big and clear nucleus, bundles of tonofibrils in the cytoplasm and desmosomes (Fig. 1). The nuclear chromatin displays uniform distribution. Usually a single nucleolus is visualized, and in rare instances — more than one. Most frequently, their structure represents a transition from homogeneous to reticular nucleolus. The dark epithelial cells exhibit an elongated shape, and have long cytoplasmic processes, penetrating in between adjacent thymocytes, and thereby building up a reticulum within the medulla. Their cytoplasm is rich in cell organelles. Inflated cisterns and vacuoles of the granular endoplasmic reticulum are observed (Fig. 2 a, b), as well as numerous free ribosomes within the cytoplasm. The Golgi complex is particularly well developed with marked vacuolization, and not infrequently, several Golgi fields are formed. The paranuclear space is usually extended. The richness and great variety of dense lysosomal bodies and secretory granules characterizes the dark medullary epithelial cells (Fig. 3). The clear epithelial cells have rather rounded shape, not infre-

quently with eccentrically situated nucleus, and adequately developed Golgi apparatus (Fig. 4) in the neighbourhood of which a varying number of vacuoles are situated. It is of interest to note the clear epithelial cells with numerous vacuoles, occupying totally the cytoplasm. These vacuoles are restricted by a basement membrane, exhibit variable sizes, and are most fre-

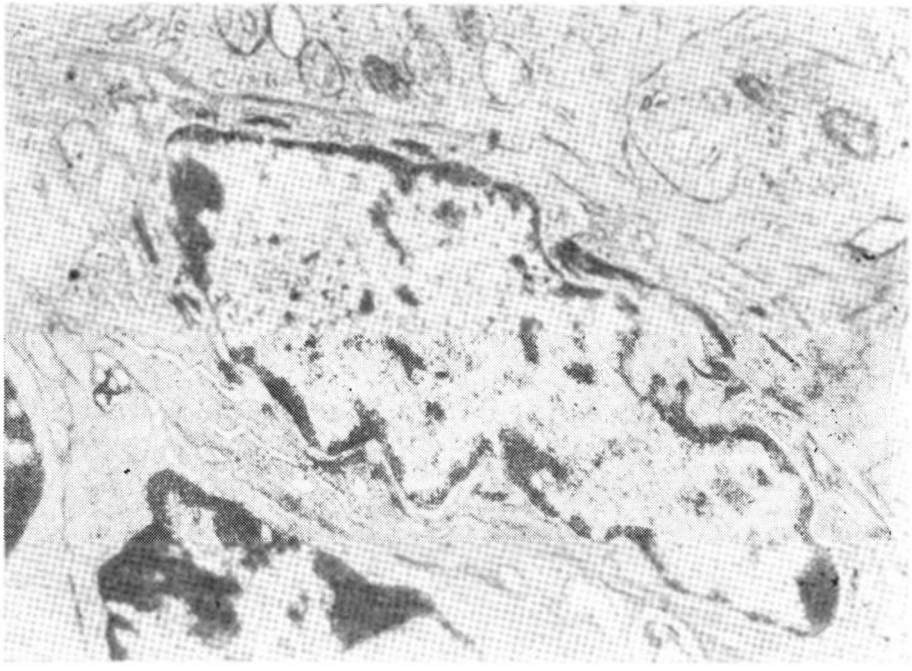

Fig. 1: Medullary epithelial cell; N-nucleus (electr. micr. magnif. ×5000).

quently filled up with electron clear, homogeneous material. Single vacuoles reach larger sizes, and form intracellular cysts with microvilli protruding into the lumen.

The cytological features of the medullary epithelial cells, taking part in the build up of Hassall's corpuscles, are not object of the present study.

The results of our investigation show that the medullary epithelial cells of thymus possess common ultrastructural features, which lay emphasis on their epithelial nature (10, 13, 19, 21, 23). The distinction of two types of epithelial cells is most likely conditioned by the greater abundance of cytoplasmic organelles within the dark cells, imparting a darker appearence to the cytoplasm, being furthermore in agreement with the data concerning the presence of similar cells in the guinea pig (I. Töro and I. Oláh, 1966) and golden hamster (T. Ito, 1966) thymus.

A cytological characterization of the dark epithelial cells was done, and it gives us sufficient reason to accept that in addition to the role of a supporting reticulum (12), they should be also considered as cells with enhanced functional activity. The richness and diversity of dense lysosomes indicate that the representatives of the vacuolar apparatus participate in the control of the processes connected with substance synthesis (2).

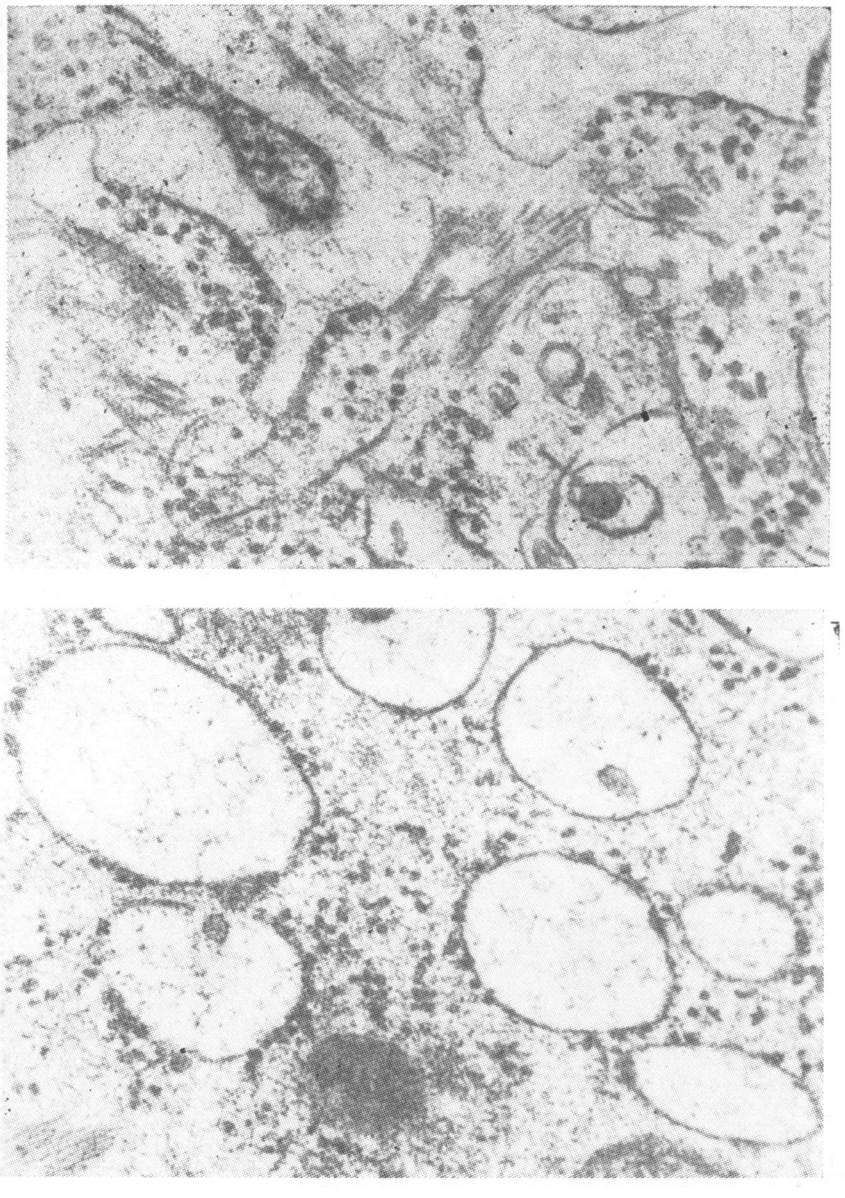

Fig. 2: Dark medullary epithelial cell; a — dilated cisterns, b — vacuoles of the granular endoplasmic reticulum (electr. micr. magnif. ×35 000).

Our data demonstrate a relationship between the vacuoles of the clear cells and the Golgi apparatus elements, although we have no sufficient ground to make a definitive statement concerning their genesis. Most of the investigators point out that the content of these vacuoles yields a PAS (+) reaction for acid mucopolysaccharides or glycoproteins (9, 11, 26), and accordingly

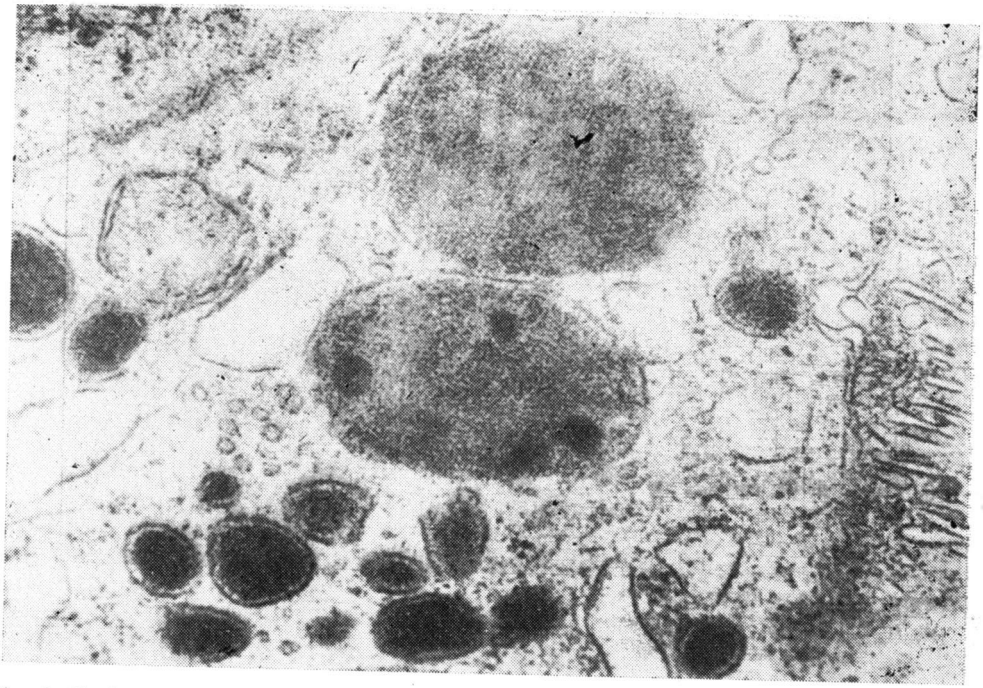

Fig. 3: Dark medullary epithelial cell — dense bodies and secretory granules (electr. micr. magnif. ×30 000).

Fig. 4: Cytoplasm of a clear medullary epithelial cell — Golgi apparatus (electr. micr. magnif. × 40 000).

identify it as a thymus lymphopoietic factor (8, 15) related to the secretory function of the thymus (5, 18). The presence of a correlation between secretory activity of the medullary epithelial cells and lymphocyte mitosis, established autoradiographically and during experimental involution and regeneration (8), the dependence of thymocyte appearance upon the differentiation and organization of epithelial cells (27), and the evidence of thymocytes penetrating the medullary epithelial cells' cytoplasm (26) are all facts in support of the statement made. Presumably the thymus factors stimulate the proliferation of lymphocytes (8, 9), but nevertheless, the exact mechanism of action is by no means elucidated. It is possible that the medullary epithelial cells assume the role of stem cells for the cortical epithelium which may in turn control thymus lymphopoiesis (14, 26), or else exert influence on the differentiation of thymocytes after a beforehand effect of cortical epithelial cells on the stem bone-marrow cells (22). It is by no means ruled out that medullary lymphopoiesis is independent from the cortical one (9).

Our results show that medullary epithelial cells possess certain cytological signs of active synthesis, contributing to their participation (independent or not) in the intrathymus production of immunocompetent cells.

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УЛЬТРАСТРУКТУРНЫЕ ОСОБЕННОСТИ МЕДУЛЯРНЫХ ЭПИТЕЛИАЛЬНЫХ КЛЕТОК И ИХ СВЯЗЬ С ТИМУСНЫМ ЛИМФОПОЭЗОМ

Ц. Маринова, Е. Бошнакова

РЕЗЮМЕ

Исследована тонкая структура эпителиальных клеток в мозговом слое вилочковой железы у четырехмесячных мышей Сюисс. Применена фиксация 5% глутаральдегидом, послефиксация в 1% осмиевом тетраоксиде и включение в Durcupan ACM. Разграничены два основных вида клеток, различающиеся по форме, степени развития гранулированного эндоплазматического ретикулума и аппарата Гольджи, богатству и особенностям мембраноограниченных вакуолей, гранулям и плотным лизосомальным телам. Описан особый вид светлых эпителиальных клеток.

Обсуждается связь между ультраструктурными особенностями медулярных эпителиальных клеток и внутритимусной продукцией иммунокомпетентных лимфоидных клеток.