FUNCTIONAL MORPHOLOGY OF THYMIC EPITHELIAL CELLS

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Thymic epithelial cells (ECs) play a key role in the processes of T-lymphocyte proliferation and differentiation as well as in selection of various lymphocyte clones (6, 12, 18, 23, 24). These are rather complex and definitely unclarified processes realized by direct receptor-mediated intercellular interactions and by production of locally and distantly acting biologically active substances, the so-called thymic factors synthesized in ECs. There are numerous investigations by many authors of EC morphological heterogeneity concerning various systematic classes of vertebrata and man. As a result, more than 20 morphological types of ECs have been described (1, 2, 4, 5, 7, 9—19, 22, 23, 25, 26). These investigations are unequivalent in volume and data obtained are hardly comparable mainly because of terminological discrepancies.

The purpose of the present work is to study comparatively the forms of EC structural organization and the cellular composition of thymic epithelial reticulum with representatives of various vertebrata classes chiefly with a view to clarify the more essential phylogenetic differences in the representation of EC morphological types and their involvement in formation of cellular complexes together with other thymic cells.

Material and methods

Thymi from nine biological species representatives of various systematic classes of Chordata type were studied. The following species were examined: class Osteichthyes (Mugil auratus), class Amphibia (Rana ridibunda), class Reptilia (Coluber jugularis, Vipera ammonites), class Aves (Columba livia), class Mammalia (Vespertilio pipistrelus, Mus musculus (Swiss, DBA/2), Lepus europeus, Homo sapiens). Standard electron microscopic (15) and immunohistochemical technique using two kinds of monoclonal antikeratin antibodies (BH11 and BC3) as well as anticytokeratin polyclonal antiserum and ABC kit according to the methods of Hsu (1981) and Takacs et al. (1987) was applied in our study.

Results and discussion

EC kind predominates among non-lymphoid thymic cells in all the biological species studied. Most ECs form a supporting meshwork for lymphoid cells, the so-called epithelial reticular cells. The rest insignificant part of ECs is presented by structural elements of thymic components of epithelial nature — Hassal's corpuscles and intercellular cavities. ECs of these species studied possess some common and constant morphological signs—desmosomes, intermediate filaments, contact with a basement membrane when subseptal and subcapsular

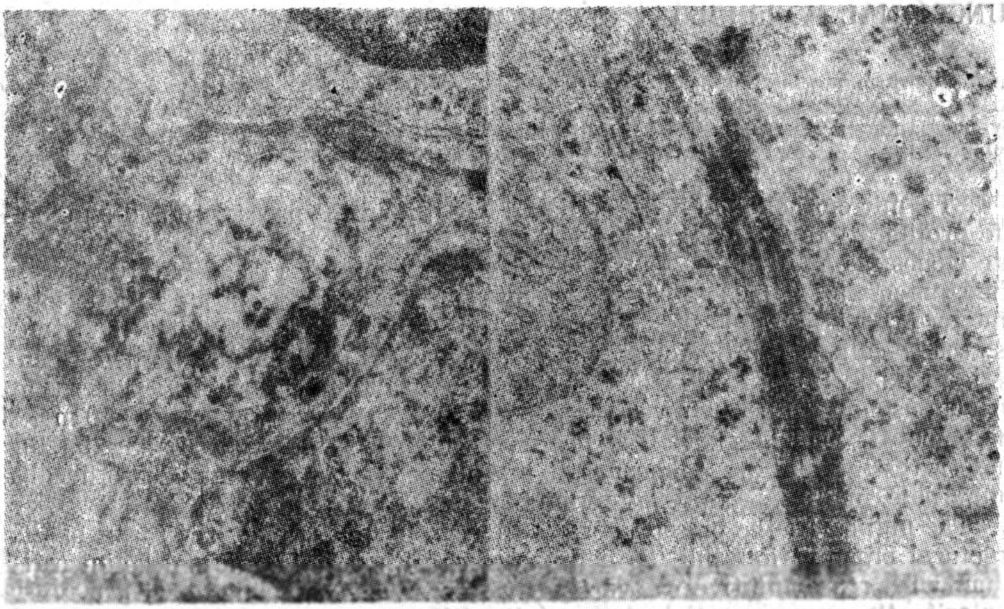

Fig. 1. -a, b. Parts of cortical epithelial cells (ECs), type 1.
Fig. 1-a. Mammalian thymus. Magn. x 20000.
Fig. 1-b. Osseus fish thymus. Magn. x 40000.

ECs are concerned. At the same time, they are characterized by certain ultrastructural peculiarities related to differences of the relative share and ultrastructure of cell organelles. They enable to dfstinguish four main morphological EC types:

Type-1 («classical») EC. Most cortical and some medullary ECs in the thymus of these species studied belong to this type. Bundles of intermediate filaments and desmosomes present their typical ultrastructural features (fig. 1-a, b). In lower-class vertebrata (fishes, amphibia) thymi desmosomal contacts between ECs, type-1 are relatively numerous. The presence of membrane-limited vacuoles with electron-dense floculated content is a constant feature of the same EC type in thymi of birds and mammals.

Type-2 («granular») EC. They can be found in thymic medulla of all the species studied. They contain secretory granules. Granular diameter varies between 200 and 300 nm in different systematic groups. Granular halo and limiting membrane as well as electron density of granular core is diferent, too. Protein-synthesizing apparatus of ECs, type-2 is very well-developed in all cases.

Type-3 («ciliary» and «microvillous»). EC. Commonly, they participate at epithelial cavity formation and possess cilia and/or microvilli. They are characteristic of thymi of amphibia, reptilia, aves, and mammalia. EC, type-2 cytoplasmic organelles are polarized and have morphological signs of synthetic activity.

Type-4 («vacuolar») EC. They are typical of avian and mammalian thymic medulla. Rough endoplasmic reticulum and Golgi apparatus both are well-developed. Relatively numerous Golgi vacuoles polarize often ECs, type-4 and lend them a labyrinth-like shape.

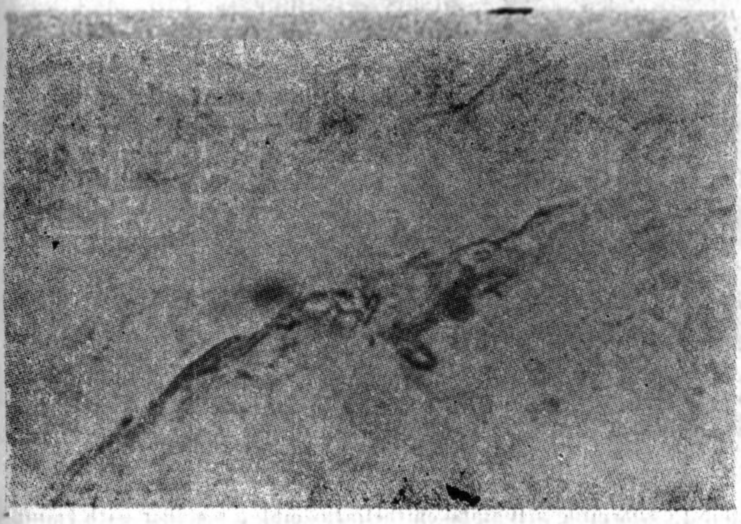

Fig. 2. Immunoreactive keratin-positive ECs, type 1, with network-like localization in the thymic cortex. Reptilian thymus. Microphoto 10 x 100.

The afore-described morphologfcal EC types are involved to a different extent in the formation of complexes (associations) with other thymus cell kind.s Usually, ECs, type-1, are singly scattered. The immunohistochemical labelling of their tonofilament-bound keratin antigens by polyclonal anticytokeratin antiserum enables their visualization in the form of keratin-positive ECs forming an epithelial network of differeht density in thymic cortex and medulla (fig. 2). The participation at the formation of lympho-epithelial complexes together with lymphoid cells is characteristic of the same morphological EC type. For instance, in the outer part of thymus cortex and most often subcapsularly in DBA/2 mouse thymus complexes of BH11-positive, ECs, type-1 and lymphoid cells differing in size and shape can be established (fig. 3). ECs, type-2 participate frequently at formation of myo-epithelial complexes together with myoid cells which are typical of reptilian thymic medulla when our material is concerned.

Data presented and purposeful systematization of literature data available indicate that ECs are a constant component of thymocyte microenvironment in all vertebrata classes and in man. They possess certain common and stable morphological signs determining their epithelial nature and distinguishing them from the rest cells of thymic reticulum.

The epithelial component of thymic reticulum is arranged according to a common principle in the thymus of any vertebrata that could be considered a morphological precondition for community in the functional activity of thymus epithelium. For example, EC, type-1 supporting function concerning lymphoid cells proves to be universal for thymus of any systematic groups studied. ECs with morphological signs of synthetic activity can be observed in thymus of all the species studied, too. In this sense, our results obtained are in concordance

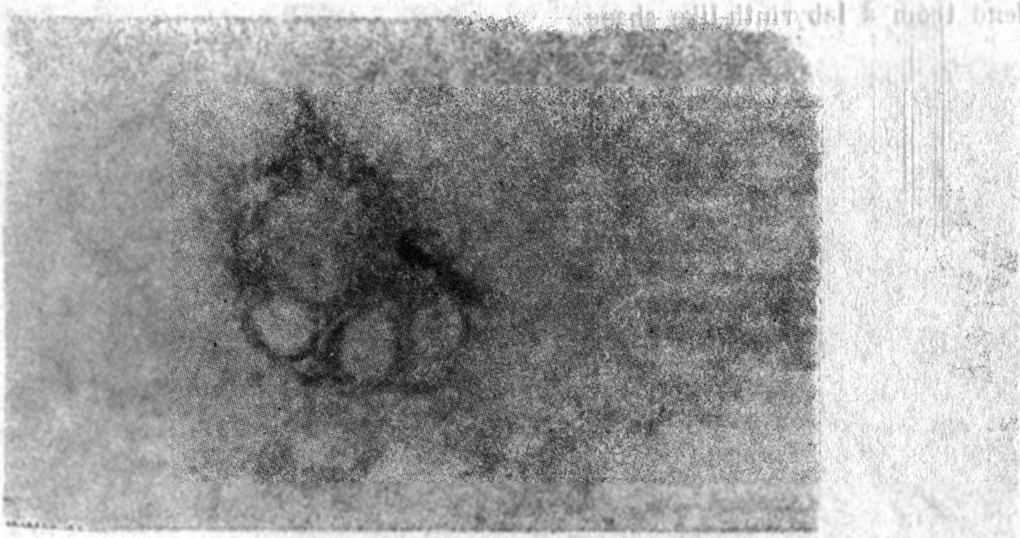

Fig. 3. BH11 keratin-positive ECs forming a lympho-epithelial complex together with immunonegative lymph cells in the subcapsular region of mouse thymus. Microphoto 10 x 100.

with literature data that ECs are a secretory system of the thymus (12, 16). Besides, in direction from lower-class to higher-class vertebrata morphological preconditions for perfecting of the mechanism of thymic factor secretion can be observed in the thymus. Elements of protein-synthesizing apparatus are thus engaged to a different extent, too.

On the other hand, data obtained argue for phylogenetic differences in the representation of morphological EC types and their ultrastructure. On this basis, an attempt is made to differentiate four cardinal morphological EC types probably specialized to perform various funcfions: a supporting one, a synthetic one, and a structure-forming one. In direction from lower-class to higher-class vertebrata morphological variety of EC types increases. As a rule, thymus medulla is richer in morphological EC types than thymus cortex does. Morphological EC types are differently represented in various systematic groups of vertebrata probably because of heterogenous origin of thymic rudiment in different biological species, of some regional and ontogenetic peculiarities as well as of the influene of diverse ecological, stress and other factors (3, 12, 13, 16, 26). These phylogenetic differences in EC morphological heterogeneity are most probably a precondition for phylogenetic ones in thymic epithelium function with lower-class and higher-class vertebrata.

ECs are heterogenous concerning their involvement in formation of associations with other thymus cells, too. There are differences within one and thn same biological species: Subcapsular and subseptal ECs, type-1, take part i lympho-epithelial complex formation. These complexes present one of the most essential stages of the process of intrathymic T-lymphocyte differentiation (6,24). Some differences in a phylogenetical aspect are also found out: lympho-epithelial complexes are observed in the thymus of birds and some mammals (6, 12, 24) but myo-epithelial ones — in the thymus of amphibia, reptilia and in myasthenic human thymus (12, 20). These literature data and our own observations allow us to assume that in ascending systematic order the tendency towards EC involvement into formation of complexes with other cells increases.

The results presented about the phylogenetic peculiarities of EC morphological heterogeneity correlate well with literature data about differences of the chemical composition and of the number of thymic factors isolated from thymi of various vertebrata (3, 11, 12, 16, 25). They are the reasons to suppose that processes of intrathymic T-lymphocyte proliferation and differentiation are morphologically assured in various systematic groups of organisms by means of morphologically heterogenous ECs involved to a different extent and by a different mechanism of action in these processes.

REFERENCES

1. Champan, Jr. W. *Z. Zellforsch.*, 114, 1971, 220—222. — 2. Frazier, J. A. *Z. Zellforsch.*, 136, 1973, 191 — 194. — 3. Froehly, M. F., P. A. Deschaux. *Thymus*, 8, 1986, 235—237. — 4. Gorgollon, P., O. Anaya. *Acta Anat.*, 100, 1978, 136—141. — 5. Gorgollon, P. *J. Morphol.*, 117, 1983, 25—28. — 6. Hirai, H., Y. Nishi, H. Kaneshima, Y. Buma, Y. Nichizuka. *Experim. Hematol.*, 13, 1985, 215—221. — 7. Hoshino, T. *Z. Zellforsch.*, 59, 1963, 513—519. — 8. Hsu, S. — M., I. Raine, H. Fanger. *J. Histochem. Cytochem.*, 29, 1981, 577—580. — 9. Isler, H. *J. Cell Sci.*, 20, 1976, 135—137. — 10. Ito, T., T. Hoshino. *Z. Zellforsch.*, 69, 1966, 311—314. — 11. Kapa, E., I. Olah, I. Toro. *Acta Biol. Acad. Sci. Hung.*, 19, 1968, 203—206. — 12. Kendal, M. D. The Thymus Gland. London, etc., Academic Press, 1981, 218 p. — 13. Leceta, J., A. Zapatal *Developm. Compar. Immunol.*, 9, 1985, 653—655. — 14. Marinova, Tz. *Verh. Anat. Ges.*, 80, 1986,633—634, — 15. Marinova, Tz. *C. R. Acad. Bulg. Sci.*, 39, 1986, 91—93. — 16. Nabarra. B., I. Andrianarison. *Thymus*, 9, 1987, 95—107. — 17. Nagata, S. *Cell Tissue Res.*, 179, 1977, 87—94. — 18. Olah, I., P. Rohlich, I. Toro. Ultrastructure of Lymphoid Organs. An Electron-microscopic Atlas. Paris, Masson, 1975. 317 p. — 19. Pereira, G., Y. Clermont. *Anat. Rec.*, 169, 1971, 613—618. — 20. Raviola, E, G. Raviola. *Amer. J. Anat.*, 121, 1967, 623—628. — 21. Reynolds, E. *J. Cell Biol.*, 17, 1963, 208—212. — 22. Singh, J. *J. Anat.*, 132, 1981, 444—447. — 23. Takacs, L., W. Savino, E. Monostroni, I. Ando, J. F. Bach, M. Dardenne. *J. Immunol.*, 138, 1987, 687—694. — 24. Wijngaert, F. P. van de, L. H. Rademakers, H. — J. Schuurman, R. A. Weger, L. Kater. *J. Immunol.*, 130, 1983, 2348—2352. — 25. Wijngaert, F. P. van de, M. D. Kendall, H. — J. Schuurman, L. H. Rademakers, L. Kater. *Cell Tissue Res.*, 237, 1974, 227—231. — 26. Zapata, A. *Developm. Compar. Immunol.*, 4, 1980, 459—463.

ФУНКЦИОНАЛЬНАЯ МОРФОЛОГИЯ ЭПИТЕЛИАЛЬНЫХ КЛЕТОК ВИЛОЧКОВОЙ ЖЕЛЕЗЫ

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РЕЗЮМЕ

С помощью электронномикроскопической и иммуногистохимической техники проведено исследование на материале вилочковой железы представителей различных систематических групп позвоночных животных (Pisces, Amphibia, Reptilia, Aves, Mammalia).

Были исследованы формы структурной организации тимусных эпителиальных клеток, а также клеточный состав тимусного эпителиального ретикулума.

Описано несколько более существенных филогенетических различий представительства морфологических типов эпителиальных клеток, а также их ультраструктура и участие в образовании лимфо-эпителиальных и мио-эпителиальных комплексов вилочковой железы.