Institute of Experimental Morphology and Anthropology

Acta morphologica et anthropologica, 10
Sofia • 2005

Presence and Distribution of *NGF* and *BDNF* Immunopositive Cells in Human Hyperplastic Thymus

*Ts. Marinova**, *K. Velikova***, *D. Petrov****, *G. Chaldakov*****, *L. Manni******, *L. Aloe******

* *Departments of Medical Biology, Medical University, Sofia*
** *Departments of General and Clinical Pathology, Medical University, Sofia*
*** *Departments of Surgery, Medical University, Sofia*
**** *Division of Cell Biology, Department of Forensic Medicine, Medical University, Varna*
***** *Institute of Neurobiology and Molecular Medicine, Rome*

We investigated, both at light and electron microscopic levels, the cellular distribution of immunoreactivity for *NGF (Nerve Growth Factor) and BDNF (Brain-Derived Neurotrophic Factor)* in myasthenic thymus. NGF and BDNF were expressed in human thymus and their expression was enhanced in the thymus of patients affected by Myasthenia gravis (histopathological findings-thymic hyperplasia), compared to the control subjects. The increased distribution of NGF and BDNF immunopositive cells correlated with thymocyte microenvironment alterations during myasthenic thymic transformation.

Key words: NGF, BDNF, thymus, hyperplasia, microscopy.

Introduction

Recent evidence indicates that some thymic cells produce NGF and BDNF, and express their low- and high-affinity receptors (p75, TrkA and TrkB) under both normal and pathological conditions, including autoimmune diseases [1, 2, 4, 7]. The present study was focused on: (i) cellular distribution of NGF and BDNF immunoreactivity, and (ii) structural alterations in the thymocyte microenvironment of human hyperplastic myasthenic thymus at both light and electron microscopic level.

Material and Methods

Thymus specimens from healthy subjects (n=7) and patients affected by Myasthenia gravis (MG), (histopathological findings-thymic hyperplasia, n=16, age range, 22 to 53 years) were obtained from surgery cases and used for histological and immunohistochemical analysis. Some kinds of antibodies (Ab), namely: Anti-NGF Ab (r)-(NGF H-20, sc-548), Anti-BDNF Ab (m)-(C-9, sc-8042), Anti-TrkA Ab (m)-(p-

TrkA E-6, sc-8058), Anti-TrkB Ab (r)-(794, sc-12) and Anti-Pan cytokeratin (m)-(C 1801), as well as ABC Staining System (r), ABC Staining System (m), Anti-rabbit IgG- and Anti-mouse IgG (whole molecule, 5nm or 10 nm gold granules) were used. Routine light microscopy, indirect immunoperoxidase method, transmission electron microscopy, immunoelectron microscopy, and immunogold-silver staining procedure were performed according to the standard protocols that we have previously described [3, 5]. Staining specificity was assessed by control tests. Labomikroscop Axioskop 20 (Fb Carl Zeiss Opton) and electron microscope Hitachi H500 were used.

Results

All types of medullary epithelial cells and some cortical epithelial cells displayed NGF and BDNF immunoreactivity in both pathological and normal thymuses. Strong immunoreactivity for NGF and BDNF was found in the medullary, subcapsular/subseptal epithelial cells, as well as in some mast cells, macrophages, and interdigitating reticulum cells in the thymus of subjects with hyperplasia-associated myasthenia gravis. The increased distribution of NGF and BDNF cortical immunopositive epithelial cells, forming islands or streaks, correlated with a spectrum of epithelial microenvironment structural alterations such as giant Hassall's corpuscles with atypical localization, lympho-epithelial cell complex formations and accumulated in large intrathymic areas intermediate filaments (Fig. 1). Numerous Hassall's bodies of MG subjects displayed elevated NGF- and BDNF-positivity

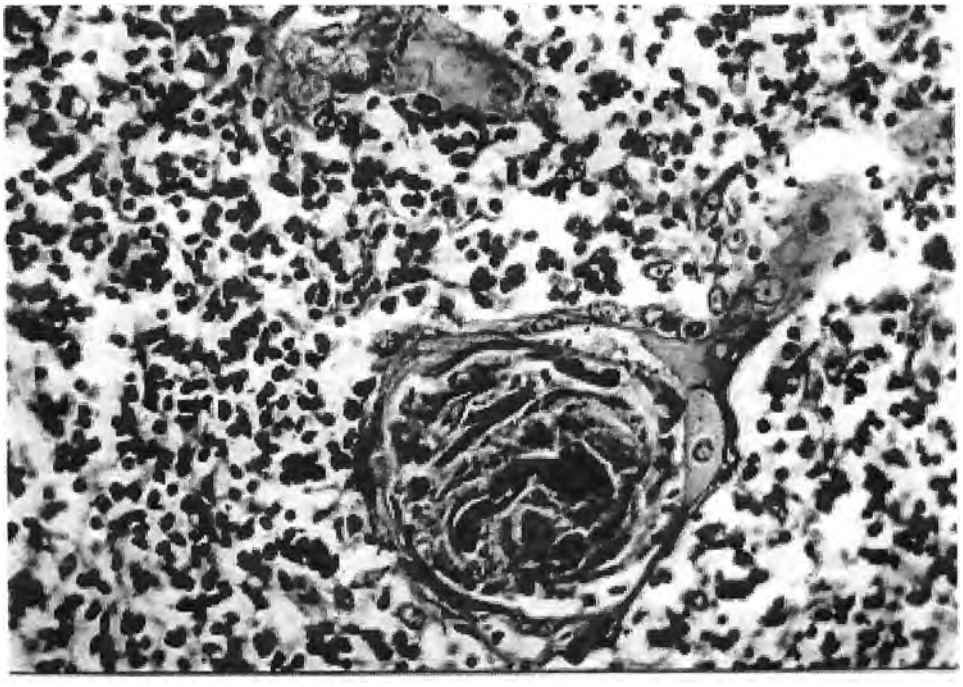

Fig. 1. Section showing a giant Hassall's corpuscle in the medullary region of hyperplastic myasthenic thymus (Magnification × 200)

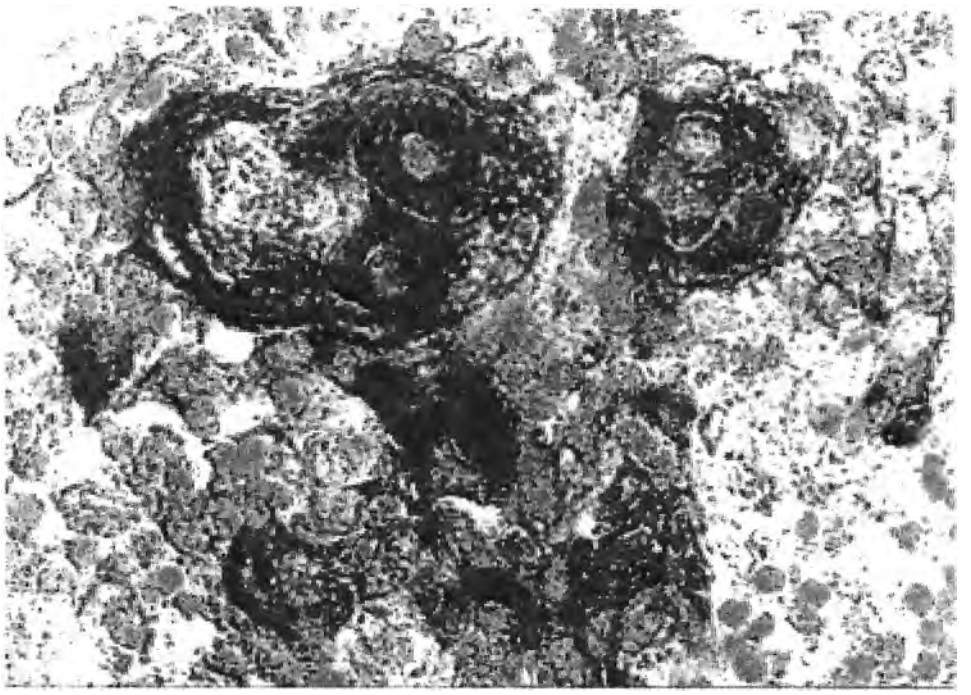

Fig. 2. Light micrograph demonstrating a strong NGF immunopositivity in the thymic medullary region, including in Hassall's corpuscles of myasthenic thymus (Magnification × 1000)

as compared to the Hassall's bodies in control thymus (Fig. 2). NGF- and BDNF-immunoreactivity was also found in the lympho-epithelial complexes of the myasthenic thymus, which were mainly localized in the cortical area.

Discussion

MG is a human autoimmune disease often leading to pathological manifestation in the thymus and in the nervous system [1, 6]. We have recently shown that the thymus of patients affected by MG expresses elevated levels of NGF suggesting a role of this neuroimmune mediator in the pathobiology of MG [5]. In the present study we detected that the thymocyte microenvironment undergoes reorganization during myasthenic hyperplasia, which includes essential regional and intracellular (structural and immunocytochemical) peculiarities. The increased expression of NGF and BDNF in thymic stromal cells suggests that these factors might be part of epithelial microenvironmental molecules implicated in thymic cell function and/or in the pathophysiology of MG. Our data raise the question of NGF and BDNF role in the local auto- and/or paracrine regulatory processes, and in the thymocyte microenvironment plasticity during myasthenic transformation.

References

1. Aloe, L. et al. Nerve growth factor and autoimmune diseases. — Autoimmunity, **19**, 1994, 141-150.
2. Aloe, L. Nerve growth factor and neuroimmune responses: basic and clinical observations. — Arch. Physiol. Biochem., **109**, 2001, 354-356.
3. Marinova, T. et al. Cellular localization of NGF and NGF receptors in aged human thymus. — Folia Biol. (Praha), **49**, 2003, 160-164.
4. Parrens, M. et al. Expression of NGF receptors in normal and pathological human thymus. — J. Neuroimmunol., **85**, 1998, 11-21.
5. Stampachiacchiere, B. et al. Altered levels of nerve growth factor in the thymus of subjects with myasthenia gravis. — J. Neuroimmunol., **146**, 2004, 199-202.
6. Turrini, P., M. L. Saccaria, L. Aloe. Presence and possible functional role of nerve growth factor in the thymus. — Cell. Mol. Biol., **47**, 2001, 55-64.
7. Vega, J. A. et al. Neurotrophins and the immune system. — J. Anat., **203**, 2003, 1-19.