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Structural and Immunocytochemical Alterations of Hassall's Bodies in Aged Human Thymus

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We investigated the structural heterogeneity and immunohistochemical profile of Hassall's bodies (HB) in human aged thymuses, obtained during cardiovascular surgery. Employing a panel of twelve antibodies, immunohistochemical methods and semiquantitative scale for detection of immunopositive cells, we observed modulation of immunoreactivity, associated with structural alterations of immunopositive HB. The results presented enrich the information about HB as antigenically distinct, functionally active, multicellular formations within the thymic medulla and raise the question of their role during age-dependent involution of the thymus.

Key words: Hassall's bodies, human thymus, immunocytochemistry.

Introduction

Hassall's bodies (HB) are unique components of the thymus which provide developing thymocytes with paracrine and juxtacrine signals to ensure their proper functional maturation during intrathymic lymphopoiesis [1, 6, 7]. Although HB have been proposed to act in both maturation of developing thymocytes and removal of the apoptotic cells, their function remains an enigma [2, 4, 10]. The purpose of the present work was to verify whether age-dependent involution of the thymus influences the presence and distribution of HB, their structural heterogeneity and immunohistochemical profile.

Material and Methods

Specimens from normal thymus (n=23) were obtained during cardiovascular surgery of old (aged 61-74 years; n=17) and young (aged 2-12 years; n=6) individuals. The thymuses collected showed no evident pathological disorders. The study was approved by the Ethics Committee of the Faculty of Medicine. Routine light microscopy, indirect immunofluorescence and immunoperoxidase techniques were performed according to standard protocols [5, 9]. Twelve primary (monoclonal and polyclonal) antibodies (Ab):

Anti-Pan cytokeratin Mo Ab (C1801, Sigma Chemical Co.), *Anti-NGF* polyclonal rabbit Ab (NGF H-20; sc-548), *Anti-TrkA* Mo Ab (p-TrkA E-6, sc-8058), *Anti-p75* Mo Ab, NGFR p75 (ME 20.4, sc-13577), *Anti-CD14* Mo Ab (UCH-M1, sc-1182), *Anti-IGF-I* Mo Ab (UBI/Biomol, Hamburg), *Anti-IGF-IR* Mo Ab (sc-N-20), *Anti-BDNF* Mo Ab (C-9, sc-8042), *Anti-bFGF* Mo Ab (UBI/Biomol, Hamburg), *Anti-CNTF* polyclonal goat Ab (R&D Systems), *Anti-EGFR* Mo Ab (clone 29.1, E-2760, Sigma St. Louis, MO), *Anti-GDNF* Mo Ab (MAB212, R&D Systems) were applied. *FITC-labeled* anti-mouse Ig (Santa Cruz Biotechnology), *Texas Red-conjugated* anti-rabbit Ig (Santa Cruz Biotechnology) as well as *ABC Staining Systems* (mouse-sc 2017 or rabbit-sc 2018) were used as secondary antibodies. Control experiments were carried out in parallel.

Results

Aged thymuses displayed large areas of adipose tissue containing scattered islands composed of epithelial cells, lymphocytes, reticular connective tissue and HB with different morphology. Four morphological types of HB were detected in the age-involuted thymus, as compared with the young specimens (data not shown): 1. Giant HB with flaky material, lymphocytes and stromal cells in the middle (Fig. 1); 2. HB with a characteristic concentric arrangement of keratinizing epithelial cells and hyaline homogenate in the center (Fig. 2); 3. "Cellular" HB with vital cells, among which individual cells exhibited the characteristics of active secretory cells, without a degenerative center; 4. Cystically degenerated HB.

HB in the young thymus displayed intensive labelling intensity after immunostaining for CK, NGF, TrkA, p75, IGF-I, IGF-IR, CD14, bFGF, BDNF, CNTF, EGFR. HB of aged thymus preserved a strong immunoreactivity for CK, p75, IGF-IR,

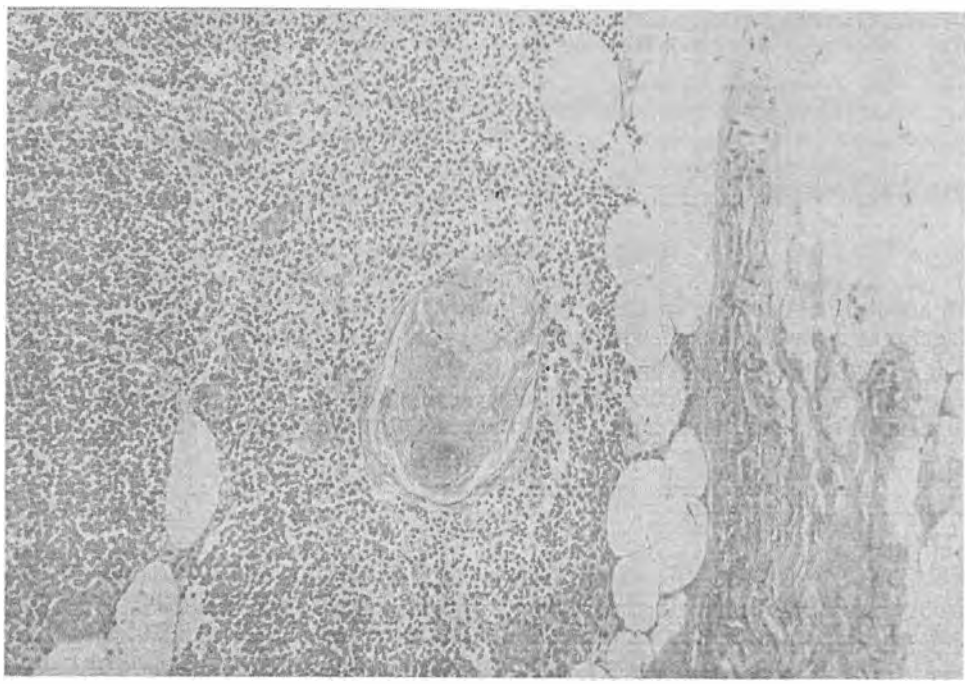

Fig. 1. Age-involuted human thymus (67-year-old male) with first type HB in the medulla. HE, × 100

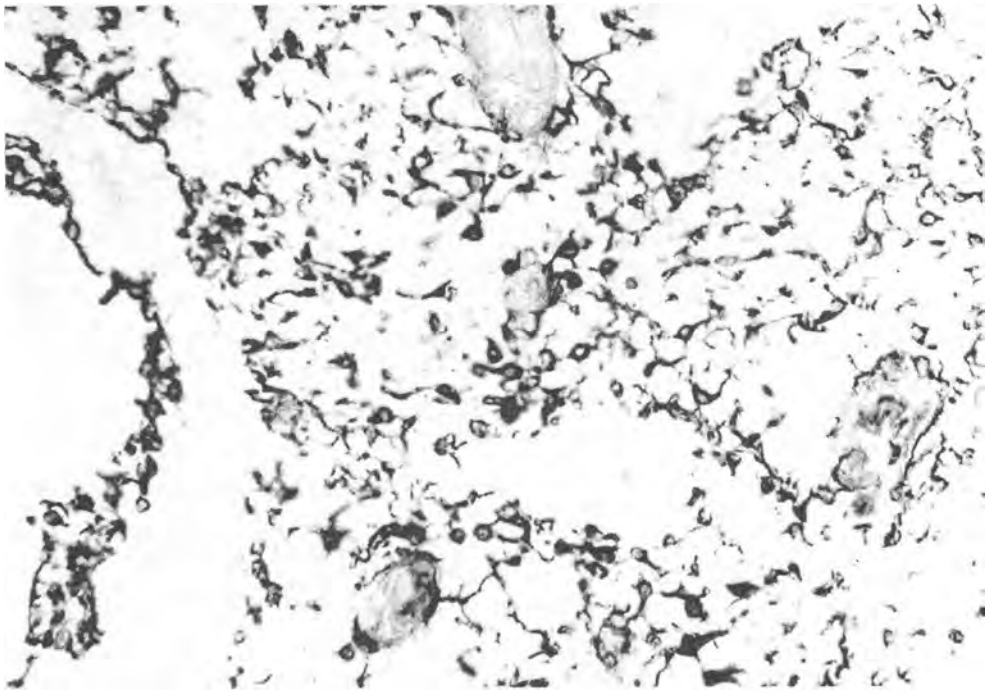

Fig. 2. Aged human thymus (68-year-old male) with strong cytokeratin immunopositive epithelial cells forming network and HB. Immunoperoxidase staining, × 400

CD14, were stained less intensely for NGF, TrkA, IGF-I, bFGF, BDNF, CNTF, EGFR and were GDNF immunonegative.

The structural components of HB displayed co-localization of IGF-I and IGF-I receptor (IGF-IR) immunoreactivity. The decreased expression of IGF-I in the aged thymus correlated with modulation of immunoreactivity of double (IGF-I/IGF-IR) positive thymic cells. In negative controls the HB were unstained.

Discussion

The thymus undergoes age-related (physiological, chronic) involution in the course of normal ontogenetic development [2, 5]. Thymic involution is particularly important in relation to immunosenescence and its various associated diseases [2, 3]. We have previously reported presence of NGF and NGF receptors immunoreactivity in age-involuted human thymus [5] and modulation of ABH antigen reactivity in senile thymus [9] in contrast with young thymus [8]. This investigation provides new structural and immunocytochemical data for immunohistochemical profile of HB suggesting that these multicellular formations are antigenically distinct and functionally active in growth factor receptor-mediated cell signalling mechanisms during physiological thymic involution. It seems likely that HB are structures, implicated in thymocytes ontogenesis, T-cell apoptosis and possibly intrathymus negative selection throughout life.

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