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Thymocyte microenvironment reorganization during leukemia transformation (structural and immunocytochemical data)

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A lot of experimental and clinical investigations of different kinds of mouse leukemia and leukemic patients have shown the important role of the thymus in the pathogenesis of lymphoid leukemia and the obligatory participation of the thymic epithelial cells in the intrathymic leukemogenesis. We have previously reported that the lympho-epithelial cell complexes seem to be a unique striking example of very close lymphoid/stromal cells interaction involved in thymic microenvironmental plasticity during chemically induced leukemogenesis. Our results provide new structural and immunocytochemical evidence for intermediate filaments alterations in cortical epithelial cells of mouse leukemia thymus. They suggest simultaneous cytokeratin/Nerve growth factor immunoreactivity modulation of thymic epithelium, which is probably involved in thymocyte microenvironment reorganization during leukemogenesis.

Key words: thymocyte microenvironment, experimental lymphoid leukemia

Introduction

Accumulating evidence shows that the thymus is involved in the pathogenesis of mouse lymphoid leukemia, but the precise role of the thymic microenvironment, including epithelial cells (EC) and their intermediate filaments (IF) proteins cytokeratins (CK) is unclear [1, 2].

We have previously reported that the lympho-epithelial cell complexes seem to be a unique striking example of very close lymphoid/stromal cells interaction involved in thymic microenvironmental plasticity during chemically induced leukemogenesis [3, 4, 5].

The present study was focused on IF reorganization and some immunocytochemical features (distribution and co-localization of CK and Nerve growth factor-NGF immunoreactivity) of the thymic microenvironment in mice with experimental chemically induced acute *L1210* lymphoid leukemia.

Material and Methods

Leukemic DBA/2 inbred mice with chemically induced L1210 lymphoid leukemia and control mice were investigated simultaneously [2, 4].

Four primary antibodies (Ab) were used for the first step of the immunocytochemical study: 1. Anti-pan cytokeratin (mouse, Mo Ab; Cat. Nr. C 1801, Sigma Chemical Co.); 2. Anti-Cytokeratin Ab (Mo, BioGenex Lab USA); 3. Anti-NGF Ab (NGF H-20, r-p, SC-548); 4. Anti-CD 14 Ab (mouse monoclonal Ab; UCH-M1, Cat. Nr. SC-1182, Santa Cruz Biotechnology); Secondary Antibodies: ABC Staining Systems (mouse-sc 2017 and rabbit-sc 2018, Santa Cruz Biotechnology) were used as secondary antibodies for immunohistochemistry at the light microscopic level. Anti-mouse IgG (coupled with 10-nm gold particles) and anti-rabbit IgG (coupled with 5-nm gold particles) gold-conjugated Ab (whole molecule, Sigma Chemical Co.) were applied for immunoelectron microscopy.

Routine methods for transmission electron microscopy, immunoperoxidase, and immunogold electron microscopy were applied according to the earlier described standard protocols [2, 4, 5]. To define the nature of the thymic cell types that expressed CK/NGF, serial tissue sections were stained with anti-pan-cytokeratin and anti-CD 14 Ab, which are known to detect epithelial cells and monocyte/ macrophages, respectively. Control experiments (negative and positive) were carried out in parallel.

Results

Intermediate filament accumulation and disassembly in large cytoplasmic areas, "elongated" desmosomes and nucleus reorganization were found in leukemia thymus (Fig. 1).

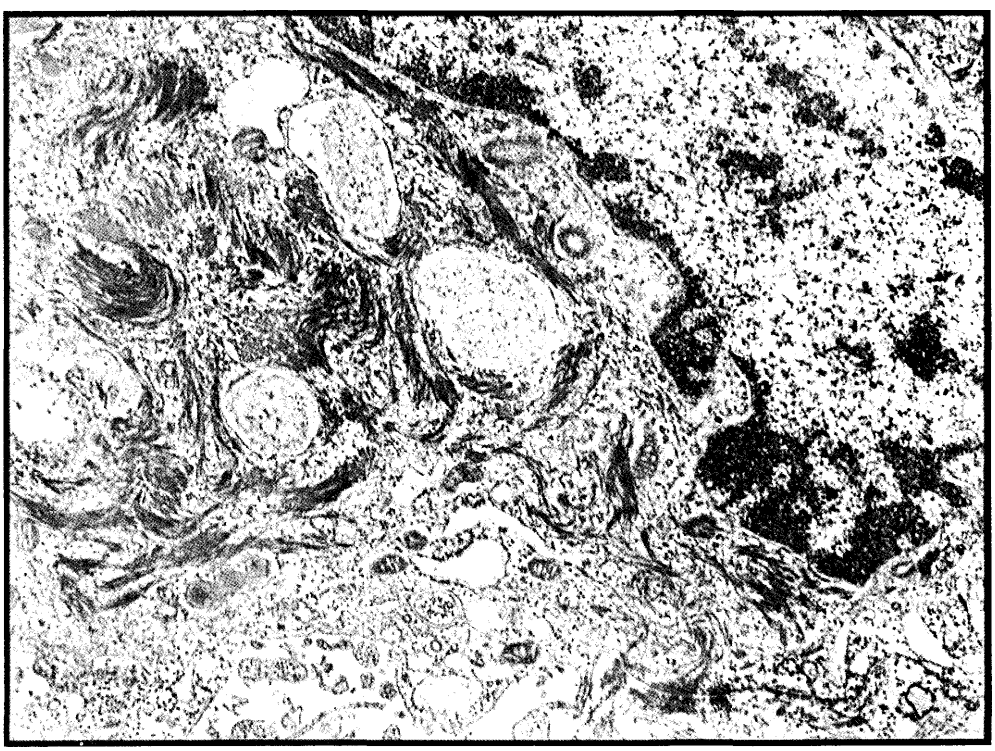

Fig. 1. Electron micrograph of EC from leukemic thymus showing IF accumulation and disassembly (Orig. magn. X 24 000).

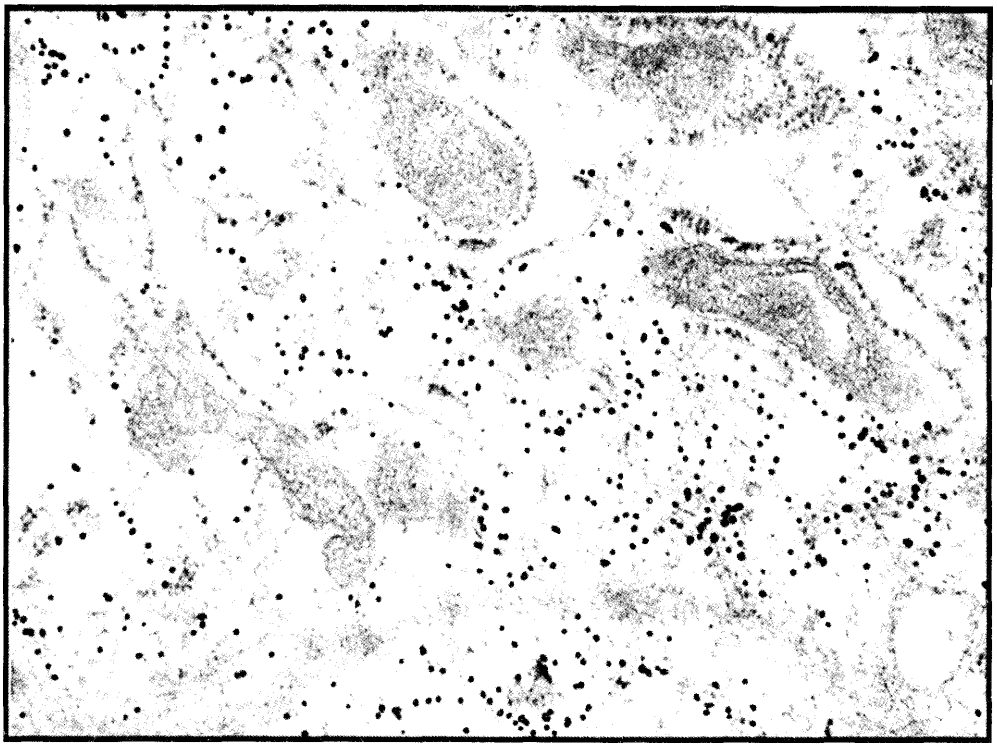

Fig. 2. Ultrastructural data for overexpression of CK in immunopositive leukemic EC. Immunogold localization of CK-binding gold granules in IF/mitochondria rich areas of EC (Orig. magn. X 28 000).

Immunogold labelling showed overexpression of CK in mitochondria rich areas of leukemic EC (Fig. 2). The increased CK immunoreactivity of epithelial cells correlated with lympho-epithelilal cells (LEC) complex formation, including thymic nurse cell-like structures and rosettes in the external cortex. Polarization of CK-binding gold granules at sites of both EC-EC and EC-LC contacts was subcapsulary and subseptaly observed. Ultrastructural data for co-localization of CK (10 nm binding gold granules) and NGF (5 nm binding gold granules) were found by immunogold electron microscopy in double binding CK/NGF-immunopositive EC processes.

Discussion

A lot of experimental and clinical investigations of different kinds of mouse leukemia and leukemic patients have shown the important role of the thymus in the pathogenesis of lymphoid leukemia and the obligatory participation of the thymic epithelial cells in the intrathymic leukemogenesis. However, the mechanisms by which EC induce "abortive" differentiation and leukemic transformation of the thymocyte precursors of the bone marrow, are still unknown [2, 6, 7].

The results of the present study provide new structural and immunocytochemical evidence for IF alterations in cortical EC of mouse leukemia thymus.

EC may be able to meet the requirements of an altered milieu by modulation their intermediate filaments proteins. The data suggest simultaneous CK/NGF

immunoreactivity modulation of thymic epithelium, which is probably involved in thymocyte microenvironment reorganization during leukemogenesis. It seems likely that the modulation of CK/NGF coexpression may be one of the mechanisms by which the double-immunopositive cortical EC are involved in local regulation of thymus plasticity and T-cell development in leukemic thymus.

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