Turk J Vet Anim Sci
26 (2002) 1171-1175

Research Article

Thymic Nurse Cells Determined *In Situ* at the Light and Electron Microscopic Level

F. Figen KAYMAZ, Attila DAĞDEVİREN
Hacettepe University, Faculty of Medicine, Department of Histology and Embryology, Ankara - TURKEY

Received: 20.11.2001

Abstract: Thymic nurse cells are isolated thymic epithelial/thymocyte complexes described *in vitro*. Their *in vivo* existence is controversial. We examined rat thymi in order to distinguish thymic nurse cells *in vivo* using the zinc iodide-osmium tetroxide (ZIO) fixation/staining technique. There were mainly two types of strongly ZIO reactive cells in the thymic cortex: macrophages and small clusters of thymic epithelial cells. The latter group was composed of thymic epithelial cells and intact thymocytes forming complexes. Thus we suggest that these structures are *in situ* equivalents of thymic nurse cells.

Key Words: Thymic nurse cells, Zinc iodide-osmium tetroxide, Light microscopy, Electron microscopy

In Situ Timik Nurse Hücrelerin Işık ve Elektron Mikroskobu ile Tanımlanması

Özet: Timik nurse hücreler, izole edilerek *in vitro* olarak tanımlanmış epiteliyal retiküler hücre/timosit kompleksleridir. In vivo varlıkları ise tartışmalıdır. Çinko iyodid-Osmiyum Tetroksit tespit ve boyama yöntemi kullanılarak sıçan timusunda timik nurse hücrelerin in vivo olarak ayırt edilerek gösterilmesi amaçlandı. Timus korteksinde çinko iyodid ile kuvvetli reaksiyon veren iki hücre tespit edildi. Bunlar; makrofajlar ve küçük gruplar oluşturan epiteliyal retiküler hücreler ile sağlıklı timositlerin oluşturduğu komplekslerdi. Bu ikinci grubu oluşturan yapıların in situ timik nurse hücrelerle benzer olduğu sonucuna varıldı.

Anahtar Sözcükler: Timik nurse hücreler, Çinko iyodid-osmiyum tetroksit, Işık mikroskopi, Elektron mikroskopi

Introduction

As the thymus is the major site for T lymphocyte maturation, its functional and structural characteristics are of special interest. The interaction of thymic epithelial cells (TECs) and/or accessory cells with maturing thymocytes is essential for T lymphocyte maturation (1,2). The term thymic nurse cell (TNC) was first introduced by investigators who were aiming to document this relation between thymocytes and TECs by isolation studies. These structures were described as packs of TECs and intact thymocytes composed of few epithelial but usually many lymphocytes. Their ultrastructure and physiological characteristics have also been studied *in vitro* (3-10). However, demonstration of their *in vivo* existence is still controversial. There are few reports describing *in situ* equivalents of TNCs in the literature (11-13). Thus we examined the structural features of these cell types in detail at light and electron microscopic levels in rat thymus.

Materials and Methods

Ten male Wistar albino rats 20 days old weighing 100-125 g were used to obtain tissue samples. The animals were decapitated and the whole thymus removed quickly and immersed in zinc iodide solution. They were kept in darkness for 24 h at room temparature. Zinc iodide-osmium tetroxide solution was prepared by mixing 2 g of metallic iodine and 6 g of metallic zinc powder with 8 ml of distilled water (14). This was then added to 80 ml of distilled water slowly as it was an exothermic reaction. The solution was filtered 5 min later in order to remove excess zinc and then added to 2% unbuffered osmium tetroxide solution at a ratio of 4 to 1. It was always prepared freshly and kept in darkness before use. Fixed and stained rat thymic tissue samples were processed for routine electron microscopy analysis. They were dehydrated, infiltrated and embedded in araldite. Semithin sections were cut with a LKB 11800 pyramitom, stained with 1% solution of methylene blue-

azur II in 1% borax, and examined and photographed under the light microscope (Olympus BH2). Ultrathin sections were cut and collected on copper grids. After staining with uranyl acetate and lead citrate, the sections were examined and photographed under the electron microscope (Zeiss EM 9S2).

Results

In semithin sections of rat thymus, known compartments of the organ, namely the cortex and the medulla, were distinguished. In the cortex, two types of strongly ZIO (+) cells were determined. The first cell group was clearly distinguished as cortical macrophages with the presence of numerous heterophagic vacuoles containing remnants of degenerating thymocyte nuclei. These cells had a large cytoplasm with an irregular outline densely stained with ZIO (Figure 1). The second cell type was ZIO reactive thymic epithelial cells. This latter type of cells had numerous slender processes extending between and surrounding thymocytes (Figure 2 and inset). Their cell body contained a rounded nucleus and a thin rim of cytoplasm which was densely ZIO reactive (Figure 3). Some of these cortical epithelial cells were enveloping groups of thymocytes resembling TNCs (Figure 2 inset, Figure 3)

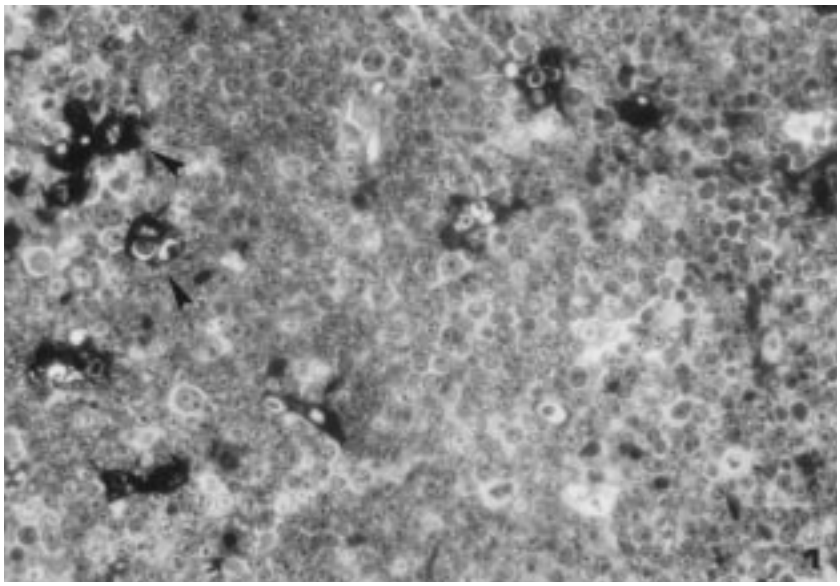

Figure 1. Several macrophages which are densly ZIO reactive were seen in the thymic cortex (arrows). In the cytoplasm of these cells, heterophagosomes containing degrading thymocytes at varying extents were clearly distinguished with their choromatin residues.

Counter stain: Methylene blue - Azur II, x100.

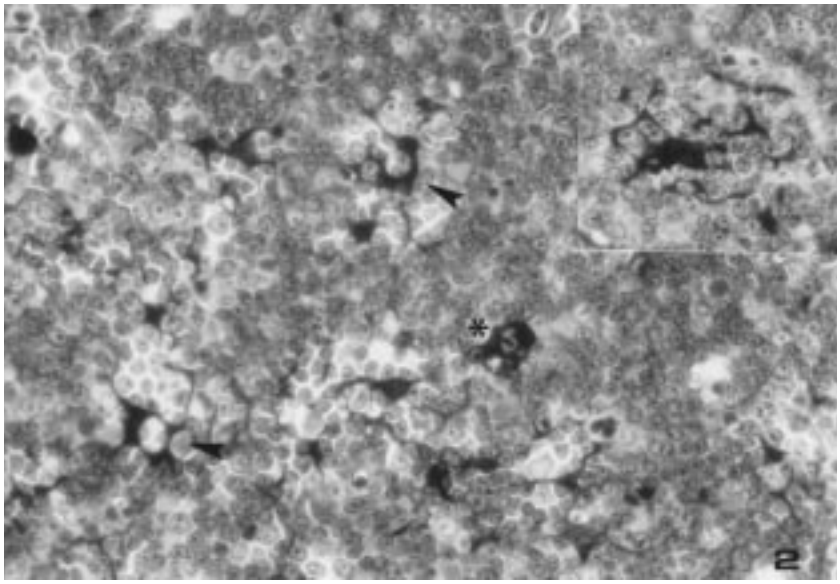

Figure 2. The cortical area consists of several ZIO epithelial cell packs (arrow) and a cortical macrophage (*). The slender processes of epithelial cells tightly surrounded cortical thymocytes appearing in contact. Inset: A ZIO (+) epithelial cell and its processes surrounding thymocytes resembling a thymic nurse cell is seen.

Counter stain: Methylene blue - Azur II, x100.

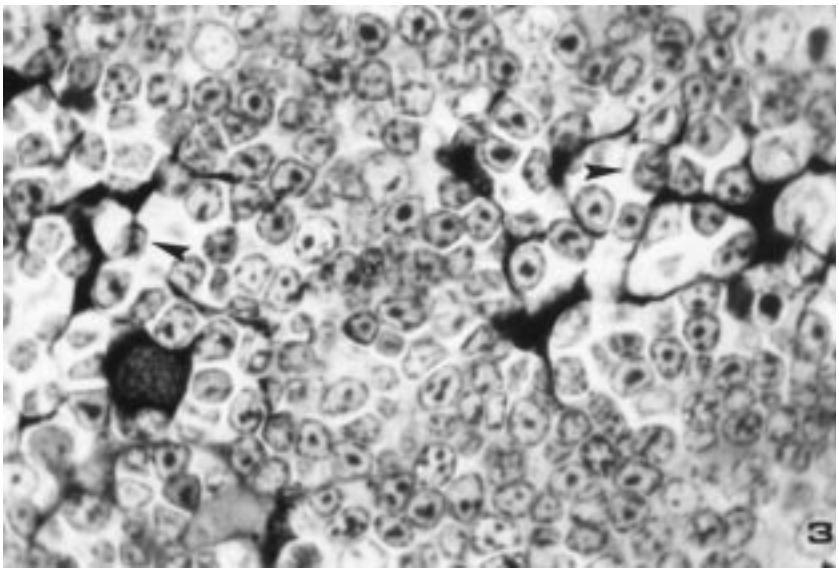

Figure 3. High magnification of thymic epithelial cells with slender processes surrounding thymocytes are seen. A mitotic figure with in one of these thymocytes packs surrounded by slender ZIO(+) processes of TECs is also distinguished (arrow).

Counter stain: Methylene blue - Azur II, x400.

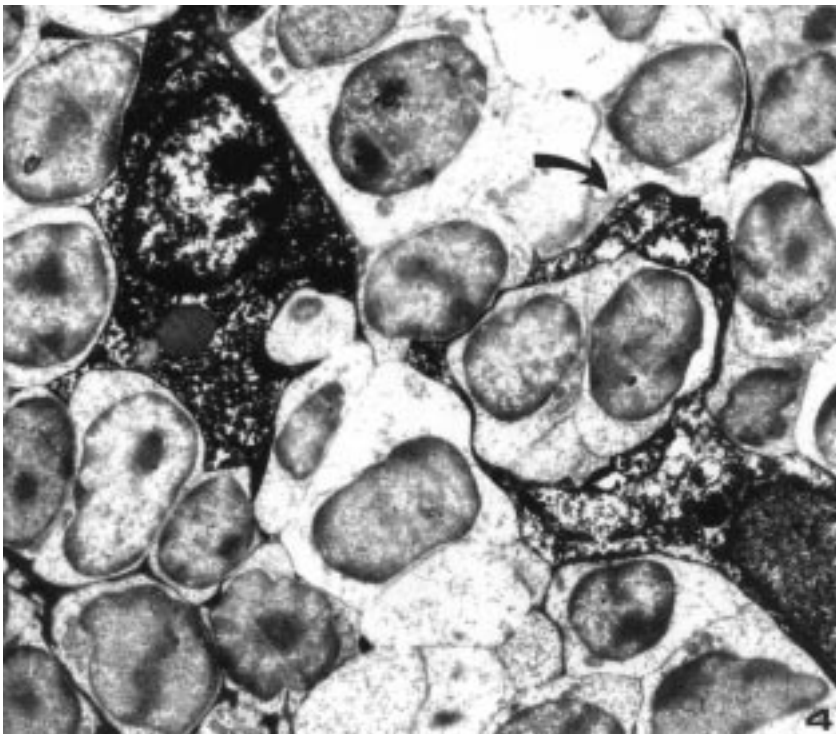

Figure 4. Low power electron micrograph of thymus cortex. Cytoplasm of a cortical macrophage was strongly ZIO (+). Slender processes extend between thymocytes (arrow).

Stain: Uranyl acetate - Lead citrate, x4500.

The above-mentioned ZIO (+) cell types were also easily distinguished at the ultrastructural level. Staining was present in all cytoplasmic elements of these cells among non-reactive thymocytes, which clearly distinguishes their relationship (Figure 4, 5). All organelles including granular endoplasmic reticulum cisterna, mitochondria, and other membranous and non-membranous organelles were ZIO (+), and were enveloped by ZIO (+) plasma membrane.

Discussion

The thymic microenvironment is mainly composed of thymic epithelial cells (TECs) and a number of mostly blood-borne haematolymphoid cell types like B cells, macrophages, interdigitating cells, Langerhans cells, eosinophils, mast cells, plasma cells, neuroendocrine cells and myoid cells, supported by a connective framework (1,2,15). Intercation of thymocytes with some members

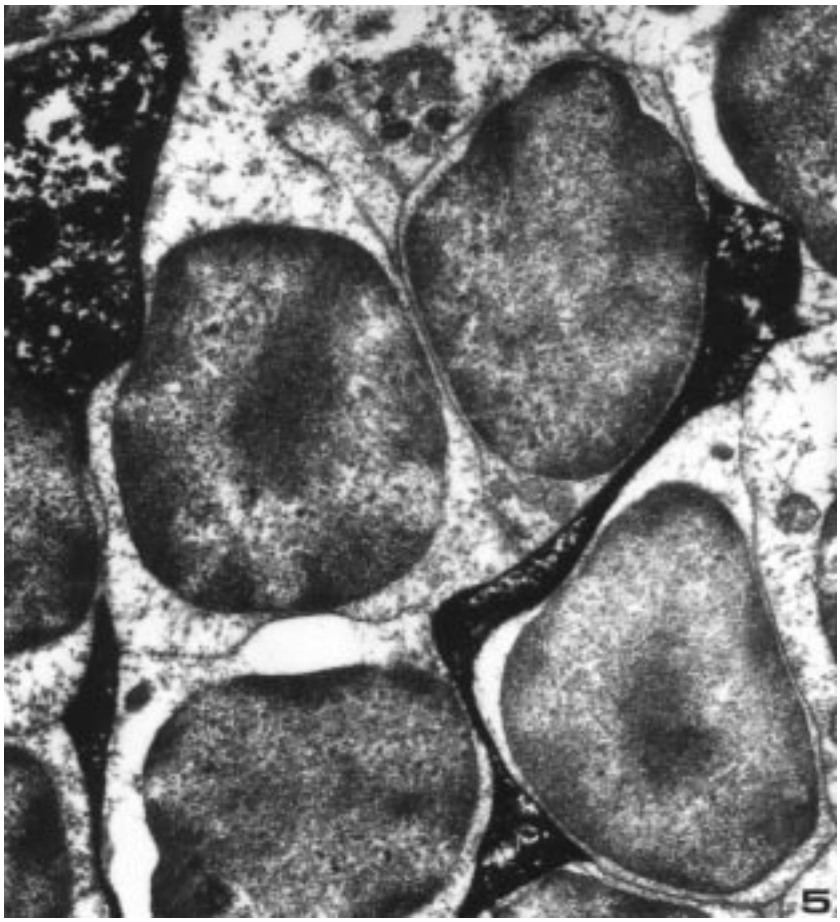

Figure 5. The structural relation between strongly ZIO(+) cell processes of epithelial cells and thymocytes is seen.

Stain: Uranyl acetate - Lead citrate, x11,250.

of this unique microenvironment is essential for the complex maturation process of developing T cells. Certain humoral factors and cytokines play important roles in this process as well as the negative and positive selection of maturing thymocytes (1-3,5,8,10). Investigators isolated thymic fragments by tryptic dissociation and subsequently performed gravity sedimentation of the organ to study in detail the TEC-thymocyte interaction and introduced the term thymic nurse cell (TNC) to describe these isolated TEC/thymocyte complexes (3,6,9). Although there is data on the TNC structure and function, their *in situ* demonstration is still controversial. Few reports on the demonstration of TNCs are found in the literature(11-13). In one of these reports the ZIO technique was reported to result in a TNC-like staining in thymic cortex and so we examined tissue samples from the rat thymus in order to obtain further evidence at light and electron microscopic level (11).

Mainly two types of ZIO (+) cells were distinctly outlined in the thymic cortex. The characterization of these cells could be done at both light and electron microscopic levels. The first group of cells were clearly macrophages as distinguished by the presence of numerous heterophagic vacuoles or secondary lysosomes containing remnants of lymphocyte nuclei. These cells had a large ZIO (+) cytoplasm with an irregular outline and sometimes short processes extending between thymocytes. The second group of cells had a very thin rim of cytoplasm surrounding their more rounded nuclei, which were characterized by the presence of slender ZIO (+) processes extending between and surrounding thymocytes.

Thymocytes surrounded or sometimes completely encircled by these thin ZIO (+) cellular processes always had normal structural features; some even showed mitotic figures. Thus these cells were considered not to be degenerating but maturing cells. The structural relation of this latter group of cells, namely thymic epithelial cells, with thymocytes was also clearly observed. As these cells occur separately rather than

being diffusely spread in the cortex representing a thymic cortical epithelial network, we suggest that these TEC/thymocyte complexes represented *in situ* equivalents of TNCs. The findings of the present study strongly suggested the *in vivo* existence of TNCs and that the ZIO technique is convenient for the demonstration of these thymocyte/TEC complexes.

References

1. Kendall, M.D.: Functional Anatomy of the Thymic Microenvironment. J. Anat., 1991; 177: 1-29.
2. Boyd, L.R., Tucek, C.L., Godfrey, D.I., Izon, D.J., Wilson, T.J., Davidson, N.J., Bean, A.G.D., Ladyman, H.M., Ritter, M.A., Hugo, P.: The Thymic Microenvironment. Immunol, Today. 1993; 14(9): 445-459.
3. Wekerle, H., Ketelsen, U., Ernst, M.: Thymic Nurse Cells. Lymphoepithelial Cell Complexes in Murine Thymuses: Morphological and Serological Characterization. J. Exp. Med., 1980; 151: 925-944.
4. Van Vliet, E., Melis, M., Van Ewijk, W.: Immunohistology of Thymic Nurse Cells. Cell. Immunol., 1984; 87: 101-109.
5. De Waal, M.R., Leene, W., Roholl, P., Wormmeester, J., Hoeben, K. T.: Cell Differentiation within Thymic Nurse Cells. Lab. Invest. 1986; 55 (1): 25-34.
6. Toussaint-Demylle, D., Scheiff, J., Haumont, S.: Thymic Nurse Cells: Morphological Study during Their Isolation from Murine Thymus. Cell Tissue Res., 1990; 261: 115-123.
7. Dipasquale, B., Tridente, G.: Immunohistochemical Characterization of Nurse Cells in Normal Human Thymus. Histochemistry. 1991; 96: 499-503.
8. Penniger, J., Wick, G.: Thymic Nurse Cell Lymphocytes React against Self Major Histocompatibility Complex. Eur. J. Immunol., 1992; 22: 79-83.
9. Toussaint-Demylle, D., Scheiff, J., Haumont, S.: Thymic Nurse Cells in Culture: Morphological and Antigenic Characterization. Cell Tissue Res., 1993; 272: 343-354.
10. Aguilar, L., Aguilar-Cordova, E., Cartwright, J., Belmont, J.: Thymic Nurse Cells Are Sites of Thymocyte Apoptosis. J. Immunol., 1994; 152: 2645-2651
11. Dağdeviren, A., Alp, H., Örs, Ü.: New Applications for the Zinc Iodide-Osmium Tetroxide Technique. J. Anat., 1994; 184: 83-91.
12. Van de Wijngaert, F.P., Rademakers, L.H., Schuurman, H.J., de Weger, R.A., Kater, L.: Identification and *in situ* Localization of the "Thymic Nurse Cell" in Man. J. Immunol., 1983; 130 (5): 2348-2351.
13. Defresne, M.P., Goffinet, G., Boniver, J.: *in situ* Characterization in Freeze-Fractured Mouse Thymuses of Lymphoepithelial Complexes Ultrastructurally Similar to Isolated Thymic Nurse Cells. Tissue Cell. 1986; 18 (3) :321-330.
14. Neibauer, G., Krawczyk, W.S., Kidd, R.L., Wilgram, G.F.: Osmium-Zinc Iodide Reactive Sites in the Epidermal Langerhans Cell. J. Cell Biol. 1969; 43: 80-89.
15. Suster, S., Rosai, J.: Histology of the Normal Thymus. Am. J. Pathol., 1990; 14 (3): 284-303.