Morphologic heterogeneity of human thymic nonlymphocytic cells

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Abstract

The thymus is the central organ of the immune system. It is essential for the development and maintenance of normal immune system, especially cell-mediated immunity. From the morphological point of view, the thymus is divided into two main compartments, cortex and medulla. The thymic microenvironment consists of a network of reticular epithelial cells and other fixed and free cells. The microenvironment of thymus is very important for the selection and maturation of T cells. T cell differentiation occurs via T cell receptors. The major histocompatibility complex participates in interactions between T cells and thymic epithelial cells, in addition to interactions between T cells and dendritic cells, macrophages and myoid cells. The neuroendocrine system regulates early T cell differentiation by the transcription of neuroendocrine genes in the stromal network and expression of cognitive receptors by immature T cells. This work briefly summarizes morphological and ultrastructural characteristics of thymic epithelial cells, dendritic cells, macrophages and myoid cells. It is accompanied by the authors' own photomicrographs and electronmicrograph from a transmission electron microscope. All of these cells play a critical role in the proliferation, differentiation and selection of precursor cells in the T-cell lineage, but the precise mechanisms not well understudood.

Introduction

The thymus is a central (primary) lymphoid organ with an important endocrine function. It is a crossroad between the immune and the neuroendocrine systems (Geenen & Brilot 2003). It is a place where the T cells precursors proliferate and mature. Mature T cells are responsible for the cell-mediated immunity. The thymus plays an important role in providing a suitable microenvironment for the proliferation, differentiation, TCR gene rearrangement and repertoire

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selection of T cells (Anderson *et al.* 2000; Staal *et al.* 2001; Guyden & Pezzano 2003; Laurent *et al.* 2004). The differentiation of T cells is a carefully orchestrated process. Mature and immunocompetent T cells leave the thymus through blood and lymphatic vessels and migrate to the peripheral lymphoid organs (Kato 1997; Dorko *et al. 1997*).

From the phylogenetical point of view, thymus played a key role in the evolution of animals during the development of adaptive immune system; therefore it is an important feature separating higher vertebrates from other animals. The development of thymus and T cells is a highly conserved process in vertebrate evolution (Bowden *et al.* 2005; Varga *et al.* 2008). During human ontogenesis, the thymus passes through marked morphological changes, including a rapid prenatal growth and postnatal, age-related involution. The highest immunological activity of the thymus is in the age of 6th months after birth, when the thymus contains the highest overall numbers of thymocytes (Weerkamp *et al.* 2005). After the 1st year of life, this organ starts to undergo changes, which become considerable in puberty and adulthood. The thymus undergoes a progressive reduction in size due profound changes in its anatomy associated with a loss of thymic epithelial cells and a decrease in thymopoiesis. These age-related changes in thymic structure are called physiological involution (Nabarra & Andrianarison 1996; Bodey *et al*. 1997; Domínguez-Gerpe & Rey-Méndez 2003; Taub & Longo 2005; Weerkamp *et al.* 2005; Cavalotti et al. 2008; Pospíšilová *et al*. 2008). Even though, the remaining tissue is able to produce immunocompetent T cells until a later age. It is therefore probable, that the thymus is important throughout lifetime, as an organ with an immune and endocrine function. Age-associated involution is reversible. For example, castration of the old rats and mice result in the regeneration of the atrophic thymuses (Dorko *et al.* 1998; Heng *et al*. 2005).

Thymic microenvironment

Thymus is composed of a cortical and a medullar part. Stained histological sections show a dark cortex coating a pale medulla. The cortex of the thymus lobes is darker because of the presence of numerous closely packed maturating T cells. An area with abundant blood vessels lies between the cortex and the medulla. It is termed as the corticomedullary junction (Schuurman *et al.* 1997). The medulla can form "buds", which sometimes extend deeply into the cortex. Similarly, portions of the cortex can extend to the medulla (Sainte-Marie 1974). Typical features of the medulla are the Hassall's corpuscules, which are formed mainly of concentrically arranged epithelial cells.

The thymic microenvironment is composed of a variety of stromal cells and the extracellular matrix. The thymic microenvironment constitutes a unique environment for the differentiation, maturation and selection of T cells. It is composed of:

Fixed cells: numerous morphologically and phenotypically heterogenic thymic epithelial cells (Fig. 1). A significant role in the thymus played also myoid cells and fibroblasts,

Free (wandered) cells: macrophages, dendritic cells and other cells of hemopoietic origin (eosinophilic and neutrophilic granulocytes, B cells, plasma cells, mastocytes and erythrocytes; Fig. 2, 3).

Both cortex and medulla of the thymus contain epithelial cells that resembles the shape of the mesenchymal fibroblastic reticular cells (Polak *et al.* 2009). Therefore they are termed as **reticular epithelial (thymic epithelial) cells**. Population of developing T cells (thymocytes) lies between processes of these cells, and constitute up to 90% of the weight of the thymus. The thymic epithelial cells have a stellate shape (*Fig. 1, 6*). Cytoplasmic extensions are connected to other cells by desmosomes. These cells contain a large, pale, oval shaped nucleus with 1 or 2 nucleoli. The cytoplasm contains a poorly developed Golgi complex, rough endoplasmic reticulum, mitochondria and intermediary cytokeratin fibrils. Those demonstrate the epithelial nature of these cells in contrast with the mesenchymal origin of the stroma of other lymphatic organs. Within the thymus, only the capsule and septa are of mesenchymal origin (Schuurman *et al.* 1997).

The epithelial primordium of the thymus is derived from the epithelial proliferation of the endoderm of the 3rd and partially the 4th pharyngeal pouch, too. The epithelial proliferation of the ectoderm of the third pharyngeal cleft forms a complex with endodermal proliferation. Important role of thymic development have also mesenchymal cells migrated from the neural crest (Kuratani & Bockman 1990; Bockman 1997; Slípka *et al*. 1998; Varga *et al*. 2008). Gordon *et al*. (2004) experimentally deny the hypothesis of the dual origin of the epithelial cells. Isolated pharyngeal endoderm transplanted in another body location in mice results in the development of a normal thymus, differentiated into a cortex and a medulla, even though there are no ectodermal cells. This conclusion contradicts the earlier findings of Cordier & Haumont (1980) of the development of the thymus in nude mice. In these immunodeficient mice the ectoderm of the 3rd pharyngeal cleft involutes during early intrauterine development, and the originated thymus does not become a lymphatic organ.

The T cells immunocompetence and specificity takes place in the thymic cortex. The microenvironment in the course of thymocyte maturating program, including proliferation, differentiation, and their education or induction of apoptosis of potentially autoaggressive cells plays a key role. The T cell differentiation occurs via T cell receptors. The major histocompatibility complex (MHC) participates in interactions between T cells and thymic epithelial cells, in addition to interactions between T cells and dendritic cells, macrophages and

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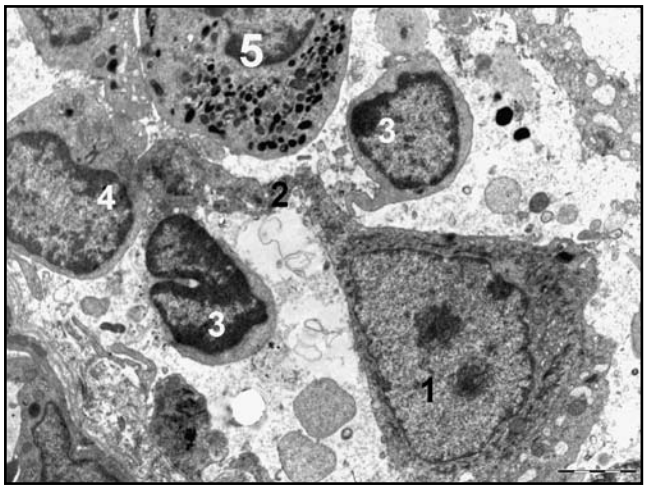

Fig. 1: The thymic medulla of a newborn. Typical stellate shaped thymic epithelial cell (morphological type 4 or 5). Containing a large, euchromatic nucleus with two nucleoli (1) and characteristic cytoplasm processes (2). Thymocytes of different sizes are in the surrounding (3, 4) a neutrophilic granulocyte (5). (TEM, line in fig. = 2×10^{-6} m)

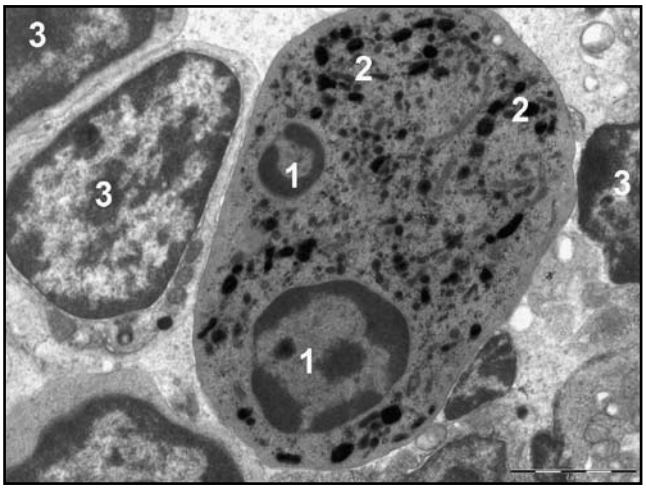

Fig. 2: Cortex of the newborns thymus. Neutrophilic granulocyte with two nuclear lobes present (1) in a denser cytoplasm, in which there are plenty of lysosomes (2). Nearby thymocytes (3). (TEM, line in fig. = 2×10^{-6} m)

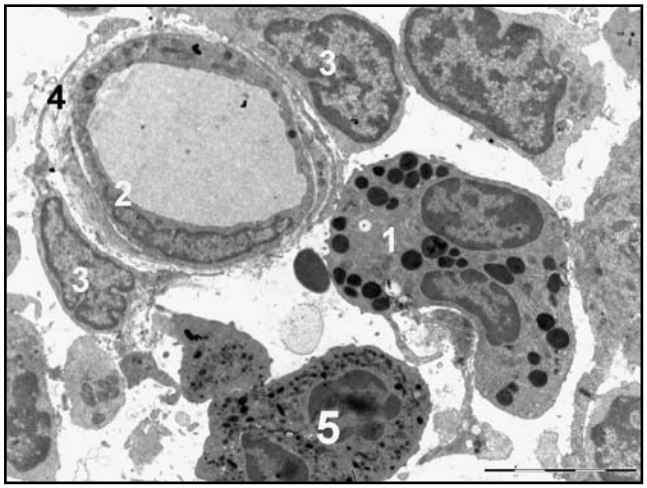

Fig. 3: Thymus of a newborn with hypoplastic left ventricle syndrome. Epithelial cells of the 1st type containing notably polymorphic nuclei (3). Their cytoplasmic processes (4) together with endothelial cells of the capillaries (2) create the blood-thymus barrier. An eosinophilic granulocyte is lies near the capillary (1) a neutrophilic granulocyte (5). (TEM, line in fig. = 5×10^{-6} m)

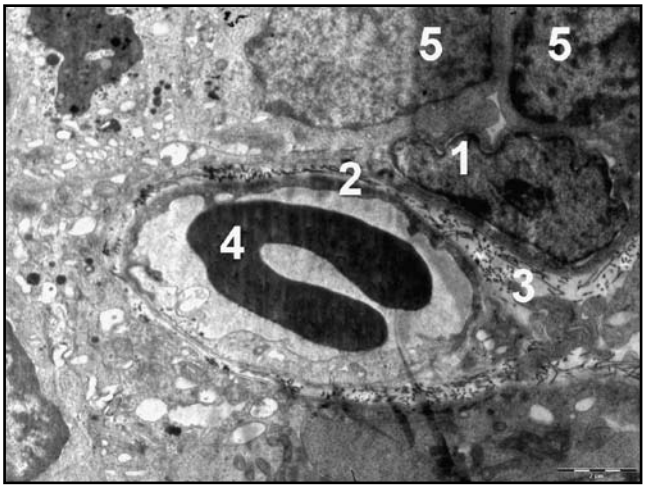

Fig. 4: Thymus of a 5 days old newborn with hypoplastic left ventricle syndrome. A prominent repithelial cell 1st type containing a polymorphic nucleus and a notable nucleolus (1) together with the capillary endothelium (2) forms the blood-thymus barrier. The space between cells is filled with collagen fibers (3). Erythrocytes are present in the vessel lumen (4), thymocytes are present nearby (5). (TEM, line in fig. = 2×10^{-6} m)

myoid cells. At each stage of the T cell differentiation in the thymus, the interactions between cells are controlled by adhesive molecules and a local gradient of growth factors (cytokines, hormones) as well as by their paracrine, cryptocrine, and autocrine influences (Kisielow & von Boehmer 1995; Anderson *et al*. 2000; Anderson & Jenkinson 2001; Brelinska 2003; Staal *et al*. 2001).

The morphological heterogeneity of the thymic epithelial cells

Many authors were concerned in a study of heterogeneity of the thymic epithelial cells, but it wasn't until Wijngaert *et al*. (1984) that they were classified in humans. The criterion was based on the **ultrastructural morphology**; although it seems that there classification is valid for all vertebrates, which have a thymus (Kendall 1991). Wijngaert *et al*. (1984) divides the thymic epithelial cells into 6 types, out of which, the first 3 occur mainly in the cortex and the remaining 3 in the medulla of the thymus:

Type 1: called as subcapsullar or perivascular cells (*Fig. 3, 4*), which separate the cortex of the thymus from the connective tissue constituting the capsule, septa and the space around vessels. These cells participate in the thymus-blood barrier. Cells of the 1st type in the subcapsullar region are composed of one or two layered border under the connective tissue of the capsule and septa (Waal & Rademakers 1997). The cells are closely

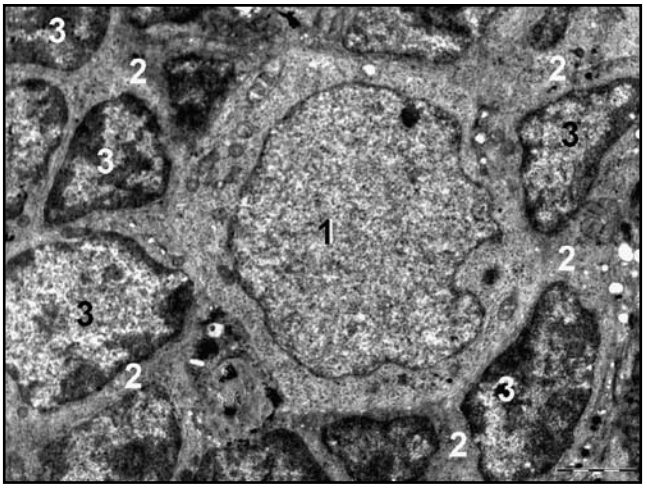

Fig. 5: The thymic cortex in a newborn. An epithelial cell (type 2 or 3, called "nurse cell") is situated in the middle containing euchromatic nucleus (1) with pale cytoplasm. Its several cytoplasmic extensions (2) surround thymocytes (3). (TEM, line in fig.= 2×10^{-6} m)

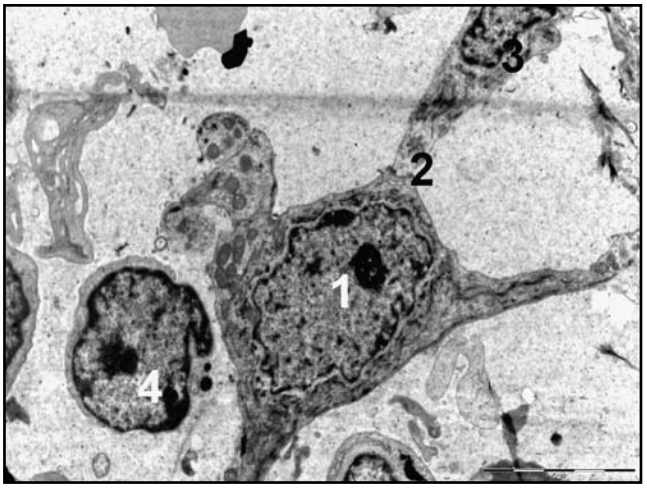

Fig. 6: Thymic medulla of a 3 week infant with transposition of great vessels of the heart. Epithelial cell of the 5th type containing a polymorphous nucleus and a prominent nucleolus (1) connected to other epithelial cells (3) with its cytoplasmic processes (2). Thymocytes present nearby (4). (TEM, line in fig. = 5×10^{-6} m)

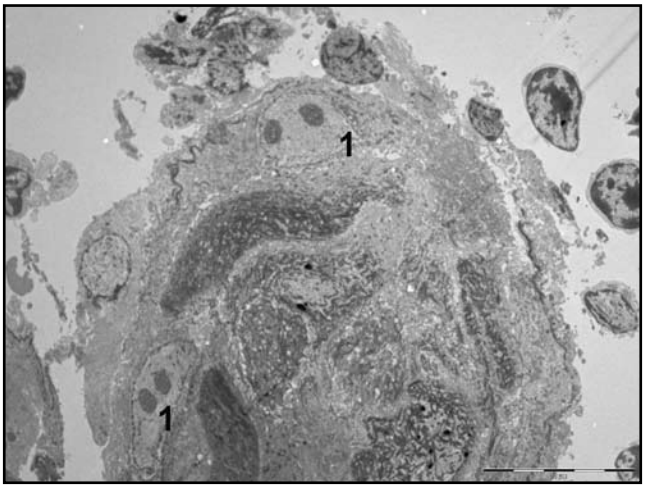

Fig. 7: An electronogram of the Hassall´s body in the thymus of a newborn with transposition of great vessels of the heart. Two nuclei containing nucleoli (1) active epithelial cells of the 6th type. (TEM, line in fig. = 10 x 10^{-6} m)

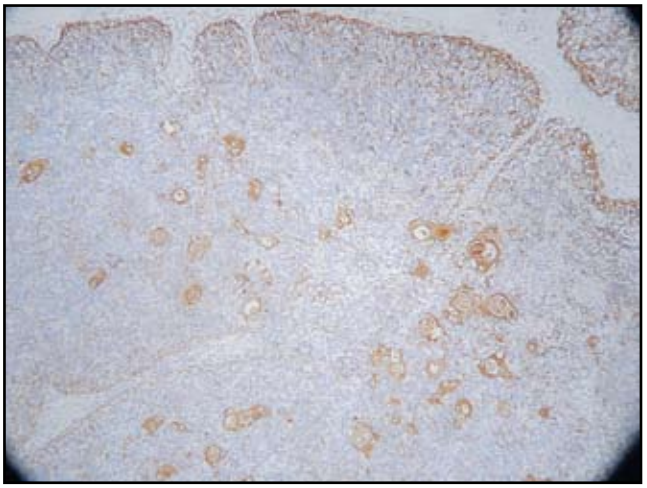

Fig. 8: Thymus in a newborn with a ventricular septum defect. Phenotypical heterogeneity of the epithelial cells, significant positivity for AE1/AE3 cytokeratin in the subcapsullar zone of the cortex and Hassall´s bodies in the medulla. (Magnif. 100x)

interconnected; these provide isolation of the thymus cortex from the surrounding tissue. The cells nuclei of the 1st type are polymorphic with prominent nucleoli,

Type 2: cells are situated in the middle part of the thymus cortex. Together with 3rd type, they constitute a dominant portion of the thymic epithelial network of the cortex. Extensions of the cells cytoplasm are connected by desmosomes; thereby form a **functional syncytium** (Kendall 1986), which is in close contact with the developing thymocytes. The cell extensions form a meshwork – cytoreticulum, which divides the thymus cortex into small separate areas. Here the developing lymphocytes are separated from the environment. Hence they are referred to as "**nurse cells**" (*Fig. 5*). Thymic nurse cells are defined as multicellular complexes of the epithelial cells and thymocytes (Brelinska & Warchol 1997). A lot of data have been generated about the multi-function of thymic nurse cells, as endocrine capability, secreting humoral factors. They support the growth of thymocytes or capacity to facilitate immature $CD4^{-}CD8^{+}$ thymocytes to differentiate into $CD4^{+}CD8^{+}$ T cells by direct interaction (Bodey et al. 2000b; Li *et al*. 2005). Nuclei of the cells of the 2nd type are large, pale with a small amount of heterochromatin. Cytoplasm is pale, rich in tonofilaments.

Type 3: cells are situated in the deeper portion of the cortex, near the area of the cortico-medullary junction. The nuclei and cytoplasm of cells are denser than the first two types. Rough endoplasmic reticulum forms dilated cisterns, which indicate active protein synthesis. The 3rd type of epithelial cells also contains cytoplasmic processes, with which they encircle developing lymphocytes. These cells anastomose with one another, but also with epithelial cells of the medulla. This provides

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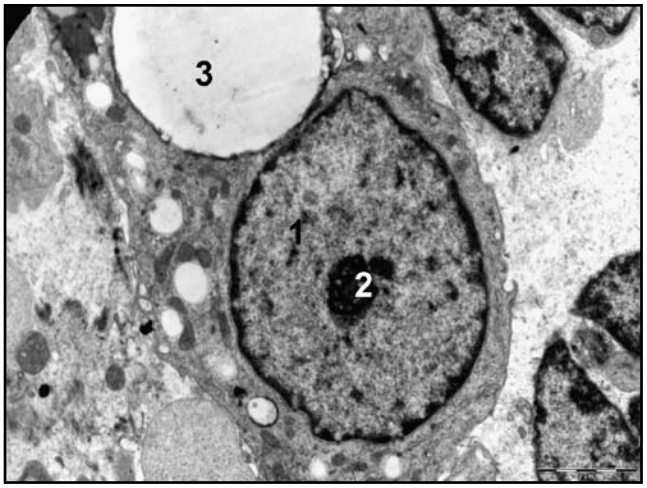

Fig. 9: Thymus of a newborn with transposition of great vessels. An epithelial cell with a large euchromatic nucleus (1), a prominent nucleolus (2) and a large vacuole (3) in its cytoplasm. (TEM, line in fig. = 2 x 10^{-6} m)

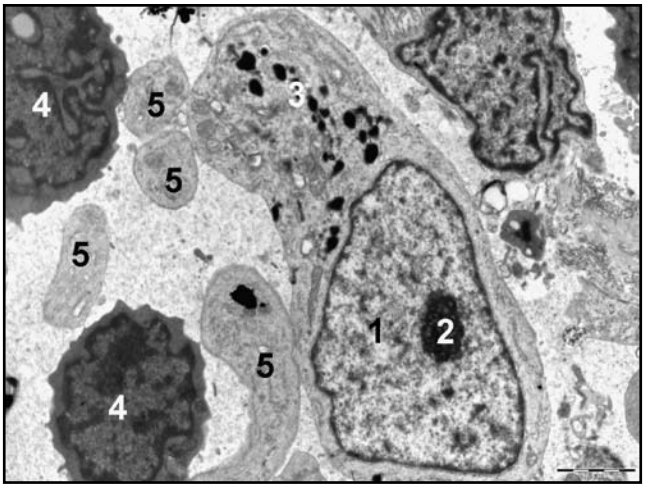

Fig. 10: An electronogram of the thymus medulla in a newborn with transposition of great vessels. In the middle is a macrophage with a pale nucleus (1), a prominent nucleolus (2) and cytoplasmic processes (5). Their pale cytoplasm contains granules with dense content (3). Thymocytes are also present (4). (TEM, line in fig. = 2 x 10^{-6} m)

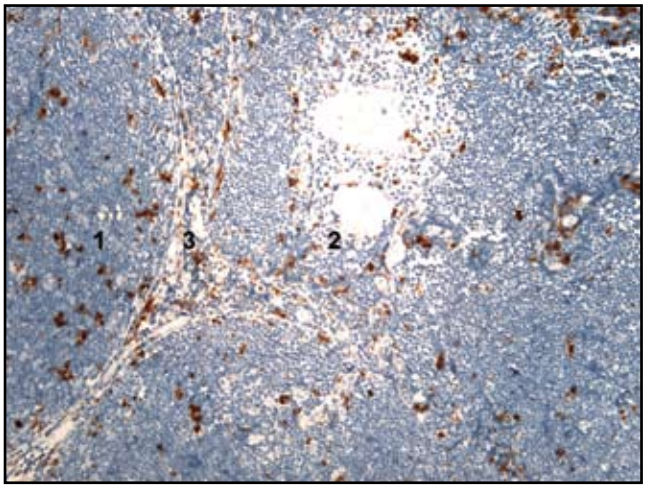

Fig. 11: Macrophages (CD 68 +) in the cortex (1), medulla (2) and in the fibrous connective tissue of the septa (3) in a newborn thymus. Clear areas of the medulla are fallen out calcified Hassall´s bodies. (Magnif. 100x)

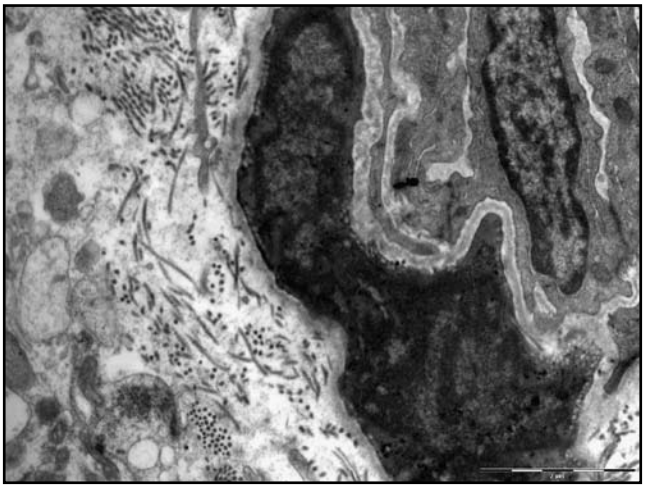

Fig. 12: Thymus of a newborn with transposition of great vessels. There is an electron dense myoid cell in the middle with typical minute pinocytotic vesicles under cytoplasmatic membrane. (TEM, line in fig. = 2 . 10^{-6} m)

the isolation of the cortex from the medulla. Thymic epithelial cells of the 2nd and 3rd type express specific surface antigens, molecules MHC I and II. They present them to developing lymphocytes, thereby they are ranked as **antigen presenting cells**.

Type 4: cells are very closely related to the 3rd type. They participate in the formation of the cortico-medullary junction and medulla. Cell nuclei contain somewhat coarse fine meshwork of heterochromatine, their electron-dense cytoplasm contains abundant tonofilaments (*Fig.1*).

Type 5: termed as medullary cells (*Fig. 6*) form the stroma of the thymus medulla. Cells nuclei are polymorphic, with well formed perinuclear chromatin and a prominent nucleolus. Few cellular organelles and abundant poly-ribosomes in the cytoplasm indicate that these cells could be stem cells (Kendall & Clarke 2000).

Type 6: cells are the prominent component in the thymus medulla. These large pale cells pile up into a spindle shape, to form the Hassall's corpuscules (*Fig. 7*). Hassall´s bodies are unique, antigenically distinct, functionally active, multicellular components of the nonlymphocytic microenviroment of the thymic medulla. They participate in the physiological activities of the prenatal and adult thymus (Bodey *et al.* 2000a).

Thymic epithelial cells are more or less resistant resistant to exogenic stimuli. Many authors refer to their persistence even after the thymus involution. Huiskamp *et al.* (1985) describes the reduction of the cortex in 24 hrs after neutron radiation of the thymus (2.5 Gy) into a thin border of vacuolated epithelial cells similar

to nurse cells. Irradiation causes the rapid depletion of lymphocytes in the thymus, but these lymphocytes are promptly restored by the proliferation of intrathymic and circulating bone marrow precursor cells. Arudchelvan *et al*. (2005) found in cortical thymic epithelial cells after irradiation (6 Gy) also cytoplasmic vacuolization with an increased amount of granular and membranous content. These features are characteristic of the hyperfunctional state of cortical epithelial cells with increased secretion activities, which suggests their important roles in the repopulation and maturation of the cortical thymocytes during recovery after irradiation (Arudchelvan *et al*. 2005).

Notwithstanding the importance of the epithelial cell network in the thymus of various animals (including humans), **epithelial-cell free areas** are also identified (called also keratin negative regions). Most frequently are localized in the area between subcapsullar epithelium and the superficial cortical layer. These regions do not contain cytoreticulum, but they can include isolated fibroblasts and several types of macrophages, which express a strong MHC II positivity. The significance of these "epithelial free areas" is not yet known. These areas are more prominent during some pathological conditions, such as pre-leukemic phase in mice and in rat thymus after the application of cyclosporine (Bruijntjes *et al*. 1993).

Phenotypic heterogeneity of the thymic epithelial cells

The heterogeneity of the epithelial cells of the thymus is manifested by a different morphology and also in **phenotype expression** of different **markers.** Division can be made on the basis of **anti-MHC II antibodies** which bind to the cytoreticulum of the cortex in human and rat thymuses. The subcapsullar zone and medulla either do not contain MHC II molecules or they are just only slightly MHC II positive (Schuurmann *et al*. 1985). Von Gaudecker *et al*. (1997) observed that the epithelial cells in the subcapsullar zone are not always MHC II negative. MHC II molecules are described on the surface of 1st type thymic epithelial cells, mainly on their cytoplasm extensions, which direct towards the deeper portions of the cortex. Thereby, pre-thymocytes in the thymus are subjected to the MHC II since their very early development.

Immunohistochemical methods are used to identify the subpopulations of epithelial cells of thymus in humans (Ritter & Haynes 1987), mice (Brekelmans & Van Ewijk 1990), rats (Kampigna & Aspinall 1990) and chicken (Boyd *et al.* 1992). Antibodies facilitate the identification of several subpopulations of thymic epithelial cells. The presence of several subpopulations urges laboratories to create common classification criteria. The international workshop in Holland: "The thymus. Histophysiology and dynamics in the immune system" provided the creation of nomenclature called "cluster of epithelial staining" (CTES) (Kampigna *et al*. 1989). CTES system was verified by Boyd *et al*. (1993), where they agree with the most substantial findings; though they differ in the model of antibody detection of epithelial cells in various animals. It seems that CTES nomenclature is equally valid for thymus of human, mice, but also rats, chicken and axolotls.

The heterogeneity of the thymic epithelial cells can be demonstrated by using antibodies against the cytokeratin polypeptide based on the **diversity of the expression of cytokeratin** in humans and other animals (*Fig. 8*). Based on the cytokeratin expression, the cortical epithelium is referred to as "simple", as it forms cytokeratin molecules of small molecular weight, whereas the medullary epithelium is "more complexed" forming keratin molecules with large molecular weight (Brekelmans & Van Ewijk 1990).

A further option for the classification of thymic epithelial cells is the presence of thymic hormones and neuroendocrinne markers in their cytoplasm. Epithelial cells of the medulla and subcapsullar zone are hormone producing cells. They can be therefore identified with antibodies against thymosine, thymuline, thymopoeitin, and the thymus humoral factor (Kendall & Stebbings 1994). Oxytocin, neurophysin, and vasopressin can also be demonstrated in the thymus. Most of neuropeptides are produced in the subcapsullar, medullar and perivascular epithelium. The entire thymic epithelium in mice is oxytocin positive (Robert *et al*. 1992).

Thymic epithelial cells and tissue engineering

Epithelial cell tissue cultures from thymuses of human and experimental animals have been used for over two decades for the investigation of the properties of epithelial cells and their significance in the selection and maturation of T cells. Tissue cultures provide data on the morphology of each type of epithelial cells, including their surface determinants. The investigation of the interactions between the epithelial cells and the developing T cells can also be performed *in vitro*. Tissue cultures also provide data on the secretion of cytokines by different cells types (Röpke 1997). Tissue cultures of different cell types play an important role in testing the effects of exogenic factors (e.g. drugs, high atmospheric pressure) (Palkovič *et al*. 2007, Danišovič *et al.* 2007, Vojtaššák *et al*. 2006). The advantage of the tissue cultures lies in the possibility of accurately defining experimental conditions and minimizing external adverse effects.

In primary cell cultures, the thymic epithelial cells are plane and arranged into one layer. This layer contains cells of two types; small cells (10–20 μm) and large cells (20–100 μm). Active DNA synthesis takes place in both types (Röpke 1997). Ultrastructurally, the tissue culture cells have an epithelial nature. Cells are connected by desmosomes and contain bundles of tono-

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filaments and intermediary filaments. Larger cells in addition to Golgi complex, mitochondria, ribosomes, and rough endoplasmic reticulum contain characteristic vacuoles (Röpke *et al*. 1990, Fig. 9). Each epithelial cell type preserves phenotypic distinctions also when cultivated. Antibodies against different cytokeratines can differentiate cortical and medullary epithelial cells. It is possible to generate a culture from both cell types (Nicolas *et al*. 1985). Accurate phenotypic cell classifications differ in various laboratories, which of course depend on the cultivated conditions.

Macrophages of the thymus

Further notable components of the thymus microenvironment are macrophages and dendritic cells. Presumably, monocytes entering the thymus differentiate into macrophages, which are situated mainly in the cortex and on the cortico-medullary junction, or into dendritic cells, which are typical for the medulla (Kaiserling *et al.* 1974). Circulating monocytes enter the thymus presumably through perivascular spaces (Vicente *et al*. 1995). It is also possible that thymic macrophages develop locally based on evidence of myelopoiesis in the thymus. Early T progenitors in the thymus have been reported to have the capacity to develop into macrophages, B cells, thymic dendritic cells, and NK cells (Lee *et al.* 2001).

Macrophages (*Fig. 10, 11*) are very effective phagocytes. They play an important role in the thymus. They contain a typical phygocyted lymphocyte material in their cytoplasm. Macrophages are observed in the thymus mainly after involution. The cortex of thymus after involution when stained with hematoxylin and eosin gives a "starry sky" image. Thymic macrophages constitute a phenotypically and ultrastructurally heterogenous cell population. Macrophages in the connective tissue of the capsule and septa are more mature, irregularly shaped, with a rough surface, containing electron-dense material in their cytoplasm. Macrophages in the thymus cortex are similar, besides containing phagolysosomes with remains of lymphocytes. Macrophages in mice are MHC II negative. In humans they can be positive. Macrophages of the corticomedullary junction differ morphologically, containing less phagocytosed material in their cytoplasm. Medullary macrophages are scarce and poor in phagocytosis, with poor expression of MHC II molecules (Boyd *et al*. 1993).

The function of macrophages during T cell development is not completely known. It seems clear that macrophages participate in the removal of apoptotic thymocytes. Less accepted is, however, their implication in the regulation of the survival, proliferation, MHC restriction, or negative selection of developing thymocytes (Wood 1985; Varas *et al*. 2003). They produce and secrete cytokines, which influence the proliferation, maturation and differentiation of thymocytes. Macrophages produce different cytokines, e.g. IL-1, IL-2, IL-4, tumor necrosis factor-α (TNF-α), and interferon-γ (Kendall 1991). Macrophages are one of the antigen-presenting cells for the thymocytes during their terminal development.

Interdigitating dendritic cells of the thymus

Interdigitating dendritic cells in the thymus are unique antigen-presenting cells originating from the bone marrow. They play an active role in the induction of immune response. In the thymus they are situated mainly in the cortico-medullary junction and medulla. They are about 20 to 30 μm long cells, containing sparse cytoplasm. They surround other cells (mainly thymocytes) with their thin cytoplasmic extensions, mainly thymocytes. They contain a well developed Golgi complex, vesiculo-tubular structures and granules of different sizes with homogenous content. The granules are adjacent to membranes. According to some authors, the cytoplasm of interdigitating cells contains the club shaped Birbeck granules. The origin and function of Birbeck granules is obscure. They can be used to identify Langerhans cells of the skin in electron microscope sections. Nuclei of the interdigitating cells of the thymus are lobular, lie excentrically, with characteristic perinuclear condensed chromatin, and a prominent nucleolus. Their typical morphology and absence of lysosomes and phagolysosomes distinguishes them from macrophages (LaFontaine *et al*. 1997; Pelletier *et al*. 1986)

The identification of the interdigitating cells is not possible using conventional light microscopy. They can be identified using monoclonal antibodies. The interdigitating cells express MHC I and II molecules, which indicates their attribution in the negative selection of T cells precursors (deletion, or functional inactivation of autoreactive thymocytes) (Schuurman *et al*. 1997).

Myoid cells of the thymus

Thymic myoid cells correspond to a muscle-like cell population present in the thymus medulla (*Fig. 12*). Their origin and biological role is not yet clear. Myoid cells hold similar characteristics as epithelial cells, therefore they were initially assumed to be of myoepithelial origin, later on speculations about their non-thymic mesodermal origin were suggested (Wakkach *et al*. 1999). Today, the hypothesis of Nakamura & Ayer-Le Liére (1986) is the most accepted, where they suggest a neuroectodermal origin of the myoid cells. That is, they are derivatives of the neural crest.

Thymic myoid cells produce many proteins, which are specific for skeletal muscles, e.g. myosin (Drenckhahn *et al*. 1979), troponin T, desmin, rapsyn and utrophin (Wakkach *et al*. 1999; Panse & Berrih-Aknin 2005). Chan (1992) describes a close relation between these

cells and non myelinated nerve fibers in the medulla of thymus in poultry. Wakkach *et al*. (1999) demonstrate the excessive production of TNF- and IL-8 in tissue cultures of myoid cells. These factors presumably protect thymocytes from apoptosis. Myoid cells play an important role in the differentiation of T cells. The protective effect of myoid cells against apoptosis of thymocytes is decribed also by Panse & Berrih-Aknin (2005).

Myoid cells have, similarly to thymic epithelial cells, surface receptors for acetylcholine (Wakkach *et al*. 1996). Thereby, it is presumable that both cell types can play an initial role in the auto-sensitization during the autoimmune disease *myasthenia gravis.*

Extra cellular matrix of the thymus

Extra cellular matrix is an important component of the microenvironment of the thymus. Generally it is composed of different collagen and reticular fibers, glycosaminoglycans and glycoproteins, including laminin, fibronectin. In the capsule and fibrous septa type I. collagen occurs, whereas type IV. collagen, laminin, and fibronectin constitutes a part of the basal lamina of the type 1. epithelial cells (Boyd *et al*. 1993).

The significance of the extra cellular substance rests in the support of the growth and development of thymocytes and epithelial cells. It provides the interactions between cells, including their migration. It also aggregate soluble cytokines to increase their local concentration.

Conclusion

The thymus was one of the last organs of our body which represented a mysterious function. Until the year 1961, controversial statements complicated most questions about the function and development, pathology and clinical significance. Even in the year 1971, The English medical dictionary, 29th ed. London stated that: "*The function of the thymus gland is unclear. It enlarges with disorders of other endocrine glands. One of the theories regarding its function is that it affects sexual maturation*". This citation was identified even though in the 50s there was already a firm evidence of the lymphopoeitic function of the thymus and its determining role in the normal function of the immunity system was confirmed in the year 1961 by Doctor Jacques Miller (Miller 1961; Miller 2002). In our work we included an outline of the actual findings of the ultrastructure and heterogeneity of the thymic stromal cells, supplemented with our own microphotographs and electromicrographs from a transmission electron microscope. All of these cells play a critical role in the proliferation, differentiation and selection of precursor cells in the T-cell lineage, but the precise mechanisms are mostly unknown.

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