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Invited Review

Cyclosporin A-induced changes of the thymic microenvironment. A review of morphological studies

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Summary. Cyclosporin A is an immunosuppressive drug, which disrupts the activation of peripheral T-lymphocyte pool and blocks the maturation of thymocytes within the thymus. Normally, thymic nonlymphoid cells provide the optimal inductive microenvironment for development of T-lymphocytes. After application of cyclosporin A the complex alterations of the thymic microenvironment occur, affecting all types of nonlymphoid cells.

All subsets of thymic epithelial cells are thoroughly changed. The subcapsular epithelial cells show the prominent enlargement of cytokeratin contents. In electron microscopy, however, these cells present the morpho-functional aspect of resting cells. The epithelial cells in deeper cortex become enlarged and stockier, whereby their cell processes appear more ramified and thicker. Thus, the cytoreticulum they create seems much denser. These cells strongly express MHC antigens. Their subcellular organization is suggestive of increased synthetic and secretory activity. The number of medullary epithelial cells is decreased. The cells with the most mature phenotype are the most prominently depleted and the ones with phenotypically and morphologically immature appearance predominate.The number of Hassall's bodies is also decreased.

The number of cortical macrophages does not increase. However, these cells become enlarged showing the prominent changes in enzyme capacity, histochemical features and ultrastructural organization. Thus, they become similar to macrophages located in the cortico-medullary zone of the normal rat thymus. Cortical macrophages increase the activity of hydrolytic enzymes, acid phosphatase and nonspecific esterase, develop the strong activity of chloroacetate esterase, the strong activity of respiratory enzyme succinic dehydrogenase and begin to show the marked presence of prostaglandin synthase. Moreover, the cytoplasmic inclusions, which are aldehyde fuchsin- and PAS-positive and show sudanophilia, appear within cortical macrophages. In electron microscopy these cells show an abundant cytoplasm a very active appearance and the variety of vacuolar cytoplasmic inclusions. The mitoses of neighboring thymocytes are often seen. The number of interdigitating cells is decreased due to reduced size of thymic medulla, but these cells do not show the substantial phenotypic changes.

The description and classification of all types of nonlymphoid cells, which constitute the normal thymic microenvironment, is also presented. The functional significance and possible mechanisms of CSA-induced changes of the thymic microenvironment are discussed.

Key words: Thymus, Thymic microenvironment, Thymic nonlymphoid cells, Cyclosporin A

Introduction

Thymus is a primary lymphatic organ, which provides the optimal inductive microenvironment for proliferation and maturation of bone marrow-derived precursor cells into functionally competent T-lymphocytes. Upon rearrangement of genes encoding the T-cell receptor (TCR), thymic lymphocytes pass through the processes of meticulous selection directed by nonlymphoid cells of the thymic milieu, which shape the T-lymphocyte repertoire, and seed the peripheral lymphatic organs (Bevan, 1997). Thymic microenvironment is composed of sessile and motile nonlymphoid cells. The former are epithelial cells and the latter are cells of mononuclear phagocyte system. In different regions of thymic tissue these cells show distinct phenotypic characteristics and each type of thymic nonlymphoid cells is believed to provide a specific type of influence within the distinct tissue niche suitable for certain stages of thymocyte maturation to occur (Boyd et al., 1993). Thymic nonlymphoid cells govern the complicated processes of T-cell production and deliver signals to maturing lymphocytes by direct cell-to-cell contacts, as well as by locally produced soluble factors. The former type of influence, in the first place, involves the interaction of self peptide/major histocompatibility complex (MHC) expressed by

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nonlymphoid cells with TCR/coreceptor molecules expressed on the surface of thymocytes (Robey and Fowlkes, 1994; Bevan, 1997). It is believed that the intensity of this interaction determines the outcome of positive and negative selection of thymocytes, which occurs in the thymus. The appropriate intensity delivers the signal to thymocytes to survive, whereas the thymocytes, which establish a weak interaction are left behind without an appropriate stimulus and destined to die by apoptosis. The cells, which develop a too strong interaction are believed to be autoreactive and are also eliminated through the process of apoptotic cell death (Vukmanović, 1996; Yamazaki et al., 1997). The latter type of influences is exerted by thymic nonlymphoid cells on thymocytes via a spectrum of hormones, cytokines and growth factors (Hadden, 1992). The intricate cellular interactions within this organ are, moreover, bi-directional and it has been shown that thymocytes exert a feedback influence on nonlymphoid cells controlling their integrity (Surh et al., 1992; van Ewijk et al., 1994), and organization (Goverman et al., 1997). The complexity of interactions, which occur between thymic lymphoid and nonlymphoid cells, makes *in vivo* studies of thymocytopoiesis very difficult.

Cyclosporin A (CSA) is a cyclic endecapeptide of fungal origin (Borel et al., 1977). Due to its immunomodulatory properties it has gained a wide clinical use for prevention of graft rejection after organ transplantation and of graft-versus-host disease after allogeneic bone marrow transplantation (Kahan, 1989).

CSA interferes with the signal transduction upon TCR engagement: it forms a complex with the intracellular binding-molecule cyclophilin and this newly formed drug-immunophilin complex binds to and inhibits the activity of calcineurin, a serine-threonine phosphatase.

This trimolecular complex inhibits the translocation of the cytoplasmic component of the NF-AT (nuclear factor of activated T-cell) transcription factor, which prevents the activation of several genes mainly involved in cytokine production and disrupts the pathways of T-lymphocyte activation (Kunz and Hall, 1993; Fruman et al., 1994). Most prominently, the production of interleukin-2 and the activation of cytotoxic T-lymphocytes are blocked, whereby the activity of suppressor T-cells is unaffected. This imbalance between subpopulations of T-lymphocytes produces immunological tolerance (Hess and Colombani, 1987) and enables the clinical use of CSA. However, in addition to strong effects on the peripheral T-lymphocyte pool, CSA also arrests the maturation of thymocytes within the thymus (Gao et al., 1988; Hiramine at al., 1988; Jenkins et al., 1988). As mentioned above, in recent years it has been shown that not only thymocytes are dependent upon signals delivered by thymic nonlymphoid cells to proceed through the process of maturation within the thymus gland. In turn, the integrity of nonlymphoid cells also depends on thymic lymphoid population (Surh et al., 1992; van Ewijk et al., 1994; Goverman et al., 1997). CSA thoroughly perturbs the process of thymocytopoiesis. Therefore, it represents a useful tool for elucidation of the delicate interplay between thymic lymphoid and nonlymphoid cells. The changed morpho-functional features of thymic nonlymphoid cells, induced by CSA *in vivo,* may also give a clue to the roles played by particular types of microenvironment elements under normal conditions. Finally, to fully understand the immunosuppressive effects of this clinically very useful drug, it is necessary to elucidate the CSA-induced changes of both lymphoid and nonlymphoid thymic cells.

Morphology of normal and cyclosporin-A-treated animals

General aspects

In normal rats the thymus is enclosed in a connective tissue capsule which gives off delicate septa dividing the parenchyma into pseudolobules, whereby the distinction between the cortex and medulla is clear due to higher lymphocyte density in the former.

The most remarkable observation, after treatment with CSA, is the strong reduction in size of thymic medulla to such an extent that only the small residual islands of remaining medullary tissue may be encountered. This has been repeatedly seen since the first studies on the effect of CSA on the thymus (Ryffel et al., 1981; Blair et al., 1982). In contrast, the overall morphological integrity of thymic cortex remains well preserved. Therefore, the thorough changes of this part of thymic microenvironment were mostly overlooked in many studies on CSA-treated thymus. The attention was focused on the alterations of thymic medulla (Beschorner et al., 1987, 1988; Hattori et al., 1987; Kanariou et al., 1989), and on medullary interdigitating cells (IDCs) in particular (Cheney and Sprent, 1985; Beschorner and Armas, 1991; De Waal et al., 1992). Therefore, the works from our laboratory were the first to announce the thorough changes of all cellular constituents of thymic cortex after CSA treatment, as well as to reveal the subtle changes which occur in the medulla (see below).

Epithelial cells

Cortex

By light microscopy, thymic epithelial cells are the most easily and instructively presented using the monoclonal antibodies directed to cytokeratin or MHC antigens, as well as by specific panepithelial monoclonals. When immunocytochemically decorated for these antigens, cortical epithelial cells of the normal thymus appear delicate and show thin, elongated cellular processes, which form a fine, spider web-like warp throughout the cortex (Figs. 1a, 2a and 2a inset). A single layer of elongated cortical epithelial cells, so-

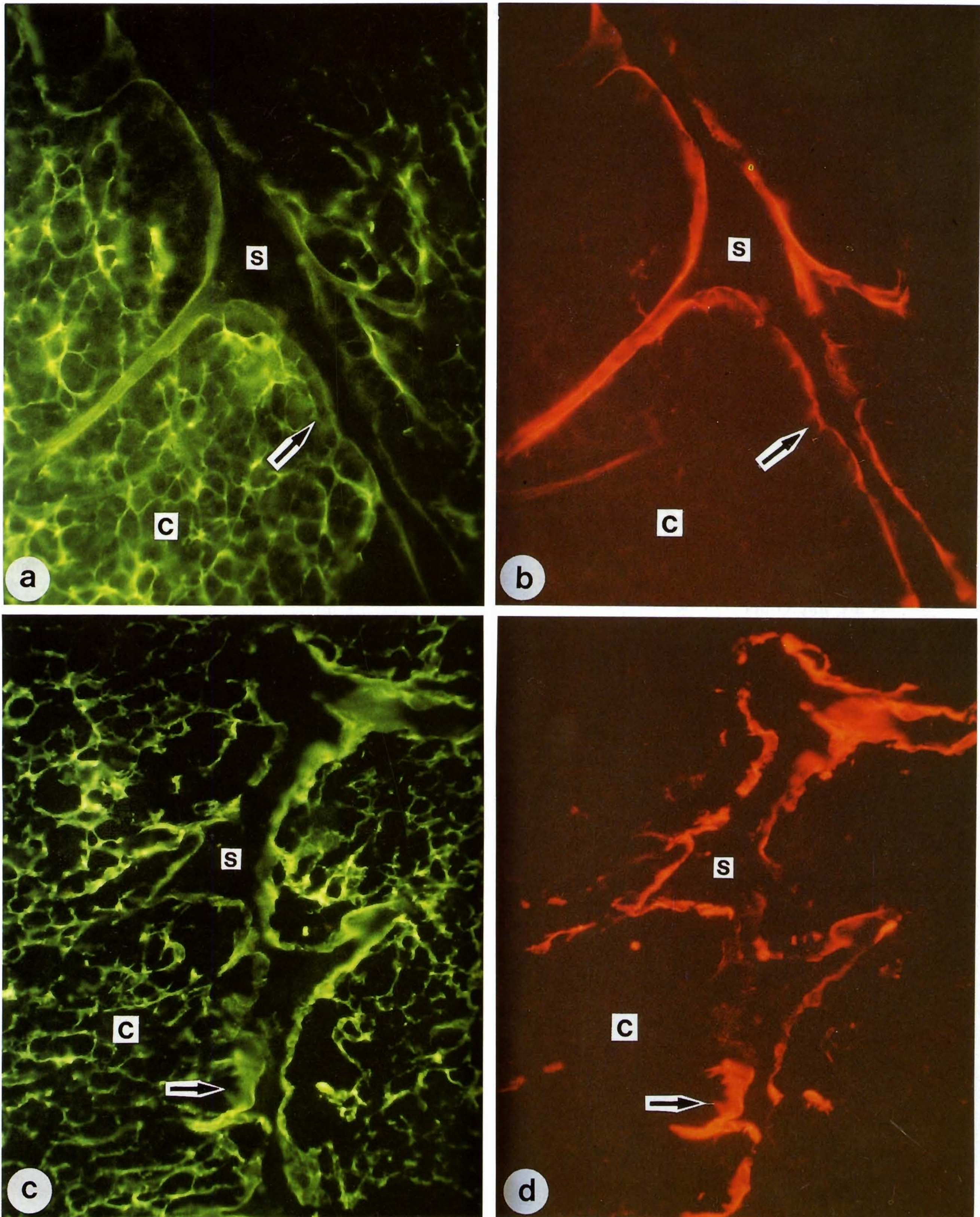


Fig.1. a and b. Normal thymus: double immunofluorescence-stained section with anti-cytokeratin 8 and 19 monoclonal antibodies, respectively. The epithelial cells in deep cortex (C) form a delicate web-like cytoreticulum. These cells are $CK8^{+}19^{-}$. The flattened epithelial cells with elongated processes (arrow) border the septal connective tissue (S). These cells are $CK8^{+}19^{+}$. **c and d.** Cyclosporin A-treated thymus: double immunofluorescence-stained section with anti-cytokeratin 8 and 19 monoclonals, respectively. Epithelial cells in deep cortex (C) are stockier, with thickened cytoplasmic prolongations. The epithelial meshwork is denser. These cells are $CK8^{+}19^{-}$. The number of $CK8^{+}19^{+}$ subcapsular epithelial cells is not reduced, but these cells also seem enlarged and stockier with thickened cellular processes (arrow). x 80. (Adapted from Milićević et al., 1992).

called subcapsular epithelium, separates the connective tissue of capsule, septa and intraparenchymal blood vessels from parenchyma proper. The delicate, elongated cytoplasmic processes of neighboring subcapsular epithelial cells are anchored to each other, forming an unbroken barrier against the stromal connective tissue. This subpopulation of thymic cortical epithelial cells, in contrast to the epithelium in deeper cortical regions, does not express MHC antigens on the surface of cytomembranes (Fig. 2a; von Gaudecker et al., 1986, Milićević et al., 1991). But in turn, it selectively stains with several antibodies (van Vliet et al., 1984; De Maagd et al., 1985; von Gaudecker et al., 1986; Kampinga et al., 1987) and shows a characteristic cytokeratin content (Fig. 1b; Čolić et al., 1989; Milićević et al., 1992).

Electron microscopy demonstrates the ultrastructural heterogeneity of cortical epithelial cells, whereby four subsets of these cells are easily distinguished in both human (van de Wijngaert et al., 1984) and rat thymus (De Waal et al., 1993; Milićević and Milićević, 1997). Type 1, "subcapsular" epithelial cells are positioned against connective tissue and always have a basal lamina. These cells are irregularly shaped and their prolongations are interconnected by desmosomes. The nucleus is mostly euchromatic and the cytoplasm has a very active appearance and contains fine bundles of keratin tonofilaments. Type 2, "pale" epithelial cells are usually positioned in the outermost regions of the thymic parenchyma. These cells are stellate-shaped, with delicate cytoplasmic prolongations and show the low electron density of the nucleus and cytoplasm. The nucleus is very large, markedly euchromatic, with a prominent nucleolus. The cytoplasm is scanty, but reflects high cellular activity. The bundles of keratin tonofilaments are delicate and sparse in perinuclear cytoplasm, but are more abundant and thicker in cytoplasmic prolongations. Type 3, "intermediate" epithelial cells are located deeper in the thymic cortex and show higher electron density of the nucleus and cytoplasm in comparison to type 2 cells. The nucleus of these cells is smaller, with a characteristic pattern of chromatin organization: small condensations of heterochromatine are evenly dispersed throughout the nucleus. These cells have ample cytoplasm and massive, sheet-like extensions, which contain abundant organelles, the most notably numerous secretory vacuoles. Fine bundles of tonofilaments are localized within the cytoplasmic prolongations. Type 4, "dark" epithelial cells (Fig. 3a) are positioned in the deep-cortex and at the cortico-medullary boundary, but may penetrate the thymic medulla. These cells show a very high electron density of the nucleus and cytoplasm. The clumps of heterochromatine are scattered all over the nucleus, which acquires a "tigerish" appearance. The nucleolus is very prominent. Cytoplasmic extensions, which are very long and delicate, contain numerous organelles, similar to the cell body. Many large secretory vacuoles and lipid droplets are seen, often discharging their contents into the intercellular space. The keratin bundles are ample and massive.

After application of CSA the morphological appearance of epithelial cells throughout the cortex is substantially changed. The use of panepithelial, anti-cytokeratin monoclonal antibodies in light microscopy shows that the epithelial cells in the deeper cortex become coarser and stockier (Fig. 1c). Cell processes appear more ramified and thicker, encompassing smaller groups of thymocytes in comparison to the control. Thus, the cytoreticulum they create is much denser (Fig.

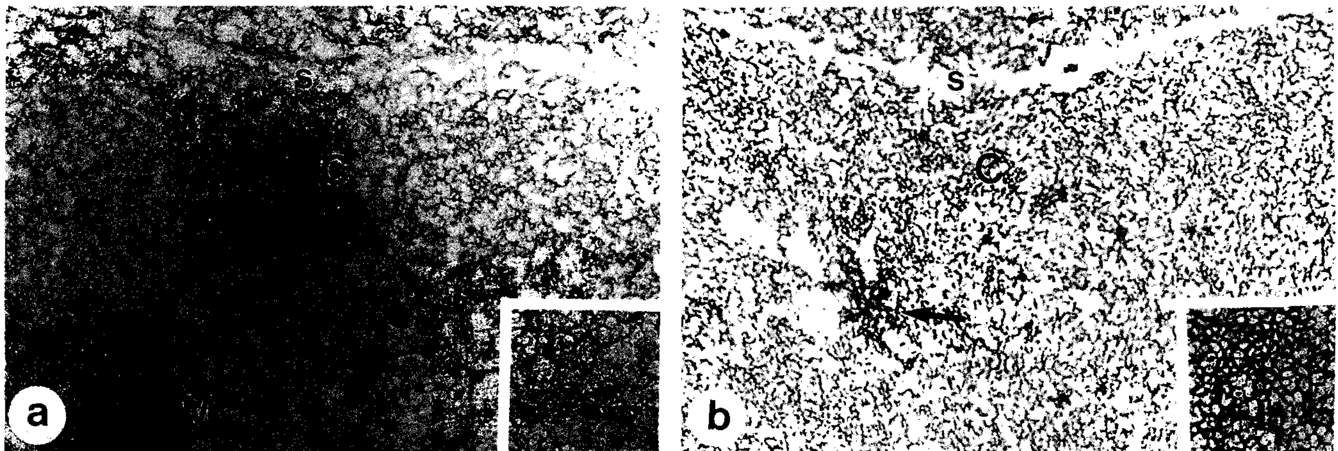


Fig. 2. a. Normal thymus. Both cortex (C) and medulla (M) are well developed. The delicate, spider web-like pattern of cortical epithelial MHC class II staining is seen. Medullary Ia-positive cells are densely arranged. x 220. Inset: delicate arrangement of cortical epithelium under higher magnification. x 460. **b.** Cyclosporin A-treated thymus. The size of thymic cortex (C) is preserved, whereas medulla (arrow) is greatly reduced. The coarse, dense pattern of cortical epithelial MHC class II staining is observed. In residual medullary tissue some Ia-positive cells are still present. x 220. Inset: dense, coarse arrangement of cortical epithelium under higher magnification. x 460. S: septum. OX-6 monoclonal antibody, two-step immunoperoxidase. (Adapted from Milićević et al., 1991, with kind permission from Kluwer Academic Publishers).

1c; Milićević et al., 1992). These cells strongly express MHC antigens on their surface membranes (Fig. 2b and 2b inset; Milićević et al., 1991). The significant morphological changes of subcapsular epithelial cells are registered by the use of anti-cytokeratin monclonals: the cells seem thickened, enlarged and dumpy, with much thicker, coarser and stockier cytoplasmic prolongations in comparison with the normal thymus (Fig. 1c,d). The continuity of these cells against connective tissue remains unbroken and their number is not decreased. These cells remain MHC-negative (Fig. 2b). Electron microscopy, however, reveals that type 1, "subcapsular" epithelial cells become flatter in shape and prominently diminished in size, whereby the volume of their cytoplasm is reduced, the number of cell organelles is decreased and the nuclear chromatin more condensed. Thus, "subcapsular" epithelial cells acquire the morpho-functional appearance of resting cells (Rhodin, 1974). On the other hand, the amount of cytokeratin is greatly increased and thick bundles are seen in the cytoplasm and cell prolongations (Milićević and Milićević, 1997). Actually, due to this increase in cytokeratin contents subcapsular epithelial cells appear hypertrophied in light microscopic immunocytochemistry (as described above). Electron microscopy, however, fully and precisely reveals the morphological changes of these cells after CSA application. In contrast to "subcapsular" epithelial cells, type 2 and 3, "pale" and "intermediate" cortical epithelial cells are markedly enlarged, whereby the amount of cytoplasm in the latter is especially increased. The nucleus is also enlarged, very euchromatic, with patent nucleolus. The number of all organelles is increased, especially of secretory vacuoles. These changes are very indicative of cellular activation, in particular of increased secretory activity. The cyto-plasmic prolongations are also thickened, more voluminous and in type 3 cells have sheet-like appearance. The amount and size of keratin filaments are increased, compared with the control tissue. Type 4, "dark" epithelial cells are the most profoundly changed

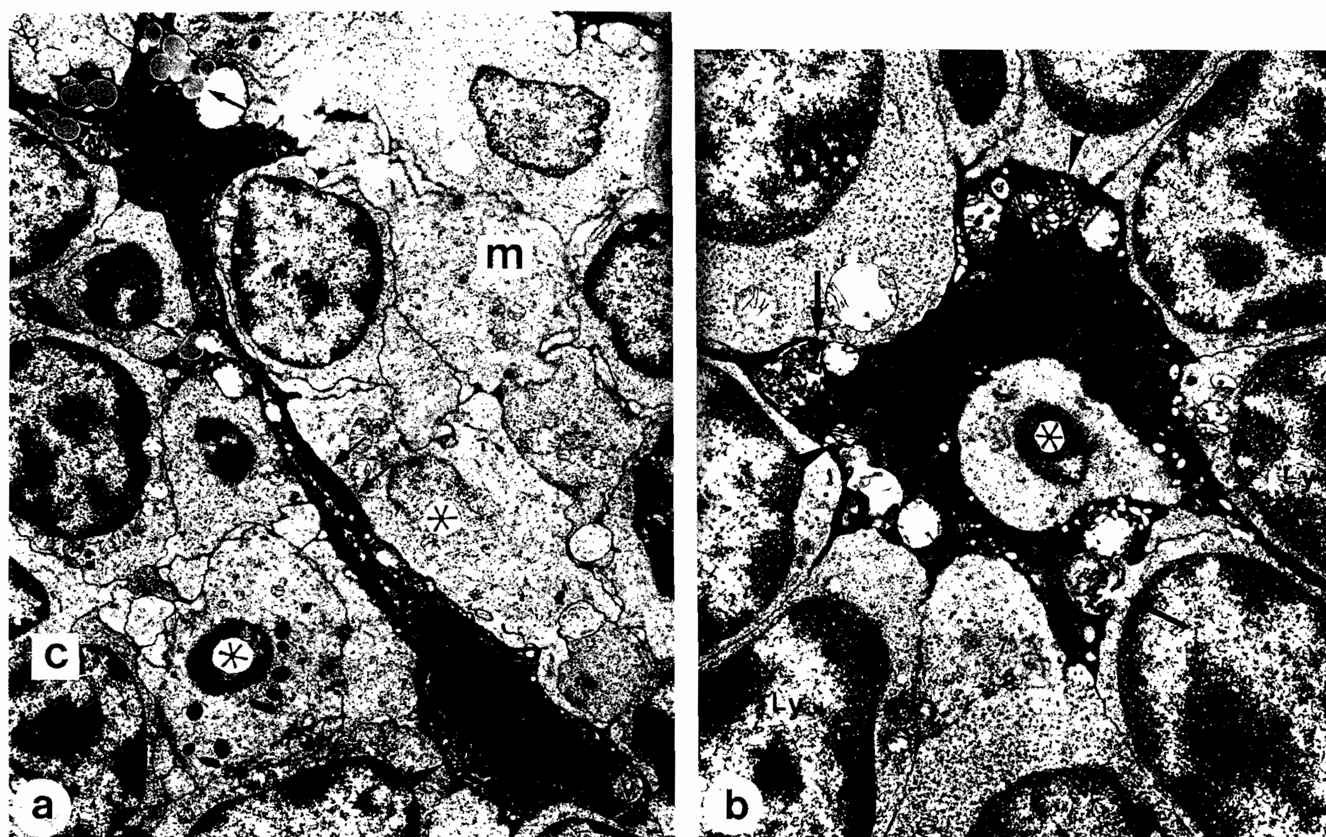


Fig. 3. a. Normal thymus. Type 4, "dark" epithelial cells positioned between the cortex (C) and medulla (M) show a very high electron-density of the nucleus (with clumps of heterochromatine) and cytoplasm. The nucleolus is very prominent. The perinuclear cistern, profiles of rough endoplasmic reticulum and well developed Golgi complex (arrowhead) show the lower electron density of their content. Large secretory vacuoles (v) and lipid droplets are seen in the cell body and in cytoplasmic extensions. Some of the vacuoles are in apposition with lipid droplets (small arrows). The keratin bundles are ample in the cell processes (large arrows). Asterisks: interdigitating cells. x 3,500. **b.** Cyclosporin A-treated thymus. Type 4, "dark" epithelial cell shows the signs of activation: numerous, large Golgi complexes (arrowheads), dilated cisternae of rough endoplasmic reticulum and secretory vacuoles of different size, which discharge their contents into the intercellular space (arrows). The surrounding lymphocytes (Ly) plunge deeply into the cytoplasm of "dark" epithelial cells, some in the form of emperipoiesis (asterisk). x 6,100. (Adapted from Milićević and Milićević, 1997).

of all cortical epithelial cells after CSA treatment (Fig. 3b). The signs of dramatic activation of these cells are seen: the nucleolus is very enlarged, numerous, very large Golgi complexes are prominent, cisternae of rough endoplasmic reticulum are dilated and secretory vacuoles are abundant, even in very remote parts of cell processes. Many of these vacuoles discharge their contents into the intercellular space (Fig. 3b). Although the bodies of these cells are not enlarged, the volume of cytoplasmic extensions and the number of their ramifications are very increased and they encompass almost every single lymphocyte within the deep cortex. Thus, the cellular network of "dark" epithelial cells in this region of the cortex is much denser than in the control. Some lymphocytes surrounding "dark" epithelial cells are deeply impressed into their cytoplasm, almost in the form of emperipoiesis (Fig. 3b). Electron microscopy confirms and extends light microscopic findings regarding type 2, 3, and 4 cortical epithelial cells, which are registered after CSA application (see above). It shows that these cells become enlarged (except type 4, "dark" cells) with thickened, stocky cell extensions and prominent morphological signs of activation, in particular of increased secretory activity (Milićević and Milićević, 1997).

Medulla

By the use of different monoclonal antibodies directed against various cytokeratins, thymic/neuroendocrine hormones etc., marked heterogeneity of medullary epithelial cells may be witnessed. On the basis of cytokeratin content several subpopulations of medullary epithelial cells may be distinguished (Čolić et al., 1989; Milićević et al., 1992). Moreover, many of the monoclonals directed against different epitopes expressed on medullary epithelial cells (Farr and Anderson, 1985; Rouse et al., 1988) in different patterns stain only a proportion of these cells in the thymic medulla. Especially frequent is that the antibodies against thymic hormones do not react with all of them (Jambon et al., 1981; Savino et al., 1982; von Gaudecker et al., 1986). Altogether, these data suggest a very complex morphological, as well as functional, heterogeneity of these cells.

Electron microscopy confirms the diversity of thymic medullary epithelial cells, as observed in light microscopy and enables the discrimination of at least three subsets of medullary epithelial cells. However, up to date is has not been possible to directly relate these findings and detect the corresponding cells in light microscopy for epithelial subsets characterized by electron microscopy and vice versa. Future research, applying modern immuno-electronmicroscopic methods, could eventually fulfil this task and also help to explain the greater morphological heterogeneity of cortical epithelial cells, as observed in electron microscopy in comparison to light microscopic findings. Such studies would, unquestionably, also contribute to a better understanding of the roles which particular subsets of epithelial cells play in the function of the thymus.

Type 5, "undifferentiated" epithelial cells are most often located at the cortico-medullary boundary. These cells are rounded in shape, with short, delicate cytoplasmic extensions and in general have an immature, blastoid appearance (van de Wijngaert et al., 1984; Milićević and Milićević, 1997). Type 6, "large" medullary epithelial cells have abundant cytoplasm and several, not very prominent, cytoplasmic prolongations. These cells obviously represent a type with high metabolic activity and several, possibly related, functions. Thus, depending on the stage/type of activity these cells may show a different appearance. Further immuno-electron-microscopical studies could reveal their true functions and elucidate the relationships between variants of these cells, as well as resolve the

Fig. 4. Cyclosporin A-treated thymus. Medullary epithelial cells (Ep) are rounded in shape, have frail extensions and show an immature, blastoid appearance. The nucleus is euchromatic, with prominent nucleoli. The cytoplasm is densely packed with polyribosomes and contains short, delicate keratin filaments (arrows). x 6,100. Inset: keratin bundles under higher magnification. x 22,000. (From Milićević and Milićević, 1997).

question if, actually, they represent different cellular subsets. The most characteristic feature of these large cells are the prominent signs of intense secretory activity: in addition to numerous transport vesicles and dilated, profiles of rough endoplasmic reticulum, solitary or grape-like clusters of vacuoles and large Golgi fields are seen (Milićević and Milićević, 1997). Type 7, "spindle-shaped" epithelial cells are small and often arranged in groups or connected to each other by large desmosomes. The cytoplasm is sparse, with scanty organelles and well-developed bundles of cytokeratin (Duijvestijn and Hoefsmit 1981; Milićević and Milićević, 1997).

After application of CSA the size of thymic medulla is reduced to such an extent that only small, residual islands of medullary tissue are seen. The loss of medullary epithelial cells is detected with panepithelial, as well as with subcapsular-medullary epithelium-specific monoclonal antibodies, and is well documented (Beschorner et al., 1987, 1988; Hattori et al., 1987; Kanariou et al., 1989; Schuurman et al., 1990). The remaining medullary epithelial cells are grouped in small clusters, but the double immunostaining shows that their phenotype is profoundly changed: most of them show the features of less mature cells, whereas the cells with the most matured phenotype are prominently depleted (Milićević et al., 1992). Very often, no epithelial cells are detected between the lymphocytes. In tissue sections immunostained for MHC antigens few positive cells are seen within the residual medullary tissue (Fig. 2b; Cheney and Sprent, 1985; Schuurman et al., 1990). Some of them represent epithelial cells and the others are very likely to be IDCs (see below). In electron microscopy, the epithelial cells with mature appearance are very rarely encountered within the residual islands of medullary tissue. The most frequent is that medullary epithelial cells with an immature appearance are seen (Fig. 4). Due to their blastoid appearance, these cells are difficult to recognize, but the bundles of keratin filaments within their cytoplasm disclose their epithelial nature (Fig. 4, inset). Number of Hassall's bodies is also decreased.

Macrophages and interdigitating cells

Cortex and cortico-medullary zone

The most convenient ways to detect macrophages within the thymic parenchyma are either immunostaining with macrophage-specific monoclonal antibodies or enzyme-histochemical demonstration of hydrolytic enzymes, for example, acid phosphatase (AcP) and nonspecific esterase (NSE). Cortical macrophages are small cells, with scanty cytoplasm and

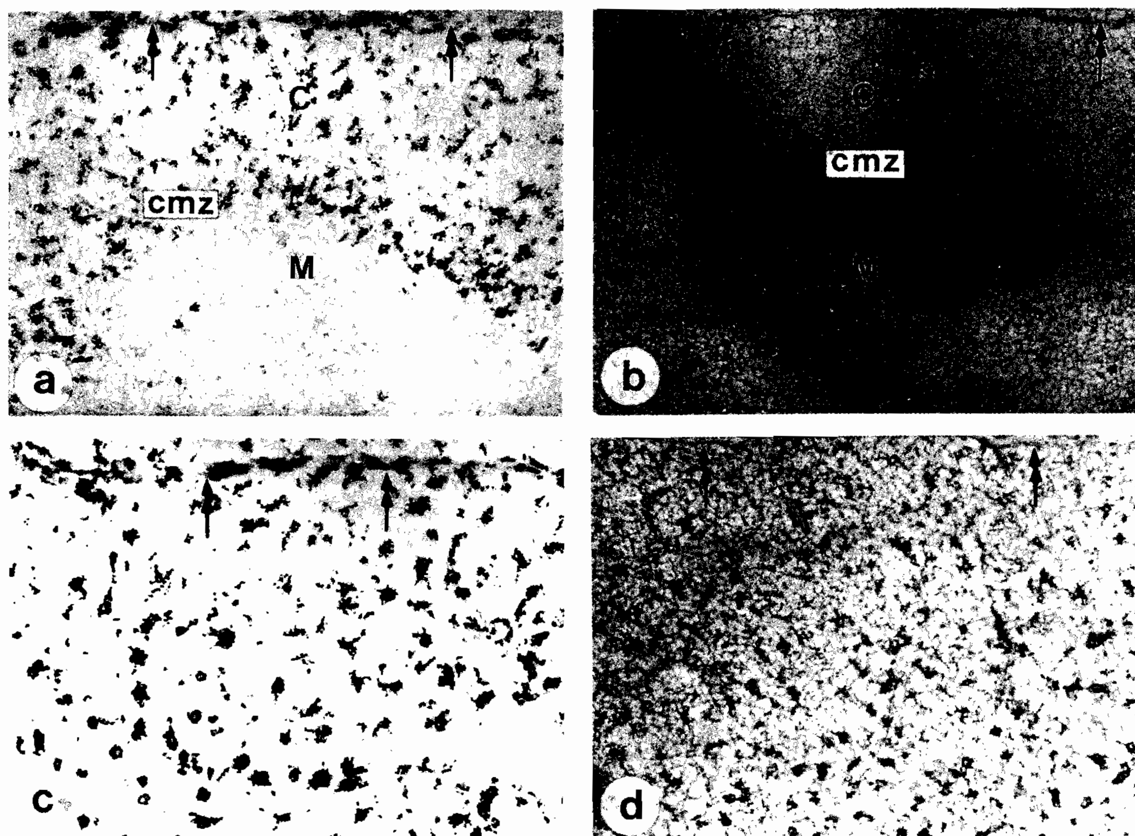
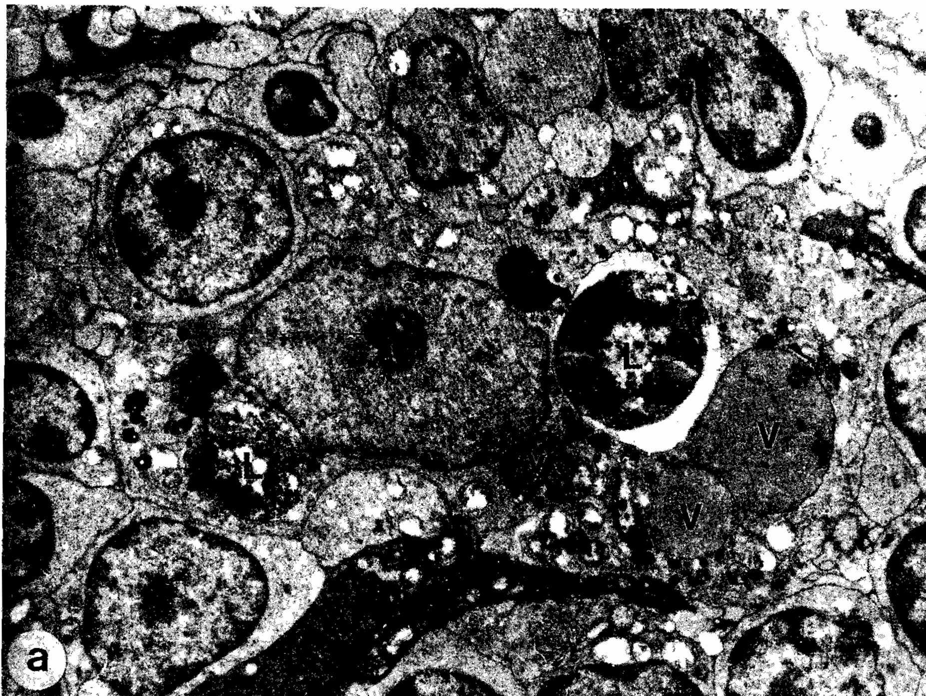


Fig. 5. a and b. Normal thymus. **a.** Smaller macrophages in the cortex and large macrophages in the cortico-medullary zone (CMZ) detected with ED2 antibody are seen. No positive cells in the medulla. **b.** Large, prostaglandin synthase-rich macrophages are localized in the cortico-medullary zone and only few smaller, weakly positive macrophages in the cortex. **c and d.** CSA-treated thymus. c. Enlarged ED2-positive cortical macrophages. d. Numerous, large, prosta-glandin synthase-rich macrophages distributed in the cortex. x 320. (Adapted from Milićević and Milićević, 1994).

are uniformly distributed throughout the tissue. These cells show very strong AcP, weak to moderate NSE activity (Milićević and Milićević, 1984), and positively stain with ED1, ED2 (Fig. 5a; Dijkstra et al., 1985), and R-MC41 (Čolić et al., 1990) monoclonal antibodies. Some of these cells also show the weak activity of



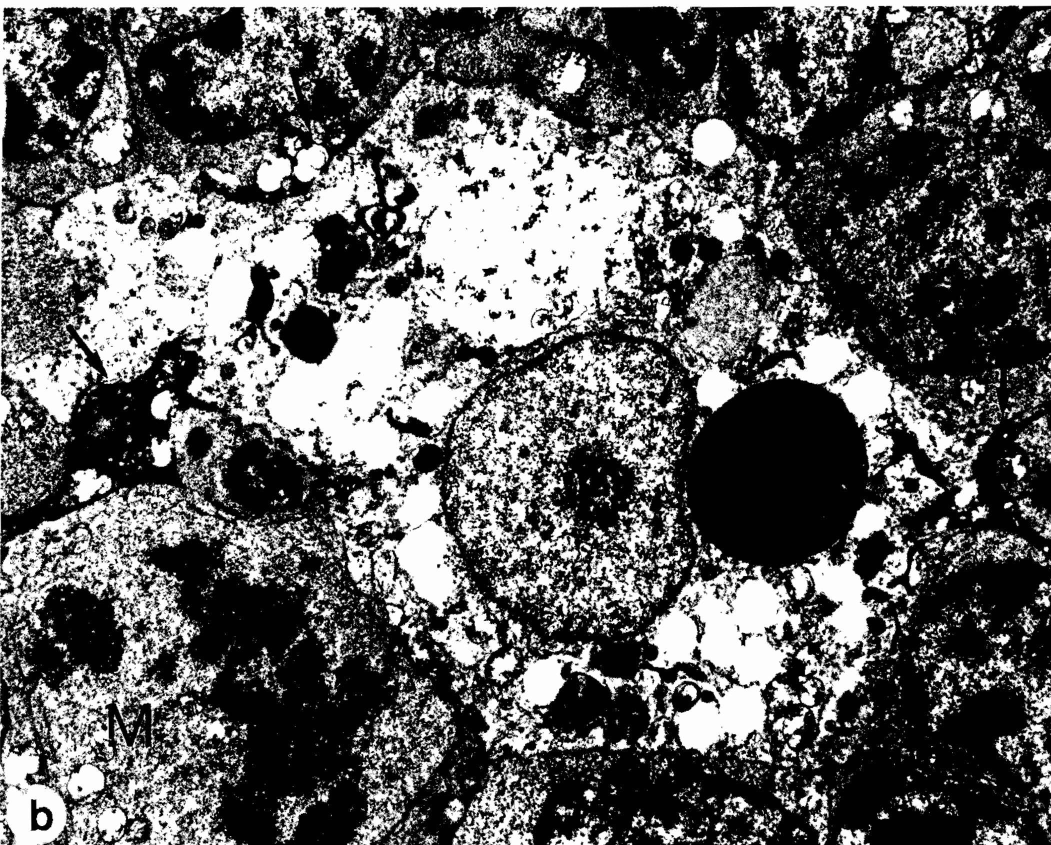


Fig. 6. a. Normal thymus. Cortico-medullary zone macrophage shows characteristic vacuolar cytoplasmic inclusions (V) and two phagocytosed dead lymphocytes (L). The nucleus is extremely euchromatic with patent nucleolus. Thiocarbohydrazide-silver proteinate, uncontrasted. x 7,840. **b.** Cyclosporin A-treated thymus. Cortical macrophage has very euchromatic nucleus and prominent nucleolus. Numerous cytoplasmic inclusions and lipid bodies, dilatations of endoplasmic reticulum, polysomes and vesicles are seen. M: mitosis. Arrows: prolongations of "dark" epithelial cells. Contrasted with uranyl-acetate and lead-citrate. x 4,725. (Adapted from Milićević et al., 1987 and from Milićević et al., 1993, with kind permission from Kluwer Academic Publishers for b).

prostaglandin synthase (PGS; Fig. 5b; Milićević et al., 1994). Electron microscopy reveals the presence of dead lymphocytes in various stages of degradation within their cytoplasm (Milićević et al., 1987). A morphologically distinct type of macrophages is positioned between the cortex and medulla, i.e., in the corticomedullary zone (CMZ) of the rat thymus. In some respects they are similar to those positioned in the cortex, but also show several characteristic features. These cells positively stain with ED1, ED2 (Fig. 5a) and R-MC monoclonals, but also have very strong activity of AcP and NSE (Milićević and Milićević, 1984; Milićević et al., 1989). In addition, they are also characterized by very strong activity of naphthol AS-D chloroacetate esterase (NASDCE; Milićević and Milićević, 1985), succinic dehydrogenase (SD; Milićević and Milićević, 1984) and PGS (Fig. 5b; Milićević et al., 1994). Moreover, these cells contain large cytoplasmic inclusions composed of unsaturated, partly oxidized lipids, which positively stain with aldehyde fuchsin (AF; Milićević et al., 1983a), Oil red O, Sudan Black B, periodic acid-Schiff (PAS) and show autofluorescence (Milićević et al., 1986; Milićević and Milićević, 1988). These cells are large and, when impregnated with silver, display the prominent cytoplasmic extensions making a garland strategically positioned between the cortex and medulla Milićević et al., 1982, 1983b; Milićević and Milićević, 1984). In electron microscopy (Fig. 6a) CMZ macrophages are characterized by vacuolar cytoplasmic inclusions, dense bodies and membrane profiles, some of which may correspond to the lipid granules observed in light microscopy. These cells have a very euchromatic nucleus, prominent nucleolus, several giant Golgi complexes and only rarely contain phagocytosed lymphocytes (Milićević, 1984; Milićević et al., 1987).

After application of CSA the number of cortical macrophages and their phenotypic characteristics, as demonstrated by the use of monoclonal antibodies, do not change significantly. However, these cells become enlarged and rounded (Fig. 5c,d; Milićević et al., 1989), showing the prominent changes in enzyme capacity, histochemical features and ultrastructural organization. The increase in activity of hydrolytic enzymes AcP and NSE is noticed, whereby cortical macrophages develop strong activity of NASDCE (Milićević et al., 1989, 1993). In contrast to what is normal, these cells also develop strong activity of the respiratory enzyme SD (Milićević et al., 1989) and begin to show a marked presence of PGS (Fig. 5d; Milićević and Milićević, 1993; Milićević et al., 1994). Moreover, the cytoplasmic inclusions, showing histochemical features similar to those of normal CMZ macrophages, appear within cortical macrophages: these granules are largely AF- and PAS-positive and show sudanophilia (Milićević et al., 1989). In electron microscopy (Fig. 6b) these macrophages show abundant cytoplasm of a very active appearance, euchromatic nucleus with very prominent nucleoli and a variety of vacuolar cytoplasmic inclusions. Most of these inclusions are filled with electron-lucent material or with a flocculent substance of moderate electron density. Very rarely the macrophages contain the engulfed lymphocyte remnants. The mitoses of thymocytes located in the immediate vicinity of these macrophages are often seen (Fig. 6b; Milićević et al., 1993).

On the basis of histochemical and ultrastructural features the cortical macrophages become very similar to CMZ macrophages. Actually, the application of CSA induces a dramatic reduction of thymic medulla, whereby only the residual islands remain preserved. The cells with morphological characteristics largely corresponding to those of normal CMZ macrophages spread throughout the cortical region; thus it becomes impossible to define the CMZ.

Medulla

In the normal thymus, several strongly AcP-positive macrophages, containing phagocytosed debris, are always scattered in the medulla. IDCs are more often positioned in the outer parts of the medullary region. These cells are ED1-positive (Dijkstra et al., 1985) and are easily recognized due to the distinct, spot-like AcP-positive reactivity in the perinuclear cytoplasm (Duijvestijn et al., 1982). Their cytoplasm is abundant, electron-lucent and contains several lysosomes and Birbeck granules. The nucleus has a characteristic bizarre shape (van Haelst, 1969; Ardavin, 1997).

The absolute number of IDCs seems decreased due to the reduction in the amount of thymic medulla. The actual loss of medullary IDCs was documented using macrophage/IDC-specific ED1 monoclonal antibody (Beschorner and Armas, 1991). Ultrastructural studies have confirmed that the decrease in number of IDCs is due to the disappearance of these cells from the thymus and not to the down-regulation of MHC class II antigens on persistent IDCs (De Waal et al., 1992). Really, the remaining MHC-positive cells are seen within the medullary islands (Fig. 2b; Cheney and Sprent, 1985; Schuurman et al., 1990). Some of these cells are keratin-positive and correspond to epithelial cells, whereas some scanty MHC-positive, keratin-negative cells (Kanariou et al., 1989), with a distinct spot-like AcP reactivity, undeniably represent IDCs (Milićević et al., 1989). Using *in vitro* assays it was also shown that the remaining IDCs retain the identical phenotypic and functional features to those of control animals (Damoiseaux et al., 1994).

Mechanisms of CSA-induced changes of the thymic microenvironment

An important question, regarding the changes of thymic nonlymphoid cells, presented herein, is whether they are a directly or indirectly induced by CSA application. On the one hand, the proliferation of thymic epithelial cells and the increased production of thymic hormones have also been registered *in vitro* under the

influence of this agent (Dardenne et al., 1987). These results suggest that CSA might exert its effects directly on thymic epithelial cells. On the other hand, it is known that thymocytes support the integrity and influence the organization of thymic epithelial cells (Surh et al., 1992; van Ewijk et al., 1994; Goverman et al., 1997). The maturation of thymocytes is affected by CSA at two levels: firstly, the development of double-positive $CD4^+CD8^+$ thymocytes, and secondly, the generation of single-positive $CD4^+CD8^-$ or $CD4^-CD8^+$ cells are blocked (Kosugi et al., 1989). Thus, it is also possible that the changes of thymic epithelial cells reflect the altered feedback influences of thymic lymphoid cells, whose physiological life cycle has been disrupted after treatment with CSA. However, as in many other instances in the biological systems, the combination of direct and indirect effects could be the true event which occurs. Very recently, the hypertrophy of human keratinocytes, combined with the delay in differentiation was observed in cultures supplemented with CSA (Prignano et al., 1996), which is very reminiscent of the changes of thymic epithelial cells registered herein. So, depending on whether distinct thymocyte subpopulations, as well as their influence on neighboring epithelial cells, are preserved or lost, the different changes of cortical (chiefly hypertrophy) and medullary (principally immaturity) epithelium occur after CSA application. Finally, it is known that the function of macrophages is largely influenced by T-lymphocytes (Doherty, 1995). Thymic macrophages are thoroughly changed after the application of CSA (Milićević et al., 1989, 1993, 1994). Thus, considering the intricate nature of cellular interactions within the thymus, it may be possible that signals, which affect the morphology and function of thymic epithelial cells, are mediated via thymic macrophages.

Functional significance of CSA-induced changes of the thymic microenvironment

Epithelial cells

Type 1, "subcapsular" epithelial cells, show prominent changes after CSA treatment. In the normal thymus, these cells produce chemoattractive factors (Imhof et al., 1988), thymic (Jambon et al., 1981; Savino et al., 1982; von Gaudecker et al., 1986) and neuroendocrine hormones (von Gaudecker et al., 1986; Kurz et al., 1996). These factors are believed to stimulate migration of T-cell precursors, and their proliferation in the subcapsular region (Imhof et al., 1988; Kurz et al., 1996). Considering that the generation of double-positive $CD4^+CD8^+$ thymocytes is blocked by CSA (Kosugi et al., 1989), the accumulation of double-negative $CD4^-CD8^-$ cells in the subcapsular region could possibly switch on the feedback loop, which down-regulates the activity of "subcapsular" epithelial cells. Further immunocytochemical work, some of which is in progress in our laboratory, could confirm this opinion. Especially, it would be interesting to investigate the changes of numerous factors, which are normally produced by these cells, after CSA treatment. This could further elucidate the role of "subcapsular" epithelial cells under normal conditions.

Other subsets of cortical epithelial cells, i.e., "pale", "intermediate" and "dark", generally show the signs of activation after CSA treatment. The morphological marks of increased synthetic and secretory activity are particularly prominent. Moreover, these cells are enlarged and appear hypertrophied, strongly expressing MHC class II antigens. Therefore, the impression is gained that intrinsic, feedback mechanisms are trying to overcome the block in maturation of thymocytes imposed by CSA (Kosugi et al., 1989) and are pushing the surrounding epithelial cells into the state of increased activity. Very similar ultrastructural signs of activation of thymic cortical epithelial cellular secretory machinery are seen after application of cyclophosphamide to rats. It is believed that these changes reflect the corrective response of cortical epithelium to the perturbation of thymocytopoiesis by this drug (Yoon et al., 1997). As mentioned above, the proliferation of thymic epithelial cells, accompanied by the increased production of thymic hormones, has been registered in cell cultures supplemented with CSA (Dardenne et al., 1987). However, in sections double-stained with anti-BrdU and anti-cytokeratin monoclonal antibodies to characterize the proliferating cells *in vivo*, similarly as in control thymuses, no incorporation of BrdU was detected in thymic epithelial cells after CSA treatment. All proliferating cells were lymphocytes (Milićević et al., 1992). Thus, the observed morphological changes of thymic epithelial cells *in vivo* do not seem to represent the proliferation, but may rather be considered as hypertrophy.

Thymic medullary epithelial cells produce a variety of hormones and active substances (for example, Savino et al., 1982; von Gaudecker et al., 1986). The prominent loss of these cells (Beschorner et al., 1987, 1988; Hattori et al., 1987; Kanariou et al., 1989), especially of phenotypically (Milićević et al., 1992) and morphologically mature ones (Milićević and Milićević, 1997), is registered after CSA treatment. However, the increase in peripheral hormone level, as well as in the number of thymulin-producing cells within the thymus, were registered in CSA-treated mice (Dardenne et al., 1987). Considering that thymic hormone-containing cells are also dispersed throughout the cortical region (Savino et al., 1982; Schuurman et al., 1985), some of the biosynthetically-activated cortical epithelial cells could be the source of hormones in CSA-treated animals. Thus, it is obvious that the complex alterations of thymic endocrine function occur, both at local and systemic levels, which deserves further attention.

Macrophages and interdigitating cells

After CSA treatment cortical macrophages become

enlarged and morpho-functionally very similar to CMZ macrophages of the normal rat thymus. These striking morphological changes are suggestive of the shift in functional activity of thymic cortical macrophages after CSA treatment. These cells accumulate unsaturated, partially oxidized lipids within their cytoplasm (which may be biochemically similar to arachidonic acid; Alberts et al., 1983). Interestingly, these cells also begin to show a strong activity of NASDCE (Milićević et al., 1989). This enzyme is characteristically present within cytoplasmic granules, similarly to other cell types which are able to metabolize arachidonic acid and produce various active substances, that is, in myeloid and mast-cells (Bancroft and Stevens, 1982). The most prominent ultrastructural feature of thymic cortical macrophages after application of CSA is the presence of cytoplasmic vacuolar inclusions (Milićević et al., 1993), similar to those of macrophages producing arachidonic acid metabolites *in vitro* (Brune at al., 1978). Ultrastructurally, ^{3}H-arachidonic acid (Dvorak et al., 1983) and PGS (Dvorak et al., 1992) have been detected within large cytoplasmic inclusions, so-called lipid bodies, of macrophages and mast-cells. Therefore, the specific ultrastructural features of thymic cortical macrophages after CSA treatment, as well as those of normal CMZ macrophages, probably reflect the production of arachidonic acid metabolites by these cells. Indeed, the presence of PGS may be demonstrated in these cells (Milićević et al., 1994). The proliferation of thymocytes is influenced by arachidonic acid metabolites *in vitro* (Delebassee and Gualde, 1988; Shipman et al., 1988). In keeping with this, mitotic figures are frequently observed in the vicinity of thymic cortical macrophages after CSA treatment. The subtle histochemical differences may be registered between macrophages positioned in the outer and inner cortical regions of CSA-treated thymuses (Milićević et al., 1989), as well as between individual macrophages of normal CMZ (Milićević and Milićević, 1984). This suggests that these cells could eventually produce different arachidonic acid metabolites, which are known to have the opposing effects on proliferation of thymocytes *in vitro* (Delebassee and Gualde, 1988; Shipman et al., 1988). Thus, depending on the prevailing secretory product, CMZ macrophages could represent the up/down regulator of kinetics of thymocyte proliferation and play a significant role in the process of normal T-cell production. Such functions may become even more evident under the conditions of disturbed thymocytopoiesis by CSA. However, considering that a large number of active substances may be secreted by macrophages (Davies and Bonney, 1979; Nathan, 1987), it seems very possible that thymic macrophages (both normal and after CSA) may produce molecules other than arachidonic acid metabolites, which could be involved in the control of thymic function.

In the normal thymus, cortical macrophages are involved in phagocytosis of apoptotic lymphocytes (Milićević et al., 1987). CSA is well known to inhibit the programmed cell death of thymocytes and T-cell hybridomas (Shi et al., 1989; Waring and Beaver, 1996). Even the earliest cellular changes related to apoptosis, so-called permeability transition of membrane and subsequent dysfunction of mitochondria, are inhibited by CSA (Savage et al., 1991; Broekemeier et al., 1992; Waring and Beaver, 1996). Therefore, the increase in apoptotic death of thymocytes is not likely to occur after the application of CSA. The morphological features of cortical macrophages in CSA-treated thymus, thus, probably do not reflect the increased phagocytic activity, although these cells retain their capability to ingest apoptotic cells, which may be seen in some of them (Milićević et al., 1989).

In recent years, the evidence showing that IDCs represent the element of thymic microenvironment responsible for elimination of autoreactive thymocytes during the process of negative selection has accumulated (for example, Brocker et al., 1997), although the involvement of medullary epithelial cells is still proposed by some researchers. Thus, the reduced deletion of autoreactive thymocytes after CSA (Gao et al., 1988; Jenkins et al., 1988) may be related to the decreased number of thymic IDCs. However, the possibility of direct action of CSA on thymocytes to prevent their elimination during negative selection (Shi et al., 1989) cannot be ruled out and further studies are necessary to shed more light on this issue.

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References

Alberts B., Bray D., Lewis J., Raff M., Roberts K. and Watson J.D. (1983). Molecular biology of the cell. Garland Publishing, Inc. New York. p 725.

Ardavin C. (1997). Thymic dendritic cells. Immunol. Today 18, 350-361.

Bancroft J.D. and Stevens A. (1982). Theory and practice of histological techniques, 2nd Ed. Churchill Livingstone. London. pp. 250-252.

Beschorner W.E. and Armas O.A. (1991). Loss of medullary dendritic cells in the thymus after cyclosporine and irradiation. Cell. Immunol. 132, 505-514.

Beschorner W.E., Namnoum J.D., Hess A.D., Shinn C.A. and Santos G.W. (1987). Cyclosporin A and the thymus. Immunopathology. Am. J. Pathol. 126, 487-496.

Beschorner W.E., Olson J.L., Hess A.D., Di Gennaro K.A. and Santos G.W. (1988). Cyclosporine-induced cell-mediated injury of the thymic medullary epithelium. Transplantation 45, 797-803.

Bevan M.J. (1997). In thymic selection, peptide diversity gives and takes away. Immunity 7, 175-178.

Blair J.T., Thomson A.W., Whiting P.H., Davidson R.J.L. and Simpson

J.G. (1982). Toxicity of the immune suppressant cyclosporin A in the rat. J. Pathol. 138, 163-178.

Borel J.F., Feurer C., Magnee C. and Stahelin H. (1977). Effects of the new anti-lymphocytic peptide cyclosporin A in animals. Immunology 32, 1017-1025.

Boyd R.L., Tucek C.L., Godfrey D.I., Izon D.J., Wilson T.J., Davidson D.J., Bean A.G.D., Ladyman H.M., Ritter M.A. and Hugo P. (1993). The thymic microenvironment. Immunol. Today 14, 445-459.

Brocker T., Riedinger M. and Karjalainen K. (1997). Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes in vivo. J. Exp. Med. 185, 541-550.

Broekemeier K.M., Carpenter-Deyo L., Reed D.J. and Pfeiffer D.R. (1992). Cyclosporin A protects hepatocytes subjected to high Ca^{2+} and oxidative stress. FEBS Lett. 304, 192-194.

Brune K., Glatt M., Kälin H. and Peskar B.A. (1978). Pharmacological control of prostaglandin and thromboxane release from macrophages. Nature 274, 261-263.

Cheney R.T. and Sprent J. (1985). Capacity of cyclosporine to induce auto-graft-versus-host disease and impair intrathymic T cell differentiation. Transplant. Proc. 17, 528-530.

Čolić M., Jovanović S., Mitrović S. and Dujić A. (1989). Immunohistochemical identification of six cytokeratin-defined subsets of the rat thymic epithelial cells. Thymus 13, 175-185.

Čolić M., Popović Lj., Gasić S., Dragojević-Simić V., Milićević N.M., Matanović D. and Dujić A. (1990). Immunohistochemical characterization of rat thymic non-lymphoid cells. II. Macrophages and granulocytes defined by monoclonal antibodies. Immunology 69, 416-422.

Damoiseaux J.G.M.C., Beijleveld L.J.J. and van Breda Vriesman P.J.C. (1994). The effects of *in vivo* cyclosporin A administration on rat thymic dendritic cells. Cell. Exp. Immunol. 96, 513-520.

Dardenne M., Savino W., Feutren G. and Bach J.-F. (1987). Stimulatory effects of cyclosporin A on human and mouse thymic epithelial cells. Eur. J. Immunol. 17, 275-279.

Davies P.D. and Bonney R.J. (1979). Secretory products of mononuclear phagocytes: A brief review. J. Reticuloendothel. Soc. 26, 37-45.

Delebassee S. and Gualde N. (1988). Effect of arachidonic acid metabolites on thymocyte proliferation. Ann. Inst. Pasteur (Immunol.) 139, 383-399.

De Maagd R.A., Mackenzie W.A., Schuurman H.-J., Ritter M.A., Price K.M., Broekhuizen R. and Kater L. (1985). The human thymus microenvironment: heterogeneity detected by monoclonal anti-epithelial cell antibodies. Immunology 54, 745-754.

De Waal E.J., Rademakers L.H.P.M., Schuurman H.-J. and Van Loveren H. (1992). Interdigitating cells in the rat thymus during cyclosporin A treatment: ultrastructural observations. Thymus 20, 163-170.

De Waal E.J., Rademakers L.H.P.M., Schuurman H.-J., Van Loveren H. and Vos J.G. (1993). Ultrastructure of cortical epithelium of the rat thymus after in vivo exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Arch. Toxicol. 67, 558-564.

Dijkstra C.D., Döpp E.A., Joling P. and Kraal G. (1985). The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. Immunology 54, 589-599.

Doherty T.M. (1995). T-cell regulation of macrophage function. Curr. Opin. Immunol. 7, 400-404.

Duijvestijn A.M. and Hoefsmit E.C.M. (1981). Ultrastructure of the rat thymus: the micro-environment of T-lymphocyte maturation. Cell Tissue Res. 218, 279-292.

Duijvestijn A.M., Sminia T., Köhler Y.G., Janse E.M. and Hoefsmit E.C.M. (1982). Rat thymus micro-environment: An ultrastructural and functional characterization. In: In vivo immunology. Histophysiology of the lymphoid system. Nieuwenhuis P., Broek A.A.V.D. and Hanna Jr. M.G. (eds). Plenum Press. New York. 441-446.

Dvorak A.M., Dvorak H.F., Peters S.P., Shulman E.S., MacGlashan D.W.Jr., Pyne K., Harvey V.S., Galli S.J. and Lichtenstein L.M. (1983) Lipid bodies: cytoplasmic organelles important to arachidonate metabolism in macrophages and mast cells. J. Immunol. 131, 2965-2976.

Dvorak A.M., Morgan E., Schleimer R.P., Ryeom S.W., Lichtenstein L.M. and Weller P.F. (1992). Ultrastructural localization of prostaglandin endoperoxyde synthase (cyclooxygenase) to non-membrane-bound cytoplasmic lipid bodies in human lung mast cells, alveolar macrophages, type II pneumocytes, and neutrophils. J. Histochem. Cytochem. 40, 759-769.

Farr A.G. and Anderson S.K. (1985). Epithelial heterogeneity in the murine thymus: fucose-specific lectins bind medullary epithelial cells. J. Immunol. 134, 2971-2977.

Fruman D.A., Burakoff S.J. and Bierer B.E. (1994). Immunophilins in protein folding and immunosuppression. FASEB J. 8, 391-400.

Gao E.-K., Lo D., Cheney R., Kanagawa O. and Sprent J. (1988). Abnormal differentiation of thymocytes in mice treated with cyclosporin A. Nature 336, 176-179.

Goverman J., Brabb T., Huseby E.S. and Farr A.G. (1997). TCR signaling regulates thymic organization: lessons from TCR-transgenic mice. Immunol. Today 18, 204-209.

Hadden J.W. (1992). Thymic endocrinology. Int. J. Immunopharmacol. 14, 345-352.

Hattori A., Kunz H.W., Gill III T.J. and Shinozuka H. (1987). Thymic and lymphoid changes and serum abnormalities in mice receiving cyclosporine. Am. J. Pathol. 128, 111-120.

Hess A.D. and Colombani P.N. (1987). Cyclosporin-resistant and -sensitive T-lymphocyte subsets. Ann. Inst. Pasteur (Immunol.) 138, 606-611.

Hiramine C., Hojo K. and Matsumoto H. (1988). Abnormal distribution of T cell subsets in the thymus of Cyclosporin A (CsA)-treated mice. Thymus 11, 243-252.

Imhof B.A., Deugnier M.A., Girault J.M., Champion S., Damis C., Itoh T. and Thiery J.-P. (1988). Thymotaxin: a thymic epithelial peptide chemotactic for T-cell precursors. Proc. Natl. Acad. Sci. USA 85, 7699-7703.

Jambon B., Montagne P., Bene M.-C., Brayer M.-P., Faure G. and Duheille J. (1981). Immunohistologic localization of "facteur thymique serique" (FTS) in human thymic epithelium. J. Immunol. 127, 2055-2059.

Jenkins M.K., Schwartz R.H. and Pardoll D.M. (1988). Effects of cyclosporine A on T cell development and clonal deletion. Science 241, 1655-1658.

Kahan B.D. (1989). Cyclosporine. New England J. Med. 321, 1725-1738.

Kampinga J., Kroese F.G.M., Duijvestijn A.M., Murawska M.B., Pol G.H. and Nieuwenhuis P. (1987). The rat thymus microenvironment: subsets of thymic epithelial cells defined by monoclonal antibodies.

Transplant. Proc. 19, 3171-3174.

Kanariou M., Huby R., Ladyman H., Čolić M., Sivolapenko G., Lampert I. and Ritter M. (1989). Immunosuppression with cyclosporin A alters the thymic microenvironment. Clin. Exp. Immunol. 78, 263-269.

Kosugi A., Zuniga-Pflucker J.C., Sharrow S.O., Kruisbeek A.M. and Shearer G.M. (1989). Effect of Cyclosporin A on lymphopoiesis. II. Developmental defects of immature and mature thymocytes in fetal thymus organ cultures treated with Cyclosporin A. J. Immunol. 143, 3134-3140.

Kunz J. and Hall M.N. (1993). Cyclosporin A, FK506 and rapamycin: more than just immunosuppression. TIBS 18, 334-338.

Kurz B., von Gaudecker B., Kranz A., Krisch B. and Mentlein R. (1996). Calcitonin gene-related peptide and its receptor in the thymus. Peptides 16, 1497-1503.

Milićević N.M. (1984). Nelimfoidne ćelije timusa pacova. D. Sc. Thesis. University of Beograd, Beograd.

Milićević N.M. and Milićević Ž. (1984). Enzyme-histochemical characterization of macrophages in the rat thymus, with special reference to metallophilic cells of the cortico-medullary zone. J. Leukocyte Biol. 36, 761-769.

Milićević N.M. and Milićević Ž. (1985). Naphthol AS D chloroacetate esterase-positive macrophages in the cortico-medullary zone of the normal rat thymus.

Milićević N.M. and Milićević Ž. (1988). On the nature of the content in the PAS-positive macrophages of the rodent thymus. Thymus 11, 257-259.

Milićević N.M. and Milićević Ž. (1993). Relationship between naphthol AS D chloroacetate esterase and prostaglandin synthase. Acta Histochem. 95, 67-70.

Milićević Ž. and Milićević N.M. (1997). Ultrastructure of different types of thymic epithelial cells in normal and cyclosporin-A-treated rats. Anat. Embryol. 196, 171-183.

Milićević N.M., Milićević Ž., Piletic O. and Mujović S. (1982). The metallophilic cells of the rat thymus. IRCS Med. Sci. 10, 950.

Milićević N.M., Milićević Ž. and Mujović S. (1983a). Specific histochemical properties of metallophilic cells in the rat thymus. IRCS Med. Sci. 11, 653.

Milićević N.M., Milićević Ž., Mujović S., Piletic O. and Ninkov V. (1983b). Reactivity of thymic metallophilic cells during the regeneration after the application of cyclophosphamide. J. Reticuloendothel. Soc. 34, 501-507.

Milićević N.M., Milićević Ž. and Mujović S. (1986). Histochemical characterization of the lipid content in the macrophages of the cortico-medullary zone of the rat thymus. Anat. Histol. Embriol. 15, 355-360.

Milićević N.M., Milićević Ž.Čolić M. and Mujović S. (1987). Ultrastructural study of macrophages in the rat thymus, with special reference to the cortico-medullary zone. J. Anat. 150, 89-98.

Milićević N.M., Milićević Ž. and Čolić M. (1989). Macrophages of the rat thymus after cyclosporine treatment: histochemical, enzymehistochemical and immunohistochemical study. Virchows Arch. (B) 57, 237-244.

Milićević Ž., Čolić M. and Milićević N.M. (1991). Organization of thymic epithelial cells in cyclosporine-treated rats. Light microscopic immunohistochemical study. Thymus 17, 75-79.

Milićević Ž., Živanović V., Todorović V., Čolić M., Kamperdijk E.W.A., Hoefsmit E.C.M. and Milićević N.M. (1992). Differential effects of cyclosporin application of epithelial cells of the rat thymus. Immunohistochemical study. J. Comp. Pathol. 106, 25-35.

Milićević N.M., Milićević Ž., Čolić M., Kamperdijk E.W.A. and Hoefsmit E.C.M. (1993). Ultrastructural study of macrophages of the rat thymus after cyclosporin treatment. Thymus 22, 35-44.

Milićević N.M., Appasamy P.M., Čolić M. and Milićević Ž. (1994). Immunohistochemical demonstraiton of prostaglandin synthase (cyclooxygenase) in thymic macrophages of normal and cyclosporin-treated rats. Immunobiology 190, 376-384.

Nathan C.F. (1987). Secretory products of macrophages. J. Clin. Invest. 79, 319-326.

Prignano F., Domenici-Lombardo L., Gerlini G., Pimpinelli N. and Romagnoli P. (1996). Cyclosporin-A affects the organization of cytoskeleton of normal human keratinocytes in culture. Histol. Histopathol. 11, 889-894.

Rhodin J.A.G. (1974). Histology. A Text and Atlas. Oxford University Press. London. pp. 150-151.

Robey E. and Fowlkes B.J. (1994). Selective events in T cell development. Annu. Rev. Immunol. 12, 675-705.

Rouse R.V., Bolin L.M., Bender J.R. and Kyewski B.A. (1988). Monoclonal antibodies reactive with subsets of mouse and human thymic epithelial cells. J. Histochem. Cytochem. 36, 1511-1517.

Ryffel B., Deyssenroth H. and Borel J.F. (1981). Cyclosporin A: Effects on the mouse thymus. Agents Actions 11, 373-379.

Savage M.K. Jones D.P. and Reed D.J. (1991). Calcium- and phosphate-dependent release and loading of glutathione by liver mitochondria. Arch. Biochem. Biophys. 290, 51-56.

Savino W., Dardenne M., Papiernik M. and Bach J.-F. (1982). Thymic hormone-containing cells. Characterization and localization of serum thymic factor in young mouse thymus studied by monoclonal antibodies. J. Exp. Med. 165, 628-633.

Schuurman H.-J., van de Wijngaert F.P., Delvoye L., Broekhuizen R., McClure J.E., Goldstein A.L. and Kater L. (1985). Heterogeneity and age dependency of human thymus reticulo-epithelium in production of thymosin components. Thymus 7, 13-23.

Schuurman H.-J., Van Loveren H., Rozing J., Van Dijk A., Loeber J.G. and Vos J.G. (1990). Cyclosporin and the rat thymus. An immunohistochemical study. Thymus 16, 235-254.

Shi Y., Sahai B.M. and Green D.R. (1989). Cyclosporin A inhibits activation-induced cell death in T-cell hybridomas and thymocytes. Nature 339, 625-626.

Shipman P.M., Schmidt R.R. and Chepenik K.P. (1988). Relation between arachidonic acid metabolism and development of thymocytes in fetal thymic organ cultures. J. Immunol. 140, 2714-2720.

Surh C.D., Ernst B. and Sprent J. (1992). Growth of epithelial cells in the thymic medulla is under the control of mature T cells. J. Exp. Med. 176, 611-616.

van de Wijngaert F.P., Kendall M.D., Schuurman H.J., Rademakers L.H.P.M. and Kater L. (1984) Heterogeneity of epithelial cells in the human thymus. An ultrastructural study. Cell Tissue Res. 237, 227-237.

van Ewijk W., Shores E.W. and Singer A. (1994). Crosstalk in the mouse thymus. Immunol. Today 15, 214-217.

van Haelst U.J.G. (1969). Light and electron microscopic study of the normal and pathological thymus of the rat. III. A mesenchymal histiocytic type of cell. Z. Zellforsch. 99, 198-209.

van Vliet E., Melis M., van Ewijk W. (1984). Monoclonal antibodies to stromal cell types of the mouse thymus. Eur. J. Immunol. 14, 524-529.

von Gaudecker B., Steinmann G.G., Hansmann M.-L., Harpprecht J.,

Milićević N.M. and Müller-Hermelink H.-K. (1986). Immunohistochemical characterization of the thymic microenvironment. A light-microscopic and ultrastructural immunocytochemical study. Cell Tissue Res. 244, 403-412.

Vukmanović S. (1996). The molecular jury: deciding whether immature thymocyte should live or die. J. Exp. Med. 184, 305-309.

Waring P. and Beaver J. (1996). Cyclosporin A rescues thymocytes from apoptosis induced by very low concentrations of thapsigargin: effects on mitochondrial function. Exp. Cell Res. 227, 264-276.

Yamazaki T., Arase H., Ono S., Ohno H., Watanabe H. and Saito T. (1997). A shift from negative to positive selection of autoreactive T cells by the reduced level of TCR signal in TCR-transgenic CD3ξ-deficient mice. J. Immunol. 158, 1634-1640.

Yoon S., Yoo Y.H., Kim B.S. and Kim J.J. (1997). Ultrastructural alterations of the cortical epithelial cells of the rat thymus after cyclophosphamide treatment. Histol. Histopathol. 12, 401-413.