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Ultrastructural alterations of the cortical epithelial cells of the rat thymus after cyclophosphamide treatment

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Summary. A single dose of cyclophosphamide (CY) was administered to Sprague-Dawley rats to investigate the effects of CY on thymic cortical epithelial cells (TCE) at the ultrastructural level. The most striking finding among the alterations in the TCE after CY treatment was a cytoplasmic vacuolization with an increased amount of granular and membranous content. The granular content appeared not only as dense bodies but also as loosely aggregated forms or finely dispersed granules. The membranous structures appeared in various forms including vesicular, tubular, vacuolar and irregular membranous structures and myelin figures. Some of the membranous structures contained granular material. Several vacuoles were closely associated with the endoplasmic reticulum (ER). The morphological alterations of the ER were also remarkable. The Golgi apparatus, mitochondria and vesicles increased in number. The cytoplasm became densely granulated due to an increased number of ribosomes and an increased amount of granular material. The tonofilaments lost their original array and increased in amount. The cell surface exhibited many cytoplasmic processes like microvilli. It seems that the above features result not only from some damage by CY, but are also signs of a hyperfunctional state of the TCE, probably due to their important functions in repopulation and maturation of the cortical thymocytes during recovery after CY-induced acute thymic involution, including the secretion of some humoral factors.

Key words: Thymic cortical epithelial cell, Cyclophosphamide, Ultrastructure, Vacuole, Rat

Introduction

Cyclophosphamide (CY) is a nitrogen mustard-derivative alkylating agent widely used in the treatment of various neoplastic diseases (Gershwin et al., 1974; Ahmed and Hombal, 1984). In addition, it has also been widely used as a potent immunosuppressive agent in organ transplantation (Starzl et al., 1973; Huber et al., 1984) as well as in the treatment of various autoimmune diseases (Gershwin et al., 1974; Kovarsky, 1983; Ahmed and Hombal, 1984). It is well known that CY exhibits a selective suppressive action on various populations and subpopulations of lymphoid cells (Turk and Poulter, 1972; Ozer et al., 1982; Zhu et al., 1987).

Thymic epithelial cells constitute a major component of the thymus. It is presently accepted that the thymus is the site of origin of some humoral factors regulating the proliferation, maturation and differentiation of thymocytes and that these humoral factors originate in thymic epithelial cells (Bach et al., 1973; Dardenne et al., 1974; Trainin, 1974; Monier et al., 1980; Jambon et al., 1981; Savino et al., 1982; Berrih et al., 1984; Savino and Dardenne, 1984; Haynes, 1990; Kendall, 1991). It has also become increasingly obvious in recent years that MHC (major histocompatibility complex) determinants on thymic epithelial cells are involved in thymocyte maturation (Rouse et al., 1979).

There is some controversy about the classification of thymic epithelial cell types. Traditionally, thymic epithelial cells have been divided into cortical and medullary epithelial cell types. Van de Wijngaert et al. (1984) classified human thymic epithelial cells into six types according to their ultrastructural characteristics: type 1 (subcapsular/perivascular); type 2 (cortical); type 3 (intermediate); type 4 (dark); type 5 (undifferentiated); and type 6 (large medullary). Moreover, Nabarra and Andrianarison (1987b) classified mouse thymic epithelial cells into three types according to their ultrastructural features: type 1 (classical); type 2 (alveolar labyrinth); and type 3 (intracytoplasmic cavity). In general, however, the thymic epithelium can be broadly subdivided into subcapsular, cortical and medullary epithelial cells and Hassall's corpuscles by immunocytochemical methods (Haynes, 1984; Rouse et al., 1988). Among these various cell types of thymic epithelial cells, thymic cortical epithelial cells (TCE) were selected as the subject of this study not only because of the heterogeneity of thymic epithelial cells

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but also because of the important function of TCE in the maturation of thymocytes and their possible role as a secretory element (Schmitt et al., 1980, 1982; Auger et al., 1982; Nabarra and Andrianarison, 1987a; Haynes, 1990).

Although there are several studies about the effects of CY on the thymus (Turk and Poulter, 1972; Scheper et al., 1975; Sharma and Lee, 1977; Loc et al., 1981; Anton, 1987), little is known about the effect of CY on the ultrastructure of TCE. Hypothetically: (1) CY may produce some cytotoxic effects on TCE which can be demonstrated at the ultrastructural level, although thymic epithelial cells have been believed to be resistant to the cytotoxic effect of CY at the light microscopic level (Turk and Poulter, 1972; Sharma and Lee, 1977); and (2) CY may cause TCE to exhibit some features that influence the proliferation and maturation of the cortical thymocytes during recovery from CY-induced acute thymic involution. Thus, the present study has been performed to investigate the changes in the ultrastructure of the TCE after CY treatment.

Materials and methods

Male Sprague-Dawley rats weighing 200-250 g were used. They were maintained in standard wire cages at room temperature on a 14-hour light to 10 hour dark cycle and were provided with food and water ad libitum. The animals were given a single oral dose of CY (150 mg/kg body weight) in distilled water and were sacrificed in groups of three at 1, 3, 7 and 14 days thereafter. Rats given the same amount of distilled water were used as controls.

For electron microscopic investigation, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (5 mg/kg body weight). The thymuses were fixed by vascular perfusion at a rate of 5 ml/min in a peristaltic pump. The ascending aorta was cannulated with a 16-gauge polyethylene catheter via the left ventricle. The blood vessels were first washed out with 0.1M phosphate buffer (pH 7.4) and then fixed for about 20 min with 1.6% paraformaldehyde and 1.7% glutaraldehyde solution in 0.1M phosphate buffer (pH 7.4). After perfusion fixation, the thymus was excised, cut into slices with a razor blade and further fixed by immersion in the same fixative for a total fixation time of 2 hr at 4 ºC. The tissue blocks were rinsed in 0.1M phosphate buffer (pH 7.4), then postfixed with 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.4) for 90 min at 4 ºC. All specimens were processed according to a standard procedure through upgrade alcohols and propylene oxide and then embedded in Epon 812 resin. Ultrathin sections were cut with a diamond knife on an ultramicrotome (NOVA, LKB). Sections showing gray to silver interference colors were mounted on unsupported 200-mesh copper grids and were double-stained first with uranyl acetate and then with lead citrate. The sections were examined in a JEOL-1200 EX II transmission electron microscope.

Results

The fine structure of thymic cortical epithelium of normal rats

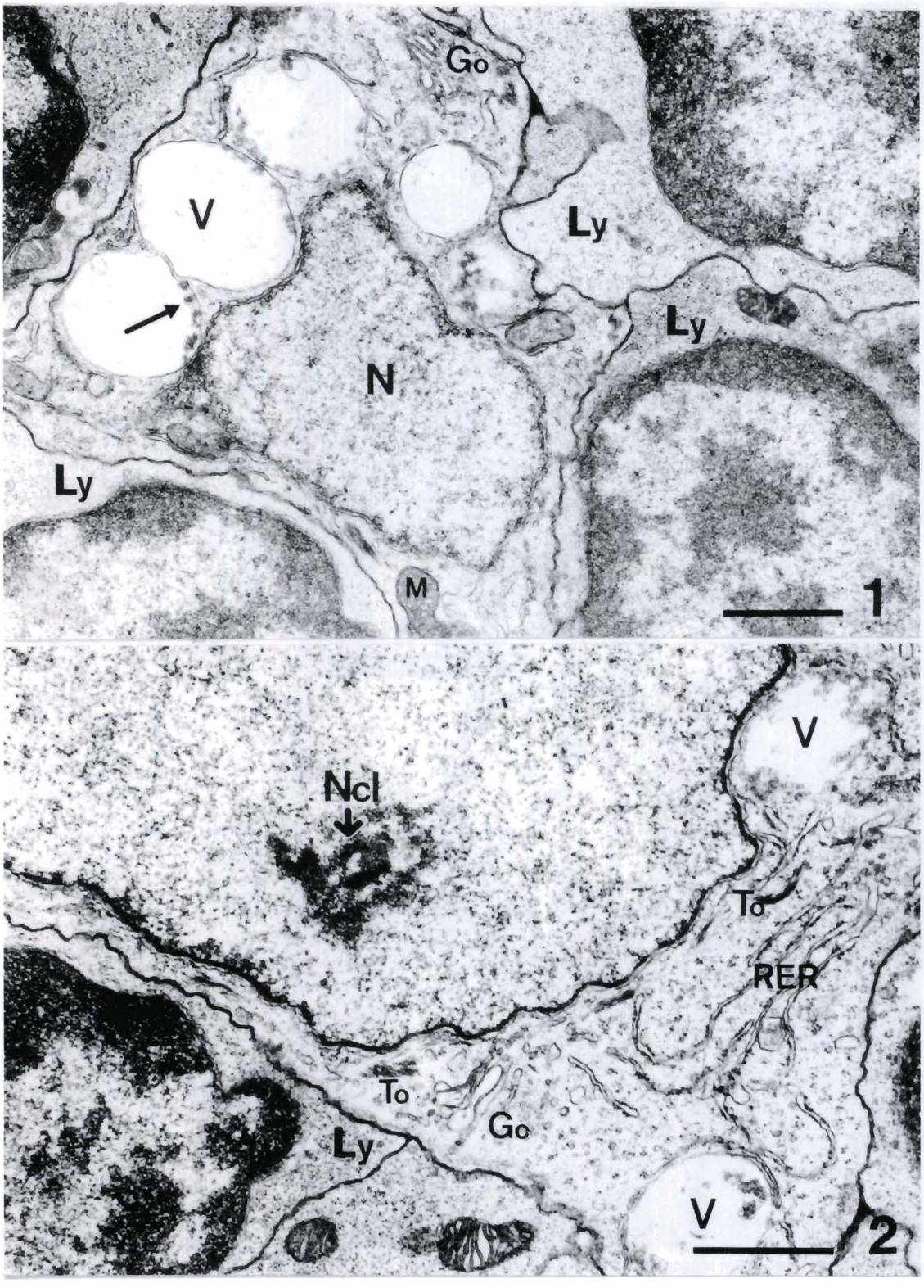
The fine structure of the thymic cortex from control groups was similar to that described previously in various mammalian species (Clark, 1963; Van Haelst, 1967; Mandel, 1968; Gorgollon and Ottone-Anaya, 1978; Van de Wijngaert et al., 1984; Nabarra and Andrianarison, 1987b; Kendall, 1991). The epithelial cells were easily identified by the presence of tonofilaments and desmosomes, which are the two specific characteristics of thymic epithelial cells. TCE were spidery in shape with long cytoplasmic processes extending in various directions and bordering the connective septae, the vessels and the thymocytes in a more or less sparse and continuous anastomosed network. The large nucleus had a clear, dispersed chromatin which was more euchromatic than that of surrounding thymocytes and also had a thin rim of heterochromatin along the nuclear envelope (Figs. 1, 2). The nucleolus was prominent and reticulated (Fig. 2). The cytoplasm contained vacuoles, dense bundles of tonofilaments and relatively sparse cellular organelles (Figs. 1, 2). The vacuoles were generally electron-lucent, but contained granular electron-dense material located mainly near their limiting membranes. Sometimes, the vacuoles also contained small empty vesicles (Fig. 1). As a rule, the Golgi apparatus and rough endoplasmic reticulum (RER) were rather poorly developed (Figs. 1, 2). There were several mitochondria and a few free ribosomes scattered throughout the cytoplasm (Figs. 1, 2).

Cyclophosphamide-induced alterations

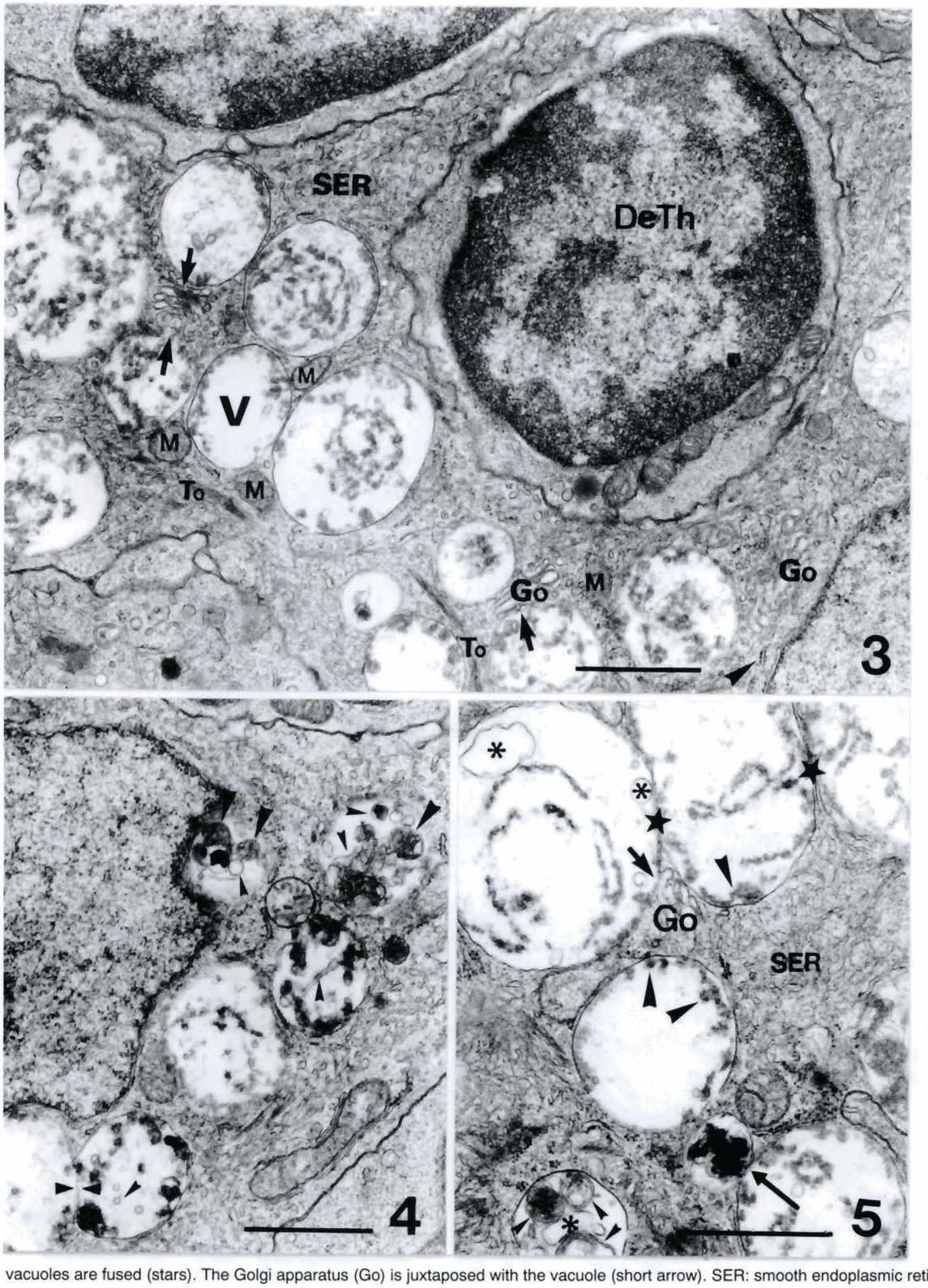
The morphological alterations in the thymic cortex were prominent during the first week after CY administration. In particular, the TCE of the CY-treated group showed the characteristics of hypertrophic changes, i.e., (1) an increase in the amount of cytoplasm, (2) the appearance of well-developed and increased number of various cytoplasmic organelles including the Golgi apparatus, endoplasmic reticulum (ER), vesicles, vacuoles, ribosomes and mitochondria (Figs. 3-12). These increased numbers of cellular elements made the cell appear more dense. Accordingly, these altered TCE will henceforth be referred to as hypertrophic TCE (HTCE) in the present study.

The most striking finding among the alterations was an extensive cytoplasmic vacuolization (Figs. 3-12). This vacuolization included the increase not only in the number and size of vacuoles, but also in the amount of their granular and membranous content (Figs. 3-12). Some of these vacuoles were fused to form larger vacuoles, and sometimes giant vacuoles (Figs. 3-12). The granular content of the vacuoles appeared not only as dense bodies formed by the clumping of electron-

dense granules but also as loosely aggregated forms or finely dispersed granules (Figs. 3-12). Particularly, the dense bodies were the most common form of the granular material. In contrast to the usually empty vesicular pattern of membranous structures within the vacuoles of normal TCE (Figs. 1, 2), those of the CY-



Figs. 1-2. Normal rat thymic cortex. The thymic cortical epithelial cells are characterized by tonofilaments (To), a prominent nucleus (N) and a nucleolus (Ncl) as well as large electron-lucent vacuoles (V) containing electron-dense granular material along their inner aspect of the limiting membranes (arrow).
M: mitochondria; RER: rough endoplasmic reticulum; Go: Golgi apparatus; Ly: lymphocytes. Bar: 1 μm.



Figs. 3-5. The cortical epithelial cells one day after CY treatment.
Fig. 3. Note that the number and electron-dense granular content of cytoplasmic vacuoles (V) are greatly increased. The cytoplasm shows an extraordinary hypertrophy of the smooth endoplasmic reticulum (SER). The RER (arrow head) is overwhelmed by the SER and tends to be short. An increased number of the Golgi apparatus (Go) is also noticed. Some of the Golgi stacks are closely associated with the vacuoles (arrows).
M: mitochondria; To: tonofilaments; DeTh: degenerating thymocytes.
Bar: 1 μm.
Fig. 4. Note the various membranous structures (small arrow heads) within the vacuoles. Structures resembling degenerating mitochondria (large arrow heads) are observed within some vacuoles. Some vacuoles are in the process of fusing with each other (triangles). Bar: 1 μm.
Fig. 5. The vacuoles contain various structures such as electron-dense flocculent material (large arrow heads), empty vesicular structures (asterisks) and myelinoid structures (small arrow heads). A phagolysosome-like vacuole (long arrow) contains a large electron-dense body. Two adjacent vacuoles are fused (stars). The Golgi apparatus (Go) is juxtaposed with the vacuole (short arrow). SER: smooth endoplasmic reticulum. Bar: 1 μm.

treated group appeared in various forms including vesicular, tubular, vacuolar and irregular membranous structures or myelin figures (Fig. 7). Some of the membranous structures contained granular material, while others seemed to be empty (Figs. 6, 7). In some areas of the cytoplasm, the vacuoles were closely

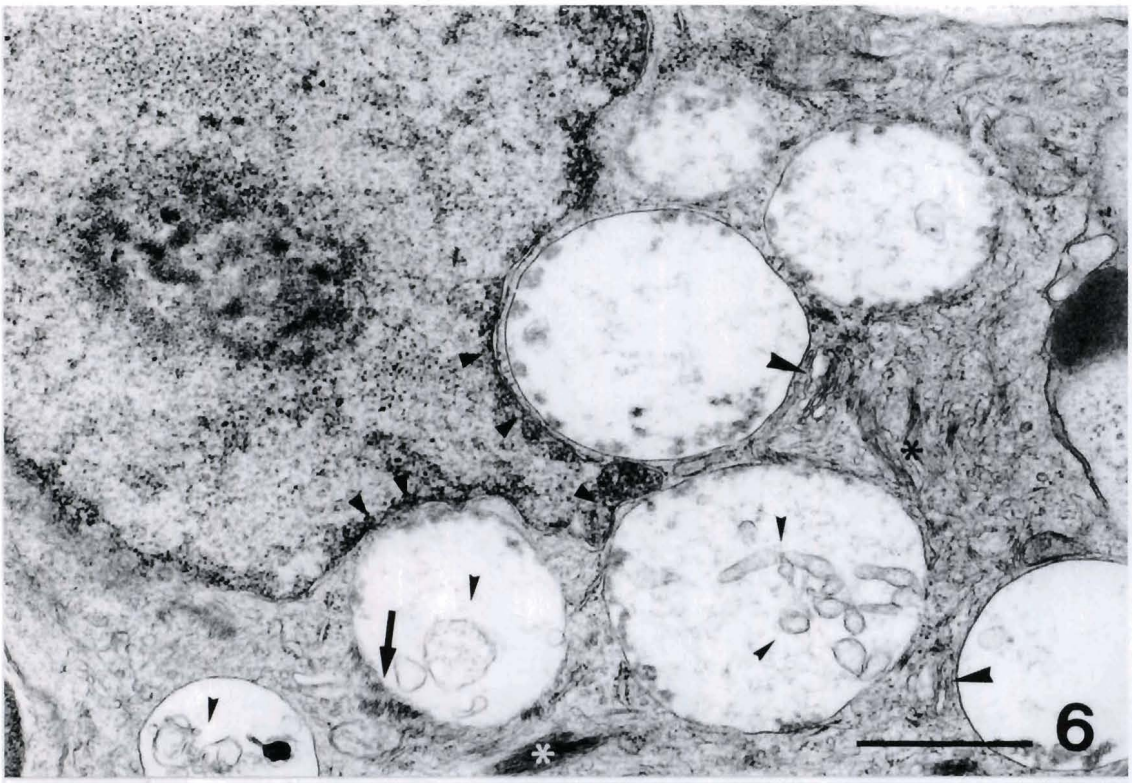


Fig. 6. The cortical epithelial cell one day after CY treatment. There are well-developed tonofilaments (asterisks) and some of them are attached at the margin of some vacuoles (arrow). The Golgi apparatus is located in close proximity to a vacuole (large arrow heads). Some vacuoles are located in indentations of the nucleus (triangles). The membranous structures (small arrow heads) are contained within some vacuoles. Bar: 1 μm.

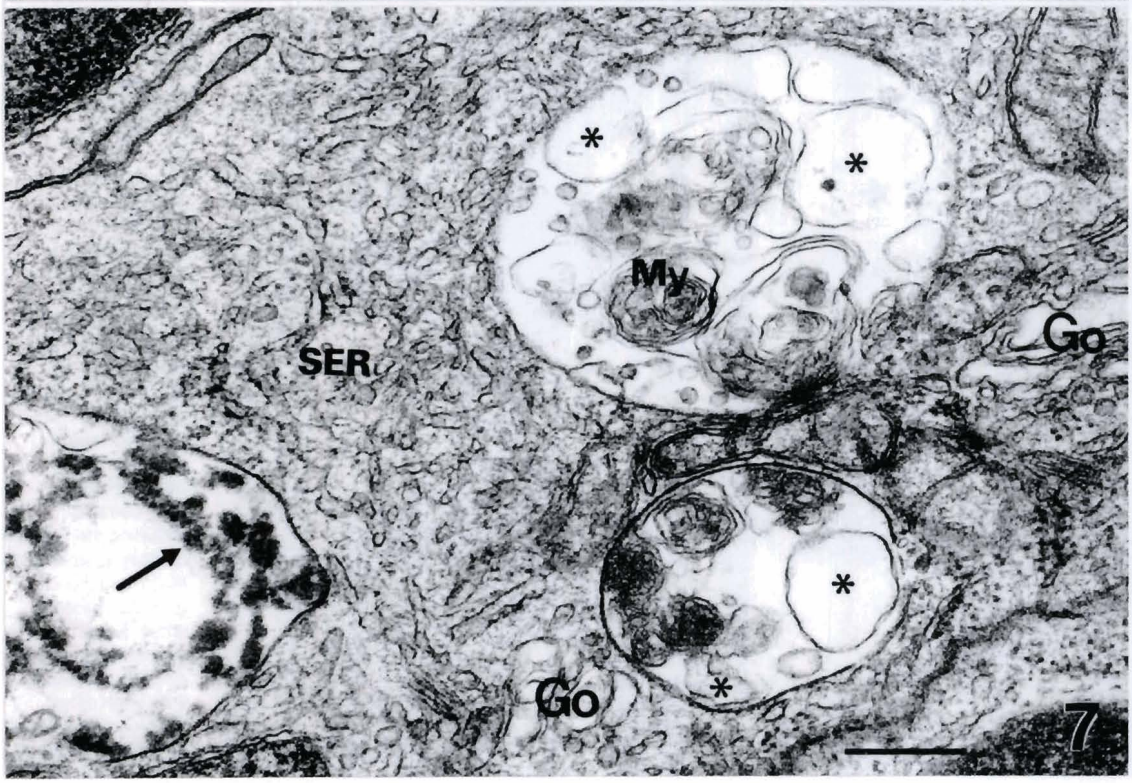
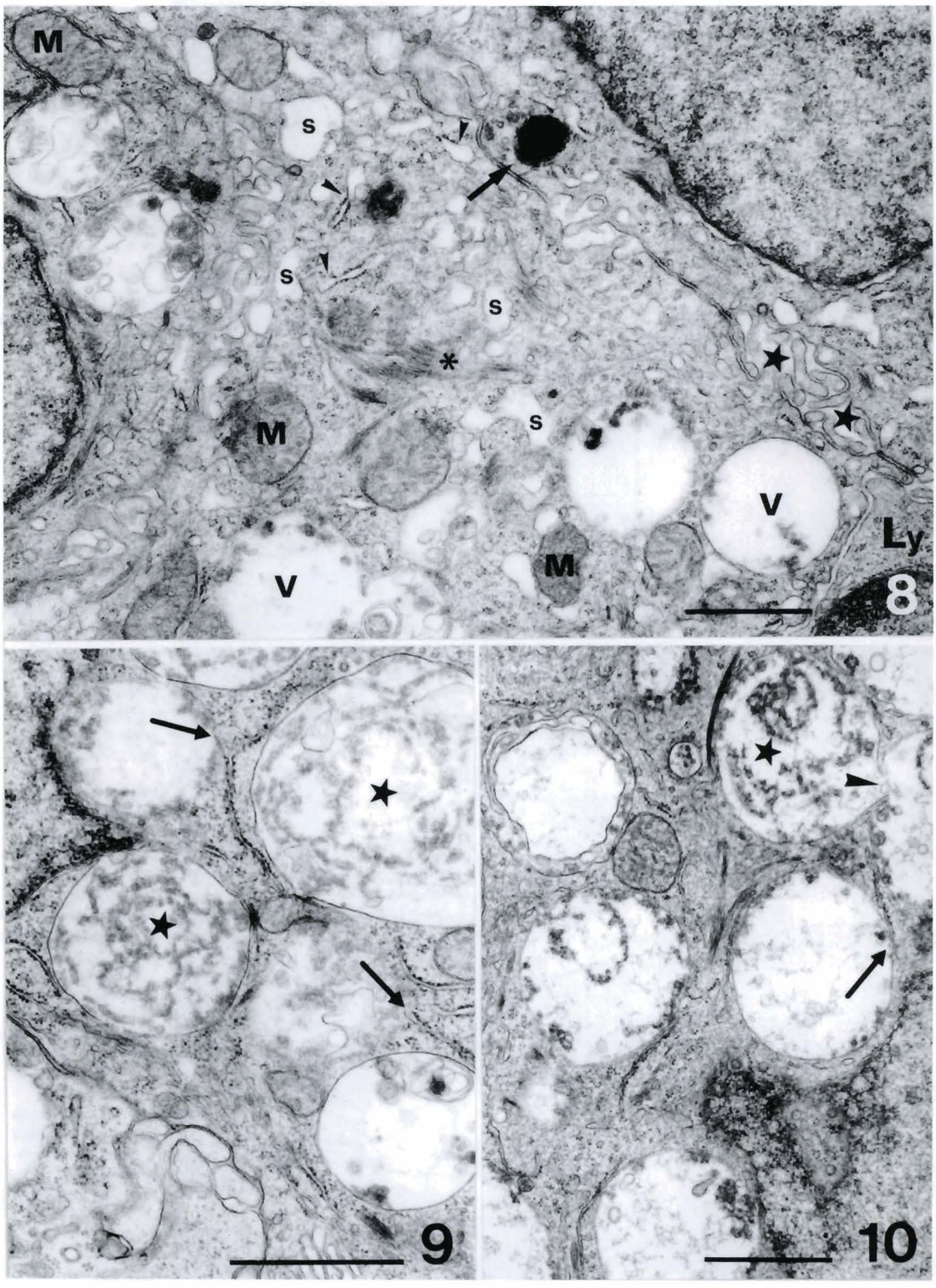
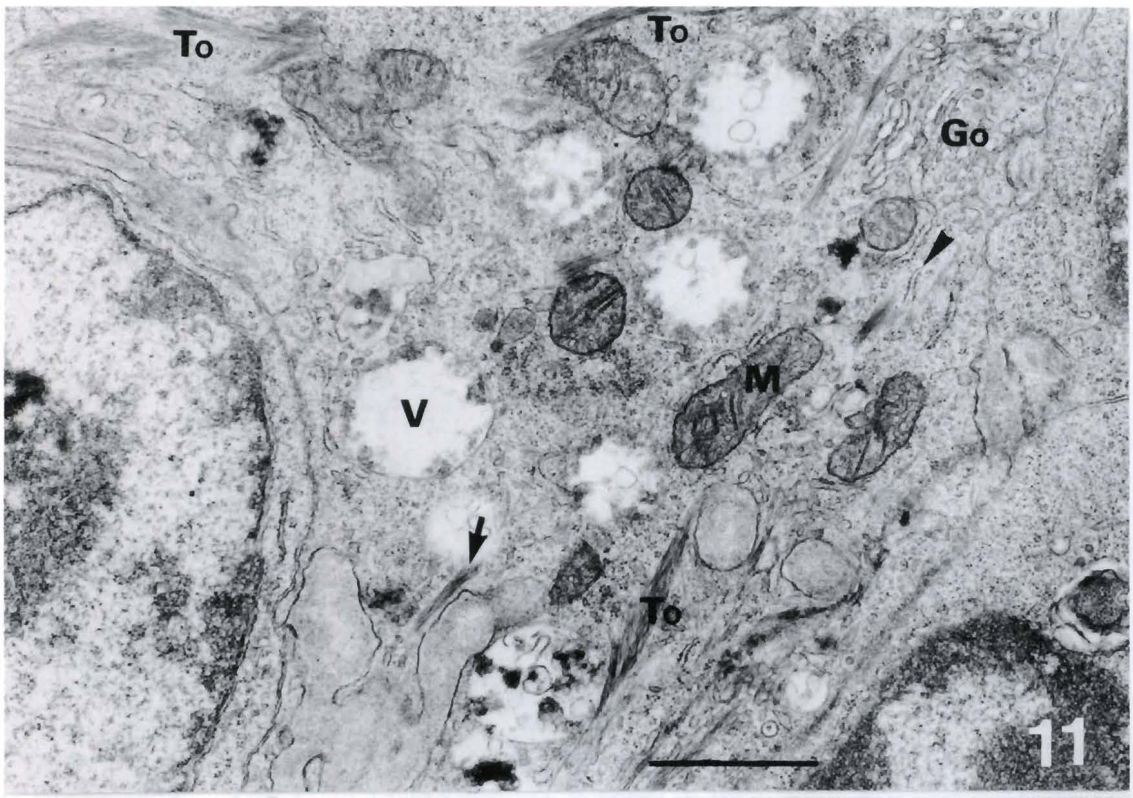


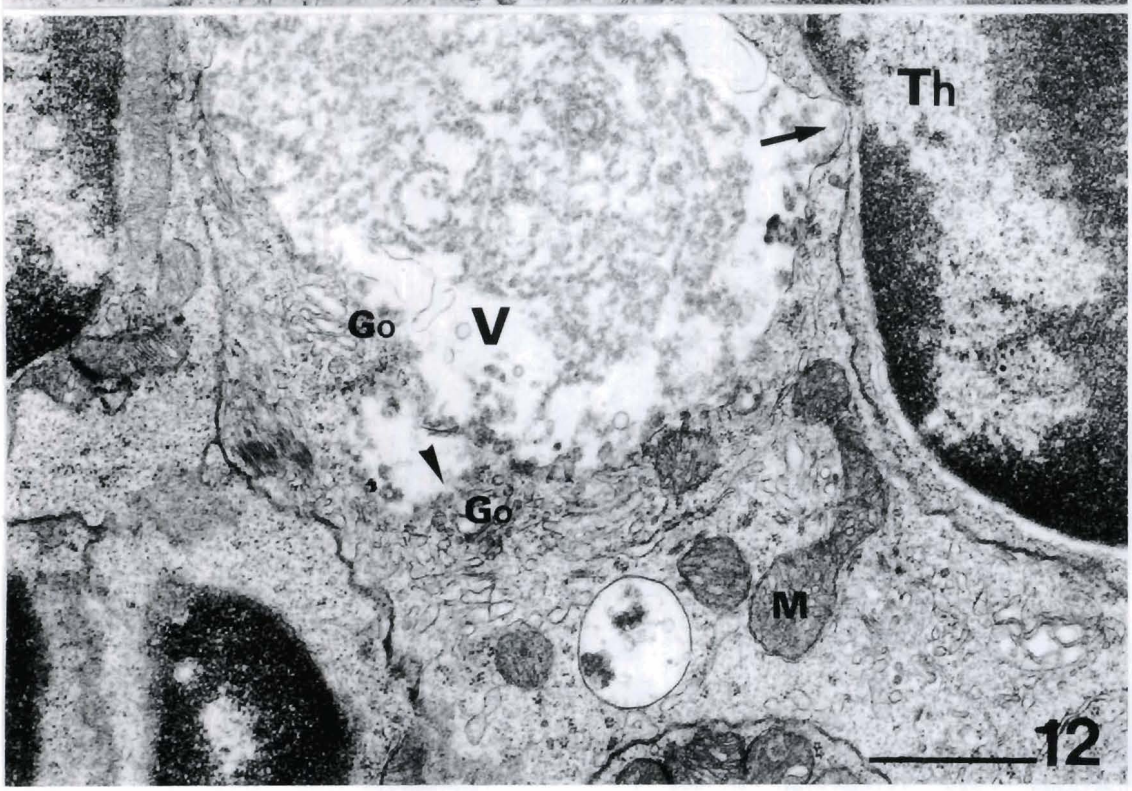
Fig. 7. The cortical epithelial cell three days after CY treatment. Variations in the appearance of the content are seen among vacuoles. The most common type is the one containing dense bodies in the vacuole (arrow). All of the vacuoles are filled with amorphous material. Myelin figures (My) and vesicular and vacuolar structures (asterisks) are also commonly seen in the vacuoles. Go: Golgi apparatus; SER: smooth endoplasmic reticulum. Bar: 200 nm.

associated with the ER, and it was sometimes found that RER was in contact with the vacuoles (Figs. 9, 10). In particular, some vacuoles located near the cell boundary appeared to be in the process of exocytosis since the vacuoles filled with a granular material were often seen near the cytoplasmic membrane of the TCE in close



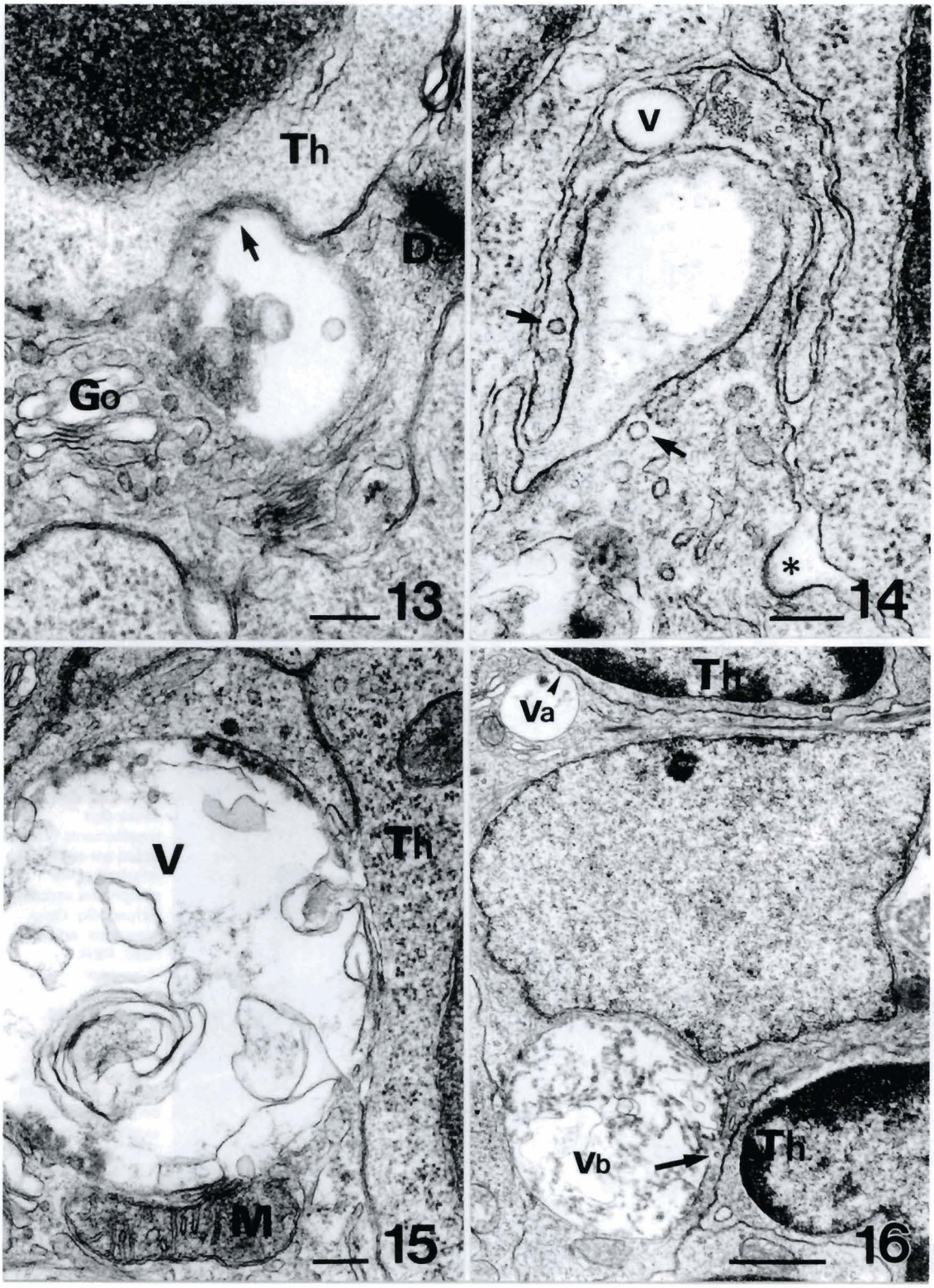
Figs. 8-10. The cortical epithelial cells three days after CY treatment.
Fig. 8. Dilatation of the endoplasmic reticulum (ER), especially of the smooth ER (s) and transitional ER (arrow heads) is noticeable. The cell boundary has short, membrane-bound, irregularly-arrayed cytoplasmic processes (stars) like microvilli. A lysosome-like structure is observed (arrow).
M: mitochondria; Ly: lymphocytes; V: vacuoles; asterisk: tonofilaments.
Bar: 1 μm.
Fig. 9. Some vacuoles show several areas of communication between them and the ER (arrows). Some vacuoles are filled with abundant electron-dense granular material (stars). Bar: 1 μm.
Fig. 10. Several vacuoles display different patterns of their content, including the vacuole containing abundant electron-dense granular material (star). Some vacuoles are fused and communicate with each other (arrow head). RER contacts a vacuole (arrow). Bar: 1 μm.





Figs. 11-12. The cortical epithelial cells and thymocytes one week after CY treatment.
Fig. 11. The cytoplasm contains numerous ribosomes and many mitochondria (M). There are also well-developed tonofilaments (To) which are sometimes attached at the margin of a vacuole (arrow). Go: Golgi apparatus; arrow head: RER; V: vacuole. Bar: 1 μm.
Fig. 12. A giant vacuole (V) containing a large amount of granular material appears to be in the process of exocytosis (arrow). The Golgi apparatus (Go) is very close to the vacuole (arrow head). Th: thymocytes; M: mitochondria. Bar: 1 μm.

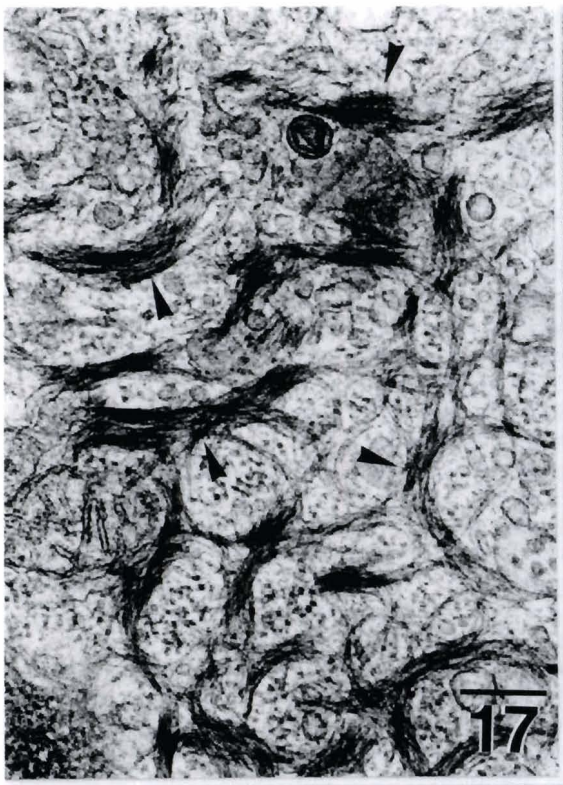
contact with the cell membrane of adjacent thymocytes, and sometimes the vacuoles seemed fused with the cell membrane of the TCE (Figs. 12-16). The increased vacuolization was first noticed as early as one day after CY treatment and reached the highest degree on the third day, but the extent of vacuolization gradually decreased

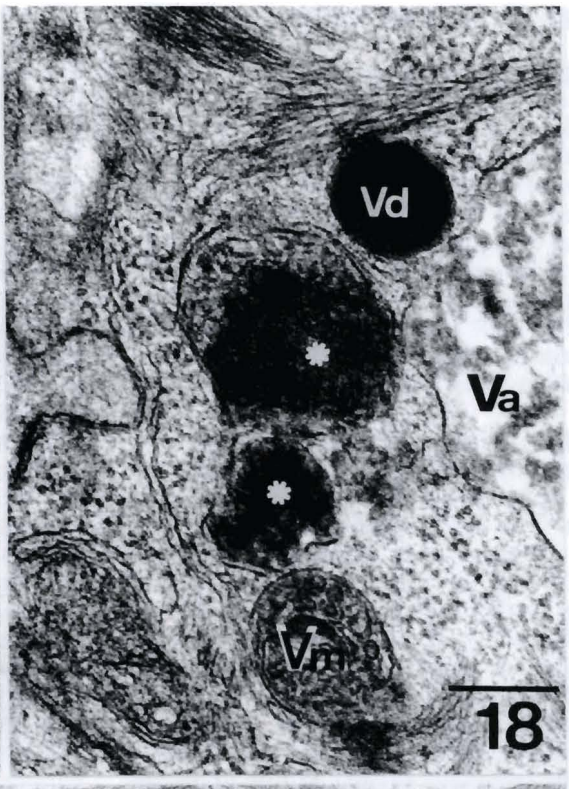


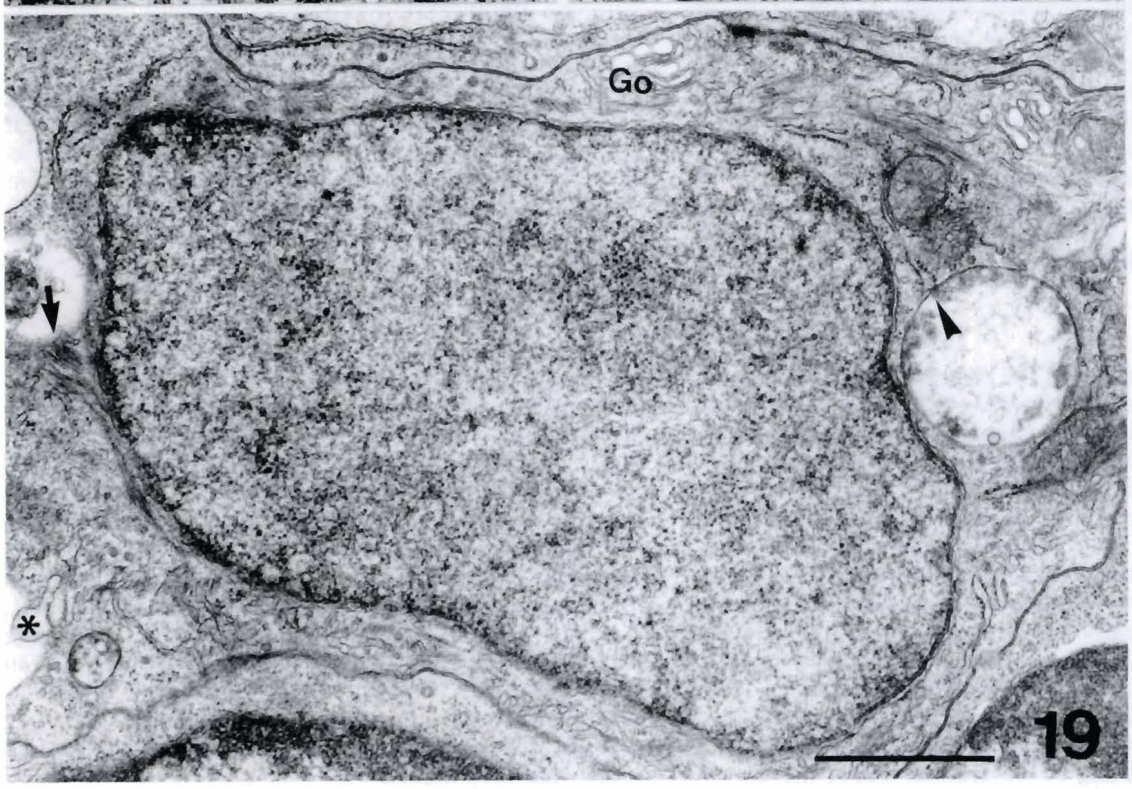
Figs. 13-16. The cortical epithelial cells and thymocytes one week after CY treatment. **Fig. 13.** A vacuole is in the process of exocytosis (arrow). The Golgi apparatus (Go) is in close proximity to the vacuole.
Th: thymocyte;
De: desmosome.
Bar: 200 nm.
Fig. 14. Some vesicles (arrows) and a vacuole (V) are located near the cell membrane and a vacuole shows the appearance of exocytosis (asterisk).
Bar: 200 nm.
Fig. 15. A vacuole (V), possibly in the process of exocytosis, seems to be fused with the cell membrane of a thymocyte (Th).
M: mitochondria.
Bar: 200 nm.
Fig. 16. Note a vacuole (Va) which is located in close proximity to the cell membrane of a thymocyte. Note also a vacuole (Vb), which seems to be in the process of secreting its granular content into the cell membrane of a thymocyte (arrow).
Th: thymocytes.
Bar: 1 μm.

thereafter.

There were remarkable changes of tonofilaments. The tonofilaments lost their original array and configuration, being scattered in the cytoplasm after the administration of CY (Figs. 3-12). Some of them were closely associated with certain cellular components







Figs. 17-18. The cortical epithelial cells one week after CY treatment.
Fig. 17. Note the prominent and well-developed tonofilaments (arrow heads). Bar: 200 nm.
Fig. 18. There are several types of vacuoles. Va: a vacuole showing the usual pattern of the vacuoles of normal TCE; Vm: a vacuole resembling a multivesicular body; asterisks: the vacuoles resembling phagolysosomes; Vd: a membrane-bound dense oval body resembling a lysosome or a secretory granule. Bar: 200 nm.

Fig. 19. The cortical epithelial cell two weeks after CY treatment. The vacuoles are closely associated with the tonofilaments (arrow), RER (arrow head) and SER (asterisk). Go: Golgi apparatus. Bar: 1 μm.

including the vacuoles and cell membrane, and were attached at several points on the surface of those cellular organelles (Fig. 6). The amount of the tonofilaments reached the maximum in the first week after CY administration (Figs. 17, 18), and decreased thereafter (Fig. 19).

The morphological alterations of the ER were also remarkable. One day after CY treatment, abundant smooth endoplasmic reticulum (SER), rarely seen in the normal TCE, filled the cytoplasm (Figs. 3-10). Three days after CY treatment, the cisternae of the ER were considerably dilated and vesiculated, especially in the SER and transitional endoplasmic reticulum (TER) (Fig. 8). The HTCE had plenty of vesicular and tubular forms of the ER cisternae extensively dispersed throughout the entire cytoplasm. The RER also increased in the HTCE but tended to be short in length (Figs. 3-12). Two weeks after CY administration, these phenomena declined (Fig. 19).

Three days after CY administration, the mitochondria seemed to increase to some extent in number and size and they contained a dense granular matrix (Figs. 8, 11, 12). The Golgi apparatus was well developed, and increased in number after the administration of CY (Fig. 3). There were many vesicles including the Golgi vesicles (Figs. 3, 11). In addition, there were a few lysosome-like structures and phagolysosome-like structures (Figs. 8, 18). Occasionally, multivesicular bodies were also observed in the TCE after the administration of CY (Fig. 18). The cytoplasm became densely granulated due to the increased number of ribosomes and the increased amount of fine granular material (Fig. 10). Numerous short, cylindrical and membrane-bound cytoplasmic processes like microvilli were often found at the cell surface of the HTCE, particularly at three days after CY administration (Fig. 8). These processes were arranged irregularly (Fig. 8).

In general, most of the above features began to appear from the first day and reached the maximum on the third day, but one week after CY administration, these phenomena declined.

Discussion

The salient features of the morphological alterations in the TCE, after administration of CY, were the increase in number and size of the vacuoles, and the increase in amount of their content, especially of the granular content. It is difficult, at present, to know the exact nature and role of these vacuoles. However, it is considered that at least some of them may be storage vacuoles of secretory products, although they do not have the ordinary morphology of secretory granules. It has been demonstrated that the thymic epithelial cells secrete biologically active factors that regulate the proliferation, maturation and differentiation of thymocytes, including thymic hormones such as thymulin, thymopoietin and thymosins (Bach et al., 1973; Dardenne et al., 1974; Trainin, 1974; Monier et al., 1980; Jambon et al., 1981; Savino et al., 1982; Berrih et al., 1984; Savino and Dardenne, 1984; Haynes, 1990; Kendall, 1991), multiple types of cytokines such as IL-1, IL-6, IL-7, TGF and leukemia inhibitory factor (Haynes, 1990; Le et al., 1991; Chen et al., 1992), chemotactic factors for bone marrow-derived thymocyte progenitors (Champion et al., 1986), and granulocyte and macrophage colony stimulating factors (Le et al., 1988; Nanno et al., 1991). Strong evidence has been accumulated that the vacuole in the normal thymic epithelial cell represents the site where thymic hormones are stored and where they mature (Savino and Dardenne, 1986; Nabarra and Andrianarison, 1987a). Especially, it has been demonstrated by immunoelectron microscopy that the vacuoles containing electron-dense granules are preferentially labeled with an anti-thymulin antibody, and it has been suggested that these vacuoles are a form of secretory granule (Schmitt et al., 1980, 1982; Auger et al., 1982). The presence of zinc in these vacuoles also supports this idea because zinc is an integral component of thymulin (Bach et al., 1973; Dardenne et al., 1982; Nabarra et al., 1984). It was reported that the dense granular material observed in the vacuoles of the normal thymus is the final form of thymulin binding to zinc, and that the immature form is electron-lucent (Nabarra and Andrianarison, 1987a). The fact that those vacuoles increased in size during the blockage of secretion by anti-thymulin antibodies, by colchicine or by monensin is also in favor of this hypothesis (Savino and Dardenne, 1986; Nabarra and Andrianarison, 1987a). There is some controversy about the secretory process of the peptidic hormone produced in thymic epithelial cells. It has been suggested that the intracellular pathways of thymulin secretion follow a classical peptide secretion model (Savino and Dardenne, 1986). On the other hand, Nabarra and Andrianarison (1987a) suggested that TCE had a peculiar secretory mechanism. They pointed out that the immature form of secretory material might pass to the transitional element of the ER, and this secretory product would then pass directly from the transitional element to the vacuoles (Nabarra and Andrianarison, 1987a). Although the morphological features of the TCE after CY treatment in the present study suggested their active synthetic and secretory activity, the overall results of the present study made it difficult to clarify this controversial secretory mechanism because on the one hand, it was partly in agreement with the classical peptide secretion model in that the Golgi apparatus was not only well developed and had increased in number but was also closely associated with the vacuoles in some regions. On the other hand, it was also partly in agreement with the Nabarra and Andrianarison's model in that the vacuoles were closely associated with the ER, especially with the transitional ER, and there were the communication sites between the ER and the vacuole in some regions. Whichever of the two models is correct, the vacuoles seem to be storage vacuoles of secretory products since the present study provides some evidence suggesting they are secreted by a process of exocytosis;

i.e., the vacuoles filled with a granular material were often seen next to the cell membrane of the TCE in close contact with the thymocyte membrane, and sometimes the vacuoles seemed fused with the cell membrane of the TCE.

Moreover, there were several additional important findings supporting the hypothesis that the vacuoles may be storage vacuoles of secretory products. (1) There was too small a number of lysosomes in comparison with the number of vacuoles. If those vacuoles had been phagosomes, there should have been more lysosomes, (2) There were abundant, well developed components for the synthesis, intracellular transport and secretion of protein, such as the Golgi apparatus, ER, ribosomes, vesicles and mitochondria. (3) The tonofilaments increased in amount, and they were closely associated with certain cellular components including the vacuoles and cell membranes. Although the exact role of tonofilaments in the thymic epithelial cells is not well understood, our results and those of Kato et al. (1981) suggest that the tonofilaments may be closely associated with intracellular transport of secretory products and of cell organelles and that they are possibly associated with the exocytosis of the vacuoles in the thymic epithelial cells.

Thus, TCE may play a crucial role in the regeneration of thymocytes in the thymic cortex after CY-induced destruction of thymocytes by secreting certain humoral factors. It is believed that the origin of the repopulating thymocytes after a depletion of thymocytes in the thymus is bone marrow, and these bone marrow-derived precursors are continuously recruited from the circulation to maintain the thymocyte population (Turk and Poulter, 1972; Basch and Kadish, 1977; Boersma et al., 1981; Lepault and Weissman, 1981; Ezine et al., 1984). These thymocyte precursors are considered to be influenced by thymic epithelial cells to become mature thymocytes.

There are other possible interpretations on the nature and role of the vacuoles which are observed to increase in number and size after administration of CY. These vacuoles may be associated with the cytotoxic effect of CY or may be the autophagic vacuoles of the TCE affected by CY, by which damaged cellular components are removed from the cytoplasm. Sobhon et al. (1977) observed degenerative bodies in the crypt epithelial cells of the small intestine in CY-treated mice, and they suggested that the degenerative bodies occurred as a result of damage to epithelial cells. Stella et al. (1990) found microvacuolization of some urothelial cells in patients who were treated with CY. Hopkins et al. (1982) observed myocytic cytoplasmic vacuolization in the inbred ACI rat after CY treatment, although the dose of CY was much higher than in the present study. Shirota and Tavassoli (1991) reported that a salient feature of cellular injury, caused by CY, was membrane instability, and they supposed that the subsequent membranous sloughing led to the generation of membrane structures or myelin figures within the vacuoles. In agreement with that study, we could also observe membrane structures and myelin figures within the vacuoles. In addition, Shirota and Tavassoli (1991) reported that membrane instability was also manifested by a dilatation of the cisternae of the ER, which was observed in the present study. They suggested that the injury by CY could be due to the alterations of lipid or protein components of the membrane, by which lipid peroxidation could occur, leading to increased fluidity of membranes and consequently increased membrane permeability (Shirota and Tavassoli, 1991). Therefore, it is plausible that at least some alterations of the TCE after CY administration, including the increase in the number of cytoplasmic vacuoles, especially those containing membranous structures, might be due to damage by the cytotoxic effects of CY. The lysosomes present near the vacuoles may support this idea, although they were only few in number.

The vacuoles might be the phagocytic vacuoles which engulf adjacent dead thymocytes and thereby participate in the final breakdown and resorption of the destroyed cells. Although Pinkel (1968) reported that many of the thymic epithelial cells showed an evidence of phagocytic activity in that what appeared to be the parts of lymphocyte nuclei were present within their vacuoles, other investigators have suggested that the thymic epithelial cells are not capable of phagocytic action (Clark, 1963; Fawcett, 1986). The evidence of phagocytosis, such as the presence of phagocytized material in the process of degradation, could not be found in the present study. In addition, the number of lysosomes was too small.

The vacuoles may be the transformed mitochondria or transformed ER. In agreement with the observation of Nabarra and Andrianarison (1987a), membrane fragments which resembled degenerating mitochondria were found in these vacuoles, and there were the images which could represent an intermediary stage between normal mitochondria and certain types of vacuoles. There were also structures that could represent an intermediate stage between normal ER and certain types of vacuoles, especially in the early stage of this experiment.

Lastly, the vacuoles may be composed of a mixed form of vacuoles produced by the combined effects of several causes including those described above. Some of these vacuoles, especially those which contain granular material, may be storage vacuoles containing secretory products. Other vacuoles, especially those which contain membranous structures, may be formed by the cytotoxic effects of CY, or they may be the autophagic vacuoles containing parts of damaged cellular components.

However, it is too early to draw a final conclusion, and additional experiments are required. If the nature and origin of the content of the vacuoles are elucidated, it will be also possible to determine whether the HTCE represent a hyperfunctional state of TCE or a damaged state of TCE due to the cytotoxicity of CY.

Evidence obtained in the present study suggests that

the morphological alterations of TCE after CY administration may occur due to increased cellular activities. The TCE, after CY treatment, exhibited hypertrophic changes resulting in an increase in the size of the cytoplasm and the presence of well-developed and increased numbers of the Golgi apparatus, ER, ribosomes, vesicles, vacuoles and tonofilaments. In addition, these HTCE showed numerous cytoplasmic processes like microvilli on the cell surface. This finding suggests that the HTCE can increase the contact with the thymocytes through these cytoplasmic processes promoting the intercellular communication between the HTCE and thymocytes. Since CY has toxic effects mainly on rapidly proliferating cells, it is believed that the CY-induced alterations of TCE are not entirely attributable to the cytotoxic effects of CY because TCE are not rapidly proliferating cells. Chin and Hudson (1974) reported that the murine peritoneal macrophage following CY administration tended to be stimulated instead of being damaged. The features of the HTCE in the present study resemble the hypertrophic thymic epithelial cells of a study of splenectomized chickens showing various signs of secretory activity with large and dense cytoplasm containing abundant RER and vesicular elements, and a large number of mitochondria and ribosomes in the thymus (Rusu et al., 1981). These authors strongly suggested that the hypertrophic epithelial cells have an intensive metabolic activity and that they synthesize protein. Therefore, these facts might suggest that the HTCE, during the recovery of thymic cortical thymocytes after being destroyed by CY, may have a considerable influence on the process of proliferation, maturation and differentiation of the thymocyte precursors.

In conclusion, it seems that the morphological features of the TCE after CY treatment result from hyperfunctional activities of the TCE as well as from some damage by CY. It is considered that the TCE after CY treatment may play a role in the regeneration of thymocytes probably through the secretion of some humoral factors, while the cytotoxic effect of CY on TCE may also play a role in the immunosuppressive action of CY. Further studies may provide more insights into these facts.

References

Ahmed A.R. and Hombal S.M. (1984). Cyclophosphamide (Cytoxan): a review on relevant pharmacology and clinical uses. J. Am. Acad. Dermatol. 11, 1115-1126.

Anton E. (1987). Delayed toxicity of cyclophosphamide in normal mice. Br. J. Exp. Pathol. 68, 237-249.

Auger C., Monier J.C. and Dardenne M. (1982). Identification of FTS on thymus ultrathin sections using monoclonal antibodies. Immunol. Lett. 5, 213-216.

Bach J.F., Dardenne M., Pleau J.M. and Bach M.A. (1973). Isolation, biochemical characteristics, and biological activity of a circulating thymic hormone in the mouse and in the human. Ann. N.Y. Acad. Sci. 249, 186-210.

Basch R.S. and Kadish J.L. (1977). Hematopoietic thymocyte precursors: II. Properties of precursors. J. Exp. Med. 145, 405-419.

Berrih S., Savino W., Azoulay M., Dardenne M. and Bach J.F. (1984). Production of anti-thymulin (FTS) monoclonal antibodies by immunization agaisnt human thymic epithelial cells. J. Histochem. Cytochem. 32, 432-438.

Boersma W., Betel I., Daculsi R. and Van Der Wesen G. (1981). Post-irradiation thymocyte regeneration after bone marrow transplantation. I. Regeneration and quantification of thymocyte progenitor cells in the bone narrow. Cell Tissue Kinet. 14, 179-196.

Champion S., Imhof B.A., Savagner P. and Thiery J.P. (1986). The embryonic thymus produces chemotactic peptides involved in the homing of hemopoietic precursors. Cell 44, 781-790.

Chen W.F., Fan W., Cao L.X. and Zhang P.X. (1992). Multiple types of cytokines constitutively produced by an established murine thymic epithelial cell lines. Eur. Cytokine Netw. 3, 43-52.

Chin K.N. and Hudson G. (1974). Ultrastructural changes in murine peritoneal cells following cyclophosphamide administration. Br. J. Pathol. 55, 554-561.

Clark S.L. Jr. (1963). The thymus in mice of strain 129/J, studied with the electron microscope. Am. J. Anat. 112, 1-33.

Dardenne M., Papiernik M., Bach J.F. and Stutman O. (1974). Studies on thymus products. III. Epithelial origin of the serum thymic factor. Immunology 27, 299-304.

Dardenne M., Pleau J.M., Nabarra B., Lefrancier P., Derrien M., Choay J. and Bach J.F. (1982). Contribution of zinc and other metals to the biological activity of the serum thymic factor. Proc. Natl. Acad. Sci. USA 79, 5370-5373.

Ezine S., Weissman I.L. and Rouse R.V. (1984). Bone marrow cells give rise to distinct cell clones with the thymus. Nature 309, 629-631.

Fawcett D.W. (1986). Bloom and Fawcett: a textbook of histology. 11th ed. WB Saunders. Philadelphia. pp 436-445.

Gerswin M.E., Goetzl E.J. and Steinberg A.D. (1974). Cyclophosphamide: use in practice. Ann. Intern. Med. 80, 531-540.

Gorgollon P. and Ottone-Anaya M. (1978). FIne structure of canine thymus. Acta. Anat. 100, 136-152.

Haynes B.F. (1984). The human thymic microenvironment. Adv. Immunol. 36, 87-142.

Haynes B.F. (1990). Human thymic epithelium and T cell development: current issues and future directions. Thymus 16, 143-157.

Hopkins H.A., Betsill W.L., Hobson A.S. and Looney W.B. (1982). Cyclophosphamide-induced cardiomyopathy in the rat. Cancer. Treat. Rep. 66, 1521-1527.

Huber C., Niederwiser D., Schonitzer D., Gratwohl A., Buckner D. and Margreiter R. (1984). Liver transplantation followed high-dose cyclophosphamide, total-body irradiation, and autologous bone marrow transplantation for treament of metastatic breast cancer. Transplantation 37, 311-312.

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.

Kato K., Ikeyama S., Takaoki M., Shino A., Takeuchi M. and Kakinuma A. (1981). Epithelial cell components immunoreact with anti-serum thymic factor (FTS) antibodies: possible association with intermediate-sized filaments. Cell 24, 885-895.

Kendall M.D. (1991). Functional anatomy of the thymic microenvironment. J. Anat. 177, 1-29.

Kovarsky J. (1983). Clinical pharmacology and toxicology of cyclo-

phosphamide: emphasis on use in rheumatic diseases. Semin. Arthritis. Rheum. 12, 359-372.

Le P.T., Kurtzberg J., Brandt S.J., Niedel J.E., Haynes B.F. and Singer K.H. (1988). Human thymic epithelial cells produce granulocyte and macrophage colony stimulating factors. J. Immunol. 141, 1211-1217.

Le P.T., Lazorick S., Whichard L.P., Haynes B.F. and Singer K.H. (1991). Regulation of cytokine production in the human thymus: epidermal growht factor and transforming growth factor alpha regulate mRNA levels of IL-1 alpha, IL-1 beta, and IL-6 in human thymic epithelial cells at a post-transcriptional level. J. Exp. Med. 174, 1147-1157.

Lepault F. and Weissman I.L. (1981). An in vivo assay for thymus-homing bone marrow cells. Nature 293, 151-154.

Loc N.K., Olah I., Takacs L., Kittner Z. and Toro I. (1981). Effect of cyclophosphamide on the rat thymus. Acta Morphol. Acad. Sci. Hung. 29, 319-333.

Mandel T. (1968). Ultrastructure of epithelial cells in the cortex of guinea pig thymus. Z. Zellforsch. 92, 159-168.

Monier J.C., Dardenne M., Pleau M. and Schmitt D. (1980). Characterization of facteur thymique sérique (FTS) in the thymus. I. Fixation of anti-FTS antibodies on thymic reticulo-epithelial cells. Clin. Exp. Immunol. 42, 470-476.

Nabarra B. and Andrianarison I. (1987a). Pattern of secretion in thymic epithelial cells: ultrastructural studies of the effect of blockage at various levels. Cell Tissue Res. 249, 171-178.

Nabarra B. and Andrianarison I. (1987b). Ultrastructural studies of thymic reticulum. I. Epithelial components. Thymus 9, 95-121.

Nabarra B., Halpern S., Kaiserlian D. and Dardenne M. (1984). Localization of zinc in the thymic reticulum of mice by electron-probe microanalysis. Cell Tissue Res. 238, 209-212.

Nanno M., Takahashi T., Sakata T., Horikawa T., Sako T., Suzuki R., Itoh T. and Kumagai K. (1991). Production of granulocyte-macrophage colony-stimulatign factor or GM-CSF-like growth factor by rat thymic epithelial cell line. J. Leukoc. Biol. 50, 561-567.

Ozer H., Cowens J.W., Colvin M., Nussbaum-Blumenson A. and Sheedy D. (1982). In vitro effects of 4-hydroperoxy-cyclophosphamide on human immunoregulatory T subset function. I. Selective effects on lymphocyte function in T-B cell collaboration. J. Exp. Med. 155, 276-290.

Pinkel D. (1968). Ultrastructure of human fetal thymus. Am. J. Dis. Child 115, 222-238.

Rouse R.V., Van Ewijk W., Jones P.P. and Weissman I.L. (1979). Expression of MHC antigens by mouse thymic dendritic cells. J. Immunol. 122, 2508-2515.

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.

Rusu V.M., Constantin C. and Duca C. (1981). Ultrastructural changes of the hypertrophic epithelial cells in the thymus of splenectomized chickens. Thymus 3, 213-222.

Savino W. and Dardenne M. (1984). Thymic hormone-containing cells. VI. Immunohistologic evidence for the simultaneous presence of thymulin, thymopoietin and thymosin 1 in normal and pathological human thymuses. Eur. J. Immunol. 14, 987-991.

Savino W. and Dardenne M. (1986). Thymic hormone-containing cells. VIII. Effects of colchicine, cytochalasin B, and Monensin on secretion of thymulin by cultured human thymic epithelial cells. J. Histochem. Cytochem. 34, 1719-1723.

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. 156, 628-630.

Scheper R.J., Baak J.P., Hiisivara S. and Veldhuizen R.W. (1975). RRBC and EAC rosette formation after DNCB contact sensitization and cyclophosphamide treatment of guinea pigs. Immunology 29, 909-919.

Schmitt D., Monier J.C., Dardenne M., Pleau J.M., Deschaux P. and Bach J.F. (1980). Cytoplasmic localization of FTS in thymic epithelial cells. An immunoelectron microscopical study. Thymus 2, 177-181.

Schmitt D., Monier J.C., Dardenne M., Pleau J.M. and Bach J.F. (1982). Location of FTS (facteur thymique sérique) in the thymus of normal and auto-immune mice. Thymus 4, 221-231.

Shirota T. and Tavassoli M. (1991). Cyclophosphamide-induced alterations of bone marrow endothelium: implication in homing of marrow cells after transplantation. Exp. Hematol. 19, 369-373.

Sharma J.M. and Lee L.F. (1977). Suppressive effect of cyclophosphamide on the T-cell system in chickens. Infect. Immun. 17, 227-230.

Sobhon P., Wanichanon C. and Sretarugsa P. (1977). Morphological changes induced by cyclophosphamide in crypt epithelium of the small intestine in mice: light and electron microscopic studies. Am. J. Anat. 149, 563-584.

Starzl T.E., Groth C.G , Putnam C.W., Corman J., Halgrimson C.G., Penn I., Husberg B., Gustafsson A., Cascardo S., Geis P. and Iwatsuki S. (1973). Cyclophosphamide for clinical renal and hepatic transplantation. Transplant. Proc. 5, 511-516.

Stella F., Battistelli S., Marcheggiani F., De Santis M., Giardinini C., Baronciani D., Manenti F., Mattioli S. and Troccoli R. (1990). Urothelial cell changes due to busulfan and cyclophosphamide treatment in bone marrow transplantation. Acta Cytol. 34, 885-890.

Trainin N. (1974). Thymic hormones and the immune responses. Physiol. Rev. 54, 272-315.

Turk J.L. and Poulter L.W. (1972). Selective depletion of lymphoid tissue by cyclophosphamide. Clin. Exp. Immunol. 10, 285-296.

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 Haelst U.J.G. (1967). Light and electron microscopic study of the normal and pathological thymus of the rat. I. The normal thymus. Z. Zellforsch. 77, 534-553.

Zhu L.P., Cupps T.R., Whalen G. and Fauci A.S. (1987). Selective effects of cyclophosphamide therapy on activation, proliferation, and differentiation of human B cells. J. Clin. Invest. 79, 1082-1090.

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