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EARLY ULTRASTRUCTURAL CHANGES OF THYMOCYTES IN T-2 TOXICATED MICE

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Abstract: Subcellular changes of mouse thymocytes after T-2 intoxication were observed for up to 48 hours after injection (HAI). The following changes were observed mainly in cortical lymphocytes at 8 and 16 HAI: (1) nuclear projections, (2) nuclear macrocleft, and (3) characteristic features of apoptosis. Conclusively, it is suggested that apoptosis is partially involved in the early development of thymic lesions by T-2 toxin. (J Toxicol Pathol 6: 109～112, 1993)

Key words: Mice, T-2 toxin, Thymus, Ultrastructure

T-2 toxin, a fungal metabolite produced by species of the genus Fusarium, is well known to induce cytotoxicosis in lymphoid and hematopoietic organs in many animal species[1]. Previous studies on murine lymphoid organs[2-5] treated with T-2 toxin have been carried out mainly to describe gross and light microscopic progress of the lesions. Although Glavits reported the ultrastructure of fully developed lesions of lymphoid tissues in mice[2], subcellular changes in the early stage are still obscure. The purpose of this study was to clarify the early ultrastructural changes of thymus in mice intoxicated with T-2 toxin.

Forty 5-week-old ICR : CD-1 male mice weighing about 30 g (Charles River Japan, Kanagawa) were used in this study. Animals were maintained under controlled conditions (temperature 22±2 °C; humidity 55±5%) and fed MF pellets (Oriental Yeast Co. Ltd., Tokyo) and water *ad libitum* throughout the exprimental period. After overnight fasting, 20 mice were injected orally with 10 mg/kg b.w. of T-2 toxin (Lot No. 117 F4j078, Sigma) in 20% ethanol in phosphate buffered saline. The remaining 20 mice served as control. Five animals of each group were sacrificed by heart puncture under ether anesthesia at 8, 16, 24, and 48 hours after injection (HAI), respectively. Thymus was fixed in 10% neutral buffered formalin for light microscope and 4 μm paraffin sections were stained with hematoxylin and eosin. Small pieces of thymus were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), postfixed in 1.0% osmium tetroxide in the same buffer, and processed for electron microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and observed under a JEM-1200EX (JEOL Co. Ltd., Tokyo).

Light microscopically, solitary or focal necrosis of cortical lymphocytes and phagocytosis of necrotic lymphocytes by cortical macrophages were detected at 8 HAI. At 16 HAI, necrotic lymphocytes were observed diffusely in the whole cortex and multifocally in the medulla. At 24 and 48 HAI, depletion of lymphocytes was marked and the demarcation of cortex and medulla became indistinct. In addition, proliferation of epithelial reticular cells was also observed in the medulla, especially at 48 HAI.

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Ultrastructurally, in the fully developed lesions from 16 HAI on, degenerated cells had homogeneously condensed and highly electron-dense nuclei, and cellular and nuclear membranes exhibited lytic changes (Fig. 1) as previously described by Glavits[2]. The earlier ultrastructural changes were observed mainly in cortical lymphocytes at 8 and 16 HAI as follows.

Nuclear projections presenting as pedunculated nodules were observed in the cells, of which

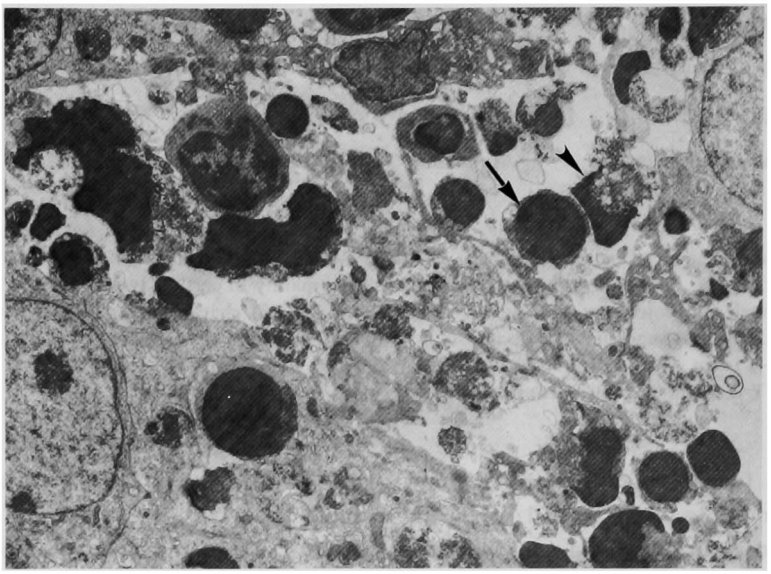

Fig. 1. Cortex at 24 HAI. Almost all lymphocytes exhibit strong degenerative changes. Pyknosis (arrow) and karyorrhexis (arrowhead). Cellular and nuclear membranes are indistinct. ×3,600.

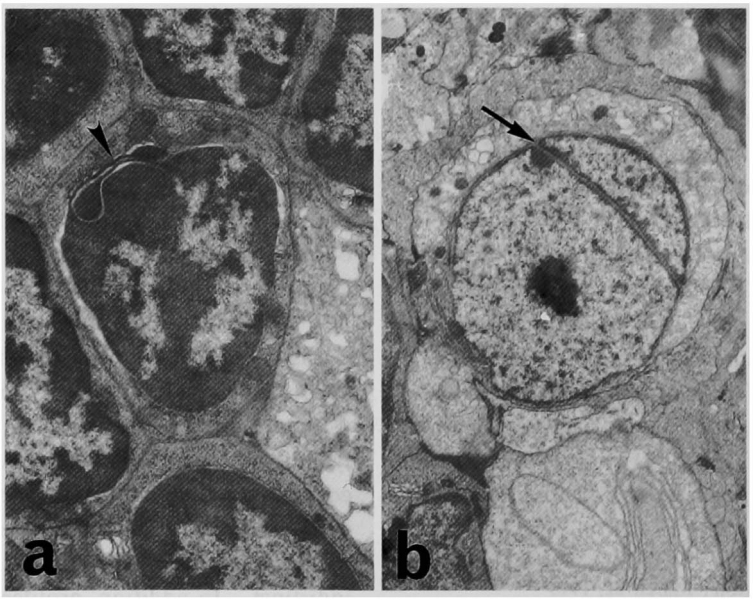

Fig. 2. a. Cortex at 8 HAI. Pedunculated nodules (arrowhead) from nuclear surface form a nuclear pocket. ×9,000. b. Cortex at 16 HAI. Macrocleft (arrow) completely cleaves a nucleus into two separate portions. ×4,400.

cytoplasm and organella looked normal. They sometimes formed so-called "nuclear pockets" that contained nuclear or cytoplasmic materials and was demarcated by single or several chromatin bands (Fig. 2a). These structures are seen in a variety of lymphoma or leukemia[6] and considered to be the morphologic expression of chromatin dislocation resulting from chromosomal abnormalities[7]. Further studies on human acute leukemic cells have suggested a correlation between the occurrence of aneuploidy and a high frequency of nuclear pockets[8]. Nuclear cleavage, i.e. so-called "macrocleft", was also found. It completely cleaved a nucleus into two separate portions in a few cases (Fig. 2b). This feature is also reported to be seen in leukemia[6]. Although the real meaning of the above-mentioned nuclear changes is not clear, they seem to be an early expression of the nuclear damage by T-2 toxin.

Fragmentation of nucleus and crescent-formation of condensed chromatin were observed in cortical lymphocytes, and some of these cells formed "apoptotic bodies" which were roughly spherical or ovoid cytoplasmic fragments containing remnants of cytoplasm or pyknotic nuclei (Figs. 3 and 4b). They are consistent with the typical ultrastructural characteristics during apoptosis or physiological cell death[9,10]. It was also reported that apoptosis was induced in macrophages and T lymphoblasts by mycotoxin, gliotoxin and sporidesmin *in vitro*[11]. In the early stage of T-2 toxin intoxication, it is proposed by this observation that apoptosis might be involved in parallel with or prior to "accidental cell death" or necrosis.

At 16 and 24 HAI, in addition to the above-mentioned early nuclear changes, interesting features were also observed in the cytoplasm of reticulocytes mainly in the medulla. Namely, reticulocytes had aggregated intracytoplamic cysts containing a few microvilli (Fig. 4). Various terms such as intracellular or intracytoplamic "canaliculi", "lumina", "alveolus", etc. have been used to describe such intracytoplasmic cysts as mentioned above. These structures were reported to be seen in normal and diseased (including neoplastic ones) epithelial cells[12]. They are also frequently seen near the thymic corpuscles of the guinea pigs and mice and suggested to be one of the manifestations of cell degeneration[13]. Although Glavits reported reticulocyte degeneration was developed after the severe depletion of

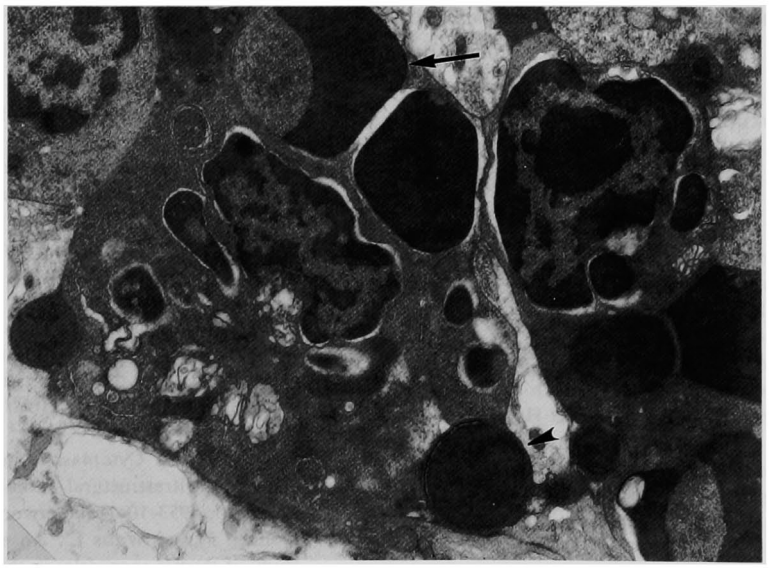

Fig. 3. Cortex at 16 HAI. Fragmentation of nucleus and crescent-shaped formation of condensed chromatin (arrow) are seen. Apoptotic body (arrowhead) contains remnants of pyknotic nucleus. ×8,000.

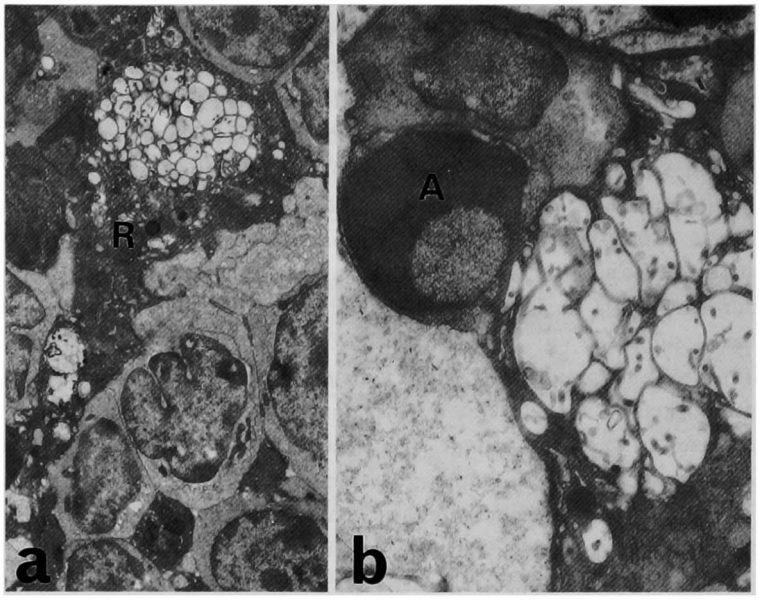

Fig. 4. a. Medulla at 16 HAI. A reticulocyte (R) has aggregated cysts in cytoplasm. ×3,000. b. Cortex at 16 HAI. High magnification of another reticulocyte adjacent to apoptotic body (A). Intracytoplasmic cysts have a few microvilli. ×10,400.

lymphocytes[2], alteration of reticulocytes and lymphocytes could be detected simultaneously in this study. In the more severe lesions, reticulocytes in the cortex and medulla contained a large amount of bundles or sheaves of intracytoplasmic tonofilament.

In conclusion, this study clarified the interesting early ultrastructural changes of thymocytes and suggested that apoptosis might be partially involved in the early development of thymic lesions by T-2 toxin.

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