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Ultrastructural Studies of Involuting Bursa and Thymus of Giriraja Birds (*Gallus domesticus*)

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Abstract: The ultra structural changes of involuting cells of bursa of fabricius and thymus could be classified as nuclear and cytoplasmic changes. Major cytoplasmic changes include arrangement of cytoskeletal filaments in parallel arrays, cytoplasmic membrane blebbing and dilatation of endoplasmic reticulum. Nuclear changes included clumping of chromatin, elongation and appearance of convolutions of the nuclear membrane with karyorrhexis, karyolysis and nucleolar disintegration. There is consequent cell volume reduction, detachment of cell from surroundings, formation of several membrane bound apoptotic bodies and its ingestion by neighboring cells.

Key words: Ultrastructure, involution, bursa, thymus

INTRODUCTION

The bursa of Fabricius and thymus are primary lymphoid organs in birds where B and T cells differentiate and participate in humoral and cell mediated immune responses respectively. Physiological involution of lymphoid organs has been extensively studied in morphological, histological and other aspects. However previous reports on the ultrastructural features of lymphoid cells during involution are scanty. Apoptosis is a form of programmed cell death serving physiologic and homeostatic functions often seen in the lymphoid organs where it is triggered by withdrawal of growth factors (Tizzard, 2000). It has been reported during thymic involution (Robb and Schneide, 1997), however ultrastructural studies of apoptosis in the involuting lymphoid organs of birds are scant. In spite of all the recent advances, apoptosis recognition is still commonly based on morphological criteria (Negoescu, 1997).

Hence an attempt has been made to study the ultrastructure of these organs at the involution stage and arrive at the normal involuntary picture of lymphocytes. The most popular strain of chicken in Karnataka "Giriraja" was used for the study. Girijaja birds, a domestic dual-purpose breed of chicken was developed at University of Agricultural Sciences, Bangalore, by crossing White Plymouth Rock, Red Cornish and New Hampshire.

MATERIALS AND METHODS

Giriraja birds were procured from the poultry farm of University of Agricultural Sciences, Bangalore. A total of 72 birds were reared from day old to 24 weeks. The bursa of Fabricius and thymus were collected from six birds each every alternate week. Only those age groups of birds showing pronounced involuntary changes in light microscopy were selected for ultrastructural studies. Ultrastructural studies were undertaken to highlight the involuntary changes. Hence, the tissues were fixed using glutaraldehyde in phosphate buffer. The standard procedure for processing tissues used for transmission electron microscopy was followed according to Luft (1961). Ultrathin sections on copper grids stained by uranyl acetate were examined under electron microscope model JEOL 100S. The electron micrographs of the areas of interest were observed and analyzed.

RESULTS AND DISCUSSION

Based on studies in light microscopy, tissues from bursa (12 and 14 weeks) and thymus (22 and 24 weeks) were selected for ultrastructural studies. The bursa of Fabricius at the stage of involution composed of normal lymphocytes with a narrow rim of cytoplasm, heavily clumped chromatin, randomly located mitochondria and other organelles. Lymphoblasts with substantial amount of cytoplasm, less clumping of chromatin and a prominent nucleolus were also seen.

Apart from the lymphocytes and lymphoblasts, certain cells with peculiar morphological features were observed. These features were classified as cytoplasmic changes and nuclear changes.

The cytoplasmic membrane was showing blebbing (Fig. 1) at many sites. The cytoplasm of such cells was comparatively condensed with vacuoles and cytoskeletal filaments in parallel arrays. The endoplasmic reticulum

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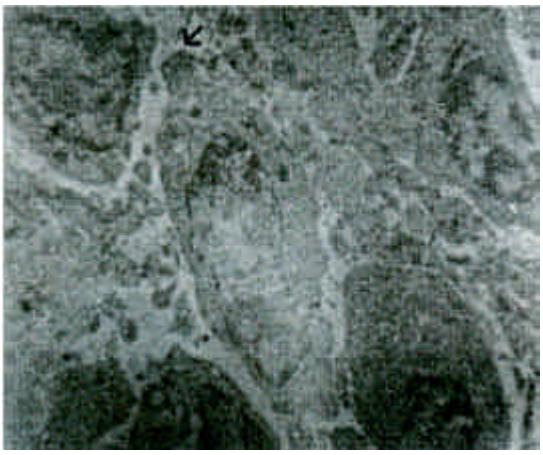

Fig. 1: Cytoplasmic blebbing

Fig. 2: Elongated nucleus

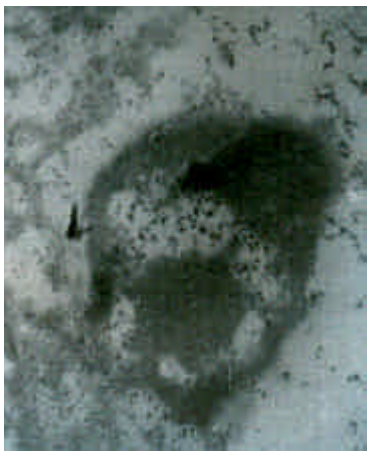

Fig. 3: Heterochromatin clumping

of such cells was dilated and free ribosomes were arranged in semicrystalline arrays.

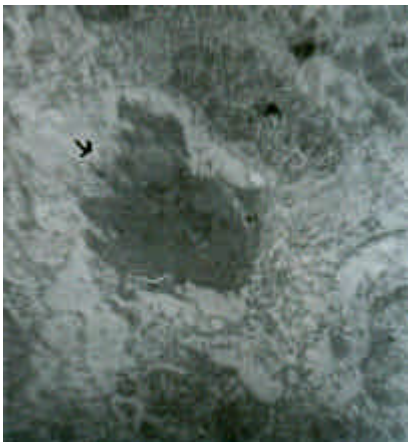

Fig. 4: Karyorrhexis

Fig. 5: Formation of membrane bound bodies

The nuclear membrane showed various modifications like pronounced elongation (Fig. 2) and heavy convolutions. Condensed and clumped chromatin (Fig. 3) was observed under the nuclear membrane. Karyorrhexis (Fig. 4) and karyolysis were noted along with nucleolar disintegration.

Together with this reduction in total cell volume and detachment of these cells from surroundings were seen. Fragmentation of such cells formed several membrane bound bodies (Fig. 5). Ingestion of such bodies by normal surrounding cells (Fig. 6) was also observed. Nicholas *et al.* (2009) explained that these may probably be apoptic bodies which are recognized by its viable neighbour cells as a target for phagocytosis.

Reticuloepithelial cells were seen as a star shaped cells having nucleus with clear nucleolus. Cytoplasm of these cells had small vacuoles, a number of

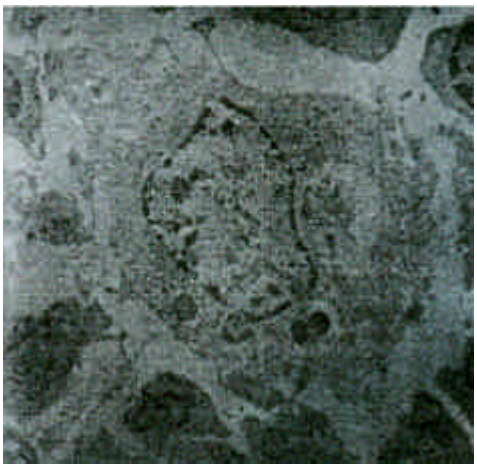

Fig. 6: Ingestion of apoptotic bodies by phagocytes

mitochondria, free ribosomes, a few vesicles of endoplasmic reticulum and microvilli. In addition, in the cytoplasm of the reticuloepithelial cells, filament bundles were observed and represent immature intermediate filaments of cytokeratin as reported by Sanchez *et al.* (1996). Apoptotic features were less pronounced in these cells.

In the thymus, in addition to others, cells with scanty cytoplasm were identified as round forms of myoid cells. Numerous micro filaments in radial arrays were appreciable in these cells slightly resembling the cytoplasm of muscle cells. These cells were found to increase in number during involution.

These peculiar features observed concur with the description of apoptotic cells by Machacaka and Compton (1993) and Wyllie and Duvall (1992). On the basis of ultrastructural studies it may be concluded that cells of the involuting bursa and thymus die by a process of apoptosis.

Although molecular techniques evolved to characterize cellular events, ultrastructural studies still has its own importance as a direct method of confirmation.

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REFERENCES

Luft, A., 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytolol., 9: 409.

Machacaka, A. and M. Compton, 1993. Analysis of thymic lymphoyte apoptosis using *in vitro* techniques. Dev. Comp. Immunol., 17: 263-276.

Negoescu, A., 1997. TUNEL: Improvement and evaluation of the method for *in situ* apoptotic cell identification. Biochemica, 2: 12-17.

Nicholas, J.W., D.J. Edward, A.P. Courtney and J.L. Roger, 2009. Apoptosis in the colonic crypt, colorectal adenomata and manipulation by chemoprevention. Cancer Epidemiol. Biomarkers, 18: 1680-1687.

Robb, M.W. and M.D. Schneide, 1997. Death by design. Circ. Res., 81: 137-144.

Sanchez, R., E. Ciriaco, A. Germana, G. Germana and J.A. Vega, 1996. Age-related changes in the medullary reticular epithelial cells of the pigeon bursa of Fabricius. Anat. Rec., 246: 473-480.

Tizzard, I.R., 2000. Veterinary Immunology-an introduction. 6th Edn., WB Saunders Company; Philadelphia, pp: 161-162.

Wyllie, A.H. and E. Duvall, 1992. Oxford Textbook of Pathology, Vol 1, Oxford University Press. Oxford, UK.