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HISTOLOGICAL AND ULTRA STRUCTURAL STUDIES OF THYMIC MYOID CELLS IN NANDANAM CHICKEN

T.A. Kannan[1], Geetha Ramesh[2], S. Ushakumari[3], Sabiha Hayath Basha[4]

[1]Professor, Centre for Stem Cell Research and Regenerative Medicine, Madras Veterinary College, Chennai, India; [2,3,4]Professor, Department of Veterinary Anatomy, Madras Veterinary College, Chennai- 600 007, India.

ABSTRACT

Light and transmission electron microscopic studies of thymic myoid cells in Nandanam Chicken was done in various age groups ranging from day-old to forty weeks. Thymus is the central lymphoid organ in fowl, provides a complex environment essential for T-cell development and maturation. The thymic gland in chicken was covered by a thin connective tissue capsule. The connective tissue septa divided the gland into lobules. Thymic parenchyma consisted of an outer darker cortex and inner pale medulla. Under light microscope, numerous large cells with eosinophilic cytoplasm were observed in the medulla and also in the cortico-medullary junction, the myoid cells. Number of myoid cells increased as age advances. Under electron microscope, the cytoplasm of the myoid cell showed myofibrils as in skeletal muscle fibre. Cytoplasm also contained few mitochondria, free ribosome and smooth endoplasmic reticulum. Small bundles of unmyelinated nerve fibres were observed in the thymic medulla.

Key Words: Histology, Ultrastructure, Thymus, Myoid cell, Chicken

INTRODUCTION

Thymus is the central lymphoid organ occurs in all vertebrate species, situated on either side of the neck, close to the jugular vein (King, 1983). Bone marrow-derived T-cell precursors undergo differentiation, maturation eventually leading to migration of positively selected thymocytes to the peripheral lymphoid organs such as the spleen (Savino and Dardenne, 2000 and Varga *et al.,* 2009) and GALT including the caecal tonsil and the lymph nodes (Ciriaco *et al.,* 2003; Gail Pearse, 2006 and Karen Staines *et al.,* 2013).

Thymus is unique among the lymphoid organs in being an epithelial organ and well known for its cellular organization (Gail Pearse, 2006). Thymic epithelium shows subpopulation heterogenicity in both capsule and thymic parenchyma that form the three-dimensional framework of the thymus (Mohammad *et al*., 2007). Among these heterogenous cell population, there is an unique and interesting myoid cells of the thymus.

Myoid cells are first described in Amphibians (Mayer, 1888) and they have been found to vary considerably due to cell turnover in the thymus in different vertebrates (Raviola and Raviola, 1967; Nakamura et al., 1986 and Geetha Ramesh and Vijayaragavan, 1997). In domestic fowl, they are identified as large cells in the thymic medulla and characterised by the presence of myofibrils in their cytoplasm similar to those found in skeletal muscle fibre (Gilmore and Bridges, 1974). Thymic myoid cells plays an important role of protecting thymocytes from apoptosis (Panse and Berrilh-Aknin, 2005).

Although, fine structure of myoid cells has been described by Frazier (1973) in fowl, little details are available of their presence in Nandanam chicken of different age groups. Hence, an attempt has been made to study the histological and ultrastructural details of this unique cell type of thymic parenchyma of Nandanam chicken. It is a dual purpose, colored variety with good disease resistance and most popular among poultry farmers due to its adaptability to backyard farming. Nandanam strain was developed in Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai.

MATERIALS AND METHODS

Over 36 specimens of thymic tissue were collected from six different age groups such as day-old, four, eight, twelve,

Corresponding Author:
T.A. Kannan, Professor, Centre for Stem Cell Research and Regenerative Medicine, Madras Veterinary College, Chennai, India,
E-mail: kanns2000@gmail.com, kannan@tanuvas.org.in

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twenty and forty weeks. Six birds were used in each age group. The thymus was removed immediately after high cervical dislocation and fixed for light and electron microscopy (Gilmore and Bridges, 1974).

For light microscopy, tissue pieces were fixed in 10% neutral buffered formalin, dehydrated in alcohol, cleared in xylene and embedded in paraffin wax. Five micron thick sections were made and stained with Haematoxylin and Eosin (Bancroft and Stevens, 2007) and Masson's trichrome for collagen and muscle fibres (Kiernan, 1981).

For electron microscopy, small pieces of tissue were fixed for 2 hours in 3 % glutaraldehyde buffered to pH 7.4 with 0.1 M sodium cacodylate buffer at 4^0C. The tissue was washed overnight in three changes of buffer at 4^0C, post-fixed in 1 % osmium tetroxide in 0.1 M sodium cacodylate buffer at 4^0C for 2 hours and washed overnight in buffer. Dehydration in ethanol and propylene oxide was followed by embedding in propylene oxide: epoxy resin mixture and embedded in Epon-araldite mixture after the method of Glauert (1967). Semi thin (1 micron) sections were stained by toluidine blue. Ultra thin sections (600 A^o to 900A^o) were prepared on Leica ultracut microtome, mounted on uncoated copper grids and stained with saturated solution of uranyl acetate and lead citrate. Screening of ultra thin sections were made with transmission electron microscope (Phillips - Teknai-10) operated at 60-kilowatt ampere (KVA). The images were documented and processed in computer.

RESULTS

Light microscopy

In the present study, thymic gland was surrounded by a thin connective tissue capsule composed mainly of collagen and few elastic fibes. Fine trabeculae / septa extended from the capsule and divided the parenchyma into lobules.

The parenchyma was observed to have a dark outer cortex and a pale inner medulla within the lobule in day-old, four weeks and ten weeks of age groups. The ratio of cortex to medulla of the thymus in day-old chick reversed in adult age groups. The cortex consisted of numerous small, medium and large lymphocytes lying within a meshwork of reticuloepithelial cells. Whereas the medulla contained predominantly retiucloepithelial cells with a fewer lymphocytes.

In all the age groups, myoid cells were most numerous in the medulla, less in cortex. Also found in the cortico-medullary junction. The extramedullary location often found in young age groups rather than adult birds.

The myoid cells were large and of varying shape in different age groups studied. From day-old to 20 weeks of age, they were seen as elongated, spindle shaped cells with striations (Fig. 1). In 40 weeks of age, the cells appeared ovoid or round. The nucleus was large and oval in shape with a single, discrete nucleolus. The cytoplasm appeared homogenous under light microscope and striations noticed in some cells. The cytoplasm was red in colour after Masson's trichrome stain.

The myoid cells were fewer in the thymic medulla on day-old and increased in number upto 40 weeks of age, in the present study.

Electron microscopy

Under transmission electron microscope, the cytoplasm of the myoid cell contained myofibrils of skeletal muscle (Fig.2). However, the overall arrangement of those myofibrils were not as in skeletal muscle fibre. The cytoplasm contained few mitochondria and smooth endoplasmic retiuclum and also free ribosomes and glycogen granules. The nucleus contained dispersed chromatin. In younger age groups, the myofibrils were oriented along the long axis and changes in the orientation was noticed in 40 weeks of age.

The myoid cells of the chicken thymus were found mainly in the medulla in all the age groups studied. The number of myoid cells increased as age advances in the present study.

Small bundles of unmyelinated nerve fibres were observed in the thymic medulla in the present study (Fig.3). However, no nerve fibres were seen to terminate directly on the surface of the myoid cell, although some fibres lay in close association with them.

DISCUSSION

Light microscopy

Though there are literatures available on the histoarchitecture of avian thymus, the detailed study about the thymic myoid cell in Nandanam chicken is scanty. Hence, the present study was therefore designed to observe the light and electron microscopic details of myoid cells in Nandanam chicken.

In chicken, thymic gland was surrounded by a connective tissue capsule and fine trabeculae / septa extended from the capsule and divided the parenchyma into lobules (Hodges, 1974; Firth, 1977; Bhattacharya and Binaykumar, 1983; Gail Pearse, 2006; Leena *et al.*, 2008). These septa are considered as important structure for immigration and emigration of T-lymphocyte precursor cells and distribution of antigens within the thymus. They are also thought to guide the invasion of interdigitating reticular cells, macrophages and act as a pathway for blood vessels and nerves to and from the thymus (Seifert and Christ, 1990 and Ritter and Crispe, 1992).

In all the age groups, the parenchyma was observed to have a dark outer cortex and a pale inner medulla and the cellular composition of the cortex was in agreement with the findings of Hodges (1974), Gilmore and Bridges, (1974) in fowl and King and Mc Lelland (1981) in birds.

The extramedullary location of the myoid cells in the present study supports the ontogenic similarities between lower and higher vertebrates (Kendall, 1980; Saad and Zapata, 1992; Bowden *et al.,* 2005 and Mohamed *et al.,* 2007). A similar finding was observed by Gilmore and Bridges (1974) and Kendall (1980) in chicken and Geetha Ramesh and Vijayaragavan (1997) in buffalo calves. But Robert *et al.* (1978) described that myoid cells were rare in human thymus.

The change in shape of the myoid cells between age groups in the present study was assumed to be change in cell morphology to be an expression of degeneration corresponding to the degeneration of the thymus as postulated by Raviola and Raviola (1967) and van de Velde and Friedman (1967). The presence of striations in the cytoplasm recognised the presence of muscular elements (Itoh, 1983 and Leena et al., 2008).

Increase in number of myoid cells from day-old to forty weeks of age in the present study leads to a postulation that these myoid cells directly or indirectly related with the involution of the organ, which is functional synchronization with the amount of sex hormone present in the blood (Vijayaragavan, 1988). Whereas Bockman (1968) assumed that the myoid cells were involved in the mechanism of tolerance to muscle self-antigen in human, but Mandel (1968a and b) opined that the thymic striated muscles may act as a local source of antigen for the self recognition of skeletal muscle in guinea pig.

Electron microscopy

Under transmission electron microscope, changes noticed in the cell organelles and orientation of myofibrils indicted the degenerating changes in involuting thymus (Van de Velde and Friedman, 1970 and Leena et al., 2012).

The presence of myoid mainly in the medulla in all the age groups studied was similar to thymic tissue from humans, amphibians, reptiles, birds and various mammals (van de Velde and Friedman, 1966, 1967; Strauss *et al.,*1966; Henry,1968; Toro *et al.,* 1969 and Bridges *et al.,* 1970). However, Morris (1971) and Sugimura (1972) have reported their presence in the medulla of the ten to twelve month old Ox, and they are present in the normal adult human thymus (Henry, 1968). The myoid cells seen in the present study are structurally similar to those described in the frog thymus (Toro *et al.,* 1969).

Two main theories have been put forward to explain the presence of myoid cells within the thymus: (1) that aberrant mesodermal elements from the branchial arches become incorporated accidentally into the thymus during its embryological development (van de Velde and Friedman, 1966 and Frazier, 1973) and (2) they develop within the thymus as an intrinsic part of the organ (Kapa *et al.* 1968; Bockman and Winborn, 1969 and Toro *et al.,* 1969). These latter authors suggested that the myoid cells develop from the epithelial cells of the thymic cytoreticulum. Toro and Olah (1967) and Toro *et al.* (1968) have also observed this process in the development of myoid cells during the *in vitro* culture of embryonic rat thymus tissue.

The number of myoid cells increased as age advances in the present study does not support the first theory that they represent the outcome of an embryological accident. Hence, the present study supports the second theory that the cells develop as an integral part of the normal thymus and as such it is reasonable to suggest that they play a physiological role therein.

The function of the myoid cells is still unknown. However, Raviola and Raviola (1967) and Rimer (1980) found that myoid cells play no physiological role because myofilaments are organized in random directions and myoid cells have no anchoring apparatus. But in the present study, myofilaments were shown to arrange regularly, and cross striations were clear. Hence, we are inclined to think that myoid cells push lymphatic cells to circulation around hatching as Toro *et a1*. (1969) suggested, though anchoring apparatus was not found in this study.

CONCLUSIONS

Thymic parenchyma was made up of an outer darker cortex and inner pale medulla. The myoid cells were numerous in the medulla and were large cells with eosinophilic cytoplasm. They were also observed in the cortico-medullary junction. Under electron microscope, the cytoplasm of the myoid cell showed myofibrils as in skeletal muscle fibre. Cytoplasm also contained few mitochondria, free ribosome and smooth endoplasmic reticulum. Small bundles of unmyelinated nerve fibres were observed in the thymic medulla. Number of myoid cells increased as age advances.

Conflict of Interests

The authors declare that they have no competing interests among them.

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