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Research Article

Electronmicroscopic Studies of Thymus in Chicken (*Gallus domesticus*)

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ABSTRACT

Electron microscopic studies on thymus of layer chicken were done in various age groups ranging from day-old to forty weeks. The thymic gland in chicken showed a thin connective tissue capsule. In thymic parenchyma, lymphocytes or thymocytes, retiucloepithelial cells, myoid cells and macrophages were the predominant component and the other cell types occasionally observed were erythrocytes, granulocytes, mast cells and plasma cells. Three types of reticuloepithelial cells were observed. The Hassall's corpuscles were composed of concentrically arranged reticuloepithelial cells. The centre of the corpuscles appeared either solid or cystic. The myoid cells were found mainly in the medulla. Intracellular and intercellular cysts were observed in association with the Hassall's corpuscles in all the age groups. The onset of involution was observed in twenty week-old birds and marked involutary changes were noticed in forty weeks.

Key words: Electronmicroscpic study, Thymus, Chicken

INTRODUCTION

The thymus gland is a central lymphoid organ in which 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 and Karen Staines *et al.,* 2013). Education of T cells by self- recognition is presumed to take place in thymus as in mammals (Kruisbeek *et al*., 1984) and defects in this process produce autoimmunity (Karen Staines *et al.,* 2013 and Van de Water *et al*., 1990).

Though there is extensive work done on the light and electronmicroscopic microscopic details of mammalian lymphoid organs, a little work was done about the ultrastructural studies of the thymus in Chicken. Hence, the present study was designed to explore the ultrastructural details of thymus in the layer chicken of different age groups.

MATERIALS AND METHODS

Thymic gland for transmission electronmicroscopic studies were collected from six different age groups such as day-old, four, eight, twelve, twenty and forty weeks. Six birds were used in each age group. Small pieces of thymic tissue (1-2 mm thickness) were collected and prefixed at 3 per cent glutaraldehyde and stored at 4^{o}C. Subsequently, the tissues were washed, three changes (each 30 minutes) in cold sodium cacodylate buffer solution (pH 7.4) and post fixed in 1 per cent osmium tetroxide for two hours at 4^{o}C. The tissues were then dehydrated in ascending grades of alcohol (50, 70, 80, 90, 95 per cent and absolute ethyl alcohol), propylene oxide:epoxy resin mixture and embedded in Epon-araldite mixture. Semi thin (1 micron) sections were stained by toluidine blue (Kannan *et al.,* 2015). 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. The ultra thin sections were examined under Phillips (Teknai-10) computer augmented transmission electron microscope operated at 60-kilowatt ampere (KVA).

RESULTS AND DISCUSSION

Capsule

In the present study, the thymic gland was surrounded by a thin connective tissue capsule composed mainly of collagen fibres and a few elastic fibres as reported by Hodges (1974) in birds and Bhattacharya and Binaykumar (1983) in chicken.

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Parenchyma

The parenchyma was composed of lymphoid cells or thymocytes, reticuloepithelial cells, myoid cells and macrophages as the predominant component of the chicken thymus in all the age groups. The other cell types observed were granulocytes, mast cells and plasma cells which were occasionally seen (Frazier, 1973) in chick.

Lymphocytes were more numerous in the cortex than in the medulla. These cells had a thin rim of cytoplasm around a nucleus with clumped chromatin. Small and medium lymphocytes were round cells with a narrow rim of cytoplasm which contained a few mitochondria and rough endoplasmic reticulum (Fig. 1). Ribosomes were observed more and lysosomes were occasionally seen. However, the medium lymphocytes had moderately wide band of cytoplasm with better developed Golgi complex which was observed smaller in the small lymphocytes. Nuclear chromatin was found densely packed at the periphery of the nucleus which was more condensed in the small lymphocyte than the medium lymphocyte (Fig. 2).

A greater proportion of large lymphocytes were seen in the medulla of the thymus in the present study. The nuclei of these cells contained one or more nucleoli and the chromatin was found to be less condensed. The cytoplasm was observed to be pale and a few strands of rough endoplasmic reticulum were present. The cytoplasmic-nuclear ratio was larger than that of small and medium sized lymphocytes as reported by Maxwell (1974), Hodges (1977) in chicken.

Three types of reticuloepithelial cells were observed in the present study. The pale reticuloepithelial cells in the cortex were similar in morphology to those present in the thymus of rat (van Haelst, 1967), the mouse (Hoshino, 1963), the guinea pig (Izard, 1966) and the monkey (Chapman and Allen, 1971). The dark reticuloepithelial cells with a relatively pale nucleus in the cortex and medulla of the chick thymus are somewhat unusual and have rarely been observed in the mammalian thymus (Fig. 3). A third type of epithelial cell was observed in the cortico-medullary junction and in the medulla. These cells had a pale, oval nucleus which contained one or two nucleoli. Indentation of the nuclear membrane was not observed. The cytoplasm had mitochondria, ribosomes, a few rough endoplasmic reticulum and small, dark granules (Fig. 4). Mandel (1968a and b) observed a similar type of cell in guinea pig. These undifferentiated epithelial cells possibly represent a reserve of epithelial cells which are able to differentiate and replace some of the other, more differentiated forms.

The myoid cells of the chicken thymus were found mainly in the medulla in all the age groups studied. Similar type of striated muscle cells or myoid cells have been found in thymic tissue from humans, amphibians, reptiles, birds and various mammals (Toro *et al.,* 1969; Bridges *et al.,* 1970 and Kendall, 1991). The cytoplasm of these myoid cells contained skeletal muscle fibres). The other organelles observed in the cytoplasm were few mitochondria and smooth endoplasmic reticulum. The myofibrils occupied greater part of the cell. The nucleus was found to contain dispersed chromatin with condensed chromatin as described in the frog thymus by 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; Mandel, 1968; 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. 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 protect thymocytes from apoptosis and could also modulate their differentiation process as suggested by Le Panse and Berrih-Aknin (2005).

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.

Macrophages were observed both in the cortex and medulla of all the age groups. The cytoplasm had vacuoles, phagocytosed materials, granules, mitochondria and endoplasmic reticulum. The nucleus appeared round to oval in shape with little chromatin. The plasma cells were observed within the connective tissue septa of all the age groups. The cells showed a very large Golgi region and well developed rough endoplasmic reticulum within the cytoplasm. Conspicuous euchroamtin of the nucleus was a common feature of the plasma cell. Mast cells in the chicken were small cells with a few secretory granules in the cytoplasm as reported by Frazier (1973) in chick thymus.

Small and large eosinophils, basophils and heterophils were found in the thymus of chicken, as in mallards, starlings, house sparrow and red billed queleas, in addition to the normal cellular constituents of the gland as described by Kendall (1980). Eosinophils in the chicken had a few small round mitochondria in the cytoplasm and characteristic bilobed nucleus which is similar to the findings of Maxwell and Siller (1972) in pigeon. The erythrocytes were commonly seen in the medulla which was found to have irregular shapes in the present study. This is in confirmation with the concept forwarded by Ward (1972) and Kendall (1975), who were of the opinion that haemopoiesis takes place in the thymus on increased demand for blood during breeding season.

The centre of the Hassall's corpuscle was either solid or cystic under electron microscope as reported by Robert *et al.* (1978). They were composed of concentric rings of squamous epithelial cells interconnected by many desmosomes. The association of dieing cells and macrophages with Hassall's corpuscles in birds proved beyond doubt that Hassall's corpuscles were the repository

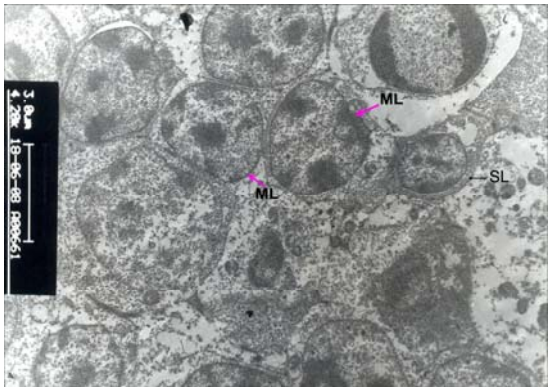

Fig.1: Transmission electron micrograph of thymus of a twenty week-old chicken showing the small and medium sized lymphocytes in the cortex x 4200 ML - Medium sized lymphocyte SL - Small sized lymphocyte

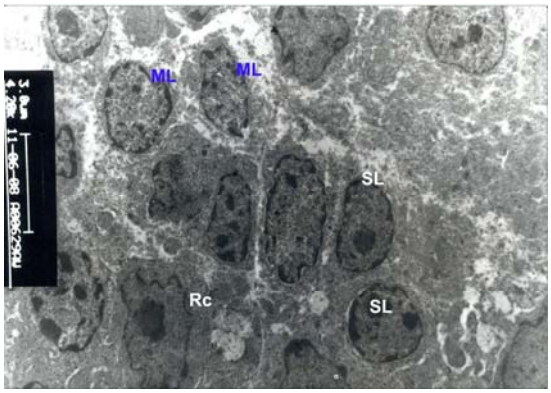

Fig. 2: Transmission electron micrograph of thymus of a day-old chick showing the lymphocyte population x 4200 ML - Medium sized lymphocyte Re - Reticuloepithelial cell SL - Small sized lymphocyte

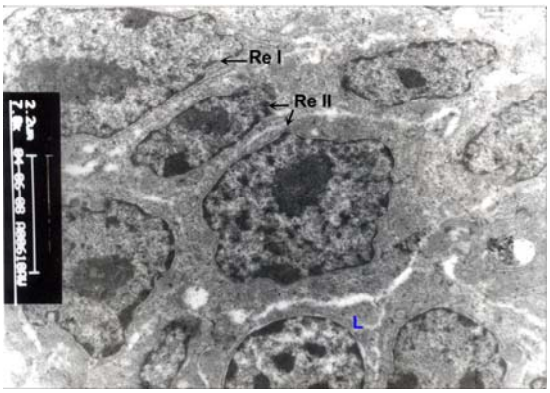

Fig. 3: Transmission electron micrograph of thymus of a day-old chick showing the reticuloepithelial cells in the cortex x 7000; Re I - Type I Reticuloepithelial cell; Re II - Type II Reticuloepithelial cell; L - Lymphocyte

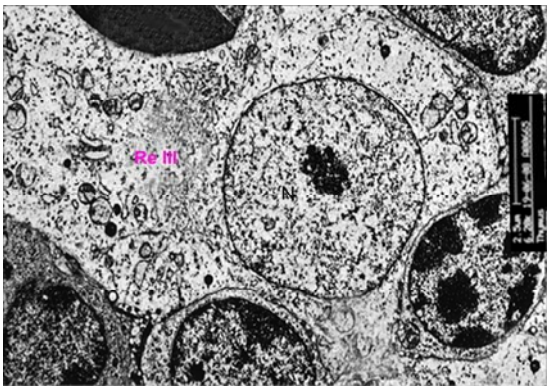

Fig. 4: Transmission electron micrograph of thymus of a day-old chick showing the third type of reticuloepithelial cell x 7000; N - Nucleus of reticuloepithelial cell; Re III - Type III reticuloepithelial cell

for a great number of old cells as opined by Blau (1973) and Olsson and Classon (1975). This finding is contrary to the findings of Senelar *et al.* (1976) in guinea pig that were of the opinion that Hassall's corpuscles are the privileged areas for maturation of the medullary lymphocytes.

The presence of unicellular/intracellular and multicellular/intercellular cysts in association with the Hassall's corpuscles in all the age groups was similar to the findings of Chan (1986) in chicken. The cells of the intercellular cysts contained dense secretory granules which are similar to the findings of Frazier (1973) in chicken. This is good evidence to support the idea that the thymus is an endocrine gland, and secretes a hormone that induces lymphoid cells to acquire immunological competence (Goldstein *et al.,* 1970). Whereas, Hoshino (1963), van Haelst (1967) and Mandel (1968a and b) are of the opinion that cystic epithelial cells show cytoplasmic features suggestive of a secretory function.

Marked involutary changes were noticed in forty weeks of age and was characterized by thickening of the capsule of the thymus with more collagen fibres, depopulation of cortical lymphocytes, pyknotic nucleus, increased number of Hassall's corpuscles and cysts as reported by Jacy Gameiro *et al.,* (2010) in human and Ciriaco *et al.* (2003) in birds.

Conclusion

The thymic gland in chicken showed a thin connective tissue capsule composed mainly of collagen fibres and a few elastic fibres. These septa divided the gland into lobules with a dark outer cortex and a pale inner medulla. Lymphocytes or thymocytes, reticuloepithelial cells, myoid cells and macrophages were the predominant cellular component of the chicken thymus in all the age groups. The other cell types occasionally observed were erythrocytes, granulocytes, mast cells and plasma cells.

Three types of reticuloepithelial cells were observed. The Hassall's corpuscles were composed of reticuloepithelial cells interconnected by many desmosomes had an abundance of cytoplasmic fibrils, a few mitochondria and ribosomes. Their number increased as age advanced.

The myoid cells of the chicken thymus were found mainly in the medulla in all the age groups studied. In the present study, two types of vesicles or cysts, intracellular and intercellular cysts were observed in association with the Hassall's corpuscles in all the age groups. The onset of involution was observed in twenty week-old birds and marked involutary changes were noticed in forty weeks.

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