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RESEARCH ARTICLE

Light and electronmicroscopic details of Thymic Reticuloepithelial cells in Nandanam Chicken (Gallus domesticus)

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Abstract

A light and electron microscopic study on the structure of thymic reticuloepihelial cells (RECs) was done in Nandanam chicken of various age groups ranging from day-old to forty weeks. The RECs were stellate shaped with large nucleus in an eosinophilic cytoplasm. Found to be more in number in medulla than the cortex. RECs in the cortex were smaller with long cytoplasmic processes and those in the medulla were larger with short processes. Under electron microscope, three types of RECs were observed in all the age groups studied. The first type were paler cells and the second type cells were darker cells. The first type were commonly observed in the cortex whereas the second type were also observed in the medulla. A third type of REC was observed in the cortico-medullary junction and in the medulla. These cells had a pale nucleus.

INTRODUCTION

In all vertebrate species, thymus is the central lymphoid organ which plays an important role in hosting and providing a suitable microenvironment for the development and production of functionally competent T-lymphocytes (Mohammad et al., 2007). This differentiation of T-cells occurs while they are progressing through the different compartments in the thymus (Bodey *et al.,* 2000 and Panse and Berrih-Aknin, 2005).

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 the different cell ypes in the parenchyma, it is the reticuloeithelial cells (RECs) of the thymus that are presumably playing a crucial role in the differentiation of T-lineage lymphoid cells (Le Douarin, 1977; Owen, 1977; Loor, 1979; Boyd *et al.,* 1983). These RECs have features of both epithelial and reticular cells. RECs sub-populations are distinguished by their location and function among different species. For example only one type of REC has been recognised in teleosts (Zapata, 1981; Gorgollon 1983); three types of RECs in birds (Frazier, 1973). Bodey (2007) observed four functional sub-types and six sub-types of RECs are described by Van de Wijngaert *et al.*(1985), Von Gaudecker (1986) and Mohammad et al. (2007) under transmission electro microscope.

By understanding the structural and fnctional importance of RECs, the present study was designed to explore the histological and ultrastructural details of thymic RECs in Nandanam chicken of different age groups.

Materials and Methods

Thymic lobes for light and transmission electron microscopic 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.

For light microscopic study, tissue pieces were collected from thymus and processed as per the standard paraffin (Cat. No. 8002-74-2 Sigma-Aldrich, India) embedding technique (Bancroft and Stevens, 2007). Tissue sections were cut at 3-5 micron thickness and used for the routine Haematoxylin-eosin staining method (Singh and Sulochana, 1978).

For electronmicroscopic study, small pieces of thymic tissues (1-2 mm thickness) were collected and prefixed at 3 per cent glutaraldehyde (Cat. No. G-5882, Sigma-Aldrich, India) and stored at 4^0C. 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^oC. 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 as per 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

Light microscopy

In the present study, in all the age groups studied, the RECs were stellate shaped with large nucleus and eosinophillic cytoplasm (Figure-1). Though, the cells were distributed both in cortex and medulla, comparatively more in number in medulla (Weiss, 1964; Firth, 1977).

RECs in the cortex were smaller, stellate in shape and had long cytoplasmic processes which formed a supportive network throughout the cortical region. But the RECs in the medulla were larger, oval shaped with shorter, spatula-like cytoplasmic processes in all the age groups of the study (Ritter and Crispe, 1992; Dellmann and Brown, 1988).

Apart from the RECs, the other major cellular population observed in this study were small, medium and large lymphocytes which found to be numerous and tightly packed in the cortex than the medulla in all the age groups studied. Thymic myoid cell, macrophages, mast cells, various forms of granulocytes and Hassall's corpuscles were also observed in all the age groups studied (Kannan *et al*., 2012).

Electron microscopy

Three types of reticuloepithelial cells were observed in the present study. The first type of epithelial cells seen in the cortex had long cytoplasmic processes. They had a pale nucleus which contained one or two nucleoli. The nuclear membrane was observed to be intended (Figure-2). The cytoplasm also appeared pale with a few mitochondria, Golgi apparatus, ribosomes and vacuoles. A similar finding was observed in the thymus of rat (van Haelst, 1967), the mouse (Hoshino, 1963), the guinea pig (Izard, 1966a and b) and the monkey (Chapman and Allen, 1971).

The second type of epithelial cell observed in the cortex had a much darker nucleus with an irregular outline and the nucleus was observed to be elongated (Figure-2). The cytoplasm was darker. However, vacuoles similar to the one present in the pale reticuloepithelial cells were observed in this type. The dark reticuloepithelial cells in the cortex and medulla of the chick thymus are somewhat unusual and have rarely been observed in the mammalian thymus. However, Izard and Harven (1968) described a "dense reticular cell" present in low numbers in the thymus and lymph nodes of mice; the number of these cells was greatly increased in leukemic mice.

The first type of reticuloepithelial cells were more commonly observed in the cortex whereas the second type was also observed in the medulla.

A third type of reticuloepithelial cell was also 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 (Figure-3). The cytoplasm had mitochondria, ribosomes, a few rough endoplasmic reticulum and small, dark granules. Mandel (1968) 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.

List of Plates and Legends

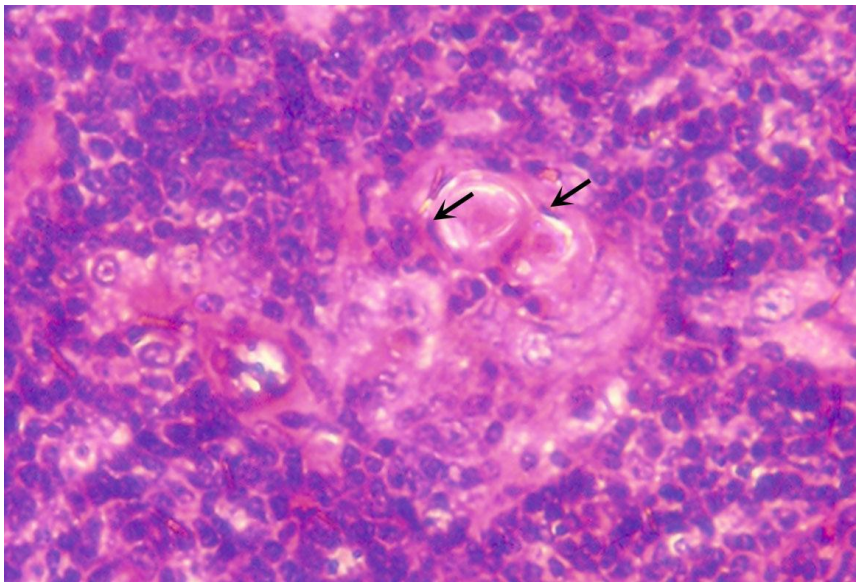

Figure-1. Photomicrograph of thymus of a day-old chick showing the reticuloepithelial cells (arrows) in the medulla H & E x 400

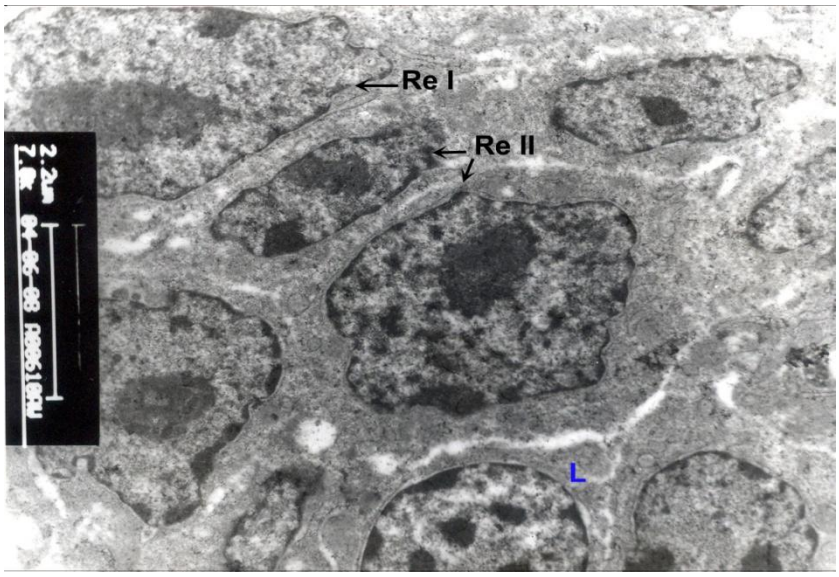

Figure-2. Transmission electronmicrograph of thymus of a day-old chick showing the reticuloepithelial cells in the cortex

Re I- Type-I Reticuloepithelial cell
Re II- Type-II Reticuloepithelial cell
L- Lymphocyte x 7000

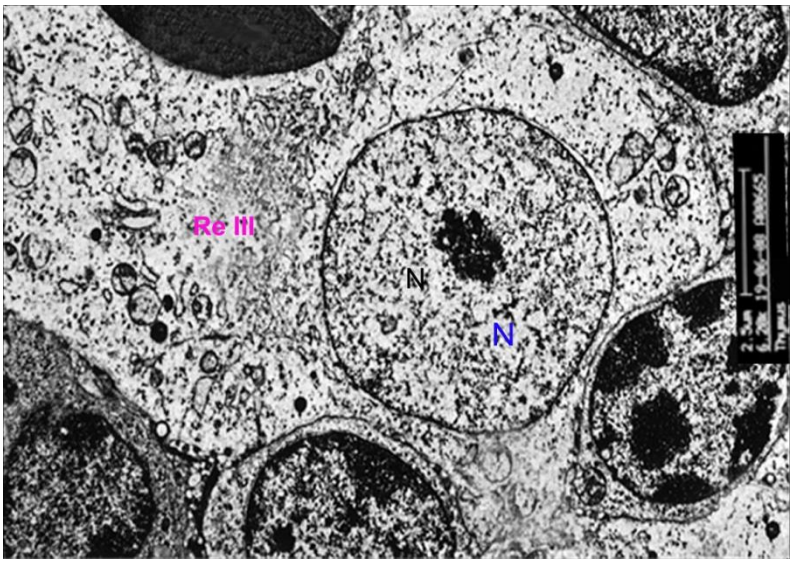

Figure-3. Transmission electronmicrograph of thymus of a day-old chick showing the third type of reticuloepithelial cell
Re III- Third type of reticuloepithelial cell
N- Nucleus of type-III reticuloepithelial cell x 7000

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Conflict of Interest Statement

Hereby**, t**he authors declare that they have no competing interests.

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