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Age related ultrastructural changes in lymphoid organs of Nandanam layer chicken (*Gallus domesticus*)

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Abstract

Transmission electron microscopic studies on thymus, spleen and caecal tonsil of layer chicken was done from day-old to forty weeks of age. The thymic gland showed a thin connective tissue capsule. In thymic parenchyma, lymphocytes, reticuloepithelial cells, myoid cells and macrophages were the predominant component. Erythrocytes, granulocytes, mast cells and plasma cells were also observed. The Hassall's corpuscles and myoid cells were also noticed. Marked involutory changes were noticed in forty weeks. The spleen was encapsulated by a connective tissue capsule with ill developed trabeculae. The white pulp was composed of lymphoblasts, lymphocytes, follicular dendritic and reticulum cells. Red pulp was composed of pulp cords consisted of erythrocytes and other cellular population. In caecal tonsil, the capsule of the germinal centre consisted of many layers of flattened reticular cells separated with an intercellular substance. The germinal centre consisted of lymphoblasts, lymphocytes, reticular cells, plasma cells, mast cells and macrophages. In forty week-old birds, the lymphocytic population was observed to be comparatively reduced and more of fibroblasts and collagen fibres were noticed.

Keywords: Age related changes, Ultrastructure, lymphoid organs, layer chicken

1. Introduction

Avian immune system resembles that of mammals since both evolved from a common reptilian ancestor and have inherited many commonalities ^[1]. They have also developed a number of different strategies that are unique to birds. Most avian immunology research has been carried out on the domestic chicken, *Gallus gallus domesticus* ^[2].

Various lymphoid organs functions to differentiate into avian immune cells: the thymus and Bursa of Fabricius are primary lymphoid organs in birds whereas the spleen, mucosal associated lymphoid tissues (MALT), germinal centers, and diffuse lymphoid tissues are secondary lymphoid organs. Birds do not have lymph nodes. The thymus, is a central lymphoid organ where T cells precursors undergo differentiation, maturation eventually leading to migration of thymocytes to peripheral lymphoid organs like spleen ^[3, 4]. Bronchial associated lymphoid tissue (BALT) and gut associated lymphoid tissue (GALT) such as caecal tonsils are found along the bronchus and intestines, respectively ^[5]. Though, Spleen is not considered as primary lymphoid organ, its importance in disease resistance is accentuated by the scarcity of avian lymph nodes. The avian spleen does not function as a reservoir of blood as in mammals and its function is not oriented towards the supply of oxygen ^[6]. It also plays an important role in the destruction of erythrocytes, phagocytosis and antigen-antibody interactions ^[7].

The chicken is a foundational model for immunological research and continues to be a valuable animal model for insights into immune function. Nandanam chicken is a dual purpose, colored variety with good disease resistance and most popular among poultry farmers due to its adaptability to backyard farming. This strain was developed in the Institute of Poultry Production and Management, Tamil Nadu Veterinary and Animal Sciences University, Chennai.

By considering the immunological and physiological importance, extensive research work has been carried out on avian lymphoid organs using light microscopy. Hence, the present study was designed to explore the age related ultrastructural changes in lymphoid organs such as spleen, thymus and caecal tonsils in Nandanam layer chicken.

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2. Materials and Methods

Tissue pieces of spleen, thymus and caecal tonsil for 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 the transmission electron microscopic study, small pieces of tissues (1-2 mm thickness) were collected and prefixed at 3 per cent glutaraldehyde and stored at 4 °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°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. Ultra thin sections (600 A° to 900A°) 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).

3. Results and Discussion

3.1 Thymus

The thymic gland was surrounded by a thin connective tissue capsule composed mainly of collagen fibres and a few elastic fibres as reported by Bhattacharya and Binaykumar⁽⁸⁾ in chicken.

In all the age groups, the parenchyma was composed of lymphoid cells or thymocytes, reticuloepithelial cells, myoid cells and macrophages as the predominant component of the chicken thymus. The other cell types observed were granulocytes, mast cells and plasma cells which were occasionally seen as reported by Frazier^[9] in the 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 as reported by Maxwell^[10], Hodges^[11] 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 monkey as mentioned by Chapman and Allen^[12]. The dark reticuloepithelial cells with a relatively pale nucleus in the cortex and medulla of the chick thymus (Fig.3). A third type of epithelial cell was observed in the cortico-medullary junction and in the medulla as per Mandel^[13, 14] 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 as per Kendall^[15] in amphibians, reptiles, birds and mammals. 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.

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 euchromatin 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^[9] in the chick thymus.

Small and large eosinophils, basophils and heterophils were found in the thymus of chicken as described by Kendall^[16]. The erythrocytes were commonly seen in the medulla as per Ward^[17], who was 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.*^[18]. The association of dying cells and macrophages with Hassall's corpuscles in birds proved beyond doubt that Hassall's corpuscles were the repository for a great number of old cells as opined by Olsson and Classon^[19]. This is contrary to Senelar *et al.*^[20] in guinea pig who were of the opinion that Hassall's corpuscles are the privileged areas for maturation of the medullary lymphocytes.

Marked involuntary 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 *et al.*^[21].

3.2 Spleen

In all the age groups, the splenic capsule was composed of collagen bundles and a few elastic fibres. The capsule was also observed with smooth muscle cells and stellate shaped fibroblasts with fibrillar cytoplasm and mitochondria as per Burke and Simon^[7]. The presence of collagen fibres increased as age advanced in the present study as per Moore *et al.*^[22].

3.2.1 White pulp

In all the age groups studied, white pulp of the spleen was observed with predominant lymphocytes of various sizes and reticulum cells. These cells were arranged in the form of clumps (Fig.4) separated by a meshwork composed of collagen, fibroblasts and reticulum cells as reported by Olah and Glick^[23].

In day-old and four week-old birds, the lymphoblasts and small lymphocytes were observed more. In eight week-old birds, all the types of lymphocytes such as small, medium and large lymphocytes were noticed. Whereas in twenty week-old birds, the existence of large lymphocytes predominated and in forty week-old birds, the depletion of lymphocytes and the amount of collagen was maximum in the stroma.

The lymphoblasts were characterised by large euchromatic

nuclei and predominance of free poly-ribosomes. The small and medium sized lymphocytes were found to be round to oval shaped and were seen with high nuclear-cytoplasmic ratio (Fig.5). The cytoplasm was sparsely seen with occasional little perinuclear rough endoplasmic reticulum, a few ribosomes and mitochondria as per Cross and Mercer [24]. The reticulum cells were stellate shaped, they had smaller nuclear-cytoplasmic ratio and more organelles in the cytoplasm (Fig.5). Rough endoplasmic reticulum was found to be more. Well developed mitochondria, ribosomes and a prominent Golgi zone were also observed. The presence of reticulum cells were commonly observed in all the age groups studied as mentioned by Burke and Simon [7].

The follicular dendritic cells were seen in all the age groups and confirmed by their large and irregular shaped nucleus as per Banchereau and Steinman [25]. The plasma cells were observed with well developed rough endoplasmic reticulum. There were larger and denser mitochondria and numerous ribosomes as per Ogata *et al.* [26]. These plasma cells synthesize and secrete antibodies that bind specifically to the antigen that initially activated the precursor B lymphocyte.

The macrophages were ovoid or stellate shaped, the cytoplasm contained vacuoles and phagocytosed materials in the cytoplasm with heterochromatic nucleus as per Burke and Simon [7]. These macrophages in the spleen were the important site of erythrocyte destruction which was evident by the presence of several partially digested fragments of old erythrocytes. According to Weiss [27], it also played a role in antigen presentation and secretion of mediators of the immune response.

3.2.2 Red pulp

The splenic red pulp was composed of anastomosing sinuses lined by endothelial cells were noticed. These sinuses were found to be separated with each other by the pulp cords. These pulp cords consisted of erythrocytes, reticular cells, lymphocytes of various sizes, macrophages, granulocytes, plasma cells and mast cells (Fig.6). Macrophages, reticulum cell and lymphocytes were also observed as reported by Abe *et al.* [28].

3.3 Caecal tonsil

The capsule of the germinal centre of the caecal tonsil of chicken consisted of many layers of flattened reticular cells separated with an intercellular substance. The cytoplasm of the reticular cells showed a number of smooth surfaced vesicles (Fig.7) as per Olah and Glick [29].

The germinal centre consisted of reticular cells, large and small lymphocytes and macrophages. The structure of the reticular cells were similar to that of the reticular cells of the capsule. The number of reticular cells in the periphery of the germinal centre was higher than the central area as per Kitagawa *et al.* [30].

The large lymphocyte had a small amount of cytoplasm with ribosomes and few mitochondria. The nucleus was found to be heterochromatic and a well developed nucleolus. The small lymphocytes were round cells which had numerous ribosomes in their cytoplasm. A patchy chromatin pattern was observed in the nucleus (Fig.8). The other cellular components of the germinal centre included fibroblasts, plasma cells, macrophages and mast cells in all the age groups. In forty week old-birds, the lymphocytic population was observed to be comparatively reduced and more number of fibroblasts and collagen fibres were noticed.

List of Plates and Legends

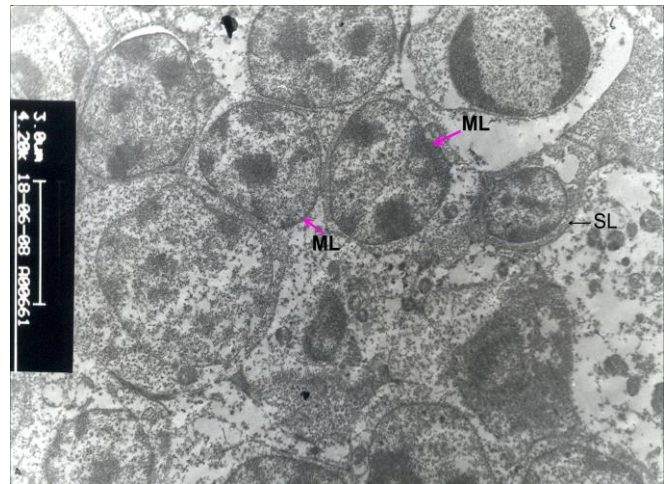


Fig 1: Transmission electron micrograph of thymus of a twenty week-old chicken showing the small and medium sized lymphocytes in the cortex x 4200 ML - Medium sized lymphocyte SL - Small sized lymphocyte

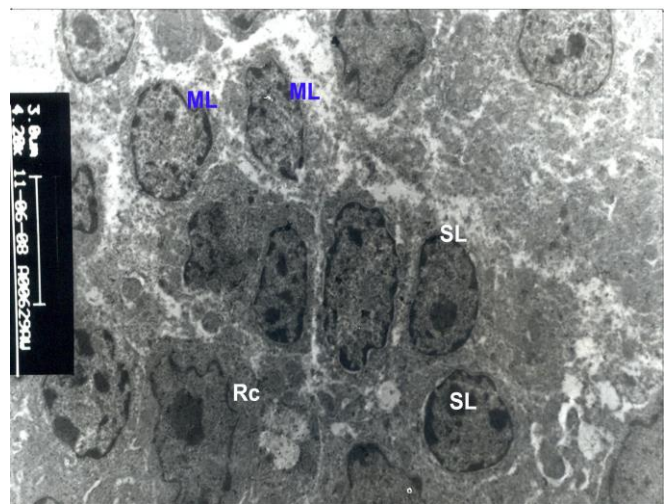


Fig 2: Transmission electron micrograph of thymus of a day-old chick showing the lymphocyte population x 4200 ML - Medium sized lymphocyte Re - Reticuloepithelial cell SL - Small sized lymphocyte

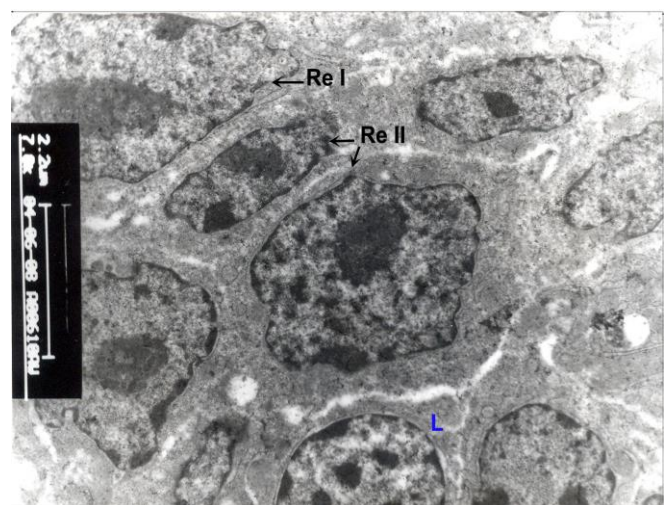


Fig 3: Transmission electron micrograph of thymus of a day-old chick showing the reticuloepithelial cells in the cortex x 7000 Re I - Type I Reticuloepithelial cell Re II - Type II Reticuloepithelial cell L - Lymphocyte

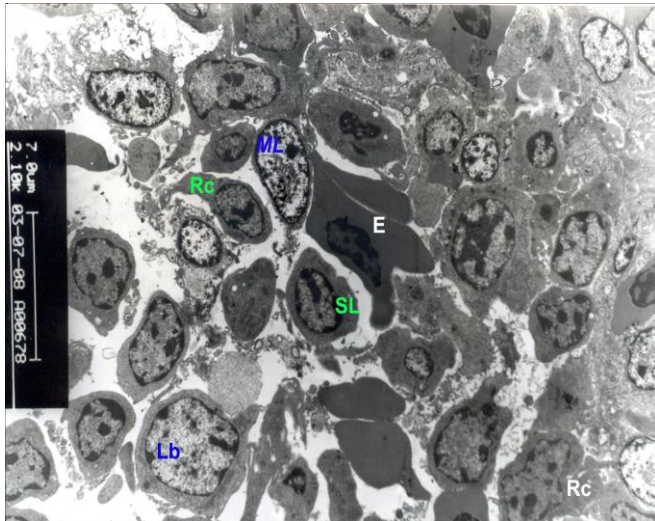


Fig 4: Transmission electron micrograph of spleen of a four week-old chicken showing the cellular components of the white pulp x 2100 E – Erythrocyte Lb – Lymphoblast ML - Medium sized lymphocyte SL - Small lymphocyte Rc - Reticular cell

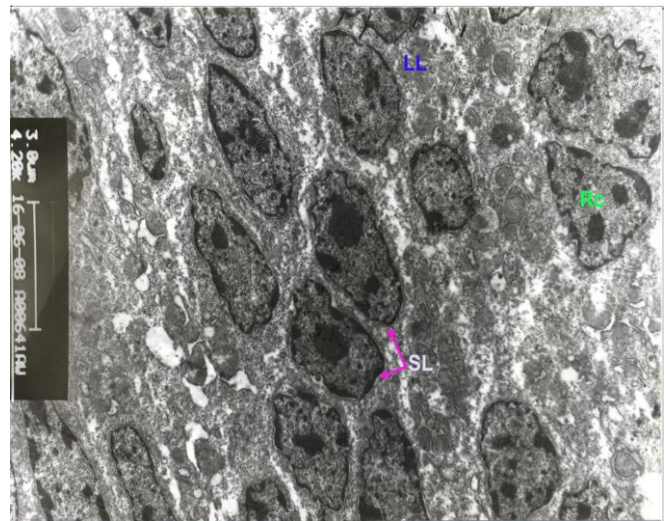


Fig 7: Transmission electron micrograph of caecal tonsil of a day-old chick showing the capsule of the germinal centre x 8900 Ca – Capsule N - Nucleus of reticular cell Rc - Reticular cell V - Vesicle



Fig 5: Transmission electron micrograph of spleen of an eight week-old chicken showing the details of lymphoblast and reticulum cell in white pulp x 7000 LbN - Nucleus of Lymphoblast Rc - Reticular cell

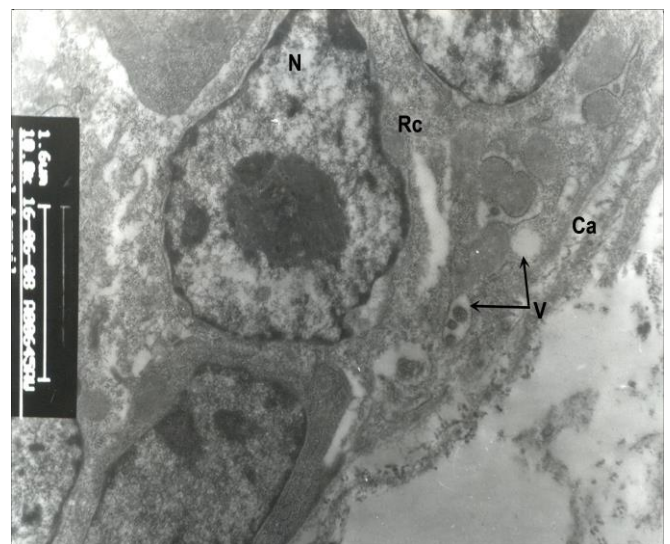


Fig 8: Transmission electron micrograph of caecal tonsil of a day-old chick showing the cellular components of the germinal centre x 3700 LL -Large lymphocyte SL - Small lymphocyte Rc - Reticular cell



Fig 6: Transmission electron micrograph of spleen of a day-old chick showing the cellular components of the red pulp x 4200 E – Erythrocytes H – Heterophil L – Lymphocytes Rc - Reticular cell

4. Conclusion

Transmission electron microscopic studies on thymus, spleen and caecal tonsil were done in layer chicken of 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, reticuloepithelial cells, myoid cells and macrophages were the predominant component and the other cell types occasionally observed were erythrocytes, granulocytes, mast cells and plasma cells. The Hassall's corpuscles were composed of concentrically arranged reticuloepithelial cells. The myoid cells of the chicken thymus were found mainly in the medulla. The onset of involution was observed in twenty week-old birds and marked involutory changes were noticed in forty weeks. The spleen was encapsulated by a connective tissue capsule. The major cellular population of the white pulp included lymphoblasts, lymphocytes of various sizes, follicular dendritic cells and reticulum cells. The splenic red pulp was composed of pulp cords consisted of erythrocytes, reticular cells and lymphocytes of various sizes, macrophages,

granulocytes, plasma cells and mast cells. The caecal tonsil revealed two types of lymphoid aggregations (germinal centre). The germinal centre consisted of lymphoblasts, lymphocytes of various sizes, reticular cells, plasma cells, mast cells and macrophages.

5. Acknowledgement

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6. References

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