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Light and Electron Microscopic Details of blood-Spleen Barrier in Nandanam Chicken (*Gallus domesticus*)

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Abstract: ***Light and electron microscopic studies of blood-spleen barrier was done in various age groups of Nandanam Chicken ranging from day-old to forty weeks. The spleen is the largest secondary lymphoid organ which can perform filtration of blood and also the primary site for imunocyte proliferation and differentiation. The results showed that the parenchyma was madeup of white and red pulp. The white pulp appeared as islands enclosed by red pulp and there was no distinct marginal zone between the red and white pulp. The trabeculae were not evident in all the age groups studied. The arterioles from the periphery of the white pulp observed to enter the red pulp as sheathed capillaries. These capillaries were found to be surrounded by reticular cells and macrophages which formed the ellipsoids or Sheathed capillaries. Under transmission electron microscope, the ellipsoids were found to have a meshwork of stellate reticular cells, reticular fibres and a few macrophages.***

Keywords: Light microscopy - electron microscopy - blood-spleen barrier – Chicken

1. Introduction

The spleen is the largest secondary lymphoid organ in Chicken, containing around 25% of the total number of lymphocytes and also the primary site for immune cell proliferation and differentiation [29] and is a specialised organ to perform filtration of blood [29]. The splenic parenchyma plays a crucial role in immune responses such as exposure to blood-borne antigens [5,29] due to the scarcity of lymph nodes and poorly developed lymphatic vessels in avian species [16,22,27,29,20].

The blood-spleen barrier [BSB] was first described in Mice by Weiss [Weiss *et al.*, 1986] and it has been confirmed by light and electron microscope [26] and also functional studies with various extracellular tracers [15]. In mouse, the BSB is composed of endothelial cells, macrophages, reticular tissue and fibres [10]. Whereas, Zhang et al. [2015] described that BSB in Chicken spleen is madeup of ellipsoids and peri-elliposoidal lymphatic sheath [PELS].

The ellipsoid or Schweigger-Seidel sheath, is a specialised capillary segment in the spleen of chicken [19]. Ellipsoids are common in avian species, turtles, cat, dogs, human but not in rodents and lagomorphs [11,21,19,24]. However, the structure of ellipsoid varies between species.

2. Literature Survey

Although, there are several detailed reports available on histoarchitecture of Chicken spleen, the study on structural details of BSB in Nandanam Chicken is very limited. Nandanam strain of chicken is a dual purpose, coloured variety with good disease resistance, developed in Institute of Poultry Production and Management, TANUVAS. Hence, the present work was aimed to study the location and structural details of BSB using light and electron microscopy.

3. Materials and Methods

Over 36 specimens of thymic tissue 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. The spleen was removed immediately after high cervical dislocation and fixed for light and electron microscopy [8].

For light microscopy, tissue pieces were fixed in 10% neutral buffered formalin, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Five micron thick sections were made and stained with Haematoxylin and Eosin [1].

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. 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 [18]. The stained ultra thin sections were examined under Phillips [Teknai-10] computer augmented transmission electron microscope operated at 60-kilowatt ampere [KVA].

4. Results and Discussion

Light Microscopy

In the present study, the parenchyma of the spleen was observed to have stroma composed of red pulp, white pulp and blood vessels. The white pulp appeared as islands enclosed by red pulp and the demarcation between the two was not distinct [2,29]. This could probably the reason for less clear zonal demarcation of white and red pulp and absence of marginal zone as in the spleen of rodents and absence of marginal zone as in the spleen of rodents [12,14,17]. The trabeculae were not evident in all the age groups studied. The arterioles from the periphery of the white pulp observed to enter the red pulp as sheathed capillaries. These capillaries were found to be surrounded by reticular cells and macrophages which formed the ellipsoids or sheathed capillaries.

Electron Microscopy

Under transmission electron microscope, the ellipsods were found to have a meshwork of polymorphic reticular cells, reticular fibres and a few macrophages [23]. These ellipsoids may act as an antigen trapping zone of the spleen [4]. The reticulum cells were stellate shaped, they had smaller nuclear-cytoplasmic ratio and more organelles in the cytoplasm [Fig-1]. Rough endoplasmic reticulum was found to be more [Fig-2]. Well developed mitochondria, ribosomes and a prominent Golgi zone were also observed. The macrophages were ovoid or stellate shaped and showed vacuoles and phagocytosed material in the cytoplasm. The nucleus was heterochroamtic [Fig-3].

The presence of reticular cells and macrophages helps the ellipsoids to regulate the movement of cells and antigens between the blood vessels and white pulp [6,9]. It is hypothesised that the ellipsoid together with PELS may be comparable to the marginal zone or comparable with that of high endothelial venules of lymph node / spleen [12,25].

In the present study, BSB was located in the ellipsoid and PELS as reported by [29,3] and in contrast to the findings of [7] in rodents. There were no structural changes noticed in ellipsoid of spleen in different age groups studied.

5. Conclusion

The present study on Chicken spleen described the location, light and electron microscopic details of blood-spleen barrier in different age groups. The ellipsoids were found to have a meshwork of polymorphic reticular cells, reticular fibres and a few macrophages. The structure of BSB was similar in all the age groups studied and it may provide a structural basis for homing of lymphocyte in chicken. There were no structural changes noticed in ellipsoids and periarterial lymphatic sheath of spleen in different age groups. This basic study helped in better understanding of the immune function of the spleen.

6. Future Scope

Though, the present study described the location and structure of BSB, quantitative and qualitative estimation of the T-lymphocyte subsets viz., CD-4 and CD-8 cells in ellipsoid and periellipsoidal lymphatic sheath will help in better understanding the immune function of spleen in Chicken. These observations will form a basis for further immunohistochemical and flow cytometric studies in chicken spleen.

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List of Plates and Legends

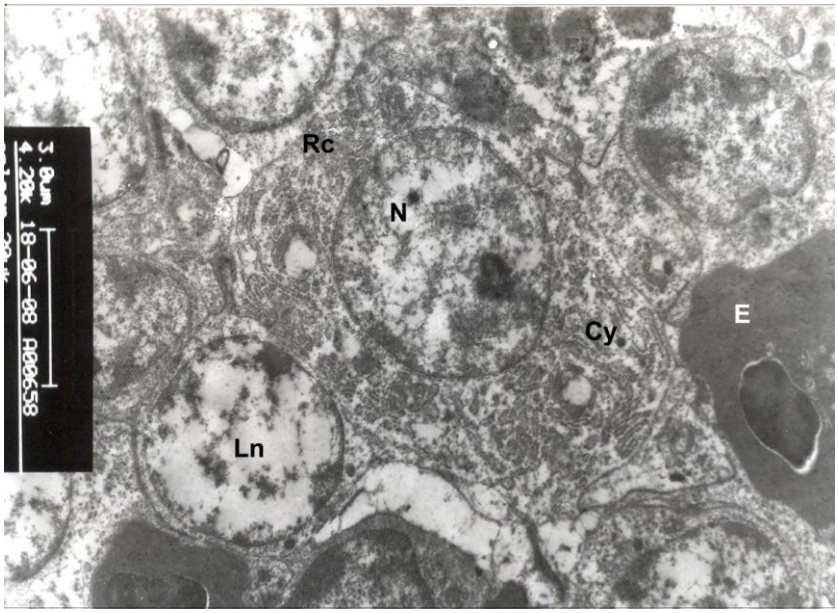

Figure 1: Transmission electron micrograph of spleen of a fortytwenty week-old chicken showing the details of reticulum cell in white pulp x 4200

Cy - Cytoplasm N - Nucleus of reticulum cell
E - Erythrocyte Ln - Nucleus of Lymphocyte
Rc - Reticulum cell

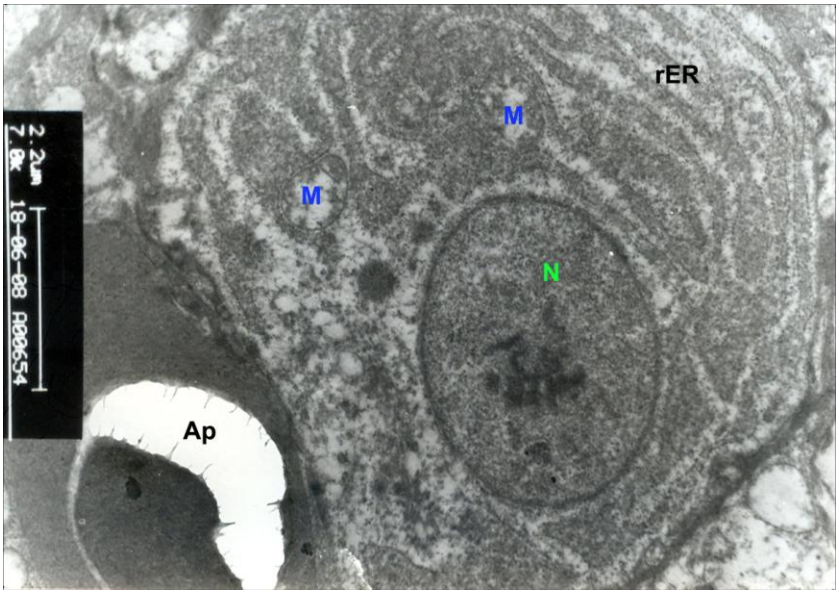

Figure 2: Transmission electron micrograph of spleen of a forty week-old chicken showing the cytoplasmic organelles of reticulum cell in white pulp x 7000

Ap - Apoptotic cell M - Mitochondria
N - Nucleus rER - Rough endoplasmic reticulum

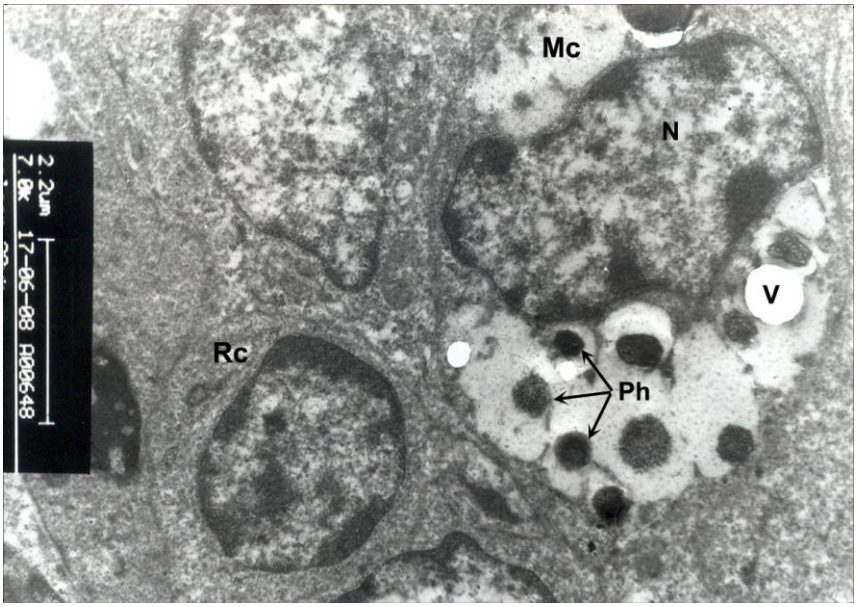

Figure 3: Transmission electron micrograph of spleen of twenty week-old chick showing a macrophage in the white pulp x 7000

Mc - Macrophage N - Nucleus of macrophage
Ph - Phagocytosed material Rc - Reticulum cell
V - Vacuole