

Electron Microscopic Studies of Spleen in Chicken (*Gallus domesticus*)

Kannan T.A., Geetha Ramesh, Ushakumari S., Dhinakarraj G. and Vairamuthu S.

Department of Veterinary Anatomy and Histology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Correspondence should be addressed to Kannan T.A., kannan@tanuvas.org.in

Publication Date: 5 May 2015

Article Link: <http://scientific.cloud-journals.com/index.php/IJAVST/article/view/Sci-270>



Copyright © 2015 Kannan T.A., Geetha Ramesh, Ushakumari S., Dhinakarraj G. and Vairamuthu S. This is an open access article distributed under the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract Electron microscopic studies on spleen of layer chicken were done in various age groups ranging from day-old to forty weeks. The spleen was encapsulated by a connective tissue capsule and the trabeculae were poorly developed in all the age groups studied. 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 arterioles that continued into the red pulp formed sheathed capillaries or ellipsoids.

Keywords *Electron Microscopy; Spleen; Chicken*

1. Introduction

Spleen is the principal organ of systemic immunity and its importance in disease resistance is accentuated by the scarcity of avian lymph nodes. The avian spleen functions as a major blood filtering organ and is the major source of antibody production. It does not function as a reservoir of blood as in mammals and its function is not oriented towards supply of oxygen (Jeurissen, 1991). The spleen also plays an important role in erythrocyte destruction, phagocytosis and antigen-antibody interactions (Burke and Simon, 1970). Though there is extensive work done on the light microscopic details, a little work was done about the ultrastructural studies of the spleen in Chicken. Hence, the present study was designed to explore the details of spleen in the layer chicken of different age groups.

2. Materials and Methods

Spleen 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 electron microscopic study, small pieces of splenic tissue (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 Å to 900 Å) 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. Capsule

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 (Burke and Simon, 1970). External surface of the capsule was observed to be lined by a single layer of mesothelial cells with small microvillous projections at their free border. The inner surface of the capsule was found to have subcapsular sinus lined by endothelial cells, filled with erythrocytes. The presence of collagen fibres increased as age advanced in the present study (Moore, et al., 1964).

3.2. Parenchyma

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 (Figure 1). These clumps were separated by a meshwork composed of collagen, fibroblasts and reticulum cells (Olah and Glick, 1982).

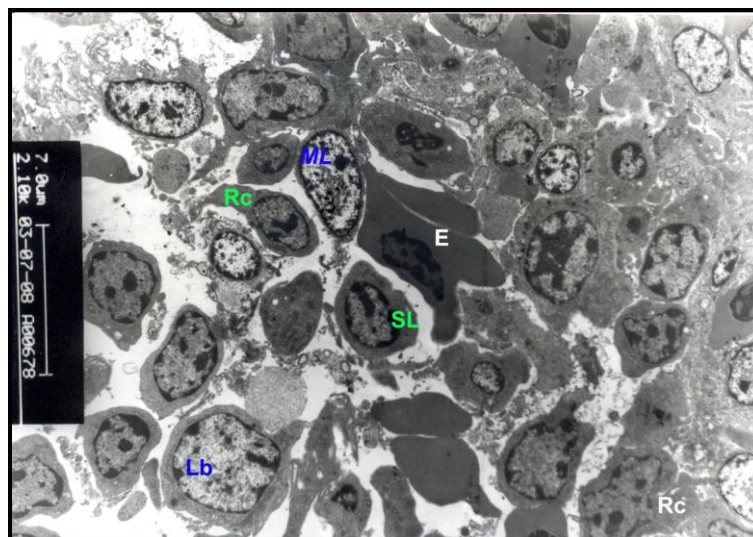


Figure 1: Transmission Electron Micrograph of Spleen of a Four Week-Old Chicken Showing the Cellular Components of the White Pulp X 2100

E - Erythrocyte Lb - Lymphoblast
ML - Medium sized lymphocyte SL - Small lymphocyte
Rc - Reticular cell

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 characterized 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 (Figure 2). The cytoplasm was sparsely seen with occasional little perinuclear rough endoplasmic reticulum, a few ribosomes and mitochondria. The smooth endoplasmic reticulum was seen in the form of small vesicles dispersed in the cytoplasm and the Golgi zone was not prominent. Nucleus was observed mostly round or slightly indented with heterochromatin (Cross and Mercer, 1993).

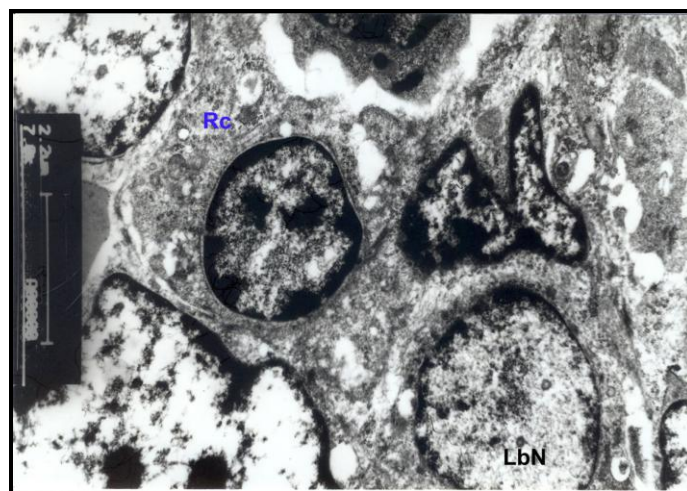


Figure 2: Transmission Electron Micrograph of Spleen of a Eight Week-Old Chicken Showing the Details of Lymphoblast and Reticulum Cell in White Pulp x 7000

LbN - Nucleus of Lymphoblast

Rc - Reticular cell

The reticulum cells were stellate shaped; they had smaller nuclear-cytoplasmic ratio and more organelles in the cytoplasm (Figure 3). 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 (Burke and Simon, 1970).

The fibroblasts appeared flat and stellate with long cytoplasmic prolongations. They had rough endoplasmic reticulum seen filled with a fine flocculent material. The smooth endoplasmic reticulum also had similar material and some of them appeared fibrillar (Galindo and Freeman, 1963).

The follicular dendritic cells were seen in all the age groups and confirmed by their large and irregular shaped nucleus. It had a little heterochromatin. The cytoplasm was found to be ramified as very thin processes in many directions. Some of the processes were observed long and straight. Mitochondria, rough and smooth endoplasmic reticulum, vesicles were rarely seen within the cytoplasm (Banchereau and Steinman, 1998).

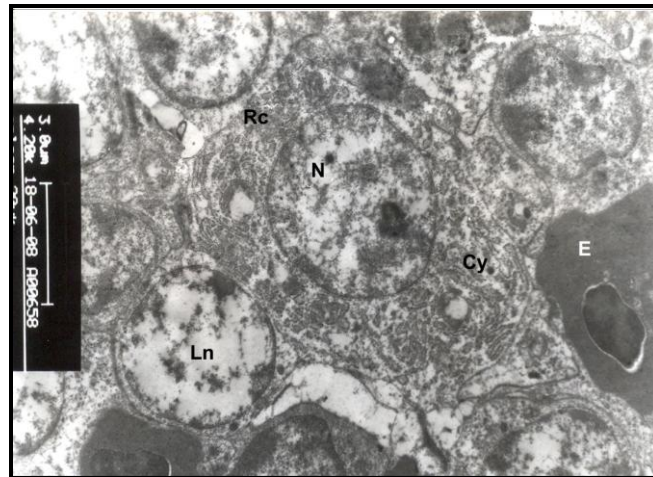


Figure 3: Transmission Electron Micrograph of Spleen of a Twenty Week-Old Chicken Showing the Details of Reticulum Cell in White Pulp x 4200

CY - Cytoplasm
 E - Erythrocyte
 Rc - Reticular cell
 N - Nucleus of reticulum cell
 Ln - Nucleus of lymphocyte

The plasma cells of the spleen were observed with well-developed rough endoplasmic reticulum. There were larger and denser mitochondria and numerous ribosomes. The heterochromatic nucleus was eccentrically placed (Ogata, et al., 1977). These plasma cells synthesize and secrete antibodies that bind specifically to the antigen that initially activated the precursor B lymphocyte. Antigen-antibody binding is a major means of immune defense.

The macrophages were ovoid or stellate shaped; the cytoplasm contained vacuoles and phagocytosed materials in the cytoplasm. The nucleus was heterochromatic (Burke and Simon, 1970). Antibodies synthesized within the rough endoplasmic reticulum are processed and packaged within the Golgi prior to secretion. 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 (1964 and 1990), it also played a role in antigen presentation and secretion of mediators of the immune response.

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 (Figure 4).



Figure 4: 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

The erythrocytes were present both in the sinuses and in the pulp cords which had different shapes. Macrophages were seen with phagocytic vacuoles which contained the degraded erythrocytes or leukocytes. The structure of reticulum cell and lymphocytes were similar to that present in the white pulp (Abe, et al., 1989).

The arterioles from the periphery of the white pulp were found to enter into the red pulp. In these arterioles, the lumen was surrounded by muscle cell and these arterioles continued into the red pulp and formed sheathed capillaries or ellipsoids (Olah and Glick, 1982). These ellipsoids were found to have a meshwork of polymorphic reticular cells, reticular fibres and a few macrophages.

4. Summary

The splenic capsule was composed of collagen bundles with a few elastic and smooth muscle fibres, smooth muscle cells and fibroblast in all the age groups. As age advanced the thickness of the capsule increased. The trabeculae were poorly developed in all the age groups studied.

The major cellular population of the white pulp included lymphoblasts, lymphocytes of various sizes and reticulum cells arranged in the form of clumps separated by a meshwork composed of collagen, fibroblasts and reticulum cells in all the age groups studied. The follicular dendritic cells had a little heterochromatin. The mitochondria, rough and smooth endoplasmic reticulum were sparse in the cytoplasm.

The splenic red pulp was composed of anastomosing sinuses lined by endothelial cells. The pulp cords consisted of erythrocytes, reticular cells, lymphocytes of various sizes, macrophages, granulocytes, plasma cells and mast cells.

Acknowledgement

The authors are thankful to the Professor and head, Department of Animal Biotechnology and Professor and Head, Centralised Instrumentation Laboratory, Madras Veterinary College for providing facilities to carry out this work.

References

- Abe, M., Takehana, K., Iwasa, K. and Hiraga, T. *Scanning Electron Microscopic Studies on the Red Pulp of the Mink Spleen*. Nippon Juigaku Zasshi. 1989. 51; 775-81.
- Banchereau, J. and Steinman, R.M. *Dendritic Cells and the Control of Immunity*. Nature. 1998. 392; 245-52.
- Burke, J.S. and Simon, G.T. *Electron Microscopy of the Spleen. I. Anatomy and Microcirculation*. Am. J. Pathol. 1970. 58; 127-55.
- Cross, P.C. and Mercer, K.L., 1993: *A Text Book on Cell and Tissue Ultrastructure*. New York: W.H. Freeman and Company.
- Galindo, B. and Freeman, J.A. *Fine Structure of Splenic Pulp*. Anat. Rec. 1963. 147; 25-42.
- Jeurissen, S.H.M. *Structure and Function of the Chicken Spleen*. Res. Immunol. 1991. 142; 352-55.
- Moore, R.D., Mumaw, V.G. and Schoenbeg, M.D. *The Structure of the Spleen and Its Functional Implications*. Exp. Mol. Path. 1964. 3; 31-50.
- Ogata, K., Sukumura, Y. and Kudo, N. *Developmental Studies on Embryonic and Post-Hatching Spleens in Chicken with Special Reference in Development of White Pulp*. Jap. J. Vet. Res. 1977. 25; 83-92.
- Olah, I. and Glick, N. *Splenic White Pulp and Associated Vascular Channels in Chicken Spleen*. Am. J. Anat. 1982. 165; 445-80.
- Weiss, L. *The White Pulp of the Spleen. The Relationships of Arterial Vessels, Reticulum and Free Cells in the Periarterial Lymphatic Sheath*. Bull Hopkins Hosp. 196. 115; 99-173.
- Weiss, L., 1990: *Mechanism of Splenic Clearance of the Blood: A Structural Overview of the Mammalian Spleen*. In *the Spleen: Structure, Function and Clinical Significance*. Bowdler, A.J. (ed.) London: Chapman and Hall Medical. 103-26.