ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Cytoarchitecture of Periarterial lymphatic sheath (PALS) in Chicken Spleen – Light and Transmission electronmicroscopic study

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Manuscript Info

Manuscript History:

Received: 15 September 2015
Final Accepted: 21 October 2015
Published Online: November 2015

Key words:

Periarterial lymphatic sheath - light and electron microscopic study - different age groups - Chicken

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Abstract

A light and transmission electron microscopic study on the structure of splenic periarterial lymphatic sheath (PALS) was done in chicken of various age groups ranging from day-old to forty weeks. The PALS was noticed in all the age groups except day-old. PALS were observed as a diffuse lymphatic sheath adjacent to the central artery in all the age groups studied. It consisted of closely packed small lymphocytes and several medium to large sized lymphocytes and reticular cells. The lymphocytes of all sizes appeared to have high nuclear cytoplasmic ratio. Few macrophages and plasma cells were also found towards the periphery of the PALS. The macrophages contained vacuoles, phagocytosed materials and granules in the cytoplasm. The plasma cells showed a very large Golgi region and well developed rough endoplasmic reticulum within the cytoplasm.

INTRODUCTION

The avian spleen differed from mammalian spleen by not having germinal centres in the white pulp and by the absence of lymph vessels (Lucas *et al.,* 1954). On the contrary, Cooper *et al.* (1967) described that in birds, the germinal centres of the white pulp and the corona of the lymphocytes, which surrounded them, were B-dependant. Two distinctly different types of lymphoid tissue are present in the spleen of chicken. One is seen along the arteries and arterioles as sheaths of small lymphocytes or as clusters of large and small lymphocytes, lymphoblasts and primitive reticular cells, which are thymus dependent. Second type is circumscribed round or oval lymphoid follicles, enclosed by a thin fibrous membrane, which always laid juxtaposition to a small artery. They resembled morphologically the follicle of the bursa of Fabricius and they are bursa dependant.

The white pulp of spleen appears as islands enclosed by red pulp and the distinction between the two pulps are not marked in Chicken (Miyamoto *et al.,* 1980; King and Mc Lelland, 1981). This could probably be the reason for clear zonal demarcation of white and red pulp and absence of marginal zone in chicken as in the spleen of

rodents (Jeurissen *et al.*, 1994). However, Ford (1975) opined that a marginal zone is present at the junction of white and red pulp in birds.

White pulp in the chicken is divided into four elements, such as periarterial lymphatic sheath (PALS), perivenous lymphatic tissue, periellipsoidal lymphatic tissue and germinal centres. The first elements appeared two days after hatching. The second, third and fourth elements appeared on the sixth day, third week and fourth week respectively (Ogata *et al.*, 1977). It is suggested that the lymph flow may starts from the region surrounding the PALS, and that the peripheral region of the PALS may also be another route for lymphocyte migration.

By understanding the structural and functional importance of PALS, the present study was designed to explore the histological and ultra structural details of the cellular components of PALS in chicken of different age groups.

Materials and Methods

Splenic tissue samples from 36 birds of six different age groups such as day-old, four, eight, twelve, twenty and forty weeks were collected. Six birds were used in each age group. The spleen was removed immediately after high cervical dislocation and fixed for light and electron microscopy (Kannan *et al.*, 2015). For light microscopic studies, tissue pieces were fixed in 10 % neutral buffered formalin and processed for paraffin embedding technique. Tissue sections were cut at 5 micron thickness and used for routine Haematoxylin-eosin staining method (Bancroft *et al.*, 2013).

For electron microscopic study, small pieces of splenic tissue (1–2 mm thickness) were collected and prefixed at 3 % glutaraldehyde and stored at 4°C. Subsequent processing, sectioning of tissue and staining were done as per Kannan *et al.* (2015). 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, the periarterial lymphatic sheath (PALS) was noticed in all the age groups except day-old as reported by Ogata *et al.* (1977) because they appeared only two days after hatching. PALS were observed as a diffuse lymphatic sheath adjacent to the central artery in all the age groups studied. It consisted of closely packed small lymphocytes and several medium to large sized lymphocytes and reticular cells (Fig.1). Few macrophages and plasma cells were also found towards the periphery of the PALS (Olah and Glick, 1982).

Transmission electron microscopy

Under electron microscope, the white pulp contained the central artery which was observed to have smooth muscle layer. The lumen of the artery was lined by endothelial cells and muscle cell. Adjacent to the central artery, a diffuse lymphatic tissue, the PALS was noticed with densely packed lymphocytes of various sizes and was also infiltrated with reticular cells (Fig.2). There were no structural changes noticed in the PALS of spleen in different age groups as reported by Raviola (1994) and Dellmann (1998).

Small and medium sized lymphocytes of PALS were round cells with a narrow rim of cytoplasm which contained a few mitochondria and rough endoplasmic reticulum (Fig.3). 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 lymphocyte (Fig.4) (Olah and Glick, 1982).

The large lymphocytes of PALS contained the nuclei with one or more nucleoli and the chromatin was found to be less condensed (Fig.5). 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. The lymphocytes concentrated close to the central artery were might be thymus dependent cells while those in the peripheral portion along with the plasma cells and macrophages are Bursa dependent lymphocytes as reported by Raviola (1994) and Dellmann (1998) in animals.

The cytoplasm of macrophages had vacuoles, phagocytosed materials, granules, mitochondria and endoplasmic reticulum. The nucleus appeared round to oval in shape with little chromatin. 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 (Burke and Simon, 1970). It also played a role in antigen presentation and secretion of mediators of the immune response in laboratory animals (Weiss, 1990).

The plasma cells showed a very large Golgi region and well developed rough endoplasmic reticulum within the cytoplasm (Fig.6). Conspicuous euchroamtin of the nucleus was a common feature of the plasma cell (Ogata *et al.*, 1977). Kopp (1990) reported that the heterochromatic nuclei did not reflect the inactivity as the small part of the genome that was euchromatic, was exceedingly active in maintaining the synthesis of many copies of a single antibody. Further, each plasma cell synthesizes and secretes antibodies that bind specifically to the antigen that initially activated the precursor B lymphocyte. Antigen-antibody binding is a major means of immune defense. Antibodies synthesized within the rough endoplasmic reticulum are processed and packaged within the Golgi prior to secretion.

Very few reticular fibres formed a meshwork around the periarterial lymphatic sheath separated it from the adjacent red pulp. Nerve fibres were seen in close association with the arteries of the white pulp as reported in Raviola (1994).

List of Plates and Legends

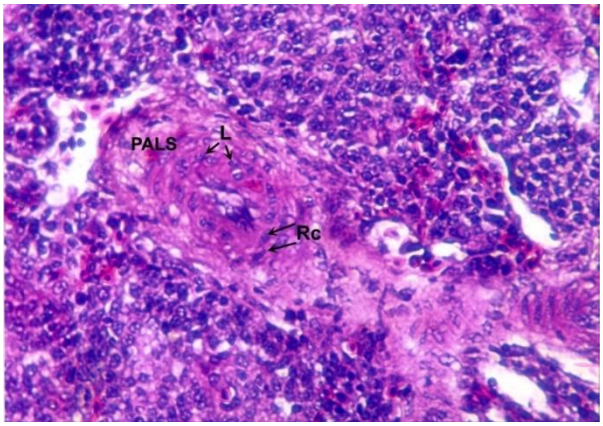

Figure-1 Photomicrograph of spleen of a day-old chick showing the PALS H & E x 400
L- Lymphocytes PALS- Periarterial lymphatic sheath Rc- Reticular cells

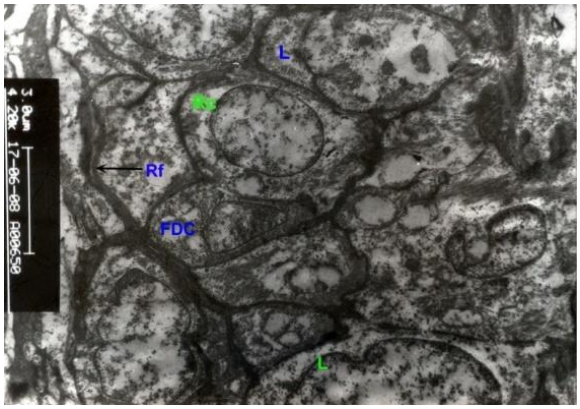

Figure-2 Transmission electronmicrograph of spleen of a twenty week-old chicken showing the tightly packed lymphocytes in the PALS x 4200
FDC- Follicular dendritic cell L- Lymphocyte
Rc- Reticular cell Rf- Reticular fibre

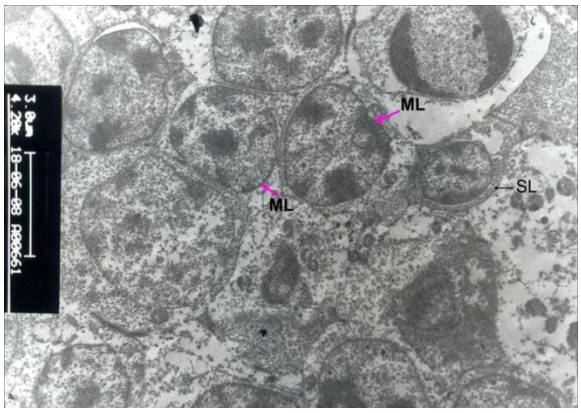

Figure-3 Transmission electronmicrograph of spleen of a twenty week-old chicken showing the small and medium sized lymphocytes in the cortex x 4200
ML- Medium sized lymphocytes SL- Small sized lymphocyte

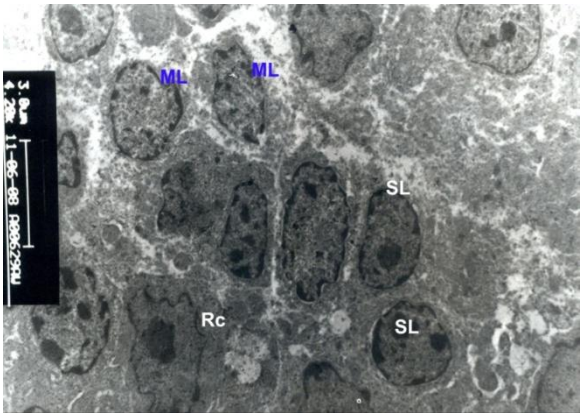

Figure-4 Transmission electronmicrograph of spleen of a day-old chick showing the Lymphocytic population x 4200
ML- Medium sized lymphocytes Rc- Reticuloepithelial cell SL- Small sized lymphocytes

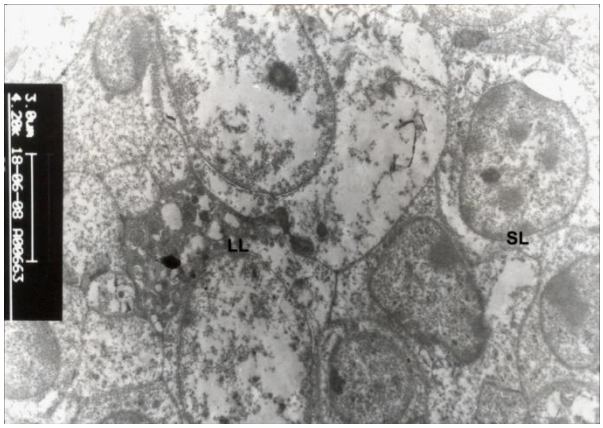

Figure-5 Transmission electron micrograph of spleen of a twenty week-old chicken showing the lymphocytes x 4200
LL- Large sized lymphocyte SL- Small sized lymphocyte

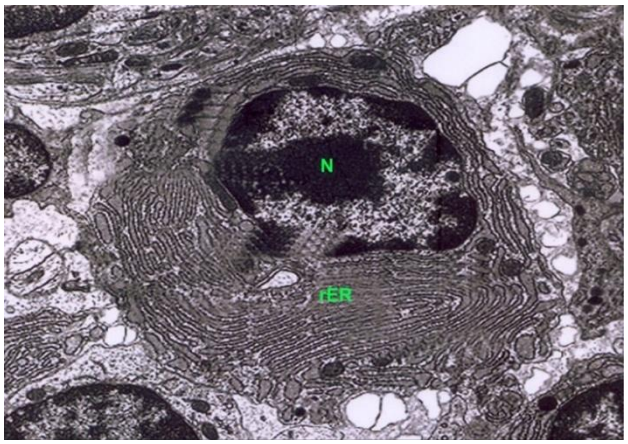

Figure-6 Transmission electronmicrograph of spleen of an eight week-old chicken showing the plasma cell x 7000

N- Nucleus of plasma cell rER- Rough endoplasmic reticulum

Acknowledgement

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

Conflict of Interest Statement

Hereby, the authors declare that they have no competing interests.

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