International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 4, Issue 2, pp: 53-57, 2019
https://doi.org/10.5281/zenodo.2589971
http://www.ijzab.com

Research Article

HISTOMORPHOLOGY AND CELL TYPES OF THE IMMUNE ORGAN (SPLEEN) IN *MYSTUS VITTATUS*

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Article History: Received 10th September 2018; Accepted 12th February 2019; Published 10th March 2019

ABSTRACT

The aim of this study was to investigate the morphologic and microscopic features of spleen as the important lymphatic organ on immune system of *Mystus vittatus* were recognized. A total of 60 adult fishes of both sexes, weighing 42-45 g (standard length 12-15 cm) collected from Vellar estuary, Parangipettai, Tamilnadu. After removing the spleen, it was immediately fixed in Bouin's solution and transport to the laboratory. The 5-6 µm sections were made using paraffin embedding techniques and stained by Haematoxylin and Eosin. The spleen as an elongated organ located on the middle part of digestive canal and microscopically was covered by a capsule of connective tissue and an epithelial layer. Spleen included white and red pulps. There are some similarities and some differences between spleen of this species and mammals. Spleen as the biggest and the most important lymphatic organ and microscopically very similar to spleen of mammals has many functions such as lymphatic cell production. Histomorphology of spleen shows that in *M. vittatus* is organized inside spleen and like some fishes, splenopancreas structure is present.

Keywords: *Mystus vittatus*, Spleen, Histology, Morphology, Histomorphology.

INTRODUCTION

The lymphoid organs can be classified roughly into two types: primary or central (antigen-independent) and secondary or peripheral (antigen-dependent). This classification is based on the antigen- dependence of cell proliferation and differentiation. It does not hold for lower vertebrates, including fish. The bone marrow in higher vertebrates is a primary organ, in which are found the pluripotent haematopoietic stem cells which differentiate into progenitors of myeloid cells (which in turn differentiate into granulocytes, monocytes, erythrocytes, and platelets) and lymphoid progenitors. Fishes are the earliest vertebrates that have a well developed immune system and lymphomyeloid tissues consist of mixed lymphoid and myeloid elements and are regarded as being less specialized in structure and function than corresponding mammalian tissues (Pitchappan, 1980). The main fish lymphoid organs are thymus glands, anterior kidney, spleen, and blood (Ankomah *et al*., 1995; Corbel, 1975; Ellis, 1980; Lin *et al*., 2005) which are commonly used as a source of lymphoid cells and in vitro experiments have been generally performed on heterogenisis population (Lymphocyte, monocytes, polymorphs, macrophages) but the yield of cells recovered from each organ had not been studied.

In adult anurans and all other higher vertebrates, hemopoietic stem cells, capable of reconstituting all lymphoid organs, are found in the bone marrow (Cooper, 1980). Fish do not possess bone marrow. However, hemopoietic bone marrow-like microenvironments are well developed in the kidney and spleen of the teleosts and in the chondrichthyes (sharks and rays), a variety of other organs such as the oesophageal wall (Leydig's organ), the liver and the gonads (Ankomah *et al*., 1995; Ellis, 1988; Fange & Mattisson, 1981). There is no available experimental data concerning the source of stem cells in fish but in teleosts, the kidney would appear important on ontogenetic and histological criteria. Heamopoiesis is first seen in the kidney early in ontogeny, through erythrocytes and macrophages are present before differentiation of the kidney so the yolk sac in probably the earliest organ of limited haemopoiesis (Ellis, 1980).

Lymphocytes do not appear until the thymus has

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become lymphoid. In the salmonidae, this is a few days before hatching (Grace & Bunney, 1980) whereas in the place (pleuronectes platessa) this does not occur until the time of metamorphosis (Lele, 1933). The information available on the structure and function of the spleen is from the studies conducted largely on mammals, and less is known of the spleen in lower Vertebrates. The spleen in fishes is generally similar to that of mammals. However, differences exist between mammalian Spleen and fish spleen with regard to the structure and functions. For example, capsular trabeculae system characteristic of mammalian spleen is not distinct in fish spleen (Ankomah *et al*., 1995). The distinction of the red and white pulps of the spleen Parenchyma is not clearly defined in fish. (Yoffey, 1985). The fish spleen serves several functions, most of which are poorly understood. According to Ferguson, (1976) in the teleost spleen macrophages transport materials from the ellipsoids to the so called melenomacrophages centres. One function of the ellipsoids in fish spleen may be trapping of antigen or antigen-antibody complexes, as a step in immune responses and in connection with degradation of material by macrophages (Fange & Nilsson, 1985).

The interactions observed between melanomacro-phages centres and lymphoid Issue indicate that these centres are also involved in immune responses (Fange & Nilsson, 1985). The structure and function of the ellipsoids and the melons macrophages centre; and the relationship between these structures and the lymphoid tissues of the fish spleen are insufficiently known and ought to be the subject of further investigation (Fange & Nilsson, 1985). One of the functions of mammalian spleen is haemopoisis (McCuskey, 1985). In teleosts, haemopoiesis goes on predominantly in the pronephros (head kidney) or in the kidney, but in some species the spleen is haemopoietically active (Ferris *et al*., 1965) Lymphocytopoiesis probably takes place in lymphoid areas of the spleen of both elasmobranches and teleosts. The present study described the structure of spleen in light microscopic level.

MATERIALS AND METHODS

Collection of fish

Live specimens of *Mystus vittatus* of the family Bagridae were collected from Vellar estuary, Parangipettai. A total of 60 adult fishes of both sexes, weighing 42-45 g (standard length 12-15 cm), was used in the present study. A stock of fish was maintained in the laboratory in a large tank. After acclimatization for at least three days, the fishes were transferred to smaller aquarium tanks with continuous aeration. They were fed with bits of earthworms and tubifex.

Morphology and Histology

The fishes were immobilized by immersion in ice-cold water for a few minutes and dissected. The location of spleen, its relation to other visceral organs and its blood supply were studied under binocular microscope. The spleen from the fish was dissected and fixed in Bouin's fluid for about 24 hours, processed, embedded in paraffin wax and cut into sections of 6μm thickness. The sections were stained by the following methods.

Heamatoxylin and Eosin

The sections were deparaffinised in xylene and hydrated through graded series of alcohol to distilled water. They were stained in aqueous Delafield's Haematoxylin for 10 minutes as suggested by Humason and washed in running water for about 10 minutes. For following dehydration through graded Series of alcohol, the sections were counter stained with eosin for 15 seconds. After further dehydration and clearing in xylene. The sections were mounted in DPX.

Preparation of imprints and Leishman stain

The imprints were prepared on a clean micro slide from cut surface of freshly dissected spleen and head-kidney. They were air-dried and stained by the Leishman stain. The slides with imprints were covered with 1 ml of Leishman stain for five minutes. During this period precaution was taken not to allow the stain to dry up. The stain on the imprints was diluted with distilled water and the mixture was allowed to stand for ten minutes and then drained off the slide. The stained imprints were washed in running tap water for two minutes, air-dried and mounted in DPX.

Cell Measurement

Calibration of the microscope was made using the stage and ocular micrometers. Measurement of the dimensions of different types of cells was made from the histological slides.

RESULTS AND DISCUSSION

The spleen of *M. vittatus* is a reddish-brown, elongated, thick and flattened structure, lying along the intestine and in the proximity of the pancreas, trunk-kidney and the air-bladder. It measures about 5-10 mm in length and 3-5 mm in width, and weighs about 0.5-0.9 g. At one point on the surface of the spleen is a deep indentation, the hilum, which appears to divide the spleen into two halves. This marks the entrance and exit of the splenic artery and vein, respectively. The splenic artery, a branch of the coeliaco mesenteric artery, enters the organ through the hilum. Immediately, it divides into two, each traversing the respective half of the spleen. On entering into the splenic parenchyma, the arteries branch off repeatedly into a number of arterioles that ultimately end in capillaries. Several venules arising from different parts of the parenchyma merge into a larger vein that emerges out through the hilum and joins the hepatic portal system. The veins and arteries run close to each other in most of the parenchyma (Figure 1).

The Spleen is surrounded by a connective tissue capsule consisting of fibroblasts and collagen fibres and a single layer of mesothelial cells. The capsule projects small trabeculae into the outer area of the splenic parenchyma.

Through the anterior end of the splenic capsule, the collagen fibres representing the trabeculae transverse the splenic pulp carrying blood vessels. This splenic blood vessel is trunk-like. The splenic parenchyma except the subscapular region is composed of red pulp and white pulp white pulp with outer clear boundaries (Figure 1 & 2). The interior of the organs contains red and white pulp. The red pulp is fully erythroid with very few lymphocytes. The white pulp comprises reticular center around the blood vessel. It is small and poorly developed, so no identifiable accumulation of lymphocytes is found with the stroma (Figure 1 & 2).

Red pulps is an extensive interconnecting system of splenic cords and sinusoids and constitute most of the splenic parenchyma some areas are occupied predominantly by erythrocytes and others show granulocytes, thrombocytes and macrophages Eosonophilic granulocytes are meagerly seen in the splenic parenchyma. Erythrocytes are found in groups. Ellipsoid is generally spherical to ovoid in shape (Figure 1 & 2), measure about 70-200 µm in diameter. Delineation of an ellipsoid from the surrounding sinus or from an adjacent ellipsoid is not easy though the presence of thin connective tissue strands could be seen in many places. An ellipsoid consists of a darkly staining central capillary surrounded by closely packed 3-4 cell-thick layer of macrophages and lymphocytes enmeshed in a network of reticular cells. In most of the ellipsoids, a clear space could be distinguished between the central capillary and the surrounding layer of cells, and similarly, between the cell layers and the surrounding trabecular connective tissue strand. In many instances, the ellipsoid capillary and the surrounding area appear depleted, and consequently, the reticular cells and their fibers become more evident.

Melanomacrophage centers (MMCs) in the spleen of *Mystus vittatus* are aggregations of closely packed macrophages that contain yellow to dark-brown colored pigments (Figure 3). The size of these centers are highly variable ranging from very small (39 µm × 17 µm) consisting of a few cells to very large (131 µm × 103 µm) with hundreds of cells. The shape is mostly spheroid though elongated and bean-shaped structures are not uncommon. The number of the MMCs is also variable, and it depends on the size i.e., if they are larger they are fewer and if smaller, numerous. The MMCs are invariably located close to the ellipsoid, sometimes, occupying almost the entire lymphoid area around the core capillary (Figure 2 & 3). There seems to be no definite capsule around these structures. Numerous melanomacrophages centers MMC or pigment nodules close to the blood vessels are dispersed thought out the red pulp which is numerous in the he Laboratory maintained fishes. The histological and imprint preparation of spleen consists of various cell types' namely small, medium and large lymphocytes, macrophages, granulocytes and erythrocytes (Figure 3 & 4).

Erythropoiesis taken place in red pulp where proerythrocytes basephilic and acidophallic erthroblasts can be distinguished. Proerythrocytes show a round shape with a mean diameter of 9µm have a scantly basophilic cytoplasm, the nucleus is large and measuring aunt 3.9 µm. Erythrocytes are formed by the lengthening of the proerythrocytes and becomes the mature erythrocytes. Lymphocyto poiesis takes place in which pulp lymphoblasts are 12 µm in average diameter its cytoplasm is scanty and basophilic. In the mature larger lymphocyte the nucleus is eccentric in position, which measures about 14 µm in average diameters. The macrophages are irregular shape and size will vary defense upon the size of the spleen. The nucleus is darkly stained and yellowish and some time block in colour. The granulocyte is round in shape with acidophilic cytoplasm and centrally located small nucleus (Figure 4). The location of the spleen in *Pomacanthus semicirculatus* is similar to that of the teleosts, where the spleen is located near and extends along the left side of the midgut is a feature in salmonidae. Whereas in *P. semicirculatus* the length is 6 to 8 cm and thickness 2 to 4 cm which is comparable to the measurement of the spleen of teleosts.

According to Foldi *et al*. (1983); Fange & Nilsson (1985); Lamers & De Haas (1985); Glenney & Petrie-Hanson (2006) as a rule there are no musucle cells in the capsule and tuberculae of the teleostean spleen which frequently contain islets of pancreas tissue and the lobules of the salmoidean spleen are sheathed off separately are placed in compartments of connective tissue. Whereas in catfish the splenic capsule is well defined and made up of collagen fibres (Deivasigamani, 2007). The spleens of domestic animals such as cat, pig, cattle, sheep and dog, and of man, in that order, are transitional types between the extreme types of horse and rabbit. They are scaled according to their relative weight, content of trabeculae and smooth muscles and the red and white pulps. There is a negative correlation between the per cent muscle in the trabeculae and the effectiveness of release of blood cells (Deivasigamani, 2007).

The Melamnomcrophage centres (MMCs) and ellipsoids cells are present in many tissue sites in fish especially in the mesenteries as free cells and in the splenic ellipsoids, the kidney and, at least in some species like the place, in the atrium of the heart, as reticuloendothelial cells (Ellis, 1980) in vivo and in vitro studies have demonstrated these cells to be highly phagocytic for inert and antigenic material (Ellis, 1988; Kohler *et al*., 1977). The Macrophage migration inhibition test has been used with positive results so presumably lymphokines like MIF may exert physiological influences on macrophages in fish. In the case of lymphoid tissue, melanin may protect against free radicals produced by phagocytic cells particularly neutrophils. Histology of spleen consists of various cell types namely lymphocytes, plasma cells granulocytes monocytes and erthrocytes (Deivasigamani, 2007; Sailendri & Muthukkaruppan, 1975). According to Corte and Sliney (1987), the imprints of spleen pro-erythro blasts and erthroid cells show a round shape.

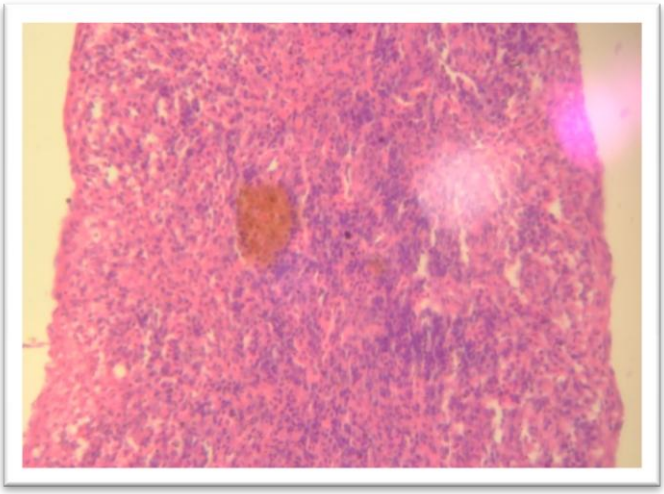

Figure 1. Section of the spleen of *M. vittatus* showing the presence of a definite connective tissue capsule (arrow), consisting of elastic fibers, melanomacrophage center (M), white pulp (W), red pulp (R). Bouin (HE) × 600.

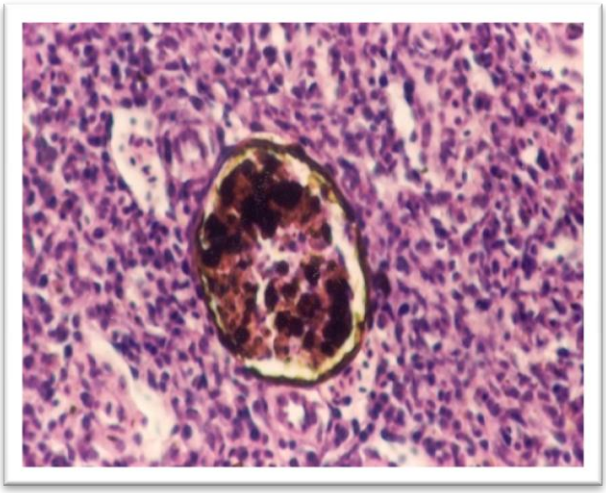

Figure 2. Section of the spleen of *M. vittatus* showing higher magnification of melanomacrophage center (arrow), L (lymphocyte), M (Macrophage), E (erythrocyte). Bouin, Bouin (HE) × 1500.

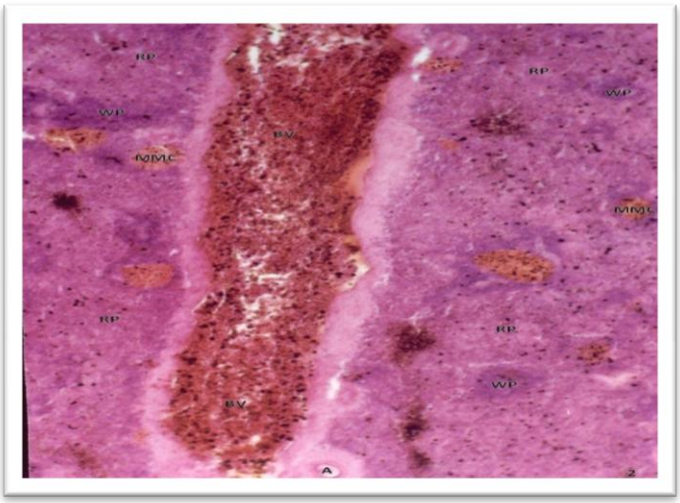

Figure 3. Section of the spleen of M. vittatus showing higher magnification of melanomacrophage center (MMC), artery (A), white pulp (WP), red pulp RP (), E (ellipsoids), BV (Blood vessels). Bouin, HE. × 1500.

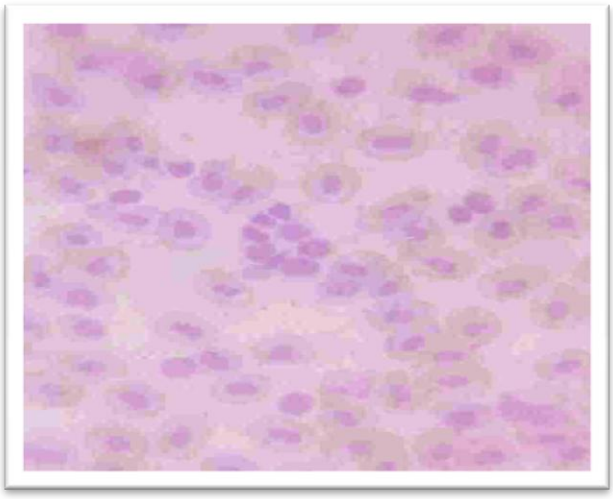

Figure 4. Imprint showing the different size of the lymphocytes (L) (small, medium and large), macrophages (M), granulocytes (G) and erythrocytes (E). Leishman stain. X. 1500.

Basophillic erthroblasts show a decrease in size ion the course of their maturation, they are smaller than proerythroblasts, more numerous erythroid cells and have the nucleus with clumped chromatic and nucleous not evident. Erythroblastic cells are clustered both in prints and histological sections of the spleen. Erythropoiesis takes place in red pulp where pro-erythrocytes, hasophillic and acidophilic erythroblasts can be distinguished. Pro-erythrocytes show a round shape with a mean diameter of 9 µm and have a scanty internally basophilic cytoplasm the nucleus is large and measuring about 3.9 µm.

CONCLUSION

Pro-erythrocytes show a decrease in width in the course of their maturation into erythrocyte and the nucleus becomes elongated. The cytoplasm is basophilic. Erythrocytes are formed by the lengthening of the proerythrocytes and became the mature erythrocytes. Erythrocytes are clustered both in imprints and histological sections of spleen. Lymphocyto poiesis takes place in white pulp lymphoblasts are 12µm in average diameters, its cytoplasm is scanty and basophilic. In the mature larger lymphocyte the nucleus is eccentric in position which measures about 14µm in average diameters based on the size, the lymphocytes are known as small lymphocylic 9 µm and large lymphocyte 12µm. The granulocytes are round in shape with a acidophilic cytoplasm and centrally located small nucleus the cytoplasm possess granulated appearance. The observations in the present study on the histomorphology of

the spleen of *M. vittatus*, such as the well developed trabecular system, predominance of red pulp and close association between the ellipsoids, lymphoid tissue and MMCs full of pigmented macrophages, indicate that this organ might be involved in multiple functions such as storage and release of blood, phagocytosis, destruction of the blood cells and immune responses. However, as also opined, the present knowledge on the structure and functions of the ellipsoids and MMCs, and the relationship between these structures and lymphoid tissue of the fish spleen is insufficient and therefore further investigations on a wide variety of species are needed to throw more light on these aspects.

ACKNOWLEDGMENTS

The author is grateful thank to Dean, CAS Marine Biology, Annamalai University, for providing the facilities to carry out the work for his support and encouragement.

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