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Phagocytic activities of splenic macrophages in Asian Sea Bass (*Lates calcarifer* Bloch, 1790)

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Abstract

Phagocytic process after intraperitoneal injection of colloidal carbon in four different doses to Asian sea bass (*Lates calcarifer* Bloch, 1790) has been documented. The fish spleen was found to play a central role in the fish immune system against invading foreign substances as thin sections of spleen melanomacrophage centre showed the carbon particle accumulation. Responses of immune systems to toxic substances might prove to be efficient biomarkers to evaluate water quality.

Keywords : antigen, colloidal carbon, macrophage, phagocytosis, sea bass, spleen

INTRODUCTION

In Southeast Asia, sea bass (*Lates calcarifer* Bloch, 1790) orange spotted grouper *Epinephelus coioides* Hamilton, 1822) and mangrove red snapper (*Lutjanus argentimaculatus* Forsskål, 1775) are important species as they command a high market price. They possess the non-specific defense mechanisms of the invertebrates such as the phagocytic mechanisms developed by macrophages and granular leucocytes. Kidney, spleen and liver are considered to be the main organs in fish where endothelial cells and macrophages eliminate undesirable substances from circulation (Dalmo *et al.,* 1997). The most important cells involved in this defense are the phagocytes (Esteban and Meseguer, 1997; Esteban *et al.,* 1998). Of the vast majority of cells circulating in the blood of fish, granulocytes and monocytes are the most important in the cellular aspects of the defense mechanism. During a histological study of blood cells Boomker (1981) found a cell resembling monocytes with a number of intracellular bacteria in bream (*Sarotherodon mossambicus* Peters, 1852). During an *in vitro* study, Ellis (1977) discovered that phagocytosis of yeast particles was performed principally by monocytes. Meseguer *et al.* (1994) and Herraez and Zapata (1986), described the morphology, formation and possible function of elanomacrophages and MMCs in the HK, together with those in spleen and liver, of the teleosts, sea bass *(Dicentrarchus labrax* Linnaeus, 1758) and gilthead sea bream (*Sparus aurata* Linnaeus, 1758).

The aims of the present study were to determine the phagocytic response of the splenic macrophages to intra-peritoneal injection of colloidal carbon in seabass (*Lates calcarifer* Bloch, 1790).

MATERIALS AND METHODS

Fish

Twenty juvenile Asian sea bass, (*Lates calcarifer* Bloch, 1790) weighing between 45- 80 g were obtained from RGCA Hatchery, Karaikal, Tamil Nadu. Fishes were acclimatized in fiber tanks with UV treated sea water with a salinity range of 25-30 ppt and temperature between 25° and 30° C. The fish were fed with commercial fish pellets (4% body weight). The water in the tank was continuously aerated and re-circulated using a biofilter. Daily 15% of the total water volume was replaced by fresh aerated sea water. Natural photoperiod was maintained during the acclimatization period and no mortality occurred during this period.

Carbon injection

A carbon suspension containing 100 mg/ml carbon was prepared according to modified method suggested by Deivasigamani (2002). The suspension was diluted using distilled water to make dilutions containing 2 mg, 4 mg, 8 mg, 16 mg and 32 mg carbon/ml. Carbon dilutions were sterilized by autoclaving, and cooled. Fifteen sea bass fish were divided into five groups and were marked based on the dose of carbon to be administered. Control fish were injected with 0.2ml of sterile distilled water. One test fish and controlled fish were sacrificed at 1, 6, 12, 18, 24h intervals after injection.

Histopathology

The abdominal cavities were then opened; spleens were removed. The organs were fixed in phosphate buffered formalin. Paraffin sections at 6 µm were stained by the standard hematoxylin and eosin (Deivasigamani, 2007). Calibration of microscope was made using stage and ocular micrometers. Measurements of the dimensions of the different types of cells and those of MMCs (melanomacrophage centers) were made under light microscope.

RESULTS AND DISCUSSION

Asian sea bass (*Lates calcarifer*) is a marine species with great economic importance, particularly in Asian countries. However numerous pathogenic viruses, bacteria, fungi and parasites affect the species causing various infectious diseases. Phagocytosis is the

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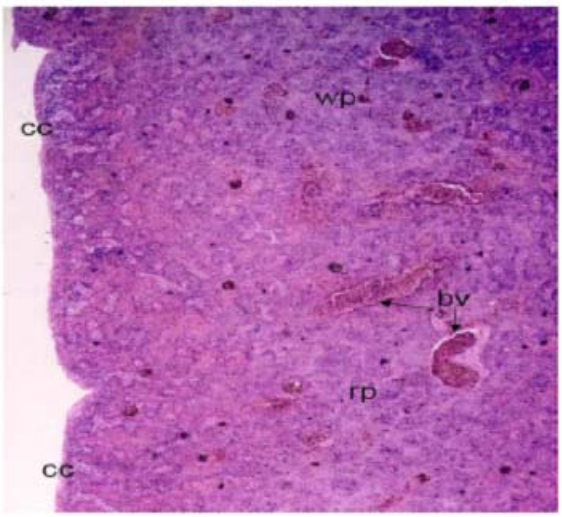

Figure 1. Control section of the spleen of *Lates calcarifer* showing the presence of a definite connective tissue capsule (cc), red pulp (rp), white pulp (wp), blood vessel (bv) consisting of elastic fibers. Bouin H & E. X 230

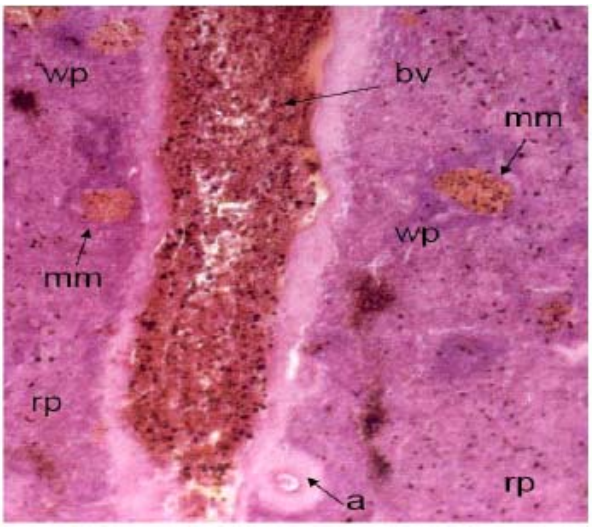

Figure 2. Control section of the spleen of *Lates calcarifer* showing melanomacrophage centres (mm), artery (a), red pulp (rp), white pulp (wp), blood vessel (bv) Bouin, HE. X 600.

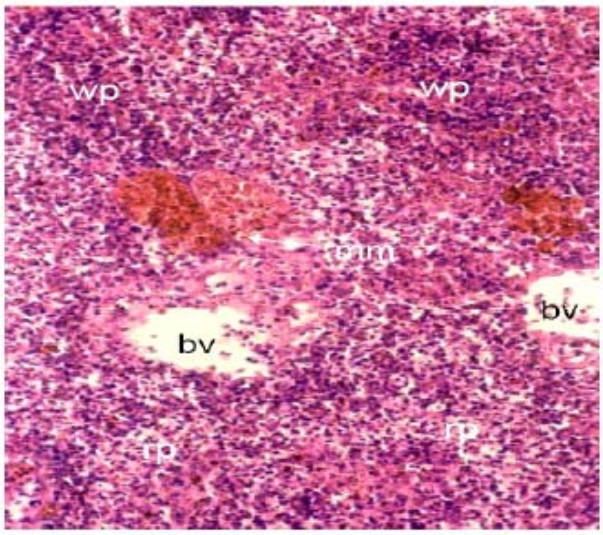

Figure 3. Carbon particles injected fish spleen showing enlarged melanomacrophage centres (mm), red pulp (rp), white pulp (wp), blood vessel (bv) Bouin, H & E. X 230

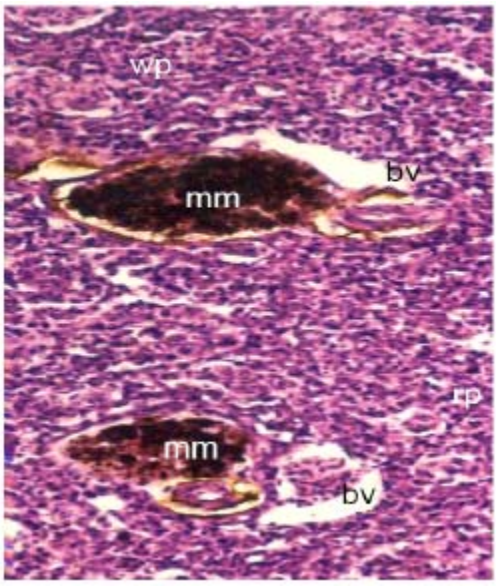

Figure 4. Carbon particles injected fish spleen showing higher magnification of enlarged melanomacrophage centres (mm) and blood vessel (bv), red pulp (rp) and white pulp (wp). Bouin, H & E. X 1000.

principal function of the teleosts fishes and their non-specific immunity is similar to those in higher vertebrates, mainly melanomacrophage centers, neutral granulocytes and thrombocytes (Adeyemo *et al.,* 2002).

The spleen of sea bass is a reddish-brown, elongated, thick and flattened structure, lying along the intestine and in the proximity of the pancreas, trunk-kidney. It measures about 8-12 mm in length and 3-5 mm in width, and weighing about 0.5-0.9 g. The spleen of sea bass is covered by a very definite capsule of connective tissue (Fig. 1).

The spaces within the connective tissue framework are filled with splenic pulp, the distinction of which into red pulp and white pulp is rather difficult in histological preparations. The pulp in general consists of large venous sinuses and their branches, representing the red pulp (RP), white pulp (WP), nodule-like ellipsoids of lymphoid tissue and dark-staining melanomacrophage centers (Fig. 2).

The MMCs are invariably located close to the ellipsoid, sometimes, occupying almost the entire lymphoid area around the core capillary (Fig. 2). There seems to be no definite capsule around these structures. The splenic pulp of sea bass includes a plexus of red pulp, ellipsoids and MMCs representing the white pulp. The venous sinuses and the red pulp are erythrocytic in nature though a number of other cells such as monocytes, eosinophils etc. could be identified. Since no mitotic cells or blast cells could be observed in this study, a function of haemopoiesis cannot be attributed to the spleen of this species. However, in a few species of elasmobranchs and teleosts haemopoiesis has been reported to occur in the spleen. For example earlier Fey (1965) and Zapata (1980) reported that erythrocytes and thrombocytes are produced in the splenic red pulp of elasmobranchs.

In the present study after injection of carbon particles, fishes were sacrificed with different time intervals (every 6 hours) and dissected to remove their spleen which were preserved in 10% formalin. Highly crowded white pulp and red pulp areas observed in thin sections showed that the immune response and proliferation of red blood cells and lymphocytes, respectively (Fig. 3). The melanomacrophage centre was found to consist of highly packed carbon particles (Fig. 4). Thus study showed that the spleen plays a vital role in sea bass immune system *i.e.* in the destruction of foreign particles. Meseguer (1997) and Esteban *et al*. (1998) also opined that fish monocyte-macrophages may be considered the most active phagocytic cell type. The phagocytic process of *O. niloticus* splenic leucocytes was found to be show similar ultrastructural features to those described for sea bass *D. labrax* (Mesequer *et al.,* 1994; Esteban *et al.,* 1998).

This study had clearly demonstrated that macrophages and leucocytes are responsible for phagocytic activities in the spleen in sea bass. Such toxic responses of the immune system may serve as biomarkers to monitor pollution and water quality and as such fish immunotoxicology might possibly be a new tool to evaluate the water quality.

REFERENCES

Adeyemo, O.K., Agbede, S.A. and Magaji, A.A. 2002. Clearance of colloidal carbon from the blood of tilapia (*Oreochromis niloticus L.*). *Vet. Archiv*., 72: 109-118.

Boomker, J. 1981. The haemocytology and histology of the haemopoietic organs of South African freshwater fish III. Onderstepoort. *J. Vet. Res*., 48: 185-193.

ELLIS, A.E. 1977. The leucocytes of fish: a review. *J. Fish Biol*., 11: 453-491.

Dalmo, R.A., Ingebrigsten, K. and Bogwald, J. 1997. Non-specific defence mechanisms in fish, with particular reference to the reticulo-endothelial system (RES). *J. Fish Dis*., 20: 241-273.

Deivasigamani, B. 2002. Histomorphology and hitophysiology of immune organs of the balgrid catfish *Mystus gulio* (Hamilton). Ph.D. dissertation, University of Madras, Chennai, India.

Deviasigamani, B. 2007. Structure of immune organ in edible catfish, *Mystus gulio*. *J. Environ. Biol*., 28: 757-764.

Esteban, A. and Meseguer, J. 1997. Factors influencing phagocytic response of macrophages from the sea bass (*Dicentrax labrax* L.) an ultrastructural study. *Anat. Rec*., 248: 533-541.

Esteban, M.A., Mulero, V., Munoz, J. and Meseguer, J. 1998. Methodological aspects of assessing phagocytosis of *Vibrio anguillarum* by leucocytes of gilthead sea bream (*Sparus aurata L.*) by flow cytometry and electron microscopy. *Cell Tissues Res*., 293: 133-141.

Fey, F. 1965. Haematologiche Untersuchungen der blut- bildenden Gewebe niederer Wirbeltiere. *Folia. Haemat*., 84: 122-146.

Herraez, M.P. and Zapata, A.A. 1986. Structure and function of the melano macrophage centers of the gold fish, *Carassisus auratus*. *Vet. Immunol. Immunopathol*., 12: 117-126.

Meseguer, J., Ruiz, A.L. and Esteban, M.A. 1994. Melano-macrophages of the seaqater teleosts, sea bass (*Dicentrax labrax*) and gilthead Sea bream (*Sparus aurata*): Morphology, formation and possible function. *Cell Tissue Res*., 277: 1-10.

Zapata, A. 1980. Splenic erythropoiesis and thrombopoiesis in elasmobranches: An ultra structural study. *Acta. Zool*., 61: 59-64.