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STUDY OF MACROPHAGES FROM SPLEEN OF TILAPIA (*Oreochromis* sp.) TO EVALUATE IMMUNE MECHANISMS IN FISH

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ABSTRACT

Increased understanding of fish antimicrobial host defences is an important step in aquaculture setting. Much of the information of macrophage biology comes from research in mammalian models, where distinct macrophage subsets have been noticed, including classically activated cells (M1) and alternatively activated cells (M2) which are associated with “kill” or “heal” response. In teleosts, the best characterized macrophage phenotype is that comparable to the M1 activation state, which act to induce inflammatory responses. Recent fish immunology research has focused on fish macrophage biology. In present research, the understanding of teleost macrophage morphology, study of phagocytosis, cell aggregation and functional heterogeneity has been addressed. Analysis from tilapia (*Oreochromis* sp.) splenic macrophages showed numerous macrophages as free or in aggregates (MAs). Formation of filopodia like extension, attachment of charcoal particles on cell surface, cell fusion, poorly organized cell aggregation was noticed. Numerous studies have shown an increase in number and size of macrophage aggregates (MAs), Melano-Macrophage Centres (MMCs) in fish collected at contaminated sites. These results can be used to study the role of macrophages against various immunomodulators.

Keywords: Macrophage aggregates (MA), Spleen, Phagocytosis, Cell – cell fusion

INTRODUCTION

A range of environmental pollutants target the immune system of vertebrates including fish. Human activities have impacted on the ecosystems for a long time and the situation tends to worsen on a global scale [1]. Aquatic ecosystems are among the most polluted ones, resulting in drastically decreased biodiversity and impaired health of the aquatic biota, including fish [2].

Fish can bioaccumulate chemical toxicants, and react to environmental changes by altering immune system [3]. Macrophages play a significant role in defense mechanisms of all vertebrates against pathogens. Two macrophage subsets have been noticed in vertebrates, including classically activated cells (M1) and alternatively activated cells (M2) which are related to “kill” or “heal” response [4, 5]. In teleosts, the best characterized macrophage phenotype is that comparable to the M1 state, which is associated to inflammatory responses [5, 6].

The aim of the present study was to investigate the morphology, phagocytic behavior, and aggregation of macrophages from spleen in teleosts. Macrophage aggregation (MA) have been used in biomonitoring studies, as because fish can indicate about the pollution status of their ecosystems [7].

Wolke R.E. (1992) stated macrophage aggregates (MAs), or melano-macrophage centers (MMC), are not confined to fish and have been observed in other poikilothermic vertebrates. The aggregations are most commonly found in the spleen, head kidney and liver, especially in relation to inflammation [8]. In the lower fishes (Agnatha, Chondrichthyes) the pigmented macrophage cells are found to be solitary or in small, irregularly shaped aggregations in liver tissue. In the Osteichthyes, greater numbers of cells and aggregates are noticed than in the lower fishes. These aggregates are nodular, and they occur more commonly in the spleen and kidney rather than the liver [8].

MATERIALS AND METHODS

Alive fish samples of tilapia (*Oreochromis* sp.) were collected from local markets and dissected in lab. Tissues from spleen were removed from tilapia (*Oreochromis* sp.) (Fig. 1) and mashed in (0.1) M phosphate buffer saline (pH 7.2) in presence of trypsin- EDTA and tryton X 100. Cell suspension was smeared on glass slides and incubated at 37 °C. After incubation the nonadherent cells were removed by washing with PBS. The adherent macrophages were fixed by methanol and stained by Giemsa to study free or macrophage aggregation on slides. Activated charcoal particles in normal saline (0.9% NaCl) was used for phagocytosis study.



Figure 1: Isolation of tissues from spleen of tilapia in the laboratory

RESULTS

Result showed numerous macrophages as free (**Figure 2**) or in aggregates (MAs) on slides. Splenic macrophages (SM) showed the property of phagocytosis like formation of pseudopodia and filopodia like extension (**Figure 2**), attachment of charcoal particle on

macrophage surface, internalization of charcoal particles (**Figure 3**) and formation of food vacuoles/endosomes (**Figure 4**). Tendency of cell-cell fusion was noticed (**Figure 5**). Present result showed poorly organized and irregularly shaped SM aggregations (**Figure 6**).

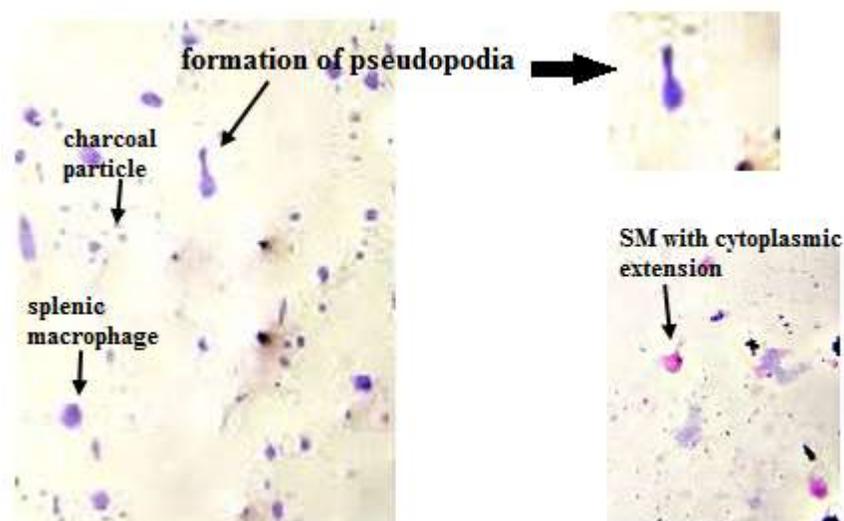


Figure 2: Formation of pseudopodia or filopodia like extension in splenic macrophages (SM) (x100)

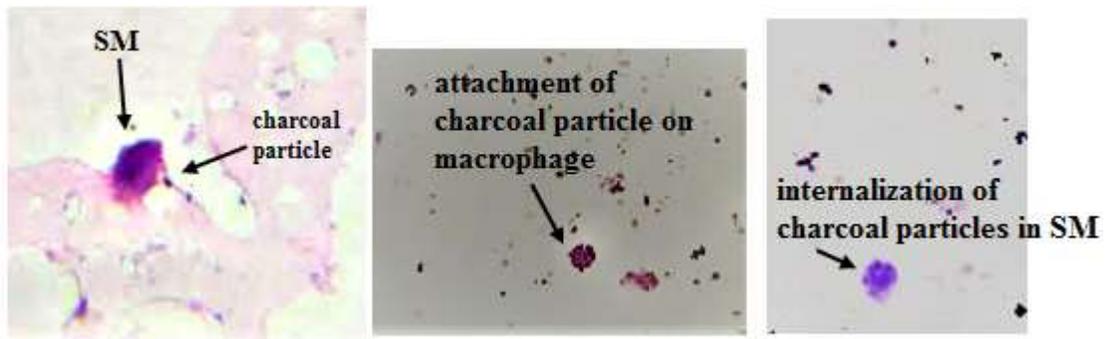


Figure 3: Attachment of charcoal particle on macrophage surface, internalization of charcoal particles in SM (x400)

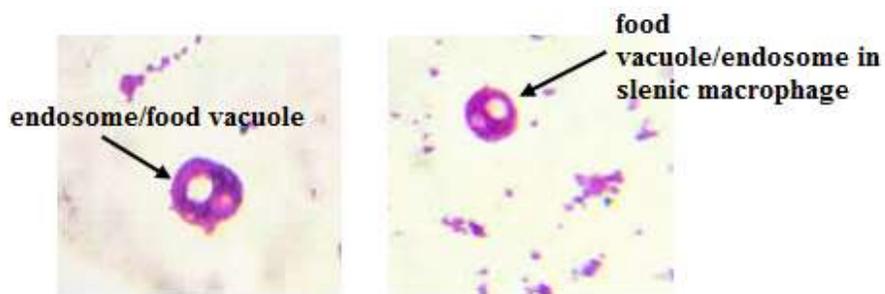


Figure 4: formation of food vacuoles indicated by arrow (x400)

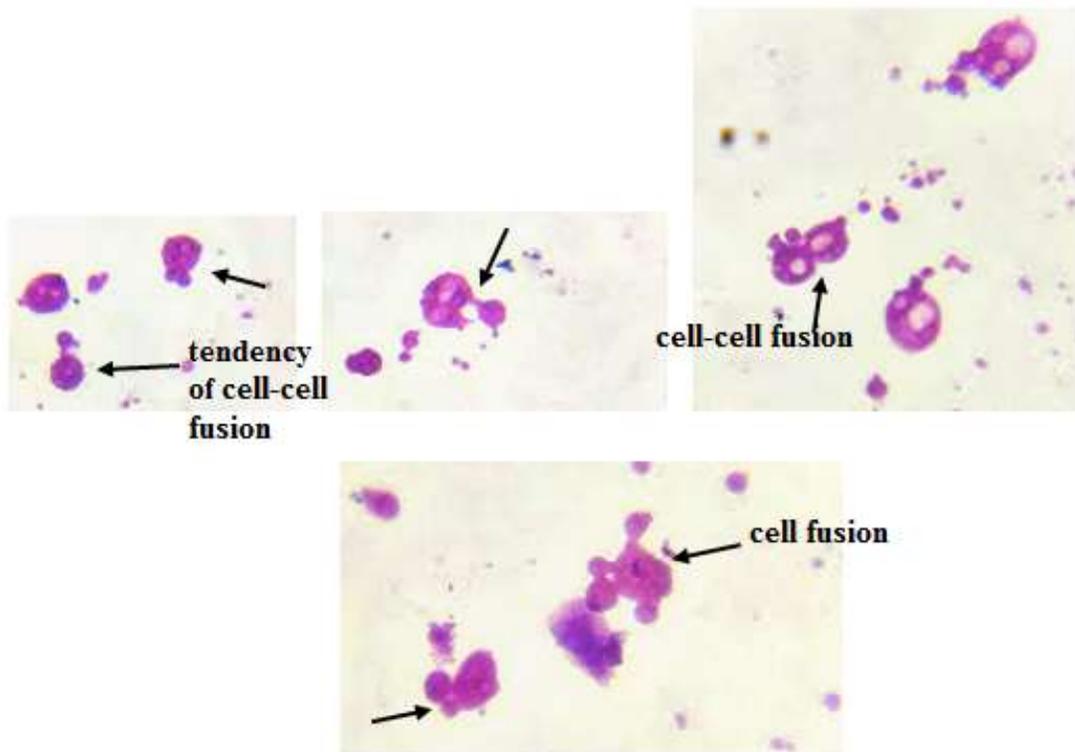


Figure 5: Formation and progression of cell-cell fusion indicated by arrow (x400)

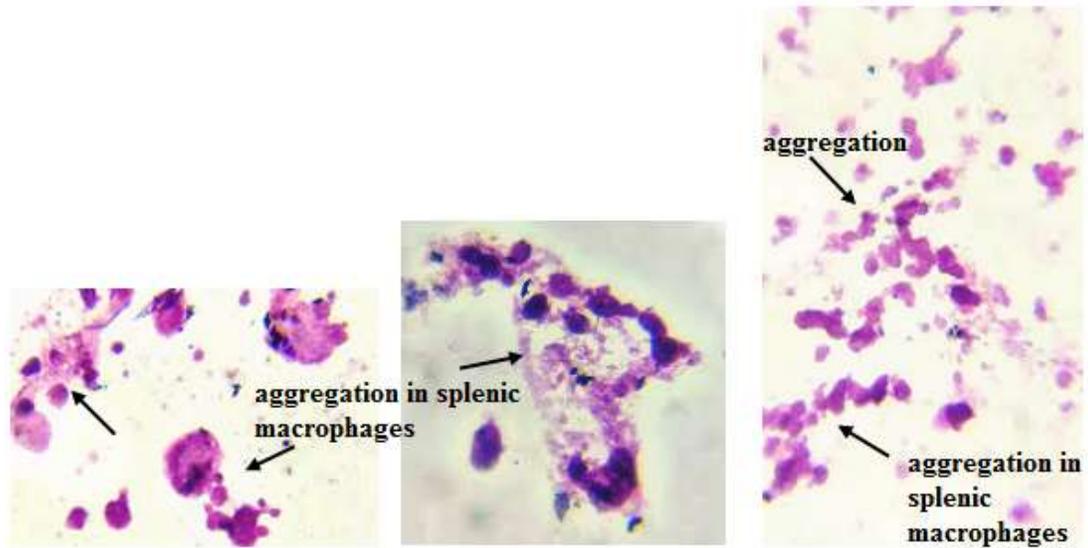


Figure 6A: Giemsa-stained stages of aggregation in splenic macrophages (SM) in tilapia (x400)

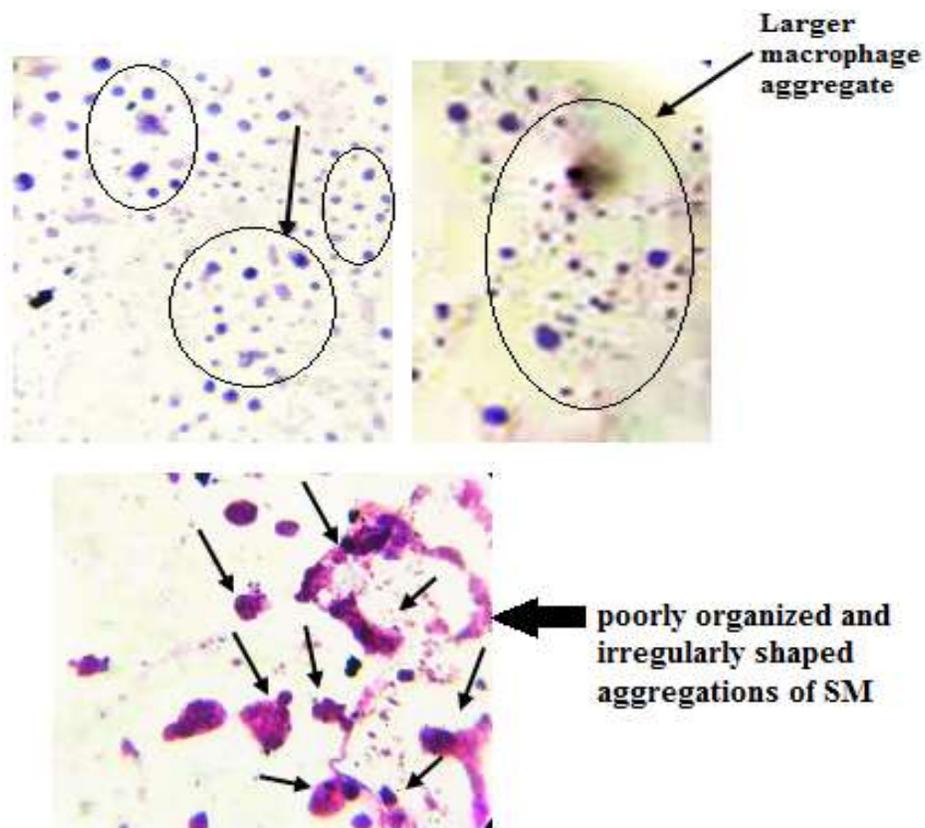


Figure 6B: Larger macrophage aggregates tended to be ovoid in shape, and smaller irregular in shape, poorly organized macrophage aggregates found in tilapia (x400)

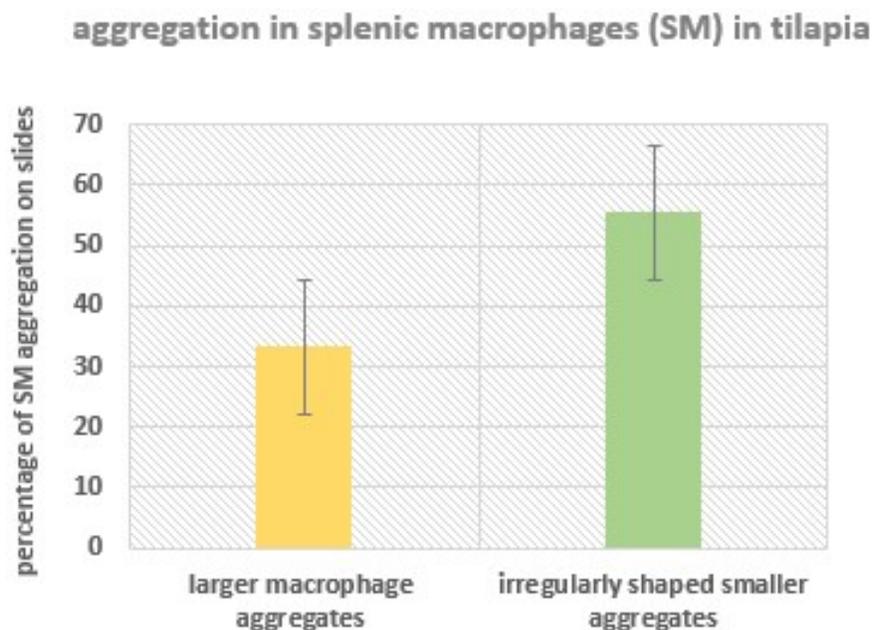


Figure 6C: Mean percentages of larger macrophage aggregates and smaller irregular in shape, poorly organized macrophage aggregates noticed on glass slides
Values are expressed as Mean \pm SEM

DISCUSSION

It is well known that negative effects of pollutants in water ecosystems have influences the whole immune system, and specifically including macrophage aggregates (MAs) [9]. Macrophage aggregates (MAs) are accumulations of pigmented macrophages occurring in hematopoietic and hepatic tissues of teleost fishes [8]. They can also occur in other organs including gonads of fishes captured from degraded and polluted environments [10]. MA are stimulated by noxious foreign materials, poor nutrition, infection, and anthropogenic contamination [2].

Researchers used MAs as an indicator of environmental stress, which increased with

starvation [2, 11]. Fishes are used as sentinel species in ecotoxicology studies and the utility of MAs as a biomarker for contaminant exposure has been demonstrated in several studies [12, 13].

Evidence indicates macrophage aggregates are associated with immune, and inflammatory responses, storage, destruction, or detoxification of exogenous and endogenous substances [8].

In present result, SM showed formation of pseudopodia, attachment of charcoal particle on surface, internalization of charcoal particles and formation of food vacuoles or endosome (Figure 2, 3, 4). In the present result, formation and progression of cell-cell fusion and cell aggregation was noticed

(Figure 5, 6A, 6B). The result also showed higher percentage of smaller, irregularly shaped, poorly organized macrophage aggregates than larger macrophage aggregates on glass slides (Figure 6C).

CONCLUSION

These results can be used to study the role of splenic macrophages against various immunomodulators. These results corroborate the previous studies [14]. Morpho-functional alteration of macrophages can be used as bio indicator to environmental pollution [14].

Variations in the number of MAs indicate stress on the fish. Although MAs occur in the spleen, head kidney, and liver of most teleosts, it is splenic MAs that best serve as a reliable histopathological bioindicator of fish health and environmental degradation.

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