

Elasmobranchs have a low incidence of disease and their immune cells have been identified as possible sources of novel tumour cell inhibitors. The basic anatomical structure of the elasmobranch gut is similar to that of other vertebrates, with a striking exception of the presence of a spiral intestine that provides an enlarged surface area for digestion and absorption of food by means of spiral folds. During recent years, considerable number of studies have described the immune system of teleosts, whereas little effort has been directed towards studying immunity in elasmobranchs. The lack of knowledge on immune cells in the intestine of elasmobranchs prompted us to carry out present study. During 2018, 13 blackmouth catshark *Galeus melastomus* and 15 specimens of the thornback ray *Raja clavata* were provided by a trawl fleet from the Gulf of Asinara (Sardinia, western Mediterranean Sea). Histochemical, immunohistochemical and ultrastructural observations were conducted on a subsample of spiral intestine of these fish species. Regarding *R. clavata*, three types of granular cells were identified; type I in epithelium, types II and III in lamina propria-submucosa, with each of them containing cytoplasmic granules with distinct ultrastructural characteristics. Immunostaining of intestinal sections showed the reactivity of granular cells: type I cells were positive for lysozyme, mast cell tryptase and tumor necrosis factor- α (TNF- α based on antibody staining; type III cells were immune-reactive to anti-interleukin 6 (IL-6) antibody, whilst type II cells were negative to all the antibodies used. Additionally, in *G. melastomus*, our studies identified unique, large granular cell type in intestinal epithelium. Electron microscopy showed that the epithelial granular cell type made intimate contact, by means of junctional complexes, with adjacent epithelial and mucous cells. Histochemical staining showed the cytoplasmic granules to be strongly eosinophilic and stained positively to periodic acid-schiff and Alcian blue. Immunostaining of intestinal sections revealed immunoreactivity of the granular cell to TNF- α antibody. However, no reactivity to inducible-nitric oxide synthesis, IL-6, interleukin IL-1 β , lysozyme, serotonin 5-HT antibodies was detected. Comparison of each granular cell type in both elasmobranch species with immune cells of teleosts or mammals and a hypothesis on their nature and function would be described.

Keywords: Elasmobranchs; spiral intestine; lysozyme; cytokines; transmission electron microscopy

Corresponding author.

E-mail address: dzb@unife.it (B. Sayyaf Dezfuli).

* These authors have contributed equally to this work.

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Liza ramada innate immunity against intestinal microparasite, *Myxobolus mugchelo* (Myxozoa)

B. Sayyaf Dezfuli^{1,*}, G. Bosi^{2,*}, M. Lanzoni¹, F. Pironi¹, G. Castaldelli¹, L. Giari^{1,*}.

¹ Department of Life Sciences & Biotechnology, University of Ferrara, St. Borsari 46, 44121 Ferrara, Italy

² Department of Health, Animal Science and Food Safety "Carlo Cantoni", Università degli Studi di Milano, St. Trentacoste 2, 20134 Milan, Italy

Abstract

Thinlip mullet *Liza ramada* is the most abundant of mullet species that live in the Comacchio lagoons (Northern Adriatic Sea, Italy). Histological and ultrastructural sections of the intestine of *L. ramada* in 30 specimens evaluated, showed that over 80% of specimens were infected with intestinal parasite of mullets, *Myxobolus mugchelo* (Myxozoa). The 'Myxozoa Grassé, 1970', is a phylum composed of over 2400 species. With reference to parasites of mullets, records on myxozoans species encountered in intestine and intestinal mesentery of worldwide mullets revealed that among 12 species of myxosporeans that infect *Liza ramada*, five belong to genus *Myxobolus*. In histological sections of the infected intestine, plasmodia of *M. Mugchelo* appeared rounded to ovoid and spindle shaped. *M. mugchelo* conspicuous plasmodia were encysted often in muscle and submucosa layers. In the muscle layer, there was no intense host inflammatory response. Plasmodia containing mature spores were situated closer to mucosal folds and were surrounded by numerous immune cells, mainly mast cells (MCs). Mature spores were generally oval in shape, they were noticed in paracellular space among the enterocytes or within them. Near the infected epithelial cells, several MCs, rodlet cells and few neutrophils were noticed. Indeed, degranulation of the MCs was very frequent in this region. In intestinal epithelium, some large cells with intracellular spores of *M. mugchelo*, resembling macrophages were documented. Some of these macrophages appeared foamy in aspect and possess elongate striated granules and frequently with enclosed necrotic debris. In some parasitized intestines, sections of epithelium were either completely substituted by spores of *M. mugchelo* or the spores were noticed inside the damaged enterocytes with spores exuded into the intestinal lumen due to destruction of these cells. These findings enabled us to postulate upon the life cycle of *M. mugchelo* and the migration of the mature spores through the intestinal wall to reach the lumen. Immunohistochemical analysis on sections of intestinal tissue of uninfected and infected *L. ramada* revealed positivity of epithelial macrophages to anti-histamine, -leuencephalin or -serotonin antibodies. The macrophages often showed at the plasma membrane and were seen close to the mucous cells and epithelial cells with intracellular spores of *M. mugchelo*. In the areas of epithelium infected with spores, epithelial cells positive to proliferating cell nuclear antigen (PCNA) were also observed. The current study is the first record on occurrence of intraepithelial macrophages that engulfed myxozoan spores. **Keywords:** Mullet, *Liza ramada*; intestine, microparasite, mast cells, macrophages

Corresponding author.

E-mail address: dzb@unife.it (B.S. Dezfuli).

* These authors have contributed equally to this work.