Abstract

The immune system is responsible for maintaining homeostasis by the initiation and control of the cellular and humoral inflammatory mechanisms in response to inherent or external factors. Innate immune system repertoire, as the first response system, is used as indicator of fish immune status. Most studies performed have been focused on the acute responses of the innate immune mechanisms of fish after inflammation and therefore few data exist about their long-term response. Therefore, this study intends to gather deeper insights on the European seabass (*Dicentrarchus labrax*) immune responses to chronic inflammation in a way to assess new biomarkers for this health condition. A total of 48 European seabass juveniles (initial body weight: ± 300 g) were randomly distributed by 2 tanks (24 individuals/tank) and injected with 100 µl of Hank's Balanced Salt Solution (Control-CTRL) or 100 µl of FIA in the peritoneal cavity, after a 24 h fasting period. Fish were fed two times a day (1.5% total biomass) during 21 days. Six European seabass were sampled from each tank at 7, 14 and 21 days post-injection. Fish hematology (total red and white blood cells and hematocrit), total peritoneal leucocytes counting, analysis of plasma humoral parameters (lysozyme, proteases activities, immunoglobulin M and proteins) and blood respiratory burst were performed. Regardless time, fish from FIA presented an increased proteases activity compared to CTRL. At 7 days following intraperitoneal infection, individuals from FIA increased the lysozyme activity compared to CTRL. Regarding to respiratory burst activity, values from European seabass injected with FIA increased at 21 days post-injection compared to individuals sampled at 7 and 14 days. Moreover, a higher activity was also observed in individuals from FIA treatment at 21 days compared to those from CTRL. Total peritoneal cells also increased in European seabass injected with FIA from 7 to 21 days, compared to CTRL individuals. Results from the present study suggest that European seabass immune response to FIA is still increasing after 21 days with higher levels of cells migration to the peritoneal cavity and increased blood respiratory bust activity at that time. Other molecular and metabolic markers are being assessed to get deeper insights on the response of European seabass to chronic inflammation.

keywords: Chronic inflammation, immune status, neutrophils, leucocytes, peritoneal cells.

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P-030.

Molecular characterization of a pattern recognition protein LGBP highly expressed in the early stages of mud crab *Scylla paramamosain*

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Abstract

The early developmental stages of the mud crab *Scylla paramamosain* suffer from high mortality caused by pathogen infections; however, few immune associated factors are known. Lipopolysaccharide and β-1,3-glucan-binding protein (LGBP) functions as a typical pathogen recognition receptor and plays an important role in the innate immune system of invertebrates. In this study we characterized a LGBP gene (SpLGBP) which was highly expressed in the late embryonic, zoea I larval stage and hepatopancreas of S. paramamosain.. It encodes 364 amino acids, composed of several conserved domains like the bacterial glucanase motif. The recombinant SpLGBP protein (rSpLGBP) was obtained through the *E.coli* expression system, in which two 6.His-tags were added to both C and N terminals during vector construction for the improvement of purification efficiency. In vivo the study showed that the SpLGBP mRNA was significantly up-regulated under *Vibrio parahaemolyticus* and a lipopolysaccharide (LPS) challenge in the hemocytes and hepatopancreas. The ELISA binding assay in vitro indicated that the rSpLGBP was capable of binding to LPSs and peptidoglycan (PGN). The rSpLGBP could agglutinate both G+ and G- bacteria in the presence of Ca2+. Our results suggest that SpLGBP may play an immunological role against pathogenic infection in the early developmental stages of *S. paramamosain*.

keywords: Ca2+ dependent, lipopolysaccharide and β-1,3-glucan-binding protein (LGBP), LPS, recognition, *Vibro parahaemolyticus*

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P-031.

The spleen in the humoral immune response of turbot (*Scophthalmus maximus*) to vaccination with the ciliate parasite *Philasterides dicentrarchi*

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Abstract

Fish spleen is an organ rich in lymphocytes, particularly, in B lymphocytes, playing a relevant role in the adaptive immune response after vaccination. After fish vaccination by intraperitoneal injection, there is a strong migration of antigen containing cells to the spleen, where antigen presentation occurs. In the present study, we have analysed the B cell populations (IgM, IgT or IgD positive cells) and the gene expression (IgM, IgT, IgD, MHCII, and several immune related genes) in the spleen of turbot immunized with a vaccine containing an oleous adjuvant and a particulated *Philasterides dicentrarchi* antigen. Fish were immunized at days 0 and 30, and samples were obtained at dasy 3, 7, 33, 37 and 60. The vaccine provoked a significant increase in specific and total serum IgM at 37 dpi and at 60 dpi, but the specific IgT levels did not vary significantly in vaccinated fish. No significant regulation of sIgT, mIgT, sIgM, mIgM and IgD was found before 37 dpi, whereas at 37 and 60 dpi, overexpression of sIgT and mIgT was detected in fish injected with adjuvant alone or with the vaccine. The identification of B lymphocytes by immunofluorescence allowed their classification in four groups: IgM+IgD-IgT- (the majority of IgM+ cells), IgM+IgD+IgT-, IgM-IgD+IgT- (very few cells; which probably correspond with lymphocytes with low levels of IgM), and IgM-IgD-IgT+ cells. The IgM+ and IgT+ cells were scattered throughout the parenchyma, and grouped around large vessels and surrounding melanomacrophage centres (MMC). IgM+ cells, IgT+ cells or a mixture of the two populations were associated frequently to MMC. Cell proliferation was estimated by using a combination of antibodies anti-IgT, anti-IgM and anti-PCNA. Proliferation of both IgM+ and IgT+ B cells was found in several areas of the spleen. In addition, vaccinated fish showed PCNA +/IgM + and PCNA+/IgT+ B cells in the mass of vaccine and cells (CVM) associated with the spleen, which may indicate an importance of the CVM during vaccination, which goes beyond a mere place of phagocytosis and exchange of material.

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keywords: Turbot, Spleen, Vaccination, B lymphocytes, Immunoglobulins

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P-032.

Field validation of immunotoxic responses in the carpet shell clam (*Ruditapes decussatus*) from contaminated sites in the South Lagoon of Tunis (Tunisia)

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Abstract

The aim of this study was to validate immunological alterations as ecotoxicological biomarkers to detect and monitor the biological effects of anthropogenic pollution in the South Lagoon of Tunis (Tunisia). Carpet shell clams (*Ruditapes decussatus*) were collected during summer and Winter from four locations: three of them within the polluted lagoon of Tunis (S1, S2 and S3) and from a clean site on the Mediterranean coast (Louza, Tunisia). To study the immunity of clams, the phenoloxidase, lysozyme, alkaline phosphatase, esterase, peroxidase, protease, antiprotease and bactericidal activities were measured in the haemolymph. Phenoloxidase activity was significantly lower in clams sampled from the three contaminated areas of the lagoon (S1, S2 and S3) than in those from the control point in both summer and winter. Lysozyme, esterase, protease and antiprotease activities were higher in the clams from site S3 than in the clams collected from the control site during winter. No significant variations were detected in the alkaline phosphatase, peroxidase and bactericidal activities of the clams collected from the four experimental sites. A significant seasonal effect was observed in clam immune status in winter. The results clearly showed that the affected biomarkers (mainly phenoloxidase) could be useful tools for biomonitoring clams in the study area.

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keywords: Biomarkers; Biomonitoring; Seasonality; Innate immunity; carpet shell clam (*Ruditapes decussatus*).

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P-033.

Hepcidin, an antimicrobial and iron-regulated peptide that provides an ability to prevent bacterial diseases in grass carp (*Ctenopharyngodon idella*).

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Abstract

Hepcidin is an antimicrobial peptide and a regulator of iron homeostasis which has three isoforms: -20, -22 and -25. While hepcidin-25 has been studied extensively, the physiological significance of other isoforms remains poorly understood. Herein, we focused on the analysis of the differences in antibacterial and iron regulatory functions of hepcidin-20 and hepcidin-25, looking for a derivative of hepcidin as a preventive drug for bacterial diseases. In this study, we examined the antimicrobial potentials of the two hepcidins in the form of synthesized peptides, hepcidin-25 and hepcidin-20. We found that hepcidin-25 and hepcidin-20 exhibited apparent bactericidal activities against both Gram-positive and Gram-negative bacteria in a dose-dependent manner. *In vitro*, the hepcidin-20 had better antibacterial activity than the hepidin-25. However, the antimicrobial activity on the cellular level has the opposite effect. We suspected that the iron-regulating function of hepcidin limits the available iron content of extracellular bacteria to enhance its bactericidal activity. Further tests indicated that only hepcidin-25 can block iron release from liver cell line L8824 via internalization and degradation of cellular iron exporter ferroportin, and restrain the use of iron in extracellular bacteria. This result also confirms our hypothesis. *In vivo*, recombinant *Ctenopharyngodon idella* hepcidin improved the survival rate of *C. idella* challenged with *Flavobacterium columnare*. In addition, the fish fed diet containing recombinant *C. idella* hepcidin had a higher survival rate than other pretreatment groups. The study showed that recombinant *C. idella* hepcidin regulated iron metabolism, causing iron redistribution, decreasing serum iron levels and increasing iron accumulation in the hepatopancreas. Immune-related genes were also evaluated, showing higher expression in the groups pretreated with recombinant *C. idella* hepcidin at an early stage of infection. In general, *C. idella* hepcidin not only has a direct killing effect on bacteria, but also reduces the available iron content of bacteria to inhibit bacterial growth. Our findings revealed a new role for hepcidin in fighting against bacterial infections and indicate a potential in controlling the bacterial infection in aquaculture.

keywords: *Ctenopharyngodon idella*; hepcidin; antibacterial;

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P-034.

A novel *CQTRIM32* from red claw crayfish *Cherax quadricarinatus* inhibits white spot syndrome virus infection

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Abstract

Tripartite motif-containing (TRIM) proteins are highly conserved molecules that participate in a variety of biological processes such as regulation of development, apoptosis, and innate immunity in vertebrates. In this report, we identified a *TRIM32* homolog (named *CqTRIM32*) in red claw