P-079.

Hematological analysis of grass carp, blunt snout bream and yellow catfish: Morphology, ultrastructure, cytochemistry and quantification of peripheral blood cells

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Abstract
The grass carp (*Ctenopharyngodon idella*), blunt snout bream (*Megalobrama amblycephala*) and yellow catfish (*Pelteobagrus fulvidraco*) are economically important fishes in China. Fish hematological features, especially the type and number of peripheral blood cells, are crucial for the evaluation of fish health state and the diagnosis of fish diseases. Since the automatic blood cell count equipment for human is not suitable for fishes, the manual method is critical in the quantification of fish blood cells. To make sense of the comparison and interpretation of the blood cell count studies in different articles, the standardization of blood cell classification is necessary. In this study, erythrocytes (RBC), thrombocytes (TC) and leucocytes (i.e. white blood cells, WBC, including lymphocytes, neutrophils and monocytes) were well distinguished in blood smears with Giemsa staining and confirmed by transmission electron microscopy. RBC, TC and WBC were directly counted with an improved Neubauer counting chamber in a modified diluting solution. The differential leucocyte count (DLC) was carried out in blood smears. In view of the labeling characteristics of peroxidase (PO) positivity in neutrophils and non-specific esterase (α-ANAE) positivity in monocytes, PO positive cell percentage and α-ANAE positive cell percentage were also determined in cytochemical staining smears. No difference was found for the percentages of neutrophils and monocytes between Giemsa staining and cytochemistry staining. The standardized classification, normal count ranges and sizes of the peripheral blood cells by the present systemic studies will provide useful references for monitoring the health status of grass carp, blunt snout bream and yellow catfish.
keywords: Grass carp (*Ctenopharyngodon idella*); Blunt snout bream (*Megalobrama amblycephala*); Yellow catfish (*Pelteobagrus fulvidraco*); Hematological parameters; Morphology.

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P-080.

Development of monoclonal antibody against IGM of large yellow croaker (*Larimichthys crocea*) and characterization of IGM+ B cells

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Abstract
In the present study, a monoclonal antibody (mAb) against large yellow croaker IgM was produced by immunizing mice with purified serum IgM. Western blotting showed that this mAb could specifically react with the heavy chain of large yellow croaker serum IgM. Indirect immunofluorescence assay (IFA) analysis suggested that the resulting mouse anti-IgM mAb could recognize membrane-bound IgM (mIgM) molecules of large yellow croaker. This mouse anti-IgM mAb also can be used for sorting of large yellow croaker IgM+ B cells through the magnetic-activated cell sorting (MACS) method, which was further confirmed by RT-PCR analysis of specific marker genes for B cells. Flow cytometry analysis showed that the percentages of IgM+ B cells in head kidney, spleen and peripheral blood lymphocytes were 29.00± 1.58%, 33.00 ± 1.64%, and 16.50 ± 2.39%, respectively. Additionally, the phagocytosis rates of IgM+ B cells for 0.5 μm beads in head kidney, spleen and peripheral blood were calculated to be 7.56 ± 0.58%, 4.053 ± 0.62% and 23.17 ± 2.26%, respectively, while only 2.36 ± 0.23%, 1.16 ±0.44% and 6.41 ± 0.45 of IgM+ B cells in these three tissues ingested 1 μm beads. Taken together, our data demonstrated that the mouse anti-IgM mAb produced in this study could be used as a tool to characterize IgM+ B cells of large yellow croaker, and rates of phagocytic IgM+ B cells varied in different tissues.
keywords: Monoclonal antibody; Immunoglobulin M; large yellow croaker (*Larimichthys crocea*); phagocytosis; IgM+ B cells

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P-081.

Fish-specific finTRIM FTR36 triggers IFN pathway and mediates inhibition of viral replication

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Abstract
The tripartite motif (TRIM) family involves many cellular processes, including fundamental functions in antiviral immunity. Antiviral activities of TRIMs are reported in a variety of patterns, and one of the most significant channels is related to the activation of the type-I interferon (IFN) pathway. In this study, we described a *fintrim* (*ftr*) gene named *ftr36*, which is mainly expressed in the gills, skin, and intestines. This study shows that *ftr36* encodes a protein affording a potent antiviral effect. *In vitro*, overexpression of FTR36 mediated an upregulated pattern of recognition receptor retinoic acid–inducible gene I (RIG-I), interferon regulatory factor 3/7(IRF3/7), IFN, and IFN-stimulated genes (ISGs) expression. Thereby, FTR36 expression could afford host defense against the spring viremia of carp virus (SVCV) and the giant salamander iridovirus (GSIV). With the deletion of the RING domain or B30.2 domain separately, the antiviral ability of FTR36 was abolished partially and almost lost its ability to activate the IFN-pathway. These findings indicate that both RING and B30.2 domains are indispensable for the antiviral activity of FTR36. Altogether, this study described a finTRIM FTR36, which can activate IFN-pathways and stimulate ISGs to provide host defense against viral infections.
keywords: FTR36; finTRIM; antiviral; interferon; mechanism

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