MONOCLONAL ANTIBODY DEVELOPMENT FOR IFN-α1 OF ATLANTIC SALMON

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ABSTRACT

IFN-α1 is a type I interferon primarily produced and secreted by viral infected cells. This cytokine increases its own expression, acts as a signal to other cells and induces the expression of key proteins that antagonize virus proliferation. All nucleated cells can produce type I IFNs, although during a primary infection most of it originates from dendritic cells (DCs), especially plasmacytoid DCs (pDCs). Type I IFN genes have been described in several fish including zebrafish, channel catfish, fugu, rainbow trout and Atlantic salmon, but so far, no antibodies are available to identify the protein, evaluate expression at the protein levels or study its physiological role as antiviral cytokine. The aim of this study was to produce and characterize a monoclonal antibody against IFN-α1 of Atlantic salmon to be able to quantify the cytokine secretion and identify fish cells secreting IFN-α1. Thus, we first produced the recombinant protein (rIFN-α1) based on the published sequence of salmon IFN-α1. The gene amplified by PCR was cloned into an expression vector, E. coli BL21 (DE3) were transformed, the gene expression was induced and the protein purified. To assess the bioactivity of recombinant protein, head kidney leukocytes were stimulated in vitro for 2, 4 and 6 h with different concentrations of rIFN-α1 and the mRNA expression level of Protein Kinase RNA-activated (PKR) and myxovirus resistance gene (Mx) were analyzed. As expected, rIFN-α1 was bioactive. Then, monoclonal antibodies against rIFN-α1 were produced and three clones were selected for further characterization. The monoclonal antibody allowed us to detect IFN-α1 by ELISA and Western blot on cell supernatants and to detect IFN-α1-secreting cells by flow cytometry.

KEYWORDS

Atlantic salmon, cytokines, recombinant protein, monoclonal antibodies, flow cytometry.

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