CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF INTERLEUKIN-12 IN AMBERJACK SERIOLA DUMERILI.

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ABSTRACT

Interleukin-12 is proinflammatory cytokine mainly produced by monocytes, macrophages and dendritic cells. In mammal, IL-12p70 is composed of IL-12p35 and IL-12p40, is known to play crucial role in promoting cell-mediated immunity (CMI) through Th1 differentiation and IFNγ production. Although fish possess two types of IL-12p35 (p35a, p35b) and three types of IL-12p40 (p40a, p40b and p40c) genes, the knowledge on functional characteristics of teleost IL-12 is still limited. In the present study, we have cloned two types of IL-12p35 and three types of IL-12p40 genes from amberjack. To elucidate the role of amberjack IL-12p70 in CMI, six types of recombinant amberjack IL-12p70 protein generated in insect cells HighFive, were purified by Ni-NTA column. Head kidney leukocytes were cultured with formalin-killed Nocardia seriolae and rIL-12. As a result of gene expression analysis after stimulation, IFN-γ was induced whereas IL-10 expression was suppressed in head kidney leucocytes stimulated with four types of rIL-12 (p35a/p40a, p35a/p40c, p35b/p40a, p35b/p40c). On the other hand, two types of rIL-12 (p35a/p40b, p35b/p40b) only elicited down-regulation of IL-10 expression. In addition, leukocytes stimulated with formalin-killed N. seriolae (without rIL-12) showed suppression of IFN-γ and up-regulation of IL-10 gene. These data suggest that all amberjack IL-12p70 isoforms are involved in promotion of CMI with different manner. Furthermore, there is no modulation of IFN-γ and IL-10 genes in the leukocytes stimulated with amberjack rIL-12 alone, indicating that induction of CMI by IL-12 requires antigen stimulation. This is the first report to show that in teleosts multiple IL-12 protein are involved in induction of CMI.

KEYWORD

IL-12, IFNγ, Amberjack, cell-mediated immunity, IL-10

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