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The effects of cytokines on the regulation of adaptive immunity of Japanese flounder Paralichthys olivaceus

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博士学位論文内容要旨
Abstract

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論文題目 Title	The effects of cytokines on the regulation of adaptive immunity of Japanese flounder *Paralichthys olivaceus*		

Vaccination strategies have traditionally been used as preventative against disease in uninfected fish. There are lots of evidences that cytokines could increase antigen specific immune response such as the Interleukin-1β (IL-1β) and IL-8 on the humoral immune response of fishes. However, the immune-adjuvant mechanism of cytokines has not been elucidated yet.

IL-1β is known as a pro-inflammatory cytokine and expressed by many cells. In the first study, the effects of IL-1β on immune responses in Japanese flounder *Paralichthys olivaceus* were investigated. To investigate the effects, eukaryotic expression plasmid carrying Japanese flounder IL-1β (pcDNA- IL-1β) was co-injected into the muscle with BSA (as the antigen). The pcDNA- IL-1β group had a significantly higher IL-1β expression than other groups in the muscle at day 1, but then IL-1β expression significantly decreased after 3 days. On the other hand, the mRNA level of TNFα was significantly increased after 3 days. Moreover, pcDNA- IL-1β group significantly stimulated the gene expression of IL-1β in the kidney at days 1 and 3. The pcDNA- IL-1β and BSA injected group showed the highest antibody titer against BSA at 30 days after injection. Furthermore, to determine the adjuvant effect of the IL-1β on DNA vaccine, the plasmid carrying JFIL-1β was co-injected into the muscle of Japanese flounder with eukaryotic expression plasmid carrying green fluorescent protein (GFP) (as the DNA vaccine model). The antibody titer against GFP of the IL-1β and GFP plasmids injected group was higher than the other groups. These results, thus, indicated that eukaryotic expression plasmids carrying JFIL-1β have immune-adjuvant effects not only on protein antigen but also on DNA vaccine model.

The IL-1β showed an adjuvant effect in Japanese flounder. In contrast, two novel IL-1β-like genes in Japanese flounder expressed sequence tag (EST) database were identified. In the second study, the genes in Japanese flounder *Paralichthys olivaceus* (JFIL1β-L1 and JFIL1β-L2) were characterized. JFIL1β-L1 was homologous to Nile Tilapia IL-1β-like gene and Arctic char IL-1β, and JFIL1β-L2 showed homology to hypothetical protein LOC100699119 of Nile Tilapia and rainbow trout *Oncorhynchus mykiss* IL-1 receptor agonist (RA). The deduced amino acids sequences of these IL-1β-like genes showed very low identities to the Japanese flounder IL-1β (JFIL-1β). Phylogenetic analysis confirmed that JFIL1β-L1 and -L2 were distinct from JFIL-1β. The gene encoding the

predicted ORF of JFIL1β-L1 and -L2 is divided into 6 exons and 7 exons, respectively. Transcripts of JFIL1β-L1 were detected in gills, intestine, kidney and spleen, and those of JFIL1β-L2 were detected in gills, intestine and spleen. The mRNA levels of JFIL1β-L1 and -L2 were not effect or slightly decreased by treatment with LPS and the formalin-killed cells of *Edwardsiella tarda* whilst mRNA levels of JFIL1β were significantly increased in the kidney and spleen at 6 hours by these treatments.

In the third study, IL-12 was also examined because it plays a central role in the regulations of adaptive immunity. It induces the production of interferon-γ from T cell and natural killer cell, and enhances cytolytic function of cytotoxic T cell. IL-12 is a heterodimeric molecule composed of two covalent subunits, p35 and p40. In Japanese flounder, the coding region of JFIL-12p35, JFIL-12p40a and JFIL-12p40b were 612, 1074 and 951 nucleotides encoding 203, 357 and 316 amino acids, respectively. Phylogenetic analyses confirmed the two different JF p40 subunits which both were homologues p40 of other fish species. JFIL-12p35 and IL-12p40a expressions were found in all used tissues. On the other hand, JFIL-12p40b transcripts were detected in the gill, head-kidney, trunk kidney, heart and spleen. The JFIL-12p40a transcripts were slightly increased post *E. tarda* and VHSV infection. The JFIL-12p40b expression was up regulated at after *S.iniae, E. tarda* and VHSV infection. In treated PBLs, IL-12 p35 and p40b were induced after treated with LPS. Moreover, IL-12 p40a and p40b expression were up-regulate after Poly I:C treated. In additionally, the pCI-neo inserted with IL-12 could express in muscle after injection. The pCI-neo-IL-12 p35 and the combination of pCI-neo-IL-12 p35 + pCI-neo-IL-12 p40b could induce the expression of IFN-γ and IL-2.

This study showed that JFIL-1β has immuno-adjuvant effects in both protein antigen and DNA vaccine model that mediates humoral immunity. The functions of IL-1β-like proteins have distinct from the function of IL-1β, even though they have a conserved IL-1β domain. Moreover, JFIL-12 also has the potential to be used as adjuvant. The cytokines could induce the expression of adaptive immunity that has the adjuvant activities in Japanese flounder.