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Short Communication

CD3γ/δ in sea bass (*Dicentrarchus labrax*): Molecular characterization and expression analysis

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ABSTRACT

The CD3 complex is the common marker on the surface of both αβ and γδ T cells and is essential for formation of the T-cell receptor complex and for T-cell activation.

In this paper, we report the gene cloning and molecular characterization of a CD3γ/δ homologue in sea bass (*Dicentrarchus labrax*), the analysis of transcription levels in lymphoid and non-lymphoid organs and the gene regulation after *in vitro* stimulation with LPS and PHA.

Four cysteine residues in the extracellular domain, involved in the constitution of immunoglobulin-like domain, are present in sea bass CD3γ/δ sequence and they are conserved both in number and position from mammals to teleost sequences. Similar to other known CD3γ/δs, in sea bass CD3γ/δ there is also a conserved immunoreceptor tyrosine-based activation ITAM motif that could be responsible for its individual signal transduction capacity.

The real time RT-PCR basal analysis shows the highest level of CD3γ/δ mRNA in thymus, followed by peripheral blood leucocytes, spleen, gills, gut, liver, head kidney, brain and muscle. The expression analysis under stimuli condition reveals a significant decrease of CD3γ/δ expression after LPS stimulation and a significant increase after PHA-L stimulation, in agreement with mammals results.

In conclusion, these data allow us to affirm that sea bass CD3γ/δ can be used as a T cell marker and will help in adding new insight on the immune response mechanisms of sea bass.

1. Introduction

In mammals, the T-cell receptor (TCR) complex consists of either α/β or γ/δ TCR heterodimers non-covalently bound to a group of three different CD3 dimers (δ–ε, γ–ε, ζ–ζ, ζ–η) [1,2]. Therefore, the CD3 complex is the common marker on the surface of both αβ and γδ T cells. The γ-, δ- and ε-polypeptides belong to the immunoglobulin (Ig) superfamily [3], comprising an extracellular Ig-like domain, a negatively charged transmembrane helix and a cytoplasmic tail, which contains a single immunoreceptor tyrosine-based activation motif (ITAM), that interacts with tyrosine kinases during the signal transduction [4]. The structure of the ζ-chain is different from the other showing a shorter extracellular domain and a longer cytoplasmic tail containing three ITAMs. In CD8α and CD4 co-receptors binding sites for a lymphocyte-specific protein tyrosine kinase (Lck) are present that after stimulation of the TCR phosphorylates ITAMs.

In birds, amphibians and teleosts, a unique CD3 named CD3γ/δ seems to play the role of both CD3γ and CD3δ subunits [5–9]. Infact, to date, non-mammalian CD3γ/δ homologues have been identified in chicken [5], in amphibians [6,7] and teleosts such as in Japanese flounder [8,10], fugu [9], carp [11], halibut [12], salmon [13,14] and starlet [15].

In the present study, we report the gene cloning and molecular characterization of a CD3γ/δ homologue in sea bass (*Dicentrarchus labrax*), the analysis of transcription levels in lymphoid and non-lymphoid organs, and the gene regulation after *in vitro* stimulation with LPS and PHA.

Thymus leucocytes were obtained from a juvenile sea bass (150 g of weight) as described by Scapigliati et al. [16] and total RNA was isolated using Tripure (Roche). CD3γ/δ sequence was identified after extensive Expressed Sequence Tag (EST) sequencing of a sea bass thymus normalized cDNA library constructed as described by De Pittà et al. [17], Venier et al. [18] and previous papers [19,20]. Single pass DNA sequencing from plasmids was performed at the local sequencing service of Laboratory of Genetics, in the Department of Life Science at the Trieste University [17]. Generated sequences were analysed for similarity with other known sequences using the BLAST program [21]. Annotation was examined by annot8r software, a web tool for the annotation of protein or nucleotide sequences from non-model organisms with Gene Ontology terms, EC numbers and KEGG biochemical pathways. EST sequences were submitted to EMBL databank receiving the numbers from FN565576 to

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