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Short communication

A CD83-like molecule in sea bass (*Dicentrarchus labrax*): Molecular characterization and modulation by viral and bacterial infection

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ABSTRACT

The CD83 cell surface marker is an important and intriguing component of immune system. It is considered the best marker for mature human dendritic cells, but it is also important for thymic development of T cells, and it also plays a role as a regulator of peripheral B-cell function and homeostasis. A CD83-like molecule was identified in sea bass (*Dicentrarchus labrax*) by EST sequencing of a thymus cDNA library; the CD83 cDNA is composed of 816 bp and the mature CD83 peptide consists of 195 amino acids, with a putative signal peptide of 18 amino acids and two possible N-glycosylation sites. The comparison of sea bass CD83 sequence with its homologues in other fish species and mammals shows some differences, with two cysteine residues conserved from fish to mammals and a high variability both in the total number of cysteines and in mature CD83 sequence polypeptide length. Basal transcripts levels of CD83 mRNA are highest in liver, followed by thymus. The *in vitro* treatment of head kidney leukocytes with LPS resulted in a down-regulation on CD83 mRNA leves both after 4 and 24 h, whereas with poly I:C an up-regulation after 4h followed by a down-regulation at 24 h was observed. An *in vivo* infection of sea bass juveniles with *nodavirus* induced an increase of CD83 expression on head kidney leukocytes both after 6 and 24 h and a decrease after 72 h. On the other hand, an *in vivo* infection with *Photobacterium damselae* bacteria induced a decrease of CD83 transcript levels after 6 and 24 h and an increase after 72 h. These findings suggest in sea bass CD83 expression could be modulated by viral and bacterial immune response.

1. Introduction

The cell surface marker CD83 has been the subject of intense studies in mammals for its importance in immune responses, mostly due to its close trascription relationship with costimulatory molecules like CD80 and CD86, and for its fundamental importance as a marker of antigen-presenting dendritic cells (DCs) [1]. CD83 is a glycosylated member of the immunoglobulin superfamily (IgSf) and it is considered as predominantly expressed in human and mouse on dendritic cells (DCs) [1,2]. CD83 has been classified as a sialic acid-binding Ig-like lectin (siglec), after the demonstration that the binding with the CD83 ligand was broken by a sialidases [3]. CD83 expression is also detectable in human on B cells (for a review see [4]), and on T cells after TCR-based activation [2,5,6]. CD83 expression is found in mouse lymphocytes as well, but at higher levels than in human [7,8]. The *in vivo* functional role of cell surface CD83 has been studied using CD83-deficient mice and the results evidenced its involvement in $CD4^+$ thymocyte maturation [9,10]. Mature human CD83 is composed of 205 amino acids and is formed by a single extracellular V-type Ig domain, a transmembrane domain and a cytoplasmic tail [5]. A soluble form of CD83, showing a size similar to cell surface CD83, has been evidenced in the serum of healthy adults and in activated dendritic cells [11]. The function of this soluble form has been studied in human both *in vitro* and *in vivo*, and a possible immunosuppressive role have been indicated (for a review see [12]).

In fish, CD83 homologues have been evidentiated in nurse shark (*Ginglymostoma cirratum*) [13] and rainbow trout (*Oncorhynchus mykiss*) [13] and, successively, in other teleost fishes such as Japanese flounder (*Paralichthys olivaceus*) [14], sea bream (*Sparus aurata*) [15] and turbot (*Scophthalmus maximus*) [16].

With the aim to add new insights in the European sea bass (Dicentrarchus labrax) "immunome" gene panel and to identify a possible marker for dendritic cells, we describe in this paper the initial characterization of a CD83 molecule. The expression of CD83 has been studied both at basal conditions in different tissues and after *in vitro* stimulation of head kidney leukocytes. In addition, we investigated the regulation of CD83 expression after *in vivo* infection

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of sea bass with a bacterial pathogen (*Photobacterium damselae* subsp. *piscicida*) and a viral pathogen (nervous necrosis virus, NNV).

2. Material and methods

2.1. Sea bass CD83 sequence identification and analysis

CD83 sequence was identified after Expressed Sequence Tag (EST) sequencing of a sea bass thymus cDNA library as described previously [17]. The library was normalized to facilitate the identification and analysis of rare transcripts. In brief, the cDNAs were size selected by Sepharose CL-4B SPUN COLUMN (GE Healthcare) and cloned in the vector pDONR221 (Invitrogen). The plasmids were than inserted in DH10B *E. coli* cells and cDNAs were directly amplified with universal primer M13 forward and M13 reverse. 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. Dnastar software was used to remove vector contamination and poly(A), and to perform the trimming of low quality sequences with high stringency parameters. Generated sequences were analysed for similarity with other known sequences using the BLAST program. Afterwards EST sequences were submitted to EMBL databank receiving the numbers from FN565576 to FN566839. The CD83 sequence was contained in the EST with accession number FN565978, and the final sequence received the accession number FN687466.

The sea bass CD83 sequence was analysed for the presence of a signal peptide, using SignalP software [18], for N- (with the NetNGlyc 1.0 Server) and O-linked (NetOGlyc 3.1 Server) glycosylation sites [19] and for phosphorylation sites (with the NetPhos 2.0 Server) [20]. The CD83 nucleotide and amino acid sequence was compared with counterparts in other vertebrate species with the EMBOSS Pairwise Alignment tool. Alignment of the sea bass CD83 amino acid sequence to other known molecules from other species was carried out using MEGA 4.1 Software [21]. A phylogenetic tree was constructed by the "neighbour-joining" method with MEGA 4.1 Software [21] on full-length amino acid sequences and 10000 bootstrap values calculated.

2.2. Basal CD83 expression analyses

To study the CD83 basal expression, six sea bass juveniles were sampled and leucocytes from different tissues (muscle, thymus, head kidney (HK), liver, spleen, gills, brain, peripheral blood leucocytes (PBL), gut) obtained as described before [22]. Total RNA was isolated from each tissue separately with Trisure (Bioline), resuspended in DEPC treated water and used for real-time quantitative PCR without pooling the tissue samples coming from the different fishes. For reverse transcription, the BioScript RNase H minus (Bioline) enzyme was used with the protocol described previously [23]. The expression level of CD83 transcript was determined with a Mx3000P™ real-time PCR system (Stratagene) equipped with version 4.1 software and using the Brilliant SYBR Green Q-PCR Master Mix (Agilent Technologies) following the manufacturer's instructions, with ROX as internal passive reference dye. Specific PCR primers were designed for the amplification of about 200 bp products from CD83 (CD83FW: 5′-GATTCAGGCACCTACGAG-3′; CD83RV: 5′-GATACGAAAAGCTCGAATAAC-3′) and 18S ribosomal RNA (18SFW: 5′-CCAACGAGCTGCTGAcc-3′; 18SRV: 5′-CCGTTACCCGTGGTCC-3′), used as a house-keeping gene. 10 ng of cDNA template was used in each PCR reaction. The PCR conditions were 95 °C for 10 min, followed by 35 cycles of 95 °C for 45 s, 52 °C for 45 s and 72 °C for 45 s. Triplicate reactions were performed for each template cDNA and the template was replaced with water in all blank control reactions. The analysis was carried out using the endpoints method option that causes the collection of the fluorescence data at the end of each extension stage of amplification. A relative quantitation has been performed, comparing the levels of the target transcript (CD83) to a reference transcript (calibrator, the tissue with the lowest CD83 expression, in this case the muscle). A normalizer target (18S ribosomal RNA) is included to correct for differences in total cDNA input between samples. The results are expressed as the mean ± SD of the results obtained from the six considered fishes.

2.3. In vitro CD83 expression analyses after stimulation

The *in vitro* CD83 expression was studied using leukocytes isolated from six juveniles sea bass (150 g of weight) head kidney (HK) cells cultured in L-15 medium (Gibco) containing 10% FCS, adjusted to 1×10^5 cells/ml and incubated at 18 °C for 4 h and 24 h with: 1) 5 μg ml^{-1} of lipopolysaccharide (LPS from *E. coli* 0127:B8, Sigma); 2) 50 μg ml^{-1} poly I:C (Sigma). The cell control samples were stimulated with L-15 alone. Total RNA was isolated with Tripure (Roche), resuspended in DEPC treated water and used for real-time quantitative PCR without pooling the samples coming from the different fishes. The primers and the real-time PCR conditions were the same as described in the above section, except that the calibrator for this experiment was the time 0 control.

The results were expressed as the mean ± SD of the results obtained from six fishes and the differences from the control were considered significant if $p < 0.05$ using the two-way ANOVA analysis following by Bonferroni's post test.

2.4. CD83 expression analyses after infection with nervous necrosis virus and Photobacterium damselae

The *in vivo* CD83 expression analysis was performed on sea bass samples experimentally infected with nervous necrosis virus (NNV), otherwise known as viral encephalopathy and retinopathy virus (VERV), and with *P. damselae* subsp. *piscicida*. Details of pathogen infection have been described previously [24,25]. Briefly, sea bass individuals (30–40 g) kept in the IFAPA Center *El Toruño*, Cádiz (Spain) were used. Two weeks prior infection, fish were transferred to the quarantine facilities for adaptation and distributed into 4,000 L tanks. Fish (200 individuals) in the control tank were injected intramuscularly (i.m.) with PBS, whereas all fish from other tanks (200 + 200 individuals) were injected i.m. with NNV (10^4 $TCID_{50}$ ml^{-1}) and with *P. damselae* subsp. *piscicida* (10^5 cfu/ml, 1 h) by bath immersion. The virus and bacteria dose was tested to induce low mortality in sea bass by previous *in vivo* tests (not shown). For gene expression analyses, 5 fishes for group were sampled at each time point. From each individual fish the head kidney was removed after infection at hours: 0, 6, 24 and 72. The tissue was immersed in 1 ml of Trizol (Invitrogen), labelled, immediately frozen in liquid nitrogen and stored at −80 °C until further use. Total RNA extraction, cDNA preparation and real-time quantitative PCR (the calibrator for this experiment was the time 0 control) were performed as described above.

The results were expressed as the mean ± SD of the results obtained from six fishes and the differences from the control were considered significant if $p < 0.05$ using the two-way ANOVA analysis following by Bonferroni's post test. One asterisk indicates when $p < 0.05$ with respect to the time 0 control; two asterisks indicate when $p < 0.01$ with respect to the time 0 control and three asterisks indicates when $p < 0.001$ with respect to the time 0 control.

3. Results and discussion

3.1. Sea bass CD83 sequence characterization

The identified sea bass CD83 cDNA consists of 816 bp (accession number FN687466), containing a 588 bp ORF, a 35 bp 5′-UTR and