Fish & Shellfish Immunology 34 (2013) 378–382

Contents lists available at SciVerse ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Short communication

# First molecular cloning and gene expression analysis of a teleost CD200 (OX-2) glycoprotein from rock bream, *Oplegnathus fasciatus*

Seong Don Hwang [a], Ju-Won Kim [a], Mu-Chan Kim [a], Do-Hyung Kim [b,**], Chan-Il Park [a,*]

[a] Institute of Marine Industry, College of Marine Science, Gyeongsang National University, 38 Cheondaegukchi-Gil, Tongyeong, Gyeongnam 650-160, Republic of Korea
[b] Fish Health Center and Department of Aqualife Medicine, Chonnam National University, Yeosu 550-749, Republic of Korea

ARTICLE INFO

*Article history:*
Received 2 August 2012
Received in revised form
26 September 2012
Accepted 14 October 2012
Available online 2 November 2012

*Keywords:*
CD200
*Oplegnathus fasciatus*
*Edwardsiella tarda*
*Streptococcus iniae*
Red seabream iridovirus

ABSTRACT

CD200 plays an important role in delivering an immunoregulatory signal to the immune system through interaction with its receptor. However, CD200 has not been characterized and its function in teleosts is unknown. In this study, the rock bream (*Oplegnathus fasciatus*) CD200 gene (RbCD200) was cloned and its expression profile was analyzed after infection with *Edwardsiella tarda*, *Streptococcus iniae* or red seabream iridovirus (RSIV). The coding region of RbCD200 cDNA was 855 bp, encoding 284 amino acid residues. The gene consisted of two extracellular Ig-like domains and a transmembrane domain. RbCD200 was highly expressed in the brain, erythrocytes, intestine and stomach of healthy rock bream. In the spleen, RbCD200 gene expression was down-regulated until 48 h after *E. tarda* exposure, except at 12 h RbCD200 gene expression was down-regulated then up-regulated at 12 h and 24 h after infection with *S. iniae* and RSIV, respectively. In the whole kidney, the RbCD200 gene was down-regulated in response to infection with *E. tarda* and *S. iniae*. However, RSIV infection increased RbCD200 gene expression in whole kidney until 48 h. These results suggest that RbCD200 is differentially expressed in the spleen and whole kidney after infection with different pathogens.



## 1. Introduction

The immune system has evolved complex mechanisms to provide rapid responses against evolving pathogens whilst controlling these responses to prevent self-damage [1]. A variety of cell surface and soluble molecules, including immunoglobulin superfamily (IgSF), are involved in controlling immunological responses. One member of the IgSF, CD200, plays an important role in the modulation of immune responses.

CD200 (also known as OX2) is a highly glycosylated type I membrane protein [2–4]. CD200 contains two extracellular immunoglobulin superfamily domains and a transmembrane domain, but it lacks a cytoplasmic signaling motif, structure particularly common in proteins mediating cell–cell interactions [3,4]. In mammals, CD200 is ubiquitously expressed in a wide range of cells, including thymocytes, activated T cells, B cells, follicular dendritic cells, neurons and vascular endothelium [5,6]. The functional properties of CD200 depends upon coperation with a receptor (CD200R) expressed on other cell types.

The receptors for CD200, CD200 receptors (CD200Rs), are glycosylated type I membrane proteins having multiple isoforms. The structure of CD200R is very similar to CD200 such that both have immunoglobulin superfamily domains and a transmembrane doamin, but CD200R have an additional cytoplasmic signaling motif containing three tyrosine residues that can be phosphorylated [7,8]. The expression of CD200R is restricted to myeloid cell, including macrophages, dendritic cells, neutrophils, mast cells, and T cells [3,8].

The interaction of CD200 with CD200R on the cell surface, depend on the cell type [9]. Their interaction is involved in the activation of anti-inflammatory pathways, induction of immune tolerance and the prevention of tissue rejection. The CD200-CD200R complex triggers RAS/mitogen-activated protein kinase (MAPK) signaling pathway, which in turn induces suppression of mast cell degranulation [7,8] and decreases macrophage-induced cytokine production of interleukin 13 (IL-13), tumor necrosis factor α (TNF α), interferon α (IFN α) and IL-17 [10–12]. The complex is also involved in regulatory T cell induction [13], cytokine profile switching from Th1 to Th2 [14] and inhibition of tumor-specific T cell immunity [15].

Due to its immunosuppressive role, CD200 overexpression in cancer tissues is able to escape from immune response. The gene expression of CD200 also provides a useful marker for several

* Corresponding author. Tel.: +82 55 772 9153; fax: +82 55 772 9159.
** Corresponding author. Tel.: +82 61 659 7177; fax: +82 61 659 7179.
*E-mail addresses:* kimdh@chonnam.ac.kr (D.-H. Kim), vinus96@hanmail.net (C.-I. Park).

1050-4648/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fsi.2012.10.024

cancers [16], lymphoid malignancies [17,18] and human hair-follicle-bulge stem cells [19]. Lack of CD200R signaling increases resistance to bacteria infection. CD200 antibodies are a suitable therapeutic for blocking its immunosuppressive effect [15].

To date, CD200 and CD200R have been cloned in mammals and functionally characterized in mouse and human [20–23]. Although the seqeunces of zebrafish and catfish fish CD200 are available in GenBank, characterization and function of CD200 in teleosts is unknown. Therefore, molecular cloning and characterization of the fish CD200 should contribute to help elucidate the mechanism of immunomodulation in fish. Here, we report the identification and molecular characterization of rock bream (*Oplegnathus fasciatus*) CD200 (RbCD200). Furthermore, RbCD200 expression was analyzed by quantitative real-time polymerase chain reaction (PCR) following infection with *Edwardsiella tarda*, *Streptococcus iniae* or red seabream iridovirus (RSIV).

## 2. Materials and methods

### 2.1. Molecular cloning of RbCD200

A partial sequence of rbCD200 cDNA was obtained by analyzing expressed sequence tags (ESTs) in the cold shock-stimulated rock bream erythrocytes library (unpublished data). This analysis revealed that the partial sequence was homologous to known CD200, and was therefore denoted as RbCD200. Primer-walking methods were conducted using an ABI 3730 automatic DNA sequencer (Life Technologies, Carlsbad, CA, USA) and an ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit to identify the full-length RbCD200 cDNA (Life Technologies). To identify the functionally important domain of RbCD200, extracellular domains and transmembrane domain were analyzed by the Pfam and SMART program, respectively. The deduced amino acid sequence of RbCD200 gene was compared with those of known CD200 genes in the GenBank database using the Clustal W program. A phylogenetic tree analysis based on the entire CD200 amino acid sequence in rock bream and other species was constructed by the neighbor-joining algorithm in the MEGA Software version 4.0. The bootstrap sampling was performed with 1000 replicates.

### 2.2. Detection of RbCD200 gene expression in healthy fish

Peripheral blood leukocytes (PBLs) and erythrocytes were isolated using Percoll density gradients. The brain, eye, gill, erythrocytes, head kidney, heart, intestine, liver, muscle, PBLs, skin, spleen and stomach were isolated from healthy rock bream. Total RNA was extracted using TRIzol reagent (Life Technologies). cDNA was synthesized from the RNA template using a first-strand cDNA synthesis kit (Takara, Kyoto, Japan). Real-time PCR was performed with SYBR Green Master Mix (Takara), following the manufacturer's protocol. Real-time PCR was carried out with cDNA templates of each organ and specific primer sets of RbCD200 (Table 1). Relative expression levels of RbCD200 mRNAs were determined using the rock bream β-actin gene as an internal reference by the comparative Ct ($2^{-\Delta\Delta Ct}$) method, according to the Thermal Cycler DICE Real-Time System (Takara). Significant differences in gene expression among tissues were determined by analysis of variance (ANOVA) compared to values for the kidney.

### 2.3. Effects of S. iniae, E. tarda and RSIV infection on RbCD200 gene expression

Healthy rock bream (approximate body length: 11–13 cm) were challenged by intraperitoneal injection with pathogenic *S. iniae*, *E. tarda* or RSIV, which were adjusted to $3 \times 10^6$, $2 \times 10^6$ cell/fish and $10^6$ copies/fish in phosphate-buffered saline (PBS), respectively [24]. Control fish were injected with PBS alone. Bacteria- and virus-infected fish were kept in seawater at 23 °C. At 6, 12, 24, and 48 h post-infection, whole kidney and spleen were collected from three fish in each group and pooled. The RNA extraction, cDNA synthesis and real-time PCR were performed as described above. Relative expression levels of RbCD200 gene were normalized to the expression of β-actin gene, and were expressed as fold changes relative to the control value. The significance differences between gene expression levels of the infected and control groups were evaluated by ANOVA.

**Table 1**
Hwang et al.

| Primer | Sequence (5′–3′) |
|---|---|
| RT-qPCR amplification | |
| CD200F | CTGAAGGTGCTCTCACAGGT |
| CD200R | GACACGACTGCCTCTTCAGT |
| β-actin-F | TCATCACCATCGGCAATGAG |
| β-actin-R | TGATGCTGTTGTAGGTGGTC |
| RSIV quantification | |
| MCPL-F | CCCTATCAAAACAGACTGGC |
| MCPL-R | TCATTGTACGGCAGAGACAC |
| MCPS–F | CTGCGTGTTAAGATCCCCTCCA |
| MCPS-R | GACACCGACACCTCCTCAACTA |

## 3. Results and discussion

### 3.1. Characterization of RbCD200 cDNA

The length of the RbCD200 cDNA coding region was 855 bp, which encoded 284 amino acid residues (GenBank accession no. AB6653530) (Fig. 1). The 5′ untranslated region (UTR) started 24 bp upstream of the putative ATG start codon. The 3′ UTR was 1037 bp with two polyadenylation signals (AATAAA) and a poly-A tail. The RbCD200 gene had a typical CD200 structure [4], which consisted of two extracellular Ig-like domains, (*i.e.* Ig-like V-domain, and CD80-like Ig-like C2-domain) and a transmembrane domain (Fig. 1). Five glycosylation sites were found on the extracellular side of the membrane (at residues 87, 137, 170, 179 and 196). The structure and characterization of RbCD200 gene was similar to that of the mammalian gene. Therefore, RbCD200 is likely functional and capable of responding to mammalian CD200.

To investigate the molecular evolution between CD200 and other CD genes, phylogenetic analysis was conducted using the neighbor-joining distance method. The phylogenetic analysis revealed that RbCD200 clustered with CD200 homologs of Nile tilapia and zebrafish (Fig. 2). Fish CD200s were closer to those of mammals than CD200R or viral CD200. This indicates that fish CD200s are closely related to those of mammals. RbCD200 shared a relatively high level of amino acid sequence identity with its counterparts in Nile tilapia (58%). The deduced amino acid sequence of RbCD200 was relative low identical to that of mammalian sequences.

### 3.2. Gene expression of RbCD200

In healthy fish, RbCD200 was significantly expressed in brain, erythrocytes, intestine and stomach (28.9-, 840-, 64.6- and 29.5-fold, respectively) and weakly expressed in head kidney, liver, muscle and spleen (2.9-, 2.05-, 6.44-, and 5.9-fold, respectively) as compared to the kidney (Fig. 3). In mammals, expression of CD200 was detected in smooth muscle, scattered blood vessels, gut nerve bundles and vascular endothelia in kidney, liver and spleen [6,15,20]. Mammalian CD200 was also expressed by various