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CD83 is required for the induction of protective immunity by a DNA vaccine in a teleost model

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ABSTRACT

In mammals, CD83 is a surface marker on mature dendritic cells and vital to lymphocyte activation. In teleost, studies on the function of CD83 are very limited. In this study, we examined the potential involvement of turbot (*Scophthalmus maximus*) CD83, SmCD83, in vaccine-induced immunity. For this purpose, turbot were immunized with pORF75, a DNA vaccine against megalocytivirus, in the presence or absence of pSmCD83, a plasmid that constitutively expresses SmCD83. Immune response and protection analysis showed that the presence of pSmCD83 significantly (i) enhanced the activation of head kidney macrophages (HKM) and immune gene expression, (ii) inhibited viral replication in fish tissues following megalocytivirus challenge and increased the survival of the vaccinated fish, and (iii) stimulated production of specific serum antibody and the cytotoxicity of peripheral blood leukocytes. To further examine the effect of SmCD83, pORF75 was administered into turbot in which SmCD83 was knocked down. Subsequent analysis showed that in fish with SmCD83 knockdown, vaccine-induced HKM activation and antibody production were severely reduced, and, consistently, the protectivity of pORF75 was drastically decreased. Taken together, these results indicate for the first time that teleost CD83 is required for the induction of protective immune response by DNA vaccine.

1. Introduction

CD83 is a glycosylated transmembrane protein belonging to the immunoglobulin (Ig) superfamily (Kretschmer et al., 2008). Human CD83 consists of an N-terminal extracellular V-type Ig-like domain, a hydrophobic transmembrane domain, and a short C-terminal cytoplasmic domain (Kozlow et al., 1993). In humans and mice, CD83 is expressed on dendritic cells (DCs) and considered a cell surface marker of antigen-presenting DCs (Pinho et al., 2014; Prechtel and Steinkasserer, 2007; Zhou and Tedder, 1995; Zhou et al., 1992). DCs constitute a fundamental bridge between innate immune recognition of pathogen-associated molecular patterns (PAMPs) and acquired immunity. Activation of DCs by PAMPs upregulates the expression of MHC class II (MHCII), CD80, and CD86, which are required for DC interaction with naive T cells (Lee et al., 2011). In addition to DCs, CD83 is also expressed in other types of cells such as activated B cells and T cells, natural killer cells, and thymic cells (Breloer et al., 2007; Fujimoto et al., 2002; Kretschmer et al., 2007; Mailliard et al., 2005; Wolenski et al., 2003). In B cells, CD83 is an early activation marker that is known to be upregulated following engagement of Toll-like receptor 4 by lipopolysaccharide (LPS) (Kretschmer et al., 2007), while in T cells, CD83 promotes T cell survival in the periphery (Lüthje et al., 2008; Prazma et al., 2007). Elevated CD83 expression increased the priming of human $CD8^+$ T cells, and stimulated $CD8^+$ T cell lines and allogenic T cells *in vitro* (Aerts-Toegaert et al., 2007; Hirano et al., 2006). Studies with murine models showed that CD83 played a key role in thymic selection of double positive thymocytes to CD4 single positive T cells, and that CD83-deficient mouse displayed a drastically reduced amount of CD4 single positive T cells in the thymus and an equally reduced amount of $CD4^+$ T helper cells in the periphery, which was restored to normal level by expression of CD83 on the thymic epithelium (Lüthje et al., 2006). The $CD4^+$ T cells that finally matured in the absence of CD83 displayed reduced proliferation and IL-2 secretion upon *in vitro* stimulation, and reduced delayed type hypersensitivity reaction *in vivo* (Fujimoto et al., 2002; Garcia-Martinez et al., 2004). CD83 expressed on murine thymic epithelial cells is thought to deliver a crucial signal to double positive thymocytes, thus allowing them to mature to immune competent $CD4^+$ T cells (Lüthje et al., 2006).

In fish, CD83 homologues have been reported in several teleosts (Aoki and Hirono, 2006; Donate et al., 2007; Ohta et al., 2004); however, studies on the function of fish CD83 are very limited. In

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a previous study, we characterized the expression profile of a CD83 homologue (named SmCD83) from turbot (*Scophthalmus maximus*), an economic fish farmed worldwide. We observed an upregulated expression of SmCD83 during immunization with different types of vaccines (Hu et al., 2010a). In the present study, we aimed to investigate the immune effect of SmCD83 in the context of vaccination. For this purpose, we overexpressed or knocked down SmCD83 in turbot and examined the ensuing impact on the protective immune response induced by a DNA vaccine.

2. Materials and methods

2.1. Fish

Clinically healthy turbot (i.e. (i) no positive results were obtained when 10% of the stock were examined for viral or bacterial presence in blood, spleen, kidney, and liver; (ii) the fish exhibited no observable clinical signs of any kind) (9.7 ± 1.7 g) were purchased from a local fish farm. The fish had no history of prior infection. The fish were fed daily with commercial dry feed pellets and maintained at 20 °C in aerated seawater. For tissue collection, fish were euthanized with tricaine methanesulfonate (Sigma, St. Louis, USA), followed by severing the spinal cord of the fish with a scalpel.

2.2. Plasmid construction and preparation

The DNA vaccine plasmid pORF75 was created as follows. ORF75 of megalocytivirus RBIV-C1 (Zhang et al., 2013a) was amplified by PCR with the primers ORF75F1 and ORF75R1 (Table 1), and the PCR products were inserted into the SmaI site of pCN3, which was created by inserting linkers L86 and L87 (Jiao et al., 2009) into pCI-neo (Promega, USA) between XhoI/SmaI. pCN8 was created by inserting the Myc-tag (5'- GGGGAACAAAAACTCATCTCAGAAGAGGA TCTGTGA -3') into pCI-neo at the SmaI site. pSmCD83, which expresses Myc-tagged SmCD83, was constructed as follows: the coding sequence of SmCD83 was amplified by PCR with the primer pair SmCD83F/SmCD83R (Table 1), and the PCR products were inserted into pCN8 at the SmaI site. Endotoxin-free plasmid DNA was prepared using Endo-Free Plasmid Kit (Omega Bio-tek, Doraville, USA). The quality of the DNA was analyzed by determining $A_{260/280}$ and $A_{260/230}$ absorbance ratios using NanoDrop 2000 (Thermo Scientific, Waltham, USA). The integrity of the plasmid DNA was assessed by agarose gel electrophoresis.

2.3. Preparation of recombinant P444 (rP444)

To obtain recombinant P444, the protein encoded by ORF75, the plasmid pEtP444, which expresses His-tagged P444, was constructed as follows. The coding sequence of P444 (residues 24–230) was amplified by PCR with the primers ORF75F2 and ORF75R2 (Table 1). The PCR products were ligated with the T-A cloning vector pEASY-Simple-T (TransGen Biotech, Beijing, China), and the recombinant plasmid was digested with EcoRV to retrieve the fragment containing ORF75, which was inserted into pET259 (Hu et al., 2010b) at the SwaI site, resulting in pEtP444. For protein preparation, *Escherichia coli* BL21 (DE3) (TransGen Biotech, Beijing, China) was transformed with pEtP444. The transformant was cultured in Luria-Bertani broth (LB) medium at 37 °C to mid-log phase, and expression of the recombinant protein was induced by adding isopropyl-β-D-thiogalactopyranoside to a final concentration of 0.4 mM. Growth was continued at 37 °C for 5 h, and the recombinant protein was purified using Ni-NTA agarose (QIAGEN, Valencia, USA) as recommended by the manufacturer. The purified protein was dialyzed for 24 h against PBS. The concentration of the protein was determined using BCA Protein Assay Kit (Sangon Biotech, Shanghai, China).

Table 1
Primers used in this study.

Primer	Sequence (5'-3')[a]
ORF75F1	GATATCGCCACCATGGATTCCCTTATCGACCT (EcoRV)
ORF75R1	GCGCGATATCTAGTGTTTTATATACCCCAAAGTCA (EcoRV)
ORF75F2	GATATCATGGATGAGTACAATGCAGAGGGCT (EcoRV)
ORF75R2	GATATCACACATGCCCATGTCAACAT (EcoRV)
SmCD83F	GATATCGCCACCATGTTCCCACATCACCTGA (EcoRV)
SmCD83R	CGATATC AACATACACGGGCTTCCC (EcoRV)
ORF75F3	TGACCACCGTGACAGACTTTC
SmCD83F1	CTGATGCTCGCCCTTGTAATA
CNR2	GCTCGAAGCATTAACCCTC

[a] Underlined nucleotides are restriction sites of the enzymes indicated in the parentheses at the end of the sequence.

2.4. Vaccination

pORF75, pSmCD83, and pCN3 were diluted with PBS to 300 μg/ml. To prepare the vaccine formulation composed of pORF75 plus pSmCD83 (pORF75 + pSmCD83), pORF75 and pSmCD83 were diluted with PBS to 600 μg/ml and mixed at an equal volume. Turbot were divided randomly into five groups (N = 80) and injected intramuscularly (i.m.) with 50 μl pORF75, pORF75 + pSmCD83, pSmCD83, pCN3, or PBS (control). At one month post-vaccination, 39 fish were removed from each group and infected via intraperitoneal (i.p.) injection with 50 μl megalocytivirus RBIV-C1 (Zhang et al., 2013a) that had been suspended in PBS to 2×10^5 copies/ml. Nine fish were used for the examination of viral loads in spleen at 3 days (d), 6 d, and 9 d post-challenge (3 fish per time point) by absolute quantitative real time PCR as reported previously (Zhang et al., 2013b); the remaining fish were monitored daily for mortality over a period of one month, with moribund fish being euthanized as described in Section 2.1. Dying fish were also randomly selected for the examination of virus in liver, kidney, and spleen as above. The vaccination experiment was performed two times.

2.5. Detection of vaccine gene expression in vaccinated fish

Muscle, kidney, and spleen were taken from vaccinated fish at 7 d post-vaccination. Total RNA was extracted from the tissues with EZNA Total RNA Kit (Omega Bio-tek, Doraville, USA) and treated with RNase-free DNaseI (Omega Bio-tek, Doraville, USA). Three micrograms of RNA were used for cDNA synthesis with RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Waltham, USA), and RT-PCR was performed with the primer pairs ORF75F3/CNR2 and SmCD83F1/CNR2 (Table 1). The forward primer is specific to the target gene (i.e. ORF75 or SmCD83), while the reverse primer is specific to the plasmid. Detection of vaccine protein by immunocolloidal gold electron microscopy was performed as reported previously (Jiao et al., 2009).

2.6. Quantitative real time reverse transcription-PCR (qRT-PCR)

Head kidney was taken from the vaccinated fish (5 from each group) at 7 d post-vaccination. Total RNA extraction and cDNA synthesis were as described above. qRT-PCR was carried out in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) using SYBR Premix Ex Taq Kit (Takara, Dalian, China) as described previously (Zhang et al., 2013b). Melting curve analysis of amplification products was performed at the end of each PCR to confirm that only one PCR product was amplified and detected. The expression levels of the immune genes were analyzed using comparative threshold cycle method ($2^{-\Delta\Delta CT}$) with RNA polynerase subunit D (RPSD) as an