Developmental and Comparative Immunology 55 (2016) 1–11

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

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

Molecular characterization and expression analysis of B cell activating factor from rock bream (*Oplegnathus fasciatus*)

G.I. Godahewa [a, b, 1], N.C.N. Perera [a, b, 1], Navaneethaiyer Umasuthan [a, b], Qiang Wan [a, b, **], Ilson Whang [b], Jehee Lee [a, b, *]

[a] Department of Marine Life Sciences, School of Marine Biomedical Sciences, Jeju National University, Jeju Self-Governing Province, 690-756, Republic of Korea
[b] Fish Vaccine Research Center, Jeju National University, Jeju Self-Governing Province, 690-756, Republic of Korea

ARTICLE INFO

Article history:
Received 18 June 2015
Received in revised form
5 October 2015
Accepted 5 October 2015
Available online 9 October 2015

Keywords:
TNF ligands
B cell activating factor (BAFF)
Immune responses
Recombinant protein
Lymphocyte viability

ABSTRACT

B cell activating factor (BAFF) is a member of the tumor necrosis factor (TNF) ligand family. BAFF has been shown to induce survival and proliferation of lymphocytes. We characterized the gene encoding BAFF (*RbBAFF*) in rock bream (*Oplegnathus fasciatus*), and attempted to determine its biological functions upon immune responses. *In silico* analysis of *RbBAFF* demonstrated the presence of common TNF ligand family features, including a TNF domain, a D-E loop, and three cysteine residues that are crucial for trimer formation. Amino acid sequence alignment confirmed that *RbBAFF* and its homologs were conserved at secondary and tertiary levels. Transcriptional analysis indicated that *RbBAFF* mRNAs were ubiquitously expressed in wide array of tissues. The higher levels of constitutive expression were observed in the kidney, head kidney and spleen, suggesting an important physiological relationship with lymphocytes. Under pathological conditions, *RbBAFF* mRNA levels were significantly elevated. The role of *RbBAFF* in lymphocyte survival and proliferation was confirmed by MTT assays and flow cytometry. Recombinant RbBAFF protein (10 μg/mL) was able to prolong the survival and/or enhance the proliferation of rock bream lymphocytes by approximately 30%. Transcription of *IL-10* and *NFκB-1* was significantly stimulated by *RbBAFF*. Our findings provide further information regarding fish BAFF gene and its role in adaptive immunity.

1. Introduction

The tumor necrosis factor (TNF) family of ligands plays a vital role in regulating inflammation and tissue homeostasis. They can stimulate the downstream caspase signaling pathway, and activate the mitogen-activated protein kinases or extracellular regulatory kinases (Shu et al., 1999). Furthermore, several TNF ligands can boost cell proliferation, differentiation, and survival in contrast of apoptosis (Tribouley et al., 1999).

The cytokine B cell activating factor (BAFF; also known as BlyS, THANK, TNFSF13b and TALL-1) belongs to the TNF ligand superfamily 13B (Ai et al., 2011; Liang et al., 2010; Mukhopadhyay et al., 1999). It is thought to be responsible for the survival, proliferation, and maturation of B cells (Bossen and Schneider, 2006; Schneider et al., 1999). It has been shown that BAFF-deficient mice are almost completely devoid of B cells (Schiemann et al., 2001). In addition, BAFF plays crucial roles in immunoglobulin secretion (Ai et al., 2011; Liang et al., 2010), T cell activation, and B cell mediated autoimmune pathogenesis (Cheema et al., 2001; Mariette et al., 2003).

In vertebrates, BAFF is expressed as a homotrimeric transmembrane protein, and is anchored on the surface of various cell types including macrophages, dendritic cells, monocytes, T lymphocytes, and non-lymphoid cells (Mackay and Browning, 2002; Vogt et al., 2005). The trans-membrane form of BAFF can result in another functionally important soluble protein fragment upon proteolytic processing by furin-like proteases (Moore et al., 1999; Schneider et al., 1999). The soluble region of BAFF is designated

* Corresponding author. Marine Molecular Genetics Lab, Department of Marine Life Sciences, Jeju National University, 66 Jejudaehakno, Ara-Dong, Jeju 690-756, Republic of Korea.
** Corresponding author. Marine Molecular Genetics Lab, Department of Marine Life Sciences, Jeju National University, 66 Jejudaehakno, Ara-Dong, Jeju 690-756, Republic of Korea.
E-mail addresses: oneqiang@msn.com (Q. Wan), jehee@jejunu.ac.kr (J. Lee).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.dci.2015.10.004
0145-305X/

the D-E loop, or "Flap", and contains conserved cysteine (Cys) residues and N-glycosylation sites. Three types of B cell receptors are involved in mediating the *in vivo* activity of BAFF: B cell maturation antigen (BCMA), trans-membrane activator and CAML interactor (TACI), and BAFF receptor (BAFFR) (Gross et al., 2000; Schneider et al., 1999; Tribouley et al., 1999). Of these, BAFFR is the principal receptor for BAFF (Liang et al., 2010), while BCMA and TACI possess higher potential for binding to other TNF family ligand members (Bossen and Schneider, 2006). The BAFF-B cell receptor system provides positive and negative feedback signals to influence B cell developments (Nguyen and Morris, 2014). The BAFF and its receptors are one of the important members in 'apoptosis and survival-APRIL and BAFF signaling pathway' and play a role in the B cell and T cell arms of immune system (Bossen and Schneider, 2006; Mackay and Leung, 2006). The signals from the BAFF receptors activate the NF-κB signaling cascades, and stimulate different regulatory proteins including IL-10, MIP-1β, Bcl-2, Bcl-XL, CD23 and COX-2, and in turn mediate the inflammation, and division, survival, differentiation and maturation of B-cells (Bossen and Schneider, 2006; Claudio et al., 2002; Xu et al., 2002).

The BAFF gene has been identified in mammals (Guan et al., 2008; Shen et al., 2012), avians (Chen et al., 2009), amphibians (Yang et al., 2013), and several fish species including zebrafish (Liang et al., 2010), spiny dogfish (Li et al., 2012), mefugu (Ai et al., 2011), miiuy croaker (Meng et al., 2015) and yellow grouper (Xiao et al., 2014). A growing body of evidence suggests that BAFF is an important gene with respect to understanding the evolution of the innate and adaptive immune systems in teleosts. Rock bream is one of the most economically valuable fish species in Korea. However, the rock bream aquaculture industry has been adversely affected by frequent outbreaks of bacterial and viral diseases in recent years (Li et al., 2011; Lipton and Kim, 2010). In this present study, we aimed to elucidate the function(s) of BAFF in rock bream and clarify its role during immune responses to bacterial and viral pathogens through characterization of the structural features, spatial and temporal transcriptional expression profiling in different tissues and after immune challenges, and the biological activity assay of recombinant protein. Our work may contribute to reducing the economic losses currently experienced by the rock bream aquaculture industry in Korea.

2. Materials and methods

2.1. Experimental animals, pathogens and chemicals

Rock breams were obtained from the Ocean and Fisheries Research Institute of Jeju Special Self-Governing Province (Jeju, Republic of Korea). Rock bream iridovirus (RBIV) was isolated from infected rock bream kidney samples as previously described (Godahewa et al., 2014). A strain of *Streptococcus iniae* was obtained from the Department of Aqualife Medicine at Chonnam National University (Korea). Oligonucleotide primers used in this study were synthesized by Integrated DNA Technologies, Inc, USA. All chemicals used in this study were molecular biology grade, and purchased from Sigma, USA. Kits for PCR purification, gel purification, and plasmid extraction were obtained from Bioneer, Korea. Taq polymerase, SYBR Ex Taq, molecular markers, and restriction enzymes were purchased from Takara, Japan.

2.2. Rock bream transcriptome library construction and RbBAFF sequence identification

A rock bream cDNA sequence database was constructed using Roche's GS-FLX automated sequencing technology, as described previously (Umasuthan et al., 2011). The cDNA sequences that shared similarity with known BAFF homologs were mined using the Basic Local Alignment Search Tool (BLAST) on NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3. Bioinformatic analysis of RbBAFF

The DNAssist version 2.2 was employed to obtain the putative coding sequence (CDS) of *RbBAFF* cDNA, and to derive the corresponding protein sequence. Functional domains and motifs were identified using the SMART proteomics database (Letunic et al., 2009), Motif Scan tools, PROSITE profile database (De Castro et al., 2006), and the Conserved Domain Database (CDD) (http://www.ncbi.nlm.nih.gov/cdd). Amino acid identity and similarity percentages with known BAFF homologues from different species were calculated by using EMBOSS Needle Pairwise sequence alignment. ClustalW2 (Thompson et al., 1994) was used to perform multiple sequence alignments. The three-dimensional structure of RbBAFF was predicted using the SWISS-MODEL protein modeling server (http://swissmodel.expasy.org/) and examined using PyMOL v1.5 software. Evolutionary relationship was assessed by applying the Neighbor-Joining (NJ) method in MEGA 5.0 (Tamura et al., 2011). A phylogenetic tree was constructed using the protein sequences from selected BAFF members from different taxonomic classes, with 5000 bootstrap replicates.

2.4. Experimental animal rearing and tissue sampling

Healthy rock breams (mean body weight ~50 g) were reared in 400 L tanks filled with sand-filtered aerated seawater (salinity 34 ± 1 psu; pH 7.6 ± 0.5; 24 ± 1 °C). Fish were acclimated to laboratory conditions for 1 week prior to experiments. Static laboratory environmental conditions were maintained throughout the experiment. Blood samples (1 mL fish^{-1}) were collected from the caudal vein using a 22 G sterile syringe, and hematic cells harvested by centrifugation (3000 × *g*, 4 °C, 10 min). Other tissues, including the kidney, head kidney, brain, gills, liver, spleen, skin, intestine, muscle, and heart were collected from three healthy fish. All tissue samples were snap frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

2.5. Stimulation of immune responses

To study the immune response of *RbBAFF* in selected tissues, fish were divided into five groups and challenged with various substances: phosphate-buffered saline (PBS); Streptococcus iniae; RBIV; lipopolysaccharide (LPS); and polyinosinic:polycytidylic acid (poly I:C). Briefly, fish in the various groups were administered 100 μL of challenge substance (Table 1) *via* intra-peritoneal and/or intra-muscular routes. Three individuals were randomly collected at 0, 3, 6, 12, 24, and 48 h post infection (p.i.). Then, spleen tissues were recovered, snap frozen in liquid nitrogen, and stored at −80 °C.

Table 1
Summary of the immune challenges used in the current study.

Pathogen	Source	Mode	Dose/Fish	Volume
LPS	*E. coli* 055:B5, sigma	Intra-peritoneal	125 μg	100 μL
S. iniae	CNU[b], Korea	Intra-peritoneal	1×10^7 CFU	100 μL
RBIV	Infected kidney	Intra-muscular	10^2 $TCID_{50}$[a]	100 μL
Poly I:C	Sigma	Intra-peritoneal	150 μg	100 μL
PBS (control)	–	Intra-peritoneal	–	100 μL

[a] $TCID_{50}$, 50% tissue culture infectious dose.
[b] Obtained from the Department of Aqualife Medicine at Chonnam National University (Republic of Korea).