Fish & Shellfish Immunology 41 (2014) 586–592

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

LECT2 improves the outcomes in ayu with *Vibrio anguillarum* infection via monocytes/macrophages

Jiong Chen [a, b, *], Qiang Chen [a, b], Xin-Jiang Lu [a], Chang-Hong Li [a]

[a] Laboratory of Biochemistry and Molecular Biology, School of Marine Sciences, Ningbo University, Ningbo 315211, China
[b] The Donghai Sea Collaborative Innovation Center for Industrial Upgrading Mariculture, Ningbo University, Ningbo 315211, China

ARTICLE INFO

Article history:
Received 9 August 2014
Received in revised form
8 October 2014
Accepted 9 October 2014
Available online 18 October 2014

Keywords:
Cytokine expression
Histological damages
LECT2
Monocyte/macrophage depletion
Survival rate

ABSTRACT

Leukocyte cell-derived chemotaxin 2 (LECT2) is reported to be a cytokine involved in the immune response against pathogenic microorganisms in fish. However, its accurate function in whole fish remains unclear. In this study, we provide the first report on the effect of LECT2 on fish defenses against pathogens *in vivo*. The administration of recombinant LECT2 improved the survival rate of *Vibrio anguillarum* infected ayu. The bacterial burden of *V. anguillarum* infected ayu was decreased in LECT2-treated ayu blood, liver, spleen, and kidney compared with saline control. In bacteria-infected ayu, LECT2 treatment altered the mRNA expression of cytokines, including TNFα, IL-1β, and IL-10, which are all important for the inflammatory response in fish. LECT2 treatment also reduced histological damage in bacteria-infected ayu, and increased peritoneal monocytes/macrophages in both healthy and infected ayu at 12 h post infection. When ayu monocytes/macrophages were depleted by clodronate-liposomes treatment, LECT2 treatment did not increase the survival rate of bacteria-infected fish compared with healthy control fish. Thus our results suggest that LECT2 can modulate host defense in ayu and mediate antibacterial protection against *V. anguillarum* through monocytes/macrophages.

1. Introduction

The ayu or sweetfish, *Plecoglossus altivelis*, is a teleost fish found in streams and coastal waters in regions of Asia. It is the sole member of the Osmeriformes family Plecoglossidae, which is most similar to rainbow smelt, *Osmerus mordax*. Because of their good flavor profile, they are considered a popular and highly valued edible fish in Asia. Artificially raised ayu are susceptible to bacterial infection due to high-rearing densities, and disease is now a primary constraint to their culture [1]. Fish likely develop a specific immune system suitable for water environments [2]. Moreover, the innate immunity of fish plays a fundamental role in host defense because of the intrinsic inefficiency of the acquired immune response in fish [3]. It is therefore important to study the modulation of the fish immune system and its response against pathogens.

LECT2, firstly identified as a chemotactic factor in mammals [4], consists of 151 amino acids and three intra-molecular disulfide bonds [4]. LECT2 is mainly produced in the liver and secreted into blood in humans and other mammals [5]. It plays a multi-functional role in processes involved in disease conditions or immune regulation, such as hepatocarcinogenesis, sepsis, and natural killer T (NKT) cell homeostasis [6]. In addition to mammals, a large number of LECT2 genes have also been cloned and characterized in teleosts such as ayu (*P. altivelis*) [7], croceine croaker (*Pseudosciaena crocea*) [8], rainbow trout (*Oncorhynchus mykiss*) [9], and zebrafish (*Danio rerio*) [10]. Fish LECT2 expression increases dramatically upon pathogen infection or stress [10,11], and the polymorphisms of LECT2 genes show significant associations with bacterial disease resistance [12]. Recently, we found that LECT2 could interact with CD209-like protein which is a pattern recognition receptor mainly expressed in the head kidney and leukocytes [13], suggesting that the function of LECT2 in the fish immune system could be directly related to immune cell function. Most recently, we determined that recombinant ayu LECT2 (rPaLECT2) treatment could increase phagocytosis and bacterial killing in ayu monocytes/macrophages *in vitro* [7], and alter the expression of a variety of genes mainly implicated in actin cytoskeleton, pattern recognition receptors, and cytokines [7]. Together, these results suggest that fish LECT2 may be directly involved in innate immunity against bacterial infection. However, the effects of teleost LECT2 on host defenses against pathogens *in vivo* are still unclear.

* Corresponding author. Laboratory of Biochemistry and Molecular Biology, School of Marine Sciences, Ningbo University, Ningbo 315211, China. Tel.: +86 574 87609571; fax: +86 574 87600167.
E-mail address: jchen1975@163.com (J. Chen).

http://dx.doi.org/10.1016/j.fsi.2014.10.012
1050-4648/

The inflammatory reaction, essential for defense against infection, initially leads to the upregulation of the cytokines, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) [14,15]. During systemic inflammation, IL-1β and TNFα induce fever, inflammation, tissue destruction, and cell death [14]. The inflammatory response to systemic infection is in part linked to IL-1β activity [16]. Loss control of dramatic upregulation of IL-1β may be a mechanism explaining the immune disorder in infectious disease [16]. TNFα is mainly produced by macrophages in response to inflammation, infection, and other environmental stresses [17,18]. TNFα induces a variety of biological effects as a marker of severe infection [19]. Infection also increases bacterial burden, which triggers multiple inflammatory mechanisms. Bacterial endotoxins, such as LPS, will impair normal cellular functions [20]. The proteases produced by bacteria will stimulate activated leukocytes to generate enzymes such as matrix metalloproteinases, which are linked to stalled healing [21]. Therefore, bacterial clearance in organs is one of the most important beneficial consequences of inflammation against infection [22]. Monocytes/macrophages participate in the inflammatory response and inflammation resolution during infectious conditions [23] by internalizing pathogens [24], and releasing inflammatory cytokines (e.g. TNFα) and chemokines (e.g. CXCL8, CCL2) to trigger leukocyte infiltration to kill pathogens.

In the present study, we determined the effects of rPaLECT2 on mortality, bacterial burden, cytokine gene expression, and peritoneal cell counts in ayu upon *Vibrio anguillarum* infection. Furthermore, clodronate-liposomes were employed to deplete monocytes/macrophages in ayu, and rPaLECT2 effects on survival were measured in monocyte/macrophage depleted ayu after *V. anguillarum* challenge.

2. Materials and methods

2.1. Fish

Healthy ayu weighing 20–25 g each were obtained from a commercial farm in Huangtan Reservoir, Ningbo city, China. The fish used in this investigation were pathogen-free and maintained in stock tanks supplied with filtered water at 20–22 °C. Fish were fed a diet of commercial fish food once a day. The experiments were approved by the Committee on Animal Care and Use and the Committee on the Ethics of Animal Experiments of Ningbo University.

2.2. Survival assay

rPaLECT2 with bioactivity was produced as previously reported [25]. rPaLECT2 in 100 μl saline (0, 0.005, 0.05 or 0.5 μg/g body weight) was injected intraperitoneally (ip) to ayu immediately after infection ip with 1.2×10^4 colony forming units (CFU) *V. anguillarum* per fish. Another group of ayu with saline treatment was injected with *V. anguillarum*. Morbidity was monitored for 96 h after challenge, and the results were recorded every 12 h.

Based on the results obtained from the above experiment, pretreatment or post-treatment with rPaLECT2 (0.05 μg/g body weight) was used to detect the effect of rPaLECT2 on the survival of ayu upon *V. anguillarum* infection. Briefly, rPaLECT2 (0.05 μg/g body weight) was administrated ip to ayu 1 d before or after *V. anguillarum* challenge. Morbidity was monitored for 96 h after challenge, and the results were recorded every 12 h.

2.3. Bacterial burden assay

rPaLECT2 (0.05 μg/g body weight) was administrated ip to ayu that were infected simultaneously with *V. anguillarum*. Bacterial burden was measured as colony-forming units per mg tissue as previously described [6,26]. Briefly, the blood, liver, and spleen, kidney were harvested aseptically from ayu at 24 h post infection (hpi). The tissues from each ayu were weighed and homogenized in 1 ml of sterile PBS (pH7.2). Homogenates and blood were serially diluted in sterile PBS (pH7.2) and then plated onto separate Thiosulfate Citrate Bile Salts (TCBS) agar plates for 18 h at 28 °C. CFU were then calculated in all plates and multiplied by the dilution factor. Data in liver, spleen and kidney samples were presented as CFU per mg, while data in blood samples were expressed as CFU per 50 μl.

2.4. Determination of cytokine transcript levels associated with LECT2 administration

rPaLECT2 (0.05 μg/g body weight) was administrated ip to ayu, which was injected ip simultaneously with 1.2×10^4 CFU *V. anguillarum* per fish. Liver, spleen and head kidney were collected at 4, 8, 12, or 24 h after rPaLECT2 treatment to determine the mRNA expression of *TNFα*, *IL-1β*, and *IL-10* in various ayu tissues. Tissues were frozen in liquid nitrogen, and stored at −70 °C until use for RNA extraction.

Changes of *TNFα*, *IL-1β*, and *IL-10* mRNA expression in ayu liver, spleen, head kidney were determined by real-time quantitative PCR (RT-qPCR) as previously described [27]. Specific primers were designed according to the cDNA sequences of *TNFα*, *IL-1β*, and *IL-10* (Table 1). The housekeeping *β-actin* mRNA was selected as an internal control, and primers pActin2F and pActin2R were expected to amplify a 231-bp *β-actin* specific fragment. Total RNA was isolated from ayu tissues using RNAiso reagents (TaKaRa, Dalian, China), after which, the first strand cDNAs were synthesized using AMV reverse transcriptase (TaKaRa). RT-qPCR reaction was performed using SYBR® Premix Ex Taq™ (Perfect Real-Time) (TaKaRa) in a StepOne™ Real-Time PCR System (Applied Biosystems, Foster City, USA). RT-qPCR was carried out with an initial denaturation step at 95 °C for 5 min, followed by amplification of the target cDNA (35 cycles of denaturation at 95 °C for 25 s, annealing at 58 °C for 30 s, and extension at 72 °C for 45 s). The mRNA expression of the *TNFα*, *IL-1β*, and *IL-10* was normalized against that of the *β-actin*.

2.5. Histological observation

rPaLECT2 (0.05 μg/g body weight) was administrated ip to ayu, which was injected simultaneously with 1.2×10^4 CFU *V. anguillarum* per fish. Liver, spleen, and kidney were collected at 24 h after LECT2 treatment to determine the histological changes. Tissue samples were processed according to Procopio et al. [28]. In brief, the samples were dissected out and fixed in Bouin's solution. After 24 h fixation, the samples were placed in 70% ethanol, dehydrated in a graded ethanol solutions up to pure ethanol and then embedded in paraffin wax. After being dewaxed and

Table 1
Oligonucleotide primers used in this work.

Primer	Gene	Accession number	Nucleotide sequence (5′ → 3′)	Amplicon length (base pairs)
PaTNFαF	*TNFα*	JP740414	ACATGGGAGCTGTGTTCCTC	115
PaTNFαR			GCAAACACACCGAAAAAGGT	
PaIL-1βF	*IL-1β*	HF543937	TACCGGTTGGTACATCAGCA	104
PaIL-1βR			TGACGGTAAAGTTGGTGCAA	
PaIL-10F	*IL-10*	JP758157	TGCTGGTGGTGCTGTTTATGTGT	73
PaIL-10R			AAGGAGCAGCAGCGGTCAGAA	
pActin2F	*β-actin*	AB020884	TCGTGCGTGACATCAAGGAG	231
pActin2R			CGCACTTCATGATGCTGTTG	