Cloning and Expression Analysis of Two Pro-Inflammatory Cytokines, IL-1β and Its Receptor, IL-1R2, in the Asian Swamp Eel Monopterus albus

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Abstract—Interleukin-1β (IL-1β) is the prototypic pro-inflammatory cytokine, whose functions are mediated through interaction with its receptors (IL-1R1 and IL-1R2). Herein, we cloned the full-length cDNA and genomic DNA of IL-1β and IL-1R2 in the Asian swamp eel (Monopterus albus). The eel IL-1β cDNA encodes a putative polypeptide of 246 amino acids. The protein sequence includes a typical IL-1 family signature, but lacked an interleukin-converting enzyme cleavage site. The genomic DNA of eel IL-1β was 2520 bp and comprised five exons and four introns. The eel IL-1R2 cDNA encoded a putative propeptide of 423 amino acid residues, comprising a signal peptide, a transmembrane region and two Ig-like domains in the extracellular region. Similar to other vertebrates, the genomic DNA of the eel IL-1R2 has nine exons and eight introns. Real-time PCR analysis indicated that IL-1β and IL-1R2 were constitutively expressed in all tissues, especially in the liver and immune-related organs. After infection with Aeromonas hydrophila, the transcript levels of IL-1β and IL-1R2 were induced in the head kidney and spleen, reaching their highest levels at 6 h post injection. In vitro, IL-1β and IL-1R2 mRNA levels were also upregulated rapidly at 1h post infection with A. hydrophila. Furthermore, acanthocephalan Pallisentis (Neosentis) celatus could induce the expression of both genes in the head kidney and intestine. In infected intestines, the transcript levels of IL-1β and IL-1R2 were increased by 21.4-fold and 20.8-fold, respectively, relative to the control. The present study indicated that IL-1β and IL-1R2 play an important role in inflammation and host defense, especially in the anti-acanthocephalan response.

Keywords: interleukin-1β, IL-1β, IL1 receptor 2, gene structure, inducible expression, Asian swamp eel, Monopterus albus

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INTRODUCTION

Asian swamp eel (Monopterus albus) is found in Southeast Asian, and cage-cultured in central and southern China. In recent years, Asian swamp eel has become a highly appreciated commodity in the Chinese fish market, and has been selected as a target species for Chinese aquaculture because of its rapid growth, high survival rate and adaptability to cage-culture conditions. Illness frequently occurs during artificial breeding, which has resulted in extensive losses to eel farming industries. Bacteria have emerged as important pathogens, among which Aeromonas sp. causes hemorrhagic septicemia, dropsy and stigmato- sis [1, 2]. In addition, parasites are also harmful to the eel. For example, Gnathostoma spinigerum often infects in Asian swamp eel, and a maximum of 698 larvae were recovered from one eel [3, 4]. The acanthocephalan Pallisentis (Neosentis) celatus also infects the eel, with the highest infection rate (45.81%) in spring, which leads to serious pathological changes, such as visceral adhesion and intestinal wall perforation [5]. Eustrongylides, which infect the viscera of eels, cause visceral damage, hemorrhage, and changes to physiological and biochemical indicators [5]. Recently, a lethal rhabdovirus has been identified in Asian swamp eel, which causes a systemic hemorrhagic disease with high mortality [6].

Interleukin-1β (IL-1β) is an important cytokine that mediates immune regulatory and inflammatory responses by binding to receptors on the cell surface.
There are two primary IL-1 receptors (IL-1R) in mammals including the type I IL-1 receptor (IL-1R1) and the type II receptor (IL-1R2) [8]. All of the known biological activities of IL-1β are mediated by IL-1R1, while IL-1R2 is a well-documented endogenous inhibitor of IL-1β activity [9].

Although teleost IL-1R2 could inhibit IL1β expression and participate in signal transduction through IL-1β to regulate the process similarly to mammals, there are also some differences [10]. For example, mammalian IL-1R2 contains three Ig-like domains, while fish IL-1R2 only has two Ig-like domains in the extracellular region [10]. IL-1β in mammals, as well as in birds and amphibians, has a six coding exon structure, which differs from the IL-1β genes in teleost species. Type I IL-1β genes in teleosts are similar to the IL-1β gene from tetrapod and type II IL-1β lack one or two coding exons at their 5' end [11]. Thus, studies on fish IL-1β and IL-1R2 would provide a valuable insight into the control of diseases during fish farming. Little data have been reported on Asian swamp eel immune system. In this study, we reported the molecular cloning of Asian swamp eel immune system. During fish farming, little data have been reported on Asian swamp eel immune system, and the type II receptor (IL-1R2) [8].

Experimental

Fish. Asian swamp eels, weighing approximately 80 g, were maintained in aerated fiberglass tanks supplied with a continuous flow of recirculating freshwater. Fish were fed twice daily with commercial pellets, and were given a 2-week acclimatization period before treatment.

Cloning of IL-1β and IL-1R2 cDNA in the Asian swamp eel. Based on the conserved sequences of IL-1β homologs from Japanese parrotfish, perch and fugu (GenBank accession nos. FJ155360, AY383480 and NM_001280090, respectively) and IL-1R2 homologs from sea bass, flounder, tilapia and fugu (GenBank accession nos. FJ155360, AY383480 and NM_001280090, respectively), a pair of degenerate primers, IL-1β-Fd/IL-1β-Rd and IL-1R2-Fd/IL-1R2-Rd, were designed to obtain the partial sequence of IL-1β and IL-1R2 (Table 1). cDNA was prepared as a template for the PCR from head kidney that had been stimulated with 25 μg/mL of poly I:C (Sigma, USA) for 24 h. The resultant products were cloned into vector pMD18-T (Takara, Japan) and transformed into Escherichia coli strain DH5α competent cells, following manufacturer’s instructions. Putative clones were screened by PCR and the selected clones were sequenced. 5’-Rapid amplification of cDNA ends (RACE) and 3’-RACE were performed with gene-specific primers and adaptor primers, as described previously [12].

Sequence analysis. Protein prediction was performed using software at the ExPASy Molecular Biology Server (http://expasy.pku.edu.cn/). The putative open reading frames (ORFs) were analyzed for the presence or absence of signal peptides using Signal P 3.0 (http://www.cbs.dtu.dk/services/SignalP/). Putative domains were identified by PROSITE (http://ca.expasy.org/prosite). A multiple alignment was generated using the Clustal W 1.83 program. Sequences were printed and shaded using BOXshade in the EMBnet services (http://embnet.vital-it.ch/). Global sequence comparisons were performed using the MatGAT program (V2.02) with the scoring matrix BLOSUM60, with a gap open penalty of 10 and gap extension penalty of 1. Phylogenetic analysis was performed using the Maximum-likelihood method within the Mega 5.0 software. The degree of confidence for each branch point was determined by bootstrap analysis (1,000 times).

Cloning of genomic sequence. Genomic DNA was purified from the eel spleens using a Wizard Genomic DNA Purification Kit (Promega, USA). Based on the cDNA full-length sequence, primers were designed to obtain the full-length genomic sequence of IL-1β and IL-1R2. PCR was performed using the primers listed in Table 1. The exon/intron structures of the identified genomic sequences were determined by alignment of the full-length cDNA to the genomic sequence using BLAST2.

Real-time PCR analysis of gene expression. For the cDNA templates of IL-1β and IL-1R2, DNase I was used to treat the total RNA before the RNA was reverse-transcribed using a cDNA Synthesis Kit (Fermentas). The expressions of IL-1β and IL-1R2, as well as the housekeeping gene β-actin, were quantified by real-time PCR, as described previously [12, 13].

A standard was constructed using a mixture of equimolar amounts of purified PCR products amplified from cDNA for each gene to be studied. A serial dilution of the standards was run along with the cDNA samples in the same 96-well PCR plate and served as a reference for quantification. The expression level of each gene was calculated as arbitrary units normalized to the expression of β-actin. The fold changes were calculated as the average expression of the treatment groups divided by that of the relevant control group.

Tissue distribution of IL-1β and IL-1R2 in the healthy Asian swamp eels. Four healthy Asian swamp eels (mean ± SEM = 120 ± 16 g) were anesthetized, killed and ten tissues, including the head kidney, caudal kidney, spleen, intestine, brain, heart, muscle, liver, skin and blood, were sampled. The RNA preparation and RT-PCR analysis was performed following the instructions of the TransScript II Green One-Step qRT-PCR SuperMix kit (TRAN). In all cDNA samples, the expression of each gene was calculated rela-