Fish and Shellfish Immunology 93 (2019) 863–870

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

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

Short communication

Comparative analysis of the expression patterns of IL-1β, IL-11, and IL-34 in golden pompano (*Trachinotus ovatus*) following different pathogens challenge

Ying Wu[a,b,1], Yongcan Zhou[a,1], Zhenjie Cao[b], Yun Sun[a,b,*], Yang Chen[b], Yajing Xiang[b], Lu Wang[a], Shengnan Zhang[a], Weiliang Guo[b]

[a] State Key Laboratory of Marine Resource Utilization in South China Sea, Hainan University, PR China
[b] Hainan Provincial Key Laboratory for Tropical Hydrobiology and Biotechnology, College of Marine Science, Hainan University, PR China

ARTICLE INFO

Keywords:
Interleukin
Trachinotus ovatus
Expression patterns
Pathogens challenge
Immune defense

ABSTRACT

Interleukins (ILs) are a subgroup of cytokines, which are molecules involved in the intercellular regulation of the immune system. These cytokines have been extensively studied in mammalian models, but systematic analyses of fish are limited. In the current study, 3 IL genes from golden pompano (*Trachinotus ovatus*) were characterized. The IL-1β protein contains IL-1 family signature motif, and four long helices (αA - αD) in IL-11 and IL-34, which were well conserved. All 3 ILs clustered phylogenetically with their respective IL relatives in mammalian and other teleost species. Under normal physiological conditions, the expression of IL-1β, IL-11, and IL-34 were detected at varied levels in the 11 tissues examined. Most of the 3 ILs examined were highly expressed in liver, spleen, kidney, gill, or skin. Following pathogenic bacterial, viral, or parasitic challenge, IL-1β, IL-11, and IL-34 exhibited distinctly different expression profiles in a time-, tissue-, and pathogen-dependent manner. In general, IL-1β was expressed at higher levels following challenge with all pathogens examined than was observed for IL-11 and IL-34. Furthermore, *Streptococcus agalactiae* and *Cryptocaryon irritans* caused higher levels of IL-1β and IL-11 expression than *Vibrio harveyi* and viral nervous necrosis virus (VNNV). The increased expression of IL-34 caused by VNNV and *C. irritans* were higher than that caused by *V. harveyi* and *S. agalactiae*. These results suggest that these 3 ILs in *T. ovatus* may play different effect pathogen type specific responses.

1. Introduction

Interleukins are a group of cytokines that serve as regulatory proteins that regulate which specific immune system pathway will be initiated [1]. It was initially thought that ILs only signaled between leucocytes. However, it has been demonstrated that ILs are produced by a wide variety of cell types, including $CD4^+$ T helper cells, macrophages, monocytes, and endothelial cells [2]. In general, cytokines are produced at the site of entry of a pathogen and propagate inflammatory signals that regulate the capacity of resident and newly arrived phagocytes to destroy the invading pathogens [3]. Since ILs were first described over 30 years ago [4], a total of 37 ILs have been discovered at the time of publication. While the majority of research has been conducted in mammals, recent studies have expanded into lower vertebrates, including fish.

Both genes and proteins of IL-1β, IL-11, and IL-34 have been identified in mammals, and both the loci and genes were observed to be highly conserved. In mammals. Interleukin-1β is a pleiotropic cytokine regulating numerous immune and inflammatory responses. One of the earliest proinflammatory cytokines to be expressed in response to infection, IL-1β activates monocytes, macrophages, and neutrophils. In addition, IL-1β also induces T helper 1 cell (Th1) and T helper 17 cell (Th17) adaptive cellular responses [5–10]. Like IL-1β, IL-11 is also a multifunctional cytokine. IL-11 belongs to the IL-6 family that serves to stimulate hematopoietic progenitor cells and exerts a series of important immunomodulatory effects [11–13]. Interleukin 34 was identified through a systematic functional screening of the extracellular proteome as a protein that binds to the extracellular domain of colony-stimulating factor-1 receptor (CSF-1R). Activation of this receptor has been shown to promote monocyte survival and proliferation [14]. It has been suggested that IL-34 may play essential roles in the pathological mechanisms of autoimmune disorders, inflammation, infection, and

* Corresponding author. College of Marine Sciences Hainan University, 58 Renmin Avenue, Haikou, 570228, PR China.
E-mail address: syshui207@126.com (Y. Sun).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.fsi.2019.08.018
Received 23 April 2019; Received in revised form 4 August 2019; Accepted 8 August 2019
Available online 15 August 2019
1050-4648/

cancer [15,16].

Although the functions of the IL family of proteins are well characterized in mammals, little is known about these proteins in fish. To date, identification and analyses of IL-1β, IL-11, and IL-34 in fish have been reported in sea bass (*Dicentrarchus labrax*), Atlantic halibut (*Hippoglossus hippoglossus* L), Atlantic cod (*Gadus morhua*), rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*), carp (*Cyprinus carpio*), Japanese flounder (*Paralichthys olivaceus*), fugu (*Takifugu rubripes*), Atlantic salmon (*Salmo salar*), catfish (*Ictalurus punctatus*), and zebrafish (*Danio rerio*) [17–24]. Among these reports, the expression of these 3 ILs is modulated by pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides (LPS) and poly I:C has been examined [17,19,23,24].

In China, *Trachinotus ovatus* (golden pompano) is an important aquaculture species [25]. As the culture density has increased, many diseases have emerged, resulting in serious economic losses in *T. ovatus* [25–28]. Although *IL-1β*, *IL-11*, and *IL-34* belong to different IL families, they are all sensitive to pathogenic microbial stimuli. In the present study, we aimed to compare the expression patterns of the three genes in response to challenge with various categories of pathogens (*Vibrio harveyi*, *Streptococcus agalactiae*, viral nervous necrosis virus, and *Cryptocaryon irritan*). The results of the systematic analysis of IL-1β, IL-11, and IL-34 following pathogenic bacterial, viral, and parasitic infections in *T. ovatus* enhance the current understanding of the function of these 3 ILs, and their role in the immune defense against pathogenic insults.

2. Materials and methods

2.1. Fish

Trachinotus ovatus (average weight, 15 g ± 0.7 g) were purchased from a commercial fish farm in Chengmai, Hainan province, China. Prior to experimentation, fish were housed at 26 °C in aerated and circulating seawater for one week to acclimate to the laboratory environment. Fish were randomly sampled to confirm that they were in fact free of bacterial, viral, or parasitic infections. At the time of sample collection, fish were euthanized with tricaine methanesulfonate (Sigma, St. Louis, MO, USA), dissected, and tissues were collected as previously reported [29].

2.2. Sequence analysis and cloning

The full-length sequence of three *T. ovatus* interleukin genes (IL-1β, IL-11, and IL-34) were obtained from our transcriptome database. The amino acid sequences of IL-1β, IL-11, and IL-34 (GenBank accession numbers: AZL87291.1, AZL87292.1, and AZL87294.1, respectively) were analyzed using the BLAST program hosted by the National Center for Biotechnology Information (NCBI, National Institute of Health, Bethesda, Maryland, USA). A domain search was performed using the conserved domain search program of NCBI. Multiple sequence alignments were created using DNAMAN (Lynnon Biosoft, USA) software. Phylogenetic analysis was performed using ClustalX along with the Neighbor joining algorithm of MEGA 6.0. Gaps were removed by pairwise deletion, and 1000 bootstrap replicates were performed in the phylogenetic analysis.

The coding sequences of *T. ovatus IL-1β*, *IL-11*, and *IL-34* were amplified by PCR with the primers IL-1β-F/R, IL-11-F/R, and IL-34-F/R, respectively (Table S1). The purified PCR products were ligated into the pEASY®-T1 Simple Cloning Vector (Transgen, Beijing, China) and transformed into Trans1-T1 Phage Resistant Chemically Competent Cell (Transgen, Beijing, China), then the positive clones were separately sequenced.

2.3. Challenge of fish with bacterial, viral, or parasitic pathogens

The fish pathogens *V. harveyi* [26] and *S. agalactiae* [30] were isolated from diseased fish in the Hainan province of China. The bacteria were cultured at 28 °C in LB broth. Each of 20 fish in a single tank was intraperitoneally infected with 2×10^5 CFU of *V. harveyi*. Another 20 fish were infected with PBS as a control. At each of 6, 9, 12, and 24 h post-infection (hpi), 5 fish were randomly selected from each group for sacrifice and sampling of liver, spleen, kidney, gill, and skin. To examine the effects of *S. agalactiae* on ILs expression, the fish were challenged as above with 1×10^4 CFU, and sampled in the same manner as well at 6, 12, 24, and 48 hpi. VNNV is a neuropathogenic virus and was obtained from a mortality in juvenile golden pompano in Hainan province of China. VNNV was suspended in PBS and adjusted its concentration to 1×10^7 copies/ml. A tank of 20 fish was intraperitoneally injected with the virus (1×10^6 copies per fish). Five fish were randomly selected at 1, 3, 5, and 7 dpi for sacrifice and tissue collection. A local strain of *C. irritans* was isolated from pompano obtained from an aquafarm in Lingao County, Hainan province, China. The parasite was passaged and propagated in pompano in a laboratory. Theronts of *C. irritans* were released from the tomonts at 27.0 ± 0.5 °C, were collected immediately, and used within 1 h of collection. For the *C. irritans* experiment, 35 fish were exposed to 1.75×10^5 *C. irritans* theronts in 100 L seawater. Five fish were randomly selected for sacrifice and tissue collection at each of the 6 and 12 hpi, as well as at 1, 2, 3, 5, and 7 dpi. For each of the time points sampled, total RNA was extracted from the tissues for qRT-PCR analysis.

2.4. qRT-PCR analysis of IL-1β, IL-11, and IL-34 expression

To determine basal expression levels of IL-1β, IL-11, and IL-34 in the absence or presence of infections, total RNA was extracted from the blood, muscle, kidney, stomach, liver, spleen, intestine, heart, brain, gill, and skin, and from 5 healthy or infected *T. ovatus* from 30 mg tissue samples with the Eastep® Super Total RNA Extraction Kit (Promega, Shanghai, China). The quality of the RNA was examined by determining 260/280 or 260/230 absorbance ratio using a NanoDrop2000 (Thermo Scientific, USA) and gel electrophoresis. All extracted samples had an A260/280 ratio or 260/230 ratio of approximately 2.0. The purified RNA was adjusted to 0.1 μg/μl with nuclease-free water. One microgram of total RNA was used for first-strand cDNA synthesis with Eastep® RT Master Mix Kit (Promega, Shanghai, China) according to manufacturer's instructions. Next, qRT-PCR was conducted using a QuantStudioTM Real-Time PCR machine (Applied Biosystems) using the SYBR ExScript qRT-PCR Kit (LS2062, Promega, Shanghai, China). The primers used for the reactions are listed in Table S1, and primers B2M-F/B2M-R of β-2 microglobulin (*B2M*, GenBank accession number KX987233) was used as an internal reference (Table S1). The qRT-PCR thermocycling parameters were adopted from a previously published protocol [31]. All experiments were repeated three times.

2.5. Statistical analysis

Statistical analyses were carried out using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Data were analyzed using analysis of variance (ANOVA), and statistical significance was defined as $P < 0.05$.

3. Results

3.1. Sequence and phylogenetic analyses

The IL-1β, IL-11, and IL-34 proteins are comprised of 261, 200, and 217 amino acid residues, respectively. The signature motif of the IL-1 family, which is reasonably well conserved, was in fact observed in the *T. ovatus* IL-1β. Furthermore, IL-11 and IL-34 possess the four main α-

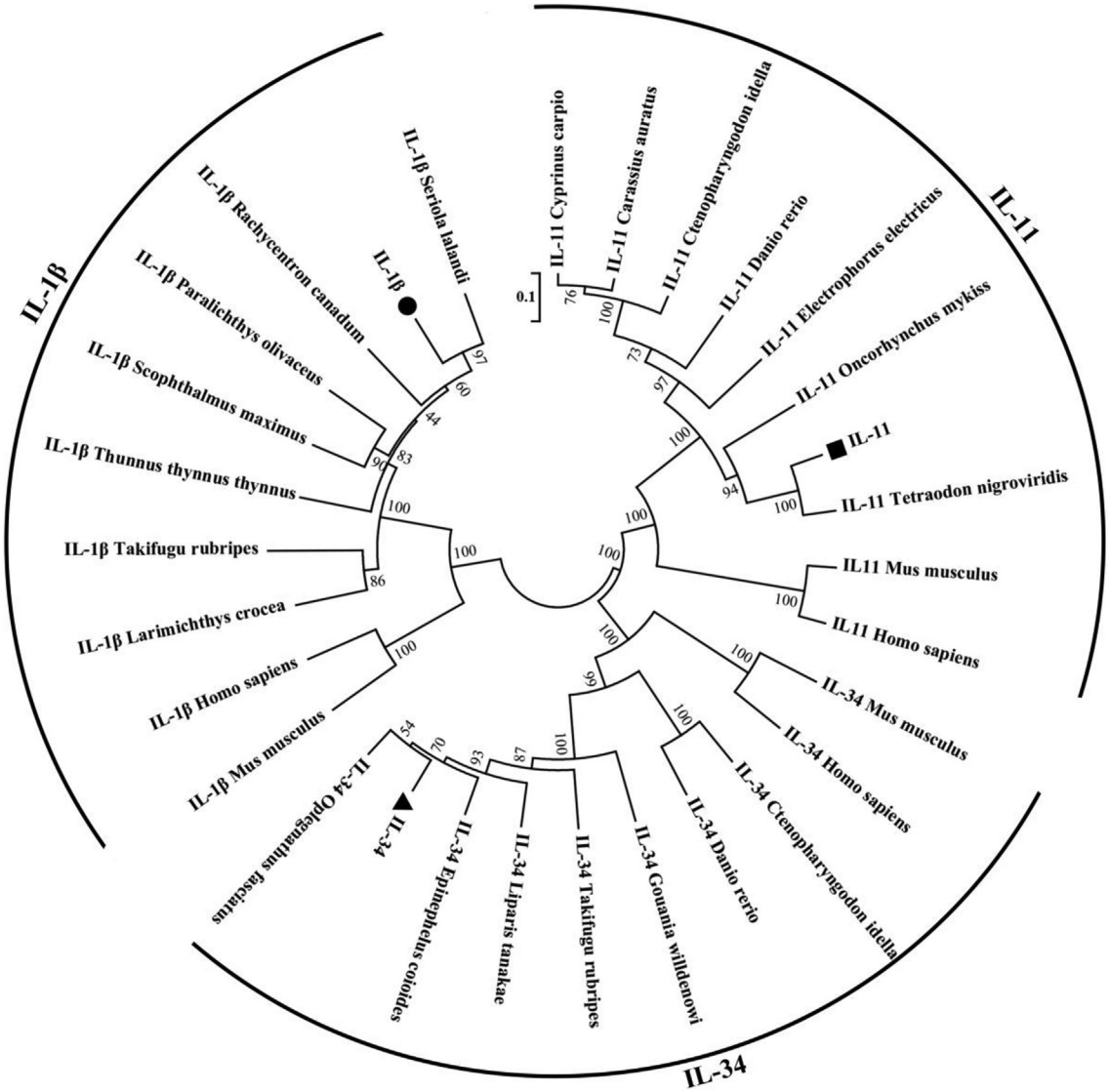

Fig. 1. Phylogenetic analysis of IL-1β, IL-11, and IL-34. Neighbor joining tree was drawn from a ClustalX-generated multiple sequence alignment of the *Trachinotus ovatus* three ILs proteins and other ILs orthologues and paralogues. The GenBank accession numbers are listed in Table S2.

helix regions characteristic of these proteins from other species (Fig. S1). Multiple sequence alignments revealed that IL-1β, IL-11, and IL-34 share 55%–75%, 49%–85%, and 45%–85% overall sequence identities, respectively, with the corresponding ILs in other teleost species. The proteins shared approximately 30%, 25%, and 28% overall sequence identities, respectively, with their human counterparts (Fig. S1).

Phylogenetic analysis of the IL homologues of teleosts and mammals resulted in the formation of 2 main clades, one containing IL-11 and IL-34, and the other containing IL-1β. Close relationships between *T. ovatus* and *Seriola lalandi* IL-1β, *T. ovatus* and *Tetraodon nigroviridis* IL-11, and *T. ovatus* and *Oplegnathus fasciatus* IL-34 were evident in the tree topology (Fig. 1).

3.2. Expression profiles of IL-1β, IL-11, and IL-34 under normal physiological conditions

The expression of IL-1β, IL-11, and IL-34 in *T. ovatus* were examined in 11 tissues: blood, muscle, kidney, stomach, liver, spleen, intestine, heart, brain, gill, and skin by qRT-PCR. The results showed that each of the ILs were expressed in the examined tissues, although the expression patterns differed. The expression of IL-1β was detectable in all tissues examined, with the highest in the gill, followed by the intestine and kidney (Fig. 2A). For IL-11, high expression levels were detected in spleen, kidney, and liver (Fig. 2B), and relatively high levels of IL-34 expression was observed in liver, muscle, and skin (Fig. 2C). Interestingly, the blood exhibited the lowest expression levels for all the ILs examined.

3.3. Expression profiles of ILs in response to infection

Trachinotus ovatus were exposed to bacterial (the gram-negative *V. harveyi*, and the gram-positive *S. agalactiae*), viral (VNNV), and parasitic (*C. irritans*) infections. The expression levels of IL-1β, IL-11, and IL-34 were examined at different time points, all of which were relevant to the corresponding pathogen in the 5 tested tissues (liver, spleen, kidney, gill, and skin). The data suggest that the expression of IL-1β, IL-11, and IL-34 were significantly increased in response to infection in several of the tissues examined at all post-challenge times. Specifically, the expression of IL-1β was significantly up-regulated in all the tissues tested in response to all the pathogens examined in this study. Infection with *V. harveyi* resulted in significant increases in IL-1β expression in liver at all examined time points, spleen at 6 hpi, gill at 6, 9, and 24 hpi, and skin at 6 and 24 hpi (Fig. 3A). Infection with *S. agalactiae* resulted in a significant increase in IL-1β expression in liver at 24 hpi only.

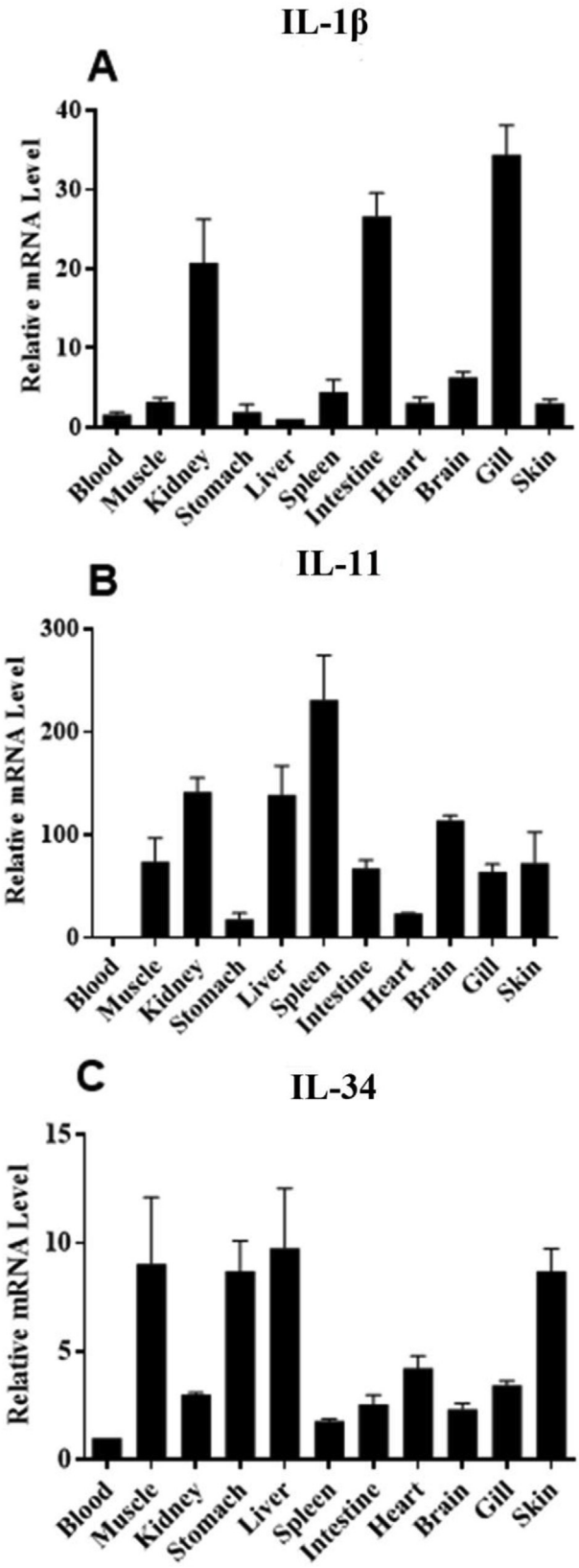

Fig. 2. IL-1β, IL-11, and IL-34 expression in fish tissues under normal physiological conditions. Constitutive expression of IL-1β, IL-11, and IL-34 in various tissues in *Trachinotus ovatus* were determined by quantitative real time PCR. For convenient of comparison, for each gene, the expression level in the tissue with the lowest expression was set as 1. Vertical bars represent means ± SE (N = 3).

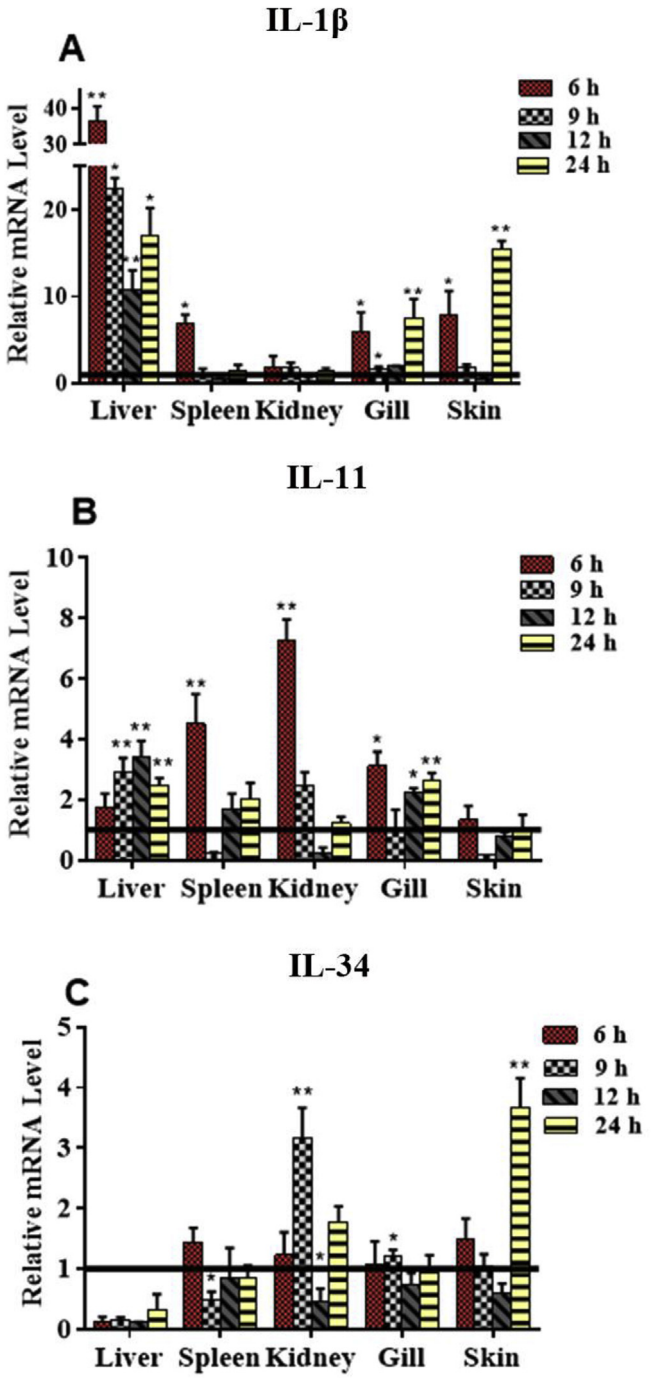

Fig. 3. IL-1β, IL-11, and IL-34 expression in response to *Vibrio harveyi* infection. Expression of IL-1β (A), IL-11 (B), and IL-34 (C) in *V. harveyi* infected or not infected (control) *Trachinotus ovatus* liver, spleen, kidney, gill, and skin were detected using quantitative real-time PCR at various time points post-challenge. At each time point, the expression level of the control fish was set as 1. Values are shown as means ± SE (N = 3). **P < 0.01, *P < 0.05.

Significant increases in IL-1β expression were also observed in the spleen and kidney at all time points examined, and gill and skin at 12, 24, and 48 hpi (Fig. 4A). Significant increases in IL-1β expression were observed in response to VNNV infection in gill at all time points, was observed in liver at 1 dpi, spleen and kidney at 3 dpi, and skin at 1 and 7 dpi (Fig. 5A). Following *C. irritans* infection, IL-1β expression was significantly increased in liver at 2, 3, and 7 dpi, in spleen at 12 hpi and 7 dpi, kidney at 7 dpi, gill at 2, 3, 5, and 7 dpi, and skin at 7 dpi (Fig. 6A).

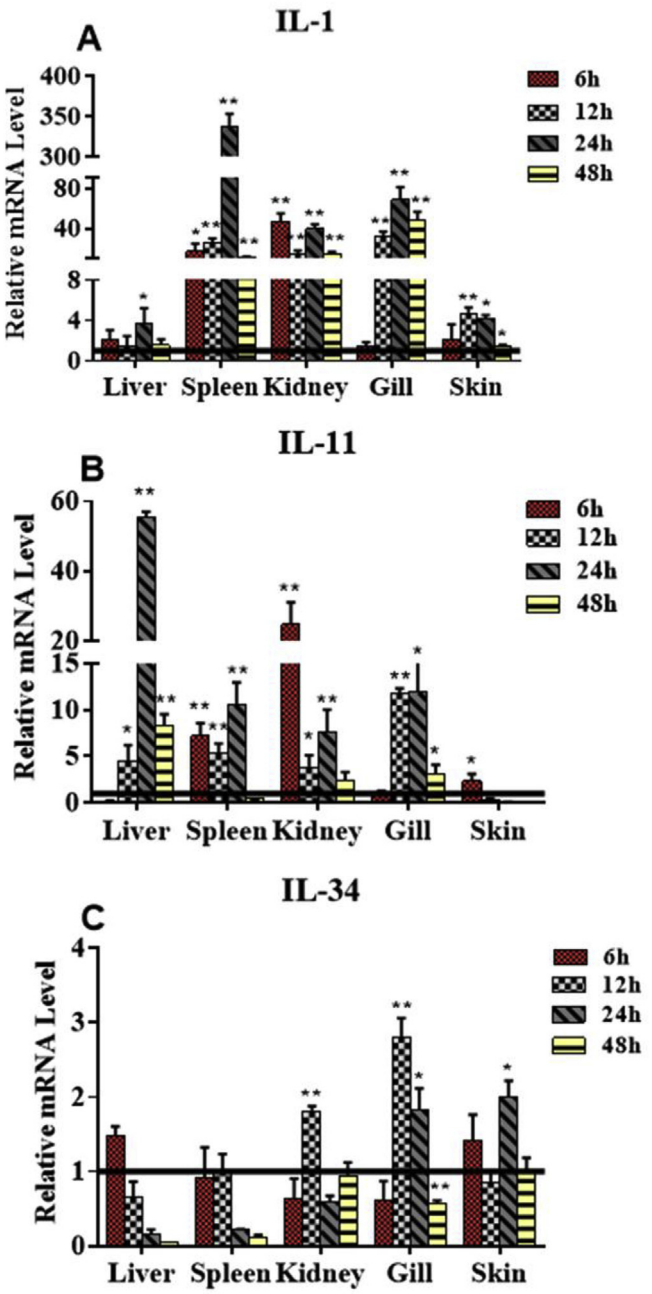

Fig. 4. IL-1β, IL-11, and IL-34 expression in response to *Streptococcus agalactiae* infection. Expression of IL-1β (A), IL-11 (B), and IL-34 (C) in *S. agalactiae* infected or not infected (control) *Trachinotus ovatus* liver, spleen, kidney, gill, and skin were detected using quantitative real-time PCR at various time points post-challenge. At each time point, the expression level of the control fish was set as 1. Values are shown as means ± SE (N = 3). **P < 0.01, *P < 0.05.

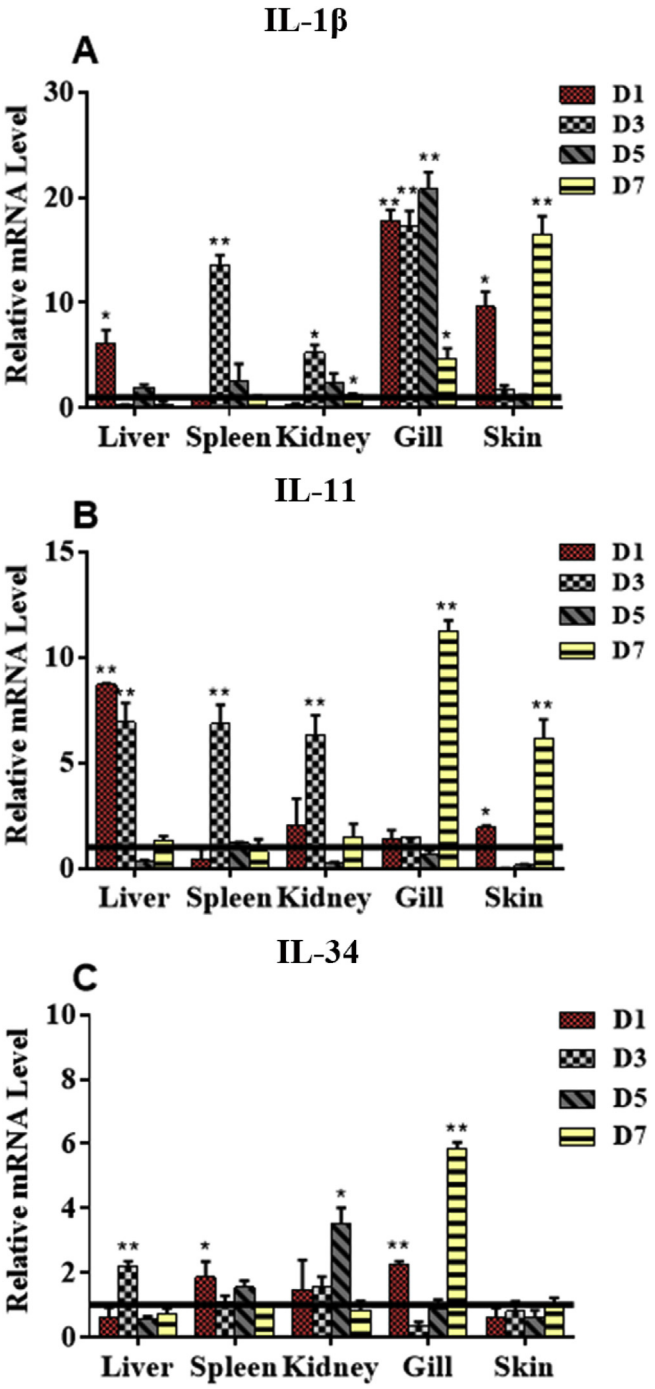

Fig. 5. IL-1β, IL-11, and IL-34 expression in response to VNNV infection. Expression of IL-1β (A), IL-11 (B), and IL-34 (C) in VNNV infected or not infected (control) *Trachinotus ovatus* liver, spleen, kidney, gill, and skin were detected using quantitative real-time PCR at various time points post-challenge. At each time point, the expression level of the control fish was set as 1. Values are shown as means ± SE (N = 3). **P < 0.01, *P < 0.05.

In response to *V. harveyi* infection, IL-11 expression was significantly up-regulated in liver at 9, 12, and 24 hpi, spleen and kidney at 6 hpi, and gill at 6, 12, and 24 hpi. No changes in expression were observed in skin (Fig. 3B). Expression of IL-11 was significantly up-regulated in all tissues examined after *S. agalactiae* and VNNV infection. Infection with *S. agalactiae* significantly enhanced IL-11 expression in skin at 6 hpi only, but significantly enhanced IL-11 expression in liver and gill at all examined time points with the exception at 6 hpi, and in spleen and kidney at all examined time points except for 48 hpi (Fig. 4B). Infection with VNNV significantly increased IL-11 expression in liver at 1 and 3 dpi, spleen at 3 dpi, kidney at 3 dpi, gill at 7 dpi, and skin at 1 and 7 dpi (Fig. 5B). After *C. irritans* infection, IL-11 expression was significantly increased in liver at 1 and 7 dpi, spleen at 5 and 7 dpi, kidney at 7 dpi, and skin at 3 dpi. However, no statistically significant changes in the expression of IL-11 in gill were observed at any time point (Fig. 6B).

For IL-34, expression was significantly up-regulated in kidney at 9 hpi and skin at 24 hpi after *V. harveyi* infection (Fig. 3C). Infection with *S. agalactiae* resulted in significant up-regulation of IL-34 expression in kidney (12 hpi), gill (12 and 24 hpi), and skin (24 hpi). Expression of IL-

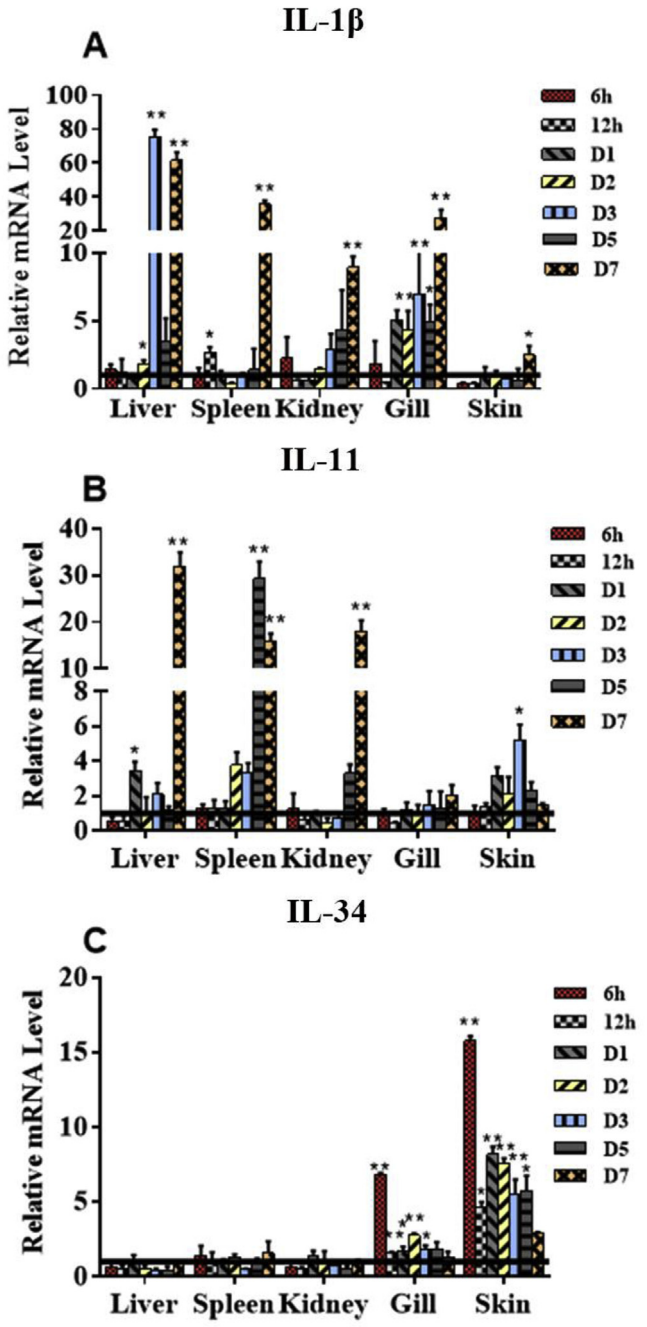

Fig. 6. IL-1β, IL-11, and IL-34 expression in response to *Cryptocaryon irritans* infection. Expression of IL-1β (A), IL-11 (B), and IL-34 (C) in *C. irritans* infected or not infected (control) *Trachinotus ovatus* liver, spleen, kidney, gill, and skin were detected using quantitative real-time PCR at various time points post-challenge. At each time point, the expression level of the control fish was set as 1. Values are shown as means ± SE (N = 3). **P < 0.01, *P < 0.05.

34 was not altered in liver and spleen (Fig. 4C). Following VNNV infection, expression of IL-34 was significantly up-regulated in liver at 3 dpi, spleen at 1 dpi, kidney at 5 dpi, and gill at 1 and 7 dpi. Expression did not significantly change in skin (Fig. 5C). Unlike IL-1β and IL-11, *C. irritans* infection significantly up-regulated IL-34 expression in gill at 6 and 12 hpi, as well as at 1, 2, and 3 dpi. In skin, expression of *IL-34* was significantly up-regulated at all examined time points except for 7 dpi. No significant changes in IL-34 expression were observed in liver, spleen, or kidney in response to *C. irritans* infection (Fig. 6C).

4. Discussion

This study, represents the first known report the cloning and characterizing IL-1β, IL-11, and IL-34 from *T. ovatus*. The IL-1 family signature motif for IL-1β, and the four long helices (αA - αD) for IL-11 and IL-34 are well conserved among *T. ovatus* and other fish and mammalian species [17,20,24,32,33]. In the phylogenetic analysis performed here, each of the IL genes clustered together with their counterparts known in other teleost species. These data suggest that the family of IL proteins is evolutionarily conserved.

Previous studies have demonstrated that IL-1β, IL-11, and IL-34 are expressed ubiquitously in tissues of rainbow trout [17,23,34], Nile tilapia [35], carp [22,36,37], Japanese flounder [24], fugu [17], Atlantic halibut [18], catfish [17,38] and zebrafish [17]. In this study, these ILs were differentially expressed in the 11 tested tissues. For example, IL-1β was dominant in gill, intestine, and kidney compared to IL-11, which was dominant in spleen, liver, and kidney. These results were similar for IL-1β in Atlantic halibut, which had the highest expression in gill. In rainbow trout, IL-11 was expressed at the highest levels in spleen and kidney [18,23]. Different from rainbow trout IL-34 which was the most highly expressed in gill, *T. ovatus* IL-34 was expressed at relatively high levels in liver, muscle, and skin. It was recently reported that in mice, IL-34 was determined to be a tissue restricted colony-stimulating factor-1 receptor (CSFR) ligand for Langerhans cells [39]. Overall, the present study showed that the 3 ILs examined were abundant in the liver, spleen, kidney, gill, and skin. These tissues comprise the major immune organs of teleosts, with many immune cells such as lymphocytes and phagocytes. These results suggest that ILs in *T. ovatus* are likely involved in the responses to infectious microbes.

Pathogenic bacteria, viruses, and parasites infect fish through breaks in the skin, and through the gills, before spreading to other internal organs, with liver, spleen, and kidney being the main target organs where massive pathogen infiltration and proliferation occur post-infection [17]. Many reports have indicated that ILs are highly expressed in gill. This can be explained by the fact that gill is a primary site where foreign insults are first encountered [17,18,23]. In this study, it was observed that, following infection with various pathogens, the expression levels of IL-1β, IL-11, and IL-34 were all significantly up-regulated in the gills, followed by the skin. In line with these results, in *Epinephelus coioides*, IL-34 was up-regulated at 6 hpi, 12 hpi, and 3 dpi in skin, and at almost all time points in gill followed by *C. irritans* infection [17]. Furthermore, in the present study, it's interesting that the expression of IL-1β, IL-11, and IL-34 were all significant higher in gill than the other four tissues (liver, spleen, kidney, and skin) in response to VNNV infection. Similarly, in *Gadus morhuaL*, IL-1β expression was significantly increased after poly I:C stimulation in gill [19]. These might suggest a conserved role for IL-1β in gill, where it functions as an inflammatory cytokine. The up-regulation of IL-1β, IL-11, and IL-34 in gill of infected *T. ovatus* may confirmed the activation of mononuclear phagocytes in sites of bacterial, viral, and parasitic infection. This is indicative of an improved antigen presentation and local antigen specific immune responses.

Following bacterial infection, IL-1β expression was significantly up-regulated in liver after *V. harveyi* infection, while *S. agalactiae* significantly enhanced the expressions of IL-1β in spleen and kidney of *T. ovatus*. Similarly, IL-11 expression was significantly induced by both bacterial pathogens in liver, spleen, and kidney. For IL-34, expression was significantly induced in kidney by *V. harveyi* and *S. agalactiae*. Interestingly, it was observed that expression of the ILs was up-regulated higher in response to *S. agalactiae* infection than to *V. harveyi*. This might suggest that ILs play a more important role in defending against gram-positive bacterial infection.

Previous studies showed that ILs were involved in antiviral immunity. For example, expression of IL-1β in Salmonids was significantly up-regulated in kidney at all time points (day 1–8) in response to viral haemorrhagic septicemia virus (VHSV) infection, which peaked at day

1 with 47.9-fold induction [20]. After stimulation of rainbow trout with poly I:C, IL-11 expression was profoundly increased at 3, 7 hpi, and reduced at 24 hpi [23]. Similarly, IL-34 was highly up-regulated at 4 hpi in the gill, liver, gonad, and spleen following poly I:C treatment [17]. In the present study, it was observed that the expression of IL-1β, IL-11, and IL-34 were most significantly up-regulated in the early time points after VNNV infected, suggesting that the cytokines are part of the first-line responses in liver, spleen, and kidney. All of the results may suggest that all three ILs play an important role in the antiviral response.

In the current study, *C. irritans* infection significantly up-regulated expression of IL-1β and IL-11 after 6 hpi mainly in liver, spleen, and kidney. In contrast, IL-34 expression significantly increased in response to *C. irritans* challenge at 6 hpi, although predominantly in gill and skin. Similarly, in *E. coioides*, IL-34 was the most strongly up-regulated in gill and skin after *C. irritans* infection, which allowed for the inference to be made that mononuclear phagocytes were activated in parasite infected sites [17]. In mammals, the IL-34 deficient mice responded poorly to skin antigen and viral infection of the central nerve system (CNS) [39]. These results suggest that IL-1β, IL-11, and IL-34 may be involved in the host immune response against parasitic infections, although the main response organs were different. Meanwhile, the results also possibly indicate that IL-34 may be a rapid response molecule to parasitic infection, primarily in gill and skin.

On the whole, when comparing expression patterns of the 3 ILs examined here following different types of pathogenic infections, it was observed that IL-1β was significantly induced to a greater degree by all pathogens examined than IL-11 and IL-34. These data may suggest that IL-1β effects broad functions in the immune response of *T. ovatus*. Furthermore, for IL-1β and IL-11, the increases of expression caused by *S. agalactiae* and *C. irritans* were markedly higher than those caused by *V. harveyi* and VNNV. For IL-34, the increases of expressions caused by VNNV and *C. irritans* were markedly higher than those caused by *V. harveyi* and *S. agalactiae*, which was primarily observed in the gill and skin. These results might suggest that IL-1β, IL-11, and IL-34 serve different functions in response to different pathogens.

In conclusion, it was demonstrated here that that IL-1β, IL-11, and IL-34 were expressed widely in *T. ovatus* tissues, especially in major immune organs, and that the 3 IL genes displayed significant and varied alterations in mRNA levels in response to bacterial, viral, and parasitic infections. Furthermore, the changes in expression were dependent on the type of pathogen, tissue type, and infection stage. These results provide the first systematic study of IL-1β, IL-11, and IL-34 against bacteria, viruses and parasites in fish, thus promoting the current understanding of the function of these ILs in the immune defense against different pathogens.

Acknowledgements

This research was supported financially by the National Natural Science Foundation of China (No. 41666006, No. 31560725) and the Nanhai Famous Youth Project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.08.018.

References

[1] G. Perez-Cordon, I. Estensoro, L. Benedito-Palos, J.A. Calduch-Giner, A. Sitja-Bobadilla, J. Perez-Sanchez, Interleukin gene expression is strongly modulated at the local level in a fish-parasite model, Fish Shellfish Immunol. 37 (2014) 201–208.
[2] C.J. Secombes, T. Wang, S. Bird, The interleukins of fish, Dev. Comp. Immunol. 35 (2011) 1336–1345.
[3] J.A. Chabalgoity, A. Baz, A. Rial, S. Grille, The relevance of cytokines for development of protective immunity and rational design of vaccines, Cytokine Growth Factor Rev. 18 (2007) 195–207.
[4] I. Gery, R.K. Gershon, B.H. Waksman, Potentiation of the T-lymphocyte response to mitogens. I. The responding cell, J. Exp. Med. 136 (1972) 128–142.
[5] C.A. Dinarello, Interleukin-1 in the pathogenesis and treatment of inflammatory diseases, Blood 117 (2011) 3720–3732.
[6] F. Martinon, A. Mayor, J. Tschopp, The inflammasomes: guardians of the body, Annu. Rev. Immunol. 27 (2009) 229–265.
[7] L. Agostini, F. Martinon, K. Burns, M.F. McDermott, P.N. Hawkins, J. Tschopp, NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder, Immunity 20 (2004) 319–325.
[8] C.T. Weaver, L.E. Harrington, P.R. Mangan, M. Gavrieli, K.M. Murphy, Th17: an effector CD^4 T cell lineage with regulatory T cell ties, Immunity 24 (2006) 677–688.
[9] C. Dong, TH17 cells in development: an updated view of their molecular identity and genetic programming, Nat. Rev. Immunol. 8 (2008) 337–348.
[10] F.L.V. de Veerdonk, M.G. Netea, C.A. Dinarello, L.A.B. Joosten, Inflammasome activation and IL-1 beta and IL-18 processing during infection, Trends Immunol. 32 (2011) 110–116.
[11] X.X. Du, D.A. Williams, Interleukin-11: a multifunctional growth factor derived from the hematopoietic microenvironment, Blood 83 (1994) 2023–2030.
[12] T. Putoczki, M. Ernst, More than a sidekick: the IL-6 family cytokine IL-11 links inflammation to cancer, J. Leukoc. Biol. 88 (2010) 1109–1117.
[13] N.S. Weich, A. Wang, M. Fitzgerald, T.Y. Neben, D. Donaldson, J. Giannotti, et al., Recombinant human interleukin-11 directly promotes megakaryocytopoiesis in vitro, Blood 90 (1997) 3893–3902.
[14] H. Lin, E. Lee, K. Hestir, C. Leo, M. Huang, E. Bosch, et al., Discovery of a cytokine and its receptor by functional screening of the extracellular proteome, Science 320 (2008) 807–811.
[15] M. Baghdadi, Y. Umeyama, N. Hama, T. Kobayashi, N. Han, H. Wada, et al., Interleukin-34, a comprehensive review, J. Leukoc. Biol. 104 (2018) 931–951.
[16] M. Baghdadi, H. Endo, Y. Tanaka, H. Wada, K.I. Seino, Interleukin 34, from pathogenesis to clinical applications, Cytokine 99 (2017) 139–147.
[17] T. Wang, T. Kono, M.M. Monte, H. Kuse, M.M. Costa, H. Korenaga, et al., Identification of IL-34 in teleost fish: differential expression of rainbow trout IL-34, MCSF1 and MCSF2, ligands of the MCSF receptor, Mol. Immunol. 53 (2013) 398–409.
[18] A.C. Overgard, I. Nepstad, A.H. Nerland, S. Patel, Characterisation and expression analysis of the Atlantic halibut (*Hippoglossus hippoglossus* L.) cytokines: IL-1beta, IL-6, IL-11, IL-12beta and IFNgamma, Mol. Biol. Rep. 39 (2012) 2201–2213.
[19] M. Seppola, A.N. Larsen, K. Steiro, B. Robertsen, I. Jensen, Characterisation and expression analysis of the interleukin genes, IL-1β, IL-8 and IL-10, in Atlantic cod (*Gadus morhua* L), Mol. Immunol. 45 (2008) 887–897.
[20] M. Husain, S. Bird, R. van Zwieten, C.J. Secombes, T.H. Wang, Cloning of the IL-1 beta 3 gene and IL-1 beta 4 pseudogene in salmonids uncovers a second type of IL-1 beta gene in teleost fish, Dev. Comp. Immunol. 38 (2012) 431–446.
[21] D.Q. Lu, J.X. Bei, L.N. Feng, Y. Zhang, X.C. Liu, L. Wang, et al., Interleukin-1beta gene in orange-spotted grouper, *Epinephelus coioides*: molecular cloning, expression, biological activities and signal transduction, Mol. Immunol. 45 (2008) 857–867.
[22] K. Fujiki, D.H. Shin, M. Nakao, T. Yano, Molecular cloning and expression analysis of carp (*Cyprinus carpio*) interleukin-1 beta, high affinity immunoglobulin E Fc receptor gamma subunit and serum amyloid A, Fish Shellfish Immunol. 10 (2000) 229–242.
[23] T. Wang, J.W. Holland, N. Bols, C.J. Secombes, Cloning and expression of the first nonmammalian interleukin-11 gene in rainbow trout *Oncorhynchus mykiss*, FEBS J. 272 (2005) 1136–1147.
[24] M.D. Santos, M. Yasuike, H. Kondo, I. Hirono, T. Aoki, Teleostean IL11b exhibits complementing function to IL11a and expansive involvement in antibacterial and antiviral responses, Mol. Immunol. 45 (2008) 3494–3501.
[25] F. Yin, P. Sun, B.J. Tang, X.M. Dan, A.X. Li, Immunological, ionic and biochemical responses in blood serum of the marine fish *Trachinotus ovatus* to poly-infection by *Cryptocaryon irritans*, Exp. Parasitol. 154 (2015) 113–117.
[26] Z.G. Tu, H.Y. Li, X. Zhang, Y. Sun, Y.C. Zhou, Complete genome sequence and comparative genomics of the golden pompano (*Trachinotus ovatus*) pathogen, *Vibrio harveyi* strain QT520, Peer J 5 (2017) e4127.
[27] Y.L. Su, H.D. Xu, H.L. Ma, J. Feng, W.G. Wen, Z.X. Guo, Dynamic distribution and tissue tropism of nervous necrosis virus in juvenile pompano (*Trachinotus ovatus*) during early stages of infection, Aquaculture 440 (2015) 25–31.
[28] Y. Peng, X. Cai, G. Zhang, J. Wang, Y. Li, Z. Wang, et al., Molecular characterization and expression of interleukin-10 and interleukin-22 in golden pompano (*Trachinotus ovatus*) in response to *Streptococcus agalactiae* stimulus, Fish Shellfish Immunol. 65 (2017) 244–255.
[29] B. Sun, Y. Lei, Z. Cao, Y. Zhou, Y. Sun, Y. Wu, et al., TroCCL4, a CC chemokine of *Trachinotus ovatus*, is involved in the antimicrobial immune response, Fish Shellfish Immunol. 86 (2019) 525–535.
[30] W.T. Hu, W.L. Guo, A.Y. Meng, Y. Sun, S.F. Wang, Z.Y. Xie, et al., A metabolomic investigation into the effects of temperature on *Streptococcus agalactiae* from Nile tilapia (*Oreochromis niloticus*) based on UPLC-MS/MS, Vet. Microbiol. 210 (2017) 174–182.
[31] Z.J. Cao, M.W. He, X.J. Chen, S.F. Wang, Y. Cai, Z.Y. Xie, et al., Identification, polymorphism and expression of MHC class I alpha in golden pompano, *Trachinotus ovatus*, Fish Shellfish Immunol. 67 (2017) 55–65.
[32] J. Felix, J. Elegheert, I. Gutsche, A.V. Shkumatov, Y.R. Wen, N. Bracke, et al., Human IL-34 and CSF-1 establish structurally similar extracellular assemblies with their common hematopoietic receptor, Structure 21 (2013) 528–539.
[33] M.J. Czupryn, J.M. McCoy, H.A. Scoble, Structure-function relationships in human interleukin-11. Identification of regions involved in activity by chemical modification and site-directed mutagenesis, J. Biol. Chem. 270 (1995) 978–985.

[34] O. Pleguezuelos, J. Zou, C. Cunningham, C.J. Secombes, Cloning, sequencing, and analysis of expression of a second IL-1beta gene in rainbow trout (*Oncorhynchus mykiss*), Immunogenetics 51 (2000) 1002–1011.
[35] D.S. Lee, S.H. Hong, H.J. Lee, L.J. Jun, J.K. Chung, K.H. Kim, et al., Molecular cDNA cloning and analysis of the organization and expression of the IL-1beta gene in the Nile tilapia, *Oreochromis niloticus*, Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 143 (2006) 307–314.
[36] M.O. Huising, C.P. Kruiswijk, J.E. van Schijndel, H.F. Savelkoul, G. Flik, B.M. Verburg-van Kemenade, Multiple and highly divergent IL-11 genes in teleost fish, Immunogenetics 57 (2005) 432–443.
[37] C.M.S. Ribeiro, T. Hermsen, A.J. Taverne-Thiele, H.F.J. Savelkoul, G.F. Wiegertjes, Evolution of recognition of ligands from gram-positive bacteria: similarities and differences in the TLR2-mediated response between mammalian vertebrates and teleost fish, J. Immunol. 184 (2010) 2355–2368.
[38] Y. Wang, Q. Wang, P. Baoprasertkul, E. Peatman, Z. Liu, Genomic organization, gene duplication, and expression analysis of interleukin-1beta in channel catfish (*Ictalurus punctatus*), Mol. Immunol. 43 (2006) 1653–1664.
[39] Y.M. Wang, K.J. Szretter, W. Vermi, S. Gilfillan, C. Rossini, M. Cella, et al., IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia, Nat. Immunol. 13 (2012) 753–760.