PAPER • OPEN ACCESS

Induction of Toll-like receptor (TLR) 2 and Myeloid88-dependent TLR-Signaling Response to Aeromonas Hydrophila Infections in Gouramy (Osphronemus Gouramy)

To cite this article: D Kusumawaty *et al* 2017 *IOP Conf. Ser.: Mater. Sci. Eng.* **180** 012270

View the article online for updates and enhancements.

This content was downloaded from IP address 167.205.22.106 on 08/01/2018 at 03:56

IOP Conf. Series: Materials Science and Engineering **180** (2017) 012270 doi:10.1088/1757-899X/180/1/012270

Induction of Toll-like receptor (TLR) 2 and Myeloid88-dependent TLR-Signaling Response to Aeromonas Hydrophila Infections in Gouramy (Osphronemus Gouramy)

D Kusumawaty[1]*, S Suhandono[2], A Pancoro[2] and INP Aryantha[2]

[1]Dept. of Biology Education, Indonesia of Education University, Jl. Dr. Setiabudi No 229 Bandung Indonesia

[2]School of Life Science and Technology. Bandung Institute of Technology (ITB), Jl. Ganesa No 10 Bandung Indonesia

*d.kusumawaty@gmail.com

Abstract. Toll-like receptors (TLRs) are a family of innate immune receptors that recognize molecular patterns associated with microbial pathogens (PAMP) and induce antimicrobial immune responses. Here we report the expression of TLR2, Myeloid differentiation primary response gene 88 (Myd88) and Interleukin 1beta (IL1β) from the Gouramy an economically freshwater fish in Indonesia. Upon stimulation with Aeromonas hydrophila, the TLR2 expression was obviously up-regulated in spleen and liver, Myd88 expression up-regulated in spleen and kidney, and IL1β expression was up-regulated in a spleen, kidney, and liver. The highest expression of TLR2 was in liver or hepatopancreas, MyD88 in kidney and IL1β was in the spleen. These findings together highlighted the contribution of TLR2 in augmenting innate immunity in fish and indicated its important role in immune surveillance of various organs during the pathogenic invasion. This study will enrich the information in understanding the innate immune mechanism in fish and will be helpful in developing preventive measures against infectious diseases in fish.

1. Introduction

Bacterial infection leads to initiation of an antibacterial innate immune response by pattern recognition receptors (PRRs) that bind pathogen-associated molecular patterns (PAMPs) of invading pathogen. One of family PRRs that play essential roles in recognition of the innate system in response to invading pathogen is Toll-like receptors (TLRs) [1].

TLRs-signaling divided into MyD88 dependent and independent pathway [2]. In MyD88-dependent-signaling pathway, ligand binding to TLR triggers sequential recruitment of MyD88, members of IRAK (interleukin-1 receptor associated kinase) family and TRAF6 that resulted in nuclear factor (NF)kB activation and induction of several inflammatory cytokines include IL-8 and TNFα [2].

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd

Some TLR activated during Aeromonas hydrophila infection in fish are TLR1 in Epinephelus coioides [3], TLR-2 on Cirrhinus mrigala [2] and Epinephelus coioides [3], TLR 3 on Pseudosciaena crocea [4], TLR4 on "Atlantic salmon" [5] and "rare minnow" Gobiocypris rarus [6] TLR5 on Cirrhinus mrigala [7] and TLR22 on Pseudosciaena crocea [8,9]. TLR22, which is unique to fish.

Oshronemus gouramy is an important aquaculture species in the West Java Provence-Indonesia, but a tremendous economic loss is often caused by gram negative bacteria, Aeromonas hydrophila [10]. A better understanding of the immune defense mechanisms of gouramy may contribute to the development of management strategies for disease control and long-term sustainability of gouramy farmer. The Oshpronemus gouramy is fresh water fish which have extended period maternal phase. So when the gouramy farming is infected Aeromonas hydrophila, the farmer will get a tremendous economic loss. Moreover, Aeromonas hydrophila, a gram-negative bacterium of the family Aeromonadaceae, is often found in association with hemorrhagic septicemia in cold-blooded animals including fish, reptiles, and amphibians [6].

In Gouramy, the innate immune mechanism is meager, and there is no information on any TLR. In this study to identify TLR 2 and associated downstream signaling molecules like MyD88 and TRAF6 and to examine their expression pattern after Aeromonas hydrophila infections

2. Materials and methods

2.1. Fish

Gouramy fingerlings (3 months of age and weighing 10 g) were obtained from the Central of Freshwater Aquaculture Semata-Tasikmalaya, and stocked in 500 L aerated tanks, with each tank containing 100 fishes. Acclimatization was carried out for 2-weeks before the start of the experiment. The fish were fed twice a day with commercial feed with daily one-third water exchange. The water temperature is 28°C.

2.2. Challenge test

One loop of Aeromonas hydrophila will culture in TSB broth 28°C for 18 hours with constant shaking. Further, 500 µl culture will subculture in five ml TSB broth 28°C for five hours with constant shaking. Fish were injected intraperitoneally Aeromonas hydrophila with a dose 2 x 106 CFU/ml. The fish observed for four days. Several tissues (liver, whole kidney, and spleen) collected from three fish at 6, 12, 24, 36, 48, 72, and 96 hours post-exposure.

2.3. RNA isolation and 1st strand cDNA synthesis

Total RNA from various organs or tissues extracted with TRIzol reagent; concentration was measured by UV-spectrophotometer and the integrity was assessed by observing the band intensity of 28S and 18S rRNA (ribosomal RNA) in 1% agarose gel. To synthesize first strand cDNA, one µg total RNA was first treated with 1 unit of DNase I and was reverse transcribed with oligo-dT primer and RevertAid first strand cDNA synthesis kit.

2.4. Cloning of gouramy TLR2 and Myd88 gene

PCR primers to amplify and clone gouramy TLR2 and Myd88 gene were designed based on the nucleotide sequences of the conserved regions of common fish in GenBank. Total RNA extracted from gouramy hepatopancreas; cDNA prepared, and PCR was carried out with one µg of cDNA in a 50 µl reaction volume. The condition of PCR based on (7) method. Cloning, sequencing and BLAST search confirmed these as TLR2 and Myd88 gene in gouramy. Further, a set of internal primers will design from an overlapping sequence of the previously amplified products. Primers for real-time PCR analysis of TLR2 gene will design from this sequence. Gene expressions of TLR2, Myd88 and IL-1β gene in healthy fish and infected fish based on [4].

2.5. Real-time PCR analysis
Real-time PCR (RT-PCR) of the target genes: The cDNA samples were diluted 20-times with distilled water. The changes of mRNA level of TLR2, MyD88, interleukin (IL1β) and the reference gene EF1 performed in ABI7300 Real-time PCR system (Applied Biosystems, USA) real-time PCR detection system. Amplifications were carried out in 20 µl reaction volume, containing five µl of cDNA, 0.25 µl of forward and reverse primer 10µl of TOYOBO 29 SYBR Green PCR master mix and 13.5 µl of PCR grade water. PCR amplifications performed under the following conditions: initial denaturation at 95°C for 10 minutes followed by 40 cycles of 94°C/10 second, 60°C for 10 seconds and 72°C for 10 seconds. The reaction carried out without cDNA was used as negative control [11]. To calculate relative gene expression of TLR2, MyD88, and IL1β with that of a reference gene, EF1. Relative expression ratios obtained by normalizing expression of the target gene, as determined by mean crossing point deviation, by that of a reference gene, b-actin following 2 –DDCT method. The data obtained from the qRT-PCR analysis was expressed as an average of three individual experiments ± standard error (s.e.), and the significant difference between control and treated.

3. Result and discussion

3.1. Tissue expression profile of OspTLR2/myd88/IL1β (figure 1)

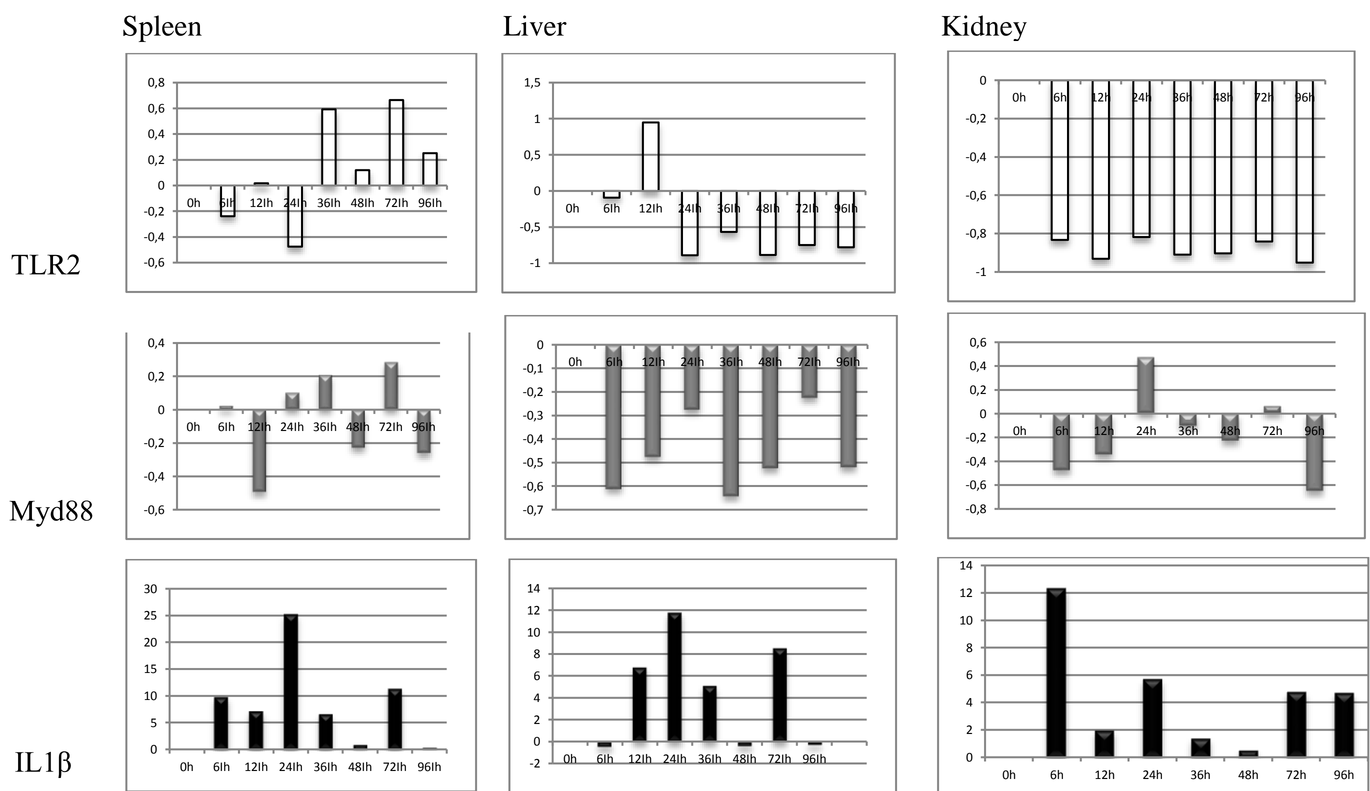

Figure 1. Modulation of TLR2, Myd88 and IL1β expressions by Aeromonas hydrophila infection.

Aeromonas hydrophila was injected into gouramy fingerlings, and after 6, 12, 24, 36, 48, 72, and 96 hours, tissues were collected and used for total RNA extraction and real-time PCR analysis. The relative expression of the TLR2, Myd88 and IL1β gene was normalized to the expression of EF1 (internal

control) and expressed as fold changes about the un-treatment control group. The mean value of three fish (n = 3).

In spleen, TLR2 expression was downregulated from 0 hour to 24 hours post infection and then fluctuating upregulated since 36 hours to 96 hours post injected with *Aeromonas hydrophila*. For Myd88 expression was downregulated to 12 hours and fluctuating upregulated from 24 hours - 72 hours [12]. Different with another IL1β expression directly upregulated from 6 hours to 96 hours post injected. In liver, pattern expression of TLR2 is similar to spleen but Myd88 expression was fluctuating downregulated (figure 1) [13-15].

In the kidney, TLR2 and Myd88 expression downregulated. IL1β have the highest expression in three organ spleen, liver, and kidney. TLR2 expression obviously up-regulated in spleen and liver, Myd88 expression up-regulated in spleen and kidney, and IL1β expression upregulated in a spleen, kidney, and liver. The highest expression of TLR2 was in liver or hepatopancreas, MyD88 in kidney and IL1β was in spleen (figure 1) [16-18].

4. Conclusion

These findings together highlighted the contribution of TLR2 in augmenting innate immunity in fish and indicated it is an important role in immune surveillance of various organs during a pathogenic invasion. This study will enrich the information in understanding the innate immune mechanism in fish and will be useful in developing preventive measures against infectious bacterial in gouramy fish.

5. Acknowledgements

The authors would like to extend gratitude to Doctoral Scholarship Programme from DIKTI (Directorate General of Higher Education Indonesia). Prof. Ikuo Hirono who has provided the opportunity to be able to work in the Laboratory of Genome Science Course of Applied Marine Biosciences Graduate School of Marine Science and Technology Tokyo University of Marine Science.

References

[1] Kawai T and Akira S 2006 TLR signaling *Cell Death & Differentiation* **13**(5) 816-825
[2] Basu M et al 2012 Induction of toll-like receptor (TLR) 2 and MyD88-dependent TLR- signaling in response to ligand stimulation and bacterial infections in the Indian major carp, mrigal (Cirrhinus mrigala) Mol. Biol. Rep. 39(5) 6015
[3] Li Y-W et al 2012 Molecular cloning of orange-spotted grouper (Epinephelus coioides) TLR21 and expression analysis post Cryptocaryon irritans infection. Fish Shellfish Immunol. 32(3) 476
[4] Huang X-N Wang Z-Y and Yao C-L 2013 Characterization of Toll-like receptor 3 gene in large yellow croaker, Pseudosciaena crocea. Fish Shellfish Immunol. 31(1) 98
[5] Tsoi S et al 2006 Identification of a transcript encoding a soluble form of toll-like receptor 5 (TLR5) in Atlantic salmon during Aeromonas salmonicida infection Veterinary Immunology and Immunopathology 109 183
[6] Su J et al 2009 Toll-like receptor 4 signaling pathway can be triggered by grass carp retovirus and Aeromonas hydrophila infection in rare minnow Gobiocypris rarus. Fish Shellfish Immunol 27(1) 33
[7] Basu M et al 2012 Inductive expression of toll-like receptor 5 (TLR5) and associated downstream signaling molecules following ligand exposure and bacterial infection in the Indian major carp, mrigal (Cirrhinus mrigala). Fish Shellfish Immunol. 32(1) 121
[8] Mu X, Pridgeon JW and Klesius PH 2011 Transcriptional profiles of multiple genes in the anterior kidney of channel catfish vaccinated with an attenuated Aeromonas hydrophila Fish Shellfish Immunol. 31(6) 1162
[9] Xiao X et al 2011 Characterization of large yellow croaker (Pseudosciaena crocea) β-actin

promoter supports β-actin gene as an internal control for gene expression modulation and its potential application in transgenic studies in fish Fish Shellfish Immunol. 30(4-5) 1072