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Functional characterization of galectin-3 from Nile tilapia (*Oreochromis niloticus*) and its regulatory role on monocytes/macrophages

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ABSTRACT

Galectin-3 is a kind of β-galactoside-binding lectin involved in host defense against pathogen infection. However, the immune functions of fish galectin-3 remain poorly understood. In this study, the roles of a fish galectin-3 (OnGal-3) from Nile tilapia (*Oreochromis niloticus*) on the binding activity on bacterial pathogens or PAMPs, the agglutinating activity on bacterial pathogens and the regulatory effects on monocytes/macrophages activity were investigated. After *in vitro* challenge of *Streptococcus agalactiae* and *Aeromonas hydrophila*, *OnGal-3* expressions were significantly up-regulated in monocytes/macrophages. In addition, recombinant OnGal-3(rOnGal-3) protein showed strong binding activity on bacterial pathogens or PAMPs. Also, rOnGal-3 agglutinated Gram-positive and Gram-negative bacteria. Moreover, rOnGal-3 could induce the inflammatory factors expressions in monocytes/macrophages and enhance phagocytosis and respiratory burst activity of monocytes/macrophages. These results suggest that fish galectin-3 participates in anti-bacterial immune response through recognizing pathogens and modulating monocytes/macrophages activity.

1. Introduction

The galectins (Gals) are a class of endogenous lectins with high affinity for polysaccharides containing β-galactoside residues [1], which can recognize non-self-glycans of microorganisms and are considered as pattern recognition receptors (PRRs) [2]. On the other hand, Gals can activate innate immune responses by recognizing pathogenic microorganisms, and then mediate downstream responses such as induction of phagocytosis, activation of complement and platelets, as well as enhancement of natural killer cell (NKC) activity [3,4]. Until now, fifteen mammalian galectins are identified and divided into three groups based on their conserved carbohydrate recognition domain (CRD): “prototype” (galectin-1, -2, -5, -7, -10, −11, −13, −14, −15), “chimera” (galectin-3) and “tandem repeat” (galectin-4, -6, -8, −9, −12) [5]. As the only chimera galectin, galectin-3 has one CRD and a long non-lectin domain. The long non-lectin domain can facilitate galectin-3 to form monomer or pentamer, which makes galectin-3 easier to bind different ligands and connect distinct networks [6]. The biological functions of mammalian galectin-3 related to cell proliferation, differentiation and apoptosis have been well-described [7–9]. However, the information of fish galectin-3 remains largely unknown.

To date, although galectin-3 has been reported in various fish species including tilapia (*Oreochromis niloticus*) [10], zebrafish (*Danio rerio*) [11], Japanese conger eel (*Conger myriaster*) [12], channel catfish (*Ictalurus punctatus*) [13], turbot (*Scophthalmus maximus* L.) [14] and half-smooth tongue sole (*Cynoglossus semilaevis*) [15], the functional characterization of fish galectin-3 is still limited. In this study, the expression profiles of OnGal-3 in monocytes/macrophages after bacterial infections were analyzed and the corresponding recombinant protein was obtained via a prokaryotic expression system (*Escherichia coli*, *E. coli*). The roles of rOnGal-3 on recognizing bacterial pathogens, inflammatory cytokines expressions in monocytes/macrophages,

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phagocytic activity and respiratory burst of monocytes/macrophages were investigated. These data indicated OnGal-3 might play important roles in the immune response of fish against bacterial infection.

2. Materials and methods

2.1. Fish

Nile tilapia (50 ± 10 g) were acquired from a local fish farm in Zhanjinag, Guangdong, China. The fish were cultured in 1000 L tank with aerated freshwater under 28 ± 2 °C for three weeks [16,17]. All experiments were conducted according to the principles and procedures of Guangdong Province laboratory animal management regulations.

2.2. Head kidney monocytes/macrophages isolation and S. agalactiae and A. hydrophila stimulation

Monocytes/macrophages were prepared as previous methods [18,19]. Briefly, healthy tilapia was anesthetized by MS222. The head kidney was carefully excised and transferred through a 40 μm stainless nylon mesh (Greiner Bio-OneGmbH, Germany). The cell suspension was suspended in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, US) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin and 25 U/mL heparin (Gibco). The cell suspension was slowly added into a 51%/34% percoll (GE Healthcare) density gradient, centrifuged at 400 g for 40 min, and the cell layer of the interface was carefully aspirated and then washed with PBS at 500 g for 10 min. Cell viability was measured using a Trypan Blue Staining kit (Sangon Biotech). The cells were cultured at 25 °C for 24 h and then removed the non-adherent cells.

S. agalactiae (ZQ0910) and *A. hydrophila* (BYK00810) used in the experiment were isolated from Nile tilapia and kept in Guangdong Ocean University. The challenge experiment were carried out in accordance with the methods of Mu et al. [20,21]. The monocytes/macrophages were stimulated by inactivated bacterial with 1×10^7 CFU/mL. The cells were collected and lysed by Trizol Reagent (Sangon Biotech) at 0 h, 3 h, 6 h, 12 h, 24 h, and 48 h post-challenges, respectively.

2.3. Quantitative real-time PCR

The Quantitative real-time RT-PCR analysis of *OnGal*-3 mRNA expression in monocytes/macrophages following *S. agalactiae* and *A. hydrophila* injection were performed on Roche LC384 LightcyclerTM (Roche, Switzerland). The first-stand cDNA was synthesized from total RNA by using EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen, China). The PCR reaction volume was 10 μL including 5 μL of FastStart Essential DNA Green Master (Roche, Switzerland Total RNA of different time points cells were extracted by using EasyPure RNA Kit (TransGen, China) according to the protocol, 1 μL of diluted cDNA, 0.5 μL of each primer (Table 1), and 3.5 μL of nuclease free water. The program was performed as follows: 1 cycle of 10 min at 95 °C, 40 cycles of 10 s at 95 °C, 15 s at 55 °C, and 15 s at 72 °C. The *β-actin* gene (housekeeping gene) was taken as internal control reference gene. The relative expression level of *OnGal*-9 mRNA were calculated using $2^{-\Delta\Delta Ct}$ method [22].

2.4. Binding of rOnGal-3 to bacterial pathogens

rOnGal-3 was produced as previously reported [23]. The binding ability of rOnGal-3 to Gram-positive bacteria (*S. agalactiae*) and Gram-negative bacteria (*A. hydrophila*) were detected by the methods of Bai et al. [24,25]. In brief, the bacteria were cultured to an OD_{600} of 0.4–0.6, 10 μL of rOnGal-3 (1 mg/mL) was incubated with 500 μL of bacteria for 1 h at 37 °C, then washed three times with PBS and eluted with 7% SDS for 1 min, centrifuged at 12,000 rpm for 10 min. The supernatant was used to run SDS-PAGE and Western blot with anti-GST antibody. Control bacterial cells were incubated with PBS or pGEX-4T-1 and performed the same treatment.

Table 1
Primers used in this study.

Primers	Nucleotide Sequence (5′→3′)	Comment
qOnGal-3-F	CTTCCACTTCAACCCTCGCT	RT-PCR
qOnGal-3-R	CCCTCTCCTCTTTGCCCCAC	RT-PCR
β-actin-F	CGAGAGGGAAATCGTGCGTGACA	RT-PCR Control
β-actin-R	AGGAAGGAAGGCTGGAAGAGGGC	RT-PCR Control
qIL-6-F	ACAGAGGAGGCGGAGATG	RT-PCR
qIL-6-R	GCAGTGCTTCGGGATAGAG	RT-PCR
qIL-8-F	GATAAGCAACAGAATCATTGTCAGC	RT-PCR
qIL-8-R	CCTCGCAGTGGGAGTTGG	RT-PCR
qIL-10-F	TGGAGGGCTTCCCCGTCAG	RT-PCR
qIL-10-R	CTGTCGGCAGAACCGTGTCC	RT-PCR
qMIF-F	CACATCAACCCTGACCAAAT	RT-PCR
qMIF-R	GCCTGTTGGCAGCACC	RT-PCR

Previous studies showed that the binding ability of galectin to bacterial was owing to the carbohydrates on bacteria cell wall, which could be competitively inhibited by other carbohydrates [26]. Here, we chose several saccharides including lipopolysaccharide (LPS), lipoteichoic acid (LTA), galactose, mannose, and maltose to explore the inhibitory capacity. The recombinant protein (1 mg/mL, 10 μL) was incubated with saccharides (1 mg/ml, 50 μL) for 1 h at room temperature, respectively. After that 500 μL of *S. agalactiae* and *A. hydrophila* were added and the binging ability were detected by Western blot as described above.

2.5. Binding assay of rOnGal-3 to carbohydrates

To further understand the competitive combination of galectin to carbohydrates, enzyme-linked immunosorbent assay (ELISA) was performed according to the methods of Zhang et al. [27,28]. In brief, LTA, LPS, galactose, mannose, and maltose were chosen and adjust to a concentration of 100 μg/mL with 20X Coating Buffer (Sangon Biotech, shanghai), and the 96-well plates were coated with these carbohydrates (100 μL) at 4 °C overnight and blocked for 2 h at 37 °C, respectively. The carbohydrates were incubated in a 10 times dilution in the next well to each other, and a blank well as control. Plates were washed with TBST three times between each step. Then the rOnGal-3 (100 μg/mL, 100 μL) were added to the well and incubated for 2 h at 37 °C. After that the Anti-GST mouse monoclonal antibody (Sangon Biotech, shanghai) was used as primary antibody, and the HRP-goat α-rabbit antibody (Sangon Biotech, shanghai) was used as second antibody. 100 μL of TMB mixed reaction solution was added to each well and incubated at 37 °C for 15 min in the dark, then use the H_2SO_4 (2 M, 50 μL) to terminate the reaction. The optical density (O.D.) rate was measured using a Microplate Reader (Thermo, USA) at 405 nm.

2.6. Bacterial agglutination assay

Agglutination assay was performed according to the previous methods [29,30]. Briefly, *S. agalactiae* and *A. hydrophila* were cultured to an OD_{600} of 0.4–0.6 and washed three times with PBS. The bacteria were re-suspended in 0.1 M Na_2CO_3 and then add FITC (Solaribo, China) to a final concentration of 0.1 mmol/L, incubated at 37 °C for 30 min. Then centrifuged three times to completely remove the FITC and incubated 10 μL of rOnGal-3(1 mg/mL) with the bacteria at room temperature for 1 h. The bacteria were applied to a glass slide and the results were observed with a fluorescent microscope.

2.7. Analysis of the expressions of inflammatory-related factors in monocytes/macrophages

The experiment was performed according to the method of Mu [31]. Briefly, the monocytes/macrophages were challenged with 50 μL of rOnGal-3(1 mg/mL), pGEX-4T-1 (1 mg/mL), and PBS, respectively. The cells were collected and the cDNA were synthesized as described in Section 2.3. The expression levels of *IL-6, IL-10, IL-8* and *MIF* (Table 1) in monocytes/macrophages were measured by qRT-PCR.

2.8. Phagocytosis assay

Phagocytosis assay was performed as previous method of Bai et al. [32]. Briefly, 200 μL of FITC-labeled *S. agalactiae* and *A. hydrophila* suspension was mixed with 190 μL of monocytes/macrophages and 10 μL of rOnGal-3 (1 mg/mL) and incubated in the dark for 1 h with shaken every 5 min. In the control group, 10 μL of PBS or pGEX-4T-1 (1 mg/mL) was used instead of rOnGal-3. Then centrifuge at 500 g for 10 min to completely remove the non-ingested bacteria. The results were analyzed using flow cytometer. The fluorescence data for this experiment is limited to a Gate to ensure the accuracy of the analysis, and all data were repeated in triplicate.

2.9. Respiratory burst assay

The effect of rOnGal-3 on respiratory burst activity of monocytes/macrophages was performed by referenced to the previous methods [33,34]. In brief, PMA stimulates monocytes/macrophages to produce reactive oxides that oxidize dihydrorhodamine to rhodamine 123, which is capable of emitting fluorescence. According to the protocol of PMN Oxidative Burst Quantitative Assay Kit (Absin Bioscience, Shanghai), 300 μL of monocytes/macrophages cells was mixed with 10 μL of rOnGal-3(1 mg/mL) and incubated at 25 °C for 24 h. Then add 50 μl of PMA as the activator and incubate at 37 °C for 15 min. Subsequently, 25 μL of dihydrorhodamine was added to the mixture and incubated at 37 °C for 5 min in the dark. 1 mL of diluted hemolysin was added at room temperature and hemolyzed for 15 min. The mixture was washed twice with PBS, centrifuged at 1500 rpm for 5 min and discarded the supernatant, then the cells were resuspended in 0.5 mL PBS for flow cytometry analysis. For the control group, rOnGal-3 was replaced with pGEX-4T-1 or PBS and the same procedure were performed.

2.10. Statistical analysis

All data in this study were displayed as means ± standard deviation (SDs). Statistical analysis were performed by the LSD (least significant difference) test using SPSS 17.0 software. Differences were considered significant at $p < 0.05$ (*) and highly significant at $p < 0.01$ (**)

3. Results

3.1. Quantitative real-time analysis of OnGal-3 response to bacterial infection

Expression patterns of *OnGal-3* in monocytes/macrophages after *S. agalactiae* and *A. hydrophila* challenge were performed by qRT-PCR. The results shown that the expression level of *OnGal-3* peaked at 24 h with higher copy multiple after challenged with *S. agalactiae* (74-fold) (Fig. 1A), while the *A. hydrophila* group peaked at 12 h (11.2-fold) (Fig. 1B).

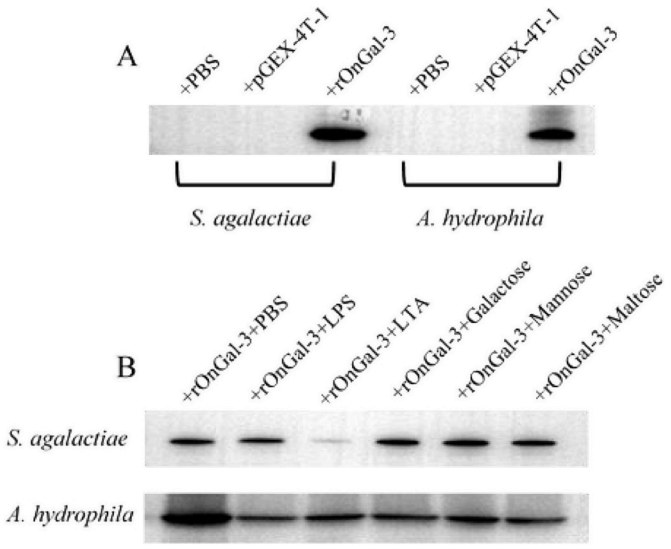

Fig. 2. (A) Western blot analysis of rOnGal-3 bind to *S. agalactiae* and *A. hydrophila*. (B) Western blot analysis of the inhibitory effect of carbohydrate on rOnGal-3 on bacterial binding activity.

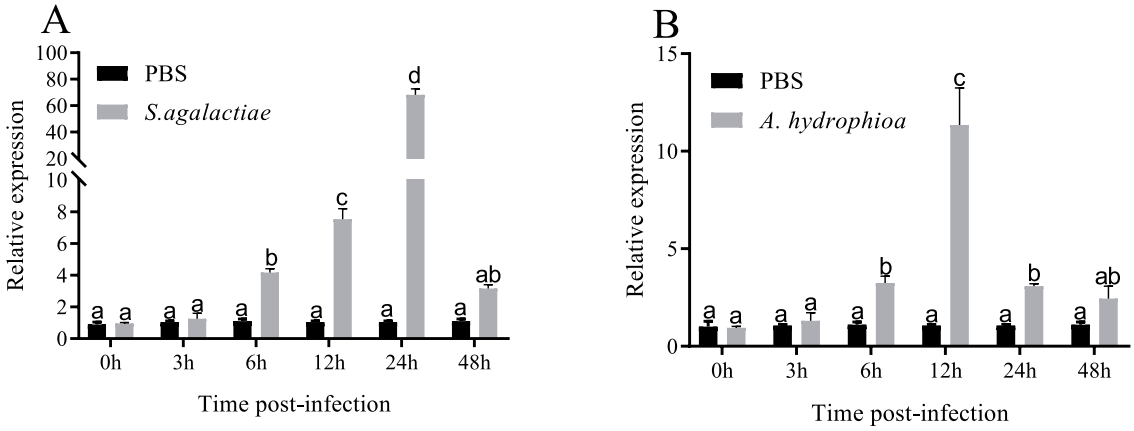

Fig. 1. The mRNA expression of *OnGal-3* in head kidney monocytes/macrophages after challenged with *S. agalactiae* and *A. hydrophila*. Data are shown as mean ± SD. The error bars represent standard deviation (n = 3) and different letters (a, b, c) depict statistical significance between groups of cells after challenges and health. ($p < 0.05$).

3.2. Binding of rOnGal-3 to bacterial pathogens

The binding ability of rOnGal-3 to *S. agalactiae* and *A. hydrophila* were investigated by Western blot. The result showed that rOnGal-3 could bind both *S. agalactiae* and *A. hydrophila*. While the pGEX-4T-1 and PBS could not bind neither of them (Fig. 2A). Additionally, the results of the competition inhibition experiment show LTA could strongly inhibit the binding of rOnGal-3 to *S. agalactiae*, and LPS showed a weaker ability to inhibit rOnGal-3 to *A. hydrophila* (Fig. 2B). However, galactose, mannose, and maltose showed no obvious inhibitory activity.

3.3. Binding assay of rOnGal-3 to carbohydrates

ELISA was performed to confirm the sugar binding specificity of rOnGal-3. The results showed that rOnGal-3 could bind all the tested carbohydrates (LTA, LPS, galactose, mannose, and maltose), and its sugar binding ability depend on the concentration of rOnGal-3 rather than carbohydrates (Fig. 3).

3.4. Agglutinating activity of rOnGal-3

FITC-labeled *S. agalactiae* and *A. hydrophila* were used to test the agglutinating activity of rOnGal-3, and after incubated with rOnGal-3, the results were checked with inverted fluorescence microscope. As shown in Fig. 4, rOnGal-3 could agglutinate both *S. agalactiae* and *A. hydrophila*, while the pGEX-4T-1 and PBS could not agglutinate any bacteria.

3.5. Effects of rOnGal-3 on the expressions of inflammatory-related factors

To investigate the effects of rOnGal-3 on the inflammatory-related

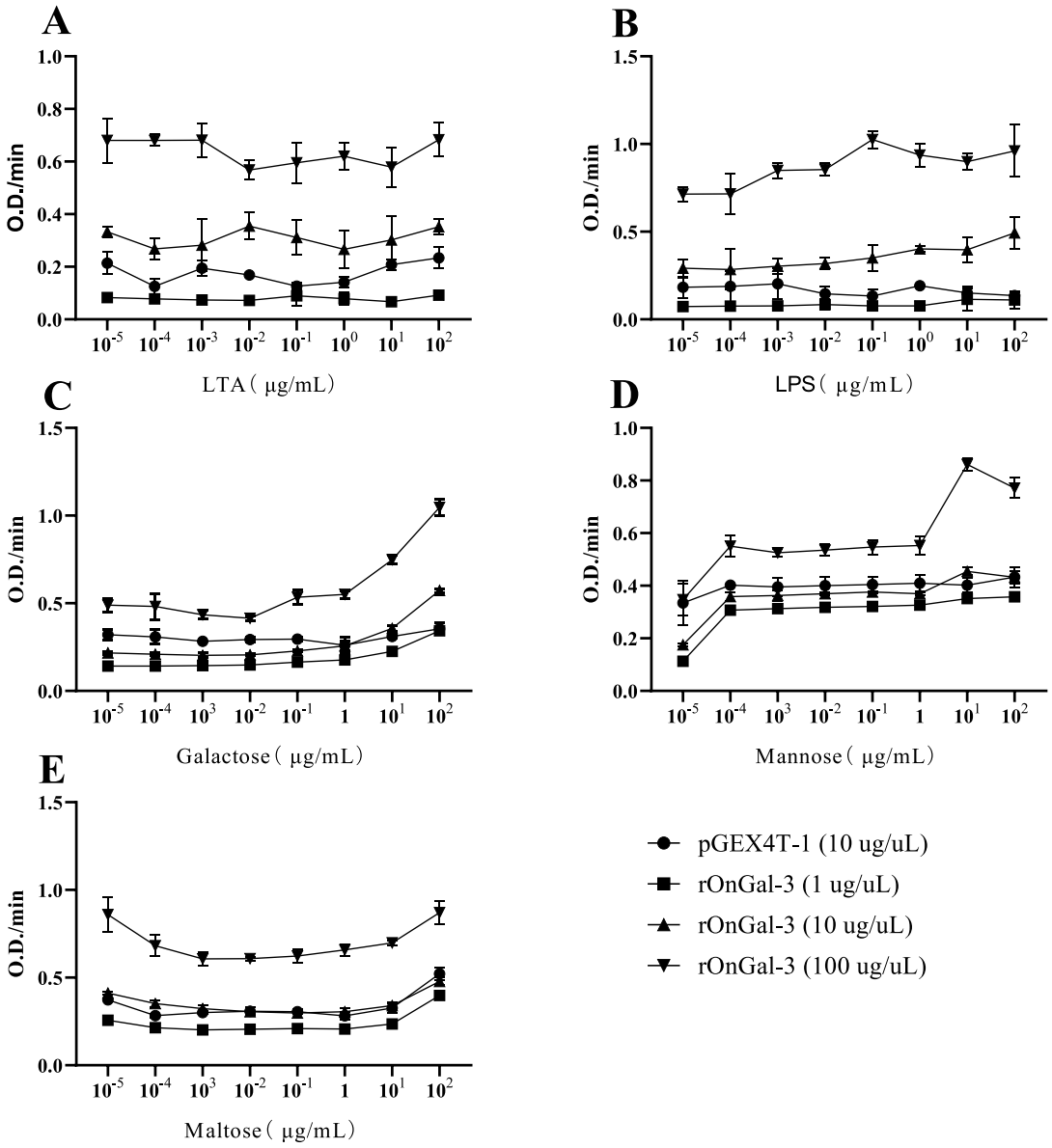

Fig. 3. ELISA analysis of rOnGal-3 incubated with LTA (A), LPS (B), galactose (C), mannose (D), and maltose(E) in different concentration, respectively. The carbohydrates were incubated in a 10 times dilution in the next well to each other, and a blank well as control.

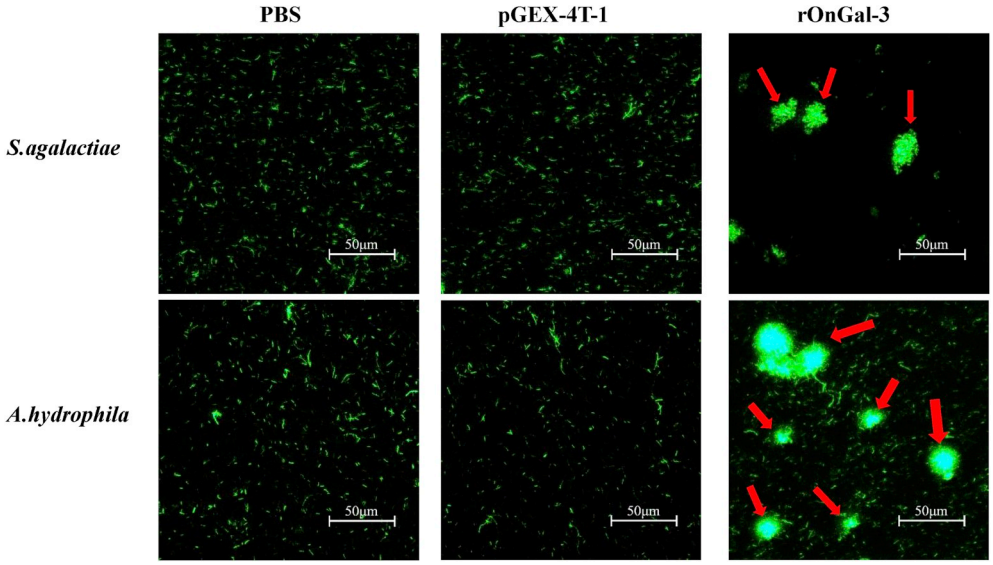

Fig. 4. Agglutinating activity of rOnGal-3 against FITC-labeled *S. agalactiae* and *A. hydrophila*. PBS or pGEX-4T-1 was incubated with bacteria as a negative control. The agglutination was presented with red arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

factors of monocytes/macrophages *in vitro*, we selected several cytokines for testing. The results indicated that the mRNA expression level of *IL-6*, *IL-10*, *IL-8*, and *MIF* were significantly up-regulated after stimulation with rOnGal-3. Among them, the transcript of *IL-6* and *IL-10* were rapidly raised at 12 h with much more significantly compared with the pGEX-4T-1 stimulation (*IL-6*, 16.3 vs. 7.2-fold, 12 h; *IL-10*, 9.7 vs 1.8-fold, 12 h) (Fig. 5 A and C), while the expressions of the *IL-8* and *MIF* reached the maximum expression level at 24 h (Fig. 5B and D).

3.6. Enhancement of phagocytosis by rOnGal-3

The ability of rOnGal-3 promote the phagocytosis of bacteria by macrophages was determined by flow cytometer. As shown in the histogram, there were almost no fluorescent signal of macrophages (Fig. 6 A). After labeled with FITC, a strong fluorescent signals of *S. agalactiae* and *A. hydrophila* could be observed in Fig. 6 B and C. The phagocytosis rates of the macrophages were detected by phagocytosing the bacteria

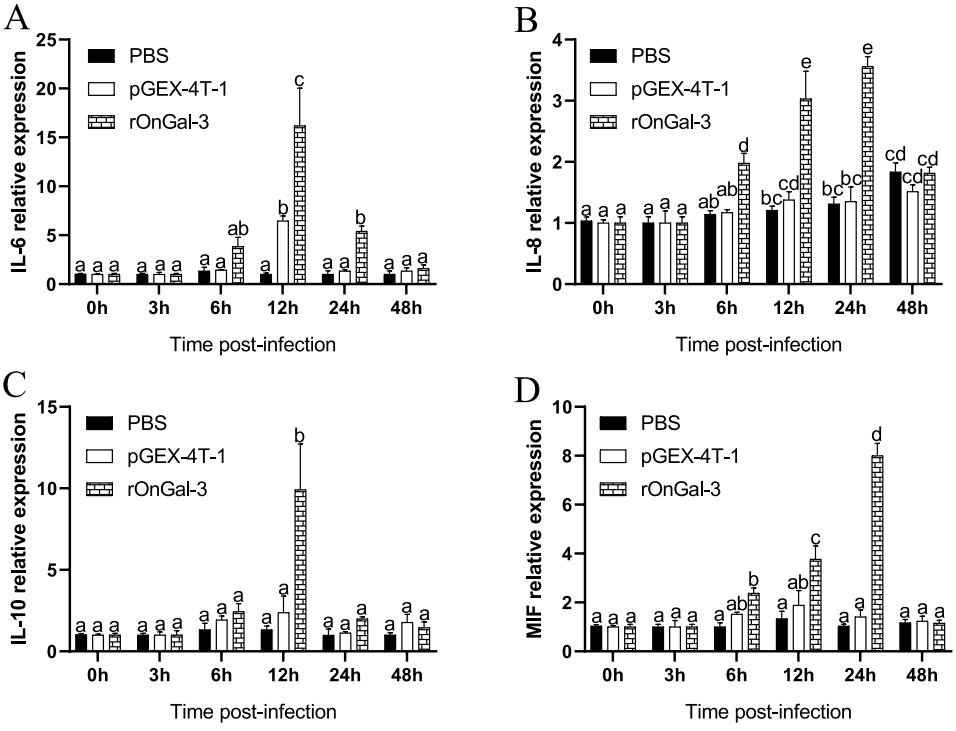

Fig. 5. The mRNA expression of *IL-6* (A), *IL-8* (B), *IL-10* (C) and *MIF* (D) from Nile tilapia in the head kidney monocytes/macrophages. Nile tilapia head kidney monocytes/macrophages were treated with PBS, pGEX-4T-1 (50 μg/mL), and rOnGal-3 (50 μg/mL). The mRNA level of *IL-6*, *IL-8*, *IL-10*, and *MIF* gene was normalized to that of *β-actin* and fold units were calculated deciding the values of the PBS treated cells. The error bars represent standard deviation (n = 3) and different letters (a, b, c) depict statistical significance between groups of cells after challenges and health. ($p < 0.05$).

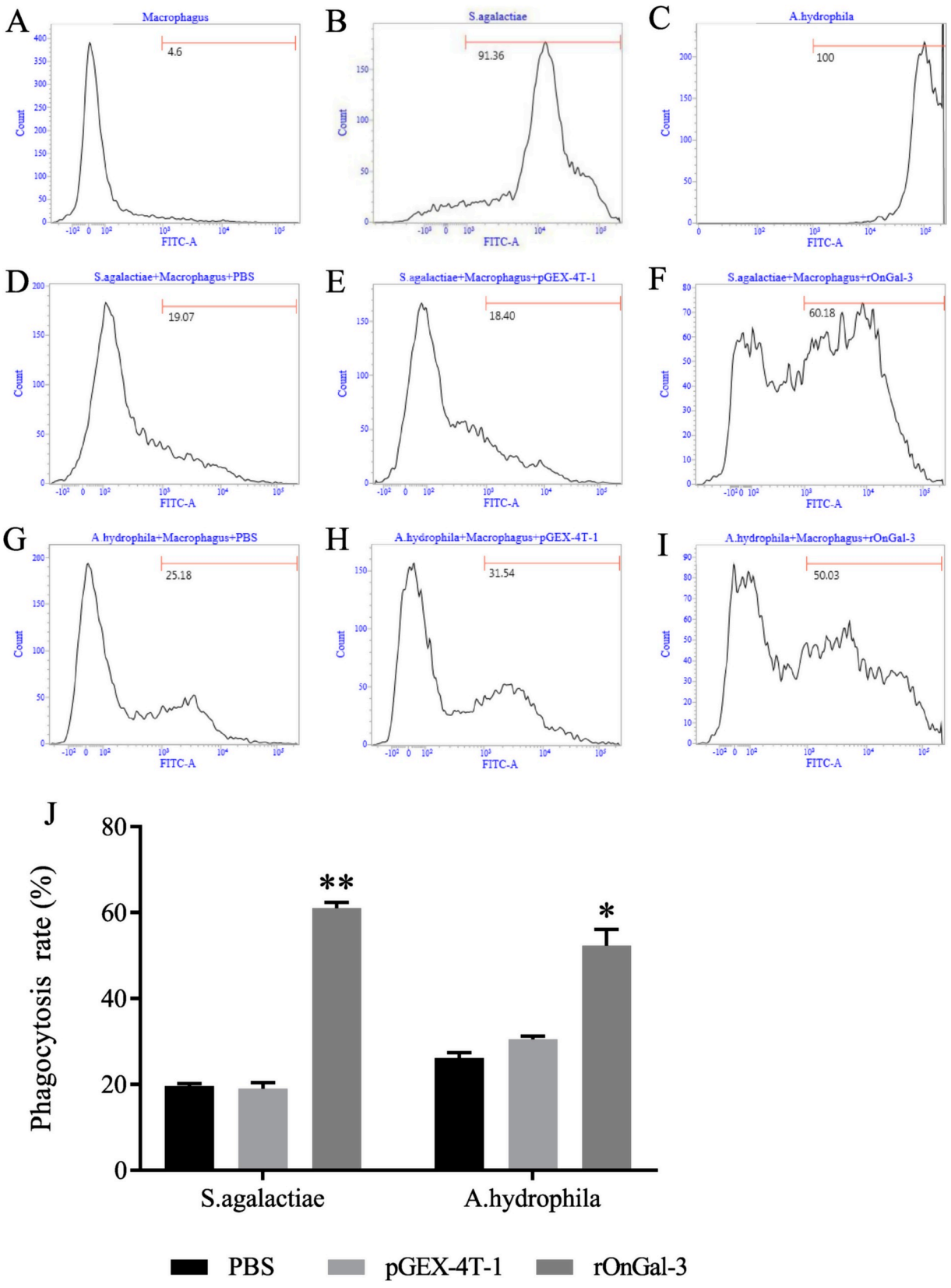

Fig. 6. Effects of rOnGal-3 on phagocytosis of Nile tilapia macrophages. Flow cytometric analyses of the macrophages phagocytosing *S. agalactiae* and *A. hydrophila*. Data show analyses of 10,000 events. The marker represented phagocytosis part. (A) The histogram of macrophages. (B and C) The histogram of FITC-labeled *S. agalactiae* and *A. hydrophila*. (D, E, F, G, H, and I) The histogram of flow cytometric analyses of the macrophages phagocytosing *S. agalactiae* and *A. hydrophila* pre-incubated with PBS, pGEX-4T-1, and rOnGal-3, respectively. The phagocytosis rates were shown near the marker. The results shown here were from one experiment out of three independent experiments. (J) The histogram of the phagocytosis rates. The average standard deviation was obtained from three experiments. The symbol * shows a significant difference from control ($p < 0.05$).

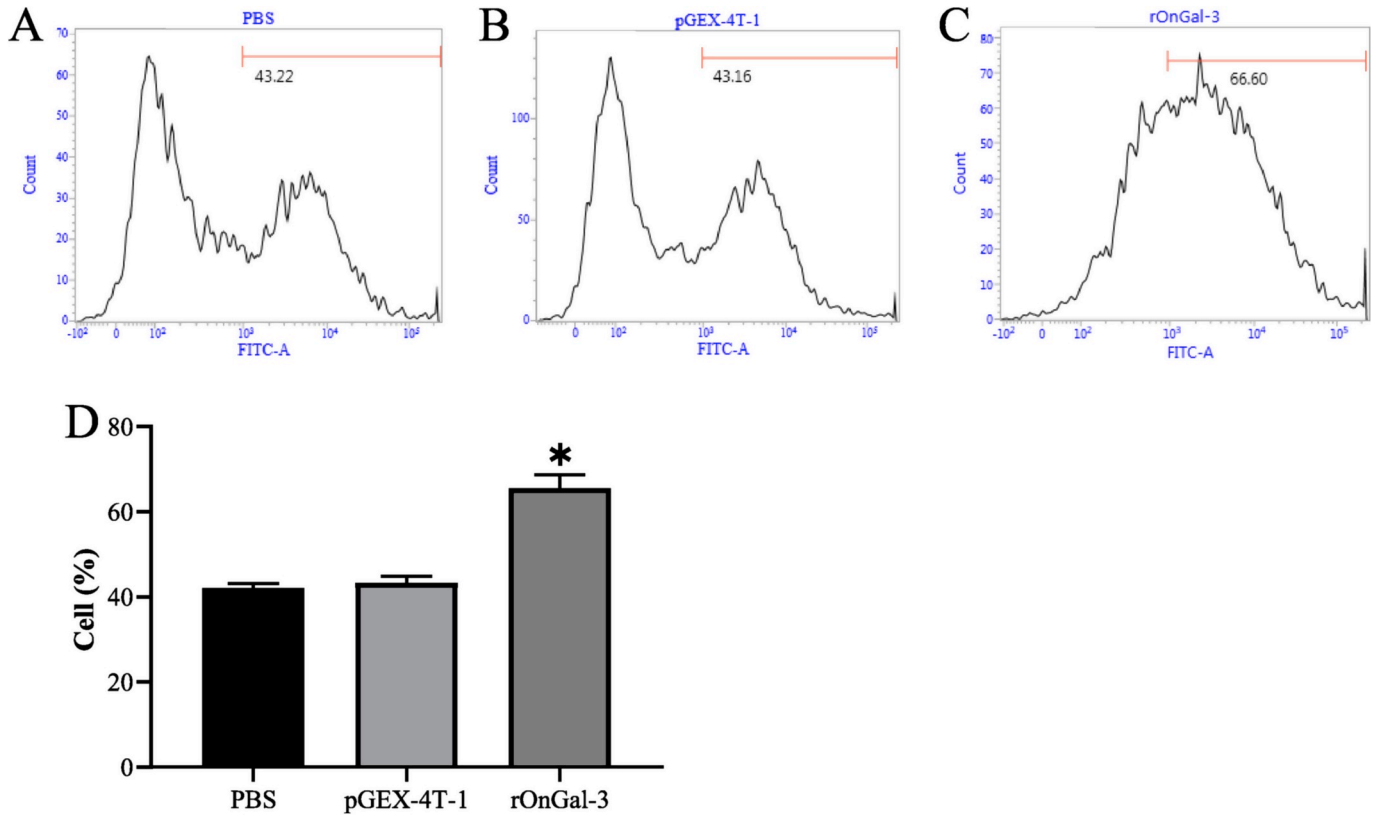

Fig. 7. Flow cytometric analyses of the head kidney monocytes/macrophages respiratory burst from Nile tilapia. (A) The histogram of flow cytometric analyses of the monocytes/macrophages respiratory burst pre-incubated with PBS, pGEX-4T-1 or rOnGal-3 (10 μg/mL). (B) The histogram of the positive cell rates. The average standard deviation was obtained from three experiments. Values are shown as mean ± SD (n = 3). ($p < 0.05$).

which were incubated with PBS, pGEX-4T-1, and rOnGal-3, respectively (Fig. 6 D–I). Statistical analysis of the data indicated that rOnGal-3-treated *S. agalactiae* and *A. hydrophila* had a significantly stronger fluorescent signal compare to those of PBS-treated and pGEX-4T-1-treated group (Fig. 6 J), hinting that rOnGal-3 could effectively promote phagocytosis.

3.7. Enhancement of respiratory burst activity by rOnGal-3

In order to detect the enhance ability of rOnGal-3 on respiratory burst, the monocytes/macrophages incubated with PBS, pGEX-4T-1, and rOnGal-3, respectively. After stimulating with PMA, the fluorescent signal was determined by flow cytometer. The results showed that the level of respiratory burst in the rOnGal-3-treated group was significantly enhanced compared with PBS-treated and pGEX-4T-1-treated group (Fig. 7).

4. Discussion

Galectin-3 has been found to be involved in many biological processes, such as cell–cell or cell–extracellular matrix adhesion [35,36], cell growth and migration [37,38], inflammation [37], pathogen recognition [16], and apoptosis [39]. However, the understanding of fish galectin-3 during immune response against pathogen infection remains limited. Here, we investigated the function of a fish galectin-3 (OnGal-3), including the binding and aggregation of pathogens, the binding of polysaccharides, and the regulation of inflammatory factors, phagocytosis and respirator bursts activity of monocytes/macrophages.

Monocytes/macrophages exert the immune protective effects through phagocytosis, antigen presentation and cytokine production in immune system [40,41]. In this study, *OnGal-3* expressions in monocytes/macrophages were up-regulated after *S. agalactiae* and *A. hydrophila* stimulation, suggesting that *OnGal*-3 might be involved in immune responses against bacterial infection, which was similar with the studies of mannose-binding lectin and collection-K1 in monocytes/macrophages [20,29]. Further *in vitro* experiments indicated that rOnGal-3 could bind *S. agalactiae* and *A. hydrophila*. LTA, LPS, galactose, mannose and maltose are considered to be PAMP present in the microbial cell wall [42]. The result showed that LTA or LPS could suppress the binding of rOnGal-3 on bacterial pathogens. Interestingly, the LTA had a stronger inhibitory activity than LPS, which was consistent with the finding of C-type lectin from *Fenneropenaeus chinensis* [26]. In addition, rOnGal-3 could bind bacterial carbohydrates including LTA, LPS, galactose, mannose, and maltose with a dose-dependent manner. Given LTA, LPS, galactose, mannose and maltose are considered to be the PAMPs present on microbial walls or membranes, the results described above suggest a potential role of rOnGal-3 on pathogen recognition. Similar with rOnGal-2 [16] and rOnGal-9 [19], rOnGal-3 also could agglutinate both *S. agalactiae* and *A. hydrophila*. These results indicate that OnGal-3 agglutinate bacterial pathogens via binding directly to carbohydrates on the surface of bacteria, which can prevent bacterial pathogens from entering cell and facilitate macrophages to eliminate bacterial pathogens [43].

It has been well-recorded that monocytes/macrophages can elicit an immune response through secreting various cytokines including interleukin 6 (*IL-6*), interleukin 10 (*IL-10*), interleukin 8 (*IL-8*), and macrophage migration inhibitory factor (*MIF*) [20,44,45]. The present study found that the transcripts of cytokines (*IL-6* and *IL-10*) and chemokines (*IL-8* and *MIF*) in monocytes/macrophages exhibited remarkably increase after incubating with rOnGal-3. These results were consistent with previous studies of MAp44 and MASP-1(C-type lectin family) in monocytes/macrophages [18,46]. Compared to *IL-6* and *IL-10*, the peak time of *IL-8* and *MIF* mRNA expression were later after rOn-Gal-3 induction. Similar result was reported in the study of Mu

et al. [46]. *IL-6* is known as pro-inflammatory cytokine and its expression can be mediated by activation of *IL-10*. *IL-8* is a neutrophil chemotactic factor that can recruit series of cells such as macrophages, mast cells, primarily neutrophils and other granulocyte to the site of infection [47]. *MIF* plays a role in the regulation of macrophage activity in host defense through suppressing anti-inflammatory effects of glucocorticoids [48–50]. Thus, the current data indicated that OnGal-3 could modulate inflammatory response and migration of immune cells. Moreover, as non-opsonic receptors, galectin can activate phagocytosis of macrophages when binding with PAMPs [51]. Our study found that rOnGal-3 could remarkably enhance the phagocytosis and respiratory burst activity of macrophages. The similar observation was also recorded in the research of Nile tilapia mannose-binding lectin, which was considered to be involve in killing bacterial pathogen [20]. These data implied that OnGal-3 play a role in pathogens clearance.

Taken together, the transcription of *OnGal-3* in monocyte/macrophages was up-regulated after bacterial stimulation. Recombinant OnGal-3(rOnGal-3 protein could bind Gram-positive/Gram-negative bacteria and polysaccharides, as well as aggregate bacterial pathogens. Furthermore, rOnGal-3 could increase the expression of inflammatory-related factors in monocytes/macrophages, enhance the phagocytosis and respirator bursts activity of monocytes/macrophages. The results in this study suggested that OnGal-3 was involved in pathogen recognition and regulatory of the activities of monocytes/macrophages.

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References

[1] F.T. Liu, G.A. Rabinovich, Galectins: regulators of acute and chronic inflammation, Ann. N. Y. Acad. Sci. 1183 (1) (2010) 158–182.
[2] S. Sato, J. Nieminen, Seeing strangers or announcing “danger”: galectin-3 in two models of innate immunity, Glycoconj. J. 19 (7–9) (2002) 583–591.
[3] U.C. Sharma, S. Pokharel, T.J. van Brakel, J.H. van Berlo, J.P. Cleutjens, B. Schroen, S. André, H.J. Crijns, H.-J. Gabius, J. Maessen, Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction, Circulation 110 (19) (2004) 3121–3128.
[4] G.R. Vasta, Roles of galectins in infection, Nat. Rev. Microbiol. 7 (6) (2009) 424–438.
[5] Z. Bai, L. Zhao, X. Chen, Q. Li, J. Li, A galectin contributes to the innate immune recognition and elimination of pathogens in the freshwater mussel Hyriopsis cumingii, Dev. Comp. Immunol. 73 (2017) 36–45.
[6] A.K. Ludwig, M. Michalak, Q. Xiao, U. Gilles, F.J. Medrano, H. Ma, F.G. FitzGerald, W.D. Hasley, A. Melendez-Davila, M. Liu, K. Rahimi, N.Y. Kostina, C. Rodriguez-Emmenegger, M. Moller, I. Lindner, H. Kaltner, M. Cudic, D. Reusch, J. Kopitz, A. Romero, S. Oscarson, M.L. Klein, H.J. Gabius, V. Percec, Design-functionality relationships for adhesion/growth-regulatory galectins, Proc. Natl. Acad. Sci. U. S. A. 116 (8) (2019) 2837–2842.
[7] M. Farhad, A.S. Rolig, W.L. Redmond, The role of Galectin-3 in modulating tumor growth and immunosuppression within the tumor microenvironment, OncoImmunology 7 (6) (2018) e1434467.
[8] J. Dumic, S. Dabelic, M. Flogel, Galectin-3: an open-ended story, Biochim. Biophys. Acta 1760 (4) (2006) 616–635.
[9] F.T. Liu, G.A. Rabinovich, Galectins as modulators of tumour progression, Nat. Rev. Cancer 5 (1) (2005) 29–41.
[10] J.J. Zhu, M. Wei, Q. Ao, Y. Tan, Y. Luo, H. Wang, H. Jiang, Q. Hu, Characterization and expression of galectin-3 after Streptococcus agalactiae and Aeromonas hydrophila challenge in GIFT strain Nile tilapia (Oreochromis niloticus), Fish Shellfish Immunol. 86 (2019) 974–980.
[11] G.R. Vasta, H. Ahmed, S.-J. Du, D. Henrikson, Galectins in teleost fish: Zebrafish (Danio rerio) as a model species to address their biological roles in development and innate immunity, Glycoconj. J. 21 (8–9) (2004) 503–521.
[12] O. Nakamura, T. Watanabe, H. Kamiya, K. Muramoto, Galectin containing cells in the skin and mucosal tissues in Japanese conger eel, Conger myriaster: an immunohistochemical study, Dev. Comp. Immunol. 25 (5–6) (2001) 431–437.
[13] S. Zhou, H. Zhao, W. Thongda, D. Zhang, B. Su, D. Yu, E. Peatman, C. Li, Galectins in channel catfish, Ictalurus punctatus: characterization and expression profiling in mucosal tissues, Fish Shellfish Immunol. 49 (2016) 324–335.
[14] M. Tian, N. Yang, L. Zhang, Q. Fu, F. Tan, C. Li, Expression profiling and functional characterization of galectin-3 of turbot (Scophthalmus maximus L.) in host mucosal immunity, Fish Shellfish Immunol. 84 (2019) 333–340.
[15] C. Chen, H. Chi, B.-g. Sun, L. Sun, The galectin-3-binding protein of Cynoglossus semilaevis is a secreted protein of the innate immune system that binds a wide range of bacteria and is involved in host phagocytosis, Dev. Comp. Immunol. 39 (4) (2013) 399–408.
[16] J.Z. Niu, Y. Huang, J.M. Niu, Z.W. Wang, J.F. Tang, B. Wang, Y.S. Lu, J. Cai, J.C. Jian, Characterization of Galectin-2 from Nile tilapia (Oreochromis niloticus) involved in the immune response to bacterial infection, Fish Shellfish Immunol. 87 (2019) 737–743.
[17] Y. Huang, Z.W. Wang, Q. Zheng, J.F. Tang, J. Cai, Y.S. Lu, J.C. Jian, Conservation of structural and interactional features of CD28 and CD80/86 molecules from Nile tilapia (Oreochromis niloticus), Fish Shellfish Immunol. 72 (2018) 95–103.
[18] L.L. Mu, H.R. Wu, K.L. Han, L.T. Wu, X. Bian, B.X. Li, Z. Guo, X.X. Yin, J.M. Ye, Molecular and functional characterization of a mannose-binding lectin/ficolin-associated protein (MAp44) from Nile tilapia (Oreochromis niloticus) involved in the immune response to bacterial infection, Dev. Comp. Immunol. 101 (2019) 103438.
[19] J.Z. Niu, Y. Huang, Y. Li, Z.W. Wang, J.F. Tang, B. Wang, Y.S. Lu, J. Cai, J.C. Jian, Characterization of a tandem-repeat galectin-9 from Nile tilapia (Oreochromis niloticus) involved in the immune response against bacterial infection, Fish Shellfish Immunol. 92 (2019) 216–223.
[20] L.L. Mu, X.X. Yin, Y.J. Yang, L.T. Wu, H.R. Wu, B. Li, Z. Guo, J.M. Ye, Functional characterization of a mannose-binding lectin (MBL) from Nile tilapia (Oreochromis niloticus) in non-specific cell immunity and apoptosis in monocytes/macrophages, Fish Shellfish Immunol. 87 (2019) 265–274.
[21] L.L. Mu, X.X. Yin, Y. Xiao, X. Bian, Y.J. Yang, L.T. Wu, J.M. Ye, A C-type lectin (CL11X1-like) from Nile tilapia (Oreochromis niloticus) is involved in host defense against bacterial infection, Dev. Comp. Immunol. 84 (2018) 230–240.
[22] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method, Methods 25 (4) (2001) 402–408.
[23] G.L. Luo, J.Z. Niu, Y. Huang, S.H. Cai, J.F. Tang, J.C. Jian, Prokaryotic expression and condition optimization of galectin-3 gene in Oreochromis niloticus, J. Guangdong Ocean Univ. 39 (4) (2019).
[24] Y. Bai, D. Niu, Y. Bai, Y. Li, T. Lan, M. Peng, Z. Dong, J. Li, Identification of a novel galectin in Sinonovacula constricta and its role in recognition of Gram-negative bacteria, Fish Shellfish Immunol. 80 (2018) 1–9.
[25] Y. Wang, F. Ke, J. Ma, S. Zhou, A tandem-repeat galectin-9 involved in immune response of yellow catfish, Pelteobagrus fulvidraco, against Aeromonas hydrophila, Fish Shellfish Immunol. 51 (2016) 153–160.
[26] X.W. Zhang, W.T. Xu, X.W. Wang, Y. Mu, X.F. Zhao, X.Q. Yu, J.X. Wang, A novel C-type lectin with two CRD domains from Chinese shrimp Fenneropenaeus chinensis functions as a pattern recognition protein, Mol. Immunol. 46 (8–9) (2009) 1626–1637.
[27] R.K. Madusanka, T.T. Priyathilaka, N.D. Janson, T.D.W. Kasthuriarachchi, S. Jung, M.D.N. Tharuka, J. Lee, Molecular, transcriptional and functional delineation of Galectin-8 from black rockfish (Sebastes schlegelii) and its potential immunological role, Fish Shellfish Immunol. 93 (2019) 449–462.
[28] D.L. Zhang, C.H. Lv, D.H. Yu, Z.Y. Wang, Characterization and functional analysis of a tandem-repeat galectin-9 in large yellow croaker Larimichthys crocea, Fish Shellfish Immunol. 52 (2016) 167–178.
[29] L.L. Mu, X.X. Yin, X. Bian, L.T. Wu, Y.J. Yang, X.F. Wei, Z. Guo, J.M. Ye, Expression and functional characterization of collection-K1 from Nile tilapia (Oreochromis niloticus) in host innate immune defense, Mol. Immunol. 103 (2018) 21–34.
[30] S. Tasumi, W.J. Yang, T. Usami, S. Tsutsui, T. Ohira, I. Kawazoe, M.N. Wilder, K. Aida, Y. Suzuki, Characteristics and primary structure of a galectin in the skin mucus of the Japanese eel, Anguilla japonica, Dev. Comp. Immunol. 28 (4) (2004) 325–335.
[31] L.L. Mu, X.X. Yin, X. Bian, L.T. Wu, Y.J. Yang, X.F. Wei, Z. Guo, J.M. Ye, Expression and functional characterization of collection-K1 from Nile tilapia (Oreochromis niloticus) in host innate immune defense, Mol. Immunol. 103 (2018) 21–34.
[32] Z. Bai, L. Zhao, X. Chen, Q. Li, J. Li, A galectin from Hyriopsis cumingii involved in the innate immune response against to pathogenic microorganism and its expression profiling during pearl sac formation, Fish Shellfish Immunol. 56 (2016) 127–135.
[33] G.H. Cha, Y. Liu, T. Peng, M.Z. Huang, C.Y. Xie, Y.C. Xiao, W.N. Wang, Molecular cloning, expression of a galectin gene in Pacific white shrimp Litopenaeus vannamei and the antibacterial activity of its recombinant protein, Mol. Immunol. 67 (2) (2015) 325–340.
[34] Z. Sha, L. Wang, L. Sun, Y. Chen, Y. Zheng, M. Xin, C. Li, S. Chen, Isolation and characterization of monocyte/macrophage from peripheral blood of half smooth tongue sole (Cynoglossus semilaevis), Fish Shellfish Immunol. 65 (2017) 256–266.
[35] H. Inohara, S. Akahani, K. Koths, A. Raz, Interactions between galectin-3 and Mac-2-binding protein mediate cell-cell adhesion, Cancer Res. 56 (19) (1996) 4530–4534.
[36] P. Pesheva, S. Kuklinski, B. Schmitz, R. Probstmeier, Galectin-3 promotes neural cell adhesion and neurite growth, J. Neurosci. Res. 54 (5) (1998) 639–654.
[37] D.K. Hsu, R.Y. Yang, Z. Pan, L. Yu, D.R. Salomon, W.P. Fung-Leung, F.T. Liu, Targeted disruption of the galectin-3 gene results in attenuated peritoneal inflammatory responses, Am. J. Pathol. 156 (3) (2000) 1073–1083.
[38] I. Cortegano, V. del Pozo, B. Cardaba, B. de Andres, S. Gallardo, A. del Amo, I. Arrieta, A. Jurado, P. Palomino, F.T. Liu, C. Lahoz, Galectin-3 down-regulates IL-5 gene expression on different cell types, J. Immunol. 161 (1) (1998) 385–389.
[39] K.K. Hoyer, M. Pang, D. Gui, I.P. Shintaku, I. Kuwabara, F.T. Liu, J.W. Said, L.G. Baum, M.A. Teitell, An anti-apoptotic role for galectin-3 in diffuse large B-cell lymphomas, Am. J. Pathol. 164 (3) (2004) 893–902.

[40] S. Han, H. Zhuang, P.Y. Lee, M. Li, L. Yang, P.A. Nigrovic, W.H. Reeves, Differential Responsiveness of Monocyte and Macrophage Subsets to Interferon, Arthritis & rheumatology, Hoboken, N.J., 2019.
[41] M. Sioud, S. Pettersen, I. Ailte, Y. Floisand, Targeted killing of monocytes/macrophages and myeloid leukemia cells with pro-apoptotic peptides, Cancers 11 (8) (2019).
[42] J. Lu, C. Teh, U. Kishore, K.B. Reid, Collectins and ficolins: sugar pattern recognition molecules of the mammalian innate immune system, Biochim. Biophys. Acta 1572 (2–3) (2002) 387–400.
[43] W.S. Thulasitha, N. Umasuthan, I. Whang, B.H. Nam, J. Lee, Antimicrobial response of galectin-1 from rock bream Oplegnathus fasciatus: molecular, transcriptional, and biological characterization, Fish Shellfish Immunol. 50 (2016) 66–78.
[44] T. Calandra, T. Roger, Macrophage migration inhibitory factor: a regulator of innate immunity, Nat. Rev. Immunol. 3 (10) (2003) 791–800.
[45] E.H. Lee, Y. Rikihisa, Absence of tumor necrosis factor alpha, interleukin-6 (IL-6), and granulocyte-macrophage colony-stimulating factor expression but presence of IL-1beta, IL-8, and IL-10 expression in human monocytes exposed to viable or killed Ehrlichia chaffeensis, Infect. Immun. 64 (10) (1996) 4211–4219.
[46] L.L. Mu, X.X. Yin, H.R. Wu, K.L. Han, L.T. Wu, M.M. Ding, X. Bian, B.X. Li, S.L. Fu, F. Liang, Z. Guo, J.M. Ye, Expression and functional characterization of a mannose-binding lectin-associated serine protease-1 (MASP-1) from Nile tilapia (Oreochromis niloticus) in host defense against bacterial infection, Fish Shellfish Immunol. 91 (2019) 68–77.
[47] Y. Itoh, T. Joh, S. Tanida, M. Sasaki, H. Kataoka, K. Itoh, T. Oshima, N. Ogasawara, S. Togawa, T. Wada, H. Kubota, Y. Mori, H. Ohara, T. Nomura, S. Higashiyama, M. Itoh, IL-8 promotes cell proliferation and migration through metalloproteinase-cleavage proHB-EGF in human colon carcinoma cells, Cytokine 29 (6) (2005) 275–282.
[48] K. Takahashi, K. Koga, H.M. Linge, Y. Zhang, X. Lin, C.N. Metz, Y. Al-Abed, K. Ojamaa, E.J. Miller, Macrophage CD74 contributes to MIF-induced pulmonary inflammation, Respir. Res. 10 (1) (2009) 33-33.
[49] F. Harry, B. Jürgen, C. Thierry, B. Richard, The macrophage migration inhibitory factor-glucocorticoid dyad: regulation of inflammation and immunity, Mol. Endocrinol. 21 (6) (2007) 1267–1280.
[50] A.A. Yousef, C.N. Metz, C.K. Fan, A. Bayan, V.P. Sonya, B. Steven, L. Hans, O. Mahendar, V.A. Pavlov, C. Thomas, Thyroxine is a potential endogenous antagonist of macrophage migration inhibitory factor (MIF) activity, Proc. Natl. Acad. Sci. U. S. A. 108 (20) (2011) 8224–8227.
[51] S. Gordon, Phagocytosis: an immunobiologic process, Immunity 44 (3) (2016) 463–475.