Fish & Shellfish Immunology 41 (2014) 380–388

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Toll-like receptor recognition of bacteria in fish: Ligand specificity and signal pathways

Jie Zhang [a, b], Xianghui Kong [a, *], Chuanjiang Zhou [a], Li Li [a], Guoxing Nie [a], Xuejun Li [a]

[a] College of Fisheries, Henan Normal University, Xinxiang 453007, PR China
[b] College of Life Science, Henan Normal University, Xinxiang 453007, PR China

ARTICLE INFO

Article history:
Received 29 May 2014
Received in revised form
5 September 2014
Accepted 14 September 2014
Available online 19 September 2014

Keywords:
Toll-like receptors
Bacteria
Fish
Ligand specificity
TLR signaling pathways

ABSTRACT

Pattern recognition receptors (PRRs) recognize the conserved molecular structure of pathogens and trigger the signaling pathways that activate immune cells in response to pathogen infection. Toll-like receptors (TLRs) are the first and best characterized innate immune receptors. To date, at least 20 TLR types (TLR1, 2, 3, 4, 5M, 5S, 7, 8, 9, 13, 14, 18, 19, 20, 21, 22, 23, 24, 25, and 26) have been found in more than a dozen of fish species. However, of the TLRs identified in fish, direct evidence of ligand specificity has only been shown for TLR2, TLR3, TLR5M, TLR5S, TLR9, TLR21, and TLR22. Some studies have suggested that TLR2, TLR5M, TLR5S, TLR9, and TLR21 could specifically recognize PAMPs from bacteria. In addition, other TLRs including TLR1, TLR4, TLR14, TLR18, and TLR25 may also be sensors of bacteria. TLR signaling pathways in fish exhibit some particular features different from that in mammals. In this review, the ligand specificity and signal pathways of TLRs that recognize bacteria in fish are summarized. References for further studies on the specificity for recognizing bacteria using TLRs and the following reactions triggered are discussed. In-depth studies should be continuously performed to identify the ligand specificity of all TLRs in fish, particularly non-mammalian TLRs, and their signaling pathways. The discovery of TLRs and their functions will contribute to the understanding of disease resistance mechanisms in fish and provide new insights for drug intervention to manipulate immune responses.

1. Introduction

The innate system, the most ancient and universal form of host defense, is an efficient first line of defense against invading microbes in invertebrates and vertebrates [1]. Fish in aquatic environments protect themselves from various microbial pathogens mostly with the help of innate or non-specific immunity [1]. Pattern recognition receptors (PRRs), part of the ancient innate arm of the immune system, are conserved in invertebrate and vertebrate lineages. They recognize the conserved molecular structure of pathogens, known as pathogen-associated molecular patterns (PAMPs), and trigger the signaling pathways that activate immune cells in response to pathogen infection [2].

Among various PRRs, Toll-like receptors (TLRs) are the first and best characterized innate immune receptors. All TLRs are type I transmembrane proteins that contain three parts: an extracellular N-terminus with leucine-rich repeat (LRR) domain, a transmembrane domain and an intracellular C-terminus with a Toll/IL-1 receptor (TIR) domain [3,4]. TLR specificity is determined by sequence variation and the number of LRR domains, which is involved in pathogen recognition. Contrary to the extracellular LRR domain, the cytoplasmic TIR domain which activates downstream signaling pathways is highly conserved not only between the different TLRs of one species but also between different animal species [5,6].

Toll receptors have originally been identified in fruit fly (*Drosophila melanogaster*) embryos as the product of the Toll gene, which controls the establishment of drosoventral polarity [7,8]. These receptors have been related to the synthesis of anti-microbial peptides and play a critical role in immunity against fungal infection in flies [9]. An ortholog of Toll from *D. melanogaster* has been identified in humans and could activate certain genes necessary for innate or adaptive immune responses. Given its structural and functional similarities to the Toll receptors, this ortholog was named “Toll-like receptor” (now known as TLR4) [8,10,11]. The

* Corresponding author. No. 46, Jianshe Road, College of Fisheries, Henan Normal University, Xinxiang 453007, PR China. Tel.: +86 373 3328928.
E-mail addresses: xhkong@htu.cn, xhkong6@gmail.com (X. Kong).

http://dx.doi.org/10.1016/j.fsi.2014.09.022
1050-4648/

Abbreviations

PRR	pattern recognition receptor
TLR	toll-like receptor
PAMP	pathogen-associated molecular pattern
LRR	leucine-rich repeat
LRRNT	N-terminal LRR capping region
TIR	Toll/IL-1 receptor
IL-1	interleukin-1
LTA	lipoteichoic acid
LPS	lipopolysaccharide
PGN	peptidoglycan
Pam2CSK4	synthetic diacylated lipopeptides
Pam3CSK4	synthetic triacylated lipopeptides
MALP-2	macrophage-activating lipopeptide-2
Poly(I:C)	polyinosinic:polycytidylic acid
LBP	LPS-binding protein
CD14	cluster of differentiation 14
MD2	myeloid differentiation protein 2
TRIL	TLR4 interactor with leucine-rich repeats
CpG DNA	DNA-containing unmethylated CpG motifs
CpG ODN	CpG-containing oligodeoxynucleotides
ER	endoplasmic reticulum
ECD	ectodomain
IFN	interferon
MyD88	myeloid differentiation primary-response protein 88
MAPKs	mitogenactivated protein kinases
IRAK	IL-1 receptor-associated protein kinase
TRAF	TNFR-associated factor
TNFR	TNF receptor
TNF	tumor necrosis factor
TAK	TGF-β-activated kinase
TGF	transforming growth factor
TAB	TAK 1 binding protein
IKK	IκB kinase
IκB	inhibitor of NF-κB
NEMO	NF-κB essential modulator
NF-κB	nuclear factor κB
AP-1	activator protein
IRF	interferon regulatory factor
TIRAP	TIR domain-containing adaptor protein
TRIF	TIR domain-containing adaptor inducing IFN-β
TRAM	TRIF-related adaptor molecule
TICAM	TIR domain-containing adaptor molecule
TANK	TRAF-family-member-associated NF-κB activator
TBK	TANK binding kinase
RIP	receptor-interacting protein

human genome contains 10TLRs, contrary to the 13TLRs in the mouse genome [12]. In mammalians, TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 are expressed in the plasma membrane, whereas TLR3, TLR7, TLR8, and TLR9 are localized within intracellular vesicles [13]. In bony fish, Sangrador-Vegas et al. [14] isolated a cDNA sequence of an interleukin-1 receptor from rainbow trout (*Oncorhynchus mykiss*), and it is the first piscine member of the interleukin-1/TLR superfamily. The first teleost TLR gene was characterized in goldfish (*Carassius auratus*) [15]. To date, at least 20 TLR types (TLR1, 2, 3, 4, 5M, 5S, 7, 8, 9, 13, 14, 18, 19, 20, 21, 22, 23, 24, 25, and 26) have been found in more than a dozen of fish species (Table 1). However, orthologs of mammalian TLR6 and TLR10 have not been identified in fish. Among all these TLRs, TLR1, TLR2, TLR4, TLR5, and TLR9 are presumed as sensors of bacterial ligands in fish [16], although some reports have suggested that the TLR7 and TLR8 in humans can also sense bacterial RNA [17,18]. In this review, the ligand specificity and signal pathways of TLRs that recognize bacteria in fish are summarized.

2. Specificity of TLRs in bacterial recognition

2.1. TLR1 and TLR2

In mammals, TLR2 recognizes various ligands from bacteria by forming homodimer or heterodimer with TLR1 or TLR6 [19]. Tri-acylated lipopeptides are considered as ligands for TLR2-TLR1, whereas diacylated lipopeptides are recognized by TLR2-TLR6 heterodimers [12,20]. In addition, TLR2 recognizes lipoteichoic acid (LTA) and peptidoglycan (PGN), which are characteristic cell wall components of Gram-positive bacteria [21,22]. In fish, similar to other vertebrates, TLR1 molecules do not have LRRNT modules in the N-terminal, which is believed to be important for dimerization with TLR2. Thus, fish TLR1 also possibly forms a dimmer with TLR2 similar to that in mammals [23]. Comparative sequence analysis showed high conservation of the position of the critical PGN recognition leucine residues in carp TLR2 LRR domain [24]. Ribeiro et al. [24] investigated the role of the TLR2 in the recognition of

Table 1
Known ligands of TLRs in fish.

TLRs	Fish species	Ligands	References
TLR1	Rainbow trout, pufferfish large yellow croaker, orange-spotted grouper	Unknown	[29–32]
TLR2	Commom carp, rohu, channel catfish, orange-spotted grouper	PGN, LTA, Pam_3CSK_4, Lipopeptides	[23,24,27,28,31]
TLR3	Fugu, zebrafish, rohu, orange-spotted grouper	dsRNA, poly(I:C)	[38,104–107]
TLR4	Channel catfish, grass carp, mrigal, zebrafish, rare minnow	Unknown	[23,39–44]
TLR5M	Japanese flounder,channel catfish, gilthead seabream, fugu, rainbow trout	Flagellin	[23,54,57–60]
TLR5S	Japanese flounder,channel catfish, gilthead seabream, fugu, rainbow trout	Flagellin	[23,54,57–60]
TLR7	Fugu, rainbow trou, Zebrafish, channel catfish	Unknown	[38,58,78,108,109]
TLR8	Fugu, channel catfish, Atlantic salmon, rainbow trout	Unknown	[38,58,78,108–110]
TLR9	Japanese flounder, cobia, zebrafish, rainbow trout, Atlantic salmon	CpG DNA	[68–75]
TLR13	Atlantic salmon, channel catfish	Unknown	[111,112]
TLR14	Lamprey, fugu, Japanese flounder	Unknown	[38,76,77]
TLR18	Zebrafish, channel catfish	Unknown	[23,78]
TLR19	Zebrafish, channel catfish	Unknown	[23,78]
TLR20	Zebrafish, common carp, channel catfish	Unknown	[23,80,81]
TLR21	Zebrafish, channel catfish, orange-spotted grouper	CpG DNA	[23,38,83,87,113–115]
TLR22	Fugu, zebrafish, grass carp channel catfish	dsRNA, poly(I:C)	[23,38,84–88]
TLR23	Fugu	Unknown	[38]
TLR24	Lamprey	Unknown	[89]
TLR25	Channel catfish, fathead minnow, Nile tilapia	Unknown	[23]
TLR26	Channel catfish	Unknown	[23]