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Review

Toll-like receptors in bony fish: From genomics to function

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ABSTRACT

Receptors that recognize conserved pathogen molecules are the first line of cellular innate immunity defense. Toll-like receptors (TLRs) are the best understood of the innate immune receptors that detect infections in mammals. Key features of the fish TLRs and the factors involved in their signaling cascade have high structural similarity to the mammalian TLR system. However, the fish TLRs also exhibit very distinct features and large diversity which is likely derived from their diverse evolutionary history and the distinct environments that they occupy. Six non-mammalian TLRs were identified in fish. TLR14 shares sequence and structural similarity with TLR1 and 2, and the other five (TLR19, 20, 21, 22 and 23) form a cluster of novel TLRs. TLR4 was lost from the genomes of most fishes, and the TLR4 genes found in zebrafish do not recognize the mammalian agonist LPS and are likely paralogous and not orthologous to mammalian TLR4 genes. TLR6 and 10 are also absent from all fish genomes sequenced to date. Of the at least 16 TLR types identified in fish, direct evidence of ligand specificity has only been shown for TLR2, TLR3, TLR5M, TLR5S and TLR22. The common carp TLR2 was shown to recognize the synthetic triacylated lipopeptide Pam_3CSK_4 and lipopeptides from gram positive bacteria. The membrane-bound TLR5 (TLR5M) signaling in response to flagellin in rainbow trout is amplified through interaction with the soluble form (TLR5S) in a positive loop feedback. In Fugu, TLR3 is localized to the endoplasmic reticulum (ER) and recognizes relatively short dsRNA, while TLR22 has a surveillance function like the human cell-surface TLR3. Genome and gene duplications have been major contributors to the teleost's rich evolutionary history and genomic diversity. Duplicate or multi-copy TLR genes were identified for TLR3 and 7 in common carp, TLR4b, 5, 8 and 20 in zebrafish, TLR8a in rainbow trout and TLR22 in rainbow trout and Atlantic salmon. The main task for current and near-future fish TLRs research is to develop specificity assays to identify the ligands of all fish TLRs, which will advance comparative immunology research and will contribute to our understanding of disease resistance mechanisms in fish and the development of new adjuvants and/or more effective vaccines and therapeutics.

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Abbreviations: CD14, cluster of differentiation 14; ER, endoplasmic reticulum; IL, interleukin; IL1R, interleukin-1 receptor; IRAK, IL1R-associated kinase; IRF, interferon regulatory factor; LBP, LPS-binding protein; LPS, lipopolysaccharide; LRR, leucine-rich repeats; MD-2, myeloid differentiation protein-2; MyD88, myeloid differentiation primary response gene/protein 88; NF-kB, nuclear factor kB; PAMP, pathogen associated molecular pattern; PRR, pattern recognition receptor; TICAM, TIR-containing adaptor molecule; TIR, Toll/interleukin-1 receptor resistance domain; TLR, Toll-like receptor; TRAF6, tumor necrosis factor receptor-associated factor 6; TRIF, TIR domain-containing adaptor inducing interferon.

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1. Foreground

This review is not the only one on Toll-like receptors in fish which has recently been published. As the recent reviews by Rebl et al. (2010) and Takano et al. (2010) provided comprehensive literature surveys and detailed descriptions of the known similarities and differences between the fish and mammalian TLR signaling systems, I chose to focus on the genomics perspective of TLRs research in fish with two primary objectives: (i) To remind the reader of the various genome duplications during vertebrate evolution and relating that to the evolution of the TLR-signaling cascade in fish; and (ii) to provide a comprehensive literature guide on teleost TLR ligands.

2. Introduction—mammalian TLRs and their known ligands

Receptors that recognize conserved pathogen molecules are part of the ancient innate arm of the immune system and are conserved in both invertebrate and vertebrate lineages. The family of Toll-like receptors (TLRs) is the best understood of the innate immune receptors that detect infections. TLRs are transmembrane proteins that recognize conserved pathogen structures to induce immune effector molecules. In vertebrates, TLRs can distinguish among classes of pathogens and serve an important role in orchestrating the appropriate adaptive immune responses (Takeda et al., 2003). TLRs contain an extracellular N-terminus with leucine-rich repeat region (LRR), a transmembrane domain and an intracellular C-terminus with a Toll/IL-1 receptor domain (TIR). An illustration of the TLRs domains is presented in a schematic diagram of several fish TLRs (Fig. 1). The cytoplasmatic TIR domain harbors conserved amino acids that have been shown to be involved in the signaling as well as in the localization of the TLR (Slack et al., 2000; Funami et al., 2004), while the LRR region is involved in pathogen recognition (Bell et al., 2003). In humans, 10 TLRs have been described and shown to identify distinct pathogen associated molecular patterns (PAMPs), which are molecules characteristic of a class of pathogens and are essential for pathogens survival (Iwasaki and Medzhitov, 2004; Pasare and Medzhitov, 2005; Roach et al., 2005; Temperley et al., 2008). Overall, 21 distinct TLR gene types have been identified to date from various animal species (Roach et al., 2005; Temperley et al., 2008).

TLRs recognize their ligands through interactions with the LRRs and trigger the activation of intracellular signaling through a cytoplasmic myeloid differentiation primary response protein 88 (MyD88)-dependent pathway or a MyD88-independent pathway. All mammalian TLRs, with the exception of TLR3, depend at least in part on the MyD88 adaptor for full signal transduction activity. In the MyD88-dependent pathway, MyD88 recruits the interleukin-1 receptor-associated kinases (IRAKs) and TNF receptor-associated factor 6 (TRAF6), which in turn activate downstream genes in TLR signal transduction. Ultimately, through the activation of NF-κB, IRF3 or IRF7, the TLR signaling pathways induce production of proinflammatory cytokines including interleukin (IL), tumor necrosis factor (TNF), and type I interferon (IFN) molecules that mediate direct defense responses and alert adaptive immune cells to the presence of a pathogen (Iwasaki and Medzhitov, 2004; Kawai and Akira, 2010). In recent years the TLR signaling cascade has been intensively studied in mammals. The discovery of several TIR-containing adaptors led to the current understanding that individual TLR types recruit distinct cytosolic adaptors which can trigger specific responses to the infecting microbes (Kawai and Akira, 2010; Akira et al., 2006). In addition, cell-type specific signaling defined by its immunological properties can alter the response triggered by the same TLR signal in different cell types (Barbalat et al., 2009).

Two major TLR subfamilies were identified in human. TLR1, 2, 4, 5, 6 and 10 are the members of the cell surface sub-family recognizing microbial lipids, sugars and proteomes (Hajjar et al., 2001; Hayashi et al., 2001; Hoshino et al., 1999; Takeuchi et al., 2001, 2002; Underhill et al., 1999; Werts et al., 2001). TLR3, 7, 8 and 9 are the members of the nucleic acid-sensing subgroup recognizing nucleotide derivatives of viral or bacterial origin (Diebold et al., 2004; Latz et al., 2004; Gorden et al., 2005; Alexopoulou et al., 2001; Gibbard et al., 2006). The nucleic acid TLRs are localized in various intracellular compartments. Three TLR genes found in mice, TLR11, 12 and 13, have been lost from the human genome, and of the three, only one ligand for TLR11 has been identified to date (Beutler, 2009; Yarovinsky et al., 2005). The ligand is Profilin, a protein from a protozoan parasite, suggesting that TLR11 is also a cell surface receptor.

The TLR1, 2, 6 and 10 genes form a phylogenetically related cluster based on sequence similarities and genomic structures (Roach et al., 2005; Temperley et al., 2008), and in their dimeric combinations they cover broad variations of bacterial peptidoglycans and lipoproteins (Medzhitov, 2001). They are primarily located on the cell surface and upon activation they induce NF-κB expression through the recruitment of IL-1R signaling molecules. (Medzhitov et al., 1998; Shimizu et al., 2005, 2007). In mammals, the synthetic diacylated (Pam_2CSK_4) and triacylated (Pam_3CSK_4) lipoproteins are known experimental agonists of TLR2/6 and 2/1 heterodimers (Takeuchi et al., 2002; Shimizu et al., 2005, 2007). In addition, TLR2 has been implicated in the recognition of zymosan from fungi, tGPI-mucin from *Trypanosoma cruzi* and the hemagglutinin protein from measles virus (Akira et al., 2006).

The mammalian TLR4 is a central protein in the receptors complex responding to bacterial lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria. The transfer of monomeric LPS to TLR4 on the cell surface is mediated through a complex of LPS-binding protein (LBP) and cluster of differentiation 14 (CD14) (Schumann et al., 1990; Wright et al., 1990). TLR4 forms a receptor complex with myeloid differentiation protein 2 (MD2), and together they function as the main cell-surface LPS-binding component (Hoshino et al., 1999; Rhee and Hwang, 2000). In addition to binding LPS, TLR4 has been implicated in the recognition of viral surface proteins (Akira et al., 2006).

TLR5 in mammals and fish has been shown to recognize the flagellin protein component of bacterial flagella and be responsible for flagellin-mediated NF-κB activation (Hayashi et al., 2001; Tsujita et al., 2004). In mammals it has also been implicated to be involved in adaptive immunity through promoting of the differentiation of helper T cells and naïve B cells into immunoglobulin A – producing plasma cells in response to flagellin (Uematsu et al., 2008).

In mammals, TLR3 has been shown to respond to double-stranded RNA (dsRNA), TLR9 to unmethylated CpG DNA and TLR7 and TLR8 were shown to be activated by synthetic antiviral imidazoquinoline compounds and were implicated in recognizing single-stranded RNA (Diebold et al., 2004; Latz et al., 2004; Gorden et al., 2005; Alexopoulou et al., 2001; Gibbard et al., 2006). These TLRs are primarily located in the endoplasmic reticulum and in lysosomal-like vesicles and are thought to have an important role