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Unique features of chicken Toll-like receptors

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ABSTRACT

Toll-like receptors (TLRs) are a major class of innate immune pattern recognition receptors that have a key role in immune homeostasis and the defense against infections. The research explosion that followed the discovery of TLRs more than a decade ago has boosted fundamental knowledge on the function of the immune system and the resistance against disease, providing a rational for clinical modulation of the immune response. In addition, the conserved nature of the ancient TLR system throughout the animal kingdom has enabled a comparative biology approach to understand the evolution, structural architecture, and function of TLRs. In the present review we focus on TLR biology in the avian species, and, especially, on the unique functional properties of the chicken TLR repertoire.

1. Introduction

Virtually all organisms have evolved sophisticated systems to sense and respond to changing environmental conditions. These systems usually consist of sensory receptors that recognize the presence of distinct environmental cues and transduce these signals to intracellular effectors that orchestrate the appropriate cellular response. The nature of the sensory receptors that link the environment to the innate and adaptive immune system has long been a mystery until the discovery of the family of Toll-like receptors (TLRs) about 15 years ago (Medzhitov et al., 1997; Medzhitov, 2009; Palm and Medzhitov, 2009). TLRs sense the presence of conserved microbial structures in the environment and instruct the eukaryotic cells to an adequate response, mainly the production of antimicrobial peptides, cytokines and chemokines (Kawai and Akira, 2007). In mammals, TLRs are expressed by most cell types, although the TLR repertoire and the level of receptor expression are dynamic and subject to regulation (van Aubel et al., 2007; O'Neill, 2008; Kondo et al., 2012).

TLRs are evolutionary highly conserved and may be present in all living multi-cellular organisms (Roach et al., 2005; Oshiumi et al., 2008). A total of 10 different TLR have been identified in humans, but many more may exist in other species. Comparative TLR biology studies largely confirm the conserved nature of the TLR family but have also revealed important functional differences among species (Werling et al., 2009). Knowledge of the natural variation in TLR specificity and function contributes to our understanding of the evolution of the immune system and disease resistance mechanisms, and may enable more rational modulation of the immune response (e.g., through vaccination). In recent years, excellent reviews on TLRs diversity among animal species have become available, often based on data from comparative genomic analysis, transcription profiling, and cell responses to TLR ligands (Oshiumi et al., 2008; Werling et al., 2009; Cormican et al., 2009; Satake and Sasaki, 2010; Palti, 2011). In the present review, we focus on the unique molecular and functional properties of the TLR repertoire of the avian species.

2. Principles of Toll-like receptor function

All identified TLRs are type I membrane proteins with a highly conserved architecture (Carpenter and O'Neill, 2009; Kang and Lee, 2011). The N-terminus of the protein contains the extracellular ligand-binding domain (ectodomain) that is composed of 19–27 leucine-rich repeats (LRRs) and is variably glycosylated. Variations in the LRR framework create binding pockets or regions with variable ligand specificity as evidenced by structural studies (Kang and Lee, 2011). The C-terminal cytoplasmic tail of the TLRs contains a highly conserved globular signaling region, which is termed the Toll/interleukin receptor (TIR) domain because of its homology with the interleukin-1 receptor signaling domain. The TIR domain

Abbreviations: CD14, cluster of differentiation 14; dsRNA, double stranded ribonucleic acid; IFN, interferon; IL, interleukin; IRAK, interleukin-1 receptor-associated kinase; IRF3, interferon regulatory factor 3; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide; LRR, leucine-rich repeats; MD-2, myeloid differentiation protein-2; MPL, monophosphoryl lipid A; MyD88, myeloid differentiation primary response gene/protein 88; NF-κB, nuclear factor kappa B; PMSF, phenylmethylsulfonyl fluoride; SNP, single-nucleotide polymorphism; ssRNA, single stranded ribonucleic acid; TIRAP, Toll-interleukin 1 receptor (TIR) domain containing adaptor protein; PRAT4A, protein associated with Toll-like receptor 4; TIR, Toll/interleukin-1 receptor resistance domain; TLR, Toll-like receptor; TRAF6, tumor necrosis factor receptor-associated factor 6; TRAM, TRIF-related adaptor molecule; TRIF, TIR domain-containing adaptor inducing interferon.

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is generally more conserved among different members of the TLR family than the ectodomain. The ectodomain and TIR domain are spatially separated by a transmembrane helix that positions the protein within the membrane. Most members of the mammalian TLR family form homodimers, although TLR2 complexes to heterodimers with either TLR1 or TLR6. Mammalian TLR4 is functional when complexed with the MD-2 co-receptor. Signaling of TLR4 and several other TLRs is most effective in the presence of the lipid scavenger protein CD14 (Lee et al., 2012).

In the absence of ligand, members of the TLR family are typically located either at the surface of eukaryotic cells (e.g., mammalian TLR1, TLR2, TLR4, TLR5, TLR6) or in the endolysosomal compartment (mammalian TLR3, TLR7–9). The localization of TLRs is determined by the presence of specific amino acid motifs in the transmembrane region (Leifer et al., 2006; Nishiya et al., 2005), but may also depend on ligand stimulation, receptor glycosylation, and a growing number of "helper" or "shuttle" proteins like UNC93B (TLR9), MD-2, and PRAT4A (TLR4) (McGettrick and O'Neill, 2010; Lee et al., 2012).

Each of the TLRs has a distinct ligand specificity. Ligands are most often of microbial origin. In general, the ligands are structurally conserved, absent in the host, and important for survival of the microbe. These features enable safe detection by the host of a wide range of bacteria, viruses or fungi with only a handful of TLRs. TLR ligands for the mammalian TLR can be grossly divided into: (i) lipidated molecules (glycolipid, lipopeptide) which are recognized by TLR4/MD-2 (LPS), and the combination of either TLR2-TLR1 or TLR2-TLR6 (di- and triacylated lipopeptides), or possibly TLR2-TLR10 (Kirschning and Schumann, 2002; Guan et al., 2010); (ii) conserved proteins which are recognized by TLR5 (flagellin) and mouse TLR11 (profillin-like protein; flagellin) (Hayashi et al., 2001; Yarovinsky et al., 2005; Mathur et al., 2012), and (iii) DNA or RNA, recognized by TLR3 (dsRNA), TLR7 and TLR8 (ssRNA) and TLR9 (DNA)and mouse TLR13 (23S rRNA) (Kumar et al., 2009; Oldenburg et al., 2012).

Signaling through TLRs is initiated by receptor dimerization that follows binding of the TLR ligand to its receptor. For instance, one molecule of dsRNA binds to the extracellular domains of two TLR3 molecules, one molecule of the synthetic lipopeptide Pam_3CSK_4 binds with two acyl chains to the ligand-binding domain of TLR2 and with one to that of TLR6, and one LPS molecule can crosslink two TLR4/MD-2 complexes (Jin and Lee, 2008). Dimerization of TLR is assumed to bring the cytosolic TIR domains in close proximity to form a scaffold that enables the recruitment of signaling adaptor molecules (Kawai and Akira, 2007). Major adaptor proteins include MyD88, TIRAP, TRIF, and TRAM (O'Neill and Bowie, 2007). Dependent on the nature of recruited adaptors, one or two main signaling routes are activated by a distinct TLR complex. In mammals, the MyD88-dependent pathway is activated by all TLRs except TLR3. This activation results via phosphorylation, (poly)ubiquitinylation, and binding of several signaling proteins including IRAK1, IRAK2, IRAK4, TRAF6 and NEMO (Kawai and Akira, 2007). This signaling cascade ultimately causes the nuclear translocation of one or more transcription factors such as NF-κB that control the transcription of mainly pro- and anti-inflammatory genes (Kawai and Akira, 2007). The second major (MyD88-independent) TLR signaling route uses the adaptor protein TRIF. This protein is recruited to the TIR domain of TLR3 and, via the adaptor protein TRAM, to the TIR domain of TLR4. Recruitment of TRIF and TRAM results in the translocation of the transcription factor IRF3 to the nucleus to induce expression of IFNβ and IFN-inducible genes.

3. The TLR repertoire of chicken

Soon after completion of the chicken genome sequence in 2004, bioinformatics analysis predicted the presence of putative TLR orthologs and their downstream signaling and effector molecules in the avian species (International Chicken Genome Sequencing Consortium, 2004; Yilmaz et al., 2005). Genome scanning of the putative chicken TLR (chTLR) repertoire revealed the presence of both similar and unique groups of TLRs compared to the human TLR family which can be divided into five groups of TLR, that is, TLR2/1/6/10, TLR3, TLR4, TLR5, TLR7/8/9 (Temperley et al., 2008). The chicken is predicted to have two TLR2 isoforms (chTLR2 types 1 and 2), two TLR1/6/10 orthologs, and a single chTLR3, chTLR4, chTLR5, and chTLR7. In addition, chickens have two TLRs that appear absent in the mammalian species, namely chTLR15 and chTLR21. One of the chTLR1/6/10 orthologs carries the ligand specificities of mammalian TLR1 and TLR6 in a single receptor (see below) and was therefore originally designated as chTLR16 (Keestra et al., 2007), but is also known as chTLR1 type 1 (chTLR1t1) or chTLR1-like protein A (chTLR1LA) (Temperley et al., 2008; Cormican et al., 2009). A truncated form of chTLR16 is named chTLR1 type 2 (chTLR1t2) or chTLR1-like protein B (chTLR1LB). This receptor has a shorter N-terminal LRR region compared to chTLR16 (Keestra et al., 2007; Higuchi et al., 2008). ChTLR8 is disrupted by the insertion of a retroviral-like insertion element (Philbin et al., 2005). Chickens lack an ortholog of mammalian TLR9. The chicken and human TLR repertoire, and the ligand specificity of the receptors are summarized in Table 1.

4. Functionality of chicken TLRs

Numerous studies on chicken tissue indicate often cell-type specific expression of the chicken TLR (e.g., Iqbal et al., 2005b). Similarly, reactivity of chicken immune cells to mammalian TLR agonists supports the presence of TLRs and functional downstream signaling pathways (Kogut et al., 2005, 2006). Direct evidence that the identified chTLRs are functional is largely based on the use of heterologous expression of recombinant chTLR proteins, construction of chimeric TLR receptors, and gene silencing in the natural background of chicken cells. This approach has revealed species-specific differences in TLR ligand specificity, interactions of chTLRs with accessory molecules, and unexpected downstream signaling events. Current evidence of the functionality of chTLR repertoire and the unique properties of chicken TLR is indicated below for each of the identified receptors and depicted in Figure 1.

4.1. Chicken TLR2 complex – more diverse but less specific than mammalian TLR2

TLR2 proteins have been identified in many species and can form functional heterodimeric complexes with TLR1 or TLR6 (Jin

Table 1
Toll-like receptors with their typical TLR ligands in humans and chicken.

Ligand	TLRs in humans	TLRs in chickens
Triacylated lipopeptides	TLR2/TLR1	TLR2t1/TLR16, TLR2t1/TLR1LB[a], TLR2t2/TLR16
Diacylated lipopeptides	TLR2/TLR6	TLR2t2/TLR16, TLR2t1/TLR1LB
Possibly triacylated lipopeptides	TLR2/TLR10	Absent
dsRNA	TLR3	TLR3
LPS	TLR4/MD-2	TLR4/MD-2
Flagellin	TLR5	TLR5
ssRNA	TLR7	Possibly TLR7
ssRNA	TLR8	Not functional
DNA	TLR9	Absent
DNA	Absent	TLR21
Protease	Absent	TLR15

[a] Alternative nomenclature: TLR16 = TLR1t1 = TLR1LA; TLR1t2 = TLR1LB.