Published in final edited form as:
Sci Signal. 2012 May 8; 5(223): pe11.

The Structure of the TLR5-Flagellin Complex: A New Mode of Pathogen Detection, Conserved Receptor Dimerization for Signaling

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Abstract

Knowledge about how Toll-like receptors (TLRs) recognize pathogenic ligands is critical to understanding how these receptors are activated and to designing therapeutic compounds that target this family of receptors for inflammatory diseases. The crystal structure of TLR5 in complex with its bacterial ligand flagellin revealed that the ligand-binding mode for TLR5 is distinct from that of previously characterized TLRs. Nevertheless, like other TLRs, TLR5 forms a dimer in response to ligand binding. This work contributes to our current knowledge of TLR function and further demonstrates the ability of TLRs to couple versatile ligand recognition to a conserved receptor signaling mechanism.

The Toll-like receptor (TLR) family in the mammalian genome was first characterized as innate immune sensors that recognize evolutionarily conserved pathogen-associated molecular patterns (PAMPs) 10 years ago (1, 2). Since then, the quest to define the structural bases of ligand recognition by TLRs and thus to understand the molecular mechanisms of their activation has been a focus of structural biology. To date, the structures have been published for TLR2-TLR1 and TLR2-TLR6 heterodimers in complex with lipopeptides (3, 4), TLR3 in complex with a double-stranded RNA molecule (5), and TLR4 in complex with MD-2 with or without lipopolysaccharide (LPS) (6, 7). Although TLRs display a conserved horseshoe-shaped leucine-rich repeat (LRR) structural fold, each receptor binds to its ligand with distinct regions depending on the nature of the ligands (Fig. 1). The lack of a common ligand-binding mode among the published TLR-ligand complexes makes it difficult to predict the ligand recognition mode for the remaining TLRs. Yoon and colleagues determined the ligand-complexed crystal structure of another member of TLR family, TLR5, adding another important piece of the puzzle of how TLRs mediate ligand recognition and immune activation (8). Human TLR5 recognizes bacterial flagellin, a principal component of bacteria motor flagella present in both Gram-positive and Gram-negative bacteria (9). Flagellin binding leads to the clustering of the cytoplasmic TIR [Toll/interleukin (IL)–1 receptor] domain of TLR5 and the activation of the MyD88 (myeloid differentiation 88) pathway, which includes the recruitment of IRAK (IL-1-receptor–associated kinase) and TRAF6 (TNF-receptor–associated factor 6) and the activation of the transcription factor nuclear factor (NF) κB, leading to the production of the proinflammatory cytokine IL-6 (10).

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Crystallization of TLR receptors has proven to be challenging, primarily due to poor expression of recombinant versions of the extracellular ligand-binding domains and the low crystallization tendency of native TLRs. The authors used two technical strategies: (i) screening homologs of human TLR5 with various extracellular truncations to find a construct with abundant expression, and (ii) fusion of a hagfish variable lymphocyte receptor (VLR) domain at the C terminus to facilitate crystallization. Both techniques have been employed previously in structure solutions of TLRs (3). In this case, Yoon *et al.* succeeded in expressing several truncated forms of the extracellular domain of TLR5 from zebrafish and validated that a human TLR5 ligand functionally activated zebrafish TLR5 using a reporter system for transcriptional activation of NF-κB. They crystallized a construct containing the 14 N-terminal LRRs from zebrafish TLR5 fused to a hagfish VLR in complex with a fragment of *Salmonella* flagellin (FliC). They also solved the structure of a shorter construct of the TLR5 alone. The 2.5 Å resolution structure of the TLR5-FliC complex revealed both the unique aspects of ligand binding to TLR5 and the common dimerization feature shared between TLR5 and other TLRs.

Yoon *et al.* described how TLR5 forms a 2:2 dimer with FliC, similar to that of TLR4:MD-2-LPS but different from the 2:1 dimers observed in complexes of TLR1-TLR2:lipopeptide or TLR3:dsRNA (double-stranded RNA). The binding interface for flagellin in TLR5 is rather extensive and involves the N-terminal and first 10 LRR regions, with a focal region centered at the LRR9 loop mediating many of the important interactions. The LRRs involved in binding of flagellin to TLR5 are different from those used by TLR2/1 in lipopeptide binding, which requires the central LRR 9 to 12 repeats, and from those involved in dsRNA recognition by TLR3, which requires the N-terminal to third repeats (NT to LRR 3) and repeats 19 to 21. Although TLR4 uses similar regions [the N-terminal to the fifth repeats (LRR NT to LRR 5) and between the eighth and tenth repeats (LRR 8-10)] to recognize MD-2, the detailed interface residues are different. Thus, depending on the ligands, different TLRs use different sets of LRRs for their ligand recognition. This enables a limited number of TLRs to recognize a diverse array of ligands. On the ligand side, TLR5 recognition involves a set of relatively conserved residues among bacteria on both the N- and C-terminal helices in the D1 domain of flagellin. These TLR5 interface residues of flagellin are normally involved in bacteria flagellin oligomer formation and, thus, are not exposed in live bacteria (11). Like other TLR-ligand complexes, the ligand-bound TLR5 forms a symmetric m-shaped dimer with the C-terminal LRR regions juxtaposed against each other, a conformation conducive to the dimerization of the intracellular TIR domains, which activate downstream signaling pathways. The dimer interface induced by flagellin binding involves residues from LRRs 12 and 13 of TLR5, and the authors demonstrated that mutations in these LRRs resulted in forms of TLR5 that failed to transcriptionally activate the NF-κB reporter system.

The work by Yoon and his colleagues represents another concrete advancement to our understanding of TLR functions. Just a few years ago, it was difficult to understand the basis of pattern recognition or how a limited number of TLRs (10 in human) can recognize a large number of pathogenic ligands. Structural studies have provided several insights into ligand recognition by TLRs. First, TLRs recognize conserved functional groups present on their ligands, whether they are amino acids, nucleotide backbone, or lipid head groups. They are true PAMP-based recognition receptors. In addition, TLRs are a family of versatile receptors that can use practically every single LRR for ligand recognition, and the variations in the shape and location of recognition LRRs are the basis for their ligand diversity. Finally, all TLRs share a conserved ligand-induced dimerization mode, supporting a common intracellular activation pathway. The structures not only provide insights into the function of these receptors, but they will also likely facilitate the design of therapeutic agents for the treatment of inflammatory diseases. Although these discoveries have been exciting, there are

also questions that remain to be answered. There is still no structural solution for TLR7 or TLR8, which recognize single-stranded RNA, or for TLR9, which recognizes CpG oligodeoxynucleotides (1). In addition, some TLRs recognize ligands that lack conserved PAMPs. For example, TLR4 recognizes heat shock proteins (such as HSP60), fibronectin domains, and some viral proteins in addition to LPS (1). It remains to be seen if TLR4 uses different sets of LRRs from those for recognition of MD-2 and LPS. It is anticipated that some of the remaining questions will be resolved in the next few years with additional structural studies.

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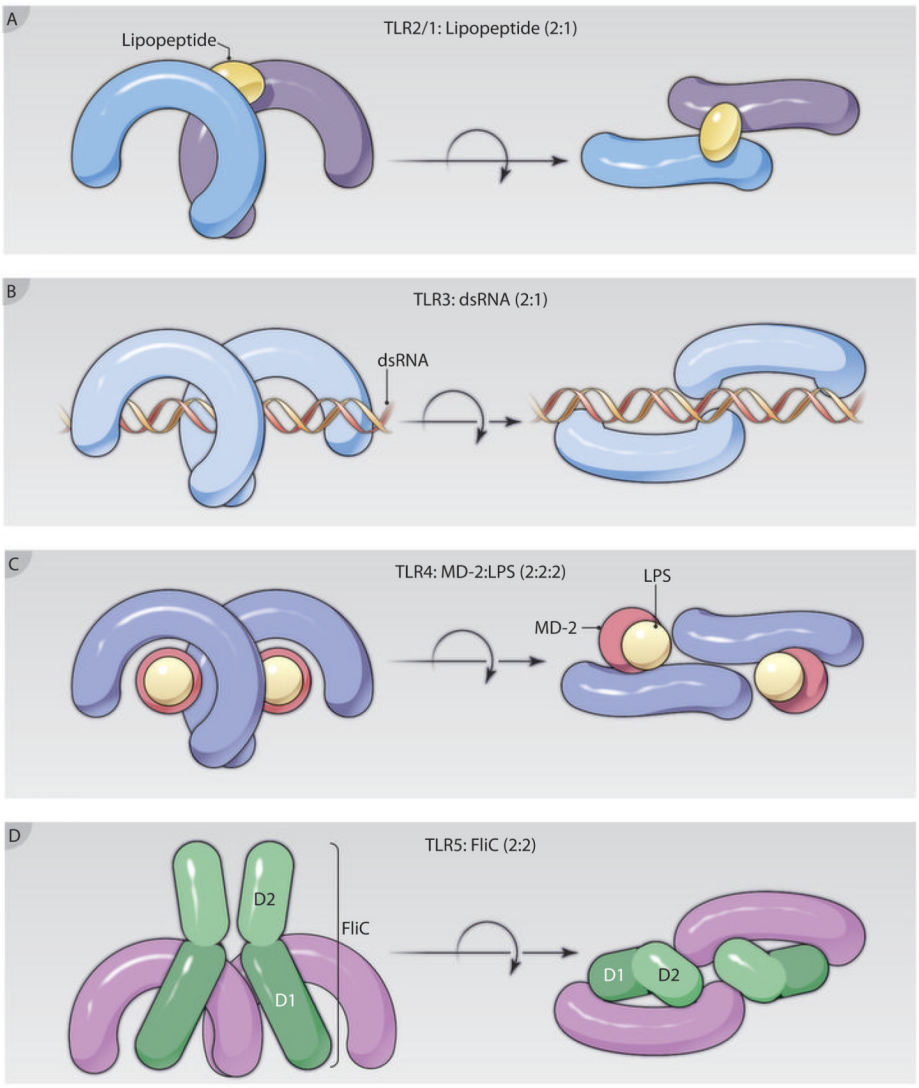

Fig. 1.

Two orthogonal views of dimerized TLR receptors bound to ligand. (**A**) TLR2/1 forms a 2:1 receptor:ligand complex with lipopeptide. The recognition interface is centered on LRRs 9 to 12. (**B**) TLR3 recognizes dsRNA in a 2:1 complex using N- (LRR NT to LRR 3) and C-terminal (LRRs 19 to 21) segments. (**C**) TLR4 forms a 2:2:2 complex with MD-2 and LPS using LRR NT to LRR 5 and LRR 8 to 10. (**D**) TLR5 recognizes flagellin fragment FliC in a 2:2 complex using the first 10 LRR repeats (LRR NT to LRR 10). All TLRs share a common dimerization mode that involves the C-terminal LRR domains.
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