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Ligand Recognition by the Toll-like Receptor Family

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Abstract: Toll-like receptor (TLR) family proteins, type I transmembrane proteins, play a central role in human innate immune response by recognizing common structural patterns in diverse molecules from bacteria, viruses and fungi. Recently four structures of the TLR and ligand complexes have been determined by high resolution x-ray crystallographic technique. In this review we summarize reported structures of TLRs and their proposed activation mechanisms. The structures demonstrate that binding of agonistic ligands to the extracellular domains of TLRs induces homo- or heterodimerization of the receptors. Dimerization of the TLR extracellular domains brings their two C-termini into close proximity. This suggests a plausible mechanism of TLR activation: ligand induces dimerization of the extracellular domains, which enforces juxtaposition of intracellular signaling domains for recruitment of intracellular adaptor proteins for signal initiation.

Key words: innate immune response, leucine rich repeat, pattern recognition receptor, toll-like receptor, hybrid LRR technique

TOLL-LIKE RECEPTOR FAMILY PROTEINS

Research of innate immunity is one of the most vigorously investigated field of immunology since Metchnikoff first introduced phagocytic theory of immunity (O'Neill, 2004; Silverstein, 2003). Innate immune system is characterized by fast and immediate response against invading pathogens. In contrast, highly specific adaptive immune system operates later in infection. Innate immune response is initiated by the limited number of receptors termed pattern recognition receptors (PRRs) present in several immune cell types including dendritic cells, neutrophils and natural killer T cells. Among these receptors, the representative key player is the TLR (Toll-Like Receptor) family.

Until now 10 human and 13 mouse TLRs have been reported since TLR4, the first TLR discovered, was identified as a homolog of *Drosophila* Toll. Toll has been shown to be critical for embryo development and antifungal immune activity (Gay and Keith, 1991; Matsushima, 2007; Medzhitov, 1997). TLRs are type I transmembrane glycoprotein composed of extracellular, transmembrane and conserved intracellular domains (Gay and Gangloff, 2007). The extracellular domains of TLRs characterized by repeated LRR (Leucine-Rich Repeat) modules are specific for pathogen-associated molecular patterns. Each TLR binds to distinctive microbial components. For example, as TLR2 complex with TLR1 or TLR6 recognizes lipoprotein or lipopeptide, TLR3, TLR4, TLR5, TLR7 (or TLR8), and TLR9 recognize viral double stranded RNA, lipopolysaccharide (LPS), bacterial flagellin, single stranded RNA, and microbial, respectively. Binding of these ligands to corresponding TLRs induces multimerization of the receptors and believed to trigger recruitment of intracellular adaptor proteins to the intracellular TIR (Toll/IL-1 Resistance) domains of TLR for signaling.

Although innate immune response against infectious microbes is an essential protective system, patients under compromised immune condition may suffer from septic syndrome due to unregulated and over-reactive immune responses mediated by TLRs and other innate immune receptors. Moreover, genetic defects in some TLRs lead to severe defects toward infectious diseases such as meningitis, tuberculosis and asthma (Echchannaoui, 2002; Ogus, 2004; Texereau, 2005). Therefore, development of therapeutic agents modulating activities of TLRs is urgently required. To date, TLR agonists including imiquimod of TLR7, monophosphorylated Lipid A (MPL) of TLR4 are successfully used for clinical treatments of the diseases or for enhancing vaccine responses (Beutner, 1998; Hemmi, 2002; Romagne, 2007). Furthermore, TLR antagonists that can bind to receptor but fail to initiate intracellular signal are being developed to control microbial inflammations or autoimmune diseases. For example, Eritoran, a potent

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antagonist of TLR4 is currently under phase III clinical trial against severe sepsis (Kanzler, 2007).

Structural studies of TLR and ligand complexes have been attractive area of research because structural information is critical for study of innate immune system as well as for development of novel drugs against several immune syndromes. In 2005, Choe et al. determined the first crystal structure of human TLR in atomic detail (Choe, 2005). However many important questions about TLRs and their roles in innate immune response had remained elusive because the structure did not contain bound ligands and therefore could not provide direct insights into ligand specificity or receptor activation. Recently four structures of the TLR-ligand complexes have been determined by high resolution x-ray crystallography; TLR1-2-lipopeptide, TLR3-dsRNA, TLR4-MD-2-Eritoran, and TLR4-MD-2-LPS. In this review we summarize (1) common structural characteristics of the TLR family, (2) structural patterns in ligands that are responsible for TLR binding and activation, and (3) structures of the TLR dimers induced by agonistic ligands.

STRUCTURE OF THE TLR FAMILY

LRR is a relatively large protein family that includes more than 6000 proteins in Pfam data base. LRR domains exist in about 300 human proteins (Kobe and Deisenhofer, 1994; Kobe and Deisenhofer, 1995; Kobe and Kajava, 2001; Matsushima, 2007). To date, approximately seventy structures of three-dimensional structures of LRR superfamily have been reported. All known structures of LRR superfamily proteins demonstrate an arc or horseshoe-like shape. This protein architecture is derived from unique sequence pattern in the LRR proteins. All LRR modules in the proteins are composed of the highly conserved and variable parts (Kajava, 1998; Kobe and Kajava, 2001). The conserved "LxxLxLxxN" motif part forms the inner concaves containing the continuous parallel β-strands. On the other hand, the variable sequences form the outer convexes surfaces formed by α-helices and/or loops. Almost all LRR proteins have the capping modules termed LRRNT and LRRCT at their both termini (Kajava, 1998). These capping modules containing cysteine clusters shield the hydrophobic core composed of conserved leucines from exposure to outside. The reported structures of TLRs demonstrate that TLR family proteins belong to the "typical" subfamily of the LRR superfamily (Bell, 2005; Choe, 2005; Jin, 2007; Kim, 2007b; Liu, 2008; Matsushima, 2007). They all contain the horseshoe-like shapes similar with the structures of other LRR containing proteins. However, some TLRs demonstrate unusual structural features distinctive from those of other "typical" subfamily proteins.

Structure of TLR3, the first reported structure of TLR, shows the flat horseshoe-like shape with an uniform b sheet conformation similar with those of other "typical" subfamily proteins (Bell, 2005; Choe, 2005). This structural property is originated from well conserved sequence pattern of TLR3 - conserved leucine residues point inward for hydrophobic interaction and asparagine ladders interact via hydrogen bonds with the amide and carbonyl groups in the neighboring LRR module. In contrast, TLR1, TLR2 and TLR4, although they clearly are members of typical subfamily, show unusual structural features different from that of TLR3 (Jin, 2007; Kim, 2007b; Park, 2009). Due to sharp structural transitions in the β sheet, structures of these TLR proteins can be divided into N-terminal, central, and C-terminal subdomains. Both N-terminal and C-terminal domains have conserved structure pattern common to other typical family proteins. However, the central domains adopt the unusual β sheet conformation with smaller inner radius and larger twist angles. These unusual structural alterations appear to be caused by irregular LRR motives concentrated on the central domains: central domains of TLR1, TLR2 and TLR4 have no conserved asparagine ladders that maintain overall curvature of the horseshoe-like shape by forming continuous hydrogen-bonding interactions. Moreover, the LRR modules of the central domains have considerable variations of amino acid lengths, ranging from 20 to 33 residues. Generally LRR modules containing fewer amino acid residues have loops in their variable convex regions, but those containing more residues tend to form helices in the variable regions. Central domains of TLR1, TLR2 and TLR4 have mixture of a helices containing regions and loop containing regions. This appears to distort the otherwise regular structures of the convex region and makes inner radii smaller and substantial deviation from the principal plane of the horse-shoe like structure. Protein regions with unusual structural alterations often play important role in function because they can provide unusual shape or flexibility necessary for activity. Consistently, the central domains of TLR1, TLR2 and TLR4 have essential roles in ligand recognition or binding of accessory protein (discussed later) (Jin, 2007; Kim, 2007b; Park, 2009).

Other TLRs can be divided into these two classes although their structures are not known yet. TLR5, TLR7, TLR8 and TLR9, which have strictly conserved asparagine ladders, probably have the flat single domain-like architectures similar with those of the traditional "typical" subfamily proteins (Matsushima, 2007). Conversely, TLR10 that has the asparagine ladders broken and the irregular LRR modules with longer lengths in the central part of the protein is likely to have distorted multi-domain architecture present in structures of TLR1, TLR2 and TLR4.

STUCTURES OF THE TLR-LIGAND COMPLEXES

To date, four structures of TLR complexes with their ligands are reported by different laboratories (Jin, 2007; Kim, 2007b; Liu, 2008; Park, 2009). This allows nice answers to controversial questions about TLRs. For instance, it is clear that most TLRs except TLR4 directly bind to their ligands, that MD-2 is absolutely necessary for ligand binding by TLR4, and that dimerization of TLRs is induced by the ligand binding. Moreover, it offers clues to how other TLRs interact with their ligands to be activated although the related structural information remains to be further investigated.

Structure of the TLR1-TLR2-lipopeptide complex

TLR2 in association with TLR1 is essential for recognizing bacterial lipoproteins, especially tri-acylated lipoproteins (Takeuchi, 2001; Takeuchi, 2002). Crystal structure of human TLR1-TLR2-Pam_3CSK_4 shows that overall shape of the complex is similar with alphabet "m" where two N-terminal domains outstretch toward opposite ends and the C-terminal domains converge in the middle (Jin, 2007). Pam_3CSK_4 is a synthetic peptide containing the conserved N-terminal cysteine acylated by three palmitate groups (Hioe, 1996). It can bind and activate TLR1-TLR2 complex like natural lipoproteins. Surprisingly, the lipopeptide-binding site is found in a highly unexpected region (Jin, 2007). It is located at the boundary of the central and C-terminal domain in the convex regions of TLR1 and TLR2. The two hydrophobic pockets form a continuous ligand binding channel by forming a ligand induced heterodimer. Two of the three lipid chains of the Pam_3CSK_4 are inserted into the TLR2 pocket, and the remaining amide-bound lipid chain is inserted into the narrower TLR1 channel. Moreover, the complex of TLR1 and TLR2 is further stabilized through protein-protein interactions in the ligand binding pocket.

Structural study of TLR1-TLR2-lipopeptide offers an opportunity to resolve the questions whether CD14 as a co-receptor of TLR2. CD14, which is one of the accessory proteins of TLR4, is also a LRR family protein (Fujihara, 2003; Miyake, 2006). CD14 forms a homodimer through interaction between the two C-terminal regions (Kim, 2005). The CD14 dimer represents the horseshoe-like structure like TLRs. Surprisingly CD14 has the hydrophobic pocket in the convex region of the LRRNT and LRR modules similar with the ligand binding pocket of TLR1-TLR2 complex. This amino terminal pocket of CD14 is predicted to be the LPS binding pocket of CD14. It has been reported that CD14 enhances the binding efficiency of lipoprotein to the TLR1-TLR2 complex (Iwaki, 2005; Manukyan, 2005). However, there are controversies whether CD14 is necessary for ligand binding as a co-receptor or only a catalyst for lipopeptide binding by TLR2 (Nakata, 2006). The crystal structure shows that the lipopeptide by itself is sufficient to form the TLR1-TLR2 heterodimer without CD14 (Jin, 2007). As CD14 can induce transfer of the monomerized LPS to the TLR4-MD-2 complex for proinflammatory responses (Fujihara, 2003; Miyake, 2003), it is conceivable that CD14 facilitates lipopeptide transfer as monomeric form to TLR1-TLR2 complex. Moreover, we found that CD14 interacts only weakly with TLR2 (unpublished data). These results suggest that CD14 is a catalyst, not a co-receptor, for lipopeptide recognition in the TLR1 and TLR2 complex.

Structure of TLR1-TLR2-lipopeptide provides us with information to predict binding mode of LTA(lipoteichoic acid) to TLR1-TLR2 and di-acylated lipopeptide to TLR2-TLR6. LTAs have been reported as potent inducers of innate immune response (Deininger, 2003; Han, 2003; Schroder, 2003). LTAs have two lipid chains attached to the glycerol backbone and phosphate containing units repeated ranging from 4 to 25 times (Morath, 2001). Structure of the TLR2-Pam_2CSK_4 complex shows that the Pam_2CSK_4 binds in similar fashion with that of Pam_3CSK_4: two lipid chains of the Pam_2CSK_4 are inserted into the TLR2 pocket and glycerol and peptide backbone is held by conserved protein residues (Jin, 2007). Because of structural homology with di-acylated Pam_2CSK_4 lipopeptides, two lipid chains of LTAs are likely to be inserted into the TLR2 binding pocket like Pam_2CSK_4. Di-acylated lipopeptide is recognized by the TLR2-TLR6 complex (Buwitt-Beckmann, 2005; Takeuchi, 2001). The structure of TLR6 can be predicted by homology modeling (Jin, 2007). The structural model suggests a clue to why TLR2-TLR6 complex is unable to interact with the tri-acylated lipoproteins. It is the two bulky phenylalanine residues blocking the potential lipid binding channel in TLR6, and thus the amide-bound lipid chain of the tri-acylated lipopeptide may not be inserted into TLR6.

Structure of the TLR3-dsRNA complex

TLR3 has been shown to recognize double stranded RNA produced by viral replication (Alexopoulou, 2001). In a recent issue, Liu et al. reported structure of mouse TLR3 bound to dsRNA (Liu, 2008). Overall structure of the TLR3 homodimer induced by dsRNA resembles "m" shaped complex of the TLR1-TLR2-lipopeptide. The dsRNA interacts with both N-terminal (LRRNT and LRR1-3) and C-terminal (LRR19-21) site on the glycan-free convex region of each TLR3. The positively charged residues of both termini of mTLR3 are the major determinants for interaction with the sugar-phosphate backbones of dsRNA. Interestingly, while the glycan-free surface is essential for recognition of dsRNA, the N-glycosyl moiety of Asn413 located on the concave surface of mTLR3 extends toward the dsRNA and directly contacts it. The protein-protein

interactions occur only at the LRRCT modules of TLR3 in the TLR3-dsRNA complex, bringing the C-termini of TLR3 homodimer into contact.

Regardless of apparent similarity of the overall shape, the TLR3 homodimer has the completely different ligand binding mode compared to that of the TLR1-TLR2 heterodimer (Jin, 2007; Liu, 2008). Although ligand binding pocket in TLR1-TLR2 complex is located at the boundary between the central and the C-terminal domains, dsRNA interaction sites in TLR3 are placed in evolutionally well-conserved N- and C-termini. Furthermore, TLR3 interacts with the sugar-phosphate backbones by mainly charge interactions, not by hydrophobic interactions as shown in TLR1-TLR2 heterodimer. The protein-protein interaction of the TLR1-TLR2 complex is localized near the ligand binding pocket, but that of TLR3 homodimer is separated far from the main ligand binding site of the TLR3-dsRNA complex.

Other intracellularly localized TLRs, TLR7, TLR8 and TLR9 interact with nucleic acid-derived ligands as well (Akira and Takeda, 2004; Gay and Gangloff, 2007; West, 2006). Their ligand bound structures can be predicted based on the structure of the TLR3-ligand complex because they share substantial sequential and functional similarities (Matsushima, 2007). As previously proposed, the glycan-free surface of TLR3 is a critical requisite for dsRNA binding (Liu, 2008). TLR3 has the fifteen N-linked glycosylation sites. Among them, four glycosylations of the inner concave surface seem to inhibit binding of dsRNA. All TLR7, TLR8 and TLR9 also have large number of potential N-linked glycosylation sites, ranging from 13 to 21. There are four or five glycosylation sites in the inner concave of TLR7-9 that can interfere with ligand binding. To predict existence of glycan-free surface in these TLRs, structure of human TLR7 was homology-modeled using the human TLR3 structure as a template. The model shows that TLR7 also contains the glycan-free surface like TLR3. Potential N-glycosylation sites of TLR8 and TLR9 are well conserved with those of TLR3. The plausible glycosylation analysis strongly suggests that ligand binding site in TLR7-9 would be the face without any glycosylations in the convex region. The LRR20 module with short insert sequence of TLR3 is one of the sites essential for interaction with dsRNA (Liu, 2008). Basic residues in LRR20 protruded from the convex region directly bind sugar-phosphate backbone of dsRNA. Surprisingly, all eighth LRRs of TLR7, TLR8 and TLR9 contain nonconsensus LRR modules with additional 16 amino acid residues, which include two cysteine-rich "CXXC" box (Matsushima, 2007). Site-directed mutagenesis conducted by Gibbard et al. demonstrated that modification of any of these four cysteine residues to alanine completely abolished signaling by TLR8 when activated by both resiquimod and ssRNA (Gibbard, 2006). This result agrees well with insertion specificity model proposed by Bell *et* al (Bell, 2003). Collectively, like TLR3, LRR motives with inserted sequence located at the glycan-free surface of TLR7-9 appears to be the sites for ligand binding.

Structure of the TLR4-MD-2-ligand complex

TLR4 in company with its co-receptor MD-2 is responsible for LPS recognition (Shimazu, 1999; Viriyakosol, 2001). LPS is a major component of the outermembrane of Gram-negative bacteria (Erridge, 2002). They are composed of lipid A, core and O-antigen sugars. Previous research demonstrated that the lipid A region with 5~7 acyl chains and phosphorylated di-glucosamine is sufficient for immunological toxicity of LPS (Tanamoto and Azumi, 2000). In an article by Kim *et al*., it has been reported that one molecule of mouse MD-2 interacts with mouse TLR4 at the concave surface provided by the N-terminal and the central domains (Kim, 2007b). MD-2 adopts a β cup fold with two antiparallel β-sheets. The LPS binding pockets are generated by separating one side of the β-sandwich and exposing the hydrophobic core residues. The LPS binding pocket of the MD-2 is lined with hydrophobic residues, except the opening region that has many positively charged residues. Therefore, the overall shape and electrostatic behavior of MD-2 seem to be suitable for binding of the amphipathic and negatively charged ligand such as LPS. The interaction between TLR4 and MD-2 is formed mainly by hydrogen bonds in two opposite charged patches named A and B patches. The negatively charged and evolutionarily well conserved A patch of TLR4 interacts with the basic residues in MD-2. On the other hand, the positively charged B patch is located in less conserved area of TLR4 and interacts with acidic residues in MD-2.

Kim *et al.* successfully crystallized human TLR4-VLR hybrid complexed with MD-2 bound to its antagonistic ligand Eritoran using a novel technique termed the "Hybrid LRR technique" (Jin and Lee, 2008; Kim, 2007b). Eritoran containing four acyl chains is a lipid A analog and a potent antagonist of TLR4. Crystallographic analysis of TLR4-ligand complexes has been hampered by several technical difficulties. Simple truncation of non essential parts of protein often improve crystallization behavior of many proteins but this method is not inappropriate for TLR family because they are composed of continuous LRR modules. Simple removal of the LRRNT or LRRCT modules protecting the hydrophobic cores of the TLR family could expose the hydrophobic core to make the structure of TLR very unstable. Hybrid LRR technique allows fusion of truncated fragments of TLRs with the LRRNT or LRRCT domains from other LRR-containing proteins at the highly conserved "LxxLxLxxN" site (Kim, 2007b). Variable Lymphocyte Receptors (VLRs) were

chosen as fusion partners because all VLR proteins have canonical LRR modules with almost infinite sequence diversity (Kim, 2007a). VLRs mediate adaptive immune responses in jawless fish and have almost unlimited sequence diversity (Pancer and Cooper, 2006). Among fifteen constructs of TLR4-VLR hybrids generated, seven of them were successfully expressed as soluble proteins. In our laboratory TLR1, TLR2, TLR5, TLR7, TLR8 hybrids with VLR fragments were produced with the similar or better rate of success (unpublished data). VLR fusion did not change native structure of TLR and the TLR-VLR hybrids retain the function. It is likely that hybrid proteins failed producing soluble and folded proteins probably have serious collision of side chains at the boundary of fusion site. Although VLRs are shown to be a successful fusion partner for TLRs, it is yet not clear if VLR is the best fusion partner for other LRR family proteins. More research is required to find optimal fusion partner for LRR hybridization.

Eritoran binds to the hydrophobic pocket in MD-2 in a fashion that acyl chains occupy most of the MD-2 pocket (Kim, 2007b). The main hydrophobic interaction is reinforced by charge interaction between the ligand and positively charged residues in MD-2. There is no direct interaction between Eritoran and TLR4. Ohto *et al* reported crystal structure of human MD-2 with lipid IVa (Ohto, 2007). The tetraacylated lipid IVa is a derivative of Lipid A and acts as an antagonist in human cells like Eritoran (Kusumoto, 2003; Mullarkey, 2003). Although their chemical structures have large difference, Eritoran and Lipid IVa show highly homologous structure when bound to MD-2 (Kim, 2007b; Ohto, 2007). As noted, both antagonistic Eritoran and lipid IVa have four lipid chains although agonistic LPS contains more or longer lipid chains. Since lipid chains of Eritoran or lipid IV occupy almost all the available volume in MD-2 pocket, LPS is proposed to bind TLR4-MD-2 complex with different structure. It is thus conceivable that LPS may be able to make the MD-2 pocket larger because one side of MD-2 pocket is opened with no any disulfide bonds. Alternatively, four chains of LPS are inserted into MD-2 pocket and the remaining two chains protrude from MD-2 pocket for interaction with another TLR4. Both proposed binding modes of LPS to MD-2 pocket need to provoke the structural changes of MD-2 that should play important roles for dimerization of the TLR4-MD-2.

Recently reported structure of the TLR4-MD-2-LPS complex supports the second hypothesis that the LPS binding does not change overall size of the MD-2 pocket (Park, 2009). Instead, LPS moves up to the solvent area by ~5Å, which generates room enough for two additional lipid chains in the MD-2 pocket. Dimerization of the TLR4-MD-2 complex is mediated by several interactions. The major contribution is derived from hydrophobic interaction between the sixth lipid chain of LPS and a hydrophobic patch in TLR4. Hydrophilic interaction between MD-2 and TLR4 surrounds this core hydrophobic interaction, and ionic interaction between the phosphate group of LPS and TLR4-MD-2 further stabilizes the TLR4 dimer. The TLR4-MD-2-LPS dimer shows the "m" shaped architecture, which further supports the hypothesis that all TLR family receptors have similar "m" shaped dimeric structure when bound to agonistic ligands.

LIGAND-INDUCED DIMERIZATION AND ACTIVATION OF TLR

Previous crystallographic studies demonstrated that homodimer or heterodimer of TLRs can be induced by binding of agonistic ligands (Jin, 2007; Kim, 2007b; Liu, 2008; Park, 2009). A model of the TLR activation mechanism is proposed based on the following structural and biological observations (Fig. 1). (1) It has been known that several TLRs form homo- or hetero-multimer before binding of their ligands (Akira and Takeda, 2004; Latz, 2007; Ozinsky, 2000; Triantafilou, 2006). These pre-ligand complexes cannot induce the signaling cascade for immune responses presumably because the intracellular TIR domains have unproductive orientation for signaling. (2) Bindings of ligands to TLRs do not result in major conformational changes (Jin, 2007; Liu, 2008; Park, 2009). Overall structures of TLRs in the TLR1-TLR2 heterodimer, TLR3 and TLR4-MD-2 homodimers are nearly identical with that of the monomers. (3) The two TIR domains should be brought into close proximity in the ligand induced homo- or heterodimer of TLRs. In TLR1-TLR2 complex, the C-termini of the extracellular domains of TLR1 and TLR2 converge in the middle (Jin, 2007). Similarly, the C-termini of the extracellular domains of TLR3 are only ~7Å apart in the ligand induced homodimer (Liu, 2008). (4) Sequence alignment shows that there are only a few residues able to act as a long and flexible linker between the extracellular and transmembrane domain or between the transmembrane and intracellular domain (Matsushima, 2007). Hence the C-termini of TLRs into close apposition appear to induce juxtaposed intracellular TIR domains for signaling. Above results provide clues to signaling mechanism via TLRs. TLRs without their ligands already exist as the preformed and weakly bound homo- or heterodimers in the cellular membrane (Fig. 1). The cytoplasmic TIR domains of these complexes are inactive because the distance or orientation between the TIR domains is not suitable for signal initiation. Binding of agonistic ligands to TLRs induce the rearrangement of the extracellular domains and bring the C-termini of extracellular domains into close distance, which leads to juxtaposition of the TIR domains for signal initiation.

In some TLRs, it has been proposed that the conformational

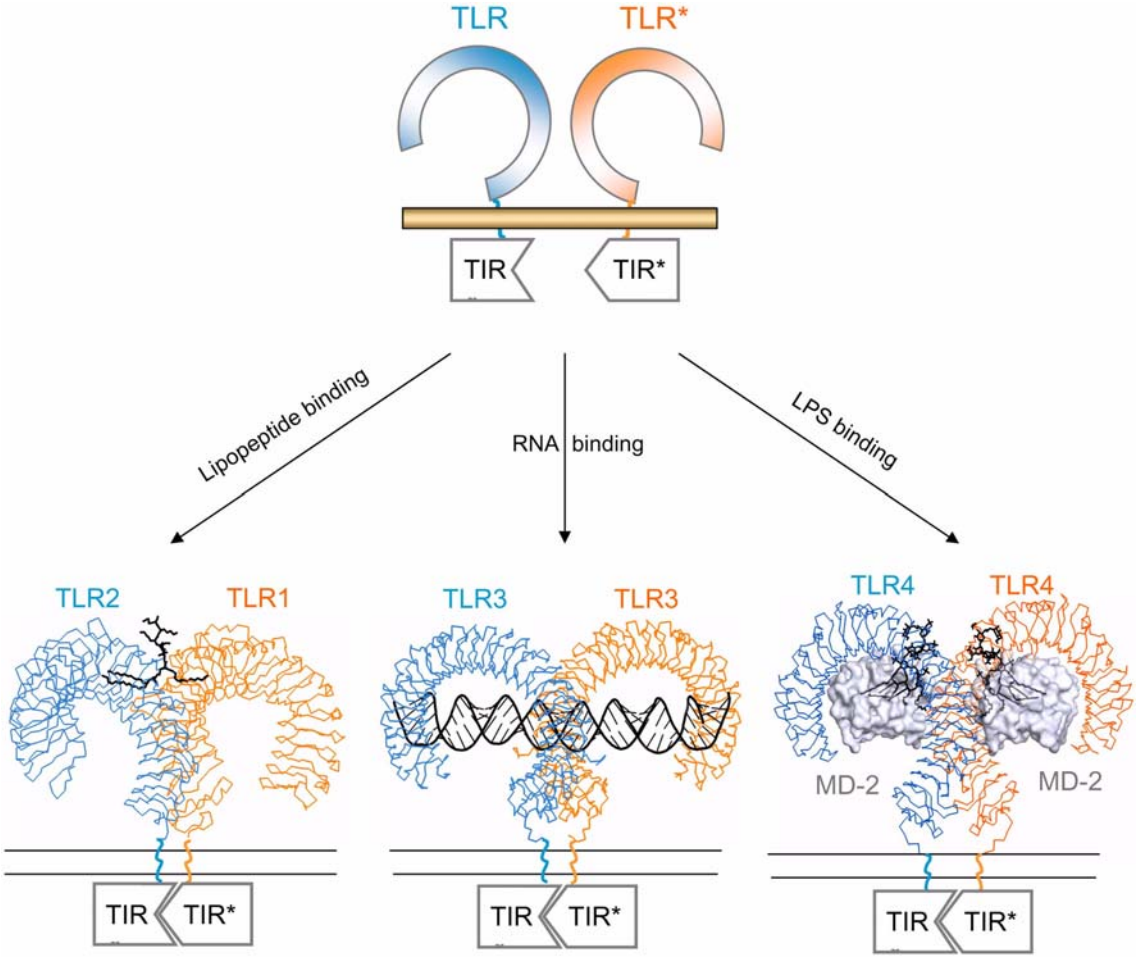

Fig. 1. Model of TLR activation by agonistic ligands. The "m"-shaped TLR dimers are induced by binding of their agonistic ligands. Structure of the pre-ligand complex is unknown. The crystal structures of TLR1-TLR2-Pam_3CSK_4 (left), TLR3-RNA (middle) and TLR4-MD-2-LPS (right) are drawn as proposed by Jin, Liu and Prak et al. (Jin, 2007; Liu, 2008; Park, 2009). TLR dimers are colored blue and orange, and ligands are colored black, respectively. Asterisks are used to mark the second TLRs or TIRs in the receptor complex.

changes induced by ligands binding are critical for receptor activation. Latz et al. provided evidences that only stimulatory DNAs lead to dramatic conformational changes in the TLR9 extracellular domain and these alterations bring the TIR domains of TLR9 closer for activation (Latz, 2007). TLR9 has the unusual LRR15 motif with 58 amino acid residues located in the central part of the extracellular domain (Matsushima, 2007). Long LRR motif appears to confer flexibility and allows conformational change in the extracellular domain of TLR9. Like TLR9, both TLR7 and TLR8 have the long LRR motifs, containing 73 residues in TLR7 and 64 in TLR8, in the central parts of the extracellular domains. Therefore, it appears that conformational changes induced by ligand binding controls signaling by TLR7 and TLR8. Similarly, the conformational change of accessory protein, not TLRs, seems to be important for signal transduction in the TLR4 system.

FUTURE PERSPECTIVES

In this review, we summarized current structural understanding of TLR-ligand interaction and models of receptor activation by the homo- or heterodimeriztion. Future structural research of other TLRs will confirm this hypothesis. The TLR family and its adaptor proteins, MyD88, MAL, TRIF and TRAM, interact each other through evolutionary conserved TIR domains (O'Neill L and Bowie, 2007). Several structure models of TIR complexes are contradictory each other probably due to lack of high resolution structural information (Dunne, 2003; Gautam, 2006; Núñez Miguel, 2007). Therefore determining experimental structures of the TIR multimers will be crucial for clearer understanding of signaling mechanism. The Hybrid LRR technique proved its usefulness in crystallographic research of the TLR family. This technique can have broader application in

structural analysis of other LRR proteins as well as generation of artificial proteins with beneficial therapeutic activities.

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