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Structural study of ssRNA sensing TLRs in innate immune system

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Toll-like receptors (TLRs) are a family of pattern-recognition receptors that recognize microbial components and initiate subsequent immune responses. Ten members of the human TLR family (TLR1 to TLR10) have been identified to date. The extracellular domains have leucine rich repeats (LRRs) and are responsible for binding so-called "pathogen-associated molecular patterns".

TLR7 and TLR8 recognize ssRNA and initiate innate immune responses. Moreover, several small molecule compounds have been identified as TLR7 and TLR8 activators. We determined the crystal structures of unliganded and ligand-induced activated human TLR8 dimers [1]. Ligand recognition was mediated by a dimerization interface formed by two protomers. Upon ligand stimulation, the TLR8 dimer was reorganized such that the two C-termini were brought into proximity. The loop between leucine-rich repeat 14 (LRR14) and LRR15 was cleaved; however the N- and C-terminal halves remained associated and contributed to ligand recognition and dimerization. Ligand binding induces reorganization of the TLR8 dimer, which enables downstream signaling processes.

To elucidate how TLR8 recognizes its natural ligand (ssRNA), as well as how the receptor can be activated by molecules as structurally and chemically different as ssRNA and the chemical ligands, we performed crystallographic studies of TLR8 in complex with ssRNAs. The resultant structures revealed that TLR8 recognizes, at distinct sites, uridine and small oligonucleotides derived from degradation of ssRNA. Uridine bound the site on the dimerization interface where small chemical ligands are recognized, whereas short oligonucleotides bound a newly identified site on the concave surface of the TLR8 horseshoe structure. Site-directed mutagenesis revealed that both binding sites were essential for activation of TLR8 by ssRNA. In addition, we structurally revealed the role of the proteolytic processing of Z-loop.

Furthermore, we biochemically demonstrated that TLR7 responds to guanosine and its derivatives. We determined the crystal structures of activated TLR7-small ligand-ssRNA complexes [3]. Similar to TLR8, TLR7 harbors two ligand-binding sites, the first and second sites. The first site is used for small-ligand binding and is essential for activation in both TLR7 and TLR8. Conversely, the second site is an ssRNA-binding site, which is spatially distinct from that of TLR8. Moreover, the ligand-recognition mode at the second site in TLR7 is completely different from that in TLR8. The first site of TLR8 accommodate uridine, but TLR7 preferentially binds to guanosine at the first site. The second site of TLR7 specifically recognizes uridine moieties in ssRNA.

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