Toll-Like Receptor 9 Contains Two DNA Binding Sites that Function Cooperatively to Promote Receptor Dimerization and Activation

Innate immunity is essential for host defense against microbial infections and relies on pattern recognition receptors (PRRs) such as Toll-like receptors (TLR) for pathogen recognition. TLR9 is known as a sensor of unmethylated CpG motif of DNA (CpG DNA) derived from pathogens. Structural and biochemical analyses demonstrate that TLR9 recognizes not only CpG DNA but also 5′-xC motif containing DNA (5′-xCx DNA) and the latter binds to TLR9 in the presence of CpG DNA and promotes dimerization and activation of TLR9. The findings of this study will help understand the mechanism of TLR9 activation and the development of therapeutic agents targeting TLR9.

Toll-like receptor (TLR) recognizes molecular patterns from pathogenic microorganisms and activates an innate immune response through inducing the production of type I interferons and pro-inflammatory cytokines. TLR is a type-I transmembrane receptor and, to date, 10 TLRs have been identified in humans. Structural analyses have revealed that ectodomains of TLRs form a homo- or hetero-dimer upon binding of ligands. Members of the TLR7 subfamily, including TLR7, 8 and 9, are localized in endosome and participate in the sensing of nucleic acids derived from microbes, namely, ssRNA (TLR7/8) and dsDNA with CpG motifs (TLR9). So far, the crystal structure of TLR9 complexed with CpG DNA (2:2 complex) has been determined [1], where the two CpG DNAs are recognized by both TLR9 protomers and contribute to the TLR9 dimerization [Fig. 1(A)]. A recent study has shown that DNAs containing the TCG or TCC motif at the 5′-end in addition to the CpG motif activate TLR9 more effectively than DNAs with the CpG motif alone [2, 3]. Length, and dimerization properties of ODNs modulate their activation of TLR9. We performed a systematic investigation of the sequence motifs of B-class and C-class phosphodiester ODNs to identify the sequence properties that govern TLR9 activation. ODNs shorter than 21 nt and with the adenosine adja-