Toll-like receptors of the ascidian, *Ciona intestinalis*: prototypes with hybrid functionalities of vertebrate Toll-like receptors

Naoko Sasaki, Michio Ogasawara, Toshio Sekiguchi, Shoichi Kusumoto and Honoo Satake


Key transmembrane proteins in the innate immune system, Toll-like receptors (TLRs), have been suggested to occur in the genome of non-mammalian organisms including invertebrates. However, authentic invertebrate TLRs have been neither structurally nor functionally investigated. In this paper, we originally present the structures, localization, ligand recognition, activities and inflammatory cytokine production of all TLRs of the ascidian, *Ciona intestinalis*, designated as Ci-TLR1 and Ci-TLR2. The amino acid sequence of Ci-TLR1 and Ci-TLR2 were found to possess a unique structural organizations with moderate sequence similarity to functionally characterized vertebrate TLRs. Ci-TLR1 and Ci-TLR2 genes were expressed predominantly in the stomach and intestine as well as in hemocytes. Ci-TLR1 and Ci-TLR2 expressed in HEK293 cells, unlike vertebrate TLRs, were localized to both the plasma membrane and endosomes. Intriguingly, both Ci-TLR1 and Ci-TLR2 stimulate NF-κB induction in response to multiple pathogenic ligands such as double stranded RNA, and bacterial cell wall components which are differentially recognized by respective vertebrate TLRs, revealing that Ci-TLRs recognize broader pathogen associated molecular patterns (PAMPs) than vertebrate TLRs. The Ci-TLR-stimulating pathogenic ligands also induced the expression of Ci-TNFα in the intestine and stomach where Ci-TLRs are expressed. These results provide evidence that the TLR-triggered innate immune systems are essentially conserved in ascidians, and that Ci-TLRs possess ‘hybrid’ biological and immunological functions, compared with vertebrate TLRs. Moreover, it is presumed that chordate TLR ancestors also acquired the Ci-TLR-like multiple cellular localization and PAMP recognition.

(Left) Structural organization of Ci-TLR1 and Ci-TLR2. Ci-TLR1 and Ci-TLR2 were found to encode 883 and 948 amino acids, respectively. SMART protein domain analyses revealed that both of deduced proteins harbor an intracellular TIR domain, a transmembrane domain, and multiple extracellular LRRs. (Right) Ligands for Ci-TLR1 and Ci-TLR2. 24-h treatment of stable Ci-TLR1 (A) or Ci-TLR2 (B) transfectants with Zymosan, HKLP, Poly(I:C) or Flagellin resulted in induction of NF-κB.