INNATE IMMUNITY

TLR4 signalling

Toll-like receptor 4 (TLR4) is unique among TLRs in its ability to activate two distinct signalling pathways — one pathway is activated by the adaptors TIRAP (Toll/interleukin-1-receptor (TIR)-domain-containing adaptor protein) and MyD88, which leads to the induction of pro-inflammatory cytokines, and the second pathway is activated by the adaptors TRIF (TIR-domain-containing adaptor protein inducing interferon-β) and TRAM (TRIF-related adaptor molecule), which leads to the induction of type I interferons. Until now, it had been believed that these two signalling pathways were activated simultaneously at the plasma membrane. Now, a study from Ruslan Medzhitov's laboratory shows that the two signalling pathways are induced sequentially and that the TRAM–TRIF pathway is only operational from early endosomes following endocytosis of TLR4.

SUPERSTOCK

The authors found it puzzling that TLR4 is the only known TLR capable of inducing the production of type I interferons from the plasma membrane so they decided to take a closer look at TLR4 signalling. First, they assessed the subcellular localization of tagged TLR4 and found that it localized to both the plasma membrane and endosomal vesicles. They found that lipopolysaccharide-induced TLR4 internalization was disrupted in the presence of a specific inhibitor of dynamin — a GTPase that regulates the endocytosis of 'pinched-off' invaginations of the plasma membrane. Disruption of endocytosis abolished the TRAM–TRIF-dependent phosphorylation of interferon-regulatory factor 3 (IRF3), a component of the pathway that leads to the production of type I interferons, whereas TIRAP–MyD88-dependent signalling proceeded normally.

Next, a deletional analysis of TRAM showed that the first 20 amino acids constituted the minimum signal for TRAM localization to both the plasma membrane and endosomes, but the first 7 amino acids were sufficient to allow TRAM to localize specifically to endosomes. These 20 amino acids constitute a bipartite localization domain — consisting of a myristoylation motif (the first 7 amino acids) and a polybasic domain — that is commonly found in other proteins that shuttle between the plasma membrane and endosomes. Mutational analysis showed that the myristoylation motif is necessary for endosomal localization but both parts of the bipartite motif are required for plasma-membrane targeting. A TRAM transgene of which the protein product resided specifically on endosomes retained the ability to signal the production of type I interferons.

This study supports a model whereby ligand engagement of TLR4 at the plasma membrane induces the TIRAP–MyD88 pathway. TLR4 is then endocytosed into endosomes to engage the TRAM–TRIF pathway. These results also confirm that TRAM, similar to TIRAP, is a 'sorting' adaptor that defines the location of subcellular TLR signalling, and that receptors that induce the production of type I interferons all signal from intracellular compartments.

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ORIGINAL RESEARCH PAPER Kagan, J. C. *et al.* TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-β. *Nature Immunol.* 24 February 2008 (doi:10.1038/ni1569)