Catching the adaptor—WDFY1, a new player in the TLR–TRIF pathway

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The innate immune system detects microbes and abnormal self through pattern recognition receptors (PRRs), which detect molecules that are either specific for microbes (such as lipopolysaccharide), present in much higher concentrations during infection (such as double-stranded RNA), or present in aberrant locations (such as cytosolic DNA) [1]. The Toll-like receptors (TLRs) are the best-described set of PRRs. TLRs are membrane-bound receptors localized on the plasma membrane and in endosomes, the ligand-binding regions of which face the extracellular environment and the endosomal lumen, respectively [1]. In this issue of *EMBO Reports*, Hu and colleagues report that WD-repeat and FYVE-domain-containing protein 1 (WDFY1) recruits the signaling adaptor TRIF to TLR3 and TLR4, thereby potentiating signaling from these PRRs (Fig 1); [2].

See also: Y-H Hu *et al* (April 2015)

Upon ligand engagement, TLRs elicit an intracellular signaling cascade that eventually leads to the expression of genes encoding antimicrobial, inflammatory, and immunomodulatory proteins [1]. Through a focused effort, four TLR adaptor proteins containing TIR homology domains were identified about ten years ago and demonstrated to play specific roles in the activation of TLR signaling [2–8]. The TIR adaptors enable signaling through TLRs by interacting with the TIR domain in the cytoplasmic tail of TLRs [1]. Among the 10 human TLRs, all except TLR3 are dependent on the TIR adaptor protein MyD88 (myeloid differentiation primary response gene 88) for signaling [1]. The MyD88 pathway activates the transcription factor NF- κ B to induce

pro-inflammatory gene expression. On the other hand, TLR3 depends on TRIF (TIR-domain-containing adapter inducing IFN- β) for downstream signaling, and TLR4 can signal both through MyD88 and TRIF. Like the MyD88 pathway, the TRIF pathway activates NF- κ B, but in addition activates the transcription factor IRF3. Therefore, the TRIF pathway stimulates the expression of both inflammatory genes and interferons with antiviral activity. The physiological relevance of TRIF is highlighted by the enhanced susceptibility of humans with loss-of-function mutations in TRIF to viral infections [9].

Given the essential role for TIR adaptor proteins in TLR signaling, it is important for cells to be able to efficiently recruit these adaptors to TLRs after sensing of a danger signal. A study published in the current issue of *EMBO Reports* by the Liu laboratory [10] describes a screen of over 10,000 cDNA expression plasmids in HEK293-TLR3 cells for the ability to increase reporter gene activation by the TLR3 agonist poly(I:C). This approach led to the identification of WDFY1—a protein localized on early endosomes—as a potentiator of the TLR3 pathway. When overexpressed, WDFY1 resulted in an increase in poly(I:C)-induced IFN- β , ISRE, and NF- κ B reporter gene expression, as well as elevated phosphorylation of the signaling proteins IRF3 and I κ B α . By contrast, Sendai virus-mediated induction of IFN- β remained unchanged, arguing for a specific role of WDFY1 in TLR3 but not RIG-I signaling. Knockdown of WDFY1 by RNAi reduced the responsiveness through the TLR3 pathway, thus providing strong evidence for a role of endogenous WDFY1 in TLR3 signaling.

At the mechanistic level, cell fractionation and co-immunoprecipitation experiments

revealed the recruitment of WDFY1 and TRIF to TLR3 upon poly(I:C) stimulation, and this was supported by microscopy data showing partial colocalization between WDFY1 and TRIF upon TLR3 ligand engagement. Since TLR3 activation requires tyrosine phosphorylation, Hu and colleagues investigated the interaction between WDFY1 and tyrosine-phosphorylated TLR3. WDFY1 binds selectively to WT TLR3 but not to two non-phosphorylatable mutants at sites Y759 and Y858. Overexpression of TRIF, TBK1, or IRF3—but not of TLR3—rescued reporter gene expression after WDFY1 depletion. Taken together, this places WDFY1 in the TLR3 pathway downstream of phosphorylation of the TLR3 TIR domain, but upstream of TRIF, and as a factor involved in recruitment of TRIF to TLR3 (Fig 1). Finally, the authors showed that the second WD domain of WDFY1 is responsible for binding to the TIR domain of TLR3. Since TRIF is also involved in signaling downstream of TLR4, the authors examined the role of WDFY1 in this pathway. Interestingly, reduced expression of WDFY1 also inhibited LPS-stimulated signaling and gene expression, suggesting this protein is also involved in the recruitment of TRIF to TLR4.

With the identification of WDFY1 as a novel player in the TLR3/4–TRIF pathway, a series of new questions arise, which will be interesting grounds for future studies. For instance, is WDFY1 specific for TRIF or is it also involved in the recruitment of other TIR adaptors? Is there a role for other WD-repeat-containing proteins in PRR signaling? Is WDFY1 essential for stimulation of TLR3/4-driven immune activation in response to microbial pathogens? Is WDFY1 involved in mounting antimicrobial and inflammatory responses *in vivo*?

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Figure 1. WDFY1, a new player in the TLR-TRIF pathway.

TLR3-stimulated signaling is fully dependent on TRIF, and TLR4 stimulates two signaling pathways, one of which is dependent on TRIF. In both cases, TRIF-dependent signaling occurs from an endosomal platform. WDFY1 is present on the membrane of early endosomes and interacts with TLR3 and TLR4 upon ligand engagement. This stimulates TRIF recruitment and initiates signal transduction, ultimately leading to the induction of gene expression. The recruitment of WDFY1 to TLR3 occurs through interaction with the TIR domain and is dependent on the presence of specific tyrosine phosphorylations.

Collectively, this work contributes important new insights on how TLRs control the recruitment of adaptor proteins to achieve full immune activation in response to the recognition of danger signals.

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