CORRESPONDENCE

Wdfy1 deficiency impairs Tlr3-mediated immune responses in vivo

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As germline-encoded pattern recognition receptors, Toll-like receptors (TLRs) play important roles in innate immune responses against various pathogens.[1] Among TLR family members, only TLR3 and TLR4 use TIR domain-containing adapter-inducing interferon-β (TRIF) as an adapter protein to trigger the production of type I interferon (IFN-I) and inflammatory cytokines.[1,2] Secreted IFNs then bind their plasma membrane receptors, further stimulating the transcription of IFN-stimulated genes (ISGs), which have multiple antiviral functions, such as restricting the replication and assembly of viral particles.[3] We previously reported an accessory protein, named WD repeat and FYVE domain-containing protein (WDFY1), that strengths the interaction between TRIF and TLR3 or TLR4.[4] At the cellular level, overexpression of WDFY1 reinforced TLR3/4-mediated innate immune responses, and the suppression of WDFY1 by RNAi had the opposite effects. We also reported that the function of WDFY1 is highly dependent on its FYVE domain, which is responsible for its localization to the early endosome.[5] However, whether Wdfy1-knockout (KO) mice are susceptible to Tlr3 or Tlr4 ligands remains unknown.

To address how mouse Wdfy1 affects innate defense, we compared the cellular immune responses of wild-type (Wdfy1$^{+/+}$) mice and Wdfy1-KO (Wdfy1$^{-/-}$) mice against poly I:C and LPS. We first checked the transcription of the *Ifn-I* gene, ISGs, and inflammatory cytokine genes in bone marrow-derived macrophages (BMDMs). The results showed that knockout of Wdfy1 markedly impaired both poly I:C- and LPS-induced transcription of *Ifnb1*, *Isg56*, *Tnfa*, *Il6*, and *Il1b* (Fig. 1a and Supplementary information, Fig. S1a). These results suggest that mouse Wdfy1 is important for both Tlr3 signaling against double-stranded RNA and Tlr4 signaling against gram-negative bacteria in immune cells.

We then performed similar experiments using mouse embryonic fibroblasts (MEFs). Knockout of Wdfy1 markedly impaired poly I:C-induced transcription of *Ifnb1*, *Isg56*, *Tnfa*, *Il6*, and *Il1b* (Fig. 1b). However, in LPS-treated MEFs, knockout of Wdfy1 impaired the transcription of *Ifnb1* but had no marked effect on the transcription of *Isg56*, *Tnfa*, *Il6*, or *Il1b* (Supplementary information, Fig. S1b). These results suggest that in nonimmune cells, Wdfy1 mainly participates in Tlr3 signaling and Tlr4-mediated production of Ifn-I but not Tlr4-mediated production of inflammatory cytokines.

To address the importance of Wdfy1 in host defense in vivo, we injected poly I:C plus D-galactose into age- and sex-matched Wdfy1$^{+/+}$ and Wdfy1$^{-/-}$ mice by the intraperitoneal (*i.p.*) route. Mouse sera were collected at the indicated times after injection, and the concentrations of Ifn-β and inflammatory cytokines in the sera were measured by ELISA. As shown in Fig. 1c, the circulating levels of Tnfα, Il-6 and Ifn-β in Wdfy1$^{+/+}$ mice were significantly increased one and three hours after poly I:C injection. Although the circulating level of these cytokines in Wdfy1$^{-/-}$ mice also increased after poly I:C injection, the concentration of cytokines in these mice was significantly lower than that in Wdfy1$^{+/+}$ mice. We also intraperitoneally injected LPS into age- and sex-matched Wdfy1$^{+/+}$ and Wdfy1$^{-/-}$ mice and assessed the production of Tnfα and Il-6 after injection. Changes in the concentrations of circulating Tnfα and Il-6 in both Wdfy1$^{+/+}$ and Wdfy1$^{-/-}$ mice at 1 and 3 h after injection showed the same trend. There were no significant differences in the changes in circulating Tnfα and Il-6 concentrations induced by LPS injection between Wdfy1$^{+/+}$ and Wdfy1$^{-/-}$ mice (Supplementary information, Fig. S1c).

We then compared the role of Wdfy1 in poly I:C- and LPS-induced inflammatory death. Among the poly I:C-injected groups, Wdfy1$^{+/+}$ mice died 5–8 h after injection, with an average 41.6 percent survival rate. In contrast, Wdfy1$^{-/-}$ mice showed delayed death, with an average 63 percent survival rate (Fig. 1d). The survival rates of Wdfy1$^{+/+}$ and Wdfy1$^{-/-}$ mice differed significantly ($p < 0.05$), suggesting that depletion of Wdfy1 protected mice from the inflammatory death induced by poly I:C. Among the LPS-injected groups, there was no significant difference in survival rate between Wdfy1$^{+/+}$ and Wdfy1$^{-/-}$ mice (Supplementary information, Fig. S1d). These results suggest that mouse Wdfy1 plays an important role in Tlr3-mediated innate responses but not Tlr4-mediated host defense.

In the present study, we compared the roles of mouse Wdfy1 in Tlr3 signaling and Tlr4 signaling in vitro and in vivo. Endosomal TLR3 recognizes dsRNA and utilizes TRIF to trigger inflammation and IFN effects.[6] Wdfy1 deficiency impairs Tlr3-mediated innate responses in both immune cells and MEFs. Consistently, in vivo infection studies show that Wdfy1 deficiency protects mice from inflammatory death induced by poly I:C injection, suggesting the important role of Wdfy1 in Tlr3 signaling. LPS-engaged TLR4 triggers both myeloid differentiation primary-response gene 88 (MyD88)-dependent signaling at the cell surface and TRIF-dependent signaling at endosomes.[7] We found no significant difference in circulating inflammatory cytokines upon LPS stimulation between Wdfy1$^{+/+}$ and Wdfy1$^{-/-}$ mice. Consistently, Wdfy1 deficiency had little effect on inflammatory death induced by LPS. These results suggest the insufficient role of endosomal Wdfy1 in Tlr4 signaling. Moreover, Wdfy1 deficiency impaired LPS-induced transcription of Ifn-I and inflammatory cytokines in immune cells but had little effect on the production of inflammatory cytokines in MEFs, suggesting that Tlr4-mediated

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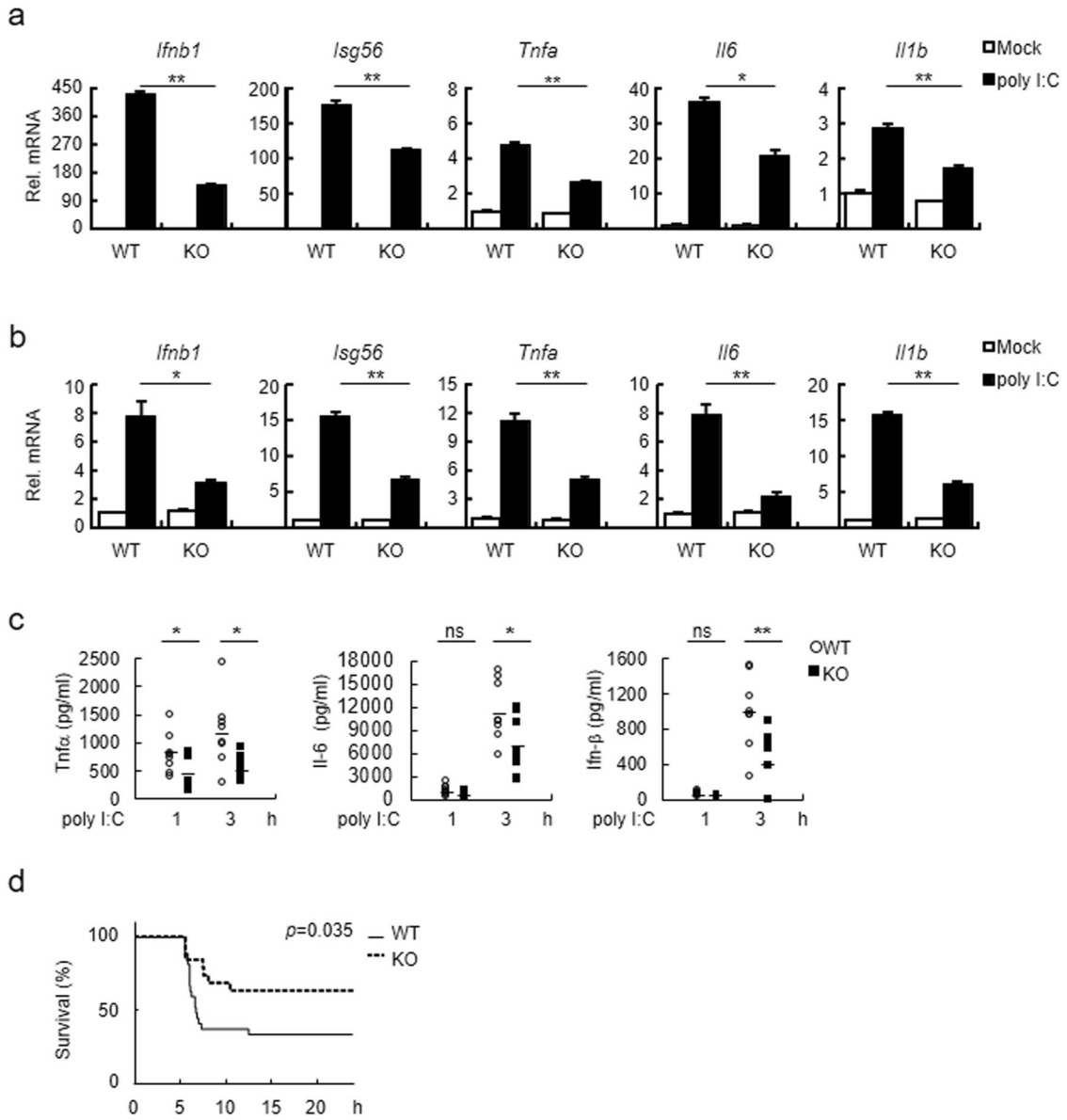

Fig. 1 Wdfy1 deficiency impaired Tlr3-mediated immune responses in vivo. a BMDMs from Wdfy1$^{+/+}$ and Wdfy1$^{-/-}$ mice were treated with poly I:C (35 μg/ml) for 4 h before qPCR was performed. Student's *t* test was used for statistical analysis. Graphs show the mean ± SD, $n = 3$. *$p < 0.05$; **$p < 0.01$. **b** MEFs from Wdfy1$^{+/+}$ and Wdfy1$^{-/-}$ mice were treated with poly I:C (20 μg/ml) for 4 h before qPCR was performed. Student's *t* test was used for statistical analysis. Graphs show the mean ± SD, $n = 3$. *$p < 0.05$; **$p < 0.01$. **c** Wdfy1$^{+/+}$ and Wdfy1$^{-/-}$ mice were injected intraperitoneally with poly I:C (4 μg/g) plus D-galactose (0.5 mg/g, 2a, $n = 8$ for each group). The concentration of circulating cytokines at the indicated times after injection was measured by ELISA. Student's *t* test was used for statistical analysis. *$p < 0.05$; **$p < 0.01$; N.S., not significant. **d** Wdfy1$^{+/+}$ and Wdfy1$^{-/-}$ mice were injected intraperitoneally with poly I:C (4 μg/g) plus D-galactose (0.5 mg/g, 2c, $n = 19$ for each group). The survival rate of the mice was monitored for the indicated duration. The log-rank test was used for statistical analysis.

production of inflammatory cytokines in nonimmune cells, unlike immune cells, relies more on the Myd88-dependent pathway.

Altogether, our results provide further evidence for the important role of Wdfy1 in Tlr3 signaling both in vivo and in vitro. As Tlr3 signaling is involved in not only antiviral immune responses but also the pathogenesis of a variety of inflammation-related diseases,[8–10] the role of Wdfy1 in related diseases needs further elucidation.

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AUTHOR CONTRIBUTIONS

Y.Y. and Y.H.H. performed the experiments and analyzed the data, L.Y. designed the research and wrote the paper.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41423-020-0461-4) contains supplementary material.

Competing interests: The authors declare that they have no conflicts of interest.

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