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Roles for LPS-dependent interaction and relocation of TLR4 and TRAM in TRIF-signaling

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Abstract

Toll-like receptor 4 (TLR4) activates two distinct signaling pathways inducing production of proinflammatory cytokines or type I interferons (IFNs), respectively. MyD88 and TIRAP/Mal are essential adaptor molecules for the former but not for the latter pathway. In contrast, TRIF/TICAM-1 and TRAM/TICAM-2 are essential for both. TIRAP is a sorting adaptor molecule recruiting MyD88 to activated TLR4 in the plasma membrane. TRAM is thought to bridge between TLR4 and TRIF by physical association. Little is known, however, how TRAM interacts with TLR4 or with TRIF during LPS response. Here, we show that TRAM recruits TRIF to the plasma membrane. Moreover, LPS induces upregulation of TLR4-association with TRAM and their subsequent translocation into endosome/lysosome. The internalized signaling complex consisting of TLR4 and TRAM colocalizes with TRAF3, a signaling molecule downstream of TRIF, in endosome/lysosome. These results suggest that TLR4 activates TRIF-signaling in endosome/lysosome after relocation from the cell surface.

Keywords: Innate immunity; Lipopolysaccharide; Toll-like receptor; Interferon

Innate immunity is the first line of defense against microbial infection [1]. The Toll family of receptors plays an essential role in innate recognition of microbial products and activation of defense responses [2]. Toll-like receptors (TLRs) are type I transmembrane proteins that contain a large, leucine-rich repeat (LRR) in an extracellular region and a Toll/IL-1 receptor homology (TIR) domain in a cytoplasmic region [3]. TLRs recognize microbial products ranging from membrane glycolipids to nucleic acids. Lipopolysaccharide (LPS) is one of the most immunostimulatory glycolipids constituting the outer membrane of the Gram-negative bacteria, and recognized by the TLR4/MD-2 receptor complex [4]. MD-2 is an extracellular molecule that is associated with the LRR in TLR4, and directly binds to LPS and triggers TLR4-clustering [4–7]. CD14 is another LPS binding protein present in the serum or on the cell surface and has a role in augmenting LPS responses. At least two distinct intracellular signaling pathways are activated by TLR4. One pathway requires two adaptor molecules, myeloid differentiation factor 88 (MyD88) and TIR domain-containing protein (TIRAP)/MyD88 adapter-like (Mal), and leads to early activation of the transcription factor NF-κB, the production of proinflammatory cytokines, and Th1 responses. A second set of adaptor molecules, TIR domain-containing adapter inducing interferon-β (TRIF)/TIR domain-containing adapter molecule-1 (TICAM-1) and TRIF-related adapter molecule (TRAM)/TIR domain-containing adapter molecule-2 (TICAM-2), are required for late NF-κB activation and LPS-induced phosphorylation and dimerization of the

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transcription factor interferon regulatory factor-3 (IRF-3), leading to interferon-β (IFNβ) production and activation of acquired immune responses [2].

MyD88-signaling is activated earlier than TRIF-signaling [8]. It is unknown why TRIF-signaling is delayed when compared with MyD88-signaling. We previously reported that TLR4 is internalized by 60–120 min after LPS stimulation [9]. Considering this kinetics, MyD88-signaling would be terminated before TLR4 is internalized. Given that LPS-induced IRF-3 phosphorylation is detectable 30–90 min after LPS stimulation, TRIF-signaling would be activated during TLR4-internalization. It is possible that TLR4-internalization is tightly linked with TRIF-signaling.

TIRAP is essential for TLR4 to initiate MyD88-signaling on the plasma membrane. TIRAP, which is required for TLR4 signaling, contains a phosphatidylinositol 4,5-bisphosphate (PIP2) binding domain that mediates TIRAP recruiting to membranes. The function of TIRAP in TLR signaling is to facilitate MyD88 delivery to activated TLR4 on the plasma membrane [10]. On the other hand, TRAM contains an N-terminal myristoylation site that is responsible for targeting TRAM to the plasma membrane. Mutation in N-myristoylation sequence of TRAM abrogated its membrane localization as well as its ability to induce cytokines in response to LPS [11]. When overexpressed, TRAM was shown to associate with TLR4 or with TRIF in the absence of LPS stimulation [12,13]. Little is known about how LPS influences the interaction of TRAM with TLR4 or with TRIF. If TLR4-internalization is linked with TRIF-signaling, TRAM may also be relocated with TLR4. We here studied the effect of LPS on TRAM interaction with TLR4 and on their subcellular distribution, and found that TRIF-signaling is tightly linked with relocation of TLR4 and TRAM from the plasma membrane to lysosme/endosome.

Materials and methods

Expression constructs and stable transfectants. Ba/F3 cells were cultured as described previously [9]. The cDNAs encoding muTLR4, muMD-2, and muCD14 were cloned as described previously [14]. The C-terminus of TLR4 was tagged with GFP (TLR4-GFP). The cDNAs encoding muTRAM, muTRIF, and muTRAF3 were cloned into retrovirus vector pMX-puro. The C-terminus of TRAM was tagged with GFP or DsRed monomer (TRAM-GFP or TRAM-DsRed). To mutate the RHIM in muTRIF was replaced with alanines by QuikChange site directed mutagenesis (Stratagene). Ba/F3 cells expressing a variety of combination of muTLR4, TLR4-GFP, muMD-2, muCD14, TRAM-GFP, TRAM-DsRed, TRIF-DsRed, and TRAF3-Flag were established by retroviral transduction. RAW264.7 cells expressing TRAM-GFP or TRAM-GFP, TRIF-DsRed, and TRAF3-Flag were established by retroviral transduction. Retrovirus transduction and establishment of stable clones were conducted as described previously [9].

Cell staining and flow cytometry. Cell staining and flow cytometry analysis were conducted as described previously [9].

Cell stimulation, immunoblotting, and immunoprecipitation. Cell stimulation, immunoblotting, and immunoprecipitation were conducted as described previously [9].

Confocal imaging. Cells were stimulated with 1 μg/ml LPS or AlexaFluor^{568} labeled LPS (Invitrogen) for 60 min. Cells were stimulated with 1 μg/ml LPS with 5 μg/ml Texas Red labeled Transferrin (Invitrogen) for 60 min to stain endosomes. Golgi complex was stained with 5 μM BODIPY FL C_5-Ceramide (Invitrogen) for 15 min at 37 °C. Lysosomes were stained with 50 nM LysoTracker Red (Invitrogen) according to manufacturer's instructions. After LPS stimulation, Cells were fixed by 4% paraformaldehyde-PBS for 10 min at room temperature and washed twice with PBS. At intracellular Flag staining, cells were permeabilized by 0.1% Triton X-100-PBS for 4 min, and traced with biotin conjugated anti-Flag (M2, Sigma) followed by Streptavidin-AlexaFluor^{647}(Invitogen). Images of live cells or fixed cells were captured with a Zeiss LSM 5PASCAL. A 63×/1.4 oil immersion objective lens was used to image cells. An argon 488-nm laser was used for GFP excitation, and a HeNe 543-nm laser was used for DsRed, Texas RED and AlexaFluora^{568} excitation and a HeNe 633-nm laser for AlexaFluor^{647}. The images were obtained with LSM image browser.

Results

CD14-dependent TLR4-internalization to endosome/lysosome

We previously reported that TLR4-clustering is followed by TLR4-internalization [9]. Lipid A induced TLR4-internalization in Ba/F3 cells expressing TLR4/MD-2 with CD14, but not in those expressing TLR4/MD-2 without CD14 (Fig. 1A). Upon stimulation with lipid A, cell surface TLR4 was gradually downregulated and disappeared from the plasma membrane within 120 min. To determine the subcellular distribution of internalized TLR4, TLR4-GFP was expressed in Ba/F3 cells together with MD-2 and CD14. Most TLR4 inside the cells colocalized with a Golgi marker, C_5-Ceramide (Fig. 1B). Interestingly, TLR4-GFP was detected in vesicles outside the Golgi apparatus after LPS stimulation (Fig. 1B, lower right). TLR4 in the vesicles colocalized with a lysosomal marker LysoTracker and LPS (Fig. 1C and D), but only partially with an endosomal marker transferrin (Fig. 1E). These results suggested that LPS induces TLR4 relocation from the plasma membrane to endosome/lysosome.

LPS-induced association between TLR4 and TRAM

TRAM localizes on the plasma membrane due to the N-terminal myristoylation [11], and was shown to associate with TLR4 without any LPS stimulation [12]. Little is known, however, how LPS influences TLR4–TRAM interaction. To address the effect of LPS on the TLR4–TRAM interaction, we studied physical association between TRAM and TLR4 in Ba/F3 cells expressing TLR4/MD-2 and GFP-tagged TRAM. Although the previous study reported, by using HEK293 cells transiently expressing TLR4 and TRAM, that TRAM is constitutively associated with TLR4 [12], we could not detect TLR4 coprecipitation with TRAM-GFP even after LPS stimulation in Ba/F3 cells expressing TLR4/MD-2 and TRAM-GFP. Because membrane CD14 was reported to be required for TRIF-signaling [15], we used Ba/F3 cells expressing CD14, TLR4/MD-2, and TRAM. We were able to detect weak but significant TLR4 coprecipitation, which was apparently upregulated after LPS stimulation (Fig. 1F). TLR4-association with

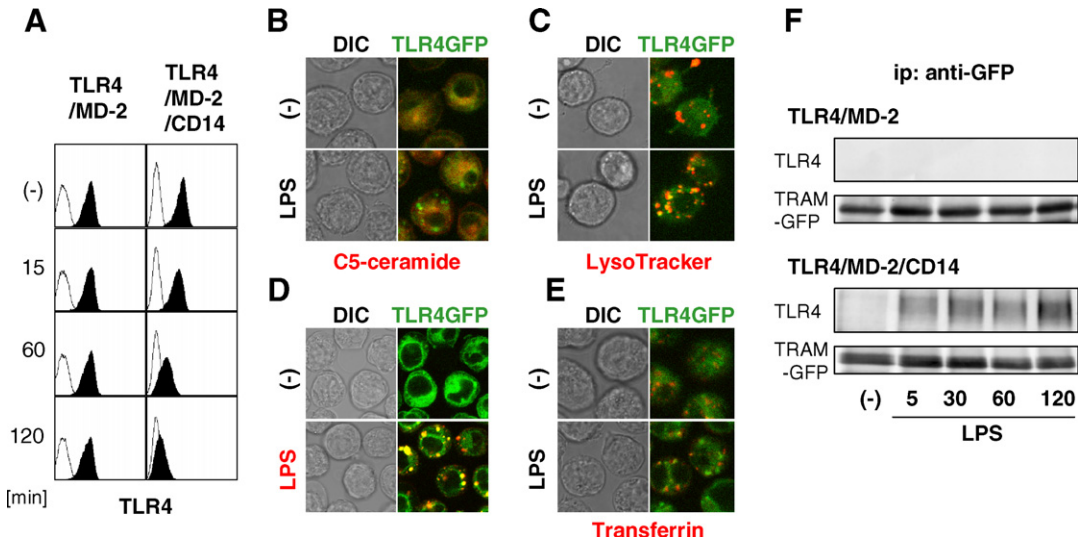

Fig. 1. LPS induces TLR4-internalization and association of TLR4 and TRAM. (A) Cell surface expression of TLR4 in Ba/F3 cells expressing TLR4, MD-2 with (right) or without (left) CD14. Cells were stimulated with lipid A at 1 μg/ml for the indicated periods of time. Open histograms show staining with the secondary reagent alone. (B–E) The subcellular distribution of TLR4 in Ba/F3 cells expressing TLR4-GFP, MD-2, and CD14. Cells were treated with medium (top) or LPS at 1 μg/ml (bottom) for 60 min, costained with 5 μM BODIPY-FL C_5-Ceramide as a Golgi marker (B), 50 nM LysoTracker Red as a lysosomal marker (C), or 5 μg/ml Texas Red-Transferrin as an endosomal marker (E). AlexaFluor^{568}-LPS (1 μg/ml) was used for stimulation in (D). (F) Ba/F3 cells expressing TLR4, MD-2, and TRAM-GFP with (bottom) or without (top) CD14 were stimulated with LPS at 1 μg/ml for the indicated periods of time. TRAM-GFP was precipitated and TRAM-GFP (bottom) and coprecipitated TLR4 (top) were detected by immunoblotting with Ab against GFP or TLR4, respectively.

TRAM was detectable as early as 5 min and lasted until as late as 120 min after LPS stimulation. These results demonstrated that the association between TLR4 and TRAM is dependent on membrane CD14 and apparently upregulated by LPS stimulation.

LPS-induced TRAM translocation

TLR4–TRAM association continued during TLR4-internalization (Fig. 1A and F), suggesting that TRAM may also be relocated upon LPS stimulation. We examined the subcellular distribution of TRAM-GFP in Ba/F3 cells expressing TLR4/MD-2 and CD14. TRAM predominantly localized on the plasma membrane in the steady state (Fig. 2A). TRAM resided in the plasma membrane and partially colocalized with CD14 but not with TLR4 (Fig. 2A). Due to myristoylation [11], TRAM was likely to colocalize with GPI-anchored CD14 in lipid rafts. Interestingly, TRAM was translocated out of the plasma membrane within 30 min after LPS stimulation (Fig. 2B). TRAM-translocation was specifically induced by TLR4 ligands LPS, but not by TLR2/TLR1 ligand Pam_3CSK_4 (Fig. 2C). TRAM translocation was not seen in Ba/F3 cells lacking expression of any of the three molecules, CD14, MD-2, and TLR4, demonstrating that all these three molecules were essential for TRAM-internalization (data not shown). Translocated TRAM showed partial colocalization with LysoTracker and AlexaFluor^{568}-labeled LPS (Fig. 2D and E). In keeping with LPS-dependent TLR4–TRAM association, internalized TLR4 and TRAM showed colocalization with each other (Fig. 2F). TRAM-GFP translocation from the plasma membrane was also seen in a mouse macrophage cell line RAW 264.7 cells (Fig. 3A) or Bone marrow derived dendritic cells (data not shown). TRAM-GFP colocalized with membrane CD14 partially in the steady state, but not any more after LPS stimulation (Fig. 3A). Internalized TRAM colocalized with endosomal markers (dextran and transferrin) and with LysoTracker (Fig. 3B). These data suggested that LPS induces TRAM translocation from the plasma membrane to endosome/lysosome. We also examined TIRAP in Ba/F3 cells expressing CD14 and TLR4/MD-2 or in RAW264.7 cells. LPS stimulation did not induce TIRAP translocation in Ba/F3 cells expressing CD14 and TLR4/MD-2 or in RAW264.7 cells (data not shown).

TRIF and TRAF3 co-localization with TRAM under stimulation

TRIF is the principal adaptor molecule in LPS-induced IFNβ production. We examined the subcellular distribution of TRIF during LPS response. Overexpression of TRIF was reported to induce apoptosis due to the conserved residues VQLG in a receptor interacting protein homotypic interaction motif (RHIM) [16]. The VQLG sequence was, therefore, replaced with alanines to prevent apoptosis due to TRIF-overexpression. Mutated TRIF does not alter LPS-signaling and resided in the cytoplasm when expressed without TRAM (Fig. 4A, middle). However, co-expression of TRAM relocated TRIF from the cytoplasm to the plasma membrane, where TRIF and TRAM colocalized with each other (Fig. 4A, bottom).

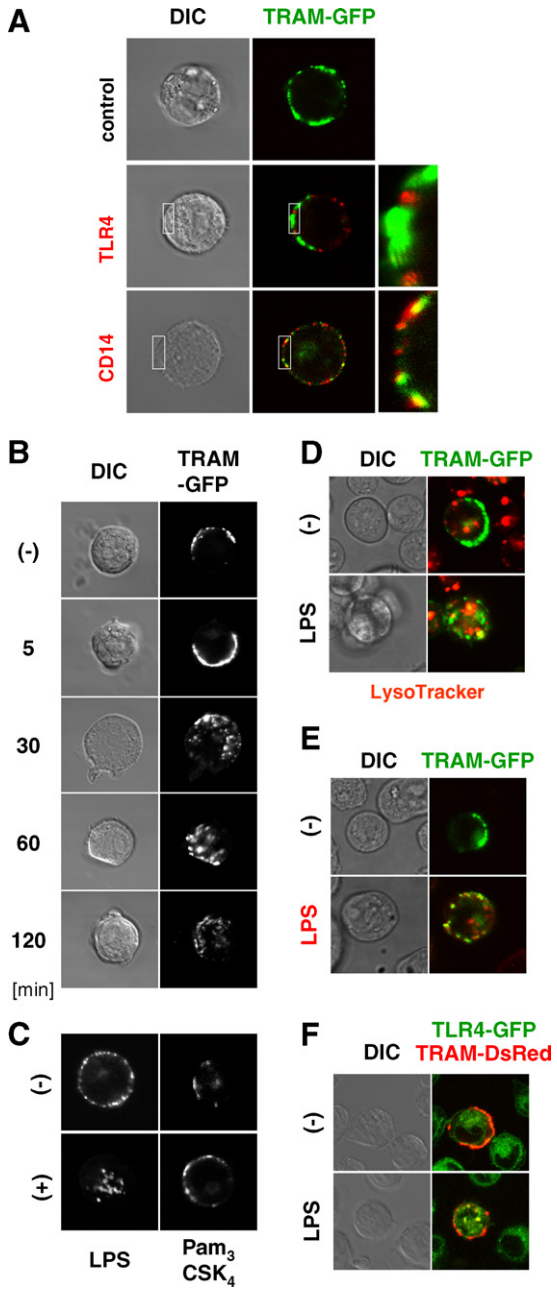

Fig. 2. LPS induces TRAM-internalization. The subcellular distribution of TRAM-GFP in Ba/F3 cells expressing TLR4, MD-2, CD14, and TRAM-GFP was observed on confocal microscopy (A–E). (A) Cell surface TLR4 (middle) or CD14 (bottom) was stained with biotinylated mAbs and streptavidin-Texas Red. The DIC images were shown in left. The magnified images of the indicated squares were shown in right. (B) Cells were treated with medium (top) or LPS at 1 μg/ml for the indicated periods of time. The DIC images were shown in left, and TRAM-GFP was shown in right. (C) Cells were treated with medium (top), LPS or Pam_3CSK_4 at 1 μg/ml for 10 min as indicated in the figure. (D) Cells were treated with medium (top) or LPS at 1 μg/ml for 60 min (bottom). Cells were costained with 50 nM LysoTracker Red. (E) Cells were treated with medium (top) or AlexaFluor^{568}-LPS at 1 μg/ml for 60 min (bottom). (F) Ba/F3 cells expressing TLR4-GFP, MD-2, CD14, and TRAM-DsRed were treated with medium (top) or LPS at 1 μg/ml for 60 min (lower). All images represent 1 μm of optical sections.

LPS stimulation induced further translocation of TRIF and TRAM. Upon LPS stimulation, TRIF and TRAM trafficked from the plasma membrane to intracellular vesicles (Fig. 4B). LPS is unable to induce type I interferon production in TRAF3 deficient cells [17]. TRAF3 is recruited to TRIF and has a critical role in TRIF-dependent production of type I IFN and IL-10. Interestingly, TRAF3 was reported to be associated with TRIF as late as 120 min after LPS stimulation [17], when the LPS receptor complex relocated to the endosome/lysosome. We examined the subcellular distribution of TRAF3 to clarify the site where TRIF-dependent signaling for type I IFN was activated. Flag-TRAF3 was expressed in Ba/F3 or RAW264.7 cells together with TRAM-GFP and TRIF-DsRed. TLR4, MD-2 and CD14 were also expressed in Ba/F3 cells. TRAF3 resided in the cytoplasm and its distribution did not change dramatically under LPS stimulation. Colocalization of TRAF3 with TRAM and TRIF was seen at inside the cells after LPS stimulation (Fig. 4C and D, shown in white). These results suggested that the LPS receptor complex was able to activate TRIF- and TRAF3-dependent LPS-signaling in endosome/lysosome.

Discussion

TLRs can be divided into two groups based on their specificity and subcellular location. TLR1/2/4/6 recognize microbial membrane components and reside on the plasma membrane, whereas nucleic acid-sensing TLR3/7/8/9 are intracellular and signal from endosome/lysosome [2]. Cell surface TLRs are distinct from intracellular TLRs in adaptor usage. TIRAP has a phosphatidylinositol 4,5-bisphosphate (PIP2) binding domain, which mediates TIRAP recruitment to the plasma membrane [10]. TIRAP then facilitates MyD88 delivery to activated TLR4 on the cell surface. TRAM, on the other hand, resides in the plasma membrane through its myristoylation [11]. TRAM is similar to TIRAP in that TRAM has a role in recruiting TRIF, instead of MyD88, to activated TLR4 on the plasma membrane (Fig. 4A). These results suggest a role for TIRAP and TRAM in recruiting MyD88 or TRIF to the cell surface, respectively. By contrast, intracellular TLRs do not require TIRAP or TRAM for activating MyD88-dependent or TRIF-dependent signaling pathways. For example, TLR3 is able to activate TRIF in the absence of TRAM.

Type I IFN production is restricted to intracellular TLRs except TLR4. The present study suggested that TRIF-dependent TLR4-signaling inducing IFNβ production occurs inside the cells, revealing an unexpected similarity of cell surface TLR4 to intracellular TLRs.

LPS-induced production of proinflammatory cytokines like TNF-α or IL-6 requires both MyD88- and TRIF-dependent pathways, since mice lacking either adaptor showed impaired production of these cytokines [18,19]. The early phase of LPS-signaling on the cell surface is followed by translocation of TRAM and TRIF into endosome/lysosome (Figs. 2–4), where TRAF3 colocalizes with TRIF and TRAM (Fig. 4C and D). Given that TRAF3 is not associated with MyD88 during LPS response [17],

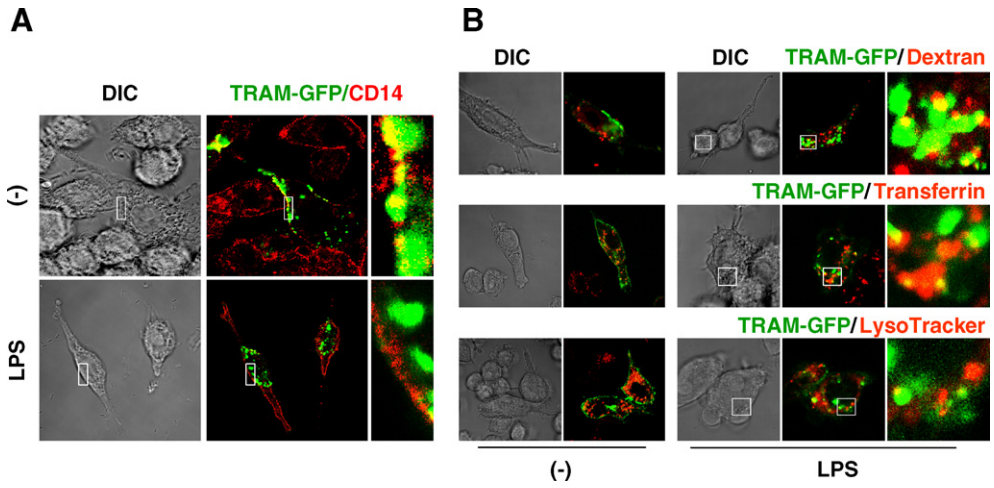

Fig. 3. LPS-induced TRAM translocation in RAW264.7 cells. (A) RAW264.7 cells expressing TRAM-GFP were treated with medium (top) or LPS at 1 μg/ml for 2 h (bottom). Cells were costained with biotinylated anti-CD14 followed by streptavidin-Texas Red. The magnified images of the indicated squares were shown in right. (B) RAW264.7 cells expressing TRAM-GFP were treated with medium (left two columns) or LPS at 1 μg/ml for 2 h (right three columns), costained with: 5 mg/ml Texas Red-Dextran (top); 5 μg/ml Texas Red-Transferrin (middle); or 50 nM LysoTracker Red (bottom). TRAM-GFP and costained markers were observed on confocal microscopy. The magnified images of the indicated squares were shown in rightmost.

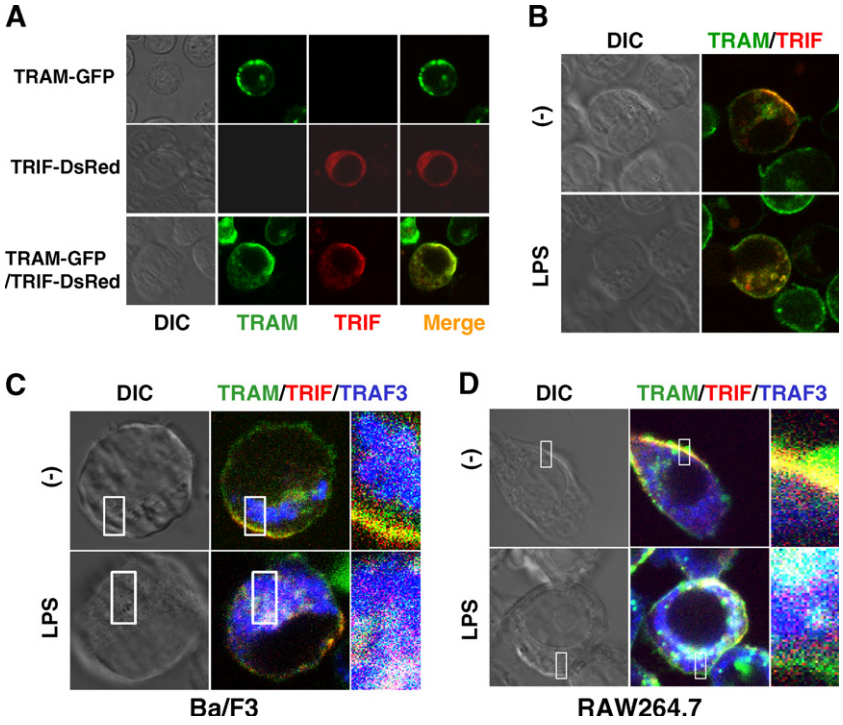

Fig. 4. The distribution of the LPS signaling complex during LPS responses. (A) TRAM-GFP (top), TRIF-DsRed (middle), or TRAM-GFP+TRIF-DsRed (bottom) were expressed in Ba/F3 cells expressing TLR4, MD-2, and CD14. The subcellular distribution of these molecules was analyzed with confocal microscopy. (B) Ba/F3 cells expressing TLR4, MD-2, CD14, TRAM-GFP, and TRIF-DsRed were treated with medium (top) or LPS at 1 μg/ml for 60 min (bottom). (C) Flag-TRAF3 was expressed in Ba/F3 cells used in (B) and Ba/F3 cells were treated with medium (top) or LPS at 1 μg/ml for 60 min (bottom). Flag-TRAF3 was detected with biotinylated anti-flag antibody followed by streptavidin-AlexaFluor^{647}. (D) RAW264.7 cells expressing TRAM-GFP, TRIF-DsRed, and flag-TRAF3 were treated with medium (top) or LPS at 1 μg/ml for 60 min (bottom). Flag-TRAF3 was detected as in (C). The magnified images of the indicated squares were shown in right in (C) and (D).

TRAF3 seems to be specifically employed for delayed activation of TRIF-signaling, leading to type I IFN production. TRAF6 shows sharp contrast to TRAF3 in that TRAF6 is required for proinflammatory cytokine production but not for type I IFN production [20]. Upon LPS stimulation, TRAF6 interacts with both MyD88 and TRIF [17,20,21]. TRAF6 association with TRIF was reported to peak as early as 15 min after LPS stimulation, whereas TRAF3 association with TRIF was detected as late as 120 min after stimulation [17,21]. TRIF-dependent LPS

signaling might be further dissected into two steps depending on TRAF3 or TRAF6. TRAF6 mediates early TRIF-signaling in the plasma membrane, whereas TRAF3 mediates late TRIF-signaling in endosome/lysosome. Delaying activation of TRAF3-dependent TRIF-signaling is likely to be due to translocation of the receptor and adaptor molecules from the plasma membrane to endosome/lysosome.

Until recently, it was believed that upon TLR4 activation, all four adapter molecules were recruited to TLR4. Evidence is mounting, however, that TRAM–TRIF can be selectively activated, depending on the nature of the ligand. Vesicular stomatitis virus (VSV) stimulates only TRIF-signaling [15,22]. The present study revealed a unique requirement for TRIF-signaling in CD14-dependent TLR4-internalization into endosome/lysosome. Our results suggest that the internalized TRIF-signaling complex is formed in endosome/lysosome to initiate specific signaling. It is interesting to study that VSV also induce internalization of TLR4 and TRAM.

In conclusion, this study has revealed a functional difference between TLR related adaptor proteins and the mechanism by which TRAM functions in the TLR4 signaling pathway. Our results also indicate that TRIF dependent signaling might be regulated by manipulating the translocation of TLR4 and TRAM.

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References

[1] C.A. Janeway Jr., R. Medzhitov, Innate immune recognition, Annu. Rev. Immunol. 20 (2002) 197–216.
[2] S. Akira, K. Takeda, Toll-like receptor signalling, Nat. Rev. Immunol. 4 (2004) 499–511.
[3] R. Medzhitov, P. Preston-Hurlburt, C.A.J. Janeway, A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity, Nature 388 (1997) 394–397.
[4] K. Miyake, H. Ogata, Y. Nagai, S. Akashi, M. Kimoto, Innate recognition of lipopolysaccharide by Toll-like receptor 4/MD-2 and RP105/MD-1, J. Endotoxin. Res. 6 (2000) 389–391.
[5] Y. Nagai, S. Akashi, M. Nagafuku, M. Ogata, Y. Iwakura, S. Akira, T. Kitamura, A. Kosugi, M. Kimoto, K. Miyake, Essential role of MD-2 in LPS responsiveness and TLR4 distribution, Nat. Immunol. 3 (2002) 667–672.
[6] U. Ohto, K. Fukase, K. Miyake, Y. Satow, Crystal structures of human MD-2 and its complex with antiendotoxic lipid IVa, Science 316 (2007) 1632–1634.
[7] R. Shimazu, S. Akashi, H. Ogata, Y. Nagai, K. Fukudome, K. Miyake, M. Kimoto, MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4, J. Exp. Med. 189 (1999) 1777–1782.
[8] T. Kawai, O. Takeuchi, T. Fujita, J. Inoue, P.F. Muhlradt, S. Sato, K. Hoshino, S. Akira, Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes, J. Immunol. 167 (2001) 5887–5894.
[9] M. Kobayashi, S. Saitoh, N. Tanimura, K. Takahashi, K. Kawasaki, M. Nishijima, Y. Fujimoto, K. Fukase, S. Akashi-Takamura, K. Miyake, Regulatory roles for MD-2 and TLR4 in ligand-induced receptor clustering, J. Immunol. 176 (2006) 6211–6218.
[10] J.C. Kagan, R. Medzhitov, Phosphoinositide-mediated adaptor recruitment controls Toll-like receptor signaling, Cell 125 (2006) 943–955.
[11] D.C. Rowe, A.F. McGettrick, E. Latz, B.G. Monks, N.J. Gay, M. Yamamoto, S. Akira, L.A. O'Neill, K.A. Fitzgerald, D.T. Golenbock, The myristoylation of TRIF-related adaptor molecule is essential for Toll-like receptor 4 signal transduction, Proc. Natl. Acad. Sci. USA 103 (2006) 6299–6304.
[12] H. Oshiumi, M. Sasai, K. Shida, T. Fujita, M. Matsumoto, T. Seya, TIR-containing adapter molecule (TICAM)-2, a bridging adapter recruiting to toll-like receptor 4 TICAM-1 that induces interferon-beta, J. Biol. Chem. 278 (2003) 49751–49762.
[13] K.A. Fitzgerald, D.C. Rowe, B.J. Barnes, D.R. Caffrey, A. Visintin, E. Latz, B. Monks, P.M. Pitha, D.T. Golenbock, LPS-TLR4 signaling to IRF-3/7 and NF-kappaB involves the toll adapters TRAM and TRIF, J. Exp. Med. 198 (2003) 1043–1055.
[14] S. Saitoh, S. Akashi, T. Yamada, N. Tanimura, M. Kobayashi, K. Konno, F. Matsumoto, K. Fukase, S. Kusumoto, Y. Nagai, Y. Kusumoto, A. Kosugi, K. Miyake, Lipid A antagonist, lipid IVa, is distinct from lipid A in interaction with Toll-like receptor 4 (TLR4)-MD-2 and ligand-induced TLR4 oligomerization, Int. Immunol. 16 (2004) 961–969.
[15] Z. Jiang, P. Georgel, X. Du, L. Shamel, S. Sovath, S. Mudd, M. Huber, C. Kalis, S. Keck, C. Galanos, M. Freudenberg, B. Beutler, CD14 is required for MyD88-independent LPS signaling, Nat. Immunol. 6 (2005) 565–570.
[16] W.J. Kaiser, M.K. Offermann, Apoptosis induced by the toll-like receptor adaptor TRIF is dependent on its receptor interacting protein homotypic interaction motif, J. Immunol. 174 (2005) 4942–4952.
[17] H. Hacker, V. Redecke, B. Blagoev, I. Kratchmarova, L.C. Hsu, G.G. Wang, M.P. Kamps, E. Raz, H. Wagner, G. Hacker, M. Mann, M. Karin, Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6, Nature 439 (2006) 204–207.
[18] T. Kawai, O. Adachi, T. Ogawa, K. Takeda, S. Akira, Unresponsiveness of MyD88-deficient mice to endotoxin, Immunity 11 (1999) 115–122.
[19] M. Yamamoto, S. Sato, H. Hemmi, K. Hoshino, T. Kaisho, H. Sanjo, O. Takeuchi, M. Sugiyama, M. Okabe, K. Takeda, S. Akira, Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway, Science 301 (2003) 640–643.
[20] J. Gohda, T. Matsumura, J. Inoue, Cutting edge: TNFR-associated factor (TRAF) 6 is essential for MyD88-dependent pathway but not toll/IL-1 receptor domain-containing adaptor-inducing IFN-beta (TRIF)-dependent pathway in TLR signaling, J. Immunol. 173 (2004) 2913–2917.
[21] S. Sato, M. Sugiyama, M. Yamamoto, Y. Watanabe, T. Kawai, K. Takeda, S. Akira, Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF) associates with TNF receptor-associated factor 6 and TANK-binding kinase 1, and activates two distinct transcription factors, NF-kappa B and IFN-regulatory factor-3, in the Toll-like receptor signaling, J. Immunol. 171 (2003) 4304–4310.
[22] P. Georgel, Z. Jiang, S. Kunz, E. Janssen, J. Mols, K. Hoebe, S. Bahram, M.B. Oldstone, B. Beutler, Vesicular stomatitis virus glycoprotein G activates a specific antiviral Toll-like receptor 4-dependent pathway, Virology 362 (2007) 304–313.