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Cutting Edge: TRAF6 Mediates TLR/IL-1R Signaling–Induced Nontranscriptional Priming of the NLRP3 Inflammasome

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NLRP3 inflammasome activiation requires two sequential signals. The priming signal 1 from TLRs or cytokine receptors induces the transcription of NLRP3 and IL-1β, and concomitantly promotes transcription-independent activation of caspase-1. The activating signal 2 can be provided by microbial products or danger signals. In this study we found that TRAF6 is necessary for the nontranscriptional priming of NLRP3 inflammasome by TLR/IL-1R derived signals. Deficiency of TRAF6 specifically inhibited TLR/IL-1R priming–initiated caspase-1 cleavage, pyroptosis, and secretion of presynthesized IL-18. Mechanistically, TRAF6 promoted NLRP3 oligomerization as well as the interaction between NLRP3 and apoptosis-associated speck-like protein containing a CARD. Of note, the nontranscriptional priming via TRAF6 did not involve mitochondrial reactive oxygen species or the phosphorylation of Jnk, Erk, and Syk, whereas the ubiquitin E3 ligase activity of TRAF6 was required. Our findings thus extended cognition on the mechanism of NLRP3 inflammasome activation, and provided a novel target for controlling NLRP3-related diseases. ***The Journal of Immunology*****, 2017, 199: 1561–1566.**

Although it has been studied extensively, the mechanism for NLRP3 inflammasome activation is still unclear (1). What has been accepted is that the activation of NLRP3 inflammasome requires two signals: signal 1 is a priming signal from TLRs or cytokine receptors; and signal 2 is the activating signal derived from danger signals or microbial products, which usually leads to cellular potassium efflux and mitochondrial reactive oxygen species (ROS) production (2). Signal 1 was previously deemed to upregulate NLRP3 and IL-1β expression (3). However, recent studies using short-time stimulation with TLR ligands revealed that a low basal level of NLRP3 is sufficient to induce robust caspase-1 activation, and in this case, activation of TLRs offers a priming signal required for inflammasome activation independent from the transcription of NLRP3, during which IRAK1is involved (4–7). IRAK1 is an important proximal molecule downstream of the TLR/IL-1R family, bridging Myd88 and the ubiquitin E3 ligase TRAF6 (8). Classical activation of the TLR/IL-1R-Myd88-IRAKs-TRAF6 cascade leads to NF-κB and AP-1 activation to regulate the transcription of a series of inflammatory genes (9). Because TRAF6 and IRAK1 are closely connected functionally, we investigated whether TRAF6 also plays a role in the activation of NLRP3 inflammasome in the current work. Indeed, TRAF6 was found to be critical for TLR/IL-1R–initiated NLRP3 inflammasome activation independent from its transcriptional regulating role.

Materials and Methods

Mice

Traf6$^{tm2a(EUCOMM)Wtsi}$ mice were from the Wellcome Trust Sanger Institute and crossed with ACTB: FLP1 transgenic mice (The Jackson Laboratory) to obtain floxed *Traf6*. The floxed mice were crossed with the lysM-Cre mouse line (the Jackson Laboratory) to obtain knockout (KO) of *Traf6* in myeloid cells. Wild type (WT) and *Nlrp3-R258W* mice had been described previously (10). *Casp1/11*$^{-/-}$ mice were from the Jackson Laboratory.

Cells and stimulation

Bone marrow–derived macrophages (BMDMs) were prepared as described previously (10). Peritoneal macrophages from naive mice were isolated via PBS containing 5% FBS. THP-1 and J774 cell lines were from the American Type Culture Collection. The working concentration of reagents used for inflammasome stimulation were as follows: LPS (500 ng/ml), Pam3csk4 (2 μg/ml), R837 (5 or 20 μg/ml), CpG DNA(1 μM), poly(dA:dT) (1 μg/ml), and nigericin (20 μM) were from InvivoGen; LLOMe (1 mM), ATP (5 mM), Mito-TEMPO (200 or 500 μM), actinomycin D (1 μg/ml), and cholera toxin B subunit (20 μg/ml) were from Sigma; TNF-α (100 ng/ml; R&D); IL-1β (400 ng/ml; Peprotech); Gramicidin (0.5 μM; Enzo); and *Salmonella typhimurium* (multiplicity of infection = 10; American Type Culture Collection).

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The online version of this article contains supplemental material.

Abbreviations used in this article: BMDM, bone marrow–derived macrophage; KO, knockout; ROS, reactive oxygen species; shRNA, short hairpin RNA; siRNA, small interfering RNA; WT, wild type.

Immunoprecipitation and Western blotting

Cells were lysed in buffer [50 mM Tris-HCl, [pH 7.4], 150 mM NaCl, 1% NP-40, protease inhibitor mixture (Roche)] for detecting protein interactions. For ubiquitination analysis, cells were lysed with buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% SDS, protease inhibitor mixture, 5 mM iodoacetamide] and boiled at 100°C for 10 min. The clarified supernatants were diluted with buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.5% NP-40, protease inhibitor mixture, 5 mM iodoacetamide] 10 times and then processed as routine immunoprecipitation. Proteins in cell culture supernatant (serum free) were precipitated with methanol and chloroform. The Abs used for Western blotting and immunoprecipitation recognize the following Ags: mouse caspase-1 p20, human caspase-1 p20, NLRP3 were from Adipogen; mouse caspase-1 p10, mouse IL-1β, ASC, β-actin, ubiquitin, TRAF6 were from Santa Cruz; FLAG, hemagglutinin were from Sigma, GAPDH (Bioworld), Erk, Jnk, p-Erk, p-Jnk, Syk, and p-Syk were from CST.

Short hairpin RNA– and small interfering RNA–mediated gene interference

Short hairpin RNAs (shRNA) in PLKO.1 vector were introduced into target cells via lentiviral transduction. Small interfering RNAs (siRNAs) were electroporated into BMDMs by Nucleofector-2b (Lonza). The targeting sequences were: mouse TRAF6: shRNA Sh1, 5′-CCTGTGAATTTCAGAGGCTTT-3′; Sh2, 5′-CCCAGGCTGTTCATAATGTTA-3′; Sh3, 5′-GGATGATACATTACTAGTG-3′, mouse TRAF6 siRNA, 5′-GGAUGAUACAUUACUAGUGtt-3′, human TRAF6 shRNA, 5′-CCACGAAGAGATAATGGAT-3′.

Immunofluorescence

Cell death was detected by 7-AAD (Sigma) staining. ASC speck formation was detected by primary anti-ASC (Adipogen) and secondary Alexa-555 goat anti-rabbit (Invitrogen) Abs, the 7-AAD positive or speck-containing cells were visualized through confocal microscopy (Olympus FV-1200) and enumerated by Image J.

Blue native PAGE analysis of NLRP3 oligomerization

Cells were lysed and centrifuged as described previously (11). Blue native PAGE Novex 3–12% Bis-Tris protein gels (Thermo Fisher) were used to detect NLRP3 oligomerization according to the manufacturer's instructions.

Results and Discussion

TRAF6 is required for the activation of NLRP3 inflammasome

Short-time priming with TLR ligands has been shown to be sufficient to activate NLRP3 inflammasome (4–7, 12, 13). These studies indicated that TLRs stimulation provides signals other than new protein synthesis to render the NLRP3 inflammasome for activation by signal 2 such as ATP treatment, and the proximal molecules downstream of TLRs such as IRAKs are involved in various phases of NLRP3 inflammasome activation (5, 6). As a well-known molecule downstream of IRAKs, whether TRAF6 plays a role in the activation of NLRP3 inflammasome is unknown. To address this question, we obtained mice deficient for TRAF6 specifically in myeloid cells, in this study referred as TRAF6 KO. Of note, when the TRAF6-deficient BMDMs were primed with different TLR ligands for 10, 30, or 270 min followed by ATP to activate the NLRP3 inflammasome, the caspase-1 cleavage was strongly compromised in comparison with that in wild type (WT) cells (Fig. 1A, Supplemental Fig. 1A). Although the transcription of NLRP3 and IL-1β was compromised to a certain extent in TRAF6 KO BMDMs after TLR stimulation (Supplemental Fig. 1B), treating the cells for a short time (40 min or 1 h) did not lead to a reduction of NLRP3 protein in TRAF6 KO cells (Fig. 1B, Supplemental Fig. 1C). Moreover, actinomycin D–mediated blockade of gene transcription did not affect short-time TLR priming–induced NLRP3 inflammasome activation in KO or WT macrophages (Fig. 1C), which further supported the nontranscriptional role of TRAF6 in licensing NLRP3 inflammasome under such conditions. Therefore, in most of the following experiments we limited the total stimulating time within 1 h to avoid potential interference on data interpretation due to a transcriptional change of NLRP3. Although it was clearly inhibited, there was still residual activation of caspase-1 in TRAF6 KO BMDMs, which was likely due to an incomplete deletion of TRAF6 in in vitro differentiated cells (Fig. 1A). Notably, when resident peritoneal macrophages were isolated and stimulated as above, TRAF6 deficiency led to a complete abolishing of caspase-1 activation, likely due to a more efficient deletion of TRAF6 in such cells (Supplemental Fig. 1D).

Consistently, silencing of TRAF6 with specific shRNAs in J774 mouse macrophages also led to a clear inhibition of the NLRP3 inflammasome (Supplemental Fig. 1E, 1F). On the contrary, when TRAF6 was over-expressed in J774 cells, the activation of NLRP3 inflammasome was enhanced (Supplemental Fig. 1G). Of note, in both TRAF6 knockdown and over-expressing J774 cells, the protein level of NLRP3 did not change (Supplemental Fig. 1E, 1G). In human monocytic THP-1 cells, knockdown of TRAF6 also inhibited TLR ligand only and TLR ligand plus ATP–induced NLRP3 inflammasome activation (Supplemental Fig. 1H). In addition to ATP, the deficiency of TRAF6 also impeded NLRP3 activation triggered by nigericin and LLOMe (Fig. 1D, 1E, Supplemental Fig. 1I).

Next, we tested the role of TRAF6 in activating other inflammasomes. However, when AIM2 or NLRC4 inflammasome activation was induced by poly(dA:dT) transfection or *Salmonella typhimurium* infection respectively, no difference on caspase-1 cleavage was observed between TRAF6 KO and WT BMDMs (Fig. 1F). When cells were stimulated with LPS plus cholera toxin B subunit to induce caspase-11 activation, we did not find a reduction of caspase-11 or caspase-1 maturation or lactate dehydrogenase release from TRAF6 KO cells (Fig. 1G, Supplemental Fig. 1J).

All these results suggest that TRAF6 is specifically indispensable for NLRP3 inflammasome activation.

TRAF6 is required for optimal secretion of NLRP3 inflammasome–dependent cytokine and pyroptosis

Unlike the absence of basal IL-1β mRNA in BMDMs, the transcription of IL-18 was constantly high in steady state (Supplemental Fig. 1K). Consistent with the reduction of caspase-1 activation in TRAF6 KO cells (Fig. 1A), a significant reduction of IL-18 secretion was observed in KO cells compared with WT in the acute activation condition (Fig. 1H).

In addition to the maturation of IL-1β and IL-18, pyroptosis is also associated with the activation of inflammasomes (14), so we monitored pyroptosis in macrophages via 7-AAD staining. It was found that the cell death in TRAF6 KO cells was significantly reduced (Fig. 1I).

These results demonstrate that TRAF6 promotes the secretion of presynthesized IL-18 and pyroptosis through regulating NLRP3 inflammasome, which is independent from its classical role in mediating NF-κB activation.

TRAF6 is required for ASC oligomerization and the assembly of NLRP3 inflammasome

The oligomerization of ASC precedes caspase-1 cleavage during NLRP3 inflammasome activation (15). Immunofluorescence staining revealed that many ASC specks appeared upon acute stimulation in peritoneal macrophages from WT but not TRAF6 KO mice (Fig. 2A). In accordance with caspase-1

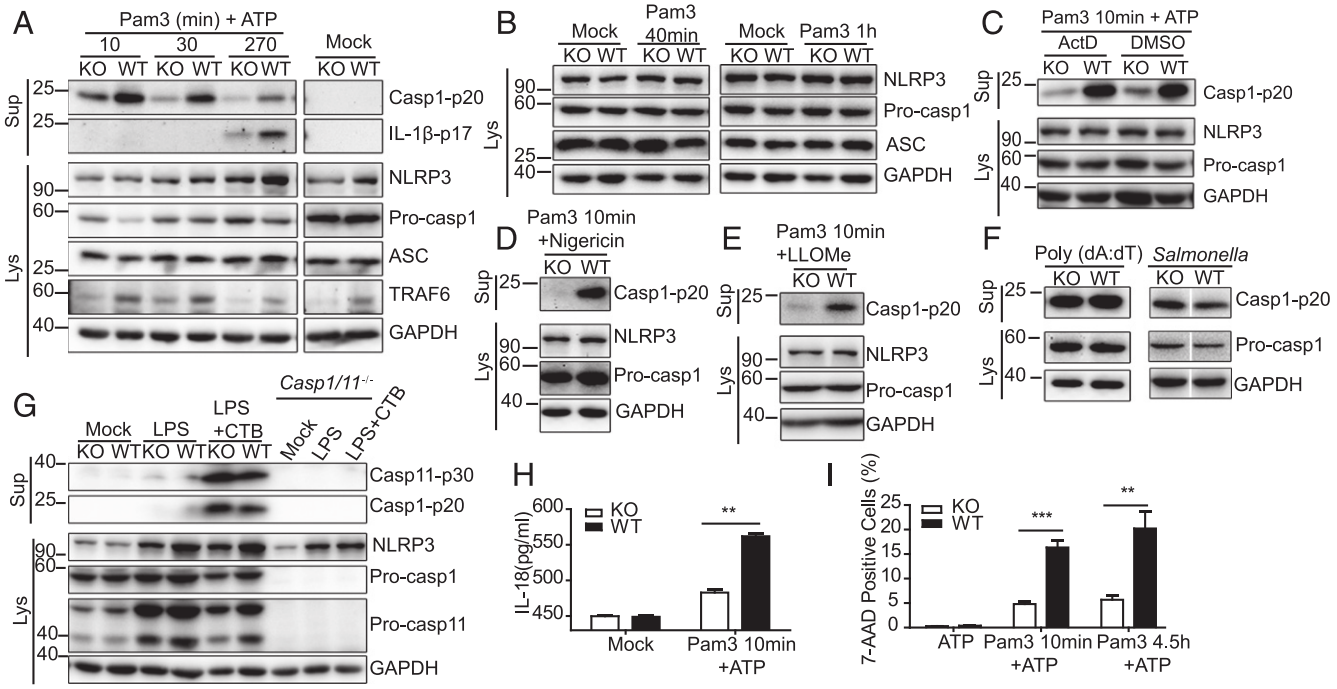

FIGURE 1. TRAF6 is required for NLRP3 inflammasome activation and function. (**A**) WT or TRAF6 KO BMDMs were primed with Pam3csk4 (Pam3) as indicated prior 30 min ATP pulse, or leave untreated (Mock). The supernatant (sup) and cell lysate (lys) were analyzed via Western blotting. (**B**) BMDMs were stimulated with Pam3 for 40 min or 1 h. (**C**) BMDMs were treated with Actinomycin D (ActD) or DMSO 30 min prior to Pam3 plus ATP treatment. (**D** and **E**) BMDMs were primed with Pam3 for 10 min before 30 min nigericin stimulation (D) or 50 min LLOMe treatment (E). (**F**) BMDMs were transfected with poly (dA:dT) for 6 h or infected with *Salmonella typhimurium* in log phase for 4.5 h. (**G**) BMDMs were treated with LPS for 4.5 h with or without cholera toxin B subunit (CTB) addition for further 8 h. (**H**) BMDMs were stimulated with Pam3 for 10 min plus ATP pulse for 30 min, the secretion of IL-18 in the cell culture supernatant were detected via ELISA. (**I**) BMDMs were primed with Pam3 as indicated prior 30 min ATP pulse, cells were stained with 7-AAD and the percentage of pyroptotic cells was measured. The white lines between the slots in (F) indicate splicing to remove unrelated samples in the same membrane. Data are representative of three biological repeats. Graphs show mean ± SD. The *p* values were determined by the Student *t* test. $^{**}p < 0.01$, $^{***}p < 0.001$.

activation, ASC oligomerization in BMDMs upon priming with different TLR ligands was also impeded by the TRAF6 deficiency (Supplemental Fig. 2A). In peritoneal macrophages, deficiency of TRAF6 even resulted in complete abolishing of ASC oligomerization (Supplemental Fig. 2B).

Because TRAF6 deficiency did not affect the activity of AIM2 inflammasome, which also engages ASC to recruit caspase-1 (Fig. 1F), we speculated that TRAF6 may be specifically used by NLRP3 to initiate the aggregation of ASC. So, we performed a coimmunoprecipitation assay in BMDMs to test whether TRAF6 was necessary for the interaction between NLRP3 and ASC. Indeed, ASC protein precipitated by NLRP3 was clearly reduced in TRAF6 KO cells compared with that in WT cells (Fig. 2B).

All these findings indicate that TRAF6 plays a critical role for the formation of a NLRP3-ASC macromolecule complex in the early stages of NLRP3 inflammasome activation.

TRAF6 mediates nontranscriptional priming in cells bearing a gain-of-function mutation of NLRP3

Our data so far has proved that TRAF6 is important for NLRP3 inflammasome activation independent from new protein synthesis. However, whether TRAF6 is involved in signal 1 or signal 2 for NLRP3 inflammasome activation is unclear. Gain-of-function mutations of NLRP3 associated with auto-inflammatory diseases can bypass signal 2 and activate inflammasome upon TLRs engagement alone (10); this unique feature of NLRP3-R258W macrophages made it a perfect tool to verify whether TRAF6 mediates the signal 1 from TLRs to activate the NLRP3 inflammasome. To this end, we applied siRNA to silence the expression of TRAF6 in BMDMs carrying the NLRP3-R258W mutation, and then stimulated these cells shortly with Pam3csk4. In the control cells (Si-luc), a clear caspase-1 cleavage was detected, whereas little caspase-1 cleavage appeared in TRAF6 silenced cells (Si-T6) (Fig. 3A). This showed that TRAF6 is crucial for the nontranscriptional priming of NLRP3 inflammasome.

TRAF6 mediates nontranscriptional priming from TLR/IL-1R for NLRP3 inflammasome activation

Potassium efflux and mitochondria ROS production have been reported as common features for the activation of NLRP3 inflammasome by different stimuli. Intriguingly, a recent study showed that gramicidin as a second signal stimulated NLRP3 inflammasome activation through potassium efflux, but without ROS production (16); whereas imiquimod (R837) and CL097 were found to activate NLRP3 in a potassium efflux–independent, but ROS-dependent, manner (17). Of note, when BMDMs were incubated with Pam3csk4 for 10 min followed with gramicidin treatment, caspase-1 cleavage was significantly reduced in TRAF6 KO cells (Fig. 3B). As R837 can provide both signal 1 through TLR7 and signal 2 through ROS production, a high-dose R837 treatment of BMDMs alone revealed that the deficiency of TRAF6 also abolished inflammasome activation under such conditions (Fig. 3C). Thus, no matter what kind of reagents serve as signal 2 and how the signal 2 works, TRAF6 is required for TLR-induced priming of the NLRP3 inflammasome.

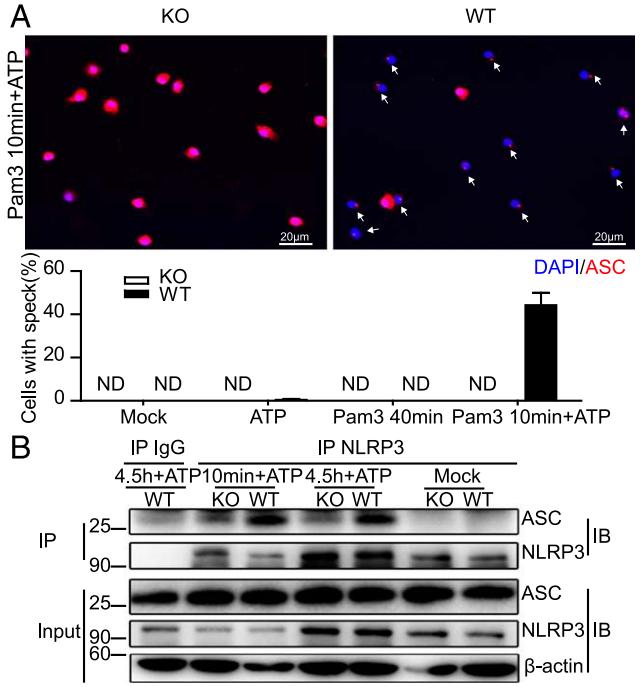

FIGURE 2. TRAF6 is necessary for ASC oligomerization and NLRP3-ASC interaction. (**A**) Resting peritoneal macrophages were treated with Pam3 or PBS for 10 min followed with or without ATP pulse for 30 min. The formation of ASC specks was monitored through ASC staining. The upper panel shows representative images (arrows indicate specks), scale bar is 20 μm, statistical analysis of speck containing cells is shown in the lower panel. (**B**) Cell lysate from Pam3 plus ATP stimulated BMDMs as indicated were incubated with NLRP3 specific Ab or control IgG overnight at 4°C, the precipitated proteins (IP) and cell lysate (Input) were detected via Western blotting. Data are representative of two biological repeats.

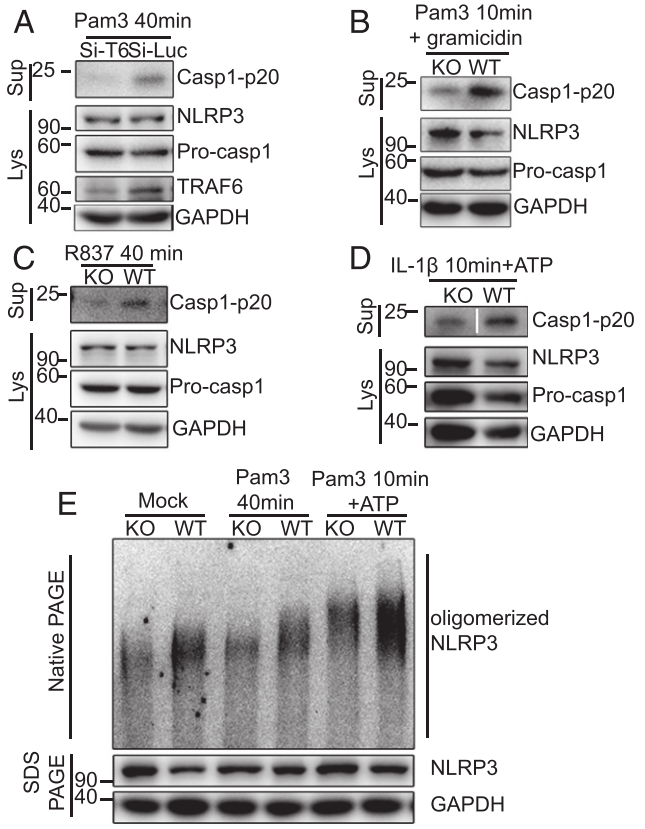

FIGURE 3. TRAF6 mediates nontranscriptional priming for NLRP3 inflammasome through promoting NLRP3 oligomerization. (**A**) BMDMs from NLRP3-R258W mutant mice were transfected with TRAF6 specific (Si-T6) or control siRNA (Si-Luc), followed with Pam3 stimulation for 40 min. (**B–D**) BMDMs were stimulated with Pam3 (B) or IL-1β (D) for 10 min followed by gramicidin (B) or ATP (D) treatment for 30 min or stimulated with high dose of R837 for 40 min (C). (**E**) BMDMs were primed for 10 min as indicated and pulsed with or without ATP for 30 min, cells were lysed with nondenature buffer and adjusted to native PAGE, part of the same samples were denatured by Laemmli buffer and adjusted to SDS-PAGE. The white line between the slots in (D) indicates the removal of unrelated samples in the same membrane by splicing. Data are representative of three biological repeats.

Moreover, TRAF6 is also well known for signal transduction downstream of IL-1 receptor (IL-1R). As expected, when BMDMs were treated with IL-1β for 10 min prior to ATP pulse, a clearly reduced caspase-1 cleavage was found in TRAF6 KO cells (Fig. 3D). Because TNF-α can also prime the NLRP3 inflammasome for activation (3, 18), but TRAF6 is dispensable for TNF-α–initiated signaling (19), we set out to test if TRAF6 was involved in TNF-α–induced inflammasome activation. In this case, short stimulation with TNF-α led to only moderate activation of caspase-1 and no reduction of caspase-1 activation in TRAF6 KO cells; in cells stimulated with TNF-α for 6 h, deficiency of TRAF6 did not reduce caspase-1 cleavage either (Supplemental Fig. 2C).

Therefore, TRAF6 is critical for TLRs and IL-1R, but not TNF-α–primed activation of NLRP3 inflammasome.

TRAF6 mediates NLRP3 oligomerization without involvement of mitochondrial ROS

An interesting question that has remained so far is whether the priming signal through TRAF6 affects the behavior of NLRP3 itself. NLRP3 could oligomerize during its activation process (11), so we studied the oligomerization of NLRP3 through the blue native PAGE method. Akin to previous reports, we also found that TLR ligand plus ATP treatment led to the formation of large molecular complex of NLRP3 (Fig. 3E) (11). More importantly, the mobility of this NLRP3 complex in TRAF6 KO cells on the gel was faster than that in WT cells, indicating the overall size of the complex was smaller in the absence of TRAF6. In addition, the density of NLRP3 signal on the native blot but not in the reduced blot was weaker in cells deficient for TRAF6, indicating that less NLRP3 complex was formed when TRAF6 was absent (Fig. 3E). Thus, TRAF6-mediated nontranscriptional priming did regulate the oligomerization of NLRP3.

TRAF6 facilitates the production of mitochondrial ROS from macrophages during bacteria engulfment and engagement of TLRs (20), and mitochondrial ROS has been suggested as important for the nontranscriptional priming of NLRP3 inflammasome (4). We thus tested whether TRAF6 mediates the priming signal through regulating mitochondrial ROS production. Notably, no mitochondrial ROS was detected in cells treated with LPS for either 40 min or 4.5 h (Supplemental Fig. 2D). Although ATP pulse alone stimulated strong production of mitochondrial ROS, preincubation with LPS for either a short or a long time had no additive effect; and under all conditions there was no drop in mitochondrial ROS in TRAF6-deficient cells (Supplemental Fig. 2D). Moreover, although the mitochondrial ROS scavenger Mito-TEMPO inhibited caspase-1 cleavage and IL-1β secretion from Pam3csk4+ATP-stimulated WT macrophages, it

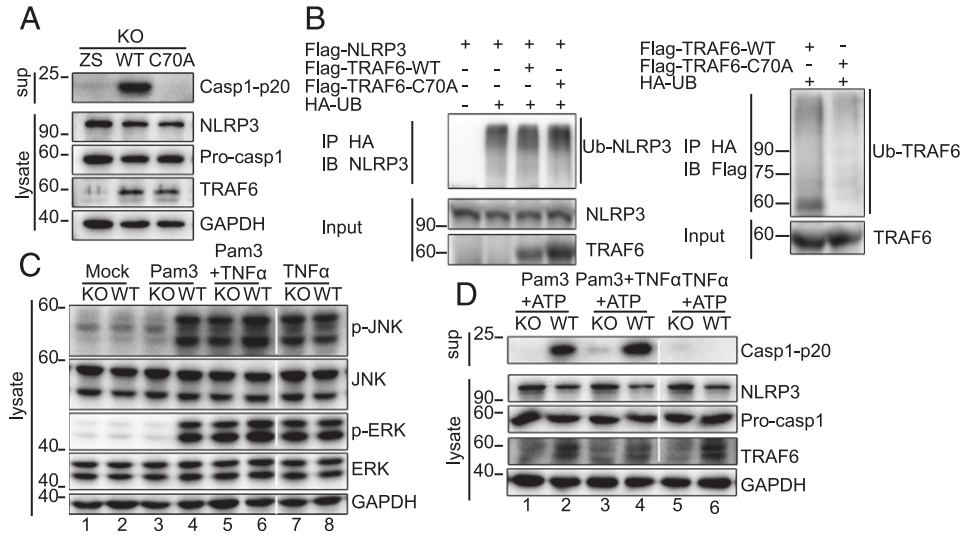

FIGURE 4. The ubiquitin E3 ligase activity of TRAF6 but not phosphorylation of Jnk, Erk, or Syk is necessary for nontranscriptional priming of NLRP3 inflammasome. (**A**) TRAF6 KO BMDMs were infected with lentivirus expressing WT, mutated (C70A) TRAF6, or Zsgreen (ZS), and treated with Pam3 for 10 min followed with ATP pulse for 30 min. (**B**) Indicated plasmids were transfected into HEK293T cells. Ubiquitinated proteins were precipitated via anti-HA Ab, followed with Western blotting analysis. (**C**) BMDM cells were treated with Pam3 or TNF-α separately or together for 10 min. (**D**) Cells were treated as in (C) with ATP added for another 30 min. The white lines between the slots in (C) and (D) indicate the removal of unrelated samples in the same membrane by splicing. Data are representative of three biological repeats.

did not interfere with the inflammasome activation in BMDMs carrying the NLRP3-R258W mutation upon Pam3csk4 treatment (Supplemental Fig. 2E–G). Because TLR ligand–initiated inflammasome activation in NLRP3-R258W cells also required TRAF6 (Fig. 3A), the failure of Mito-TEMPO to inhibit caspase-1 activation in NLRP3-R258W cells implied that mitochondrial ROS was unlikely to be involved in TRAF6-dependent priming of NLRP3 inflammasome. Thus, TRAF6 mediates the nontranscriptional priming signal upon engaging TLRs or IL-1R to induce NLRP3 oligomerization without involving mitochondrial ROS.

TRAF6 mediates nontranscriptional priming of NLRP3 inflammasome through its ubiquitin E3 ligase activity but not the phosphorylation of Jnk, Erk, or Syk

The ubiquitin E3 ligase activity is indispensable for TRAF6 to transmit the classical signal from TLRs and IL-1R (21), and we tested whether this activity was also required for priming NLRP3 inflammasome. In TRAF6-deficient BMDMs, lentiviral transduction of WT, but not an E3 ligase activity–deficient mutant TRAF6 (C70A) construct, rescued the caspase-1 cleavage (Fig. 4A). However, in HEK293T cells cotransfected with NLRP3 and TRAF6 or TRAF6-C70A led to similar ubiquitination on NLRP3, the same as in cells without any exogenous TRAF6 (Fig. 4B, left panel); even though the autoubiquitination of TRAF6 was clearly reduced when C70A mutation was introduced (Fig. 4B, right panel). Thus, NLRP3 is not the substrate of TRAF6. In accordance, we did not detect any interaction between NLRP3 and TRAF6, although as control the self-interaction between different NLRP3 molecules was clear (Supplemental Fig. 2H).

Several kinases activated by TLRs through TRAF6 have been reported to participate in the activation of inflammasomes. Jnk and Syk are necessary for NLRP3 and AIM2 activation through phosphorylating ASC to promote speck formation (22). ERK was reported as necessary for the rapid NLRP3 inflammasome activation in human monocytes (7); however, using the same ERK inhibitor, another study in THP-1 cells did not find any effect of ERK on NLRP3 inflammasome (23). Whether these molecules are involved in TRAF6-mediated nontranscriptional priming of NLRP3 inflammasome activation awaits further study. We analyzed the phosphorylation of these three kinases during the short Pam3csk4 priming plus ATP stimulation. As expected, deficiency of TRAF6 impeded the phosphorylation of Jnk and Erk, however, no phosphorylation of Syk was detected under such experimental conditions (Supplemental Fig. 2I). Costimulating TRAF6 KO cells with Pam3csk4 and TNF-α rescued the phosphorylation of Jnk and Erk to the level in Pam3csk4-stimulated WT cells (Fig. 4C, *lane* 4,5), however, costimulation failed to rescue the activation of caspase-1 in TRAF6 KO cells (Fig. 4D, *lane* 2, 3). Thus, our results indicate that the nontranscriptional priming signal mediated by TRAF6 requires its ubiquitin E3 ligase activity but not through regulating phosphorylation of Erk, Jnk, or Syk.

In summary, our current work demonstrated that TRAF6 is critical for TLRs and IL-1R–induced nontranscriptional priming of NLRP3 inflammasome. It not only revealed a novel function for the important signaling molecule TRAF6, but also shed crucial light on the understanding of the mechanism for NLRP3 inflammasome activation. Future work is needed to dissect the delicate mechanisms of how TRAF6 talks to NLRP3.

Disclosures

The authors have no financial conflicts of interest.

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