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Tripartite-Motif Protein 30 Negatively Regulates NLRP3 Inflammasome Activation by Modulating Reactive Oxygen Species Production

Yu Hu,†‡ Kairui Mao,†‡ Yan Zeng,‡ Shuzhen Chen,* Zhiyun Tao,* Chen Yang,* Shuhui Sun,‡ Xiaodong Wu,* Guangxun Meng,‡ and Bing Sun*,†

The NLR family, pyrin domain-containing 3 (NLRP3) inflammasome is critical for caspase-1 activation and the proteolytic processing of pro–IL-1β. However, the mechanism that regulates NLRP3 inflammasome activation remains unclear. In this paper, we demonstrate that tripartite-motif protein 30 (TRIM30) negatively regulates NLRP3 inflammasome activation. After stimulation with ATP, an agonist of the NLRP3 inflammasome, knockdown of TRIM30 enhanced caspase-1 activation and increased production of IL-1β in both J774 cells and bone marrow-derived macrophages. Similarly with ATP, knockdown of TRIM30 increased caspase-1 activation and IL-1β production triggered by other NLRP3 inflammasome agonists, including nigericin, monosodium urate, and silica. Production of reactive oxygen species was increased in TRIM30 knockdown cells, and its increase was required for enhanced NLRP3 inflammasome activation, because antioxidant treatment blocked excess IL-1β production. Conversely, overexpression of TRIM30 attenuated reactive oxygen species production and NLRP3 inflammasome activation. Finally, in a crystal-induced NLRP3 inflammasome-dependent peritonitis model, monosodium urate-induced neutrophil flux and IL-1β production was reduced significantly in TRIM30 transgenic mice as compared with that in their nontransgenic littermates. Taken together, our results indicate that TRIM30 is a negative regulator of NLRP3 inflammasome activation and provide insights into the role of TRIM30 in maintaining inflammatory responses.


The innate immune system is the first line of defense against pathogens. The response to pathogens (such as bacteria, fungi, viruses, etc.) in humans depends upon the ability to mount immune responses. Pattern recognition receptors (1), including TLRs (2–4), RIG-I–like receptors (5, 6), and NLRs (nucleotide-binding domain and leucine-rich repeat containing gene family) (7), are proteins used by nearly all organisms to identify pathogen-associated molecular patterns. Once activated, the innate immune system initiates the inflammatory response by secreting cytokines and chemokines, inducing the expression of adhesion and costimulatory molecules to recruit immune cells to the site of infection and to trigger the adaptive immune response.

The central role of the NLR family in the immune system has become increasingly appreciated in recent years (8, 9). Upon activation, NLRs, including NLRP1, NLRP3, and NLRC4, recruit adaptor ASC and effector caspase-1 to form large cytoplasmic complexes, defined as the inflammasome (10). The inflammasome regulates the activation of caspase-1 and subsequent cleavage of the proinflammatory mediator, IL-1β, as well as IL-18 precursors into their functional forms, which then are released from the cell. One of the best characterized inflammasomes is the NLRP3 inflammasome. NLRP3-mediated inflammasome activation occurs in response to diverse molecular entities, including bacteria (11), viruses (12, 13), fungi (14), components of dying cells (11), crystal particles (15–18), and DNA (19).

Normal activation of the NLRP3 inflammasome contributes to host defense. However, excessive activation of the NLRP3 signaling pathway leads to the pathogenesis of a spectrum of autoimmune inflammatory diseases (20, 21). Mutations in the human NLRP3 gene are associated with various autoinflammatory disorders, such as Muckle-Wells syndrome, familial cold autoinflammatory syndrome, and chronic infantile neurologic cutaneous and articular syndrome. The NLRP3 gene-targeted mice harboring mutations mimicking those causing disease in humans show very severe phentoypes, including delayed growth, increased mortality, and development of severe cutaneous lesions associated with the ac-
cumulation of inflammation markers in these tissues (22, 23). Therefore, NLRP3 inflammasome signaling must be regulated tightly to maintain immune balance.

Previous work in our laboratory has demonstrated that tripartite motif protein 30 (TRIM30) is involved in endotoxin tolerance (24). Overexpression of TRIM30 in vivo increased the survival of mice after i.p. challenge with LPS. Moreover, the critical role of the NLRP3 inflammasome in LPS-induced endotoxic shock has been documented by numerous reports in recent years (11, 25, 26). Mice with a deficiency in any component of the NLRP3 inflammasome, including NLRP3, ASC, or caspase-1, are resistant to LPS-induced endotoxic shock. Thus, we infer that TRIM30 might be involved in the regulation of NLRP3 inflammasome activation. Knockdown of TRIM30 increases ATP-induced caspase-1 activation and IL-1β production in both J774 cells and bone marrow-derived macrophages (BMDMs). More importantly, we show that TRIM30 negatively regulates the IL-1β secretion evoked by many other NLRP3 inflammasome agonists. Upon TRIM30 knockdown, we observed an increase in reactive oxygen species (ROS) production triggered by LPS plus ATP. Inhibition of ROS by antioxidant treatment diminished the amplified IL-1β production upon TRIM30 knockdown, suggesting that enhanced IL-1β production is mainly due to excess ROS production. Our results collectively demonstrate the role of TRIM30 in regulating the NLRP3-mediated inflammatory response.

Materials and Methods

Mouse strains and reagents

C57BL/6, IL-1R1−/− mice were obtained from The Jackson Laboratory (Bar Harbor, ME). TRIM30 transgenic mice were generated as described previously (24); nontransgenic littermates served as controls for the transgenic mice. All of the mice were maintained in specific pathogen-free facilities at the Animal Care Facility of the Chinese Academy of Sciences. Animal care and use were in compliance with institutional guidelines.

All of the reagents were from Sigma-Aldrich (St. Louis, MO) unless stated otherwise. Polyinosinic-polycytidylic acid (poly-IC) and CpG were prepared as in a previous report (16). Poly-IC and CpG were both obtained from R&D Systems (Minneapolis, MN), and IL-18 ELISA kits were obtained from Invivogen (San Diego, CA). Anti-NLRP3 was obtained from Alexis (Lausen, Switzerland; 804-880-C100). Monosodium urate (MSU) was prepared as described previously (16). IL-1β, TNF-α, and IL-6 ELISA kits were obtained from R&D Systems (Minneapolis, MN), and IL-18 ELISA kits were obtained from MBL International (Woburn, MA).

Cells

J774 or HEK 293T cells were maintained in humidified 5% CO2 at 37°C in DMEM supplemented with 10% (v/v) FBS, penicillin (100 U/ml), and streptomycin (100 U/ml). Lipofectamine (Invitrogen) was used for transient transfection of HEK293T cells.

BMDMs were prepared as follows: bone marrow cells were flushed from the femurs and tibias of C57BL/6 mice and subsequently depleted of red cells with ammonium chloride. Cells were cultured at 2 × 10^6 cells per well in 24-well plates in DMEM supplemented with 20 ng/ml murine M-CSF. Nonadherent cells were removed carefully, and fresh medium was added every 2 d. On day 6, cells were collected for further experiments.

Short interfering RNA synthesis and transfection

Short interfering RNA (siRNA) synthesis and transfection were done as reported previously (24).

In vitro stimulation of macrophages

J774 cells or BMDMs were stimulated as follows. Briefly, cells were stimulated with TLR agonists and then pulsed with 5 mM ATP for 30 min, 20 μM nigericin for 30 min, 250 μg/ml MSU for 3 h, or 250 μg/ml silica for 3 h. For pharmacological assessments, N-acetyl-l-cysteine (NAC; 10 mM) or diphenyleneiodonium (DPI; 12.5 μM) was added to the cell culture 15 min before the end of LPS stimulation.

Reverse transcription and quantitative real-time PCR

Total RNA was extracted from J774 cells with TRIzol (Invitrogen) according to the manufacturer’s instructions. Oligo(dT) priming and M-Moloney murine leukemia virus reverse transcriptase (Invitrogen) were used for reverse transcription of purified RNA. All of the gene transcripts were quantified by real-time PCR with SYBR Green qPCR Master Mix and a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The relative fold induction was calculated by the 2^− ΔΔCT method. The sequences of primers for real-time PCR analysis are as follows: IL-1β, forward, 5′-CAAGTCGACTACAGGCTCGG-3′, reverse, 5′-CGACAGCAACAGGCTTTTTT-3′; TNF-α, forward, 5′-AGTGTCA- GAAGCTCTGTCGC-3′; reverse, 5′-TGAGCCAGATTTCAAGCTCC-3′; IL-6, forward, 5′-TTCCATTACGGTTGCTCTTGT-3′; reverse, 5′-GAAGCGGTGGTTGTGTCACC-3′; IP-10, forward, 5′-GGGCCAGTGAGAATGAGGG-3′; reverse, 5′-CCTCGAGGATGATTCTTCA-3′; IFN-β, forward, 5′-CCACACGCTCCCTCCATACAT-3′; reverse, 5′-CAAGTGGAGACGGTGAGAACATC-3′; β-actin, forward, 5′-GTACGACCAGAACCGCACAGG-3′; reverse, 5′-GATGAGATATGCGTGGCTGTG-3′.

ROS detection

Intracellular ROS was measured with the ROS-specific fluorescent probe CM-H2DCFDA (10 μM; Molecular Probes, Eugene, OR). J774 cells were loaded for 15 min with 10 μM CM-H2DCFDA, washed twice with PBS, and exposed to ATP for 30 min, nigerin for 30 min, or MSU for 3 h. The level of fluorescence was determined by flow cytometry.

Immunoblot analysis

For immunoblot analysis, whole-cell lysates were separated by SDS-PAGE, transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA), blocked with 5% bovine serum albumin (BSA; Amresco, Solon, OH), and exposed to primary antibodies overnight. Blots were probed with a secondary HRP-conjugated antibody, followed by visualized with SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL). The overexpression level of caspase-1 was quantified by densitometry. The number of neutrophils was calculated as total cells multiplied by the percentage of Gr-1–positive cells. When IL-1β secretion was measured, 0.6 ml PBS with 1% (v/v) FBS was used, and the concentration of IL-1β in the supernatant of peritoneal lavage fluid.

In vivo peritonitis model

Mice were injected i.p. with 1 mg MSU in 200 μl PBS; control mice received PBS. Mice were sacrificed 6 h after injection. Peritoneal cells were collected with 10 ml PBS with 1% (v/v) FBS. Recovered fluid was pelleted by centrifugation, and total cells were counted. Subsequently, cells were stained for neutrophil surface marker Gr-1 and detected using flow cytometry. The number of neutrophils was calculated as total cells multiplied by the percentage of Gr-1–positive cells. When IL-1β was measured, 0.6 ml PBS with 1% (v/v) FBS was used, and the concentration of IL-1β in the supernatant of peritoneal lavage fluid.

Statistical analysis

Data are presented as the mean ± SD from three independent experiments. Statistical comparisons between different treatments were performed by unpaired Student t test, where p < 0.05 was considered statistically significant and p < 0.01 was highly significant.

Results

Knockdown of endogenous TRIM30 enhances IL-1β secretion and caspase-1 activation in J774 cells

On the basis of our previous work indicating that TRIM30 is capable of increasing the survival rate in mice suffering endotoxic shock (24), we wonder whether TRIM30 is able to modulate NLRP3 inflammasome activation. To address this question, a mouse macrophage cell line, J774, was selected, and the effect of TRIM30 on NLRP3 inflammasome activation was tested. Initially, we detected NLRP3
inflammasome activation in J774 cells treated with LPS priming and after ATP stimulation by measuring caspase-1 activation and IL-1β secretion. As shown in Fig. 1A, ATP induced caspase-1 activation (determined by immunoblotting of the p10 subunit of mature caspase-1) and IL-1β secretion in LPS-primed J774 cells. We noticed that priming of macrophages with LPS for 4–6 h induced the highest levels of caspase-1 activation and IL-1β secretion. As recently reported (28), the cells became gradually refractory to ATP stimulation when the time of LPS priming was extended (Fig. 1A). It was interesting to observe that the expression of TRIM30 reached the highest level at 6–8 h after LPS priming, the time that the caspase-1 activation began to decrease. Thus, we assumed that the enhanced TRIM30 expression might result in the depression of NLRP3 inflammasome activation. To test this hypothesis, we depleted endogenous TRIM30 expression in J774 cells by TRIM30-specific siRNA. As expected, TRIM30 siRNA (siTRIM30) reduced TRIM30 protein efficiently as compared with control siRNA (siCon). Meanwhile, caspase-1 activation was enhanced greatly after LPS plus ATP stimulation (Fig. 1B), indicating that TRIM30 negatively regulated NLRP3 inflammasome activation. To further confirm this, IL-1β secretion was measured in J774 cells in which TRIM30 had been silenced. The results showed that in response to LPS plus ATP secretion of IL-1β was increased significantly in the siTRIM30-transfected cells as compared with that in siCon-transfected cells. In contrast, production of TNF-α and IL-6 was not altered (Fig. 1C). Also, NLRP3, a LPS-inducible protein and limiting factor for robust inflammasome activation (29), was not altered in the siTRIM30-transfected cells as compared with that in siCon-transfected cells (Fig. 1B), implying that knockdown of TRIM30 did not interfere with LPS-induced NF-κB activation under these experimental conditions. Increased secretion of IL-1β may result from the enhanced mRNA level of pro-IL-1β; thus, we detected the mRNA level of the cytokines. The data showed that knockdown of TRIM30 slightly increased the levels of IL-1β mRNA by LPS stimulation (~1.5 times) but had no influence on the levels of TNF-α, IL-6, IP-10, and IFN-β (Fig. 1D). However, we did observe the great enhancement of caspase-1 activation and much more significant secretion of IL-1β protein (~4 times) when TRIM30 was silenced, suggesting that IL-1β production was enhanced largely at the posttranscriptional level in TRIM30 knockdown cells. These data indicate that knockdown of TRIM30 enhances IL-1β secretion through the regulation of caspase-1–mediated pro-IL-1β processing.

**Knockdown of TRIM30 enhances IL-1β secretion and caspase-1 activation in BMDMs**

We next address the physiological role of TRIM30 in primary cells. BMDMs were transfected with the indicated siRNA, and then IL-1β secretion and caspase-1 activation were assessed after stimulation. In response to LPS plus ATP stimulation, IL-1β and IL-18 secretion were increased significantly in TRIM30 knockdown BMDMs as compared with that in control cells (Fig. 2A). In contrast, the production of TNF-α and IL-6 was not altered (Fig. 2B). As expected, caspase-1 activation was enhanced in TRIM30 knockdown cells after LPS plus ATP stimulation (Fig. 2C). Collectively, we demonstrate that the knockdown of TRIM30 enhances NLRP3 inflammasome activation in both J774 cells and primary BMDMs.

**TRIM30 knockdown enhances IL-1β secretion and caspase-1 activation induced by various stimuli**

It is well documented that the production of mature IL-1β requires two distinct signals (30, 31), TLR priming, and inflammasome activation. We have demonstrated that TRIM30 is induced by

**Figure 1.** Knockdown of TRIM30 enhances IL-1β secretion in response to LPS plus ATP in J774 cells. J774 cells were pretreated with or without LPS (1 μg/ml) for the indicated time period (A) or 6 h (B, C) and then were stimulated further with medium or 5 mM ATP for 30 min (A–C). A, ELISA of IL-1β secretion in supernatant and immunoblot analysis of whole-cell lysates with anti–caspase-1, anti-TRIM30, or anti–β-actin (loading control). B, Immunoblot analysis with anti–caspase-1, anti-TRIM30, anti-NLRP3, or anti–β-actin of the whole-cell lysate (WCL) and supernatant (Sup) of J774 cells transfected with siCon or siTRIM30 after stimulation. C, ELISA of IL-1β, TNF-α, and IL-6 in supernatant of J774 cells transfected with siCon or siTRIM30 after stimulation. D, siCon- or siTRIM30-transfected J774 cells were treated with LPS (1 μg/ml) for 4 h. Expression levels of IL-1β, TNF-α, IL-6, IP-10, and IFN-β were quantified by real-time PCR. The relative fold induction was calculated by the 2−ΔΔCT method. Data are representative of three experiments. Error bars indicate mean ± SD between duplicates. Statistical significance was determined by the Student t test. *p < 0.05. L+A, LPS+ATP; ND, not detected; SC, siCon; ST, siTRIM30.
TRIM30 has no interaction with the components of the NLRP3 inflammasome

Because TRIM30 was seen to be a negative regulator of NLRP3 inflammasome activation, we then investigated its mechanism. Various proteins that interfere with inflammasome assembly and the processing of pro-IL-1β have been identified (32–34). They function as endogenous dominant negative proteins by preventing the recruitment of ASC or caspase-1 into the inflammasome. Therefore, we assumed that TRIM30 might regulate NLRP3 inflammasome activation by interfering with NLRP3 inflammasome assembly. To test this hypothesis, a coimmunoprecipitation assay was performed. As shown in Supplemental Fig. 1A, TRIM30 did not interact with either ASC or caspase-1. As a positive control, TAK1 was able to coimmunoprecipitate with TRIM30. In addition, we noted that TRIM30 did not regulate NLRP3 inflammasome activation by blocking the interaction between components of the NLRP3 inflammasome.

TRIM30 regulates NLRP3 inflammasome activation by modulating ROS production

ROS is believed to be a common NLRP3 activator (9). It is reasonable to ask whether TRIM30 influences ROS production, which in turn regulates NLRP3 inflammasome activation. We first measured the level of ROS production in macrophages transfected with siCon or siTRIM30 by loading with CM-H2DCFDA. As predicted, ROS fluorescence was increased more significantly after LPS plus ATP stimulation in TRIM30 knockdown cells than that of the controls (Fig. 4A). Moreover, TRIM30 knockdown potentiated ROS accumulation by MSU and nigericin (Supplemental Fig. 2). In contrast, the levels of ROS with no stimulation remained unchanged in the same conditions (Fig. 4A). It was reported that enhanced ROS production results in elevated IL-1β production (35). To further address whether ROS was involved in

FIGURE 2. TRIM30 knockdown enhances IL-1β secretion in response to LPS plus ATP in BMDMs. BMDMs were transfected with siCon or siTRIM30, pretreated with LPS for 6 h, and then pulsed with medium or 5 mM ATP for 30 min. A and B, ELISA of IL-1β and IL-18 (A) and TNF-α and IL-6 (B) in supernatant. C, Immunoblot analysis of WCL and Sup of BMDMs with anti-caspase-1, anti-TRIM30, or anti–β-actin. Data are representative of three experiments. Error bars indicate mean ± SD between duplicates. Statistical significance was determined by the Student t test. *p < 0.05.

LPS, CpG, and poly-IC stimulation (24). Thus, we tested the potential role of TRIM30 in IL-1β secretion triggered by different TLR agonists plus ATP. As seen with LPS plus ATP stimulation, IL-1β secretion and caspase-1 activation in response to CpG plus ATP stimulation were enhanced under TRIM30 knockdown conditions (Fig. 3A). However, no IL-1β secretion or caspase-1 activation was detected after poly-IC plus ATP stimulation (Fig. 3A). Next, we determined whether TRIM30 had regulatory effects on other NLRP3 inflammasome agonists. As shown in Fig. 3B, similar to ATP, a significant increase in IL-1β secretion and caspase-1 activation was observed after LPS plus MSU, silica, or nigericin stimulation. These data demonstrate that TRIM30 is a general regulator of NLRP3 inflammasome activation, which may target a common signaling pathway required for NLRP3 inflammasome activation.
elaborated. IL-1β production, siCon- or siTRIM30-transfected J774 cells were treated with an antioxidant, NAC, before ATP stimulation. As shown in Fig. 4B, knockdown of TRIM30 caused a marked increase in IL-1β secretion, and this effect was abolished when ROS production was disrupted by NAC treatment. Meanwhile, TNF-α production was not affected by NAC treatment (Fig. 4B). Similarly, the treatment of macrophages with another antioxidant, DPI, reduced the difference in IL-1β secretion between siCon and siTRIM30 groups (Fig. 4C). These data demonstrate that ROS are required for the enhancement of IL-1β secretion in TRIM30-silenced macrophages. Taken together, knockdown of TRIM30 increases the level of ROS, which promotes NLRP3 inflammasome activation.

**Overexpression of TRIM30 in vitro attenuates ROS production and NLRP3 inflammasome activation**

Because knockdown of TRIM30 enhanced NLRP3 inflammasome activation, it was assumed that overexpression of TRIM30 would reciprocally reduce NLRP3 inflammasome activation. To examine this possibility, we constructed a bicistronic TRIM30-expressing retrovirus (Fig. 5A) and infected J774 cells. Transduction of TRIM30 dramatically increased the expression of TRIM30 protein (Fig. 5C) and concomitantly led to a decrease in the level of ROS production (Fig. 5B). As a result, overexpression of TRIM30 resulted in a significant reduction of NLRP3 inflammasome-mediated caspase-1 activation (Fig. 5C) and subsequent IL-1β secretion (Fig. 5D). Meanwhile, the expression level of NLRP3 was not altered by overexpression of TRIM30 (Fig. 5C). These results indicate that the upregulated TRIM30 is able to inhibit the outcome of NLRP3 inflammasome activation. However, overexpression of TRIM30 also reduced the production of TNF-α and IL-6 (Fig. 5D). To separate the inhibition of NF-κB and inhibition of the NLRP3 inflammasome by TRIM30, we used a ring domain.
major target for the regulation of the NLRP3 inflammasome by TRIM30. Collectively, these data indicate that overexpression of TRIM30 inhibits NLRP3 inflammasome activation by attenuating ROS production.

**TRIM30 negatively regulates activation of the NLRP3 inflammasome in vivo**

To examine whether TRIM30 is involved in the inflammatory response in vivo, we used the MSU-induced peritoneal inflammatory model, which is NLRP3 inflammasome-dependent (16, 36). MSU crystals trigger acute inflammation with the hallmark of an infiltration of neutrophils at the site of crystal deposition in vivo (37). We injected MSU into the peritoneal cavity of IL-1RI–deficient or wild-type mice and evaluated the acute inflammatory response. Consistent with previous studies, MSU injection caused a marked infiltration of neutrophils into the peritoneal cavity, whereas the infiltration was impaired severely in IL-1RI–deficient mice (Fig. 6A), indicating that NLRP3 inflammasome-mediated IL-1β production is a prerequisite for MSU-induced acute inflammation. Therefore, we next determined the role of TRIM30 in the inflammatory response to MSU. Compared with nontransgenic littermates, TRIM30 transgenic mice experienced a significant reduction in neutrophil influx (Fig. 6B) and IL-1β production (Fig. 6C) induced by MSU injection. Similar to MSU, a reduction of the neutrophil influx was observed in TRIM30 transgenic mice after silica injection (Supplemental Fig. 3). These results indicate that TRIM30 negatively regulates NLRP3 inflammation activation in vivo.

**Discussion**

Our data demonstrate the negative role of TRIM30 in the regulation of NLRP3 inflammasome activation in vitro and in vivo. Knockdown of endogenous TRIM30 increased NLRP3 inflammasome-mediated caspase-1 activation and subsequent production of IL-1β and IL-18, which result from the excess production of ROS. Conversely, overexpression of TRIM30 attenuated ROS production and NLRP3 inflammasome activation. We further exploited the function of TRIM30 using transgenic mice and showed that overexpression of TRIM30 in vivo inhibited the NLRP3-mediated inflammatory response.

In this study, the production of TNF-α and IL-6 remains unchanged in TRIM30 knockdown cells compared with that in control cells by a single LPS stimulation. This is consistent with the previous work in our laboratory. Shi et al. (24) found that the difference between TRIM30 knockdown and control cells in the production of TNF-α was only detected by secondary stimulation with LPS. Moreover, previous work in our laboratory found that the ring domain of TRIM30 was required for TRIM30-mediated degradation of TAB2/3 and inhibition of NF-κB. Thus, we tested the involvement of the ring-mediated function of TRIM30 in NLRP3 inflammasome activation by using a ring dead mutation (CA) of TRIM30 with substitution of the cysteine residue at position 35 of the ring domain with alanine (24). Our data showed that overexpression of TRIM30 (CA) decreased NLRP3 inflammasome-mediated caspase-1 activation and IL-1β secretion, which was similar with overexpression of TRIM30 (Fig. 5E, 5F). Also, overexpression of TRIM30 (CA) significantly inhibited ROS production (Fig. 5G), suggesting that ROS but not NF-κB should be the major target for the regulation of the NLRP3 inflammasome by TRIM30. However, the level of IL-1β mRNA was increased slightly in TRIM30 knockdown cells after LPS stimulation (Fig. 1D). The unchanged expression of TNF-α and IL-6 (in both mRNA and protein levels, Fig. 1C, 1D) in TRIM30 knockdown cells implied that there may be an unknown mechanism for regulating the level of IL-1β mRNA besides NF-κB activation.

Our data showed that TRIM30 was a general modulator of NLRP3 inflammasome activation; therefore, TRIM30 probably targets a common signaling pathway required for NLRP3 inflammasome activation. Various proteins have been reported to inhibit caspase-1 activation by interfering with inflammasome assembly. For instance, a family of small proteins that are composed of either a CARD or a PYD only emerged as important inflammasome regulators. These CARD-only proteins and PYD-only proteins function as endogenous dominant negative proteins by preventing the recruitment of ASC or caspase-1 into the inflammasome (32). The SPRY–CARD domain interaction mediates the association of several TRIM proteins with their partners, for example, association of TRIM20 (pyrin) with caspase-1 or TRIM25 with RIG-I (34, 38). Therefore, it appears possible that TRIM30 may regulate inflammasome activation by means of interfering inflammasome assembly. However, we found no association between TRIM30 and ASC or caspase-1, regardless of the existence of SPRY (TRIM30) and CARD (ASC and caspase-1) domains (Supplemental Fig. 1). This led to looking for additional mechanisms by which the TRIM30 might regulate NLRP3 inflammasome activation.

ROS are believed to be a common NLRP3 activator (9). In our experiments, we found that ROS production was enhanced upon knockdown of TRIM30. The antioxidant treatment blocked excess IL-1β secretion induced by knockdown of TRIM30 (Fig. 4B, 4C), indicating that enhanced ROS production was required for the amplification of NLRP3 inflammasome activation. Conversely, overexpression of TRIM30 inhibited ROS production, leading to impaired IL-1β secretion and caspase-1 activation. Our data collectively suggested that TRIM30 negatively regulated NLRP3 inflammasome activation by modulating ROS production. However, the mechanism for the regulation of ROS production by TRIM30 remains unknown because the source of ROS for inflammasome activation is still uncharacterized (39). ROS can be generated in several sources, including mitochondria, NADPH oxidase, 5-lipoxygenase, and other enzymes (40), whereas the specific role for each source in NLRP3 inflammasome activation is unclear. It has been reported that the absence of autophagy results in enhanced ROS production, which in turn amplifies caspase-1 activation (35). Due to the lysosomal localization of TRIM30 and the dominant role of the lysosome in autophagosome maturation, the regulation of TRIM30 based on ROS production may be related to autophagy likewise.

In conclusion, our study demonstrates that TRIM30 is induced by TLR stimulation and in turn can restrict NLRP3 inflammasome activation. These findings may be helpful in understanding the precise regulation of NLRP3 inflammation activation and advancing the development of drugs for inflammatory diseases.

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**Disclosures**

The authors have no financial conflicts of interest.

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