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NLRP3 inflammasome contributes to endotoxin-induced coagulation

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ABSTRACT

Introduction: Excessive activation of the coagulation cascades leads to life-threatening disseminated intravascular coagulation (DIC) in sepsis. Two recent studies by our group and others have both demonstrated the noncanonical inflammasome is pivotal for the endotoxin or gram-negative bacterial-induced coagulation. Based on this, we further evaluated the function of the NLRP3 inflammasome, the most studied inflammasome, in endotoxin-induced coagulation.

Materials and methods: We established an endotoxin-induced coagulation model by intraperitoneal injection of sublethal doses of LPS in mice. Mice were sacrificed 8 h after injection and blood was collected for thrombin-antithrombin (TAT), plasminogen activator inhibitor-1 (PAI-1), prothrombin time (PT), D-dimer, IL-1β and tissue factor (TF) measurements by commercial ELISA. Lungs and livers were examined via HE staining images to determine injury scores and immunohistochemistry for TF expression and fibrin deposits. The role of NLRP3 activation was evaluated in wild-type (WT), Nlrp3$^{-/-}$, Asc$^{-/-}$ (apoptosis-associated speck-like protein containing a CARD), Caspase-11$^{-/-}$ mice and 30 min after treatment with MCC950, a potent inhibitor of NLRP3. Western blotting and Q-PCR were performed to assess TF expression in the lungs and livers. To uncover the different effects of NLRP3 and Caspase-11, we also compared the time-dependent IL-1β release in LPS-treated Nlrp3$^{-/-}$ and Caspase-11$^{-/-}$ mice. Correlation analysis of TAT, PAI-1 were estimated the relationship of coagulation and release of IL-1β, as well as IL-1β and TF.

Results: Inhibition of NLRP3 by MCC950 as well as NLRP3 or ASC deficiency decreased TAT, PAI-1, PT, D-dimer, and TF levels in blood and impaired the thrombus formation and fibrin deposition, as well as declined expression of TF in the liver and lung in endotoxin-induced coagulation but not caspase-11 deficiency. Impressively, IL-1β release is increased in LPS-treated Caspase-11$^{-/-}$ mice, but not in Nlrp3$^{-/-}$ mice. Moreover, the correlation analysis is indicated that downstream of the NLRP3 inflammasome, IL-1β expression, is positively correlated with TAT, PAI-1 and TF in blood circulation.

Conclusions: The NLRP3 inflammasome contributes to endotoxin-induced coagulation by promoting TF expression at least in part through the induction of IL-1β release. These findings broadened our understanding of the mechanism of coagulation and implicated a possible therapeutic strategy for preventing coagulation in sepsis.

1. Introduction

Coagulation is a complex physiological process that plays a crucial role in homeostasis. It is also involved in the immune response by restricting the dissemination of invading microbes [1]. Numerous studies have revealed extensive crosstalk between coagulation and inflammation, that is one system may amplify activation of the other, and dysregulation of any one component in these systems may result in

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tissue damage or even multiorgan failure, such as sepsis-associated disseminated intravascular coagulation (DIC) [2]. Thus, uncovering the detailed mechanism between coagulation and inflammation will benefit the therapeutic strategies for some diseases.

Tissue factor (TF), a type I transmembrane glycoprotein, is a pivotal trigger of the coagulation cascades [3]. Because TF can be expressed and decrypted by inflammatory events, it attracts much attention in the immune system. Two recent studies by our group and others (Zhenyu Li and coll.) have both demonstrated that bacterial endotoxin activates noncanonical inflammasome-induced pyroptosis to mediate TF release, or cell surface expression of TF, leading to the pathogenic coagulation and septic death in mice [4,5]. The two studies highlighted the role of inflammasomes in the amplification of coagulation cascades by activating TF.

Inflammasomes are protein complexes that consist of a cytosolic sensor, an adaptor protein (apoptosis-associated speck-like protein containing a CARD (ASC)), and an effector, procaspase-1, playing a pivotal role in host immune responses to infections or sterile injuries. Upon sensing pathogens or stress signals, the sensors recruit ASC, and the latter bridges the sensors to effector procaspase-1, forms a large complex and leads to the downstream responses, including the maturation and secretion of interleukin-1β (IL-1β) and IL-18, and GSDMD-mediated pyroptosis [6,7]. Inflammasomes can be divided into canonical inflammasomes and noncanonical inflammasome by inflammatory caspases, as canonical inflammasomes activate caspase-1 and noncanonical inflammasome activates caspase-11 in mice (caspase-4/5 in humans) to induce the downstream effects. Canonical inflammasomes can be further classified into NLRP1b, NLRP3, AIM2, NLRC4 and Pyrin inflammasomes [8]. The NLRP3 inflammasome is the most extensively studied inflammasome, and it can be activated by a series of stimuli, including extracellular ATP, pore-forming toxins, crystalline and several viral, bacterial, fungal and protozoan pathogens. Aberrant activation of the NLRP3 inflammasome is related to numerous diseases, such as sepsis, gouts, Alzheimer's disease, atherosclerosis and diabetes [7]. However, the role of the NLRP3 inflammasome in coagulation still remains unclear.

In this study, we found that the NLRP3 inflammasome contributes to the endotoxin-induced coagulation. Deficiency of NLRP3 or ASC, or suppression of the NLRP3 inflammasome by MCC950 [9], alleviated endotoxin-induced thrombus formation and fibrin deposition in the liver and lung. Mechanistically, the NLRP3 inflammasome amplifies coagulation by promoting TF expression, which may be correlated with the secretion of IL-1β. Our results enhanced the understanding of coagulation and inflammation and further indicated that the NLRP3 inflammasome may be a potential target for the treatment of sepsis-related coagulopathy.

2. Materials and methods

2.1. Mice

The C57BL/6J (wild-type) and *Caspase11*$^{-/-}$ mice were purchased from The Jackson Laboratory Animal Co., Ltd. (Changsha, China). The *Nrlp3*$^{-/-}$ and *Asc*$^{-/-}$ mice were provided by Dr. Rongbin Zhou and generated as previously described [10,11]. The mice in this study were all eight- to ten-week-old males and weighed 25–30 g. They were all fed in the animal center of Central South University and bred under SPF conditions with 12 h daylight and darkness, water and standard chow for at least 1 week before the study. All experimental animal procedures were approved by the Institutional Animal Care and Use Committees of Central South University.

2.2. Reagents

Standard LPS 0111: B4 (Cat. no. tlrl-eblps) was bought from Sigma; a mouse IL-1β ELISA Kit (Cat. no. 88–7013) was purchased from Thermo Fisher; the TAT Complexes Mouse ELISA Kit were from Abcam (Cat. no. ab137994); the PAI-1 Mouse ELISA Kit was bought from RD(Cat. no. DY3828-05); a mouse D-dimer assay (ELISA) kit was purchased from Cloud-Clone (Cat. No. CEA506Mu); the mouse Prothrombin time was determined with thromboplastin-D (Pacific Hemostasis, Cat. no. 100354), from Thermo Fisher; the mouse TF ELISA Kit was from ZCIBIO (Cat. no. ZC-39071); anti-mouse fibrin antibody 59D8 was from Professor Nigel Mackman; anti-mouse Tissue Factor antibody was purchased from Abcam (Cat. no. ab104513); MCC950 sodium (CP-456773 sodium) was purchased from Selleck (Cat. no. S7809).

2.3. LPS-induced endotoxemia

To establish the endotoxemia model, The C57BL/6 mice, *Nrlp3*$^{-/-}$ mice, *Asc*$^{-/-}$ mice and *Caspase-11*$^{-/-}$ mice were injected with 20 mg/kg of LPS intraperitoneally and sacrificed after 8 h. To estimate time-dependent IL-1β release in Nrlp3$^{-/-}$ and Caspase-11$^{-/-}$ mice, mice were sacrificed 1 h, 2 h, 4 h and 8 h after LPS treatment. To monitor NLRP3 inhibition, MCC950 (50 and 100 mg/kg) was injected 30 min before LPS challenge. $n = 5$ in the LPS-treated group, and $n = 3$ in the control group. Plasma, lungs and livers were collected for further investigation.

2.4. Inflammation and coagulation assays

Blood was drawn by heart puncture in syringes containing heparins (or 3.8% trisodium citrate for detecting D-dimer and PT) from anesthetized mice by intraperitoneal injection of 1% pentobarbital sodium. Isolated plasma was prepared for the detection of TAT, PAI-1, IL-1β, D-dimer, PT and TF in accordance with the instructions of commercial manufacturing kits after high-speed centrifugation (3000*g*, 10 min) at 4 °C.

2.5. Liver and lung histology and immunohistochemistry assay

After PBS perfusion to the heart, the lower lobe of the right lung and lower right lobe of the liver were cut and fixed in 4% paraformaldehyde solution for 24 h. After regular dehydration for histological sectioning, these specimens were embedded with paraffin and cut and mounted on polysine adhesion glass slides for subsequent hematoxylin and eosin staining and immunohistochemistry assays of fibrin and TF.

Liver injury was scored following the “Hepatic Injury Severity Scoring” (HISS) system introduced by Muftuoglu [12]. Typical pathological changes in the liver were spotty necrosis (scored 0 to 4), ballooning degeneration (scored 0 to 3), portal inflammation (scored 0 to 3), capsular inflammation (scored 0 to 3) and steatosis (scored 0 to 3).

Lung injury was assessed by the diffuse alveolar damage (DAD) score criteria with histological changes of interstitial pulmonary edema, alveolar edema, inflammatory infiltration, alveolar hyperemia, atelectasis or excessive expansion of the alveolar. Each of them was scored as 0 for no change, 1 for a slight change, 2 for a moderate change, or 3 for a large change [13,14].

2.6. Q–PCR

Tissue lysates (livers and lungs) were prepared. Q–PCR was performed according to the instructions for the Q-PCR kit. The mRNA level of TF was normalized to that of β-actin. The primers sequences for mouse *TF* were as follows: forward, 5′-AACCCACCAACTATACCTACACT-3′; reverse, 5′-GTCTGTGAGGTCGCACTCG-3′. The primer sequences for mouse β-actin were as follows: forward, 5′-AGT GTG ACG TTG ACA TCC GT-3′; reverse, 5′-GCA GCT CAG TAA CAG TCC GC-3′.

2.7. Western blot

For western blotting, livers and lungs were lysed with SDS (CST) supplemented with cocktail and PMSF. Protein concentrations were detected with a bicinchoninic acid assay (Pierce). Equal contents of extracts were separated by SDS-PAGE and transferred onto 0.22-mm PVDF membranes (Merck Millipore).

2.8. Statistical analysis

Graph Pad Prism 7 software was used to analyze our data. Pearson correlation analysis was used to determine the relationship of TAT, PAI-1 and IL-1β (data met the criteria of normal distribution and collinearity). Means and standard error of the mean were calculated for continuous variables. The statistical significance of the difference between two groups was determined by two-tailed Bonferroni test for parametric data. The statistical significance was set at a P value<0.05.

3. Results

3.1. Establishment of an endotoxin-induced coagulation model

To explore whether the NLRP3 inflammasome is involved in LPS-induced activation of coagulation cascades, the two important NLRP3 inflammasome component deficient mice, *Nlrp3*$^{-/-}$ and *Asc*$^{-/-}$ mice, together with the wild type (WT) mice, were subjected to 20 mg/kg LPS to induce endotoxemia. *Caspase11*$^{-/-}$mice were regarded as the positive control since our recent study has proved the dominant role of caspase-11 in endotoxin-induced coagulation [5]. To evaluate the success of the model, the cytokine IL-1β in serum, and the lung and liver injury were examined. Consistent with previous studies [5,15], LPS caused increased inflammatory cell infiltration and recruited them to blood vessels in the liver and lung, as determined by hematoxylin and eosin (H&E) staining (Fig. 1A-C), and elevated IL-1β in plasma (Fig. 1D). While NLRP3, ASC or caspase-11 were deficient, these indicators were all markedly attenuated, suggesting that the model is suitable for the further study.

3.2. NLRP3 or ASC deficiency impaired the endotoxin-induced coagulation

To monitor the LPS-induced coagulation cascades, the circulating coagulation markers including the thrombin-antithrombin (TAT) complex, plasminogen activator inhibitor type-1(PAI-1), PT and D-dimer were determined, as well as the fibrin deposits. LPS administration significantly elevated TAT, PAI-1, PT and D-dimer in plasma, and excessive fibrin deposits in livers and lungs in WT but not in *Caspase11*$^{-/-}$mice (Fig. 2A-E), as observed in our previous study [5]. Intriguingly, the levels of TAT (Fig. 2A), PAI-1 (Fig. 2B), PT (Fig. 2C), D-Dimer

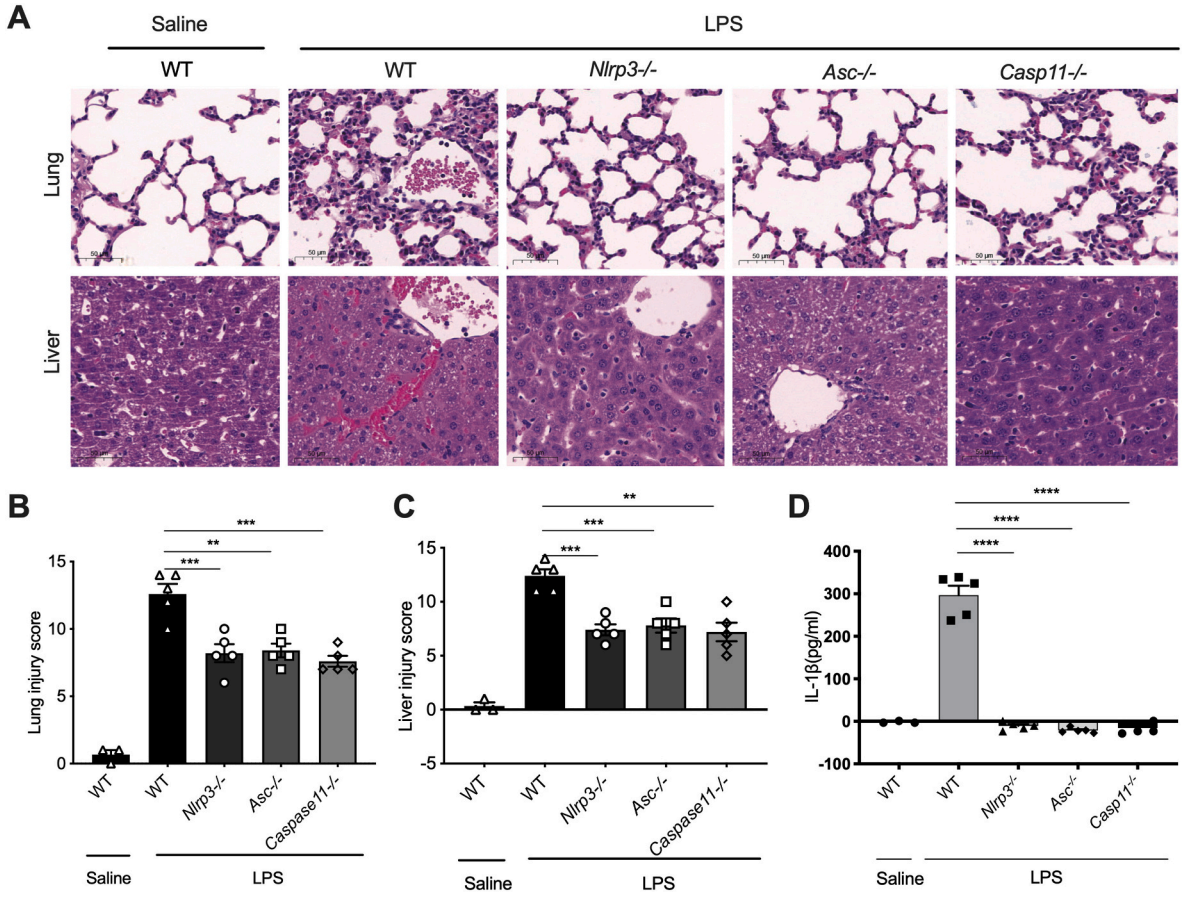

Fig. 1. Representation of the endotoxin-induced coagulation model.
Nlrp3$^{-/-}$, *Asc*$^{-/-}$, *Caspase11*$^{-/-}$ and wild-type mice were administered an intraperitoneal injection of 20 mg/kg LPS to establish the endotoxemia-induced coagulation model. The negative control group received an intraperitoneal injection of saline in wild type mice. *Caspase11*$^{-/-}$ mice were used as a positive control group. A: Representative images of HE staining in livers and lungs of endotoxemic mice versus *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$ *and Caspase11*$^{-/-}$ mice; Original magnification: 200×; B: Lung injury scores of endotoxemic mice versus the indicated knockout mice were evaluated by DAD score criteria; C: Liver injury scores of endotoxemic mice versus the indicated knockout mice were calculated by the HISS system; D: Plasma concentrations of IL-1β were measured at 8 h in endotoxemic WT mice versus the indicated knockout mice; dots represent individual mice. $n = 5$ in each LPS-treated group, and $n = 3$ in the control group. Values, mean ± SD. For statistical analysis, B, C and D were analyzed using one-way ANOVA with Bonferroni's test; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

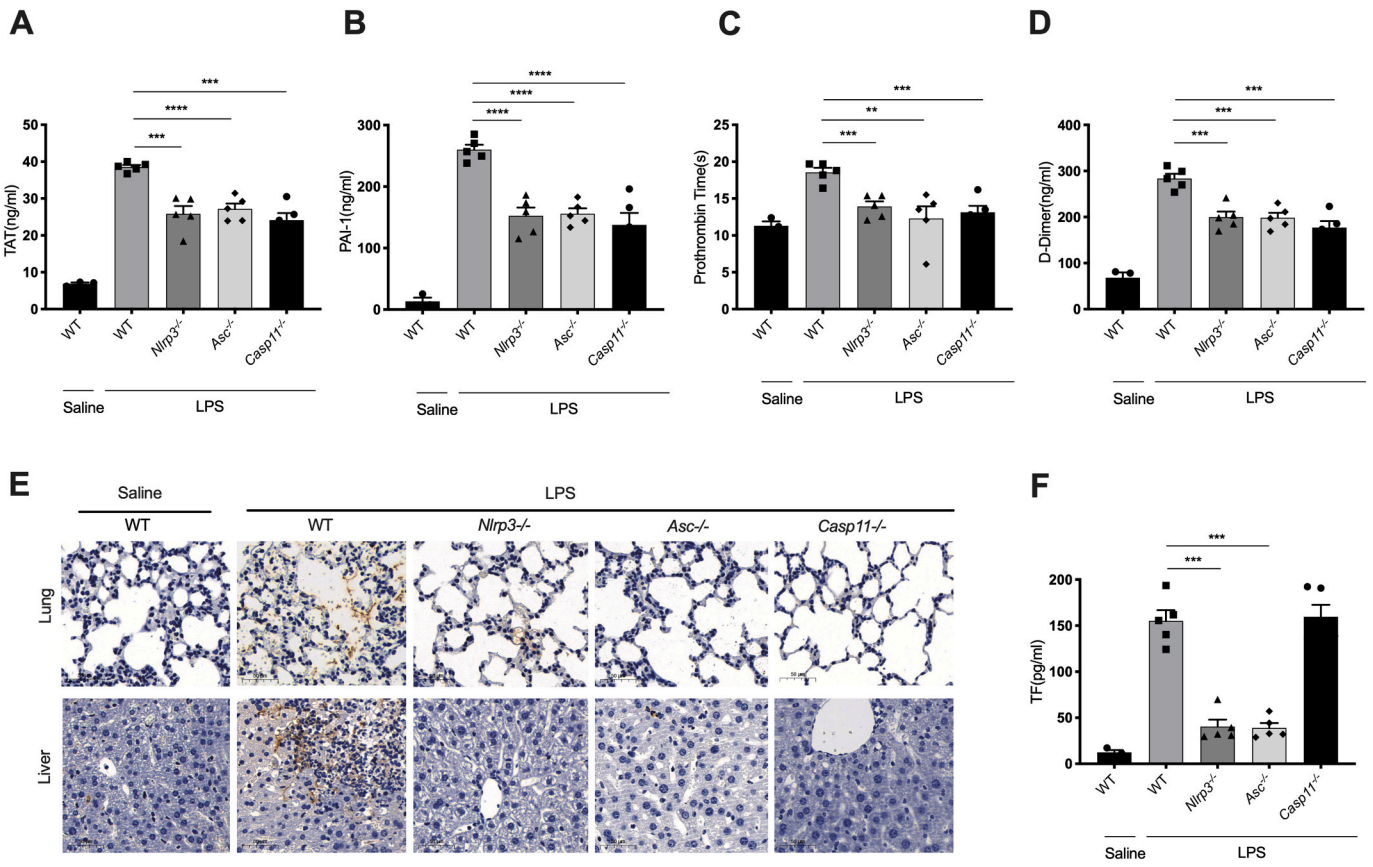

Fig. 2. NLRP3 or ASC deficiency decreased endotoxin-induced coagulation.
A-D Plasma concentrations of the TAT complex (A), PAI-1 (B), PT (C) and D-dimer (D) were measured at 8 h in LPS-treated WT mice versus *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$ *and Caspase11*$^{-/-}$ mice.
E Representative images of immunohistochemical (IHC) staining of fibrin deposits (golden) in lungs and livers. Original magnification: 200×.
F Plasma TF levels were measured at 8 h in the indicated knockout mice.
Dots represent individual mice. $n = 5$ in each LPS-treated group, and $n = 3$ in the control group. Values, mean ± SD. For statistical analysis, A and B were analyzed using one-way ANOVA with Bonferroni's test; ***$p < 0.001$; ****$p < 0.0001$.

(Fig. 2D) and fibrin deposits (Fig. 2E) were all decreased in *Nlrp3*$^{-/-}$ and *Asc*$^{-/-}$ mice, similar to those in *Caspase11*$^{-/-}$ mice, suggesting that the NLRP3 inflammasome contributes to LPS-induced activation of coagulation cascades. Since TF is a pivotal trigger of the coagulation cascade, we further detected TF levels in plasma. Impressively, the plasma TF levels were declined in *Nlrp3*$^{-/-}$ and *Asc*$^{-/-}$ mice, but not in WT and *Caspase11*$^{-/-}$ mice when treated with LPS (Fig. 2F), indicating a different role of the NLRP3 inflammasome and the noncanonical inflammasome in the expression of TF [4,5].

To further confirm the effect of the NLRP3 inflammasome on the LPS-induced coagulation cascade, MCC950, a specific inhibitor of NLRP3 was used [16,17]. Similarly, treatment with MCC950 prominently reduced the plasma levels of TAT, PAI-1, and IL-1β, as well as tissue factor (Fig. 3A-D). Thus, these data indicated that the NLRP3 inflammasome amplified the endotoxin-induced coagulation through TF expression.

3.3. NLRP3 and ASC deficiency decreased the TF expression in multiple organs

Next, we explored the TF expression in the liver and lung from *Nlrp3*$^{-/-}$ and *Asc*$^{-/-}$ mice. We extracted the livers and lungs from these endotoxinic mice, and found that transcription and translation of TF were both decreased in *Nlrp3*$^{-/-}$ and *Asc*$^{-/-}$ mice, compared to WT mice. In addition, *Caspase11*$^{-/-}$mice exhibited no obvious changes in TF expression (Fig. 4A-C), in consistence with previous studies [4,5]. Moreover, the detection of TF by immunohistochemistry in lungs and livers further supported this notion (Fig. 4D).

3.4. NLRP3 inflammasome-triggered IL-1β may contribute to TF expression

Then we explored the downstream events of the NLRP3 inflammasome in TF expression. Activation of the NLRP3 inflammasome leads to the maturation of IL-1β and pyroptotic cell death through GSDMD. Since our previous study showed that GSDMD-mediated Ca^{2+} influx is essential for the activation of TF, which activates the coagulation cascade when exposed to blood [5], we then investigated whether IL-1β was involved in the LPS-induced activation of coagulation cascades. By analyzing the correlation between the levels of IL-1β and TAT or PAI-1 in plasma from LPS-treated WT mice with or without MCC950, we observed that coagulation parameters were highly related to IL-1β (Fig. 5A-B), in line with the analysis from septic patients [5]. Moreover, the levels of IL-1β and TF were highly correlated (Fig. 5C), consistent with a previous study showing that TF expression could be rapidly upregulated by inflammatory cytokines, such as tumor necrosis factor (TNF-α) and IL-1β [2]. These results suggested that NLRP3 inflammasome-triggered IL-1β may be involved in the TF expression. We noticed that TF expression was completely different in *Nlrp3*$^{-/-}$ and *Caspase11*$^{-/-}$ mice upon treatment with LPS (Fig. 4A-C), and we then analyzed IL-1β secretion in these mice. We observed a rapid production of IL-1β at early time (1 and 2 h) in *Caspase11*$^{-/-}$ mice treated with LPS,

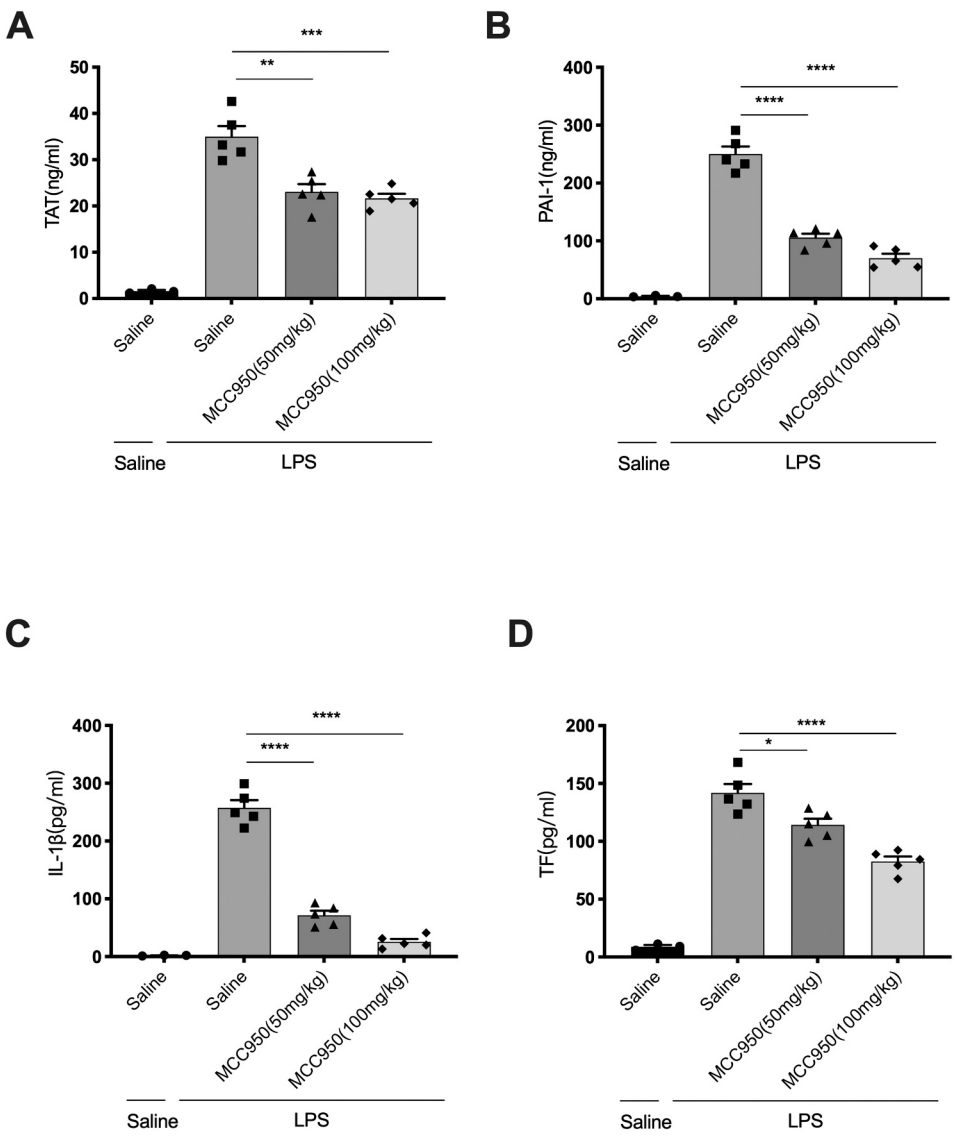

Fig. 3. MCC950 inhibited IL-1β and TF release in endotoxemia.
MCC950 was pretreated 30 min before intraperitoneal injection of 20 mg/kg LPS. Mice were sacrificed 8 h after LPS administration. Plasma levels of the TAT complex (A), PAI-1 (B), IL-1β (C) and TF (D) were estimated at 8 h in WT mice with endotoxin-induced coagulation WT mice treated with saline or MCC950 (50 mg/kg or 100 mg/kg). Dots represent individual mice. $n = 5$ in each LPS-treated group, and $n = 3$ in the control group. Values, mean ± SD. For statistical analysis, B, C and D were analyzed using one-way ANOVA with Bonferroni's test; $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

but not in *Nlrp3*$^{-/-}$ mice, although at later times (4 and 8 h), the production of IL-1β was substantially reduced in *Caspase11*$^{-/-}$ mice and similar to *Nlrp3*$^{-/-}$ mice. We supposed that the rapid secretion of IL-1β in *Caspase11*$^{-/-}$ mice may contribute to the difference (Fig. 5D).

Together, these observations indicated that the NLRP3 inflammasome contributes to the LPS-induced activation of coagulation cascades both in organs and in the blood cycle by promoting TF expression, which may be correlated with the secretion of IL-1β.

4. Discussion

Coagulation can be activated by various triggers, such as reactive oxygen species (ROS), cytokines or endotoxin, and damage-associated molecular patterns (DAMPs). Tissue factor, coagulation factor III, is located in the perivascular space but not in the intravascular space under physiological conditions. During infection or vessel injury, TF is exposed to the blood, and both full length and spliced TF are strongly expressed and sustained through the release of proinflammatory cytokines, chemokines, or other inflammatory/procoagulant microparticles by neutrophils, platelets or monocytes/macrophages that form in the immune system and endothelial cell surface, which pool of TF through thiol-disulfide exchange and protein disulfide isomerase [19], whilst induce transcriptional upregulation and messenger RNA (mRNA) splicing to increase the levels of blood-borne TF in the vascular compartment [20].

If activated, TF stimulates the generation of factor VIIa, factor Xa, and thrombin (IIa), which activate platelets and promote the release of more proinflammatory cytokines [21]. TF plays a central role as a bridge between coagulation and inflammation. Excessive inflammatory responses lead to overexpression of coagulation factors in the bloodstream and organs, promoting thrombosis in microvessels, which thus exacerbates inflammation resulting in multiorgan failure or death. Meanwhile, the NLRP3 inflammasome is pivotal for thrombosis in different diseases, such as atherosclerosis [22], hypoxia [23], intracerebral hemorrhage [24], ischemic stroke [25] and the new COVID-19 [26]. Hence, a promotive effect of the NLRP3 inflammasome is implicated in coagulation.

In this study, we showed that the NLRP3 inflammasome activates endotoxin -induced coagulation. Deletion of NLRP3 or ASC decreased the levels of TAT, PAI-1, PT and D-dimer in mouse blood plasma when treated with LPS, as well as fibrin deposits in organs. A specific inhibitor of NLRP3, MCC950 [9] further confirmed the function of the NLRP3 inflammasome in endotoxin-induced coagulation. To explore how the NLRP3 inflammasome contributes to the endotoxin-induced coagulation, we detected TF, a pivotal coagulation trigger in blood and tissues. We found deficiency of NLRP3 or ASC, or suppression of the NLRP3 inflammasome, could impair the expression, suggesting that the NLRP3 inflammasome contributes to the endotoxin-induced coagulation by

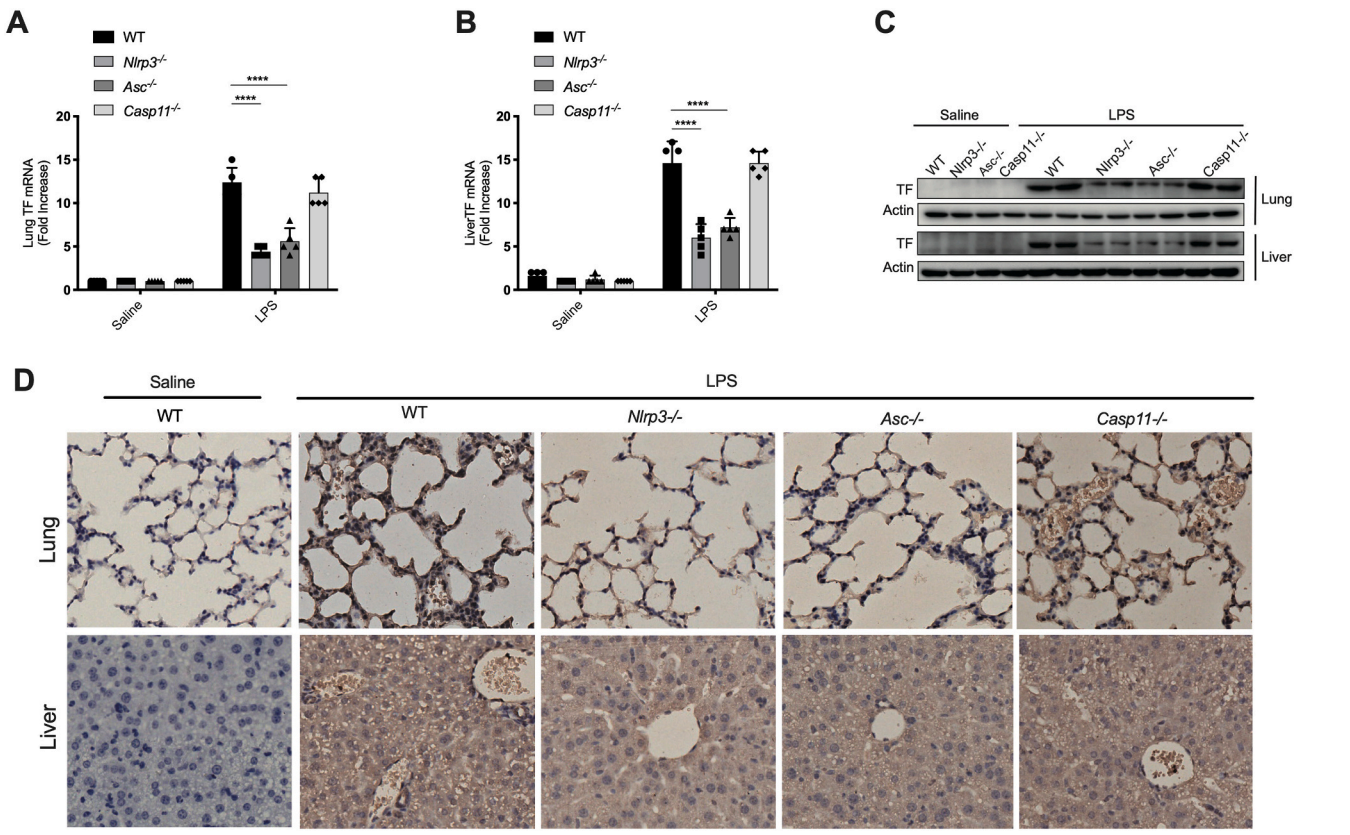

Fig. 4. NLRP3 or ASC deficiency decreased the TF expression in multiple organs.
TF mRNA was measured by Q-PCR in lungs (A) and livers (B) in WT mice versus *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$ *and Caspase11*$^{-/-}$ mice treated with LPS. The protein concentration of TF was measured in endotoxemic WT mice versus *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$ *and Caspase11*$^{-/-}$ mice (C). Representative images of immunohistochemical (IHC) staining of TF (brown) in lungs and livers (D). Original magnification: 200×. Dots represent individual mice. n = 5 in each LPS-treated group, and n = 3 in the control group. Values, mean ± SD. For statistical analysis, A-B were analyzed using two-way ANOVA with Bonferroni's test; ****$p < 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

amplifying the TF expression. To reveal the potential mechanism, we analyzed the correlation between IL-1β expression, the downstream events of the NLRP3 inflammasome, and the coagulation markers, and TF expression. We found that they were all highly related to IL-1β. Since previous studies proved that IL-1β could trigger TF release [18,27], we speculated that the NLRP3 inflammasome contributes to endotoxin-induced coagulation probably through IL-1β.

Previously, a few studies revealed the role of IL-1β in coagulation-related diseases. Pierce W et al. found that the NLRP3 inflammasome could activate platelets through the release of IL-1β to cause multiorgan injury in sepsis in a cecal ligation and puncture (CLP)-induced polymicrobial sepsis model [28]. Besides, another study indicated that an amplified IL-1β autocrine loop is required for thrombosis formation [29]. IL-1β stimulates the expression of TF in monocytes, endothelial cells or macrophages [30–32], and results in the generation of thrombin, which further activates platelets [2]. In line with previous conclusions, our study confirmed that the NLRP3 inflammasome promotes TF expression in the endotoxin- induced coagulation cascade, probably through IL-1β. Activation of the NLRP3 inflammasome also promotes the release of HMGB1 [33], which has been reported to be involved in the regulation of TF activity [34], however, in this study, we focused on the TF expression induced by the NLRP3 inflammasome, and did not further evaluate the TF activity induced by HMGB1.

Recent studies unveiled that both caspase-1 and caspase-11 dependent inflammasomes could activate coagulation through TF release or TF activation [4,5]. Activated caspase-1 or caspase-11 cleaves GSDMD to the N terminal, which forms pores on the cell membrane, leading to membrane rupture and TF release, or Ca^{2+} influx induced phosphatidylserine exposure and TF activation [35]. Thus, inflammasome activation in pyroptotic macrophages was confirmed to have a promotive role in blood clotting in thrombo-inflammatory responses. In this study, we have found that the NLRP3 inflammasome enhanced TF expression in endotoxin-induced coagulation as a supplement for the role of the inflammasome in coagulation. Despite of the NLRP3 inflammasome, other canonical inflammasomes in the thrombosis are less studied and still needs further investigation in the future.

5. Conclusions

Our study found that the NLRP3 inflammasome contributes to the endotoxin - induced coagulation and subsequent multiple organ injury. Mechanistically, the NLRP3 inflammasome promotes the TF expression, probably through IL-1β release. These findings confirmed the importance of the NLRP3 inflammasome in sepsis associated coagulation and indicated that inhibiting NLRP3 could be a new strategy for preventing coagulative complications in sepsis.

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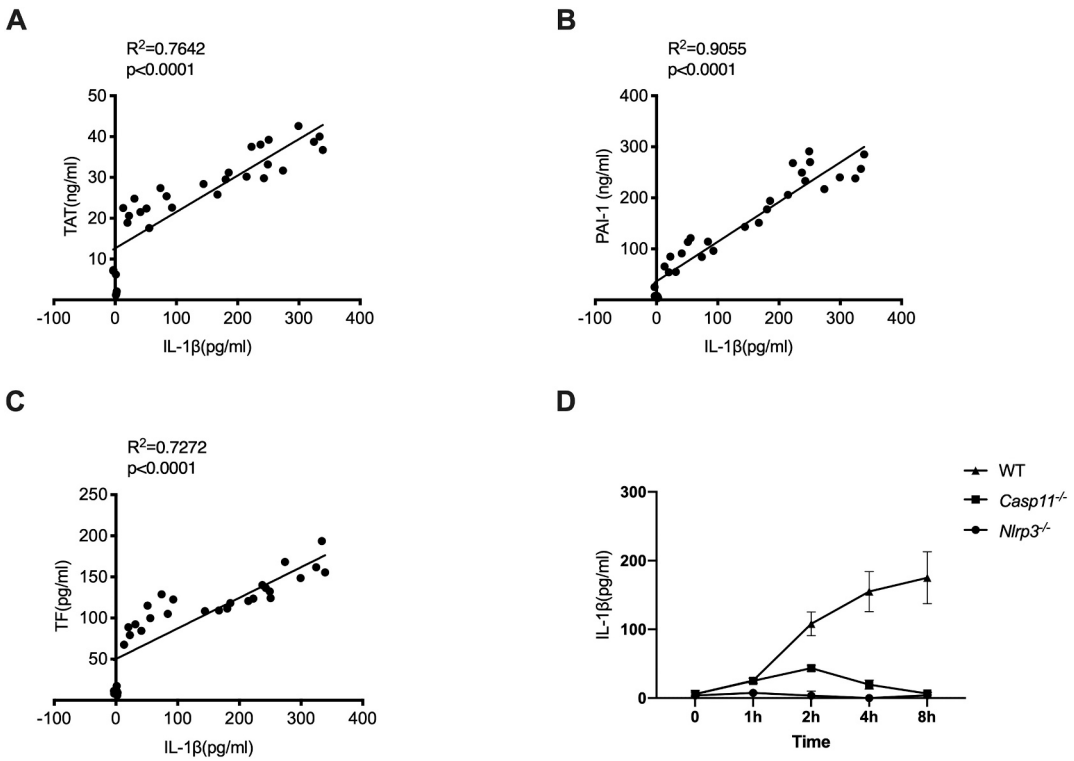

Fig. 5. IL-1β is positively correlated with TF expression in the endotoxin-induced coagulation. Plasma concentrations of TAT and PAI-1 were significantly correlated with IL-1β (A and B). The plasma IL-1β level was highly correlated with TF (C). IL-1β secretion over time in *Nlrp3−/− and Caspase11−/−* mice (D). Dots represent individual mice. Pearson correlation analysis was performed as our data met the criteria of normal distribution and colinearity.

Author contributions

Kai Zhao designed and supervised the whole project. Jie Shi and Liping Liu performed daily experiments. Yiting Tang provided technical support. Jie Shi and Kai Zhao wrote the manuscript. Jie Shi, Ni Liang and Ningjie Zhang analyzed the data. Zhongjie Yi, Fang Liang and Xinyu Yang helped with the discussion. Kai Zhao revised the manuscript. Yanjun Zhong and Wenhua Wang helped with the revision.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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