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The Endogenous Metabolite TDCA Ameliorates LPS-Driven Liver Injury via Modulation of Caspase-11/GSDMD-Mediated Pyroptosis

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Abstract

The liver is a central immunometabolic organ during endotoxemia and a major target of sepsis-related injury. Intriguingly, the liver exhibits a notable resilience to endotoxemia or septic insults, suggesting the activation of endogenous protective mechanisms. The bile acid taurodeoxycholic acid (TDCA) demonstrates hepatoprotective properties; nonetheless, its role and mechanism in lipopolysaccharide (LPS)-driven inflammatory liver injury remain elusive. This study reveals that LPS challenge induces significant reprogramming of hepatic bile acid metabolism, with TDCA being markedly elevated in LPS-challenged mice. In vitro, TDCA dose-dependently attenuated pyroptosis in bone marrow-derived macrophages, as evidenced by reduced lactate dehydrogenase (LDH) release, decreased interleukin-1 beta (IL-1 β) and interleukin-18 (IL-18) secretion, and suppressed dye Oxazole yellow uptake. Consistent with reduced non-canonical inflammasome signaling, TDCA treatment was associated with decreased activation of caspase-11 and its downstream targets Gasdermin D (GSDMD) and IL-1 β . In a lethal D-Galactosamine (D-GalN)/LPS-induced toxin-sensitized inflammatory liver injury model, therapeutic administration of TDCA (3, 6 mg/kg) profoundly improved survival rates (40% and 80%, respectively), attenuated liver injury, reduced alanine aminotransferase (ALT) and aspartate aminotransferase (AST), suppressed systemic inflammation (IL-1 β and IL-18), and ameliorated histopathological damage. Crucially, TDCA treatment reduced the activation of the caspase-11/GSDMD pathway in the septic liver. Our findings demonstrate that TDCA is an endogenously mobilized bile acid that confers protection against LPS-driven inflammatory liver injury, with effects supporting a role for modulation of the Caspase-11/GSDMD pyroptotic pathway. These observations provide hypothesis-generating implications for sepsis-associated liver injury that warrant further validation in clinically relevant sepsis models and pathway-necessity studies.

Keywords: LPS-driven liver injury; bile acid; TDCA; pyroptosis; Caspase-11; GSDMD



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1. Introduction

Sepsis, a life-threatening complication of severe trauma and infection, is a frequent occurrence in intensive care units (ICUs) [1,2]. Its mortality rate can reach nearly 25%, representing a significant challenge for healthcare systems worldwide [3,4]. The liver, as a central immune-metabolic organ, is particularly susceptible to damage during sepsis, and the development of sepsis-associated liver injury (SALI) is a strong predictor of mortality [5,6]. However, the liver is not directly “destroyed” but actively or passively undergoes “functional reprogramming; upon activation of innate immune defense constructed by endothelial cells, neutrophils, and Hepatic macrophages (Kupffer cells and monocyte-derived-macrophages (MDMs), the liver switches from metabolic function to immunogenic responses and mediates to adapt to the septic environment [7,8]. Paradoxically, despite the intense inflammatory milieu observed during sepsis or endotoxemic shock, histopathological alterations in the liver are often modest and nonspecific, frequently manifesting as bland cholestasis rather than extensive hepatocellular necrosis, even in the presence of profound clinical jaundice [6,9]. These observations suggest that robust endogenous protective and adaptive mechanisms are mobilized within the liver in response to inflammatory insults.

Bile acids (BAs), classically known for their role in lipid digestion [10], are now recognized as potent signaling molecules with diverse biological functions, including immunomodulation and cyto-protection [11–13]. Crucially, the biological activities of BAs are dictated by their specific chemical structure and concentration, exhibiting a dichotomy of effects that can range from protective anti-inflammatory signaling to pro-inflammatory cytotoxicity [14,15]. Consistently, recent clinical metabolomic studies have demonstrated significant alterations in the BA pool during sepsis, with the accumulation of specific hydrophobic BAs being inversely linked to patient survival [16,17]. These findings underscore that distinct BA species play critical, yet distinct, roles in the host’s response to sepsis [18,19]. However, while it is established that the liver reprograms its metabolic state under stress, whether this adaptation involves a protective, BA-mediated response remains an open question [20–22]. Notably, experimental endotoxemia induced by lipopolysaccharide (LPS) administration has been widely used to model the Gram-negative bacterial toxin-driven inflammatory component of sepsis, particularly the cytokine storm that contributes to SALI. Our preliminary investigations reveal that LPS challenge triggers profound transcriptional changes in hepatic BA-related genes, pointing towards an active—yet poorly understood—adaptation of BA metabolism during sepsis.

Pyroptosis, an inflammatory form of programmed cell death, has emerged as a critical driver of organ damage in sepsis [23,24]. This process is executed by the gasdermin family of proteins, primarily GSDMD [25]. In response to Gram-negative bacteria, cytosolic LPS directly activates the inflammatory caspase, caspase-11 (the murine ortholog of human caspase-4/5). Activated caspase-11 cleaves GSDMD, liberating its N-terminal fragment (GSDMD-NT), which forms pores in the plasma membrane, leading to lytic cell death and the release of pro-inflammatory cytokines such as IL-1 β and IL-18 [23,26,27]. The inhibition of this caspase-11/GSDMD pathway has been shown to confer significant protection against organ injury in experimental sepsis [28,29]. However, the endogenous regulatory mechanisms that fine-tune this destructive pathway are not fully understood [30].

Interestingly, certain bile acids, such as tauroursodeoxycholic acid (TUDCA), have demonstrated potent anti-inflammatory and hepatoprotective effects in various liver diseases [31,32]. This prompted us to investigate whether specific endogenous bile acids might function as natural inhibitors of pyroptosis during sepsis. In our study, serum BA profiling identified TDCA as one of the most significantly elevated bile acids following LPS challenge. Given that LPS-induced endotoxemia recapitulates key aspects of Gram-negative bacterial toxin exposure and the associated inflammatory cascade, this finding, together with the

observed transcriptional reprogramming of BA metabolism, led us to hypothesize that TDCA may be mobilized as an endogenous protective factor against endotoxin-driven liver injury, potentially through modulation of the caspase-11/GSDMD pathway.

Nevertheless, the specific role of TDCA in sepsis, and particularly its potential interaction with the pivotal caspase-11/GSDMD pyroptosis axis, remains entirely unexplored [33–35]. In the present study, we therefore employed LPS-induced endotoxemia and D-GalN/LPS-induced inflammatory liver injury models to specifically investigate the contribution of TDCA to the hepatic response to Gram-negative bacterial toxins. We aim to elucidate its effects on the caspase-11/GSDMD axis both in vitro and in vivo, and to evaluate its therapeutic potential. Our findings identify TDCA as a key endogenous regulator of pyroptosis and unveil a novel mechanism underlying the liver's self-defense against sepsis, positioning TDCA as a promising therapeutic candidate for LPS-driven liver pathologies.

2. Results

2.1. LPS-Induced Endotoxemia Reprograms Hepatic Bile Acid Metabolism and Elevates Serum TDCA Levels

To investigate the hepatic response to LPS-induced endotoxemia and identify potential endogenous regulatory metabolites, we performed transcriptomic analysis on liver tissues from C57BL/6J mice challenged with LPS (5 mg/kg) for 24 h. The RNA-seq data revealed that LPS challenge induced a profound transcriptional reprogramming in the liver. Gene set enrichment analysis highlighted significant alterations in bile acid (BA) metabolism-related pathways (Figure 1A). Validation via qPCR confirmed that the expression of key genes involved in bile acid synthesis and transport was significantly altered in the LPS-challenged liver compared to controls (Figure 1B).

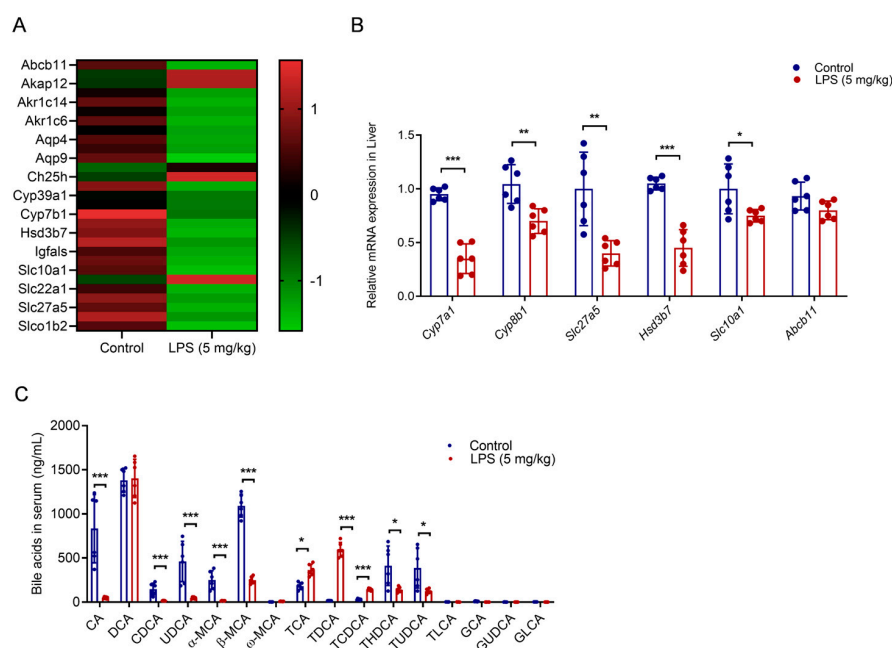


Figure 1. LPS challenge induces hepatic bile acid metabolic reprogramming and significant elevation of circulating TDCA. C57BL/6J mice were intraperitoneally challenged with LPS (5 mg/kg) for 24 h to induce endotoxemia. (A) Gene set enrichment analysis (GSEA) based on liver transcriptomic data highlighting significant alterations in bile acid (BA) metabolism-related pathways. (B) The mRNA expression of key genes involved in BA synthesis and transport was validated by qPCR. (C) Circulating BAs in serum were profiled by targeted quantitative LC-MS/MS. The data are presented as the means $\pm$ SD ($n = 6$). The data are presented as the means $\pm$ SD. Individual data points indicate biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control group (Student's t -test).

Based on these transcriptional changes, we hypothesized that the composition of the BA pool is modulated during endotoxemic stress. We subsequently performed a targeted quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay to profile circulating BAs in serum. Consistent with the transcriptomic data, the metabolomic profile was distinct between the control and LPS-challenged groups. Notably, the mean serum TDCA concentration increased from 15.95 ± 2.46 ng/mL in controls to 594.10 ± 39.85 ng/mL in LPS-challenged mice, corresponding to an ~32-fold elevation (Figure 1C, Table 1). This robust elevation suggests that TDCA mobilization may represent an adaptive host response or an endogenous protective mechanism triggered by LPS-driven endotoxemia, providing hypothesis-generating implications for sepsis-related liver injury that require validation in infection-based sepsis models.

Table 1. Circulating BAs in serum were profiled by targeted quantitative LC-MS/MS, the data are presented as the means $\pm$ SD ($n = 6$).

BAs	Control	LPS (5 mg/kg)
CA	833.36 ± 85.12	48.97 ± 31.75 ***
DCA	1381.54 ± 96.59	1402.51 ± 53.25
CDCA	147.31 ± 99.35	15.68 ± 6.53 ***
UDCA	461.19 ± 31.97	46.16 ± 29.71 ***
α -MCA	250.91 ± 83.65	14.51 ± 6.53 ***
β -MCA	1092.65 ± 42.38	249.04 ± 19.64 ***
ω -MCA	1.84 ± 1.60	8.26 ± 3.91
TCA	181.81 ± 82.17	364.84 ± 92.77 ***
TDCA	15.95 ± 2.46	594.10 ± 39.85 ***
TCDCA	30.40 ± 24.67	141.66 ± 32.73 ***
THDCA	412.32 ± 69.40	139.86 ± 85.09 ***
TUDCA	387.02 ± 93.91	123.50 ± 74.89 ***
TLCA	2.19 ± 0.96	1.57 ± 0.46
GCA	8.37 ± 7.01	1.08 ± 0.58
GUDCA	1.33 ± 1.06	0.36 ± 0.18
GLCA	2.28 ± 1.02	0.82 ± 0.36

Units: ng/mL; *** $p < 0.001$ vs. control group (Student's *t*-test).

2.2. TDCA Attenuates Intracellular LPS-Induced Pyroptosis in BMDMs

Given the pivotal role of macrophage pyroptosis in the pathogenesis of LPS-driven inflammatory responses (including endotoxemia) [36,37], we investigated whether TDCA exerts cytoprotective effects in vitro. First, to rule out potential intrinsic toxicity, we evaluated the viability of BMDMs treated with TDCA using the CCK-8 assay. As shown in Figure 2A, TDCA at concentrations ranging from 25 to 100 μ M exhibited no significant cytotoxicity; thus, these concentrations were selected for subsequent experiments.

We then utilized primary BMDMs transfected with cytosolic LPS via DOTAP to specifically activate the non-canonical inflammasome pathway [38]. Following stimulation for 16 h, BMDMs exhibited signs of extensive lytic cell death. This cytotoxicity was confirmed to be pyroptotic, as it was effectively blocked by the pan-caspase inhibitor z-VAD-FMK (positive control, 50 μ M). Importantly, treatment with TDCA significantly attenuated LPS-induced cell death in a dose-dependent manner. Specifically, TDCA treatment markedly

reduced the release of Lactate LDH, a marker of plasma membrane rupture, with the most pronounced effect observed at 100 μ M (Figure 2B).

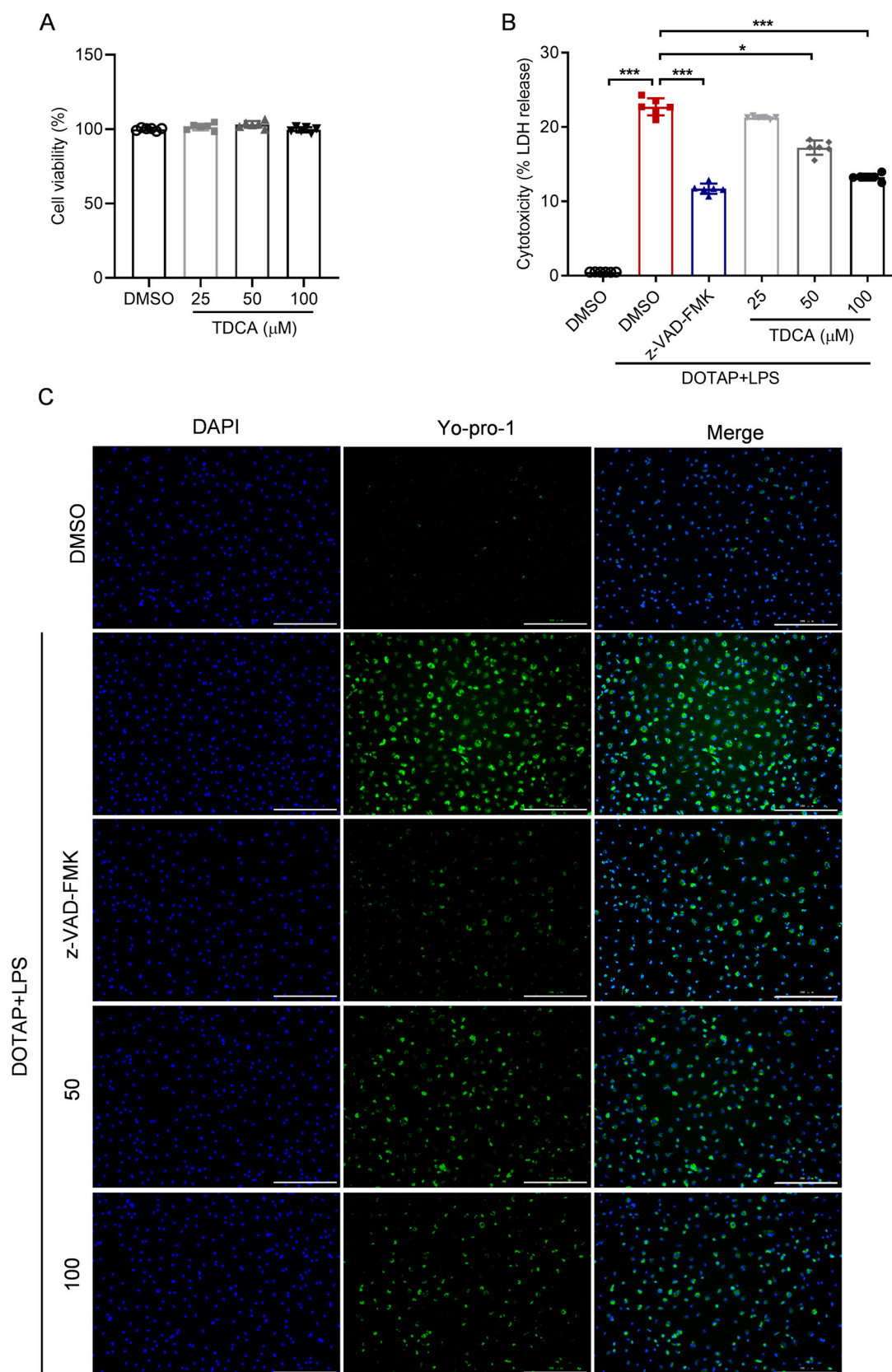


Figure 2. TDCA attenuates non-canonical pyroptosis in BMDMs. (A) The viability of BMDMs treated with increasing concentrations of TDCA (25–100 μ M) for 24 h was assessed using the CCK-8 assay.

(B) BMDMs were first primed overnight with LPS (50 ng/mL). The cells were then pretreated with the indicated concentrations of TDCA (25, 50, or 100 μ M) or the pan-caspase inhibitor z-VAD-FMK (50 μ M) for 30 min, followed by transfection with LPS (1 μ g/mL) using the DOTAP reagent for 16 h. LDH release was measured to evaluate cell death and membrane rupture ($n = 6$). Black Outline (First Bar): Control Group (DMSO). Red Outline: Model Group (DOTAP+LPS + DMSO). Blue Outline: Inhibitor Group (DOTAP+LPS + z-VAD-fmk). Grey/Black Outlines (Last 3 Bars): TDCA Treatment Groups. (C) Representative fluorescence images of YO-PRO-1 uptake indicating membrane pore formation and quantification of YO-PRO-1 positive cells (Scale bars = 200 μ m). DAPI (blue) identifies all nuclei, while Yo-pro-1 (green) labels cells undergoing pyroptosis. Similar results were obtained in at least three independent experiments. The data are presented as the means $\pm$ SD. Individual data points indicate biological replicates. * $p < 0.05$, *** $p < 0.001$ vs. control group (One-way ANOVA).

Consistent with the LDH release data, fluorescence microscopy analysis using the membrane-impermeable dye Oxazole yellow (YO-PRO-1) provided visual confirmation of membrane integrity. While the LPS + DOTAP challenge resulted in a high frequency of YO-PRO-1-positive cells, TDCA intervention significantly decreased the rate of cellular staining (Figure 2C). These findings collectively suggest that TDCA is associated with reduced membrane pore formation and pyroptosis-related cellular injury under non-canonical inflammasome activation.

2.3. TDCA Treatment Is Associated with Reduced Activation of the Caspase-11/GSDMD Signaling Axis In Vitro

To elucidate the molecular mechanism underlying the anti-pyroptotic effect of TDCA, we examined the expression and activation of key proteins in the non-canonical inflammasome pathway using Western blotting. In DOTAP + LPS-stimulated BMDMs, we observed robust activation of caspase-11, as well as the cleavage of GSDMD into its pore-forming N-terminal fragment (GSDMD-NT). Treatment with TDCA (25, 50, and 100 μ M) was associated with decreased the cleavage of caspase-11 and the subsequent generation of GSDMD-NT in a dose-dependent manner (Figure 3A,B).

Furthermore, since GSDMD pore formation facilitates the release of intracellular contents [39], we assessed the processing and secretion of proinflammatory cytokines. Immunoblotting results showed that TDCA treatment markedly reduced the processing of pro-IL-1 β into its mature bioactive form (Figure 3A). Consistent with these intracellular findings, analysis of the cell culture supernatants revealed that TDCA treatment significantly reduced the secretion of both IL-1 β and IL-18 compared to the vehicle-treated group (Figure 3C,D). Collectively, these results support that TDCA attenuates macrophage pyroptosis-associated responses and inflammatory mediator release, consistent with modulation of the Caspase-11/GSDMD signaling axis (Figure 3E), although additional pathway-necessity experiments (e.g., genetic deletion or selective functional inhibition) will be required to establish causality.

2.4. Therapeutic Post-Treatment with TDCA Protects Against D-GalN/LPS-Induced Toxin-Sensitized LPS-Driven Liver Injury and Mortality In Vivo

To validate the therapeutic potential of TDCA in vivo, we employed the D-GalN/LPS-induced model of toxin-sensitized LPS-driven acute inflammatory liver injury [40,41]. Mice were administered TDCA (3 or 6 mg/kg) or the known GSDMD inhibitor disulfiram (50 mg/kg, used as a positive control) via intraperitoneal injection 30 min after the lethal D-GalN/LPS challenge (Figure 4A).

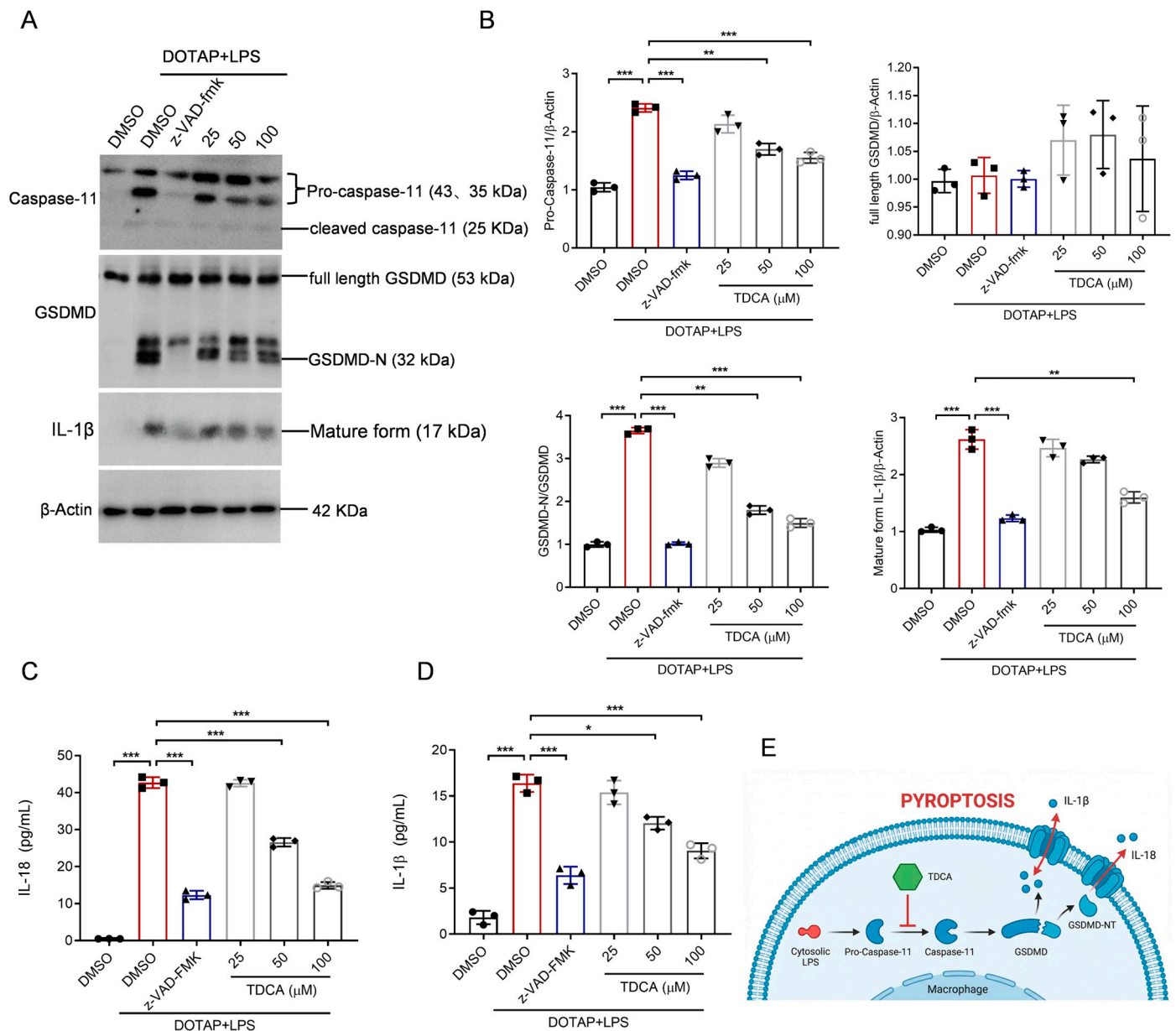


Figure 3. TDCA reduces the activation of the Caspase-11/GSDMD signaling axis and proinflammatory cytokine release in BMDMs. BMDMs were first primed overnight with LPS (50 ng/mL). The cells were then pretreated with the indicated concentrations of TDCA (25, 50, or 100 μ M) or the pan-caspase inhibitor z-VAD-FMK (50 μ M) for 30 min, followed by transfection with LPS (1 μ g/mL) using the DOTAP reagent for 2 h. (A) The protein expression levels of full-length and cleaved Caspase-11, full-length and N-terminal GSDMD (GSDMD-NT), pro-IL-1 β , and mature IL-1 β in cell lysates were analyzed by Western blotting. β -Actin was used as a loading control. (B) Quantitative analysis of the relative protein abundance of cleaved Caspase-11 and GSDMD-NT ($n = 3$ independent experiments). (C,D) The levels of secreted mature IL-1 β (C) and IL-18 (D) in the cell culture supernatants were measured by ELISA. The data are presented as the means $\pm$ SD ($n = 3$). Individual data points indicate biological replicates. Black Outline (First Bar): Control Group (DMSO). Red Outline: Model Group (DOTAP+LPS + DMSO). Blue Outline: Inhibitor Group (DOTAP+LPS + z-VAD-fmk). Grey/Black Outlines (Last 3 Bars): TDCA Treatment Groups. (E) Schematic Illustration of the Mechanism. Cytosolic LPS activates the non-canonical inflammasome pathway, leading to Caspase-11 activation and subsequent cleavage of GSDMD. The resulting GSDMD-NT fragments form pores in the plasma membrane, facilitating the release of IL-1 β and IL-18 and inducing pyroptosis. TDCA (green hexagon) treatment reduced the activation of the caspase-11/GSDMD pathway. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. LPS + DOTAP group (One-way ANOVA).

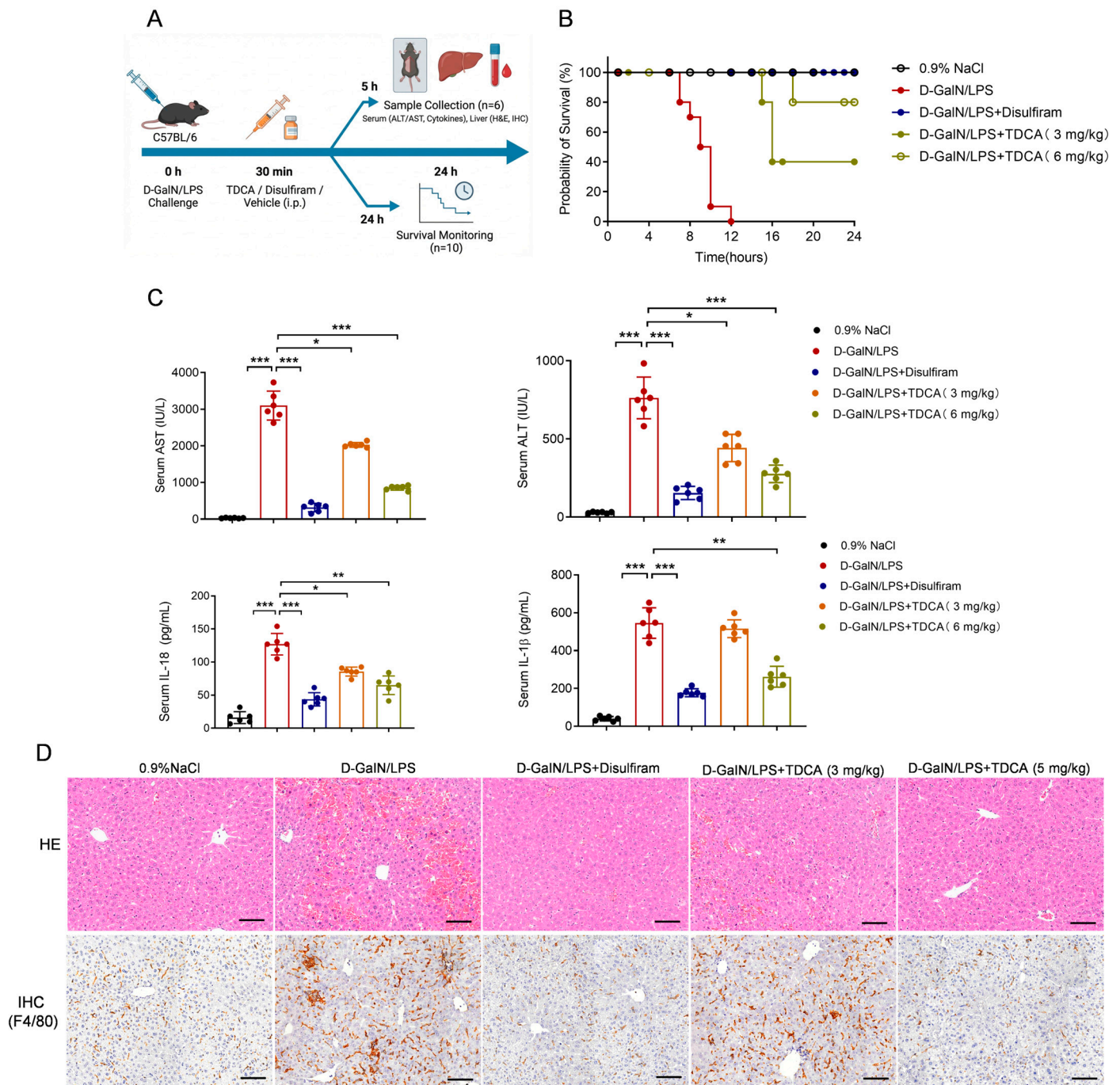


Figure 4. C57BL/6 mice were challenged with D-GalN/LPS to induce toxin-sensitized, LPS-driven acute inflammatory liver injury. **(A)** Thirty minutes post-challenge, mice received an intraperitoneal injection of vehicle, TDCA (3 or 6 mg/kg), or the GSDMD inhibitor disulfiram (50 mg/kg). **(B)** Kaplan–Meier survival curves monitored over 24 h after challenge ($n = 10$ per group). The significance of survival differences was determined by the log-rank test. **(C)** Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated to assess hepatocellular injury; the serum concentrations of pro-inflammatory cytokines IL-1 β and IL-18 were quantified using ELISA kits. **(D)** Representative hematoxylin and eosin (H&E)-stained liver tissue sections showing hepatic necrosis, hemorrhage, and inflammatory cell infiltration; representative immunohistochemical staining images showing F4/80-positive macrophage infiltration in liver sections, Scale bars = 50 μ m. Data in **(C)** are presented as the means $\pm$ SD ($n = 6$). Individual data points indicate biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. the D-GalN/LPS model group (analyzed by one-way ANOVA followed by Tukey’s post hoc test).

Survival analysis revealed that the vehicle-treated D-GalN/LPS-challenged mice succumbed rapidly to the challenge. In contrast, post-treatment with TDCA significantly improved survival rates in a dose-dependent manner, with survival increasing to 40% in the low-dose group (3 mg/kg) and 80% in the high-dose group (6 mg/kg). Notably, post-treatment with the positive control disulfiram resulted in 100% survival (Figure 4B).

We further assessed the extent of acute liver injury at 5 h post-challenge. Serum biochemical analysis showed that TDCA treatment significantly blunted the sharp elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels induced by D-GalN/LPS, indicating preserved hepatocellular integrity (Figure 4C). Additionally, TDCA treatment significantly reduced the systemic levels of key pro-inflammatory cytokines, including IL-1 β and IL-18 (Figure 4C). Histopathological examination of liver sections via H&E staining corroborated these findings; while the model group displayed extensive hepatic necrosis, hemorrhage, and massive inflammatory cell infiltration, TDCA treatment substantially alleviated these pathological changes and preserved the hepatic architecture (Figure 4D). Furthermore, immunohistochemical (IHC) staining for F4/80, a macrophage marker, revealed extensive macrophage recruitment into the inflamed livers of the model group. However, this recruitment was significantly suppressed by TDCA treatment (Figure 4D).

2.5. TDCA Reduces Hepatic Caspase-11/GSDMD Activation In Vivo

Finally, to examine whether TDCA treatment is associated with altered activation of the pyroptotic pathway identified in our *in vitro* studies, we analyzed liver tissue homogenates from the D-GalN/LPS-induced toxin-sensitized, LPS-driven acute inflammatory liver injury model. Western blot analysis revealed that the D-GalN/LPS-challenged livers were characterized by high levels of cleaved caspase-11, GSDMD-NT, and mature IL-1 β . Consistent with the *in vitro* findings, TDCA administration (6 mg/kg) was associated with a significant reduction in caspase-11 activation and the cleavage of GSDMD and IL-1 β in the liver tissues (Figure 5A,B). These data provide *in vivo* evidence that TDCA functions as an endogenous regulator that ameliorates sepsis-associated liver injury by inhibiting the Caspase-11/GSDMD pyroptosis pathway. Together, these results indicate that TDCA treatment is associated with reduced hepatic activation of the Caspase-11/GSDMD axis in this LPS-driven model of inflammatory liver injury, supporting (but not proving) a role for pathway modulation.

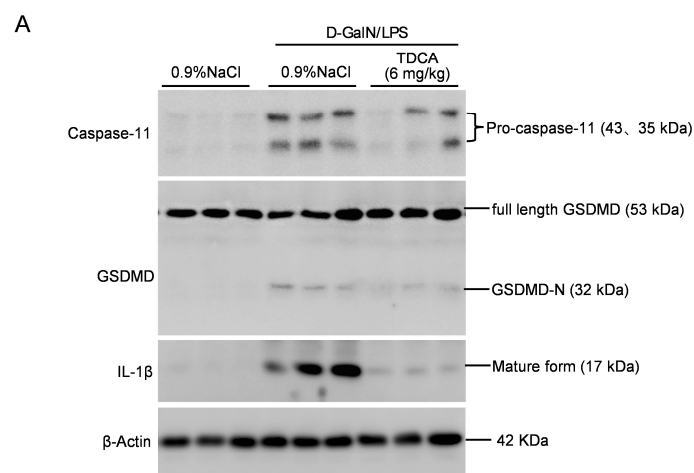


Figure 5. Cont.

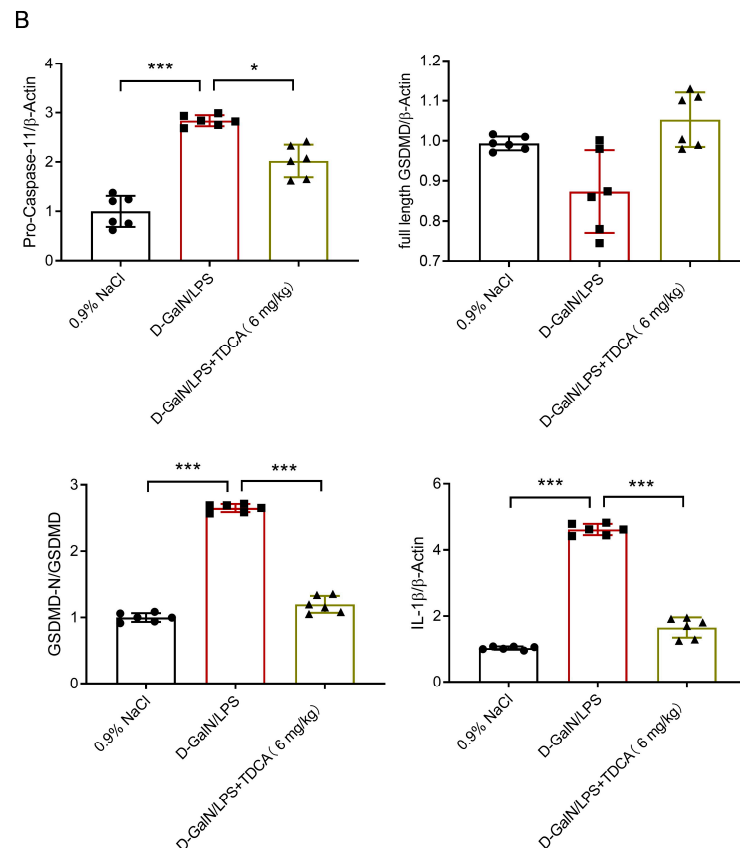


Figure 5. TDCA reduces the activation of the Caspase-11/GSDMD pyroptotic pathway in D-GalN/LPS-induced toxin-sensitized, LPS-driven acute inflammatory liver injury liver tissues. C57BL/6 mice were challenged with D-GalN/LPS to induce toxin-sensitized, LPS-driven acute inflammatory liver injury and treated with TDCA (6 mg/kg) or vehicle 30 min post-challenge. Liver tissues were collected at 5 h post-challenge for analysis. (A) Representative Western blot images showing the protein expression levels of pro-Caspase-11, full-length GSDMD and its N-terminal fragment (GSDMD-NT), and pro- and mature IL-1 β in liver tissue homogenates. Densitometric analysis of pro-caspase-11 was performed by summing the upper and lower pro-caspase-11 bands for each lane; β -Actin was used as a loading control. (B) Densitometric quantification of the relative protein abundance of cleaved Caspase-11, GSDMD-NT, and mature IL-1 β , normalized to β -Actin. Data are presented as mean $\pm$ SD ($n = 3$ per group). Individual data points indicate biological replicates. Black Outline (Bar 1): Control Group (0.9% NaCl). Red Outline (Bar 2): Model Group (D-GalN/LPS). Olive Green Outline (Bar 3): Treatment Group (D-GalN/LPS + TDCA 6 mg/kg). * $p < 0.05$, *** $p < 0.001$ vs. the D-GalN/LPS model group (one-way ANOVA followed by Tukey's post hoc test).

3. Discussion

In this study, we uncovered a novel endogenous protective mechanism orchestrating the liver's defense against endotoxemic stress. Using LPS-induced endotoxemia and D-GalN/LPS-induced toxin-sensitized, LPS-driven acute inflammatory liver injury models, which model selected inflammatory features of Gram-negative bacterial toxin-driven responses [30,42,43], we demonstrated that LPS challenge triggers a profound reprogramming of hepatic bile acid metabolism, resulting in a specific and dramatic elevation of serum TDCA. Crucially, we observed that TDCA treatment attenuated readouts consistent with reduced activation of the non-canonical inflammasome pathway. We found that TDCA treatment was associated with reduced Caspase-11 activation and GSDMD cleavage, thereby preventing macrophage pyroptosis and systemic cytokine storms. Consequently, exogenous administration of TDCA significantly improved survival and reduced hep-

atic damage in a lethal mouse model of toxin-sensitized endotoxemic liver injury. These findings suggest that the metabolic shift toward TDCA synthesis during LPS-driven endotoxemia is not merely a pathological byproduct, but an adaptive host response designed to limit excessive inflammation and organ injury, with broader implications for sepsis that remain hypothesis-generating.

The liver is the central hub for metabolic and immune integration [6]. While cholestasis and dysregulated bile acid (BA) metabolism are well-documented complications of sepsis and endotoxemia, the functional consequences of specific BA alterations remain largely obscure [9,34,44]. Our transcriptomic and targeted bile acid LC-MS/MS analyses showed that serum TDCA increased from 15.95 ± 2.46 ng/mL in controls to 594.10 ± 39.85 ng/mL in LPS-challenged mice (~32-fold, Table 1; Figure 1C). Given that LPS-induced endotoxemia selectively models the inflammatory cascade triggered by Gram-negative bacterial toxins [30,45], this aligns with recent concepts suggesting that “sickness behavior” involves metabolic rewiring to support host survival [46,47]. Unlike hydrophobic bile acids often associated with cytotoxicity, our data posits TDCA, in the context of acute LPS-driven endotoxemia, as a “danger response” metabolite. This observation parallels findings with other protective metabolites, such as itaconate, which accumulate during macrophage activation to dampen inflammation. However, the specific mobilization of TDCA identifies a liver-specific axis of immunometabolic regulation previously unrecognized in LPS-driven inflammatory liver injury. Importantly, the selective mobilization of TDCA highlights a liver-specific immunometabolic adaptation to endotoxin-driven injury rather than a generalized feature of all septic states.

A key finding of our work is the association between TDCA treatment and reduced activation of the Caspase-11/GSDMD signaling axis. Pyroptosis driven by intracellular LPS sensing via Caspase-11 is a hallmark of Gram-negative sepsis and a major contributor to septic shock and organ failure [25,48,49]. While previous studies have highlighted the anti-inflammatory properties of hydrophilic BAs like TUDCA, often attributing them to endoplasmic reticulum stress reduction [50], our study provides evidence consistent with a mechanism involving modulation of pyroptosis. By reducing the cleavage of Caspase-11 and GSDMD, TDCA effectively “plugs” the membrane pores, preventing the lytic release of LDH and key proinflammatory cytokines (IL-1 β and IL-18). This blockade breaks the vicious cycle of inflammatory cell death and recruitment that characterizes the progression of LPS-driven inflammatory liver injury. Notably, genetic or selective functional inhibition studies will be required to establish pathway necessity and causality.

Mechanistically, the anti-pyroptotic effects of TDCA may be mediated through bile acid-responsive receptors, particularly the G protein-coupled bile acid receptor TGR5 (GPBAR1), which is highly expressed in Kupffer cells and also present in cholangiocytes and hepatocytes [51–53]. Activation of TGR5 by bile acids increases intracellular cAMP levels and triggers protein kinase A (PKA) signaling [54,55], leading to suppression of NF- κ B-dependent inflammatory transcription and attenuation of mitochondrial reactive oxygen species (mtROS) production [55,56]. Given that mtROS is a critical upstream driver of NLRP3 inflammasome activation [57,58], TGR5-dependent cAMP-PKA signaling provides a mechanistic link between TDCA sensing and inflammasome restraint [55,56]. In line with this model, recent work suggests that TDCA, via a TGR5-dependent cAMP-PKA axis, orchestrates multi-level inhibition of both canonical and non-canonical inflammasome pathways, thereby preventing GSDMD-mediated pyroptosis and the ensuing cytokine storm during sepsis [59]. Although receptor-specific validation was not directly performed in the present study, our findings are consistent with this immunometabolic signaling framework and support a model in which TDCA may limit caspase-11/GSDMD-driven

pyroptosis by dampening inflammatory and mitochondrial stress responses in hepatic immune cells [27,55,59].

Importantly, the effects of bile acids on pyroptosis are highly context-dependent and vary across different tissues, cell types, and disease states [60]. Emerging evidence indicates that bile acids can exert either pro- or anti-pyroptotic effects depending on their chemical properties, concentration, target cells, and the inflammatory microenvironment [60,61]. For example, certain bile acids have been shown to promote inflammasome activation in intestinal epithelial cells or cholangiocytes during chronic injury [44], whereas hydrophilic bile acids such as TUDCA display protective effects in hepatic and metabolic inflammation [55]. In the present study, we demonstrate an anti-pyroptotic role of TDCA specifically in LPS-driven inflammatory liver injury models, primarily through modulation of macrophage-mediated inflammatory responses [27]. Therefore, the protective effects of TDCA should be interpreted within the pathological context of acute endotoxemic challenge and hepatic immunometabolic stress, rather than generalized to other disease settings [60,61].

The translational potential of these findings is underscored by our *in vivo* experiments. In the D-GalN/LPS model, which is a commonly used toxin-sensitized endotoxemia model of fulminant inflammatory liver injury, TDCA administration conferred a remarkable survival benefit (up to 80%) and preserved liver architecture. The reduction in serum ALT/AST and the suppression of hepatic neutrophil infiltration correlate well with the reduced formation of GSDMD-N formation observed in liver tissues. This is consistent with a scenario in which TDCA acts locally within the hepatic microenvironment to preserve hepatocyte and Kupffer cell integrity. Given that no specific therapies currently exist for sepsis-related liver injury other than supportive care, TDCA—or synthetic analogs optimized for this pathway—represents a promising therapeutic candidate, pending validation in clinically relevant polymicrobial sepsis models.

Despite these promising results, our study has limitations that warrant further investigation. First, BMDMs were used for *in vitro* mechanistic analyses. Although Kupffer cells are the resident macrophages of the liver and play a pivotal role in hepatic immune homeostasis, severe sepsis or endotoxemia is characterized by extensive recruitment of circulating monocytes that differentiate into inflammatory macrophages within the injured liver [62,63]. In this regard, BMDMs represent a widely accepted model of recruited macrophages during systemic inflammatory challenge; however, they do not fully capture the phenotypic and functional heterogeneity of hepatic macrophage populations [63]. Future studies employing primary Kupffer cells or liver-relevant macrophage cell lines (e.g., KUP5, RAW264.7, or THP-1) will be important to further validate our findings. Second, while we demonstrate that TDCA treatment is associated with reduced Caspase-11 activation and downstream pyroptosis, the precise upstream molecular mechanisms remain to be elucidated. It is currently unclear whether TDCA directly interacts with Caspase-11 or modulates this pathway indirectly via bile acid-responsive receptors, such as TGR5 or the nuclear receptor FXR [64,65]. Importantly, experiments establishing pathway necessity (e.g., genetic deletion or selective functional inhibition) are needed to confirm causality. Third, although LPS-based models are valuable for dissecting Gram-negative toxin-driven inflammatory pathways, they do not fully recapitulate the complexity of polymicrobial sepsis [66]. Future studies employing infection-based models, such as cecal ligation and puncture (CLP), will be necessary to determine whether the protective effects of TDCA extend beyond endotoxemic injury. Finally, species-specific differences in bile acid composition between mice and humans should be considered [67]. Although TDCA is a major component of the human bile acid pool, validation of our findings in human sepsis co-

horts will be necessary to determine the translational relevance of TDCA as a therapeutic candidate for sepsis-related liver injury.

In conclusion, our study characterizes a previously unknown “liver-protection axis” wherein LPS-induced metabolic reprogramming elevates circulating TDCA levels to counteract hyperinflammation. By identifying TDCA as an intrinsic immunometabolic regulator associated with dampened Caspase-11/GSDMD pyroptosis pathway, we provide a mechanistic link between hepatic metabolism and innate immunity. These insights not only deepen our understanding of host tolerance mechanisms but also highlight TDCA as a candidate strategy for LPS-driven inflammatory liver injury, with hypothesis-generating implications for sepsis-associated liver injury.

4. Materials and Methods

4.1. Chemicals and Reagents

TDCA was purchased from Shanghai yuanye Bio-Technology Co., Ltd. (Shanghai, China) (CAS: 1180-95-6; Cat. No.: S30708). Lipopolysaccharide (LPS, *E. coli* O111:B4) and D-Galactosamine (D-GalN) were obtained from Sigma-Aldrich St. Louis, MO, USA (LPS CAS: 93572-42-0; Cat. No.: L2630; D-GalN CAS: 59-23-4; Cat. No.: G5388). z-VAD-FMK was purchased from Med Chem Express LLC (Monmouth Junction, NJ, USA) (CAS: 161401-82-7; Cat. No.: HY-16658B). DOTAP Liposomal Transfection Reagent was purchased from Roche (Basel, Switzerland) (Cat. No.: 11202375001). Antibodies against Caspase-11, GSDMD, IL-1 β , and β -actin were purchased from Abcam (Cambridge, UK) (Caspase-11 Cat. No.: ab180673; GSDMD Cat. No.: ab209845; IL-1 β Cat. No.: ab234437; β -actin Cat. No.: AB8227). ELISA kits for IL-1 β and IL-18 were obtained from Neo Bioscience Technology (Shenzhen, China) (IL-1 β ELISA Cat. No.: EMC001b.96; IL-18 ELISA Cat. No.: EMC011.96). CCK-8 kit (Cat. No.: C0042), LDH Cytotoxicity Assay Kit (Cat. No.: C0019M), and YO-PRO-1 dye (Cat. No.: C1075M) were purchased from Beyotime Biotech (Shanghai, China).

TDCA was dissolved in DMSO for in vitro and saline for in vivo experiments. For all in vitro experiments, the final DMSO concentration was kept at 0.05% (*v/v*) and was identical in TDCA-treated and vehicle control groups. For in vivo administration, TDCA was prepared in sterile saline (no DMSO).

4.2. Animals and Experimental Establishment

Male C57BL/6J mice (6–8 weeks old) were purchased were obtained from the Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). The animals were housed in a specific pathogen-free (SPF) facility under a 12 h light/dark cycle with free access to food and water. The animal experiments protocol was approved by the local ethics committee under a project license granted by the Institutional Animal Care and Use Committee of Yunnan University of Chinese Medicine (protocol code YNUCM-XMSB-S-20241689 and 24 January 2024), and performed in accordance with the National Institutes of Health (NIH) guide for the care and use of laboratory animals.

Mice were allocated to groups by random number table. Investigators responsible for histology and immunohistochemistry (IHC) image acquisition/quantification were blinded to group allocation using coded samples. Sample sizes were chosen based on prior literature and feasibility for these models (survival: $n = 10$ /group; biochemical and histology endpoints: $n = 6$ /group), and all stated n values represent biological replicates.

LPS-induced Endotoxemia Model: To investigate the alteration of bile acid metabolism, mice were randomly assigned and intraperitoneally (i.p.) injected with LPS (5 mg/kg) challenge ($n = 6$ per group). Liver tissues and serum were collected 24 h post-challenge for RNA-seq analysis and targeted metabolomics (LC-MS/MS), respectively.

D-GalN/LPS-induced Toxin-Sensitized, LPS-Driven Acute Inflammatory Liver Injury Model: Mice were randomly divided into control, model, and treatment groups. Toxin-sensitized, LPS-driven acute inflammatory liver injury was induced by simultaneous intraperitoneal injection of D-GalN (250 mg/kg) and LPS (25 µg/kg). For the intervention groups, TDCA (3 mg/kg or 6 mg/kg) or GSDMD inhibitor disulfiram (50 mg/kg, used as a positive control) was administered intraperitoneally 30 min after the D-GalN/LPS challenge. Mortality was monitored for 24 h after the challenge ($n = 10$ per group). In a separate cohort, mice were sacrificed 5 h post-challenge ($n = 6$ per group). Serum and liver tissues were harvested for biochemical analysis, ELISA, Western blotting, and histopathological examination.

4.3. Transcriptomic Analysis (RNA-Seq)

Total RNA was extracted from liver tissues of control and LPS-challenged mice using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA integrity was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). cDNA libraries were constructed and sequenced on an Illumina NovaSeq platform (Illumina, Inc., San Diego, CA, USA). Raw data were filtered and mapped to the reference genome. Differential expression analysis was performed using the DESeq2 R package (<https://bioconductor.org/packages/release/bioc/html/DESeq2.html>, accessed on 2 May 2024). Gene Set Enrichment Analysis (GSEA) was conducted to identify significantly enriched pathways, with a focus on bile acid metabolism-related gene sets. The RNA sequencing was performed by OE Biotech Co., Ltd. (Shanghai, China).

4.4. RNA Extraction and RT-qPCR

Total RNAs from different experimental groups were extracted using an RNA Faster200 reagent (Fastagen, Shanghai, China) according to the manufacturer's instructions. RNA was reverse transcribed to generate first strand cDNAs by using the PrimeScript™ RT Master Mix Kit (Takara Bio, Shiga, Japan). The reaction conditions were 37 °C for 15 min and 85 °C for 5 s. The RT-PCR analysis was performed using an SYBR Premix Ex Taq™ (Tli RNaseH Plus) Kit (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. The expression levels of bile acid synthesis-related genes were normalized to *Gapdh*. Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method. The primer sequences used for RT-qPCR are listed in Table 2, along with their corresponding GenBank accession numbers and expected amplicon lengths.

Table 2. Primer sequences and parameters used for RT-qPCR validation.

Gene	Accession No.	Forward Primer (5'→3')	Reverse Primer (5'→3')	Amplicon Length (bp)
<i>Grapdh</i>	NM_001411842.1	GGCCGAGAATGGGAAGCTTGT	ACATACTCAGCACCGGCCTCA	102
<i>Cyp7a1</i>	NM_007824.3	GTACCTTGATGAAAGTGGGAA	ATTGCTTGATTCTTGGACAG	137
<i>Cyp8b1</i>	NM_010012.3	CTGGACAAGGGTTTTGTGCC	CCCGCATCCCCTTCAAGAAT	82
<i>Slc27a5</i>	NM_009512.2	CTACGCTGGCTGCATATAGATG	CCACAAAGGTCTCTGGAGGAT	103
<i>Hsd3b7</i>	NM_001040684.2	GGGAGCTGCGTGTCTTTGA	GTGGATGGTCTTTGGACTGGC	197
<i>Slc10a1</i>	NM_001361972.1	CAAACCTCAGAAGGACCAAACA	GTAGGAGGATTATCCCGTTGTG	108
<i>Abcb11</i>	NM_021022.3	TCTGACTCAGTGATTCTTCGCA	CCCATAAACATCAGCCAGTTGT	191

4.5. Targeted Metabolomics of Bile Acids by UPLC–MS/MS

Targeted serum bile acid profiling by UPLC–MS/MS was performed using a workflow adapted from our previously developed method [68], with study-specific details provided below.

Targeted quantification of BAs in serum was performed using UPLC coupled to a triple-quadrupole mass spectrometer (AB SCIEX QTRAP 6500, AB SCIEX, Framingham, MA, USA) operated in multiple reaction monitoring (MRM) mode. A panel of 24 bile acids was included in the analytical method; however, only 16 bile acids (CA, DCA, CDCA, UDCA, α -MCA, β -MCA, ω -MCA, TCA, TDCA, TCDCA, THDCA, TUDCA, TLCA, GCA, GUDCA, GLCA) were consistently detected and quantified above the LLOQ in mouse serum under the present experimental conditions and are therefore displayed in Figure 1.

4.5.1. Standards, Internal Standard, Calibration Curves, and Quality Controls

Stock solutions of the 24 bile acid standards were prepared at 1 mg/mL in methanol. A single isotope-labeled internal standard, CA-d4, was used for quantification (CA-d4, [vendor/cat. no. if available]). CA-d4 working solution (100 ng/mL) was prepared in 50% methanol–water, stored at -20°C in glass vials, and used throughout the study.

Calibration standards were prepared by mixing each BA working solution with an equal volume of the CA-d4 internal standard solution (final 100 ng/mL CA-d4). Calibration curves were constructed over 0.006–4000 ng/mL using the analyte/CA-d4 peak-area ratio versus concentration and $1/x^2$ weighted least-squares regression (MultiQuant V3.0.1). Quality control (QC) samples were prepared by spiking blank plasma with BA standards to yield final concentrations of 10 ng/mL (LQC), 200 ng/mL (MQC), and 2000 ng/mL (HQC) for each analyte.

4.5.2. Sample Collection and Pretreatment

Prior to analysis, serum samples (100 μL) were protein-precipitated with 300 μL methanol, vortexed for 1 min, and centrifuged at $12,000\times g$ for 15 min at 4°C . A 300 μL aliquot of the supernatant was transferred to a clean tube and evaporated to dryness under a gentle nitrogen stream. The residue was reconstituted in 100 μL CA-d4 internal standard solution (100 ng/mL), vortexed for 1 min, and centrifuged under the same conditions. Finally, 80 μL of the supernatant was transferred to an autosampler vial for UPLC–MS/MS analysis.

4.5.3. UPLC and MS/MS Conditions

Chromatographic separation was performed using a Waters CORTECS UPLC C18 column (2.1 mm $\times$ 100 mm, 1.6 μm). The column temperature was set to 45°C , with a flow rate of 0.3 mL/min and an injection volume of 5 μL . The mobile phases were A: 0.01% formic acid in water and B: acetonitrile. The gradient elution program was as follows: 0–12 min, 23–38% B; 12–26 min, 38–75% B; 26–28 min, 75–100% B; 28–32 min, 23% B.

Mass spectrometry was performed on an AB SCIEX QTRAP 6500 equipped with an electrospray ionization (ESI) source and operated in negative-ion MRM mode. Source parameters were set as follows: IonSpray Voltage (IS), -4500 V ; Curtain Gas (CUR), 35 psi; Ion Source Gas 1 (GS1), 55 psi; Ion Source Gas 2 (GS2), 55 psi; Temperature (TEM), 500°C . For each bile acid and CA-d4, the MRM transitions ($\text{Q1} \rightarrow \text{Q3}$), collision energies, and retention times are provided in Table 3.

Table 3. For each bile acid and CA-d4, the MRM transitions ($\text{Q1} \rightarrow \text{Q3}$), collision energies, and retention times.

Compound	Q1 Mass (Da)	Q3 Mass (Da)	DP (Volts)	CE (Volts)	CXP (Volts)	RT (Min)
TUDCA	498.3	79.9	−156	−149	−34	7.55
THDCA	498.3	79.9	−150	−155	−9	7.75
TCA	514.3	79.9	−134	−156	−12	8.14
GUDCA	448.3	74	−110	−87	−11	10.21

Table 3. Cont.

Compound	Q1 Mass (Da)	Q3 Mass (Da)	DP (Volts)	CE (Volts)	CXP (Volts)	RT (Min)
ω MCA	407.3	79.9	−110	−104	−11	10.19
β MCA	407.3	79.9	−120	−112	−12	11.32
α MCA	407.3	79.9	−120	−112	−12	10.72
GLCA	432.3	74	−174	−74	−32	18.80
GCA	464.3	74	−163	−91	−11	10.47
TCDCA	498.3	79.9	−150	−155	−9	11.91
TDCA	498.3	79.9	−150	−155	−9	12.78
CA	407.3	79.9	−110	−154	−11	14.13
UDCA	391.3	74	−110	−132	−12	14.55
TLCA	482.3	79.9	−101	−148	−19	16.48
CDCA	391.3	74	−110	−154	−11	17.91
DCA	391.3	74	−120	−132	−12	18.35
Cholic-d4 acid (IS)	411.3	79.9	−110	−154	−11	14.50

4.5.4. Method Validation

The analytical method was validated for linearity, limits of detection (LOD), limits of quantification (LOQ/LLOQ), precision, accuracy, stability, carry-over, matrix effects, and recovery, following our previously established targeted bile acids metabolomics workflow (Wei et al., [68]). A single isotopically labeled internal standard, CA-d4, was used as a universal internal standard for response normalization. CA-d4 (100 ng/mL) was added to calibration standards, QC samples, and study samples at a fixed level prior to analysis, and quantitative responses were calculated using analyte/CA-d4 peak-area ratios.

Calibration curves were constructed over 0.006–4000 ng/mL by plotting analyte/CA-d4 peak-area ratios versus nominal concentrations and fitting with $1/x^2$ weighted least-squares linear regression. The LLOQ was defined as the lowest calibration point meeting $RSD < 15\%$, accuracy within $\pm 20\%$, and $S/N \geq 10:1$, whereas the LOD was defined as $S/N \geq 3:1$.

Intra-day precision and accuracy were evaluated using six replicates ($n = 6$) at LQC/MQC/HQC within one day, and inter-day precision and accuracy were assessed over 48 h. Stability was assessed using MQC samples ($n = 6$) stored at 4 °C in the dark for 24 h and 48 h. Carry-over was evaluated by injecting a blank (methanol) after HQC injections ($n = 6$) and comparing the blank response with that of the preceding HQC. Matrix effects were evaluated using matrix-factor comparisons with responses normalized to CA-d4, and recovery was determined by comparing analyte/CA-d4 peak-area ratios for samples spiked before extraction versus after extraction at three QC levels ($n = 6$).

4.6. Cell Culture and Isolation of BMDMs

Bone marrow-derived macrophages (BMDMs) were isolated from the tibias and femurs of C57BL/6J mice. Cells were differentiated in DMEM supplemented with 10% FBS and 20 ng/mL M-CSF (macrophage colony-stimulating factor, Cat. No.: 315-02-50UG, Proteintech Group, Inc., Rosemont, IL, USA) for 7 days.

To activate the non-canonical inflammasome pathway, BMDMs were first primed overnight with LPS (50 ng/mL). The cells were then pretreated with the indicated concentrations of TDCA (25, 50, or 100 μ M) or the pan-caspase inhibitor z-VAD-FMK (50 μ M) for 30 min, followed by transfection with LPS (1 μ g/mL) using the DOTAP reagent. All treatment and vehicle control groups contained the same final concentration of DMSO (0.05%, *v/v*).

4.7. Cell Viability Assay, Cell Death Assays LDH Release Assay and YO-PRO-1 Staining

The cytotoxicity of TDCA on BMDMs was evaluated using the CCK-8 assay (Beyotime Biotech Inc., Haimen, China, Cat. No.: C0042). BMDMs were seeded in 96-well plates and treated with increasing concentrations of TDCA (0, 25, 50, 75, and 100 μ M) for 24 h. CCK-8 solution was added to each well, and absorbance was measured at 450 nm using a microplate reader.

Cell membrane integrity was assessed by measuring LDH release in the culture supernatants using an LDH Cytotoxicity Assay Kit according to the manufacturer's instructions (Beyotime Biotech Inc., Cat. No.: C0019M). For the visualization of membrane pore formation, cells were stained with the membrane-impermeable fluorescent dye YO-PRO-1 (Beyotime Biotech Inc., Cat. No.: C1075M). Images were captured using a fluorescence microscope (IX73; Olympus, Tokyo, Japan), and the percentage of YO-PRO-1 positive cells was quantified.

4.8. Western Blotting

Total protein was extracted from liver tissues or BMDMs using RIPA lysis buffer containing protease and phosphatase inhibitors (Thermo Fisher Scientific, Waltham, MA, USA). Protein samples were separated by SDS-PAGE and transferred onto PVDF membranes. After blocking, membranes were incubated overnight at 4 °C with primary antibodies against Caspase-11, GSDMD, IL-1 β , and β -actin. The membranes were then incubated with HRP-conjugated secondary antibodies and visualized using an ECL detection system (ECL detection kit, Thermo Fisher Scientific, Waltham, MA, USA; imaging system, Tanon Science & Technology, Shanghai, China). The bands were quantified using ImageJ software (version 1.53t; National Institutes of Health, Bethesda, MD, USA; <https://imagej.nih.gov/ij/>, accessed on 15 June 2026).

4.9. Biochemical Analysis and Enzyme-Linked Immunosorbent Assay (ELISA)

Serum Analysis: Serum levels of ALT and AST were measured using commercial assay kits according to the manufacturer's protocols (Nanjing Jiancheng Bioengineering Institute, ALT Cat. No.:C009-2-1; AST Cat. No.: C010-2-1). **Cytokine Measurement:** The levels of IL-1 β and IL-18 in serum were quantified using specific ELISA kits following the manufacturer's instructions. ELISA kits for IL-1 β and IL-18 were obtained from Neo Bioscience Technology (Shenzhen, China) (IL-1 β ELISA Cat. No.: EMC001b.96; IL-18 ELISA Cat. No.: EMC011.96).

4.10. Histopathology and Immunohistochemistry (IHC)

Liver tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned (4 μ m thickness). Sections were stained with Hematoxylin and Eosin (H&E) for pathological evaluation. For IHC, sections were deparaffinized, rehydrated, and subjected to antigen retrieval. Sections were incubated with anti-F4/80 antibody to label macrophages, followed by HRP-conjugated secondary antibody incubation and DAB staining. Images were acquired using a light microscope.

All histology and IHC image acquisition and quantification were performed with investigators blinded to group allocation using coded slides. Where quantification was performed, F4/80-positive area/cell counts were quantified using ImageJ (NIH) with consistent thresholding parameters across groups.

4.11. Statistical Analysis

Data are presented as Mean $\pm$ Standard Deviation (SD). Statistical differences between two groups were analyzed using Student's *t*-test. Comparisons among multiple groups

were performed using One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. Survival curves were analyzed using the Kaplan–Meier method and the Log-rank test. A p -value < 0.05 was considered statistically significant. Analysis was performed using GraphPad Prism software (Version 8.0).

5. Conclusions

LPS-driven endotoxemia triggers a profound metabolic reprogramming in the liver, characterized by significant alterations in bile acid synthesis and a dramatic upregulation of serum TDCA. Our study reveals that this elevation of TDCA is not merely a metabolic bystander but represents a potential endogenous self-defense mechanism against LPS-driven inflammatory liver injury. Mechanistically, TDCA treatment was associated with reduced activation of the non-canonical inflammasome, specifically attenuating caspase-11/GSDMD-mediated macrophage pyroptosis and the subsequent cytokine storm. Pharmacological administration of TDCA recapitulated protective effects in toxin-sensitized endotoxemia models, significantly improving survival and ameliorating hepatic dysfunction and structural damage in LPS-driven models. The identification of the TDCA-caspase-11-GSDMD axis highlights the pivotal crosstalk between hepatic metabolism and innate immunity. These findings provide novel insights into the liver's intrinsic capacity to limit endotoxin-driven injury and position TDCA as a promising therapeutic candidate for the management of LPS-driven inflammatory liver injury, with broader implications for sepsis remaining hypothesis-generating and requiring validation in infection-based models and pathway-necessity studies.

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Conflicts of Interest: The authors declare no conflicts of interest.

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