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Bacteria-released outer membrane vesicles promote disseminated intravascular coagulation



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ABSTRACT

Introduction: Sepsis is frequently complicated by disseminated intravascular coagulation (DIC), which promotes multiple organ dysfunctions and significantly increase the mortality of patients with sepsis. How bacteria cause DIC is not fully understood. Outer membrane vesicles (OMVs) are membrane-enclosed microvesicles released by variety of bacteria. The aim of this study is to determine whether OMVs contribute to the pathogenesis of DIC during bacterial infection.

Methods: Wild-type (WT) or Toll-like receptor 4 (TLR4) knock-out mice were intraperitoneally injected with purified *Escherichia coli* (*E.coli*) derived OMVs, or with either wild type *E.coli* or *E.coli* with genetic deletion of *ypjA*, which is critical for OMV's production. Blood samples, liver and lung tissues were collected. The development of DIC was assessed in terms of the occurrence of coagulopathy, the thrombi deposition in livers and lungs, the multiple organ injuries, and the lethality.

Results: Genetic deletion of *ypjA* significantly attenuated *E.coli*-induced coagulopathy, intravascular thrombi deposition, multiple organ injuries and mortality, whereas injection of purified *E.coli*-derived OMVs resulted in the development of DIC in a TLR4-dependent manner.

Conclusions: OMVs importantly contribute to the pathogenesis of DIC during Gram-negative bacterial infection. These findings might open a new avenue to prevent infection-associated coagulopathy by targeting OMVs production.

1. Introduction

Sepsis is often associated with coagulopathy, in its most severe form, it is manifested as disseminated intravascular coagulation (DIC) [1–4]. DIC is featured by the systemic activation of coagulation, which leads to intravascular formation of fibrin and ultimately thrombotic occlusion of small vessels [1–4]. These events can compromise the blood supply to organs and subsequently promote multiple organ dysfunctions. At the same time, the use and subsequent depletion of platelets and coagulation factors resulting from the systemic activation of

coagulation system can induce severe bleeding [1]. The occurrence of DIC significantly increases the mortality in sepsis [2]. Development of DIC is associated with the decreased plasma levels of fibrinogen and the increased plasma levels of D-dimer, thrombin-antithrombin complex (TAT) and plasminogen activator inhibitor type 1 (PAI-1) in sepsis [3]. Though it is well-known that bacterial infections are the most common cause of DIC, the underlying mechanisms are not fully understood.

Outer membrane vesicles (OMVs), released by variety of bacteria, are membrane-enclosed entities of endocytic origin [5]. OMVs are produced during all stages of bacteria growth, but are most abundantly produced on

Abbreviations: DIC, disseminated intravascular coagulation; OMVs, outer membrane vesicles; PAI-1, plasminogen activator inhibitor type 1; TAT, thrombin-antithrombin complex; *E.coli*, *Escherichia coli*; TNF, tumor necrosis factor; LPS, lipopolysaccharide; LB, Luria-Bertani broth; LAL, Limulus Amebocyte Lysate; H&E stain, hematoxylin and eosin stain; ALT, alanine transaminase; AST, aspartate aminotransferase; CRE, creatinine; DAB, 3,3-diaminobenzidine; TF, tissue factor; MPs, microparticles; PT, prothrombin time; APTT, activated partial thromboplastin time; TLR4, Toll-like receptor 4

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stressful conditions, such as temperature increase and antibiotics exposure [5,6]. Enriched in microbial components, toxins and virulent factors, OMVs play important roles in host-pathogen interaction and facilitate pathogenic bacteria to invade the host at many levels [6–11]. For examples, bacterial proteins expressed on the surface of OMVs, such as SabA and BabA, promote the adherence of bacteria to the epithelial lining of host gastrointestinal or pulmonary tracts. This not only prevents the physical elimination of pathogenic bacteria, but also facilitates their invasion to the host tissues [5,6]. OMVs-containing toxins such as leukotoxin and ClyA are more potent than their soluble forms. These toxins and other virulent factors in the OMVs facilitate the invasion of pathogenic bacteria to their host through hijacking host machinery for nutrient acquisition or impairing host immune system [6–11]. In addition, OMVs can increase the co-aggregation of bacteria in the biofilms, and thus protects bacteria from antibiotics [12,13]. Bacterial infection can result in robust release of OMVs into the circulation [5,6,14,15]. Injection of purified OMVs can trigger systemic inflammatory responses and coagulopathy [16]. Under the physiological condition, however, whether OMVs could promote DIC during bacterial infection remains unknown.

To address this unsolved question, we genetically deleted *ypjA* in *Escherichia coli* (*E.coli*) which resulted in severely impaired OMVs production. *YpjA*-knockout *E.coli* did not display growth defect as compared to the wild type (WT) strain, *ypjA* deficiency did not alter tumor necrosis factor (TNF) and interleukin (IL-6) production during *E.coli* peritonitis. However, deletion of *ypjA* gene significantly ameliorated *E.coli*-induced coagulopathy, intravascular thrombi deposition, multiple organ injuries and mortality, whereas injection of purified *E.coli*-derived OMVs resulted in severe coagulopathy. Together, these findings not only provide novel insights into how bacterial infections cause DIC, but also might open a new avenue to prevent infection-associated coagulopathy by targeting OMVs production.

2. Materials and methods

2.1. Mice

C57BL/6 and Toll-like receptor 4 (TLR4) knock-out mice were purchased from the Jackson Laboratory, and were maintained in a pathogen-free environment at the Department of Laboratory Animals of Central South University. Experimental protocols were approved by the Institutional Animal Care and Use Committees of the Central South University. All animal experiments were carried out in accordance with the U.K. Animals Act, 1986 and associated guidelines.

2.2. Reagents

Poly(I:C) (high molecular weight) and ultrapure lipopolysaccharide (LPS) (*E.coli* O111:B4) for in vivo experiments were obtained from InvivoGen.

2.3. Generation of *ypjA*-deficient *Escherichia coli*

50 base pairs of the upstream or downstream of *ypjA* gene coding sequences were selected as the homologous recombination target sequence. The long primers of kanamycin containing the *ypjA* homologous recombination target sequence were chemo-synthesized and used to produce the *ypjA* homologous recombination knock out sequences (*ΔypjA::Kan*) by polymerase chain reaction. The *ΔypjA::Kan* sequences were then transformed into the competent cells of *E.coli* which have been already transformed with plasmid pKD46. *ΔypjA* mutant strain of *E.coli* were obtained after selected by kanamycin and cultured at 37 °C.

2.4. Bacterial culture and OMVs isolation

Wild type or *ΔypjA* strains of *E.coli* were grown in Luria-Bertani broth (LB). OMVs were purified from wild type or *ΔypjA* strains of *E.coli* as previously described [15,17]. Briefly, the bacteria were grown

in 200 mL of LB till OD600 of 0.5, the bacteria-free supernatants were collected by centrifugation at 10,000 ×g for 10 min at 4 °C, filtered through a 0.45 μm filter, then subjected to centrifugation at 10,000 ×g for 10 min at 4 °C again. The supernatants were further filtered through a 0.22 μm filter and OMVs in the supernatants were pelleted by ultracentrifugation at 100,000 ×g for 4 h at 4 °C in a Beckman (optima L-100 XP) ultracentrifuge. After removing supernatant, OMVs were re-suspended in 300 μL sterile PBS. Purified OMVs were subjected to agar plating to ensure lack of bacterial contamination. Limulus Amebocyte Lysate (LAL) assay and BCA protein assay kit (Pierce) were performed according to the manufacturer's instructions to quantify protein and LPS content of OMVs.

2.5. *E.coli* peritonitis and OMVs stimulation in vivo

Male mice that were 18 to 22 g in weight were intraperitoneally injected with 10⁹ CFU live wild type or *ΔypjA* *E.coli* strains to induce bacterial peritonitis. Sterile saline was injected as control. For survival experiments, mice were monitored for up to 80 h after injection.

In OMVs stimulation experiments, 200 μg, 400 μg or 800 μg OMVs derived from wild type *E.coli* were intraperitoneally injected to mice. For priming experiments, mice were injected intraperitoneally with 0.5 mg/kg LPS (*E.coli* O111:B4) or 10 mg/kg Poly(I:C) 4 h before being challenged with OMVs (200 μg per mouse). For heparin pretreatment experiments, unfractionated heparin (Sigma) 400 IU/kg or sterile saline (control group) were injected subcutaneously 30 min before *E.coli* infection or OMVs injection. For experiments with TLR4 KO mice, *E.coli*-derived OMVs (400 μg per mouse) were injected intraperitoneally to WT or TLR4 KO mice.

Mice were sacrificed at 10 h after OMVs stimulation or 12 h after *E.coli* infection, blood samples were collected for TAT, PAI-1, Fibrinogen, D-dimer detection and biochemical assays. Serum TNF-α and IL-6 levels were assessed. Liver and lung tissues collected 12 h after OMVs stimulation or *E.coli* infection were stained with hematoxylin and eosin (H&E) or subjected to immunohistochemical staining with an anti-fibrinogen antibody.

2.6. ELISA and biochemical assays

Plasma samples were analyzed using PAI-1 (Abcam), TAT (Abcam), Fibrinogen (Abcam), D-dimer (Cusabio), TNF-α (eBioscience) and IL-6 (eBioscience) ELISA Kits. Serum levels of alanine transaminase (ALT), aspartate aminotransferase (AST), creatinine (CRE) and urea (UREA) were detected using an auto-analyzer (Sysmex DRI-CHEM3500, Fuji, Japan).

2.7. Liver and lung histopathology

Liver and lung tissues were fixed in 10% buffered formalin overnight and then placed in 75% ethanol. Fixed tissues were embedded in paraffin and cut into 4 μm sections. Sections were stained with H&E. For immunohistochemical staining, liver and lung tissues were subjected to antigen retrieval solution (0.01 M citrate buffer, PH 6.0) for 25 min at 100 °C, then incubated with the rabbit anti-Fibrinogen antibody (1:2000) for 1 h at 37 °C and overnight at 4 °C, then incubated with horseradish peroxidase-conjugated secondary antibodies (goat anti-rabbit IgG) for 30 min at 37 °C. Color development was performed with 3,3-diaminobenzidine (DAB) as a chromogen. Olympus BX43 upright microscope equipped with Olympus SC50 camera was used for digital imaging.

2.8. Measurement of prothrombin time (PT) and activated partial thromboplastin time (APTT)

Blood samples of untreated WT mice were collected in EP tubes and mixed with 0.109 mol/L sodium citrate at the ratio of 9:1. Plasma samples were then collected after centrifugation at 1200g for 15 min. 20 μL OMVs suspension was added to 300 μL plasma with final

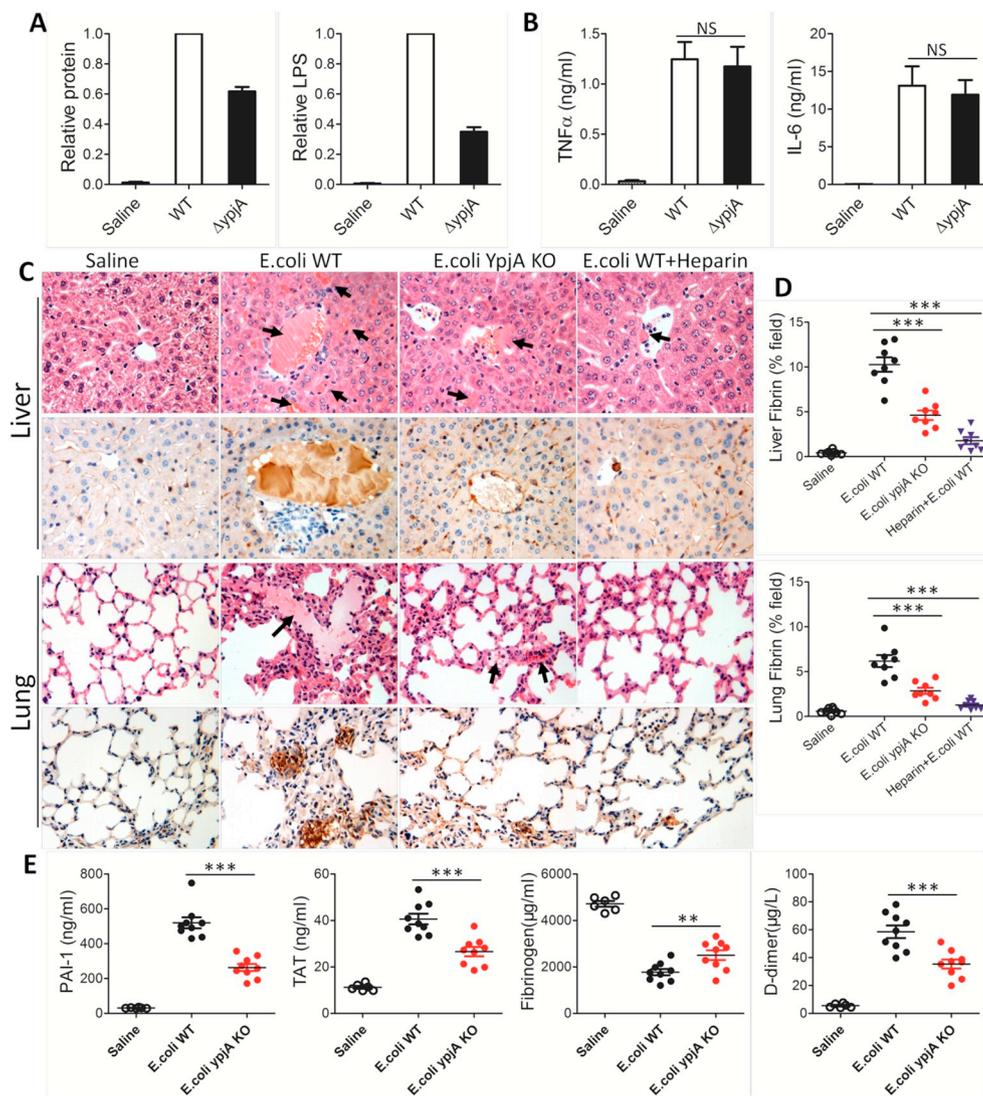


Fig. 1. Genetic deletion of *yjpA* failed to affect inflammation but attenuated *E. coli*-induced coagulopathy.

(A) OMVs production of WT or $\Delta yjpA$ *E. coli* was measured by protein- and LPS-based methods. (B). WT or *yjpA*-deficient *E. coli*-induced TNF α and IL-6 production was assessed by ELISA. (C). Representative images of liver and lung tissues from mice infected with WT or $\Delta yjpA$ *E. coli* with or without 400 IU/kg heparin pretreatment (H&E staining or immunohistochemical staining of fibrin). Thrombi formation (black arrow) and fibrin deposition were observed in liver and lung vasculatures. (D). Quantitative analysis of immunohistochemical staining by Image software. (E). Plasma levels of TAT, PAI-1, fibrinogen and D-dimer from mice challenged with WT or *yjpA*-deficient *E. coli* (10^9 cfu/mouse). Values were represented as mean \pm SEM. (***) $P < 0.001$, ** $P < 0.01$, NS = no significant, $n = 8$).

concentration of OMVs at 12.5 μ g/ml, 25 μ g/ml and 50 μ g/ml. Plasma mixed with the same volume of saline was taken as control. The PT and APTT of plasma were measured using automated coagulation analyzer (Sysmex CS-5100, Japan).

2.9. Statistical analysis

All data were analyzed using GraphPad Prism (version 5.01). The two-tailed Mann-Whitney test was used for comparison between two groups. And the Kruskal-Wallis test was used for multiple comparisons. Survival data were analyzed using log-rank test. All values were presented as mean \pm SEM, P -value < 0.05 was considered statistically significant for all experiments.

3. Results

3.1. Genetic deletion of *yjpA* mitigates *E. coli*-induced coagulopathy

Mutations in genes (e.g. *yjpA*) that encode proteins involved in OMVs production result in hypovesiculating *E. coli* strains that minimally produce OMVs without a major growth defect [15]. To investigate the role of OMVs in bacterial infection-induced coagulopathy, we generated *yjpA*-deficient *E. coli* strain. As expected, *yjpA* deficiency markedly reduced OMVs production (Fig. 1A). Mice were intraperitoneal injected with either WT *E. coli* or the *yjpA*-deficient strain

(10^9 cfu/mouse) to induce bacterial peritonitis. Though injection of purified OMVs could trigger systemic inflammation [16], deletion of *yjpA* failed to affect TNF- α and IL-6 production in *E. coli* peritonitis (Fig. 1B). Notably, *yjpA* deficiency significantly inhibited thrombi deposition in the livers and lungs, as revealed by H&E staining and immunohistochemical staining of fibrin (Fig. 1C–D). Coagulation simultaneously activates the fibrinolytic system, culminating in the elevated levels of D-dimer and the decreased plasma levels of fibrinogen [1]. Accordingly, deletion of *yjpA* significantly inhibited the elevation of serum levels of TAT, PAI-1 and D-dimer, and significantly increased plasma levels of fibrinogen (Fig. 1E). These observations clearly suggest that OMVs production during bacterial infection contributes to the development of DIC.

3.2. Genetic deletion of *yjpA* ameliorates *E. coli*-induced multiple organ injuries and mortality

DIC is an important contributing factor of multiple organ injuries and mortality in sepsis. Next we determined whether OMVs production contributes to bacterial infection-induced multiple organ injuries and mortality. Notably, deletion of *yjpA* significantly attenuated *E. coli*-induced pulmonary leukocyte infiltration and elevation of AST, ALT, CRE and UREA (Fig. 2A–B). Accordingly, *yjpA* deficiency significantly promoted survival in *E. coli* peritonitis (Fig. 2C). We also noticed that pretreatment of heparin, a well-known anti-coagulant, prevented

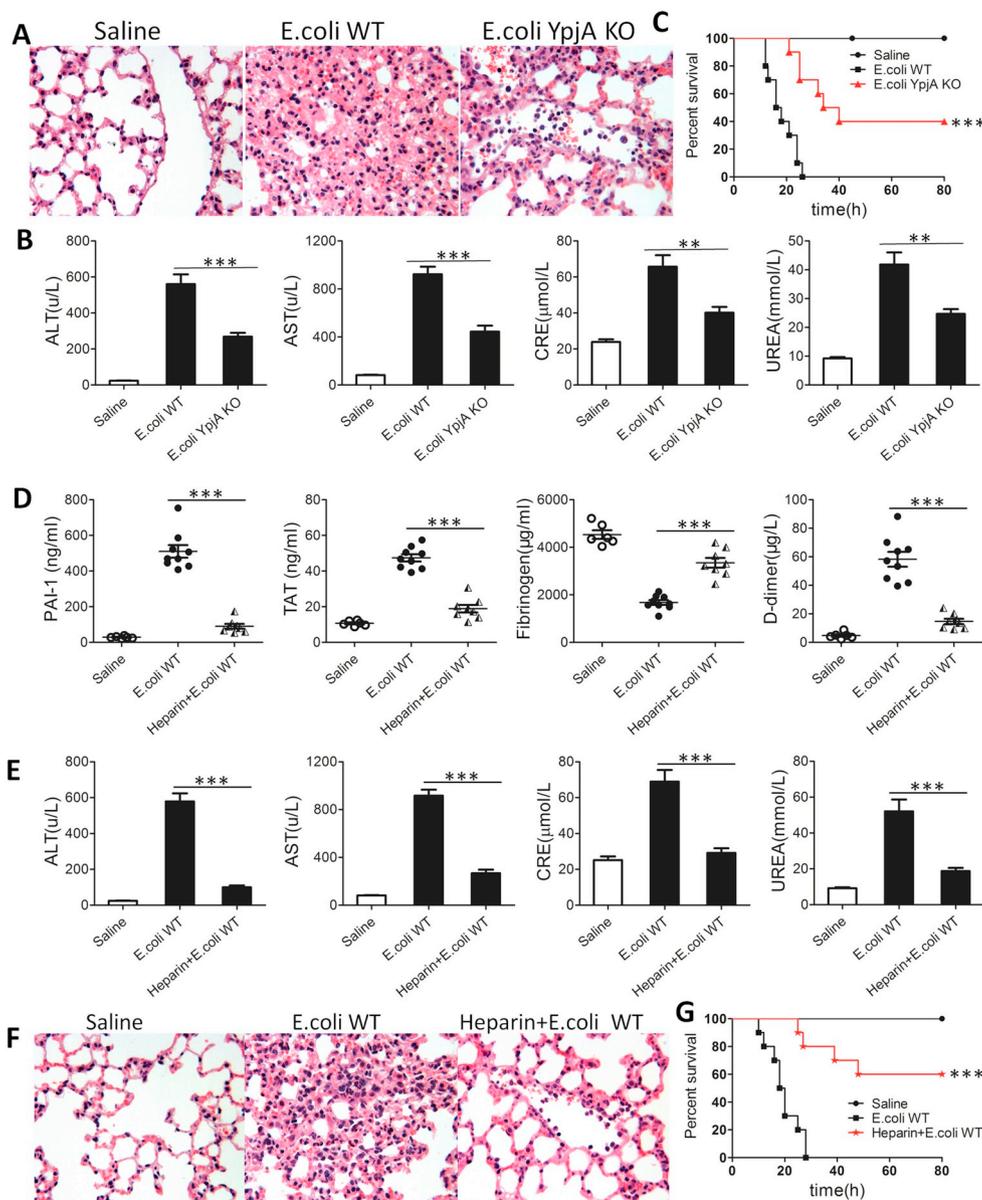


Fig. 2. Genetic deletion of *ypjA* attenuates *E. coli*-induced organ injuries, pulmonary leukocyte infiltration and mortality.

(A). Representative images of pulmonary leukocyte infiltration in mice challenged with WT *E. coli* or the *ypjA*-deficient strain (10^9 cfu/mouse). (B). Plasma levels of ALT, AST, CRE and UREA in mice challenged with WT *E. coli* or the *ypjA*-deficient strain (10^9 cfu/mouse). (C). Survival curve of mice challenged with WT or *ypjA*-deficient *E. coli*. (D–E). Plasma levels of TAT, PAI-1, fibrinogen, D-dimer, ALT, AST, CRE and UREA in mice challenged with WT *E. coli* with or without heparin pretreatment. (F) Representative images of pulmonary leukocyte infiltration in mice challenged with WT *E. coli* with or without heparin pretreatment. (G). Survival of mice challenged with WT *E. coli* with or without heparin pretreatment. Values were represented as mean \pm SEM. (***) $P < 0.001$, (**) $P < 0.01$, $n = 8$).

coagulopathy and significantly mitigated liver and kidney injuries, inhibited pulmonary leukocyte infiltration and promoted survival in *E. coli* peritonitis (Fig. 2D–G). Together with other data, these findings indicate that OMVs production during bacterial infection contributes to the development of DIC.

3.3. Injection of purified *E. coli*-derived OMVs induces DIC

Previous work shows that injection of purified OMVs could lead to the elevation of D-dimer and the decrease of fibrinogen [16]. To further prove that administration of OMVs could cause DIC, we injected the mice with purified *E. coli*-derived OMVs and measured thrombi deposition in organs. Notably, administration of OMVs induced robust thrombi deposition in the livers and lungs, as revealed by H&E staining and immunohistochemical staining of fibrin (Fig. 3A–B). OMVs injection dose-dependently increased serum levels of TAT and PAI-1, both of which are DIC markers [3] (Fig. 3C). Consistent with previous study [16], OMVs injection significantly increased plasma levels of D-dimer and decreased plasma levels of fibrinogen (Fig. 3C). Intriguingly, priming with low dose of LPS, the major cell-wall component of Gram-negative bacteria, or Poly(I:C), a double-stranded RNA mimetics,

significantly promoted OMVs-induced coagulopathy, though the priming alone was unable to induce detectable coagulopathy (Fig. 3D–E). Together, these findings indicate that injection of *E. coli*-derived OMVs into the peritoneal cavity of mice induces DIC.

3.4. Injection of purified *E. coli*-derived OMVs induces multiple organ injuries

As DIC promotes multiple organ injuries in sepsis, we next tested whether injection of purified OMVs is able to induce organ injuries. Notably, intraperitoneal injection of *E. coli*-derived OMVs led to the dose-dependent elevation of AST, ALT, CRE and UREA (Fig. 4A), which are indicative of liver or kidney injury. Injection of OMVs also resulted in pulmonary leukocyte infiltration (Fig. 4B). OMVs-induced pulmonary leukocyte infiltration and elevation of AST, ALT, CRE and UREA were much more pronounced in LPS- or Poly(I:C)-primed mice than in unprimed mice (Fig. 4B–D). Importantly, pretreatment of heparin significantly attenuated OMVs-induced liver and kidney injuries as well as pulmonary leukocyte infiltration (Fig. 4B–D). Together with other data, these findings establish that injection of *E. coli*-derived OMVs could induce DIC.

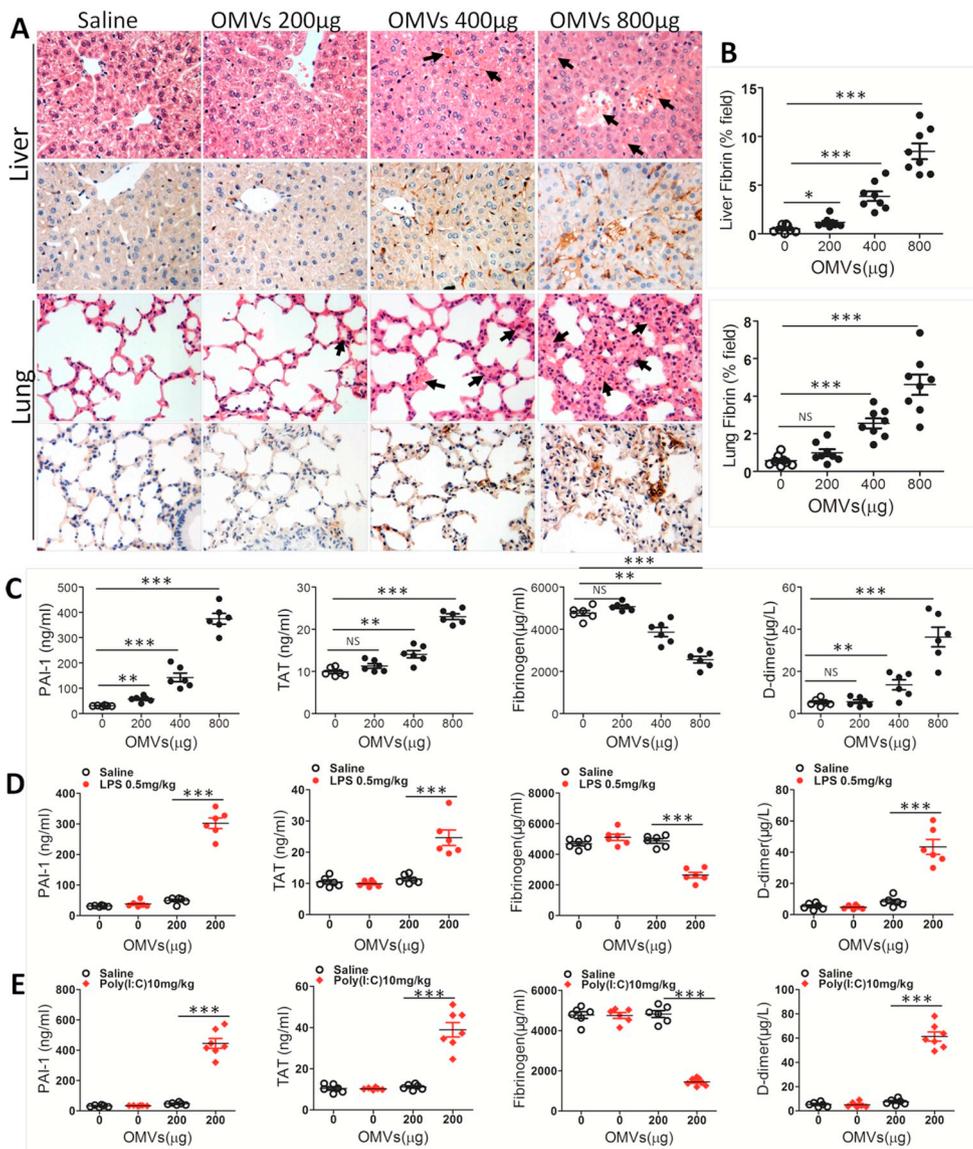


Fig. 3. Injection of *E.coli*-derived OMVs induces coagulopathy.

(A). Representative images of liver and lung tissues in mice challenged with different dose of *E.coli*-derived OMVs (H&E staining or immunohistochemical staining of fibrin). Thrombi formation (black arrow) and fibrin deposition were observed in liver and lung vasculatures. (B). Quantitative analysis of immunohistochemical staining by Image software. (C). Plasma levels of TAT, PAI-1, fibrinogen and D-dimer in mice challenged with different doses of OMVs. (D–E). Plasma levels of TAT, PAI-1, fibrinogen and D-dimer in mice challenged with OMVs (200 µg per mouse), with or without LPS or Poly(I:C) priming. Values were represented as mean ± SEM. (***) $P < 0.001$, (**) $P < 0.01$, (*) $P < 0.05$, NS = no significant, n = 6).

3.5. Injection of *E.coli*-derived OMVs cause death in LPS- or Poly(I:C)-primed mice

The occurrence of DIC significantly increases the mortality in sepsis [2]. We next determined whether injection of OMVs is able to promote lethality. Intraperitoneal injection of *E.coli*-derived OMVs caused 100% of mortality in LPS- or Poly(I:C)-primed mice (Fig. 5A–B). OMVs at this dose alone failed to cause death, neither did priming with LPS or Poly(I:C) alone (Fig. 5A–B). Further, pretreatment of heparin significantly ameliorated OMVs-induced mortality in LPS- or Poly(I:C)-primed mice (Fig. 5C–D). Taken together, these data indicate that injection of *E.coli*-derived OMVs causes death in LPS- or Poly(I:C)-primed mice, further supporting the notion that bacteria-released OMVs contributes to the development of DIC during infection.

3.6. Injection of *E.coli*-derived OMVs induces DIC in a TLR4-dependent manner

Next we investigated whether *E.coli*-derived OMVs could directly activate the coagulation system. *E.coli*-derived OMVs of different concentrations were added to the mouse plasma. Then the prothrombin time (PT) and the activated partial thromboplastin time (APTT) were measured. We observed that addition of *E.coli*-derived OMVs failed to

affect PT and APTT of WT mouse plasma (Fig. 6A), suggesting that OMVs themselves could not directly activate the coagulation system. Toll-like receptor (TLR4) is an innate immune receptor of bacterial endotoxin, which is the major component of Gram-negative bacteria-derived OMVs [5,6]. We observed that TLR4 deficiency did not display any detectable defect under the normal condition (data not show), and that genetic deletion of TLR4 failed to affect PT and APTT (Fig. 6B). However, loss of TLR4 prevented *E.coli*-derived OMVs-induced coagulopathy and elevation of TAT and PAI-1 (Fig. 6C). These data indicate that *E.coli*-derived OMVs induce DIC in a TLR4-dependent manner (Fig. 6D), further supporting the notion that OMVs themselves could not directly activate the coagulation system.

4. Discussion

Taken together, our study shows that injection of OMVs could induce coagulopathy and multiple organ dysfunctions, and that inhibition of OMVs production by deletion of *ypjA* leads to attenuated coagulopathy, multiple organ dysfunctions and lethality in *E.coli* peritonitis. As sepsis-induced DIC is characterized by coagulopathy and multiple organ dysfunctions [2], our data suggest an important role of OMVs in the pathogenesis of DIC in Gram-negative sepsis. In current study, we also found that pretreatment of heparin, an anticoagulant that prevents

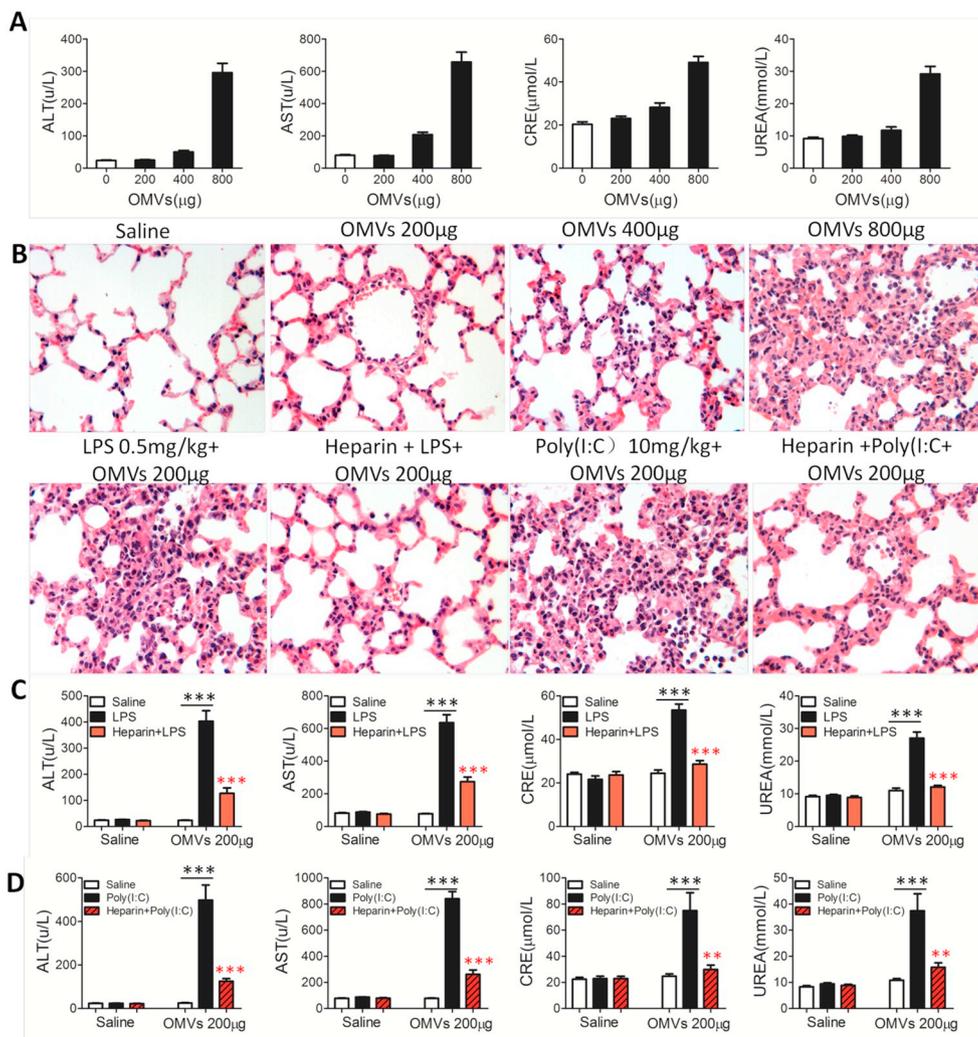


Fig. 4. Purified *E. coli*-derived OMVs induced coagulopathy-dependent multiple organ injuries. (A). Plasma levels of AST, ALT, CRE and UREA in mice challenged with different dose of *E. coli*-derived OMVs. (B). Representative Images of pulmonary leukocyte infiltration in mice challenged with OMVs with or without indicated pretreatment and priming. (C–D). Plasma levels of AST, ALT, CRE and UREA in mice challenged with OMVs (200 µg per mouse) with or without indicated pretreatment and priming. Values were represented as mean ± SEM. (***)*P* < 0.001, (**)*P* < 0.01, (*n* = 6).

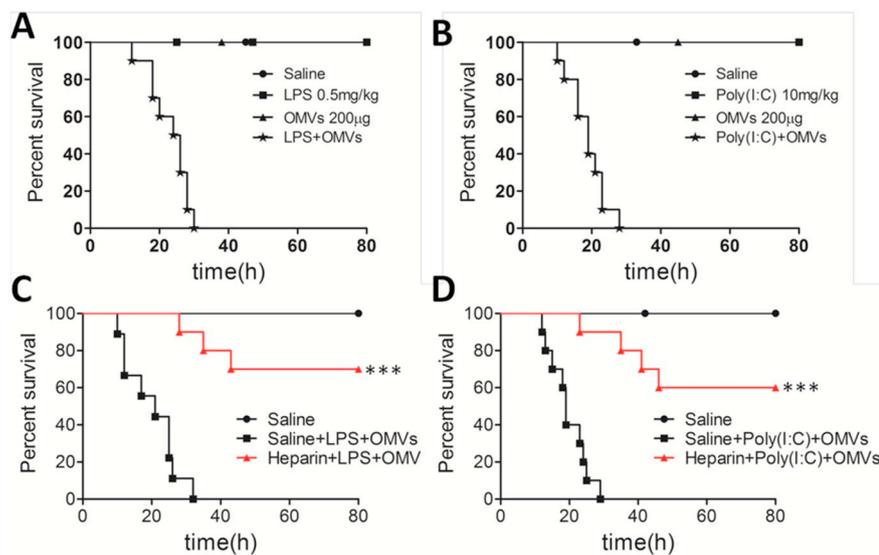


Fig. 5. Injection of *E. coli*-derived OMVs causes death in LPS- or Poly(I:C)-primed mice. (A–B). Survival of mice challenged with *E. coli*-derived OMVs with or without LPS or Poly(I:C) priming. (C–D). Survival of mice primed with LPS or Poly(I:C) and subsequently injected with *E. coli*-derived OMVs (200 µg per mouse) with or without heparin pretreatment. Values were represented as mean ± SEM. (***)*P* < 0.001, (*n* = 9).

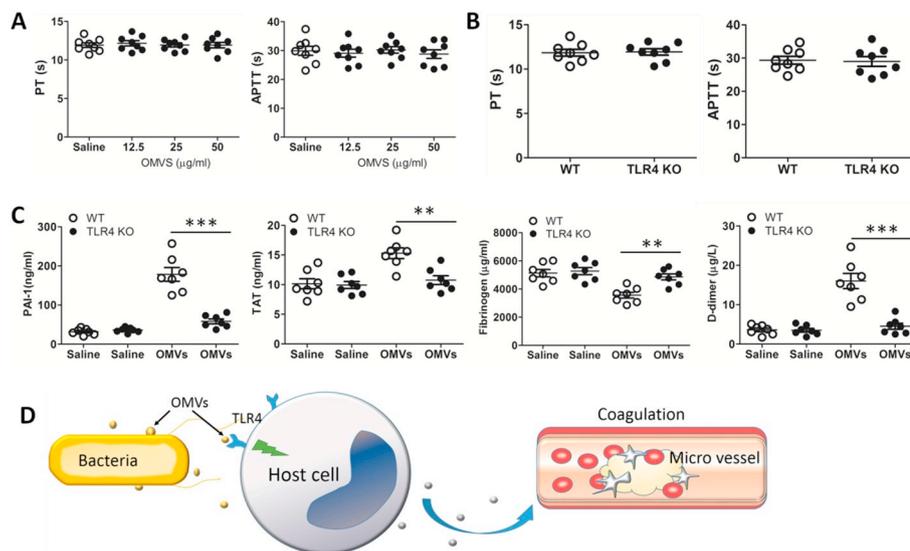


Fig. 6. Injection of *E. coli*-derived OMVs induces DIC in a TLR4-dependent manner. (A). PT and APTT of WT mouse plasma samples treated with different concentrations of *E. coli*-derived OMVs. (B). PT and APTT of plasma samples from WT or TLR4 KO mice. (C). Serum levels of PAI-1, TAT, Fibrinogen and D-dimer of WT or TLR4 KO mice intraperitoneally injected with *E. coli*-derived OMVs (400 µg per mouse) or saline. (D) The scheme of how *E. coli*-derived OMVs induce coagulation. Values were represented as mean \pm SEM. (** $P < 0.001$, ** $P < 0.01$, $n = 8$).

OMVs- or wild type *E. coli*-induced coagulopathy, significantly attenuates multiple organ dysfunctions and improves survival in *E. coli* peritonitis. These observations are in line with the accumulated clinical evidence showing that the occurrence of DIC three times increases the mortality of sepsis [2,3]. Our data suggest that OMVs could not directly activate the coagulation system. In line with this finding, *E. coli*-derived OMVs are unable to induce DIC in TLR4-deficient mice, though TLR4 is not required for the activation of coagulation system under physiological conditions. However, the mechanisms by which TLR4 signaling mediate OMVs-induced coagulopathy merit further investigations.

Though sepsis is caused by infection, persistent bacteremia is relatively rare in sepsis due to the use of broad-spectrum antibiotics [18]. However, the invading bacteria could induce host systemic immune responses by releasing their components or fragments, such as OMVs or LPS, especially after antibiotic exposure [15,17,19,20]. In addition to circulating OMVs, endotoxemia is often encountered in Gram-negative or polymicrobial sepsis [21]. In most cases of human or experimental sepsis, circulating LPS levels are not high enough to cause DIC directly [22]. Importantly, we found in this study that OMVs-induced coagulopathy, multiple organ injury and lethality were much more pronounced in low dose of LPS-primed mice than in unprimed mice. These observations clearly suggest that bacterial OMVs and endotoxemia might synergistically mediate DIC in sepsis. In line with this notion, removal of circulating LPS is beneficial to patients with sepsis [22]. As inhibition of OMVs production by deletion of *yjpA* could attenuate DIC in experimental sepsis, it is possible that targeting OMVs production might be beneficial to patients with sepsis.

One of the unsolved questions in this study is how OMVs induce DIC. OMVs contain variety of bacterial components, such as LPS, microbial nucleic acids, cyclic dinucleotides 3'-5' diadenylate (c-diAMP), 3'-5' diguanylate (c-diGMP) and toxins [5,6]. An intriguing possibility is that these microbial components might synergistically promote coagulopathy. For examples, LPS is known to stimulate the expression of tissue factor (TF), a critical initiator of coagulation cascades during infection or tissue injury [23]. Cytosolic bacterial RNA, c-diAMP and c-diGMP are able to activate the NLRP3 inflammasome, an intracellular protein complex that mediates caspase-1 activation after LPS priming [24,25]. Because OMVs could efficiently deliver their cargo into the cytosol of host cells [15,26], it is conceivable that OMVs might deliver bacterial RNA, c-diAMP and c-diGMP into the cytosol for the activation of NLRP3 inflammasome. As activated caspase-1 mediates the release of highly coagulant TF-containing microparticles (MPs) from macrophages [27], bacterial RNA, c-diAMP and c-diGMP in OMVs might promote MPs release and coagulopathy through the NLRP3 inflammasome in LPS-stimulated myeloid cells.

5. Conclusion

Taken together, our findings not only provide novel insights into how bacterial infections cause DIC, but also might open a new avenue to prevent infection-associated coagulopathy by targeting OMVs production.

Authors' contributions

EW designed and performed the experiments, analyzed the data and wrote the article; YL performed the experiments and analyzed the data; XQ performed the experiments; YT and HW designed the study and discussed the results; XX, FC supervised the study. BL conceived the project, designed the experiments, and wrote the article.

Ethics approval

Experimental protocols were approved by the Institutional Animal Care and the Medical Ethics Committee of the 3rd Xiang-ya Hospital of Central South University.

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Disclosure

The authors declare that they have no relevant conflicts of interest.

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