

Recombinant TsP53 modulates intestinal epithelial barrier integrity via upregulation of ZO-1 in LPS-induced septic mice

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Abstract. Tight junctions (TJs) are a critical component in maintaining the intestinal mucosal barrier function and gastrointestinal health of animals. Gut barrier dysfunction contributes to the initiation and development of sepsis which induces an uncontrollable systemic inflammatory response and gives rise to life-threatening clinical conditions. Excretory-secretory antigens from *Trichimella spiralis* (*T. spiralis*) have been reported to protect from sepsis in a mouse model, however the mechanism remains to be elucidated. Mice were treated with recombinant *T. spiralis* 53-kDa glycoprotein (rTsP53) at 2 or 6 h following lipopolysaccharide (LPS) injection. Survival rate, serum systemic inflammation, Chiu's score, D-lactic acid (D-LA) and diamine oxidase (DAO) as intestinal injured biomarkers, bacterial translocation and growth in peritoneal fluid (PF) and mesenteric lymph nodes (MLN), intestinal tight junction structure and protein zona occludens (ZO)-1 expression were investigated. In LPS-induced septic mice, rTsP53 was demonstrated to protract the survival and inhibit serum systemic inflammatory response, and then, allayed morphological alteration, decreased the release of D-LA and DAO from intestines. Furthermore, LPS-induced intestinal permeability, bacterial translocation and growth in PF, MLN and vital organs were significantly suppressed by rTsP53 treatment. Notably, rTsP53 treatment markedly improved the intestinal tight junction damaged in sepsis via promoting ZO-1 expression. These results demonstrated that rTsP53

ameliorated LPS-induced intestinal injury and is a potential protective agent for treatment of sepsis.

Introduction

Sepsis is a critical and life-threatening clinical condition, and is characteristic of a comprehensive systemic inflammatory response to infection. As sepsis progresses, severe pathophysiological conditions, such as multiple organ failure, shock, and death, commonly occur (1). Sepsis is positively associated with advanced age, in terms of both incidence and mortality (2). Several factors have been shown to contribute to the initiation and development of sepsis, including systemic inflammation, intestinal permeation, and a gut barrier with no integrity (3-7). Lipopolysaccharide (LPS) is commonly used for sepsis-induced systemic inflammation. It stimulates the immune system to release a series of cytokines and chemokines secreted by immune cells. In addition, LPS also causes gastrointestinal mucosal injury, involving prolongation of the transit time of intestinal epithelial cells and derangements in the intestinal barrier (3-5). Moreover, proinflammatory cytokines, oxidants, and reactive oxygen species (ROS) have been implicated as mediators of gut barrier dysfunction, such as intestinal permeation or barrier incompleteness (6,7). However, the underlying mechanism of the impact of sepsis on the gastrointestinal tract remains elusive.

Sepsis is closely associated with damage to the intestinal epithelial barrier (8,9). First, bacteria, pathogen-associated molecular patterns (PAMPs), and alarmins, and other intestinal lumen components leak into the bloodstream and, when subsequently combined with systemic inflammatory response syndrome, can bring about lethal multi-organ failure (10). Tight junctions play a critical role in the formation and maintenance of the intestinal epithelial barrier by strongly sealing adjacent intestinal mucosal cells at the apical site (11). Tight junction transmembrane proteins, such as occludins and claudins, potentiate adhesion by linking to cytoskeletal plaque proteins, for example, zona occludens (ZO)-1, -2, and -3 (12). However, the mutual regulation between sepsis and intestinal tight junctions is not completely understood.

Trichimella spiralis (*T. spiralis*) is a widely distributed parasitic nematode. A 53-kDa glycoprotein (TsP53) is a major component of the excretory-secretory antigens from

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T. spiralis and is expressed in much greater amounts in larvae (13). Interestingly, *T. spiralis* secretory products can alleviate immunological reactions in hosts with inflammatory and autoimmune diseases (14–18), such as sepsis, autoimmune encephalomyelitis, type I diabetes mellitus, and inflammatory bowel disease. The excretory-secretary antigens from *T. spiralis* can prolong survival and mitigate organ injury by decreasing sepsis-inducing inflammatory cytokines (14). Dendritic cells stimulated by excretory-secretary antigens showed significantly elevated production of interleukin (IL)-4, IL-10, and transforming growth factor- β (TGF- β) at the systemic level, and in target organs, in a model of autoimmune encephalomyelitis, which was greatly reduced in severity by the excretory-secretary antigens (15). Moreover, infection with *T. spiralis* could inhibit the initiation and development of autoimmune diabetes in non-obese diabetic (NOD) mice, which spontaneously develop autoimmune diabetes via disruption of the T-helper-mediated destruction of insulin-producing β cells (16). In addition, TsP53 can downregulate serum and colonic tumor necrosis factor- α (TNF- α) while upregulating serum T-helper 2 anti-inflammatory cytokines (IL-4, IL-13) in experimental colitis, which in turn significantly improved the colitis activity index, as well as the macroscopic and microscopic scores (17,18). The relationship between *T. spiralis* and intestinal tight junctions in sepsis is unknown.

In this study, we investigated whether recombinant TsP53 (rTsP53), a recently found protein, has effects on epithelial barrier disruption during sepsis in mice. Our results suggest that rTsP53 is a potential therapeutic protein for sepsis-stimulated systemic inflammation and intestinal epithelial barrier destruction.

Materials and methods

Animals, intravenous administration of rTsP53, and experimental design. Male BALB/c mice (weighing between 20 and 25 g) were obtained from the Experimental Animal Center of Sun Yat-sen University and randomly divided into four groups: phosphate-buffered saline (PBS) group (n=30), LPS group (n=30), LPS 2 h+rTsP53 group (n=30), and LPS 6 h+rTsP53 group (n=30). Three independent experiments were performed. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Sun Yat-set University (Guangzhou, China), which was consistent with the UK Animals Act, 1986 and EU Directive 2010/63/EU for animal experiments. The sepsis model was induced by tail intravenous injection of LPS (10 mg/kg; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) via the caudal vein.

The rTsP53 was cloned, expressed, purified, and identified by us as previously described (17). For the *in vivo* study, the experimental groups consisted of the PBS group (normal mice treated with PBS, which was the solvent used for LPS and rTsP53); the LPS group (mice that received LPS (10 mg/kg) injection by tail intravenous injection); the LPS 2 h+rTsP53 group [mice that received 50 μ g rTsP53 by tail intravenous injection 2 h after LPS (10 mg/kg) injection]; and the LPS 6 h+rTsP53 group [mice that received 50 μ g rTsP53 by tail intravenous injection 6 h after LPS (10 mg/kg) injection]. Serum, lungs, intestines, and mesentery lymph node in the

four groups were collected at 24 h after injection of PBS or LPS for further investigation.

Animal survival study. Animal survival was determined over a 72-hour time period. Animals were monitored every 12 h until the end of the 72-h study period. Mice were humanely killed upon the development of set end-point criteria (pertaining to mental state, eating and drinking, abdominal distention, respiratory distress and lethargy). All remaining animals were humanely killed at the end of the experiment.

Histopathology of the small intestine. The ileum 5 cm from the cecum was removed and opened longitudinally, and the damage was assessed histologically using a modified Chiu's score. The histological damage score was calculated on a 9-point scale (19). This scoring was done in a blinded fashion.

Transmission electron microscopy (TEM). For TEM, two 0.5x0.5 cm mini-segments of intestinal tissue from each group were excised and placed in iced PBS buffer, fixed in 2% formaldehyde and 2% glutaraldehyde in PBS overnight, washed with PBS three times for 10 min each time, post fixed in 1% osmium tetroxide in PBS, dehydrated in a graduated series of ethanol solutions, and embedded in Spurr's epoxy resin. Samples were cut into 60–80 nm sections, and stained by saturated uranium acetate and lead citrate. The sections were analyzed by electronic microscopy (H-600 TEM; Hitachi Inc., Tokyo, Japan).

Preparation of GFP-positive *E. coli*. The PEGFP-N1 eukaryotic plasmid contains a gene coding for enhanced green fluorescent protein (EGFP). The prokaryotic plasmid PET-28b(+) contains a kanamycin resistance gene. We constructed a prokaryotic plasmid expressing both EGFP and kanamycin resistance using restriction endonucleases and ligase. This new double expression plasmid was named PET28b/EGFP and transfected into competent *E. coli*. EGFP and kanamycin resistance expressions in *E. coli* were detected using a fluorescence microscope and culturing in Luria-Bertani medium containing 100 μ g/ml kanamycin. The extracted plasmid was digested by the restriction endonucleases *NotI* and *HandIII*. Agarose gel electrophoresis and ultraviolet light observation were used to identify the plasmid.

Evaluation of intestinal permeability. All animals were gavaged with kanamycin-resistant *E. coli* [2×10^7 colony-forming units (CFU) suspended in 200 ml of sterile saline]. Before the procedure, all mice were given sterile water containing 200 mg/l kanamycin for 3 days, to restrain inherent intestinal flora, and gavaged with 1.5% sodium bicarbonate to neutralize stomach acid 30 min before *E. coli* intragastric administration. At 72 h after establishment of the sepsis model, peritoneal lavage fluids, mesenteric lymph nodes (MLN), liver, spleen, kidney, and intestine from animals in each group were collected and weighed, then ground with 500 μ l of sterile saline. The tissues were cultured at 37°C on lysogeny broth (LB) agar plates containing 100 mg/ml kanamycin to measure bacterial translocation. The plates were incubated for 24 h at 37°C and the bacterial CFU were

counted. Results were expressed as CFU/ml \pm SD. Bacterial colonies were collected and amplified in LB culture medium containing 100 mg/ml kanamycin. GFP-positive *E. coli* were identified by fluorescence microscopy.

Enzyme-linked immunosorbent assay (ELISA). Serum from animals from each group was collected at 0, 6, 12, 24, 48, and 72 h after LPS injection. Blood was drawn from the caudal vein and the serum was stored at -80°C until use. Serum levels of the cytokines IL-6 and IL-10, and the chemokines D-lactic acid (D-LA) and diamine oxidase (DAO), were measured by ELISA using mouse-specific kits (R&D Systems, Inc., Minneapolis, MN, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot analysis. At 6, 12, and 24 h after LPS injection, a part of the ileum 10 cm from the cecum was obtained, weighed, and used to measure intestinal RNA and protein content. Total cellular RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) and transcribed to cDNA using the SuperScript III reverse transcriptase kit according to the manufacturer's instructions (Invitrogen). Quantitative real-time PCR was performed on an Applied Biosystems 7500 real-time PCR detection system to determine gene expression for ZO-1 using validated TaqMan gene expression assay primer/probe combinations (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). All samples were run in triplicate and relative mRNA expression levels were determined after normalizing all values to 18S RNA. ΔCt was calculated as the difference between the cycle threshold (Ct) for the gene of interest and the respective Ct for 18S. Primer sequences for ZO-1 were: Forward, 5'-GATGACCGACTC TTTCTC-3' and reverse, 5'-CAATCTCCAGTTCCTCTT-3'. Primer sequences for β -actin were: Forward, 5'-GAAATC GTGCGTGACATCAAAG-3' and reverse, 5'-TGTAGTTT ATGGATGCCACAG-3'.

Ileal protein extracts were analyzed by western blotting with anti-ZO-1 (1:1,000) or anti- β -actin (1:5,000) antibodies (Sigma-Aldrich; Merck KGaA) overnight at 4°C . After washing, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Cell Signaling Technology, Danvers, MA, USA). β -actin was used as an internal control. Densitometry was performed using ImageJ software (NIH, Bethesda, MD, USA). The optical density (OD) of the target protein was normalized to that of β -actin and presented as a percentage of the control.

Statistical analysis. Data are presented as means \pm SD. Statistical significance was determined by Student's t-test analysis or one-way ANOVA. Survival data were analyzed by the log-rank test using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). A P-value of <0.05 was accepted as statistically significant.

Results

rTsP53 intervention protected mice from LPS-induced systemic inflammation and lethality (Table I and Fig. 1)

rTsP53 treatment improved survival and organ function after LPS-induced sepsis. To investigate whether rTsP53 has

a protective effect against sepsis, we compared the rate of mortality induced by LPS in endotoxemic mice, a model of human sepsis, with or without rTsP53 treatment. The mortality rate of mice in four different groups was calculated (Table I). There were statistically significant improvements in the survival of mice in the LPS 2 h+rTsP53 and LPS 6 h+rTsP53 groups at five different time-points, compared with the LPS group. Mice in the LPS 2 h+rTsP53 group showed a significant decrease in mortality compared with the LPS 6 h+rTsP53 group. The results suggest that rTsP53 can protect against LPS-induced death in mice and treatment with rTsP53 2 h after injection of LPS was more effective.

We used the Acute Lung Injury Score to quantify the effects of rTsP53 on lung injury. The lungs of mice receiving LPS presented congested alveolar capillaries, hemorrhage, inflammatory cell infiltration, and a degree of alveolar wall thickening, which was greatly ameliorated in rTsP53-treated groups (Fig. 1A and B). Moreover, LPS remarkably elevated the concentration of liver enzymes [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)], which were released from liver cells damaged by LPS, while rTsP53 treatment could alleviate the release of ALT and AST (Fig. 1C and D). Blood urea nitrogen (BUN) and serum creatinine (Scr), as biomarkers of kidney function, were markedly decreased in the rTsP53-treated groups compared with the LPS group (Fig. 1E and F). Interestingly, the Acute Lung Injury Score, and serum ALT, AST, BUN and Scr in the LPS 6 h+rTsP53 group were much higher than in the LPS 2 h+rTsP53 group. These results indicate that rTsP53 can protect mice from LPS-induced organ damage and treatment following LPS injection was more effective at 2 vs. 6 h.

Effect of rTsP53 on plasma cytokine concentration. We hypothesized that the protection of rTsP53 in septic mice may be due to regulation of the immune response to infection. Next, we studied the proinflammatory cytokines IL-6 and TNF- α , and the anti-inflammatory cytokine IL-10. In comparison to the PBS group, the inflammatory mediators IL-6 and IL-10 reached their maximum at 12 h, while and TNF- α reached its peak value at 1 h after LPS administration. However, when mice were treated with rTsP53, particularly 2 h after LPS injection, serum levels of the proinflammatory cytokines IL-6 and TNF- α were lower compared with those of the LPS group (Fig. 1G and H). Similarly, the concentration of the anti-inflammatory cytokine IL-10 gradually increased in rTsP53-treated groups compared with the LPS group (Fig. 1I). These results suggest that rTsP53 could down-regulate LPS-induced inflammatory factors and upregulate anti-inflammatory factors.

rTsP53 treatment lessened intestinal barrier injury in LPS-induced septic mice (Fig. 2)

Changes in histopathology and Chiu's score in the small intestine. In the PBS group, histopathology revealed normal villi with an integrated structure and neat arrangement (Fig. 2A). There was distinctive damage to the intestinal mucosa in the LPS group, with massive epithelial lifting, broken tops, exposed capillaries, inflammatory cell infiltration, ulceration, and hemorrhage in the lamina propria (Fig. 2A). Compared with the LPS group, the histopathological changes

Table I. Mortality rate in four groups in the five time-points following LPS injection.

Time	PBS (%)	LPS (%)	LPS 2 h+rTsP53 (%)	LPS 6 h+rTsP53 (%)
12 h	0/30 (0)	7/30 (23.3)	1/30 (3.3) ^a	3/30 (10) ^a
24 h	0/30 (0)	14/30 (45.3)	3/30 (10) ^{a,b}	8/30 (25.7) ^a
36 h	0/30 (0)	15/30 (48.7)	4/30 (13.3) ^{a,b}	9/30 (28.7) ^a
48 h	0/30 (0)	19/30 (63.7)	5/30 (16.7) ^{a,b}	10/30 (34) ^a
72 h	0/30 (0)	21/30 (70)	6/30 (20) ^{a,b}	11/30 (38) ^a

Values are mean ± standard deviation. ^aP<0.05 vs. LPS group; ^bP<0.05 vs. LPS 6 h+rTsP53 group. Three independent experiments were performed.

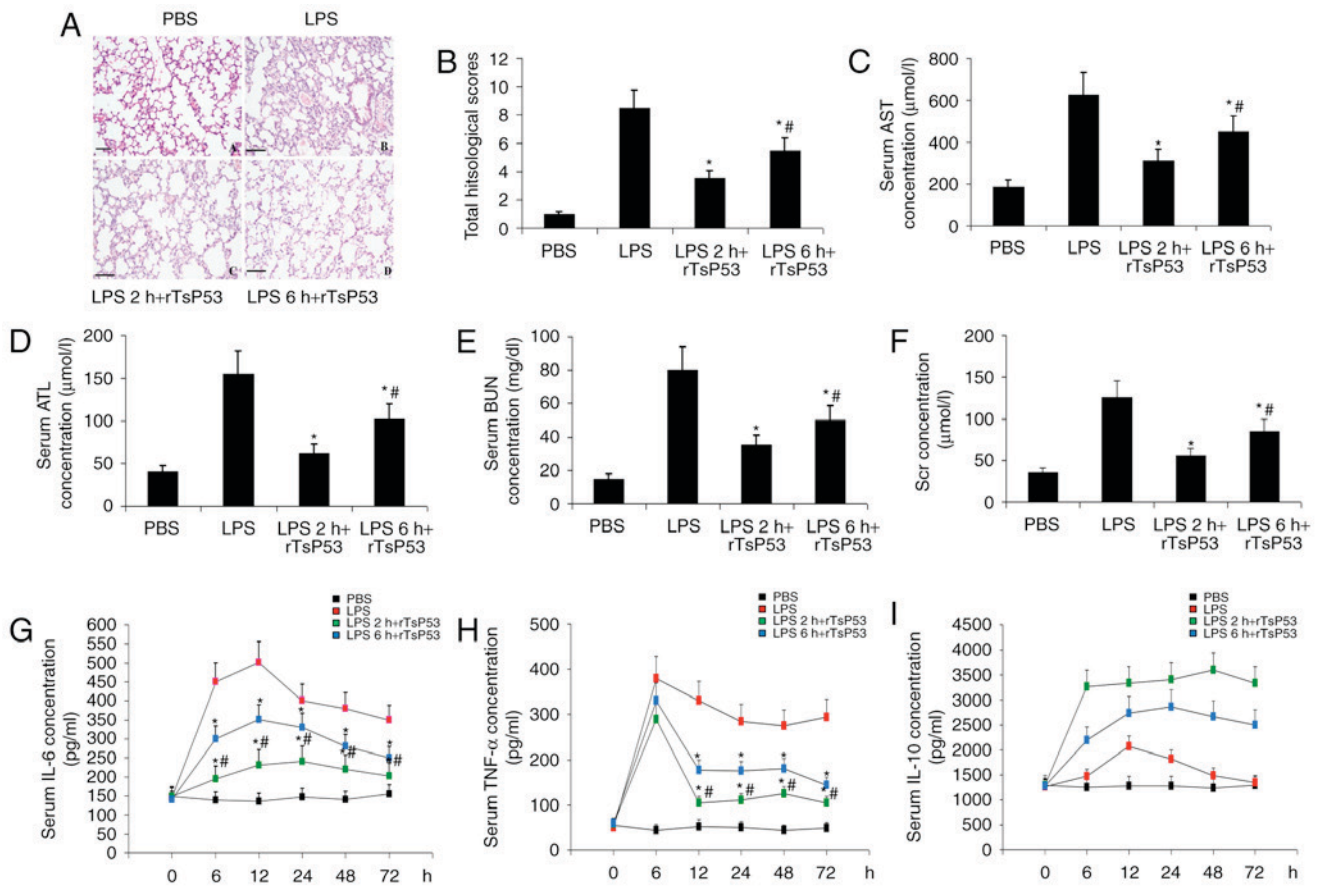


Figure 1. rTsP53 intervention protects mice from LPS-induced systemic inflammation and lethality. (A) Representative H&E stained sections of lung were shown after 24 h following injection of LPS. Original magnification, x100. (B) The severity of pneumonia was evaluated by total histological scores. Values are means ± SD, *P<0.005 vs. LPS group; #P<0.001 vs. LPS 2 h+rTsP53 group. (C and D) AST and ALT were determined after 24 h following injection of LPS. Values are means ± SD, *P<0.005 vs. LPS group; #P<0.01 vs. LPS 2 h+rTsP53 group. (E and F) BUN and Scr were accessed after 24 h following injection of LPS. Values are means ± SD, *P<0.05 vs. LPS group; #P<0.05 vs. LPS 2 h+rTsP53 group. (G-I) IL-6, TNF-α, and IL-10 were determined in the six time-points following injection of LPS. Values are means ± SD, *P<0.01 vs. LPS group; #P<0.05 vs. LPS 2 h+rTsP53 group. All statistical significance was determined by one-way ANOVA analysis.

in the lamina propria in rTsP53-treated groups were obviously improved, showing few broken tops, very slight inflammatory cell infiltration, smaller ulcers, and little hemorrhage in the lamina propria (Fig. 2A). The LPS 2 h+rTsP53 group had the lowest Chiu's score, while the LPS group had the highest. Interestingly, Chiu's score for the LPS 2 h+rTsP53 group was significantly lower than that for the LPS 6 h+rTsP53 group. These data show that rTsP53 treatment attenuated histopathological damage to the intestinal mucosa during sepsis.

Changes in the plasma concentrations of D-LA and DAO. Due to the intestinal benefit of rTsP53 in septic mice, we hypothesized that rTsP53 could regulate the serum levels of D-LA, a product of intestinal bacteria glycolysis, and DAO, a high-activity intracellular enzyme in the intestinal villi, both of which can be released into the blood from damaged intestinal mucosa. The serum level of D-LA reached a peak at 6 h following LPS injection and remained at a relatively high level until 72 h compared with the PBS group. However, rTsP53

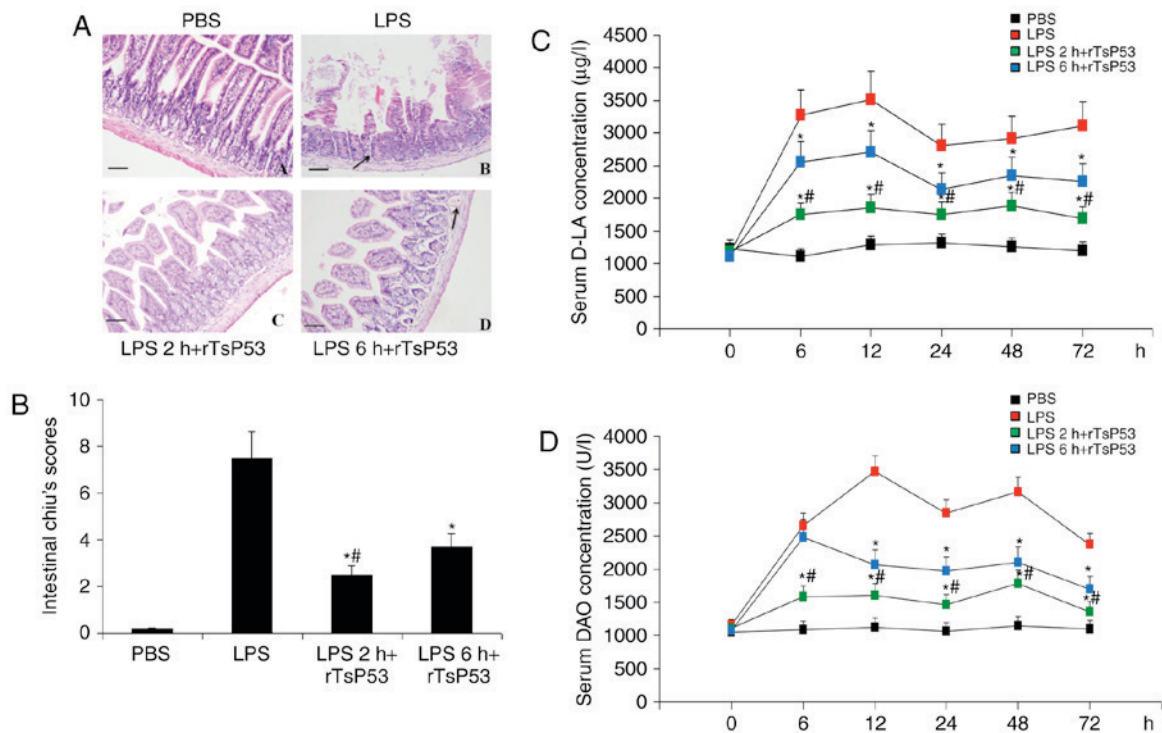


Figure 2. rTsP53 treatment improves intestinal barrier injury in LPS-induced septic mice. (A) H&E staining of small intestine sections were shown after 24 h following injection of LPS. Original magnification, x200. (B) The severity of small intestine damaged by LPS was evaluated by total histological scores. Values are means \pm SD, * P <0.05 vs. LPS group; # P <0.05 vs. LPS 6 h+rTsP53 group. The statistical significance was evaluated by one-way ANOVA analysis. (C and D) D-LA and DAO were determined in the six different time-points following injection of LPS. Values are means \pm SD, * P <0.05 vs. LPS group; # P <0.05 vs. LPS 2 h+rTsP53 group.

treatment reduced the concentration of D-LA in plasma, and the D-LA level in the LPS 2 h+rTsP53 group was much lower than that in the LPS 6 h+rTsP53 group (Fig. 2C). Similar results were observed in the alteration of DAO concentration during sepsis (Fig. 2D). These results show that rTsP53 can modulate sepsis-induced intestinal mucosal damage.

rTsP53 treatment facilitated LPS-induced intestinal permeability, bacterial translocation, and growth (Fig. 3)

rTsP53 treatment decreased bacterial translocation and growth in PF and MLN in septic mice. Mice subjected to LPS had significantly increased bacterial translocation and growth in PF and MLN compared with the PBS group. rTsP53 treatment significantly inhibited bacterial growth in PF and MLN in septic mice. Bacterial burden in PF and MLN in the LPS 2 h+rTsP53 group was lower than in the LPS 6 h+rTsP53 group (Fig. 3A and B).

rTsP53 treatment decreased bacterial translocation and growth in intra-abdominal organs. We also examined recombinant *E. coli* translocation and bacterial burden in other intra-abdominal organs, such as the liver, spleen, kidney, and intestine. Mice in the LPS group had higher levels of bacterial translocation and burden in each organ (liver, $5.5 \pm 0.89 \log_{10}$ CFU/ml/g for the LPS group vs. $0.35 \pm 0.08 \log_{10}$ CFU/ml/g for the PBS group; spleen, $4.9 \pm 0.78 \log_{10}$ CFU/ml/g for the LPS group vs. $0.4 \pm 0.09 \log_{10}$ CFU/ml/g for the PBS group; kidney, $4.5 \pm 0.72 \log_{10}$ CFU/ml/g for the LPS group vs. $0.25 \pm 0.05 \log_{10}$ CFU/ml/g for the PBS group; intestine, $9 \pm 1.44 \log_{10}$ CFU/ml/g for the LPS group vs. 2.89 ± 0.59

\log_{10} CFU/ml/g for the PBS group, P <0.05). Mice in the LPS 2 h+rTsP53 group showed a significant decrease in bacterial translocation and growth in these organs compared with the LPS group (liver, $1.3 \pm 0.22 \log_{10}$ CFU/ml/g; spleen, $1.8 \pm 0.30 \log_{10}$ CFU/ml/g; kidney, $1.3 \pm 0.22 \log_{10}$ CFU/ml/g; intestine, $2.2 \pm 0.37 \log_{10}$ CFU/ml/g), and the bacterial translocation and burden in the LPS 2 h+rTsP53 group were greatly decreased compared with the LPS 6 h+rTsP53 group (liver, $3.3 \pm 0.40 \log_{10}$ CFU/ml/g; kidney, $3 \pm 0.36 \log_{10}$ CFU/ml/g; spleen, $2.9 \pm 0.35 \log_{10}$ CFU/ml/g; intestine, $4.5 \pm 0.54 \log_{10}$ CFU/ml/g) (Fig. 3C).

rTsP53 treatment alleviated tight junction destabilization (Fig. 4)

rTsP53 treatment reduced damage to the ultrastructure of the tight junction. In the PBS group, TEM revealed intact epithelial cells in the intestinal mucosa, complete integrity of the tight junction, orderly villi of the epithelial cells, and whole tight junctions (Fig. 4A). However, in the LPS group, the epithelial cells were distributed in a disorderly manner with noticeable exfoliation, shrinkage, and disordered villi, which are characteristic of epithelial cells undergoing necrocytosis. Furthermore, other morphological changes were observed in septic mice, such as swelling of mitochondria, lysosome activation, and neutrophil infiltration, which were significantly improved in the rTsP53-treated groups (Fig. 4A).

rTsP53 treatment regulated expression of ZO-1 in intestinal tissues. ZO-1 is an important tight junction protein, which are critical structural proteins for maintaining mucosal barrier

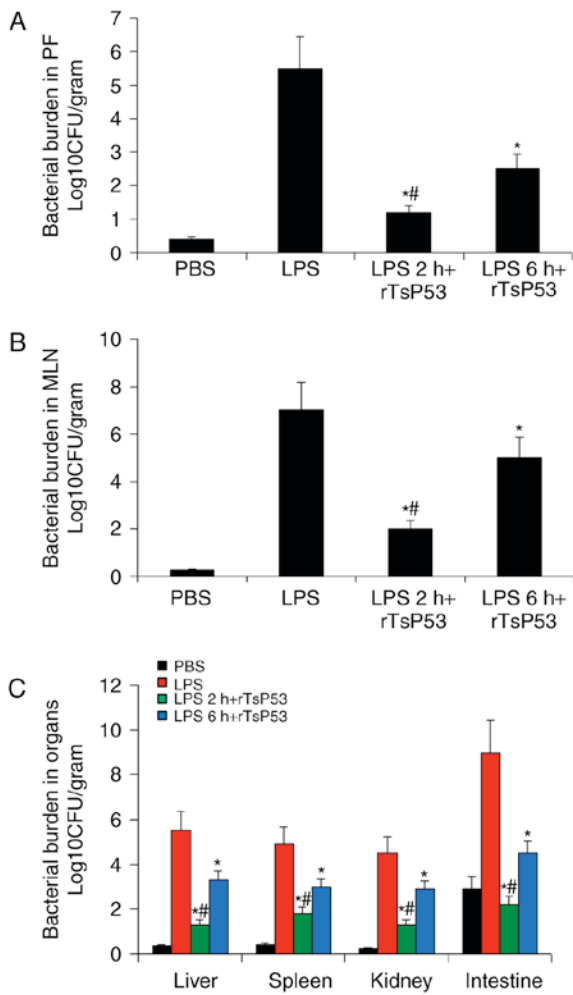


Figure 3. rTsP53 treatment protects mice from LPS-induced intestinal permeability, bacterial translocation and growth. (A-C) All animals were gavaged with 2×10^7 CFU suspended in 200 ml of sterile saline. Seventy-two hours after establishment of sepsis model, peritoneal lavage fluids (PF), MLN, liver, spleen, kidney and intestine from animals of different groups were collected. The tissues were cultured at 37°C on agar plates containing 100 mg/ml of kanamycin to measure bacterial translocation. The plates were incubated for 24 h at 37°C and then counted for bacterial colony-forming units (CFUs). Values were expressed as CFU/ml \pm SD. * $P < 0.005$ vs. LPS group; # $P < 0.05$ vs. LPS 2 h+rTsP53 group. All statistical significance was assessed by one-way ANOVA analysis.

function. Impairment of mucosal barrier function is characterized by the aberrant expression of tight junction proteins. Immunoblotting analysis was used to detect the expression of the ZO-1 protein. As shown in Fig. 4B, the expression of ZO-1 was significantly downregulated at 6 h and decreased further at 24 h after LPS administration in the small intestine. This indicates that LPS rapidly degrades ZO-1 protein and disrupts the structure of the tight junction. However, in the LPS 2 h+rTsP53 group, the expression level of the ZO-1 protein was increased significantly, and continued to increase slowly over time, compared with that of the LPS group. In the LPS 6 h+rTsP53 group, ZO-1 protein was more highly expressed than in the LPS group, but the levels were lower than in the LPS 2 h+rTsP53 group. These results suggest that rTsP53 protein has a protective effect on the tight junction in small intestines injured by LPS, especially when rTsP53 was injected 2 h after LPS administration.

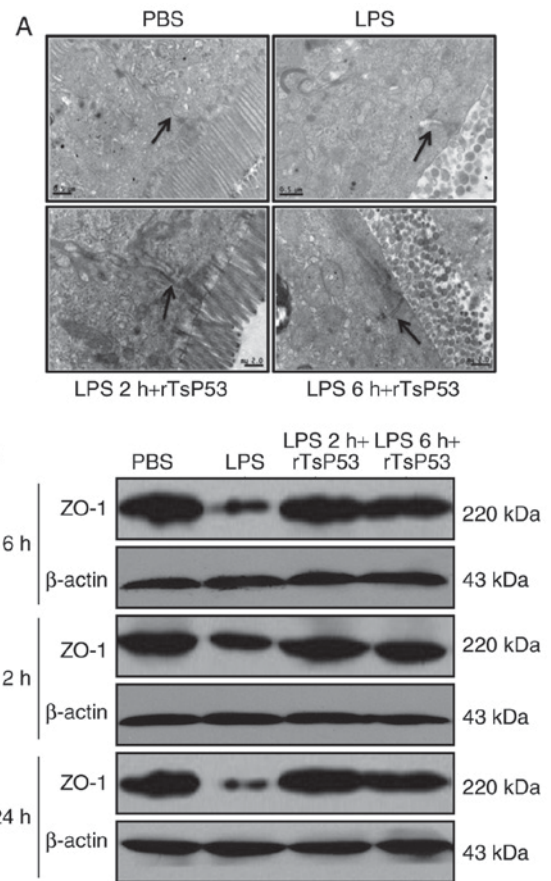


Figure 4. rTsP53 treatment contributes to alleviation of tight junction destabilization. (A) The ultrastructure of small intestines in four groups evaluated by transmission electron microscopy. Black arrow indicated the gap between villi and submucosa. (B) Western blotting of ZO-1 in small intestine mucosa from mice in three different time-points following injection of LPS. β -actin was used as the control for loading.

Discussion

Following sepsis-induced intestinal ischemia and hypoxia, ROS, cytokines, and inflammatory mediators underlie the molecular mechanisms of intestinal mucosal injury and barrier dysfunction (20). The improvement of intestinal mucosal permeability during sepsis, to avoid gut-derived secondary infection, is a crucial measure to prevent the initiation and development of sepsis. In the present study, we found that a newly discovered protein, rTsP53, could efficiently protect mice against LPS-induced sepsis. rTsP53 alleviated sepsis-induced multi-organ injuries in the lung, liver, and kidney, remarkably downregulated systematic inflammation, and mitigated intestinal permeability by decreasing ZO-1 expression, which can lead to bacterial translocation. Our results suggest that rTsP53 may be a potential mitigator in the treatment of sepsis.

The excretory-secretory antigens of *T. spiralis* were first found to have immunosuppression functions 20 years ago (21), but the mechanism remains unknown. TsP53, a 53-kDa glycoprotein, is a major component protein of the excretory-secretory antigens of *T. spiralis*. It is thought that rTsP53 can stimulate strong IgG1 production with low IgG2a induction in immunized mice, and is considered a T-cell-prone immunomodulator (22). In this study, rTsP53 significantly

downregulated TNF- α and IL-6 in septic mice and upregulated anti-inflammatory cytokine IL-10 levels. LPS-induced sepsis is a T-helper cell-driven inflammation process, in which TNF- α , IL-6, and IL-10 have a fundamental effect on triggering leukocyte activation and tissue accumulation. This may explain why rTsP53 has a major influence in LPS-induced organ damage. Our research showed that rTsP53 treatment can number the amount of congested alveolar capillaries, reduce inflammatory cell infiltration, decrease alveolar wall thickening in the lungs, and reduce concentrations of ALT and AST (indicators of liver function) and BUN and Scr (surrogates of kidney function). Therefore, we reason that rTsP53 exerts a protective effect against sepsis-induced organ injury partly through the modulation of immunological function.

It is generally thought that the intestines play a 'motor' role in systemic inflammatory response syndrome. During sepsis, massive release of inflammatory factors, intestinal ischemia and hypoxia, and cytotoxicity, impair the tight junctions between epithelial cells and cause intestinal mucosal barrier dysfunction and intestinal bacterial translocation, which can induce an endogenous 'second hit' to the body (23). The intestinal mechanical barrier is composed of mucosal epithelium, intercellular tight junctions, lamina propria, adherent junctions, and intestinal secretions. Tight junctions play an important role in maintaining the integrity of the mucosal epithelium (24,25). In our study, ZO-1, a tight junction protein, was remarkably enhanced by rTsP53 treatment in the intestinal epithelium and rTsP53 improved epithelial integrity with lower amounts of shortened villi and withered crypts. rTsP53-mediated increased ZO-1 expression, and improved mucosal permeability, contributed to reduced bacterial translocation into the blood and organs and lessened the systemic inflammatory storm. Further studies are needed to explore the upstream mechanism of ZO-1-mediated tight junction dysfunction in sepsis-induced intestinal injury.

In conclusion, this study demonstrates that rTsP53 plays a protective role in LPS-induced sepsis and prevents sepsis-induced intestinal injury via modulation of ZO-1 expression. These results have implications for the potential therapeutic use of rTsP53 in patients with sepsis.

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