

Urinary Trypsin Inhibitor Protects against Systemic Inflammation Induced by Lipopolysaccharide

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ABSTRACT

Urinary trypsin inhibitor (UTI), a serine protease inhibitor, has been widely used as a drug for patients with acute inflammatory disorders such as disseminated intravascular coagulation, shock, and pancreatitis in Japan. Recent studies have demonstrated that serine protease inhibitors may play an anti-inflammatory role beyond merely an inhibitory action on neutrophil elastase at the site of inflammation at least in vitro. To clarify the direct contributions of UTI to inflammatory condition in vivo, we analyzed its roles in experimental systemic inflammatory response induced by intraperitoneal administration of lipopolysaccharide (LPS) using UTI deficient (–/–) mice and corresponding wild-type (WT) mice. After LPS (1 mg/kg) challenge, UTI (–/–) mice revealed a significant elevation of plasma fibrinogen and fibrinogen/fibrin degradation products and a de-

crease in white blood cell counts compared with WT mice. LPS treatment induced more severe neutrophilic inflammation in the lung and the kidney obtained from UTI (–/–) mice than in those from WT mice, which was confirmed by histological examination. The protein levels of proinflammatory mediators, such as macrophage chemoattractant protein (MCP)-1 in the lungs, MCP-1 and keratinocyte chemoattractant (KC) in the kidneys, and interleukin-1 β , macrophage inflammatory protein-2, MCP-1, and KC in the liver, were significantly greater in UTI (–/–) mice than in WT mice after LPS challenge. Our results suggest that UTI protects against systemic inflammatory response and subsequent organ injury induced by bacterial endotoxin, at least partly through the inhibition of the enhanced expression of proinflammatory cytokines and chemokines.

Bacterial infection can evoke shock, acute respiratory failure, multiple organ failure, and disseminated intravascular coagulation (DIC), resulting in a high mortality rate. Lipopolysaccharide (LPS), a major component of the outer membrane of Gram negative bacteria, is one of the major toxins that initiate the cascade of pathophysiological reactions called endotoxin shock with a high mortality (Michie et al., 1988). Enhanced expression of cytokines and chemokines secreted from activated cells such as macrophages/monocytes and neutrophils is considered to be crucial in the initiation of the shock cascade (Karima et al., 1999; Underhill and Ozinsky, 2002). Besides, various molecules such as platelet-activating factor, arachidonic acid metabolites, free radicals, and proteases as well as complement fragments and coagulation

protease cascade are also implicated in the pathogenesis of endotoxin shock (Esmon et al., 1999; Karima et al., 1999; Hardaway, 2000; Bhole and Stahl, 2003). Proteases may also modulate inflammatory response elicited by LPS, because neutrophil elastase- and cathepsin G-deficient mice have been shown to be resistant to LPS-induced shock (Tkalcevic et al., 2000), and deficiency of one of serine protease inhibitors, secretory leukoprotease inhibitor, has caused a higher mortality from endotoxin shock (Nakamura et al., 2003). In addition, a recent study has suggested that pancreatic proteases may sustain systemic inflammatory response induced by LPS in vivo (Fitzal et al., 2003).

Urinary trypsin inhibitor (UTI) is a multivalent Kunitz-type serine protease inhibitor that is found in human urine and blood. UTI is recognized to be degenerated from pre- α /inter- α -trypsin inhibitors induced by neutrophil elastase during inflammation (Pratt et al., 1989). UTI has been widely

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ABBREVIATIONS: DIC, disseminated intravascular coagulation; LPS, lipopolysaccharide; UTI, urinary trypsin inhibitor; IL, interleukin; TNF, tumor necrosis factor; WT, wild type; PT, prothrombin time; FDP, fibrinogen/fibrin degradation; WBC, white blood cell; ELISA, enzyme-linked immunosorbent assays; MIP, macrophage inflammatory protein; MCP, macrophage chemoattractant protein; KC, keratinocyte chemoattractant.

used as a drug for patients with DIC, shock, and pancreatitis, especially in Japan. UTI mainly inhibits inflammatory proteases, including trypsin, α -chymotrypsin, plasmin, cathepsin G, and leukocyte elastase as well as proteases in coagulation cascade. As with other serine type protease inhibitors, UTI reportedly has anti-inflammatory properties apart from blocking of protease pathway in vitro. UTI inhibits the enhanced production of proinflammatory molecules such as prostaglandin H2 synthase-2 (Zaitzu et al., 2000), thromboxane B2 (Aibiki and Cook, 1997), interleukin (IL)-8 (Nakamura et al., 1997), and tumor necrosis factor (TNF)- α (Aosasa et al., 2001) induced by LPS in vitro. In addition, UTI ameliorates several inflammatory models such as ischemia-reperfusion injury (Yano et al., 2003), septic shock (Tani et al., 1993), hemorrhagic shock (Masuda et al., 2003), and glomerulonephritis (Koizumi et al., 2000) in vivo. In these models, however, the animals have been treated with human-derived UTI as a foreign protein; thus, the direct contribution of UTI to inflammatory diseases, including systemic inflammatory response syndrome, has never been examined in knockout mice.

In the current study, we explored the role of UTI in systemic inflammation induced by intraperitoneal injection of LPS using UTI ($-/-$) mice and WT mice. We also determined the effects of UTI deficiency on organ (lung, kidney, and liver) damage induced by LPS. Finally, we examined whether lung, kidney, and liver injury found in both genotypes was concomitant with altered profiles of proinflammatory cytokines and chemokines.

Materials and Methods

Mice. The studies were carried out in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. The Institutional Review Board approved all animal studies. The generation of mice deficient in UTI gene and normal control littermates (C57BL/6) was described previously (Sato et al., 2001). These mice were bred and maintained under a 12-h light/dark cycle in our level B pathogen-free facility. Male mice of both genotypes were used at 10 to 12 weeks of age and 27 to 31 g in weight.

Endotoxin Challenge. Both UTI ($-/-$) and WT mice were injected intraperitoneally (i.p.) with vehicle or LPS (*Escherichia coli* B55: 05; Difco, Detroit, MI) at a dose of 1 mg/kg body weight. Phosphate-buffered saline at pH 7.4 (Nissui, Tokyo, Japan) was used as vehicle for LPS.

Coagulation and Fibrinolysis Analysis and Peripheral White Blood Cell (WBC) Counts. Blood samples were collected from each mouse ($n = 10$ in each group) into 3.8% sodium citrate in a ratio of 10:1 and centrifuged at 3000g for 10 min as conducted previously (Inoue et al., 2004). The prothrombin time (PT) was evaluated by incubating 50 μ l of plasma for 5 min at 37°C and then adding 100 μ l of an equal volume mixture of simplastin (Diagnostica Stago/Roche Diagnostics, Tokyo, Japan) and 30 mM CaCl₂. Murine clottable plasma fibrinogen was determined using commercial kit (Diagnostica Stago/Roche Diagnostics), and the values were compared with a human plasma fibrinogen standard (Diagnostica Stago/Roche Diagnostics). Fibrinogen/fibrin degradation products (FDP) were measured with a commercial kit (Diagnostica Stago/Roche Diagnostics), which uses a latex turbidimetric immunoassay. All assays were measured in STA Compact (Diagnostica Stago/Roche Diagnostics) as described previously (Inoue et al., 2004). In a separate series of experiments, blood samples were collected, and WBC counts were measured ($n = 8$ in each group).

Histological Examination. After exsanguinations, the lungs were fixed by intratracheal instillation of 10% neutral phosphate-buffered formalin, pH 7.4. Livers and kidneys were fixed with the same formalin. All specimens were embedded in paraffin. Sections of 4 μ m in thickness were routinely processed with hematoxylin and eosin stain as described previously ($n = 5$ in each group) (Takano et al., 1997, 2002). In 40 \times fields, the area of inflammation was chosen randomly from different sections of each organ and measured using a videomicrometer (Olympus, Tokyo, Japan). The number of neutrophils per square millimeter in each area was counted with the micrometer under oil immersion. Results were expressed as the number of neutrophils per square milliliter of inflammatory sites. Histological sections were evaluated in a blind manner.

Enzyme-Linked Immunosorbent Assays (ELISAs) for Cytokines and Chemokines. In a separate series of experiments, the animals were exsanguinated and the lungs, kidneys, and liver were subsequently homogenized with 10 mM potassium phosphate buffer, pH 7.4, containing 0.1 mM EDTA (Sigma-Aldrich, St. Louis, MO), 0.1 mM phenylmethylsulfonyl fluoride (Nacalai Tesque, Kyoto, Japan), 1 μ M pepstatin A (Peptide Institute, Osaka, Japan), and 2 μ M leupeptin (Peptide Institute) as described previously (Takano et al., 2002; Inoue et al., 2004). The homogenates were then centrifuged at 105,000g for 1 h. The supernatants were stored at -80°C . Protein concentration was determined using the Bradford protein concentration assay kit (Bio-Rad, Hercules, CA) (Bradford, 1976). ELISAs for IL-1 β (Pierce Endogen, Rockford, IL), TNF- α (R & D Systems, Minneapolis, MN), macrophage inflammatory protein (MIP)-1 α (R & D Systems), MIP-2 (R & D Systems), macrophage chemoattractant protein (MCP)-1 (R & D Systems), and keratinocyte chemoattractant (KC; R & D Systems) in the organ tissue supernatants were conducted using matching antibody pairs according to the manufacturer's instruction ($n = 8$ in each group). The second antibodies were conjugated to horseradish peroxidase. Subtractive readings of 550 nm from the readings at 450 nm were converted to picograms per milliliter using values obtained from standard curves generated with varying concentrations of recombinant IL-1 β , TNF- α , MIP-1 α , MIP-2, MCP-1, and KC with limits of detection of 3, 9, 1.5, 1.5, 10, and 2 pg/ml, respectively.

Statistical Analysis. Data were reported as mean \pm S.E.M. using Stat View version 4.0 (Abacus Concepts, Berkeley, CA) as described previously (Takano et al., 1997). Differences were analyzed by analysis of variance followed by Fisher's protected least significant difference test (Takano et al., 1997). Significance was assigned to P values less than 0.05.

Results

Effects of UTI on Coagulatory and Fibrinolytic Changes and WBC Counts after LPS Challenge. We first evaluated coagulatory and fibrinolytic parameters and WBC counts 72 h after i.p. challenge with LPS or vehicle (Table 1). Levels of fibrinogen were significantly greater in LPS-challenged WT mice ($P < 0.05$) and LPS-challenged UTI ($-/-$) mice ($P < 0.01$) than in vehicle-challenged mice in the same genotypes. In the presence of LPS, they were significantly higher in UTI ($-/-$) mice than in WT mice ($P < 0.05$). FDP levels were increased by LPS challenge with significance in UTI ($-/-$) mice ($P < 0.01$) and without significance in WT mice. After LPS challenge, they were significantly higher in UTI ($-/-$) mice than in WT mice ($P < 0.01$). WBC counts significantly decreased after LPS challenge in UTI ($-/-$) mice ($P < 0.01$ versus other groups). LPS shortened PT compared with vehicle in UTI ($-/-$) mice without significance. In the presence of LPS, PT was significantly shorter in UTI ($-/-$) mice than in WT mice ($P < 0.05$).

Effects of UTI on Organ Damage after LPS Challenge. We next evaluated the histopathological changes in the lung, kidney, and liver obtained from both genotypes of mice 72 h after LPS challenge.

Histopathological examination revealed severe neutrophilic inflammation in the lungs obtained from UTI (–/–) mice challenged with LPS (Fig. 1A). In contrast, neutrophilic infiltration was less in LPS-treated WT mice than in LPS-treated UTI (–/–) mice (Fig. 1B). Vehicle treatment caused little histopathological changes (Fig. 1, C and D) in both genotypes of mice.

LPS challenge induced neutrophilic infiltration around glomeruli and in the interstitium in the kidney obtained from both genotypes of mice (Fig. 2, A and B). However, the severity was more prominent in UTI (–/–) mice (Fig. 2A) than in WT mice (Fig. 2B) in the presence of LPS. Vehicle treatment caused no histopathological changes (Fig. 2, C and D) in both genotypes of mice.

LPS caused widespread centrilobular vacuolation of hepatocytes and neutrophilic infiltration in the liver obtained from both genotypes of mice (Fig. 3, A and B). In the presence of LPS, there were no significant differences between both genotypes of mice. Vehicle treatment caused few histopathological changes (Fig. 3, C and D) in both genotypes of mice. The damage to the intestinal mucosal barrier in the presence of LPS was not apparent in both genotypes of mice (data not shown).

We performed morphometric analysis to quantitate the number of neutrophils in the lung, kidney, and liver tissue 72 h after LPS challenge. Compared with vehicle treatment, LPS treatment increased the numbers of neutrophils in the lung from UTI (–/–) mice with significance ($P < 0.01$) and those from WT mice without significance (Fig. 4A). In the presence of LPS, UTI (–/–) mice showed significantly increased numbers of neutrophils in the lung compared with WT mice ($P < 0.01$; Fig. 4A). Compared with vehicle, LPS significantly increased the numbers of neutrophils in the kidney from UTI (–/–) mice ($P < 0.01$) but not those from WT mice (Fig. 4B). In the presence of LPS, UTI (–/–) mice showed significantly increased numbers of neutrophils in the kidney compared with WT mice ($P < 0.05$; Fig. 4B). Compared with vehicle challenge, LPS challenge significantly increased the numbers of neutrophils in the liver from both genotypes of mice ($P < 0.01$; Fig. 4C). In the presence of LPS, there were no significant differences in the numbers between the two genotypes (Fig. 4C).

TABLE 1

Effects of UTI on the coagulatory and fibrinolytic parameters and peripheral blood cell counts after LPS challenge

Blood samples were collected 72 h after i.p. injection of vehicle or LPS. Coagulatory and fibrinolytic parameters ($n = 10$ in each group) and white blood cell counts ($n = 8$ in each group) were measured. Values are the mean \pm S.E.M.

Group & Challenge	PT	Fibrinogen	FDP	WBC
	s	mg/dl	$\mu\text{g/mm}$	per μl
WT				
Vehicle	11.9 \pm 0.1	261.7 \pm 12.9	1.8 \pm 0.4	3925 \pm 237
LPS	11.9 \pm 0.3	329.9 \pm 11.1*	2.2 \pm 0.2	3701 \pm 324
UTI (–/–)				
Vehicle	11.3 \pm 0.1	273.3 \pm 19.2	1.5 \pm 0.2	3198 \pm 255
LPS	10.1 \pm 1.0 [#]	389.1 \pm 22.4***	3.2 \pm 0.2***	2507 \pm 210***

* $P < 0.05$ versus vehicle-treated mice.

** $P < 0.01$ versus vehicle-treated mice.

[#] $P < 0.05$ versus LPS-treated WT mice.

*** $P < 0.01$ versus LPS-treated WT mice.

Effects of UTI on Organ Expression of Proinflammatory Molecules Related to LPS. Finally, we examined the protein expression of IL-1 β , TNF- α , MIP-1 α , MIP-2, MCP-1, and KC in the lung, kidney, and liver 72 h after the LPS administration. In the lung, LPS challenge caused significant elevations of the protein expression of IL-1 β , MIP-1 α , MIP-2, MCP-1, and KC in UTI (–/–) mice compared with vehicle challenge ($P < 0.01$; Fig. 5, A and C–F). In WT mice, LPS treatment induced significant enhancement in the expression of IL-1 β , MIP-1 α , MIP-2, MCP-1 ($P < 0.01$; Fig. 5, A and C–E), and KC ($P < 0.05$; Fig. 5F) compared with vehicle challenge. In the presence of LPS, the lung expression of MCP-1 was significantly greater in UTI (–/–) mice than in WT mice ($P < 0.05$; Fig. 5E).

In the kidney, LPS challenge caused significant elevations of the protein expression of IL-1 β , MIP-1 α , MIP-2, MCP-1, and KC in UTI (–/–) mice compared with vehicle challenge ($P < 0.01$ for IL-1 β , MIP-2, MCP-1, and KC, Fig. 6, A and D–F; and $P < 0.05$ for MIP-1 α , Fig. 6C). In WT mice, LPS treatment induced significant enhancement in the expression of IL-1 β , MIP-1 α , MIP-2, MCP-1, and KC compared with vehicle treatment ($P < 0.01$ for IL-1 β , MIP-1 α , and MIP-2, Fig. 6, A, C, and D; and $P < 0.05$ for MCP-1 and KC, Fig. 6, E and F). In the presence of LPS, the kidney expression of MCP-1 and KC was significantly greater in UTI (–/–) mice than in WT mice ($P < 0.05$; Fig. 6, E and F).

In the liver, LPS challenge caused significant elevations of the protein expression of IL-1 β , TNF- α , MIP-1 α , MIP-2, MCP-1, and KC in UTI (–/–) mice compared with vehicle challenge ($P < 0.01$ for IL-1 β , MIP-1 α , MIP-2, and MCP-1, Fig. 7, A and C–E; and $P < 0.05$ for TNF- α and KC, Fig. 7, B and F). In WT mice, LPS treatment induced significant enhancement in the expression of IL-1 β , MIP-1 α , and MCP-1 compared with vehicle treatment ($P < 0.01$ for IL-1 β and MIP-1 α , Fig. 7, A and C; and $P < 0.05$ for MCP-1, Fig. 7E). In the presence of LPS, the liver expression of IL-1 β , MIP-2, MCP-1, and KC was significantly greater in UTI (–/–) mice than in WT mice ($P < 0.01$ for MIP-2, Fig. 7D; and $P < 0.05$ for IL-1 β , MCP-1, and KC, Fig. 7, A, E, and F).

Discussion

The present study shows that UTI (–/–) mice reveal a significant elevation of fibrinogen and FDP and a significant decrease in WBC counts after LPS treatment compared with WT mice. LPS challenge induces more prominent neutro-

philic inflammation in the lung and the kidney obtained from UTI ($-/-$) mice than in those from WT mice. The protein levels of MCP-1 in the lung; MCP-1 and KC in the kidney; and of IL-1 β , MIP-2, MCP-1, and KC in the liver are significantly greater in UTI ($-/-$) mice than in WT mice after LPS challenge.

A number of mediators, including lipid mediators, cytokines, free radicals, complement fragments, coagulatory factors, and proteases contribute to the pathogenesis of endotoxin shock (Esmon et al., 1999; Karima et al., 1999; Hardaway, 2000; Underhill and Ozinsky, 2002; Bhole and Stahl, 2003). Among them, the products from neutrophils are recognized to play important roles. Activated neutrophils release various kinds of mediators, including proteases and

oxygen radicals (Weiss, 1989). Protease-antiprotease imbalance has been involved in a variety of inflammatory diseases (Chapman et al., 1997; Deng et al., 2001). Because neutrophil elastase exerts the most injurious effects on many kinds of substrates (elastin, type I–IV collagen, fibronectin, laminin, and proteoglycans) among the proteases produced by neutrophils, it can be a key mediator of tissue injury (Travis, 1988). Indeed, neutrophil elastase and cathepsin G-deficient mice have been shown to be resistant to lethal effects of LPS (Tkalcevic et al., 2000). Deficiency of secretory leukoprotease inhibitor also caused a higher mortality from endotoxin shock with higher production of IL-6 and high mobility group-1 (Nakamura et al., 2003). In addition, Fitzal et al. (2003) have shown that blockade of pancreatic proteases in the intestinal lumen ameliorates systemic inflammation induced by intravenous administration of LPS.

UTI is a multivalent Kunitz-type serine protease inhibitor that is found in human urine and blood. UTI is recognized to be degenerated from pre- α /inter- α -trypsin inhibitors during inflammation (Pratt et al., 1989). UTI reportedly inhibits neutrophil elastase activity in vitro (Ogawa et al., 1987; Gando and Tedo, 1995) and trypsin activity in patients with pancreatitis (Ohwada et al., 1997). Although therapeutic effects of UTI on circulatory shock have been recognized, especially in Japan, detailed explanations about the target mechanisms remains unsatisfactory. Tani et al. (1993) have reported that UTI protects against septic shock induced by Gram negative bacteria in vivo, by only estimating clinical signs such as cardiac index, blood pressure, lactic acid, blood glucose, and blood base values. Another group has shown that UTI improves hemorrhagic shock by its protective effect on myocardial mitochondrial functions (Masuda et al., 2003). However, they have not elucidated other factors regarding systemic inflammatory response. Furthermore, these studies have pivotal limits that the animals are treated with human-derived UTI as a foreign protein. In the present study, UTI ($-/-$) mice revealed a significant elevation of fibrinogen and FDP after LPS challenge compared with WT mice. Thus, our

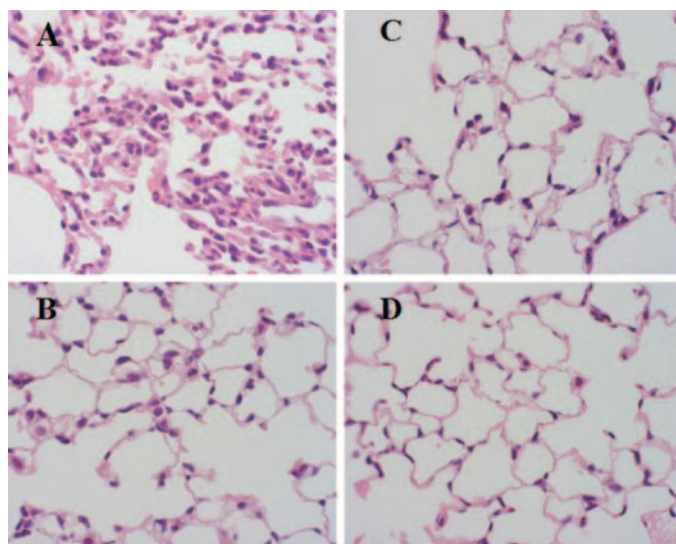


Fig. 1. Histopathological findings of the lung obtained from UTI null ($-/-$) mice injected i.p. with 1 mg/kg body weight of LPS (A), WT mice injected with LPS (B), UTI ($-/-$) mice injected with vehicle (C), and WT mice injected with vehicle (D) ($n = 5$ in each group). Seventy-two hours after injection, mice were killed and assessed. Original magnification, 400 \times .

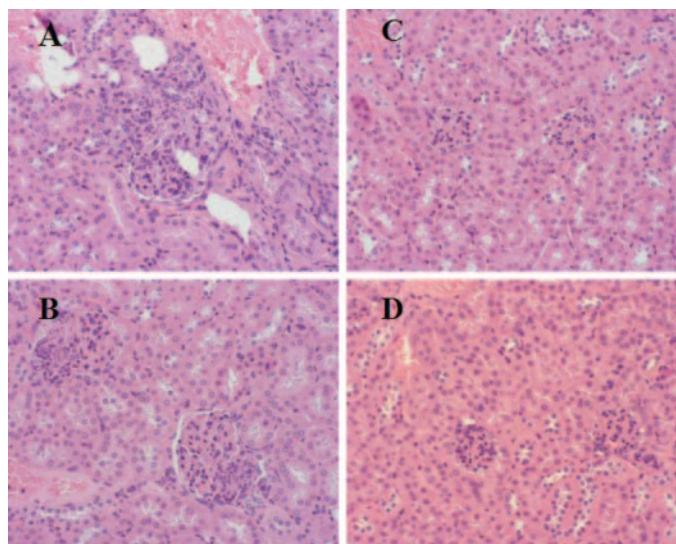


Fig. 2. Histopathological findings of the kidney obtained from UTI ($-/-$) mice injected with LPS (A), WT mice injected with LPS (B), UTI ($-/-$) mice injected with vehicle (C), and WT mice injected with vehicle (D) ($n = 5$ in each group). Seventy-two hours after injection, mice were killed and assessed. Original magnification, 200 \times .

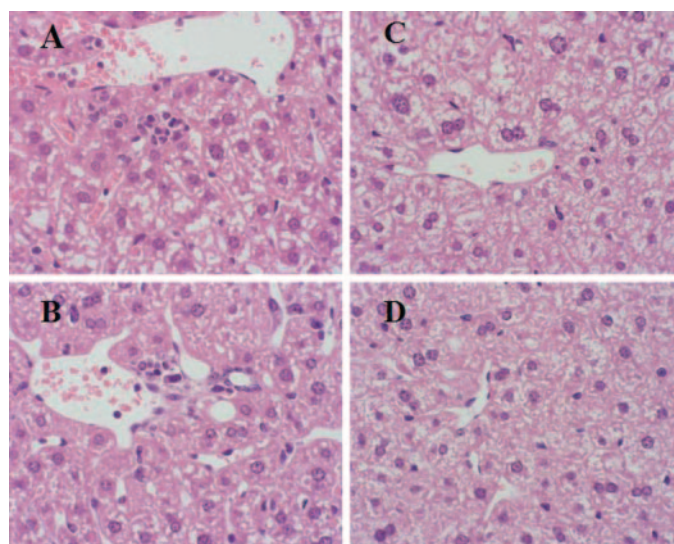


Fig. 3. Histopathological findings of the liver obtained from UTI ($-/-$) mice injected with LPS (A), WT mice injected with LPS (B), UTI ($-/-$) mice injected with vehicle (C), and WT mice injected with vehicle (D) ($n = 5$ in each group). Seventy-two hours after injection, mice were killed and assessed. Original magnification, 400 \times .

results first demonstrate the direct protective role of UTI in inflammatory response via the inhibition of synthesis for fibrinogen and its products. UTI also inhibits plasmin activity in vitro (Sumi et al., 1988). On the other hand, FDP are recognized to interact with plasmin activity (Lucas et al., 1983). Alternatively, enhanced FDP levels in UTI ($-/-$) mice compared with those in WT mice in the presence of LPS may be caused by increased plasmin activity. Inflammatory mediators reportedly induce fibrinogen synthesis, which lead direct/indirect activation of hypercoagulopathy (Michie et al., 1988). Also in our present study, PT was significantly shorter

in UTI ($-/-$) mice than in WT mice after LPS challenge. The result may support the protective role of UTI in hypercoagulopathy related to LPS. Subsequent activation of fibrinolysis after coagulation elevates FDP. These events can result in DIC and multiple organ failure, which is frequently associated with endotoxin shock (Michie et al., 1988). Together, UTI is protective against coagulatory and fibrinolytic changes that can be related to DIC caused by endotoxin shock.

The present study should be the first demonstration of the protective effects for UTI against organ damage elicited by LPS in vivo. The lungs and the kidneys are consistently the

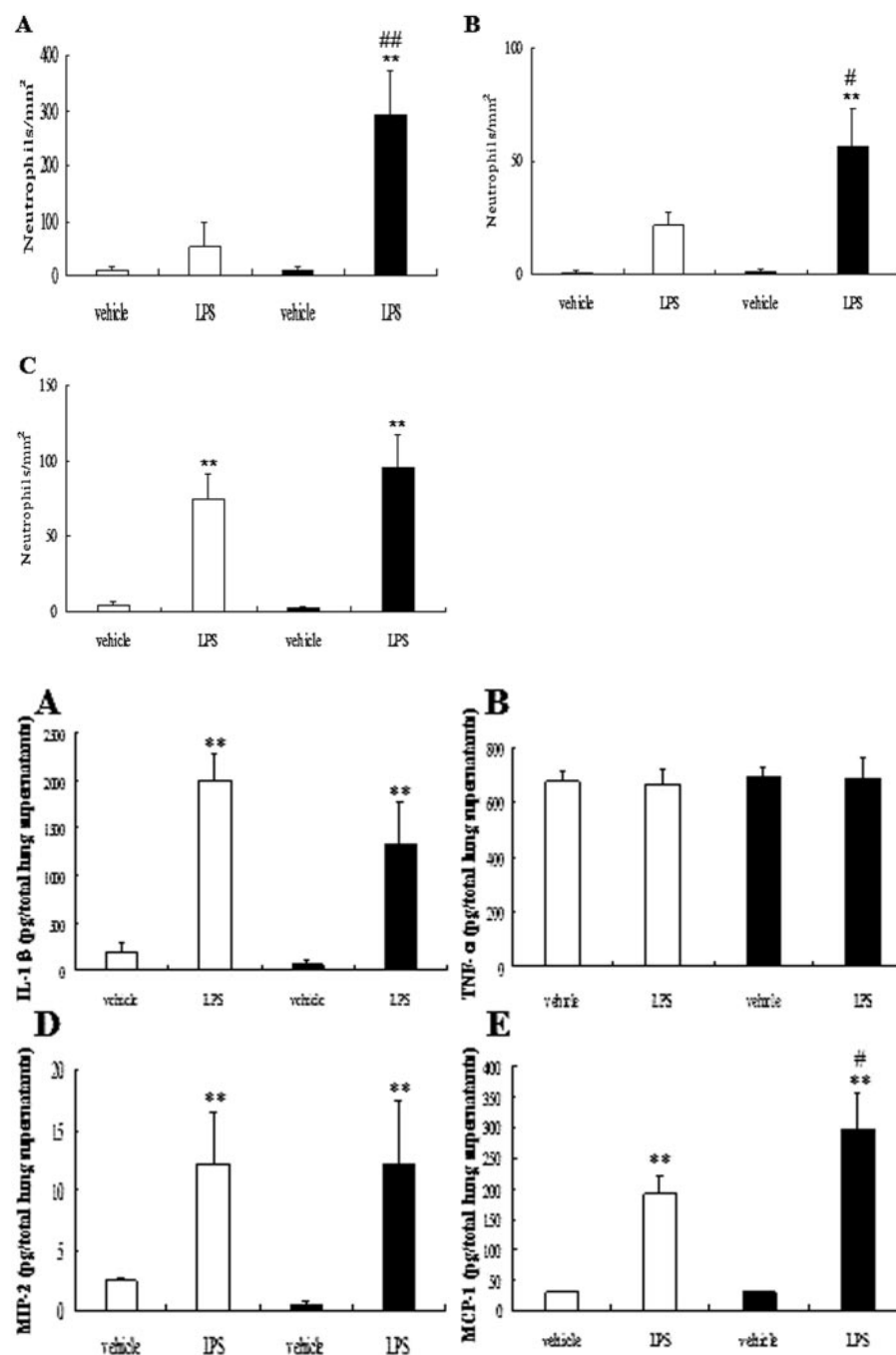


Fig. 4. Quantitative analysis of neutrophil sequestration into the different compartments from the lung (A), kidney (B), and liver (C). The organs of both WT (open symbols) and UTI ($-/-$) (closed symbols) mice were harvested 72 h after i.p. injection of vehicle or LPS ($n = 5$ in each group). The numbers of neutrophils per square millimeter in tissue sections were counted. *, $P < 0.05$ versus vehicle-treated mice; **, $P < 0.01$ versus vehicle-treated mice; #, $P < 0.05$ versus LPS-treated WT mice; ##, $P < 0.01$ versus LPS-treated WT mice. Values are the mean \pm S.E.M. in each group.

Fig. 5. Cytokine and chemokine profiles in the lung after LPS challenge. Lung tissue supernatants of both WT (open symbols) and UTI ($-/-$) (closed symbols) mice were harvested 72 h after i.p. injection of vehicle or LPS ($n = 8$ in each group). IL-1 β (A), TNF- α (B), MIP-1 α (C), MIP-2 (D), MCP-1 (E), and KC (F) levels in the lung tissue supernatants were measured by ELISA. *, $P < 0.05$ versus vehicle-treated mice; **, $P < 0.01$ versus vehicle-treated mice; #, $P < 0.05$ versus LPS-treated WT mice; ##, $P < 0.01$ versus LPS-treated WT mice. Values are the mean \pm S.E.M. in each group.

most susceptible organs in animal models of sepsis (Michie et al., 1988; Jirillo et al., 2002). On the other hand, we have previously reported that organ injury caused by LPS is concomitant with the enhanced neutrophilic sequestration in the organs accompanied by the decrease in peripheral WBC counts in vivo (Yoshikawa et al., 1994). In the present study, lung and kidney damage, including neutrophil infiltration,

were more prominent in LPS-treated UTI ($-/-$) mice than in LPS-treated WT mice. Furthermore, the reduction of WBC counts was more prominent in UTI ($-/-$) mice than in WT mice in the presence of LPS. Thus, our results suggest that UTI is protective against organ damage related to LPS possibly via the inhibition of neutrophil sequestration in the organs.

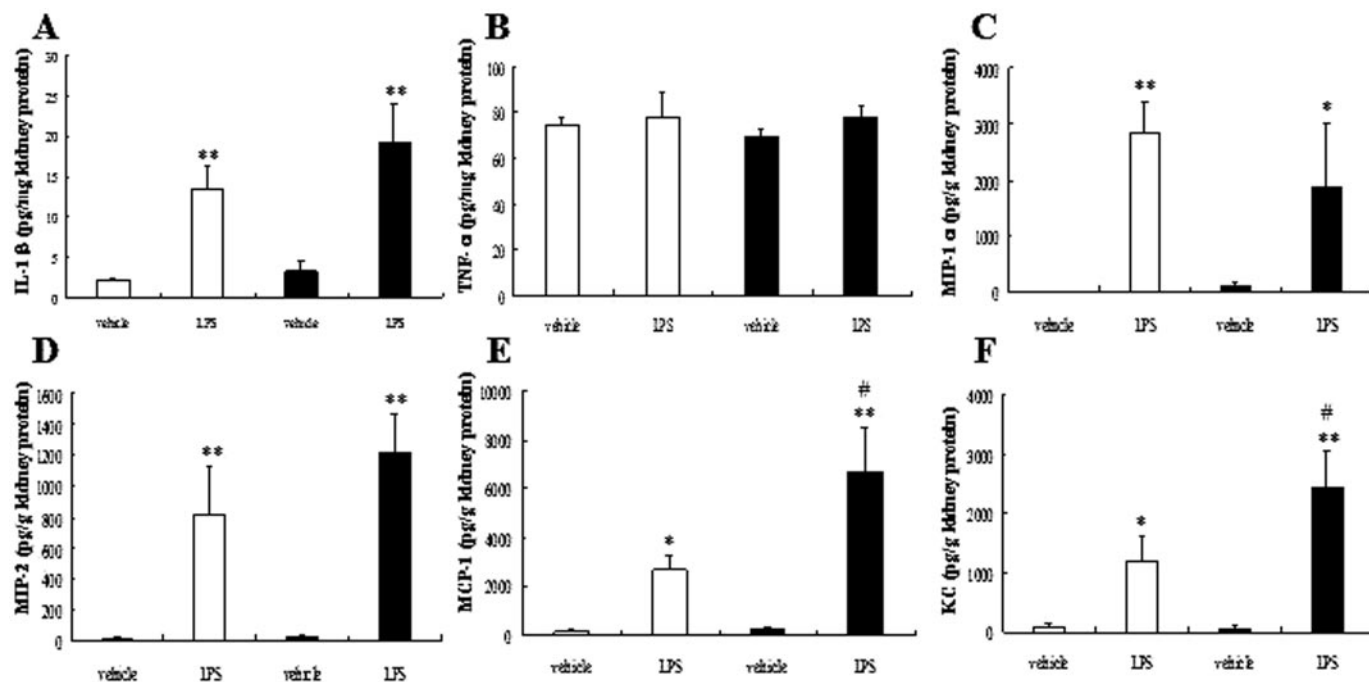


Fig. 6. Cytokine and chemokine profiles in the kidney after LPS challenge. Kidney tissue supernatants of both WT (open symbols) and UTI ($-/-$) (closed symbols) mice were harvested 72 h after i.p. injection of vehicle or LPS ($n = 8$ in each group). IL-1 β (A), TNF- α (B), MIP-1 α (C), MIP-2 (D), MCP-1 (E), and KC (F) levels in the kidney tissue supernatants were measured by ELISA. *, $P < 0.05$ versus vehicle-treated mice; **, $P < 0.01$ versus vehicle-treated mice; #, $P < 0.05$ versus LPS-treated WT mice. Values are the mean \pm S.E.M. in each group.

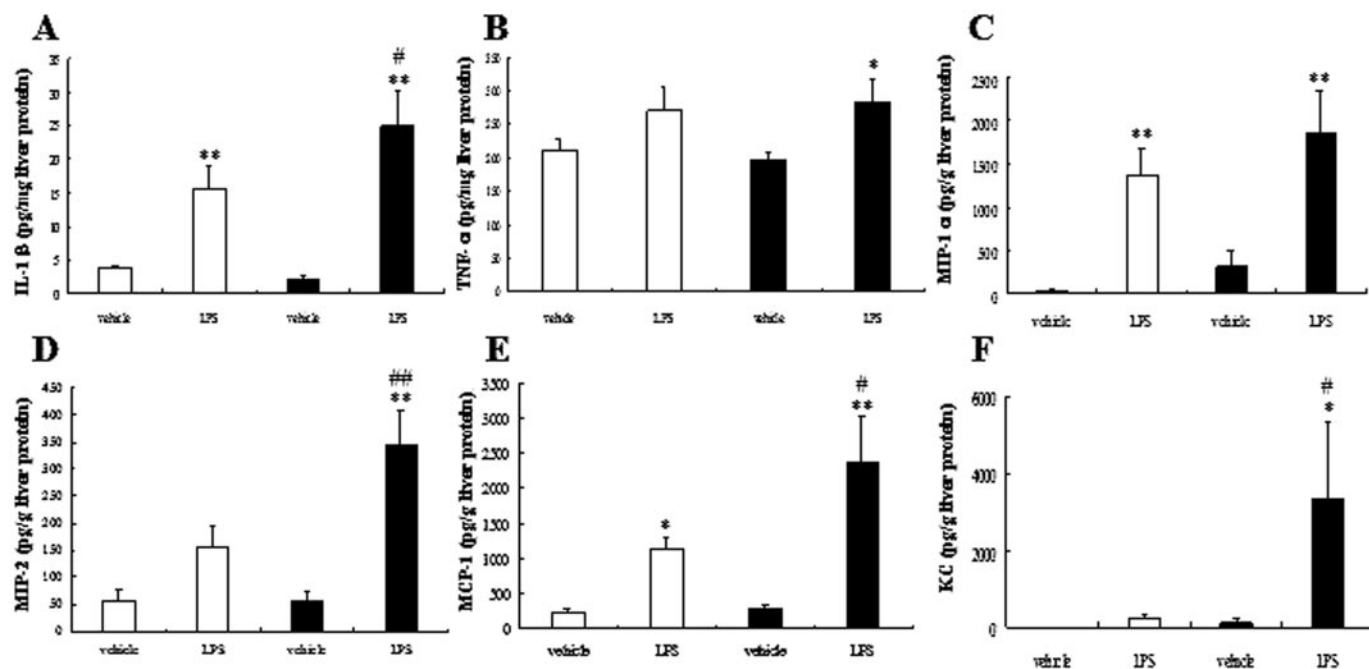


Fig. 7. Cytokine and chemokine profiles in the liver after LPS challenge. Liver tissue supernatants of both WT (open symbols) and UTI ($-/-$) (closed symbols) mice were harvested 72 h after i.p. injection of vehicle or LPS ($n = 8$ in each group). IL-1 β (A), TNF- α (B), MIP-1 α (C), MIP-2 (D), MCP-1 (E), and KC (F) levels in the liver tissue supernatants were measured by ELISA. *, $P < 0.05$ versus vehicle-treated mice; **, $P < 0.01$ versus vehicle-treated mice; #, $P < 0.05$ versus LPS-treated WT mice; ##, $P < 0.01$ versus LPS-treated WT mice. Values are the mean \pm S.E.M. in each group.

Recent studies have demonstrated that protease inhibitors may have anti-inflammatory roles other than merely suppressive effects on protease actions during inflammation. UTI inhibits prostaglandin H2 synthase-2 in neutrophils (Zaitzu et al., 2000), thromboxane B2 in monocytes (Aibiki and Cook, 1997), IL-8 in human bronchial epithelial cells (Nakamura et al., 1997), and TNF- α in monocytes (Aosasa et al., 2001). All these previous studies, however, have been conducted in vitro. In our study, the protein levels of MCP-1 in the lungs; MCP-1 and KC in the kidneys; and IL-1 β , MIP-2, MCP-1, and KC in the liver are significantly greater in UTI (–/–) mice than in WT mice after LPS challenge. Our experiments should be the first in vivo demonstration of the local (lung, kidney, and liver) anti-inflammatory role of UTI in LPS-related inflammation at the levels of protein expression of proinflammatory cytokines and chemokines. We can hypothesize that UTI protects against organ damage after LPS challenge, at least in part, via the inhibition of these proinflammatory cytokines. It is interesting that the protein levels of proinflammatory molecules were significantly greater in UTI (–/–) mice than in WT mice after LPS challenge in the liver, whereas there were no significant histological differences between the two genotypes. Expression of a variety of proinflammatory cytokines and chemokines reportedly cause the inflammatory tissue injury (Karima et al., 1999). Thus, it is possible that the enhancement in the expression of proinflammatory molecules in the liver 72 h after LPS challenge can precede that in the histological changes thereafter. Additional time course studies are needed in the future.

Finally, fibrinogen also reportedly stimulates the production of IL-1 β (Fan and Edgington, 1993; Perez and Roman, 1995) and chemokines such as IL-8, MCP-1, and MIP (Qi and Kreutzer, 1995; Harley and Powell, 1999; Walzog et al., 1999; Liu and Piela-Smith, 2000). It is also likely that UTI inhibits local cytokine expressions, at least partly, through the suppression of fibrinogen synthesis.

In conclusion, UTI protects against circulatory inflammatory response and subsequent organ damage induced by LPS, at least in part, via the modulation of proinflammatory cytokine, IL-1 β , and chemokines such as MIP-2, MCP-1, and KC. These results provide direct molecular evidence for the “rescue” therapeutic utility of UTI against systemic inflammatory response syndrome such as DIC, acute lung injury, and multiple organ dysfunction syndrome.

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