Urinary Trypsin Inhibitor Protects against Systemic Inflammation Induced by Lipopolysaccharide

KEN-ICHIRO INOUE, HIROHISA TAKANO, AKINORI SHIMADA, RIE YANAGISAWA, MIHO SAKURA, SHIN YOSHINO, HIROYUKI SATO, and TOSHIKAZU YOSHIKAWA

Inhalation Toxicology & Pathophysiology Research Team, National Institute for Environmental Studies, Tsukuba (K. I., H. T., R. Y., M. S.); Inflammation and Immunology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto (H. T, T. Y.); Department of Vaterinary Pathology, Faculty of Agriculture, Tottori University, Tottori (A. S.); Department of Pharmacology, Kobe Pharmaceutical University, Kobe (S. Y.); and Reserch Center, Mochida Pharmaceutical Company, Ltd, Shizuoka, Japan (H. S.).

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Running Title: UTI in systemic inflammation

Address for correspondence: Hirohisa Takano, MD, PhD, Inhalation Toxicology & Pathophysiology Research Team, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, 305-8506, Japan

phone and fax: +81-29-850-2334

e-mail: htakano@nies.go.jp

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ABBREVIATIONS: UTI, urinary trypsin inhibitor; LPS, lipopolysaccharide; UTI (-/-), UTI-deficient; WT, wild type; MCP, macrophage chemoattractant protein; KC, keratinocyte chemoattractant; DIC, disseminated intravascular coagulation; PHS, prostaglandin H2; TX, thromboxane; IL, interleukin; TNF, tumor necrosis factor; i.p., intrperitoneally; PBS, phosphate-buffered saline; PT, prothrombin time; FDP, fibrinogen/fibrin degradation; WBC, white blood cell; ELISA, enzyme-linked immunosorbent assays; MIP, macrophage inflammatory protein.

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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 decrease in white blood cell counts as 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 livers, 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.

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Introduction

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 (Underhill and Ozinsky, 2002; Karima et al., 1999). 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 (Karima et al., 1999; Bhole and Stahl, 2003; Hardaway, 2000; Esmon et al., 1999). Proteases may also modulate inflammatory response elicited by LPS, since 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 neutrophils elastase during

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inflammation (Pratt et al., 1989). UTI has been widely used as a drug for patients with disseminated intravascular coagulation (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 well as the 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 (PHS)-2 (Zaitsu et al., 2000), thromboxane (TX) 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 knock out 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) damages 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

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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 Institute of Health. All animal studies were approved by the Institutional Review Board. 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-hour light-dark cycle in our Level B pathogen-free facility. Male mice of both genotypes were used at 10-12 weeks of age and 27-31 g in weight.

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

Coagulation and fibrinolysis analysis and peripheral white blood cell 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 3,000 g for 10 minutes as previously conducted (Inoue et al., 2004). The prothrombin time (PT) was evaluated by incubating 50 μl of plasma for 5 minutes at 37℃ and then adding 100 μl of an equal volume mixture of Simplastin (DIAGNOSTICA STAGO, Roche, Japan) and 30 mmol/L of $CaCl_2$. Murine clottable plasma fibrinogen was determined using commercial kit (DIAGNOSTICA STARGO, Roche) and the values compared to a human plasma fibrinogen standard (DIAGNOSTICA STARGO, Roche). Fibrinogen/fibrin degradation products (FDP) were measured with a commercial kit (DIAGNOSTICA

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STAGO, Roche), which utilizes a latex turbidimetric immuno assay. All assays were measured in STA Compact (DIAGNOSTICA STAGO, Roche) as previously described (Inoue et al., 2004).

In a separate series of experiments, blood samples were collected and white blood cell (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 μm thickness were routinely processed with hematoxylin and eosin stain as previously described (n = 5 in each group) (Takano et al., 1997; Takano et al., 2002). In $\times$40 fields, the area of inflammation was chosen randomly from different sections of each organ and measured using videomicrometer (Olympus, Tokyo, Japan). The number of neutrophils per mm^2 in each area was counted with the micrometer under oil immersion. Results were expressed as the number of neutrophils per mm^2 of inflammatory sites. Histologic sections were evaluated in a blind fashon.

Enzyme-linked immunosorbent assays for cytokines and chemokines. In a separate series of experiments, the animals were exsanguinated and the lungs, the kidneys, and the livers were subsequently homogenized with 10 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM ethylenediaminetetraacetic acid (Sigma), 0.1 mM phenylmethanesulphonyl fluoride (Nacalai Tesque, Kyoto, Japan), 1 μM pepstatin A (Peptide Institute, Osaka, Japan), and 2 μM leupeptin (Peptide Institute) as described previously (Takano et al., 2002; Inoue et al., 2004). The homogenates were

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then centrifuged at 105,000 g for 1 hour. The supernatants were stored at -80 ℃. Protein concentration was determined using the Bradford protein concentration assay kit (Bio-rad Laboratories Inc., Hercules, California, USA) (Bradford, 1976). Enzyme-linked immunosorbent assays (ELISA) for IL-1 β (Endogen, Cambridge, MA), 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 manufacture'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 pg/ml 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 pg/ml, 9 pg/ml, 1.5 pg/ml, 1.5 pg/ml, 10 pg/ml, and 2 pg/ml, respectively.

Statical analysis. Data were reported as mean ± SEM using Stat view version 4.0 (Abacus Concepts, Inc., Berkeley, CA) as previously described (Takano et al., 1997). Differences were analyzed by ANOVA followed by Fisher's PLSD test (Takano et al., 1997). Significance was assigned to P values smaller 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 hours after i. p. challenge with LPS or vehicle (Table). Levels of fibrinogen

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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 as 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, the kidney, and the liver obtained from both genotypes of mice 72 hours 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 found in LPS-treated WT mice than in LPS-treated UTI (-/-) mice (Fig. 1B). Vehicle treatment caused little histopathological changes (Fig. 1C, D) in both genotypes of mice.

LPS challenge induced neutrophilic infiltration around glomeruli and in the interstinum in the kidney obtained from both genotypes of mice (Fig. 2A, 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

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changes (Fig. 2C, D) in both genotypes of mice.

LPS caused wide spread centrilobular vacuolation of hepatocytes and neutrophilic infiltration in the liver obtained from both genotypes of mice (Fig. 3A, B). In the presence of LPS, there were no significant differences between both genotypes of mice. Vehicle treatment caused few histopathological changes (Fig. 3C, D) in both genotypes of mice.

The damages to the intestinal mucosal barrier in the presence of LPS were not apparent in both genotypes of mice (data not shown).

We performed morphometric analysis to quantitate the number of neutrophils in the lung, the kidney, and the liver tissues 72 hours after LPS challenge. As compared to 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 when compared with WT mice ($P < 0.01$: Fig. 4A). As compared to vehicle, LPS increased the numbers of neutrophils in the kidney from UTI (-/-) mice with significance ($P < 0.01$) and those from WT mice without significance (Fig. 4B). In the presence of LPS, UTI (-/-) mice showed significantly increased numbers of neutrophils in the kidney as compared with WT mice ($P < 0.05$: Fig. 4B). As compared to 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).

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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, the kidney, and the liver 72 hours 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 as compared to vehicle challenge ($P < 0.01$: Fig. 5A, 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. 5A, C-E), and KC ($P < 0.05$: Fig. 5F) as compared to 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 as compared to vehicle challenge ($P < 0.01$ for IL-1 β , MIP-2, MCP-1, and KC: Fig. 6A, D-F, $P < 0.05$ 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 as compared to vehicle treatment ($P < 0.01$ for IL-1 β , MIP-1 α , and MIP-2: Fig. 6A, C, D, $P < 0.05$ for MCP-1 and KC: Fig. 6E, 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. 6E, 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 as compared to vehicle challenge ($P < 0.01$ for IL-1 β , MIP-1 α , MIP-2, and MCP-1: Fig.

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7A, C-E, $P < 0.05$ for TNF-α and KC: Fig. 7B, F). In WT mice, LPS treatment induced significant enhancement in the expression of IL-1β, MIP-1α, and MCP-1 as compared to vehicle treatment ($P < 0.01$ for IL-1β and MIP-1α: Fig. 7A, C, $P < 0.05$ for MCP-1: Fig. 7E). In the presence of LPS, the liver expression of IL-1β, MIP-2, MCP-1, and KC was significantly greater in UTI (-/-) mice than in WT mice ($P < 0.01$ for MIP-2: Fig. 7D, $P < 0.05$ for IL-1β, MCP-1, and KC: Fig. 7A, 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 as compared with WT mice. LPS challenge induces more prominent neutrophilic 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-1β, 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 (Underhill and Ozinsky, 2002; Karima et al., 1999; Bhole and Stahl, 2003; Hardaway, 2000; Esmon et al., 1999). 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

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elastase exerts the most injurious effects on many kinds of substrates (elastin, type I through 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 and co-workers have shown that blockade of pancreatic proteases in the intestinal lumen ameliorates systemic inflammation induced by intravenous administration of LPS (Fitzal et al., 2003).

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 neutrophils elastase activity *in vitro* (Gando and Tedo, 1995; Ogawa et al., 1987) 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 and colleagues 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 (Tani et al., 1993). 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 as compared with WT mice. Thus, our 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 as 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). Taken 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 damages elicited by LPS *in vivo*. The lungs and the kidneys are consistently the 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 damages including neutrophil infiltration were more prominent in LPS-treated UTI (-/-) mice than in LPS-treated WT mice.

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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 damages related to LPS possibly via the inhibition of neutrophil sequestration in the organs.

Recent studies have demonstrated that protease inhibitors may have anti-inflammatory roles other than merely suppressive effects on protease actions during inflammation. UTI inhibits PHS-2 in neutrophils (Zaitsu et al., 2000), TXB2 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-1β, MIP-2, MCP-1, and KC in the livers 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 damages after LPS challenge, at least in part, via the inhibition of these proinflammatory cytokines. Interestingly, 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 hours 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; Liu and Piela-Smith, 2000; Walzog et al., 1999). 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 damages induced by LPS, at least partly, 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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Figure legends

Fig. 1. Histopathological findings of the lung obtained from (A) urinary trypsin inhibitor (UTI) null (-/-) mice injected i. p. with 1 mg/kg body weight of lipopolysaccharide (LPS), (B) wild type (WT) mice injected with LPS, (C) UTI (-/-) mice injected with vehicle, and (D) wild type (WT) mice injected with vehicle (n = 5 in each group). 72 hours after injection, mice were killed and assessed. Original magnification ×400.

Fig. 2. Histopathological findings of the kidney obtained from (A) UTI (-/-) mice injected with LPS, (B) WT mice injected with LPS, (C) UTI (-/-) mice injected with vehicle, and (D) WT mice injected with vehicle (n = 5 in each group). 72 hours after injection, mice were killed and assessed. Original magnification ×200.

Fig. 3. Histopathological findings of the liver obtained from (A) UTI (-/-) mice injected with LPS, (B) WT mice injected with LPS, (C) UTI (-/-) mice injected with vehicle, and (D) WT mice injected with vehicle (n = 5 in each group). 72 hours after injection, mice were killed and assessed. Original magnification ×400.

Fig. 4. Quantitative analysis of neutrophil sequestration into the different compartments from the (A) lung, (B) the kidney, and (C) the liver. The organs of both WT (open symbols) and UTI (-/- : filled symbols) mice were harvested 72 hours after i. p. injection of vehicle or LPS (n = 5 in each group). The numbers of neutrophils per mm^2 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. Values are the mean ±

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SEM 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 (-/- : filled symbols) mice were harvested 72 hours after i. p. injection of vehicle or LPS (n = 8 in each group). Interleukin (IL)-1 β (A), tumor necrosis factor (TNF)- α (B), macrophage inflammatory protein (MIP)-1 α (C), MIP-2 (D), macrophage chemoattractant protein (MCP)-1 (E), and keratinocyte chemoattractant (KC: F) levels in the lung tissue supernatants were measured by enzyme-linked immunosorbent assays (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 $\pm$ SEM in each group.

Fig. 6. Cytokine and chemokine profiles in the kidney after LPS challenge. Kidney tissue supernatants of both WT (open symbols) and UTI (-/- : filled symbols) mice were harvested 72 hours 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, $^{\#\#}$ $P < 0.01$ versus LPS-treated WT mice. Values are the mean $\pm$ SEM 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 (-/- : filled symbols) mice were harvested 72 hours after i. p. injection of vehicle or LPS (n = 8 in each group). IL-1 β

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(A), TNF-α (B), MIP-1α (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 $\pm$ SEM in each group.

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

Group	Challenge	PT (s)	Fibrinogen (mg/dl)	FDP (μ g/ml)	WBC (/ μ l)
WT	vehicle	11.9±0.1	261.7±12.9	1.8±0.4	3925±237
WT	LPS	11.9±0.3	329.9±11.1 *	2.2±0.2	3701±324
UTI (-/-)	vehicle	11.3±0.1	273.3±19.2	1.5±0.2	3198±255
UTI (-/-)	LPS	10.1±1.0 #	389.1±22.4 ** #	3.2±0.2 ** ##	2507±210 ** ##

Blood samples were collected 72 hours 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. * $P < 0.05$ 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 ± SEM.