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Urokinase-Type Plasminogen Activator Potentiates Lipopolysaccharide-Induced Neutrophil Activation

Edward Abraham,* Margaret R. Gyetko,† Katherine Kuhn,* John Arcaroli,* Derek Strassheim,* Jong Sung Park,* Srerama Shetty,‡ and Steven Idell‡

Urokinase plasminogen activator (uPA) is a serine protease that catalyzes the conversion of plasminogen to plasmin. Although increased circulating levels of uPA are present in endotoxemia and sepsis, conditions in which activated neutrophils contribute to the development of acute organ dysfunction, the ability of uPA to participate directly in LPS-induced neutrophil activation has not been examined. In the present experiments, we show that uPA can enhance activation of neutrophils exposed to submaximal stimulatory doses of LPS. In particular, uPA increased LPS-induced activation of intracellular signaling pathways, including Akt and c-Jun N-terminal kinase, nuclear translocation of the transcriptional regulatory factor NF-κB, and expression of proinflammatory cytokines, including IL-1β, macrophage-inflammatory protein-2, and TNF-α. There was no effect of uPA on LPS-induced activation of p38 mitogen-activated protein kinase in neutrophils. Transgenic mice unable to produce uPA (uPA−/−) were protected from endotoxemia-induced lung injury, as determined by development of lung edema, pulmonary neutrophil accumulation, lung IL-1β, macrophage-inflammatory protein-2, and TNF-α cytokine levels. These results demonstrate that uPA can potentiate LPS-induced neutrophil responses and also suggest that such effects are sufficiently important in vivo to play a major contributory role in neutrophil-mediated inflammatory responses, such as the development of acute lung injury.

of plasminogen binding occurs with neutrophil activation and during acute inflammatory responses in vivo (34). Additionally, uPA can modulate neutrophil functions, such as chemotaxis and priming for superoxide anion release (12, 13, 19, 35). Although potentiation of neutrophil activation can occur when there is contemporaneous signaling through membrane-associated Toll-like receptor, cytokine, or G protein-coupled receptors, no studies have examined the ability of uPA to modulate LPS-induced neutrophil responses. In the present experiments, we show that uPA can enhance activation of neutrophils exposed to submaximal stimulatory doses of LPS. We also demonstrate the in vivo significance of these findings by showing that transgenic mice unable to produce uPA are protected from endotoxin-induced lung injury.

**Materials and Methods**

### Mice

Male BALB/c mice, 8–12 wk of age, were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Transgenic mice with a targeted deletion of uPA (uPA−/−) as well as control mice on the same C57Bl6/129 background have been previously described (31–33, 36). The genotypes of neutrophils, as determined by Wright’s stain, were used to confirm the neutrophils to pass through by negative selection methods. Viability, as determined by trypan blue dye exclusion, was consistently greater than 98%. Neutrophil purity, as determined by Wright’s stained cytospin preparations, was greater than 97%.

### Myeloperoxidase (MPO) assay

MPO activity was assayed, as reported previously (25, 26). Excised lungs from three mice per treatment group were frozen in liquid nitrogen, weighed, and stored at −86°C. Lungs were homogenized for 30 s in 1.0 ml 20 mM potassium phosphate, pH 7.4, and centrifuged at 4°C for 30 min at 40,000 × g. The pellet was resuspended in 200 μl 50 mM potassium phosphate, pH 6.0, containing 0.5% hexadecyltrimethyl ammonium bromide, sonicated twice for 60 s, incubated at 60°C for 2 h, and centrifuged. The supernatant was assayed for peroxidase activity and corrected to lung weight.

### Wet-to-dry lung weight ratios

All mice used for lung wet-to-dry weight ratios were of identical ages. Lungs were excised, rinsed briefly in PBS, blotted, then weighed to obtain the wet weight. Lungs were then dried in an oven at 70°C for 7 days to obtain the dry weight.

### Western blot analysis

Western blots to detect levels of phosphorylated and total p38, Akt, and JNK were performed essentially as previously described (26, 38). Neutrophils were lysed in ice-cold lysis buffer (20 mM HEPES-glutamine was obtained from Mediatech; Herndon, VA), while FBS and penicillin/streptomycin were purchased from Mediatech (Herndon, VA), while FBS and penicillin/streptomycin were purchased from Mediatech. Bicinchoninic acid (BCA) protein assay reagent was obtained from Pierce (Rockford, IL). Abs specific for p-p38, p-Akt, p-JNK (c-Jun N-terminal kinase), as well as total p38, Akt, and JNK were purchased from Cell Signaling Technologies (Beverly, MA). Custom cocktail Abs and columns for neutrophil isolation were purchased from Stem Cell Technologies (Vancouver, British Columbia, Canada). Recombinant high m.w. two-chain uPA was a generous gift from J. Henkin (Ab-bott Laboratories). The LPS concentration of the stock uPA solution (4.5 mg uPA/ml) was measured by enzyme-linked immunosorbent assay (Bio-Whittaker, Rockland, ME) and was found to be minimal, 1 pg/ml.

### Model of endotoxemia-induced lung injury

The model of endotoxemia was used, as reported previously (25, 26). Mice received an i.p. injection of LPS at dose of 1 mg/kg in 0.2 ml PBS. This dose has previously been demonstrated to produce acute neutrophilic alveolitis, histologically consistent with acute lung injury in mice (25, 26).

### Isolation of neutrophils

Neutrophils were purified from bone marrow cell suspensions, as previously described (26, 38). Briefly, to obtain the bone marrow cell suspension, the femur and tibia of a mouse were crushed with 5 ml RPMI 1640/penicillin/streptomycin, and the cells were passed through a glass wool column and pelleted by centrifugation at 1000 rpm for 10 min. The cell pellets were resuspended in 0.3% FCS/PBS and then incubated with 20 μl of primary Abs specific for cell surface markers F4/80, CD4, CD45R, CD5, and TER119 for 15 min, rotating at 4°C. This custom cocktail (Stem Cell Technologies) is specific for T and B cells, RBC, monocytes, and macrophages. Anti-biotin tetrameric Ab complexes (100 μl) were then added, and the cells were incubated for 15 min, rotating at 4°C. Following this, 60 μl of colloidal magnetic dextran iron particles was added to the suspension, 5 min on ice, and then washed twice with PBS, blotted, then weighed to obtain the wet weight. Lungs were then dried in an oven at 70°C for 7 days to obtain the dry weight.

### Pulmonary cytokine measurements

Pulmonary cytokine concentrations of murine IL-1β, MIP-2, and TNF-α were measured by ELISA (ELISA Tech, Aurora, CO), as previously described (26). Briefly, plates were coated with recombinant cytokines, and 100 μl of standards and samples were examined in duplicate. The membranes were then stripped using stripping buffer (63 mM Tris-HCl, pH 6.8, 2% SDS, 100 mM 2-ME; from Pierce) and incubated at 300 rpm for 15 min, and the supernatants were collected. Cytokine ELISAs were performed using specific Abs pairs and compared with a standard curve constructed using recombinant murine cytokine. All dilutions of standards and samples were examined in duplicate.

### Real-time quantitative RT-PCR

Quantitative RT-PCR to measure neutrophil cytokine expression was performed, as previously described (26, 30). In brief, total cellular RNA was isolated from neutrophils using the Bio-Rad Agua Pure RNA Isolation Kit (Bio-Rad), as recommended by manufacturer. Real-time RT-PCR was performed with specific primers and probes corresponding to the proinflammatory cytokine genes IL-1β, MIP-2, and TNF-α. For each mRNA detection, a fluorogenic probe and two primers (forward and reverse) for PCR were synthesized (Applied Biosystems, Foster City, CA). The internal oligonucleotide probe was labeled with the fluorescent dyes FAM at the 5′ end and TAMRA at the 3′ end. For murine IL-1β mRNA detection, the forward and reverse primers were: 5′-GCTGAAAGCTTCCACCTCAA-A-3′ and 5′-TCGGTTGCTGTCTCCACTC-3′, respectively. The internal probe was: 5′-FAM-CAGATAATACACAGATAGATCTCTATGGCGC(TAMRA)-3′. The forward and reverse primers for murine MIP-2 were: 5′-TGGTACGGCCCGAGGAGA-3′ and 5′-AATCTTTTGGACCCCTGAG-3′, respectively. The internal probe was: 5′-FAM-TGCCAGAGCAGCTGCTTGAAAGC(TAMRA)-3′. For murine TNF-α mRNA detection, the forward and reverse primers were: 5′-CTGTAAGGCGGAGTCGAGTC-3′ and 5′-CTTTGTTGTGATGATGAAATCG-3′; respectively. The internal probe was: 5′-FAM-GAGGAGCACAGCAGCAGT(TAMRA)-3′.
To optimize the primer sets, a primer optimization experiment was performed, as described in the manufacturer’s protocol. Based on primer optimization, the primer and probe concentrations for IL-1β, MIP-2, and TNF-α were 200 nM for both the primers and the probe, used in each reaction with 100 ng of total cellular RNA. In each experiment, ribosomal RNA control probe, forward primer, and reverse primer (Applied Biosystems) at concentrations of 50 nM were used to normalize for the amount of RNA in each sample.

All reagents used in the one-step RT-PCR were purchased from Applied Biosystems. Each one-step RT-PCR contained a total volume of 50 μl. The reverse-transcriptase reaction was performed for 30 min at 48°C using MultiScribe reverse transcriptase (Applied Biosystems), with a final concentration of 0.025 U/μl, was activated by an increase in temperature to 95°C for 10 min, followed by 40 cycles of amplification (95°C for 15 s and 60°C for 1 min) with a Gene Amp 5700 Sequence Detection System (ABI Prism, Foster City, CA). The quantity of cytokine mRNA was determined from a standard curve with 10-fold dilutions of known amounts of target RNA for each primer and probe set. RNA amounts were determined using software provided with the Gene Amp 5700 Sequence Detection System. Quantification was determined by dividing the target amount of each cytokine sample by the amount of 18S ribosomal RNA.

EMSAs
EMSAs were performed, as previously described (26, 30). To obtain nuclear extracts from neutrophils, cells were resuspended in buffer A containing 10 mM HEPES (pH 7.9), 1.5 mM MgCl₂, 10 mM KCl, and 0.5 mM DTT, and the samples were incubated on ice for 20 min. After cytoplasm was removed from the nuclei by 15 passages through a 25-gauge needle, the nuclei were collected by centrifugation at 600 × g for 6 min at 4°C. The pellets were suspended in buffer C containing 20 mM HEPES, 1.5 mM MgCl₂, 420 mM NaCl, 0.2 mM EDTA, 25% glycerol, and 0.5 mM PMSF. After a 30-min incubation on ice, the suspension was centrifuged at 14,000 × g for 20 min at 4°C, and the supernatant was collected. The protein concentration in the supernatants was determined using BCA protein assay kit (Pierce). Nuclear extracts (5 μg) were incubated at room temperature for 20 min in 20 μl of reaction buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM MgCl₂, 0.5 mM EDTA, 0.5 mM DTT, 50 mM NaCl, and 4% glycerol with 32P end-labeled, double-stranded oligonucleotide probe specific for the AP-1 site, 5′-AGTTGAGGGGACCTTC CCGAGG-3′ (Genekea, Burlington, VT), and 1 μg of poly(dI-dC)/poly(dI- dC). In some experiments, unlabeled NF-κB or CREB oligonucleotide (Promega, Madison, WI) was added to the samples at 200-fold excess before the addition of labeled probe and incubated for 15 min on ice. The complexes were resolved on 5% polyacrylamide gels in Tris-HCl (pH 8.0)-borate-EDTA buffer at 10 V/cm. Dried gels were exposed with Kodak Biomax MS film (Rochester, NY) for 1–24 h at −70°C.

Statistical analysis
For each experimental condition, the entire group of animals was prepared and studied at the same time. Separate groups of mice were used for Western blotting, EMSA, quantitative RT-PCR, and assessment of lung injury (i.e., poly I:C cytokine levels, MPO, and wet/dry ratios). Data are presented as mean ± SEM for each experimental group. One-way ANOVA and the Tukey-Kramer multiple comparisons test (for multiple groups) or Student’s t test (for comparisons between two groups) were used. Value of \( p < 0.05 \) was considered significant.

Results
uPA enhances LPS-induced cytokine expression by neutrophils
To assess possible interactions between uPA and LPS on neutrophil activation, neutrophils were incubated with varying concentrations of uPA (0, 1, and 10 ng/ml) and LPS (0, 1, 10, and 100 ng/ml), and mRNA levels for IL-1β, TNF-α, and MIP-2 were determined 4 h later. In these experiments, the lowest concentration of LPS had only minimal effects on neutrophil cytokine expression, while there was a dose-dependent increase in expression of all three cytokines at the higher concentrations of LPS (Fig. 1). There was no effect of uPA alone over the range of concentrations examined.

Combination of uPA with LPS resulted in enhanced expression of all three cytokines in a dose-dependent manner (Fig. 2). We excluded that the effect could be related to LPS contamination based on the minimal concentration of LPS (i.e., 1 pg/ml) that we detected in the stock uPA before dilution in the assays and the magnitude of the responses compared with those generated by considerably greater concentrations of LPS, as in Fig. 1. Although some potentiation of cytokine expression by uPA was present at both the highest and lowest concentrations of LPS tested, this effect was most evident at the mid-range concentration of LPS (Fig. 3). Combination of submaximal concentrations of LPS (i.e., 10 ng/ml) with 10 ng/ml uPA resulted in levels of cytokine expression that were not significantly different from those induced by 10-fold higher doses of LPS alone.

Effects of uPA on LPS-induced activation of p38, Akt, and JNK
Exposure of neutrophils to LPS results in activation of multiple kinases involved in proinflammatory cytokine expression. Among these, pathways leading to phosphorylation and activation of p38 mitogen-activated protein (MAP) kinase and Akt appear to be particularly important because inhibition of either of these significantly decreases LPS-induced cytokine production by neutrophils (26, 27). Recent data also show that additional kinase families that act downstream of tyrosine kinases and involve JNK are activated.
in neutrophils exposed to proinflammatory stimuli (39, 40). To examine the effects of uPA on LPS-induced activation of these kinase pathways, we coincubated LPS-stimulated neutrophils with or without uPA and then determined levels of the active, phosphorylated forms of p38, Akt, and JNK. Of note, culture of neutrophils with uPA alone had no effect on activation of p38, Akt, or JNK (data not shown).

Activation of p38 and Akt was found in neutrophils within 5 min of exposure to LPS, and continued to be present over the 60-min period examined (Figs. 4 and 5). Minimal increases in the levels of phosphorylated JNK were present starting at 5 min after LPS exposure, but only became greater than two times baseline at the 45-min time point (Fig. 6).

Coincubation of neutrophils with uPA and LPS resulted in greater activation of Akt than that found with LPS stimulation alone, particularly at the later time points examined (Fig. 4). In particular, whereas activation of Akt was relatively constant over the 60-min period after exposure to LPS, levels of phosphorylated Akt in the presence of uPA appeared to increase at the 30- and 60-min time points. In contrast, uPA had no effect on LPS-induced p38 activation (Fig. 5). Transient increases in levels of phosphorylated JNK were found 15 and 30 min after neutrophils were exposed to both LPS and uPA (Fig. 6). However, the relative increase in JNK phosphorylation induced by uPA was relatively small, being less than 30% in all cases, compared with the much greater enhancement of Akt phosphorylation, which almost 2-fold greater in neutrophils incubated with uPA and LPS for 45 min compared with that found in neutrophils cultured with LPS alone.

**FIGURE 2.** uPA potentiates LPS-induced neutrophil cytokine expression. Neutrophils were incubated without (Control) or with 10 ng/ml LPS and uPA (0, 1, or 10 ng/ml) for 4 h. Cytokine mRNA levels were determined by quantitative RT-PCR. ***$, p < 0.001$ vs control; ###, $p < 0.001$ vs LPS alone.

**FIGURE 3.** Effects of LPS concentration on uPA-associated alterations in neutrophil cytokine expression. Neutrophils were incubated with 10 ng/ml uPA and either without (Control) or with LPS (1, 10, or 100 ng/ml) for 4 h. Cytokine mRNA levels were determined by quantitative RT-PCR. Coincubation of neutrophils with 10 ng/ml uPA and 10 ng/ml LPS resulted in expression of IL-1β, TNF-α, and MIP-2 that was significantly greater than those found with 10 ng/ml uPA and 1 ng/ml LPS, and was similar to those produced by 10 ng/ml uPA and 100 ng/ml LPS, showing that the greatest potentiating effects of uPA were present at submaximal concentrations of LPS. ***$, p < 0.001$ vs control; ###, $p < 0.001$ vs 10 ng/ml uPA and 1 ng/ml LPS.

**uPA enhances LPS-induced nuclear translocation of NF-κB**

To examine the effects of uPA on transcriptional regulatory events induced in neutrophils by LPS, we determined nuclear levels of the transcriptional factor NF-κB in neutrophils stimulated with LPS alone or with both LPS and uPA. Binding sites for NF-κB are present in the promoter regions of the IL-1β, MIP-2, and TNF-α genes, and NF-κB plays an important role in the transcriptional regulation of each of these cytokines (26). Both p38 and Akt participate in enhancing nuclear accumulation of NF-κB and expression of NF-κB-dependent genes in LPS-stimulated neutrophils (26, 27).

Incubation of neutrophils with LPS resulted in nuclear translocation of NF-κB (Fig. 7). Addition of uPA to the neutrophil cultures produced further increases in LPS-induced NF-κB activation. Specificity of NF-κB binding was confirmed by demonstrating that inclusion of excess unlabeled competitor probe prevented the appearance of the specific NF-κB band. In contrast, no competition was observed with a distinct oligonucleotide specific for binding to the CREB transcriptional factor.
uPA participates in enhancing endotoxemia-induced acute lung injury

The above in vitro results demonstrate that uPA potentiates LPS-induced neutrophil activation, as shown by cytokine expression, kinase activation, and nuclear translocation of NF-κB. To determine whether these effects of uPA also have in vivo significance, we examined the development of endotoxemia-induced acute lung injury in mice with a targeted deletion of the uPA gene (uPA−/−) and background-matched control mice (uPA+/+). Of note, we have previously shown that lung injury is neutrophil dependent in this model (25).

Administration of endotoxin to control uPA+/+ mice resulted in an acute pulmonary inflammatory response, with accumulation of neutrophils into the lungs, the development of lung edema, and increased pulmonary levels of IL-1β, TNF-α, and MIP-2 protein (Fig. 8, A and B). In contrast, uPA−/− mice did not develop lung injury, as determined by interstitial edema, after endotoxin exposure. Similarly, no increase in neutrophil accumulation in the lungs was present in uPA−/− mice given endotoxin, and pulmonary cytokine levels were also significantly decreased in these animals compared with those found in uPA+/+ controls.

Discussion

In addition to its role in facilitating the cleavage of plasminogen to plasmin, uPA has been demonstrated to have additional effects on neutrophils and other cell populations that are independent of its proteolytic properties. Through interaction with uPAR and other receptors, uPA potentiates neutrophil functions important for host defense, including priming for superoxide production and chemokinesis (2, 12, 13, 19, 35). The present studies extend the spectrum of neutrophil functions in which uPA has a role by demonstrating that uPA is capable of potentiating LPS-induced neutrophil activation under in vitro and in vivo conditions. Whereas uPA itself appears incapable of activating neutrophils, combination of relatively low concentrations of uPA with LPS resulted in greater expression of proinflammatory cytokines, kinase activation, and nuclear translocation of NF-κB than that found with submaximal concentrations of LPS alone.

Although the p38 MAP kinase pathway plays a major role in LPS-associated activation of neutrophils (27), it does not appear to be involved in the potentiation of neutrophil responses induced by uPA. Coincubation of neutrophils with LPS and uPA did not result in any further increase in p38 activation beyond that found with...
LPS alone. In contrast, phosphorylation of Akt was enhanced when uPA was included to LPS-containing neutrophil cultures, suggesting that activation of Akt may be involved in uPA-induced potentiation of neutrophil responses. Phosphoinositide 3-kinases (PI3-K) and the downstream serine/threonine kinase Akt/protein kinase B have a central role in modulating neutrophil respiratory burst, chemotaxis, and apoptosis (41–44). Recent data also show that PI3-K and Akt are involved in LPS-induced neutrophil signaling (26). PI3-Ks catalyze the addition of phosphate molecules to the inositol ring of phosphoinositides. Akt is the best-characterized target of PI3-K-phosphorylated phosphoinositides (41, 42). The pleckstrin homology domain of Akt binds phosphoinositides, with activation of Akt dependent on phosphorylation of threonine 308 and serine 473 by phosphatidylinositol-dependent kinases (PDK1 and PDK2).

PI3-K is a heterodimeric complex, comprising a p110 catalytic subunit, of which there are four characterized isoforms (α, β, γ, and δ). The type IA PI3-Ks, p110α, p110β, and p110δ, associate with the p85 family of regulatory subunits, but type IB p110γ binds to a p101 adaptor molecule. Whereas type IA PI3-Ks are activated by engagement of tyrosine-phosphorylated molecules, p110γ is activated by engagement of G protein-coupled receptors (42, 45–47). In addition, PI3-Kγ can be activated by pathways independent of G proteins, such as those initiated by exposure of neutrophils to LPS (26). PI3-Kγ through its role in activating Akt appears to have an important role in modulating neutrophil functions in vivo. Recent studies in mice lacking functional PI3-Kγ showed that neutrophils from these animals are unable to activate Akt when stimulated with G protein-coupled receptor agonists, such as fMLP, C5a, or IL-8 (45–47). PI3-Kγ–/– neutrophils also demonstrate decreased phosphorylation of Akt when stimulated with LPS (26). Additionally, nuclear translocation of NF-κB and expression of proinflammatory cytokines, such as MIP-2 and TNF-α, produced by exposure of neutrophils to LPS were reduced in PI3-Kγ–/– cells compared with PI3-Kγ+/+ controls (26). Endotoxemia-induced neutrophil accumulation in the lungs, activation of NF-κB in lung neutrophils, and lung injury also were reduced in PI3-Kγ–/– mice compared with PI3-Kγ+/+ controls (26). The present findings, showing enhanced activation of Akt over that seen with LPS alone when neutrophils are exposed to both uPA and LPS, suggest that signaling through the PI3-K pathway via downstream effects on Akt and NF-κB may be involved in potentiation of LPS-induced neutrophil responses by uPA.

Transient increases in JNK phosphorylation occurred when uPA was combined with LPS. Previous studies in neutrophils indicated that proinflammatory stimuli, such as TNF-α, could activate JNK through a pathway involving the tyrosine kinases Syk and Pyk2.
with subsequent downstream activation of the MAP kinases mitogen-activated protein kinase kinase kinase 1, MAP kinase kinase 4, and MAP kinase kinase 7 (39). Despite the involvement of JNK in stress-activated responses, it is unlikely that JNK plays a major role in the uPA-associated enhancement of neutrophil responses found in the present experiments. First, the increases in JNK phosphorylation induced by the combination of uPA and LPS were transient and relatively modest in intensity. Second, and perhaps more importantly, JNK does not appear to be involved in modulating NF-κB activation, a major transcriptional regulatory factor for the cytokines examined in this study. Nevertheless, it is possible that JNK participates in uPA-induced potentiation of neutrophil activation because downstream signaling from JNK leads to activation of the transcriptional factor AP-1, which has been shown to participate in transcriptional control of TNF-α and IL-1β (48, 49).

It is unlikely that uPAR is involved in the uPA-induced enhancement of neutrophil responses found in the present experiments. Human uPA, which was used in these studies, does not interact with the murine uPAR (50). Interaction of uPA with receptors other than uPAR occurs on the neutrophil, and has been demonstrated to participate in neutrophil responses, such as chemotaxis and calcium flux (12, 13, 19, 35). Additionally, human uPA can associate with and cleave plasminogen in a species-nonspecific manner (7, 15, 24, 50). Plasmin derived from uPA-mediated plasminogen cleavage may lead to neutrophil activation through generation of proinflammatory mediators, such as IL-1β and matrix metalloproteinases, as well as through cleavage of uPAR (24, 51, 52). We are currently exploring these possibilities.

In the present experiments, uPA−/− mice were protected from LPS-induced acute lung injury. At the time point examined, lung injury is neutrophil dependent, because induced neutropenia prevents both development of pulmonary edema as well as increases in proinflammatory cytokines in the lungs (25). Previous studies (1, 20–22, 53) have shown rapid increases in circulating plasminogen activator levels as well as elevations in pulmonary concentrations of uPA after endotoxemia or bacterial infection, thereby providing the appropriate temporal relationship for interactions between LPS and uPA to enhance neutrophil activation and to contribute to the development of lung injury under in vivo conditions. Of note, pulmonary fibrinolytic activity is decreased under experimental conditions, when measured 2 h after induction of acute lung injury (54), and in patients with adult respiratory distress syndrome (55, 56), primarily reflecting increased intra-alveolar concentrations of plasminogen activator inhibitor 1 as well as various downstream antiplasmins. Therefore, it would appear that conditions allowing interactions between uPA and LPS to potentiate neutrophil activation may only be present transiently in the lungs after the initiation of endotoxemia, before being inhibited by increased expression of antiplasminolytic molecules. However, persistent elevations of plasma uPA activity have been found in septic patients (20–22), and it is possible that activated neutrophils migrate to the lungs and contribute to the development of acute lung injury after exposure to uPA and LPS in the peripheral circulation. In either case, the present experiments indicate that the potentiating effects of uPA on LPS-induced neutrophil responses are sufficiently important to play a major contributory role in the development of acute lung injury.

References


