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*J Immunol* 2006; 177:7233-7241; doi: 10.4049/jimmunol.177.10.7233
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Systemic Inhibition of the Angiotensin-Converting Enzyme Limits Lipopolysaccharide-Induced Lung Neutrophil Recruitment through Both Bradykinin and Angiotensin II-Regulated Pathways

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Recruitment of neutrophils to the lung is a sentinel event in acute lung inflammation. Identifying mechanisms that regulate neutrophil recruitment to the lung may result in strategies to limit lung damage and improve clinical outcomes. Recently, the renin angiotensin system has been shown to regulate neutrophil influx in acute inflammatory models of cardiac, neurologic, and gastrointestinal disease. As a role for the RAS in LPS-induced acute lung inflammation has not been described, we undertook this study to examine the possibility that the RAS regulates neutrophil recruitment to the lung after LPS exposure. Pretreatment of mice with the angiotensin-converting enzyme (ACE) inhibitor enalapril, but not the anti-hypertensive hydralazine, decreased pulmonary neutrophil recruitment after exposure to LPS. We hypothesize that inhibition of LPS-induced neutrophil accumulation to the lung with enalapril occurred through both an increase in bradykinin, and a decrease in angiotensin II (ATII), mediated signaling. Bradykinin receptor blockade reversed the inhibitory effect of enalapril on neutrophil recruitment. Similarly, pretreatment with bradykinin receptor agonists inhibited IL-8-induced neutrophil chemotaxis and LPS-induced neutrophil recruitment to the lung. Inhibition of ATII-mediated signaling, with the ATII receptor 1a inhibitor losartan, decreased LPS-induced pulmonary neutrophil recruitment, and this was suggested to occur through decreased PAI-1 levels. LPS-induced PAI-1 levels were diminished in animals pretreated with losartan and in those deficient for the ATII receptor 1a. Taken together, these results suggest that ACE regulates LPS-induced pulmonary neutrophil recruitment via modulation of both bradykinin- and ATII-mediated pathways, each regulating neutrophil recruitment by separate, but distinct, mechanisms. The Journal of Immunology, 2006, 177: 7233–7241.

A role for the renin angiotensin system (RAS) in cardiovascular disease has been well established, with inhibition of the angiotensin-converting enzyme (ACE) decreasing the incidence of recurrent myocardial infarction, the development of congestive heart failure, and death (1–6). Although less well characterized, several studies have also suggested a role for the RAS in pulmonary disease. Levels of ACE are elevated in bronchoalveolar lavage fluid (BALF) from subjects with sarcoidosis (7), idiopathic pulmonary fibrosis (7), and the acute respiratory distress syndrome (ARDS) (8), with mortality from ARDS positively correlated with ACE levels (9). Finally, the incidence and severity of pneumonia is decreased in subjects on ACE inhibitors (ACE-I) (10–12). Taken together, these studies suggest that ACE activity may regulate the acute inflammatory response in the lung, and in particular acute inflammation after exposure to LPS.

Although improvements in hemodynamics (i.e., blood pressure and cardiac output) are undoubtedly important mechanisms underlying the positive outcomes in subjects with cardiovascular disease treated with ACE-I, other mechanisms have also been proposed. For example, ACE has been suggested to regulate acute inflammation, as evidenced by the decrease in acute inflammation, and specifically cardiac neutrophil influx, with ACE inhibitor pretreatment in models of viral myocarditis (13–15) and cardiac ischemia/reperfusion (16), respectively. Similar findings have also been reported in noncardiovascular disease models, wherein inhibition of the RAS decreased localized neutrophil recruitment after intestinal or cerebral ischemia/reperfusion (17, 18), in a model of gastric ulceration (19), and in several models of pulmonary disease (20–24). Specifically in the lung, inhibition of ACE decreased the acute cellular inflammatory response in a bleomycin-induced model of interstitial lung disease (20), whereas inhibition of angiotensin II (ATII) decreased pulmonary neutrophil recruitment after the intratracheal administration of IMLP, Pseudomonas aeruginosa, or Bordetella bronchiseptica (21, 22). Although modulation of the RAS has been shown to decrease inflammation in the lung after exposure to formylated bacterial peptides or bacterial organisms, a role for ACE in acute lung inflammation induced by LPS and the mechanisms regulating the inhibition of pulmonary neutrophil recruitment with RAS modulation have not been examined previously.
The direct effect of ACE is to increase levels of ATII and decreases levels of bradykinin. Thus in the setting of ACE inhibition, regulation of neutrophil recruitment may occur as a result of a decrease in ATII, or an increase in bradykinin-induced signaling. Blockade of ATII decreased neutrophil influx after intratracheal challenge by Pseudomonas aeruginosa and Bordetella bronchi septica, thereby suggesting that ATII induces neutrophil recruitment and is proinflammatory (21, 22). In contrast, the role of bradykinin in regulating pulmonary neutrophil recruitment appears to be more complex, with bradykinin reported to be either pro- or anti-inflammatory depending on the stimulus and animal model used (25–30). More importantly, although modulation of ATII or bradykinin-induced signaling has been reported to modify neutrophil recruitment, the specific mechanism(s) by which this occurs is not clearly defined. One proposed mechanism is through a decrease in neutrophil capillary retention or adhesion, via decreases in CD11b or L-selectin expression (16–18). Alternatively, inhibition of ACE may modulate neutrophil recruitment via the regulation of plasminogen activator inhibitor-1 (PAI-1) expression. We have recently shown that PAI-1 regulates pulmonary neutrophil recruitment in a model of LPS-induced acute lung inflammation (31), and although not previously examined in the lung, inhibition of ACE has been shown to diminish PAI-1 expression (32–39), and although not previously examined in the lung, inhibition of PAI-1 expression in vitro and in vivo (40–47).

We undertook the present study to examine the role of the RAS in LPS-induced acute lung inflammation and to examine the mechanisms by which modulation of the RAS regulates neutrophil recruitment to the lung after exposure to LPS. Herein, we show that ACE inhibition decreased neutrophil recruitment to the lung after LPS exposure with an increase in bradykinin-, and a decrease in ATII-, induced signaling proposed as mechanisms for this diminution. Augmentation of bradykinin-mediated signaling inhibited neutrophil chemotaxis whereas inhibition of ATII-mediated signaling decreased PAI-1 expression, thereby limiting neutrophil recruitment to the lung after exposure to LPS.

Materials and Methods

Animals

Female C57BL/6 mice, or ATII type 1a receptor knockout (ATIIaR−/−) (on a B6129P2 background), age 8–12 wk, were obtained from The Jackson Laboratory. Animals were maintained in a pathogen-free environment on a 12-h light/dark cycle with full access to food and water. All animal protocols were approved by the Animal Care and Use Committee at National Jewish Medical and Research Center.

Materials

All materials used are endotoxin free with routine testing performed for the presence of endotoxin by the Limulus amebocyte lysate test (Pyrogenet; Cambrex). LPS (Escherichia coli, 0111:B4), enalapril, hydralazine, the bradykinin B1 receptor agonist [Lys-des-Arg9]-bradykinin, the bradykinin B2 receptor agonist [Hyp]-bradykinin, and the bradykinin B1 receptor antagonist [des-Arg9]-HOE 140 were obtained from Sigma-Aldrich. The bradykinin B2 receptor antagonist HOE 140 was obtained from BIOMOL. Losartan was obtained from Cayman Chemical, and IL-8 was obtained from R&D Systems. Hydroxypropylmethylcellulose was obtained from Fisher Scientific. Recombinant constitutively active PAI-1 (MPAI-191L) was obtained from Molecular Innovations, and calcine-AM was obtained from Molecular Probes.

Animal manipulation

ACE inhibition. To examine the role of ACE in LPS-induced lung inflammation, mice were pretreated with enalapril (10 mg/kg) for 6 days, as above, then administered the bradykinin B2 receptor antagonist HOE 140 (100 μg/kg, i.p.) or the bradykinin B1 receptor antagonist [des-Arg9]-HOE 140 (100 μg/kg, i.p.) 1 h before exposure to LPS, with animals sacrificed 4 or 24 h after LPS exposure. In separate experiments, mice were pretreated with the bradykinin receptor 1 agonist [Lys-des-Arg9]-bradykinin (100 μg/kg, i.v.) or the bradykinin receptor 2 agonist [Hyp]-bradykinin (100 μg/kg, i.v.) 4 h before exposure to aerosolized LPS (as above). Animals were sacrificed 4 h after LPS exposure with the recruitment of neutrophils to the alveolar space assessed.

PAI-1 repletion. To assess the role of PAI-1 in the inhibition of LPS-induced pulmonary neutrophil recruitment by systemic ACE inhibition, constitutively active PAI-1 (10 or 50 μg/mouse), or PBS as control, was administered intratracheally to enalapril pretreated mice (6 day pretreatment, as above), followed by exposure to aerosolized LPS and sacrifice 4 h after LPS exposure.

Myeloperoxidase (MPO) assay. As a marker of pulmonary neutrophil recruitment to the lung parenchyma, MPO levels were assessed from harvested lung as described previously (48).

Chemotaxis. Neutrophil chemotaxis to IL-8 was assessed using modified Boyden chambers. Isolated human neutrophils (1 × 106) were labeled with calcine-AM (1 ng/ml) for 10 min at 37°C, washed once in Kreb’s Ringers phosphate with dextrose (KRP-D), and then resuspended in KRP-D. Neutrophils were incubated with [Hyp]-bradykinin (0.1–100 μM), or KRP-D as control, at 37°C for 30 min and then placed into the upper wells of modified Boyden chambers. IL-8 (50 ng/ml) or KRP-D was placed into the lower wells with neutrophil chemotaxis, and nondirectional movement was assessed by measuring fluorescence (excitation wavelength 485, emission wavelength 528) in the lower chamber every 2 min over a 60-min time span using a fluorescent plate reader (FLX800; BioTek Instruments).

ELISA. Levels of KC, MIP-2, and PAI-1 were assessed in BALF or lung homogenates using commercially available ELISA kits as described previously (31). Specifically for PAI-1, ELISA plates were obtained from Molecular Innovations, which specifically detect total PAI-1 or active PAI-1 only with assays performed per the manufacturer’s recommendations. Lung homogenates were prepared by homogenizing previously frozen lungs with PBS with 0.1% Triton X-100 supplemented with PMSF (300 mM), aprotinin (10 μg/ml), and leupeptin (10 μg/ml). Homogenates were then centrifuged at 5000 rpm for 5 min with supernatants collected and frozen at −20°C until used in the assays.

ATII 1a receptor inhibition. The ATII 1a receptor inhibitor losartan (10 mg/kg, oral gavage), or hydroxypropylmethylcellulose as control, was administered daily to C57BL/6 mice for 3 days with a final dose given 5 h before aerosolized LPS exposure. Four or 24 h after exposure to LPS, mice were sacrificed with blood collected, BAL performed, and lungs isolated as described above.
Statistics. Statistical analysis was performed by one way ANOVA with Tukey-Kramer post hoc analysis or Student’s t test as indicated using Prism software (GraphPad Software). All graphs are presented as means ± SEM. A p value of <0.05 was considered significant.

Results
Systemic inhibition of ACE decreases pulmonary neutrophil recruitment after exposure to LPS

To examine a role for the RAS, and in particular ACE, in LPS-induced acute lung inflammation, C57BL/6 mice were administered enalapril (10 mg/kg, oral gavage) daily for 1 or 6 days, exposed to aerosolized LPS (300 µg/ml, 20 min), with neutrophil recruitment to the lung assessed at 4 or 24 h after LPS exposure. Pretreatment with enalapril decreased neutrophil recruitment to the alveolar space at both the 4 and 24 h post-LPS time points with more robust inhibition seen at 4 h (Fig. 1A). In addition, pretreatment with enalapril for 6 days, compared with a single day, was associated with a greater diminution in LPS-induced neutrophil recruitment to the alveolar compartment (Fig. 1A). Similar to the recruitment of neutrophils to the alveolar compartment, neutrophil recruitment to the lung, as assessed by MPO levels, was decreased in mice pretreated with enalapril for 6 days and then exposed to LPS compared with those exposed to LPS alone (Fig. 1B). In contrast, a single day of enalapril pretreatment was not effective in decreasing the LPS-induced recruitment of neutrophils to the lung, as assessed by MPO, compared with mice exposed to LPS alone (Fig. 1B). To exclude the possibility that the inhibitory effect of enalapril on LPS-induced pulmonary neutrophil recruitment was due to a decrease in systemic blood pressure, the antihypertensive agent hydralazine was used as a control. Mice were administered enalapril (10 mg/kg) or hydralazine (10 or 50 mg/kg) daily for 6 days, exposed to LPS, with pulmonary neutrophil recruitment assessed 4 h after exposure to LPS. Pretreatment with hydralazine, in
A decrease in neutrophil chemotacticants is not a mechanism for the diminution in neutrophil recruitment in the setting of systemic ACE inhibition

To determine whether a decrease in the level of the murine neutrophil chemotactic chemokines KC or MIP-2 was a mechanism for the decrease in pulmonary neutrophil recruitment in the setting of systemic ACE inhibition, we examined their levels in BALF. Compared with animals exposed to LPS only, there was no decrease in the levels of KC and MIP-2 in animals pretreated with enalapril and then exposed to LPS compared with those exposed to LPS alone (Fig. 3).

Pre-exposure of neutrophils to bradykinin decreases neutrophil chemotaxis

To determine a mechanism by which bradykinin-induced signaling decreased neutrophil recruitment to the lung after exposure to LPS we examined chemotaxis. Although systemic inhibition of ACE did not decrease the level of neutrophil chemotacticants after exposure to LPS (Fig. 3), the ability of neutrophils to respond functionally to these chemokines may be impaired in the setting of systemic ACE inhibition.
systemic ACE inhibition, with an augmented exposure of neutrophils to bradykinin as one possible mechanism. To examine this possibility, human neutrophils were pretreated with the B2 receptor agonist [Hyp³]-bradykinin (0.1–100 μM), or KR-P-D as control, followed by the assessment of neutrophil chemotaxis to IL-8 (see Materials and Methods). Pre-exposure of neutrophils to [Hyp³]-bradykinin decreased their subsequent chemotaxis toward IL-8 in a dose-dependent manner (Fig. 4A). In contrast, pretreatment of neutrophils with [Hyp³]-bradykinin had no effect on the nondirectional movement (chemokinesis) of neutrophils at any concentration (Fig. 4B and data not shown).

Inhibition of ACE decreases LPS-induced expression of PAI-1 in the lung

We have recently shown that PAI-1 expression increases in the lung after exposure to aerosolized LPS and that PAI-1 regulates pulmonary neutrophil recruitment in our model of LPS-induced acute lung inflammation (31). As ACE has been shown to regulate PAI-1 expression (32–39), we examined whether systemic inhibition of ACE decreased PAI-1 expression in the lung after LPS exposure. Pretreatment of mice with enalapril (10 mg/kg) for 6 days decreased the LPS-induced up-regulation of total PAI-1 in the lung and alveolar compartment, and levels of active PAI-1 in the lung, compared with LPS exposure alone (Fig. 5).

Administration of PAI-1 reverses the inhibitory effect of systemic ACE inhibition on the recruitment of neutrophils to the lung after exposure to LPS

As LPS-induced PAI-1 levels in the lung were decreased with enalapril pretreatment, we further examined the role of PAI-1 in regulating neutrophil recruitment to the alveolar space in the setting of systemic ACE inhibition. C57BL/6 mice were pretreated with enalapril (10 mg/kg, 6 days), then administered constitutively active PAI-1 (10 or 50 μg/mouse), or saline as control, 1 h before LPS exposure. The intratracheal administration of PAI-1 dose dependently reversed the inhibitory effect of enalapril on neutrophil recruitment to the lung (Fig. 5B). As PAI-1 levels were decreased in the lung with enalapril pretreatment, we also measured total and active PAI-1 in BALF (Fig. 5C). Pretreatment of mice with enalapril (10 mg/kg, 6 days), then administered constitutively active PAI-1 (10 μg/mouse), or saline as control, 1 h before LPS exposure. The intratracheal administration of PAI-1 dose dependently reversed the inhibitory effect of enalapril on neutrophil recruitment to the lung (Fig. 5).
recruitment to the alveolar compartment after LPS exposure (Fig. 6).

Inhibition of ATII-mediated signaling decreases pulmonary PAI-1 levels in LPS-exposed lungs

As administration of bradykinin agonists significantly inhibited LPS-induced pulmonary neutrophil recruitment, this suggested that bradykinin mediated signaling may also regulate pulmonary PAI-1 levels after exposure to LPS. No difference in LPS-induced PAI-1 levels, however, were found in mice pretreated with the bradykinin receptor agonists and exposed to LPS compared with those exposed to LPS alone (Fig. 7A). As ATII has been shown to up-regulate PAI-1 expression (40–47), we next examined whether ATII-induced signaling regulates PAI-1 expression after LPS exposure in our model. To examine this possibility, mice were pretreated with losartan (10 mg/kg) for 3 days and then exposed to LPS. As can be seen in Fig. 7B, pretreatment with losartan significantly decreased PAI-1 levels in the lung and alveolar compartment after exposure to LPS. To confirm these results, mice deficient in the ATII type 1a receptor, or wild-type controls, were exposed to LPS with pulmonary PAI-1 levels assessed. Similar to our results using the ATII type 1a receptor antagonist losartan, a deficiency in ATII type 1a receptor expression was associated with a decrease in pulmonary PAI-1 expression after LPS exposure (Fig. 7C).

Discussion

A role for ACE has been well established in the underlying pathophysiology of cardiovascular disease. Overexpression of ACE increases disease risk and worsens outcomes, whereas inhibition of ACE decreases the incidence of coronary artery disease and improves outcomes in congestive heart failure (1–6). Several studies have additionally implicated a role for ACE in pulmonary disease. Evidence supporting such a role for ACE includes the increased ACE levels in BALF from subjects with interstitial lung disease, sarcoidosis, pneumonia, and ARDS (7, 8); the increased incidence of pneumonia and ARDS in subjects with the ACE overexpressing polymorphism (9, 51); and the positive correlation between BALF ACE levels and clinical outcomes in ARDS (9). Although these studies strongly suggest a role for ACE in pulmonary disease, and in particular ARDS, the role of ACE in specific etiologies known

FIGURE 6. PAI-1 reverses the inhibitory effect of enalapril on LPS-induced pulmonary neutrophil recruitment. Recombinant constitutively active PAI-1 (10 or 50 μg/mouse), or PBS, was administered intratracheally to C57BL/6 mice (n = 5), pretreated with enalapril (10 mg/kg; 6 days), 1 h before exposure to LPS. Four hours after LPS exposure recruitment of neutrophils to the alveolar space was assessed as described above. * p < 0.001 LPS vs LPS plus enalapril; # p < 0.01 LPS plus enalapril plus PAI-1 (10) vs LPS plus enalapril; ** p < 0.001 LPS plus enalapril plus PAI-1 (50) vs LPS plus enalapril.

FIGURE 7. Inhibition of ATII-induced signaling, but not bradykinin-mediated signaling, diminishes the LPS-induced increase in PAI-1 levels. A, Mice (C57BL/6) (n = 5) were administered the bradykinin B1 receptor agonist [Lys-des-Arg9]-bradykinin (100 μg/kg; i.v.) (B1), the bradykinin B2 receptor agonist [Hyp3]-bradykinin (100 μg/kg; i.v.) (B2), or PBS as control, 1 h before exposure to LPS. Levels of total PAI-1 were then measured in the lung or BALF by ELISA. B, C57BL/6 mice (n = 5) were pretreated with losartan (10 mg/kg; orally), or hydroxymethylpropylcellulose as control, for 3 days and then exposed to aerosolized LPS. Total PAI-1 levels 4 h after LPS exposure in lung (left) and BALF (right) were assessed by ELISA. * p < 0.05 LPS plus losartan vs LPS. C, Mice deficient in the type 1a ATII receptor (ATII 1aR−/−) or wild-type controls (WT) were exposed to LPS with total PAI-1 levels from lung (left) and BALF (right) measured 4 h after exposure to LPS. * p < 0.01 WT vs ATII 1aR−/−.
to induce ARDS and the mechanisms by which ACE regulates the acute inflammatory response in the lung is poorly understood.

Our results presented here suggest that ACE regulates neutrophil recruitment to the lung after exposure to LPS, an early feature of the pathogenesis of ARDS. Although modulation of the RAS has been shown to decrease neutrophil influx to the lung after exposure to bacterial organisms or formylated bacterial peptides (21, 22), a role for ACE in regulating acute lung inflammation after exposure to LPS has not previously been examined. In addition, the decrease in LPS-induced pulmonary neutrophil recruitment in our study was not due to alterations in hemodynamics because pretreatment with the antihypertensive agent hydralazine had no effect on neutrophil accumulation in the lung after exposure to LPS, even though pretreatment with hydralazine decreased mean arterial blood pressure to a similar extent compared with pretreatment with enalapril (Fig. 1, C and D).

As ACE regulates levels of both angiotensin II and bradykinin, a decrease in ATII and/or an increase in bradykinin-induced signaling may be mechanisms for the diminution in pulmonary neutrophil recruitment in the setting of systemic ACE inhibition in our study. We propose that both an increase in bradykinin and a decrease in ATII-induced signaling are mechanisms regulating the decrease in pulmonary neutrophil recruitment in our model. In support for a role of bradykinin-induced signaling is that administration of bradykinin receptor agonists diminished neutrophil influx to the lung after exposure to LPS (Fig. 2A). Furthermore, pretreatment with antagonists to the bradykinin receptors reversed the inhibitory effect of systemic ACE inhibition, thereby implicating an increase in bradykinin-mediated signaling as a mechanism for the decrease in pulmonary neutrophil accumulation with enalapril pretreatment. Although not as robust, blockade of ATII signaling, via the ATII 1α receptor antagonist losartan, also diminished neutrophil recruitment to the lung, thereby suggesting that an enalapril-mediated decrease in ATII-induced signaling is an additional mechanism for the diminution in pulmonary neutrophil recruitment in our model, particularly at later time points. Other groups have also reported roles for ATII and bradykinin in regulating neutrophil recruitment to the lung. Antagonism of the ATII 1α receptor decreased cellular lung inflammation in a model of bleomycin-induced interstitial lung disease and neutrophil recruitment after intratracheal administration of ILMP, Pseudomonas aeruginosa, and Bordetella bronchiseptica (20–22). In contrast to ATII-mediated signaling, the role of bradykinin in pulmonary neutrophil recruitment appears to be agonist specific. Blockade of the bradykinin B2 receptor increased pulmonary neutrophil accumulation in both allergic and immune complex-mediated models of lung inflammation, suggesting an anti-inflammatory role for bradykinin, similar to our results presented here (Fig. 2B) (27, 29). In contrast, antagonism of the B2 receptor decreased neutrophil recruitment to the lung in models of ischemia-reperfusion, acute lung inflammation induced by i.v. Sephadex bead administration, and in a sepsis model of acute lung injury (30, 52, 53), and blockade of the B1 receptor decreased neutrophil influx induced by the localized administration of IL-1β (54).

Although bradykinin-induced signaling has been suggested to regulate neutrophil recruitment in acute inflammation, the mechanism by which bradykinin mediates these effects is at present unknown. Bradykinin, as well as ATII, are known to induce ARDS and the mechanisms by which ACE regulates the acute inflammatory response in the lung is poorly understood.

Our results presented here suggest that ACE regulates neutrophil recruitment to the lung after exposure to LPS, an early feature of the pathogenesis of ARDS. Although modulation of the RAS has been shown to decrease neutrophil influx to the lung after exposure to bacterial organisms or formylated bacterial peptides (21, 22), a role for ACE in regulating acute lung inflammation after exposure to LPS has not previously been examined. In addition, the decrease in LPS-induced pulmonary neutrophil recruitment in our study was not due to alterations in hemodynamics because pretreatment with the antihypertensive agent hydralazine had no effect on neutrophil accumulation in the lung after exposure to LPS, even though pretreatment with hydralazine decreased mean arterial blood pressure to a similar extent compared with pretreatment with enalapril (Fig. 1, C and D).

As ACE regulates levels of both angiotensin II and bradykinin, a decrease in ATII and/or an increase in bradykinin-induced signaling may be mechanisms for the diminution in pulmonary neutrophil recruitment in the setting of systemic ACE inhibition in our study. We propose that both an increase in bradykinin and a decrease in ATII-induced signaling are mechanisms regulating the decrease in pulmonary neutrophil recruitment in our model. In support for a role of bradykinin-induced signaling is that administration of bradykinin receptor agonists diminished neutrophil influx to the lung after exposure to LPS (Fig. 2A). Furthermore, pretreatment with antagonists to the bradykinin receptors reversed the inhibitory effect of systemic ACE inhibition, thereby implicating an increase in bradykinin-mediated signaling as a mechanism for the decrease in pulmonary neutrophil accumulation with enalapril pretreatment. Although not as robust, blockade of ATII signaling, via the ATII 1α receptor antagonist losartan, also diminished neutrophil recruitment to the lung, thereby suggesting that an enalapril-mediated decrease in ATII-induced signaling is an additional mechanism for the diminution in pulmonary neutrophil recruitment in our model, particularly at later time points. Other groups have also reported roles for ATII and bradykinin in regulating neutrophil recruitment to the lung. Antagonism of the ATII 1α receptor decreased cellular lung inflammation in a model of bleomycin-induced interstitial lung disease and neutrophil recruitment after intratracheal administration of ILMP, Pseudomonas aeruginosa, and Bordetella bronchiseptica (20–22). In contrast to ATII-mediated signaling, the role of bradykinin in pulmonary neutrophil recruitment appears to be agonist specific. Blockade of the bradykinin B2 receptor increased pulmonary neutrophil accumulation in both allergic and immune complex-mediated models of lung inflammation, suggesting an anti-inflammatory role for bradykinin, similar to our results presented here (Fig. 2B) (27, 29). In contrast, antagonism of the B2 receptor decreased neutrophil recruitment to the lung in models of ischemia-reperfusion, acute lung inflammation induced by i.v. Sephadex bead administration, and in a sepsis model of acute lung injury (30, 52, 53), and blockade of the B1 receptor decreased neutrophil influx induced by the localized administration of IL-1β (54).

Although bradykinin-induced signaling has been suggested to regulate neutrophil recruitment in acute inflammation, the mechanism by which bradykinin mediates these effects is at present unknown. Bradykinin, as well as ATII, are known to induce the release of neutrophil chemoattractants from endothelial cells and alveolar macrophages (55–59). As we found, however, no change in the levels of the neutrophil chemoattractants KC or MIP-2 in BALF from mice pretreated with enalapril and exposed to LPS compared with those exposed to LPS alone; a decrease in the neutrophil chemoattractant gradient in the lung is an unlikely mechanism for the inhibition of pulmonary neutrophil accumulation in the setting of systemic ACE inhibition. Our findings do suggest that bradykinin-induced signaling inhibits neutrophil chemotaxis toward a chemoattractant gradient. A previous study has suggested that bradykinin induces neutrophil chemotaxis (60); however, our findings presented here are in contrast to that previous study. Whereas we found no induction of chemotaxis with bradykinin alone (data not shown), pre-exposure of neutrophils to bradykinin decreased the subsequent chemotaxis to IL-8 without affecting the nondirectional movement of neutrophils (Fig. 4). To our knowledge this is the first description that bradykinin regulates neutrophil chemotaxis to known neutrophil chemoattractants. Although beyond the scope of the present study, the mechanisms by which bradykinin regulates neutrophil chemotaxis is not known but is the focus of ongoing studies.

Bradykinin is also known to up-regulate expression of NO, with inhibition of inducible NO expression reported to increase pulmonary neutrophil recruitment in other model systems (61–63). The possibility exists that ACE inhibition, or the administration of bradykinin receptor agonists, to LPS-exposed mice augmented levels of NO resulting in a decrease in neutrophil recruitment to the lung. Studies are currently underway to examine this possibility. Finally, ACE inhibitors have been shown recently to directly bind bradykinin receptors in vitro, thereby augmenting bradykinin-mediated signaling (64). Although a similar effect has not been shown in vivo, the possibility exists that enalapril binding to bradykinin receptors in the lung or on the neutrophil cell surface may have been a mechanism for the decrease in neutrophil accumulation to the lung after exposure to LPS in our study. Both the reversal of the effect of enalapril pretreatment by the bradykinin receptor antagonists and the more robust inhibition of pulmonary neutrophil recruitment with enalapril pretreatment, compared with pretreatment with the bradykinin receptor agonists alone, support such a mechanism. Although not the focus of the current study, such a process would provide a novel mechanism for the role of ACE inhibitors in regulating acute lung inflammation in vivo.

Similarly for ATII, the mechanisms by which ATII regulates neutrophil recruitment to areas of acute inflammation is poorly understood. Inhibition of ATII has been shown to decrease neutrophil capillary retention and adhesion through diminishing the expression of L-selectin and CD11b (17, 18). Although heretofore unexplored in acute pulmonary inflammation, and not explored herein, a decrease in L-selectin or the expression of CD11b may be mechanisms by which inhibition of ATII-induced signaling decreased LPS-induced neutrophil recruitment to the lung in our model. We do propose, however, that a decrease in PAI-1 expression, via a decrease in ATII-mediated signaling, is a mechanism for the diminution in LPS-induced neutrophil recruitment in the setting of systemic ACE inhibition. Expression of PAI-1 is dependent on the RAS. Inhibition of ACE decreases PAI-1 levels in human subjects with hypertension or the acute coronary syndrome and limits PAI-1 expression in vitro (32–39). This effect of ACE inhibition on PAI-1 expression likely occurs via a decrease in ATII levels, as ATII 1α receptor antagonists decrease serum PAI-1 levels in human subjects and in cell cultures in vitro and the administration of ATII up-regulates PAI-1 expression, both in vivo and in vitro models (40–47). Although inhibition of the RAS has been shown to decrease PAI-1 levels in cardiovascular disease, which was associated with improved outcomes, no study to date has investigated a role of the RAS in pulmonary PAI-1 expression (32–34, 39, 65). In addition, and more specifically, no study to our knowledge has examined the potential role of the RAS in regulating pulmonary PAI-1 expression after exposure to LPS and the role of RAS mediated PAI-1 expression in vivo.
pulmonary neutrophil recruitment. Our findings presented herein suggest that ACE is one factor that regulates the LPS-induced increase in pulmonary PAI-1 levels, as PAI-1 levels in the lung after exposure to LPS were diminished with systemic ACE inhibition. In addition, our results suggest that ATII-induced signaling regulates LPS-induced pulmonary PAI-1 expression as PAI-1 levels after LPS exposure were decreased both in animals pretreated with losartan and in ATII 1a receptor-deficient mice. In contrast, pulmonary PAI-1 expression after exposure to LPS does not appear to be regulated through bradykinin, as administration of bradykinin receptor antagonists to enalapril pretreated mice or pretreatment with bradykinin agonists alone did not alter PAI-1 levels after LPS exposure (Fig. 6A and data not shown). An overview of the proposed mechanisms for the regulation of LPS-induced pulmonary neutrophil recruitment by ATII and bradykinin is shown in Fig. 8.

We have recently shown that the mitogen-activating kinase JNK regulates LPS-induced pulmonary PAI-1 expression and that PAI-1 regulates the recruitment of neutrophils to the lung after exposure to LPS (31). Our current study now extends those findings and suggests that ACE activity, likely through ATII, is an additional pathway regulating PAI-1 expression in the lung after exposure to LPS and furthermore that a decrease in PAI-1 expression is a mechanism for the diminution in pulmonary neutrophil recruitment with ACE inhibition, as administration of exogenous PAI-1 reversed the inhibitory effect of enalapril on neutrophil influx to the lung after exposure to LPS (Fig. 5). Finally, our results suggest that ATII mediated signaling may regulate the activation of JNK in the lung after exposure to LPS.

Disclosures

The authors have no financial conflict of interest.

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15. delicate thrombotic mechanism that could be regulated by bradykinin, as administration of bradykinin receptor antagonists in enalapril pretreated mice or pretreatment with bradykinin agonists alone did not alter PAI-1 levels after LPS exposure (Fig. 6A and data not shown). An overview of the proposed mechanisms for the regulation of LPS-induced pulmonary neutrophil recruitment by ATII and bradykinin is shown in Fig. 8.

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