

Plasminogen activator inhibitor type-1 deficiency does not influence the outcome of murine pneumococcal pneumonia

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Urokinase-type plasminogen activator (uPA) and its receptor uPAR are components of the fibrinolytic system and are important for an adequate immune response to respiratory tract infection, in part through their role in the migration of inflammatory cells. PA inhibitor-1 (PAI-1) is the predominant inhibitor of soluble and receptor-bound uPA. To determine the role of PAI-1 in host defense against pneumococcal pneumonia, the following studies were performed: (1) Patients with unilateral community-acquired pneumonia demonstrated elevated PAI-1 concen-

trations together with decreased PA activity in bronchoalveolar lavage fluid (BALF) obtained from the infected, but not from the contralateral, site. (2) Mice with *Streptococcus pneumoniae* pneumonia displayed elevated PAI-1 protein and mRNA levels in their lungs. (3) PAI-1 gene-deficient mice, however, had an unaltered immune response to pneumococcal pneumonia, as measured by cell recruitment into lungs, bacterial outgrowth, and survival. Furthermore, plasminogen-gene-deficient mice also had an unremarkable defense against pneumococcal pneumo-

nia. These data indicate that pneumonia is associated with inhibition of the fibrinolytic system at the site of the infection secondary to increased production of PAI-1; an intact fibrinolytic response is not required for an adequate host response to respiratory tract infection, however, suggesting that the previously described role of uPA and uPAR are restricted to their function in cell migration. (Blood. 2003;102:934-939)

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Introduction

Intra-alveolar fibrin deposition is frequently found during acute inflammatory lung diseases.¹ The increased tendency to form fibrin deposits during lung inflammation probably is the result of stimulation of the coagulation system with concurrent inhibition of the fibrinolytic system. Early mediators of fibrinolysis are plasminogen activators (PAs), which activate plasminogen into plasmin, a potent protease that degrades fibrin into fibrin degradation products. PAs are controlled by specific inhibitors,² of which PA inhibitor type-1 (PAI-1) is considered most important, inactivating both urokinase-type PA (uPA) and tissue-type PA (tPA).^{3,4}

The pulmonary compartment is an important site of PAI-1 production and activity. Indeed, PAI-1 mRNA is induced in tissues of endotoxemic animals, in particular in the lung.^{5,6} In addition, bronchoalveolar lavage fluid (BALF) of patients with interstitial pulmonary fibrosis, sarcoidosis, and acute respiratory distress syndrome (ARDS) display elevated levels of PAI-1, which is held responsible for the suppressed alveolar fibrinolytic activity in these patients.⁷⁻¹¹ Recently, elevated PAI-1 concentrations were reported in BALF of patients with severe pneumonia and who required mechanical ventilation, which coincided with a profoundly suppressed fibrinolytic activity in the alveolar space.¹¹ BALF PAI-1 levels were unaltered in patients with less severe pneumonia, who were breathing spontaneously. Together these data indicate that a number of lung diseases, including pneumonia, are associated with

increased PAI-1 levels in the airways. Knowledge of the role of this endogenous PAI-1 in host defense against respiratory pathogens is highly limited, however.

Several lines of evidence indicate that PAI-1 may influence the innate immune response to pneumonia. We and others recently demonstrated that mice lacking the receptor for uPA (uPAR), which is expressed on different inflammatory cells, have an impaired leukocyte migration and are sensitive to pulmonary infections.^{12,13} On the contrary, uPA deficiency was associated with enhanced host defense and increased leukocyte influx into the inflamed area during pneumonia, suggesting that occupation of uPAR by uPA may hamper the beneficial role of this receptor in leukocyte recruitment.¹³ PAI-1 may affect leukocyte trafficking during pneumonia, and thus host defense, in several ways. Indeed, PAI-1 not only is an inhibitor of uPA in the lung, but can also interfere with cell adhesion in a more direct way.^{14,15} For example, the extracellular matrix protein vitronectin (VN) is a major binding protein for PAI-1. The binding of PAI-1 to VN competes with the binding of uPAR and integrins to VN, by which PAI-1 can inhibit integrin-mediated cell migration.¹⁶⁻²¹ Additional evidence supporting a role for PAI-1 in cell migration comes from tumor cell biology; that is, in a variety of malignant tumors, high expression of PAI-1 is predictive of more aggressive local invasion and metastasis and is a poor prognostic marker.²²⁻²⁷

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Table 1. Clinical and biochemical parameters of CAP patients

Subject	Sex	Age, y	Fever, °C	Site of infiltrate	BALF culture
1	M	42	38.6	Right middle lobe	<i>S pneumoniae</i>
2	F	47	38.9	Right middle lobe	—
3	M	40	39.1	Right upper lobe	<i>S pneumoniae</i>
4	M	36	37.8	Right middle and lower lobes	—

Clinical and biochemical parameters of 4 CAP patients with a unilateral infiltrate on chest x-ray. Site of infiltrate was determined by chest roentgenogram. — indicates no organism cultured.

The main objective of this study was to determine the role of endogenous PAI-1 in the innate immune response to bacterial pneumonia. For this, we first investigated the extent to which PAI-1 is produced and the fibrinolytic system is active in the infected lung of patients with unilateral community-acquired pneumonia (CAP). Furthermore, we evaluated the role of PAI-1 in host defense during pneumonia caused by *Streptococcus pneumoniae*, the most frequently isolated etiologic agent in CAP,²⁸ using PAI-1 gene-deficient (PAI-1^{-/-}) mice.

Patients, materials, and methods

Patient study

Study population. Four patients with a unilateral CAP were enrolled into the study. They fulfilled the following criteria: fever (above 37.7°C), PaO₂ greater than 7.5 kPa while patient is breathing room air, new unilateral infiltrate on chest roentgenogram within 2 days after admission, and no antibiotic pretreatment. Patients were excluded if they were hospitalized within 2 weeks prior to this admission or if they used immunosuppressive drugs. They were 3 men and 1 woman with a mean age of 41 ± 5 years (mean ± standard error [SE]). Ten healthy volunteers who were not taking any medication (mean age, 32 ± 8 years) served as controls. The protocol was reviewed and approved by the Medical Ethics Committee of the University of Amsterdam (The Netherlands), and written informed consent was obtained from all subjects.

BAL. BAL was performed in a standardized fashion according to the guidelines of the American Thoracic Society, with the use of a flexible fiberoptic video-bronchoscope, within 12 hours after admission. Seven successive 20-mL aliquots of prewarmed 0.9% saline were instilled in a subsegment of the lung, and each was aspirated immediately with low suction. BAL was first performed at the uninfected side in a subsegment of the middle lobe or lingula. This was followed by lavaging a subsegment of the infected lobe. Generally, 10 to 15 mL of each 20-mL aliquot was recovered. The recoveries from the infected and uninfected side did not differ.

Specimen processing. BALF was kept at 4°C until processing, which was performed within 30 minutes. The specimen was centrifuged at 3000 rpm for 15 minutes at 4°C. The first 3 recoveries of both sides were sent to the microbiology and virology department for culture and virus isolation. The remaining supernatant was stored at -80°C until assays were performed.

Assays. PAI-1 antigen and tPA antigen were measured by means of commercially available enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer's recommendations: PAI-1 was measured by TintEliza PAI-1 (Biopool, Umea, Sweden); tPA, by Asserachrom tPA (Diagnostica Stago, Asnières-sur-Seine, France). The uPA antigen was measured by ELISA as described before.²⁹ PA activity was measured by an amidolytic assay.³⁰

Mouse studies

Animals. All experiments were approved by the Institutional Animal Care and Use Committee of the Academic Medical Center, Amsterdam, The

Netherlands. Ten- to 12-week old PAI-1^{-/-} mice and plasminogen-gene-deficient (Plg^{-/-}) mice were generated as previously described.³¹⁻³³ PAI-1^{-/-} and Plg^{-/-} mice are viable and show no apparent macroscopic or microscopic histological abnormalities. Age- and sex-matched wild-type (Wt) mice were used as controls. PAI-1^{-/-} and Wt mice used in experiments were the product of 8 backcrosses to the C57BL/6J genetic background. Plg^{-/-} mice were on a mixed background of 75% C57BL/6 and 25% 129(H-2^b).

Induction of lung inflammation. Pneumococcal pneumonia was induced as described previously.^{13,34} In brief, *S pneumoniae*, serotype 3, obtained from American Type Culture Collection (ATCC 6303; Rockville, MD), were grown for 6 hours to midlogarithmic phase at 37°C with the use of Todd-Hewitt broth (Difco, Detroit, MI), harvested by centrifugation at 1500g for 15 minutes, and washed twice in sterile isotonic saline. Bacteria were then resuspended in sterile isotonic saline at approximately 4 × 10⁶ colony-forming units (CFUs) per milliliter as determined by plating serial 10-fold dilutions on sheep-blood agar plates. Then, 50 μL (2 × 10⁵ CFUs) was given intranasally. Control mice were instilled intranasally with 50 μL lipopolysaccharide (LPS)-free sterile saline.

Preparation of lung homogenates. At 24 or 48 hours after inoculation, mice were anesthetized by intraperitoneal injection with Hypnorm (Janssen Pharmaceutica, Beerse, Belgium) and midazolam (Roche, Mijdrecht, The Netherlands), and blood was collected from the inferior vena cava. Whole lungs were harvested and homogenized at 4°C in 5 vol sterile isotonic saline with a tissue homogenizer (Biospect Products, Bartlesville, OK), which was carefully cleaned and disinfected with 70% ethanol after each homogenization. Serial 10-fold dilutions in sterile saline were made from these homogenates (and blood), and 50-μL volumes were plated onto sheep-blood agar plates and incubated at 37°C. CFUs were counted after 16 hours. For cytokine measurements, lung homogenates were lysed in lysis buffer (300 mM NaCl, 15 mM Tris [tris(hydroxymethyl)aminomethane], 2 mM MgCl₂, 2 mM Triton X-100, pepstatin A, leupeptin, and aprotinin [20 ng/mL], pH 7.4) and spun at 1500g at 4°C for 15 minutes; the supernatant was frozen at -20°C until cytokine measurement.

Bronchoalveolar lavage. The trachea was exposed through a midline incision and cannulated with a sterile 22-gauge Abbocath-T catheter (Abbott, Sligo, Ireland). BAL was performed by instilling two 0.5-mL aliquots of sterile saline. Then, 0.9 to 1 mL lavage fluid was retrieved per mouse, and total cell numbers were counted from each sample in a hemacytometer (Emergo, Amsterdam, The Netherlands). BALF differential cell counts were determined on cytospin preparations stained with modified Giemsa stain (Diff-Quick; Baxter, McGraw Park, IL).

Histologic examination. After 24-hour fixation of lungs in 10% formaline and embedding in paraffin, 4-μm-thick sections were stained

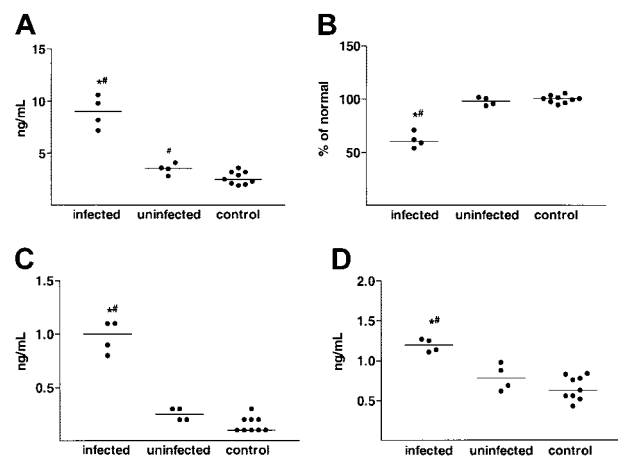


Figure 1. Fibrinolytic parameters in patients with community-acquired pneumonia. Fibrinolytic parameters (PAI-1 antigen [PAI-1Ag; panel A], PAA [panel B], uPA-Ag [panel C], and tPA-Ag [panel D]) were measured in BALF from 4 patients with CAP with a unilateral infiltrate on the chest x-ray, from the area of the infiltrate and from the uninfected site. Ten healthy subjects served as controls. Horizontal lines express medians. **P* < .05 versus healthy controls. #*P* < .05 versus uninfected lung.

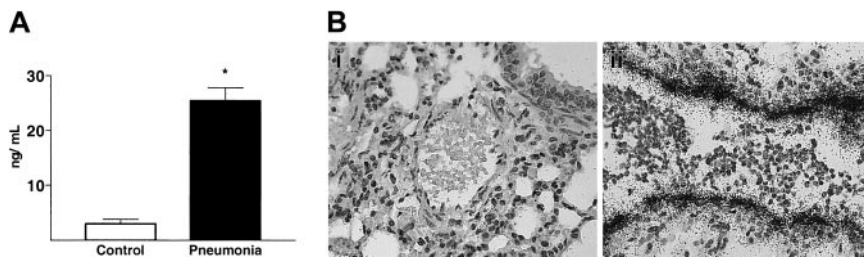


Figure 2. PAI-1 mRNA protein expression in lungs. (A) PAI-1 protein concentrations in lung homogenates measured 48 hours after intranasal administration of sterile saline (control) or *S pneumoniae* (pneumonia). Data are mean \pm SE; n = 8 for each group. * $P < .05$ versus control. (B) Localization of PAI-1 mRNA in lungs by in situ hybridization, 48 hours after intranasal inoculation with sterile saline (i) or *S pneumoniae* (ii). Slides are representative of n = 8 mice. Magnification, $\times 55$.

with hematoxylin and eosin. All slides were coded and scored by a pathologist who had no knowledge of the type of mice or treatment.

PAI-1 in situ hybridization. First, 5- μ m paraffin sections were mounted on SuperFrost Plus glass slides (Menzel-Gläser, Braunschweig, Germany) and subjected to in situ hybridization with a PAI-1 antisense riboprobe. In vitro transcription of linearized plasmid DNA was performed with the use of (35 S)-uridine triphosphate (35 S)-UTP (Amersham Pharmacia Biotech, Roosendaal, The Netherlands) to obtain the radiolabeled antisense PAI-1 riboprobe. In situ hybridization was executed by standard procedures. In situ sections were covered with nuclear research emulsion (ILFORD Imaging UK, Cheshire, United Kingdom), exposed for 2 weeks, and then developed and counterstained with hematoxylin and eosin. Four sections per specimen were used to confirm positive PAI-1 in situ hybridization signal.³⁵

Assays. Murine PAI-1 antigen levels in lung homogenates were measured by ELISA, calibrated with recombinant murine PAI-1 as described.³⁶ For this purpose, the antimurine PAI-1 monoclonal antibody H34G6 (4 μ g/mL) was coated on microtiter plates for 48 hours at 4°C. Bound PAI-1 in the samples was revealed with the use of a biotinylated and diluted (1:250) rabbit polyclonal antiserum raised against murine PAI-1, incubated for 1 hour at 37°C, and then by a peroxidase-labeled avidin-biotin complex. Cytokine levels were measured by means of commercially available ELISAs, in accordance with the manufacturer's recommendations: tumor necrosis factor- α (TNF- α), interleukin 6 (IL-6; Pharmingen, San Diego, CA), IL-1 β . Detection limits were 50 pg/mL (TNF), 37 pg/mL (IL-6), and 150 pg/mL (IL-1 β). Myeloperoxidase (MPO) activity was measured as described previously.³⁷ Briefly, lung tissue was homogenized in potassium phosphate buffer, pH 7.4. After centrifugation (4500g for 20 minutes at 4°C), pelleted cells were lysed in potassium phosphate buffer, pH 6.0, containing 0.5% hexadecyltrimethyl ammonium bromide (HETAB) and 10 mM EDTA (ethylenediaminetetraacetic acid). MPO activity was determined by measuring the H₂O₂-dependent oxidation of 3,3',5,5'-tetramethylbenzidine (TMB). The reaction was stopped with glacial acetic acid followed by reading the absorbance at 655 nm with a spectrophotometer. MPO activity was expressed as units of MPO activity per gram of lung tissue per reaction time. All reagents for the MPO assay were purchased from Sigma (St Louis, MO).

Statistical analysis. Data are expressed as means \pm standard error of the mean (SEM), unless indicated otherwise. Comparisons between groups were conducted by means of the Mann-Whitney *U* test. Survival curves were compared by log-rank test. $P < .05$ was considered to represent a statistically significant difference.

Table 2. PAI-1 deficiency does not influence leukocyte recruitment during pneumonia

	Wt mice	PAI-1 ^{-/-} mice
Cells in BALF, $\times 10^4$ /mL		
Total cells	26.5 \pm 9.3	28.1 \pm 4.2
PMNs	16.1 \pm 7.8	16.9 \pm 3.2
Lymfo	0.7 \pm 0.3	1.3 \pm 0.8
AMs	9.7 \pm 2.1	10.6 \pm 1.7
MPO activity, U/mL	3.0 \pm 0.9	4.8 \pm 0.5

Data are means \pm SEM of 6 mice per group, measured 48 hours after intranasal inoculation. PMN indicates polymorphonuclear cell; Lymfo, lymphocytes; and AM, alveolar macrophage.

Results

Alveolar fibrinolysis in pneumonia patients

Patient characteristics are presented in Table 1. All patients recovered uneventfully with antibiotic therapy. All patients underwent bilateral BAL within 12 hours after admission, first at the uninfected side and then in the area with the infiltrate on the chest x-ray, and fibrinolytic mediators were measured in BALF. BALF from healthy subjects served as control (Figure 1). PAI-1 antigen levels were increased in BALF of infected lungs compared with PAI-1 levels in BALF from uninfected lungs and healthy controls. In addition, PAI-1 antigen concentrations were higher in BALF from uninfected lungs of CAP patients than in BALF from healthy controls. Elevated PAI-1 levels were associated with a reduced bronchoalveolar PA activity, which was reduced in infected lungs of patients compared with uninfected lungs and healthy individuals. The uPA and tPA antigen levels, however, were increased in BALF of infected lungs compared with uninfected BALF. This discrepancy might be explained by the fact that the uPA and tPA antigen assays detect free PAs as well as PAs in complex with their inhibitor PAI-1.

Production of PAI-1 during murine pneumococcal pneumonia

To evaluate the role of PAI-1 in host defense during CAP, we used a pneumococcal pneumonia mouse model and compared host responses in PAI-1^{-/-} and Wt mice. To confirm PAI-1 production in this model, we measured PAI-1 levels in lung homogenates after induction of pneumonia. Intranasal administration of *S pneumoniae*

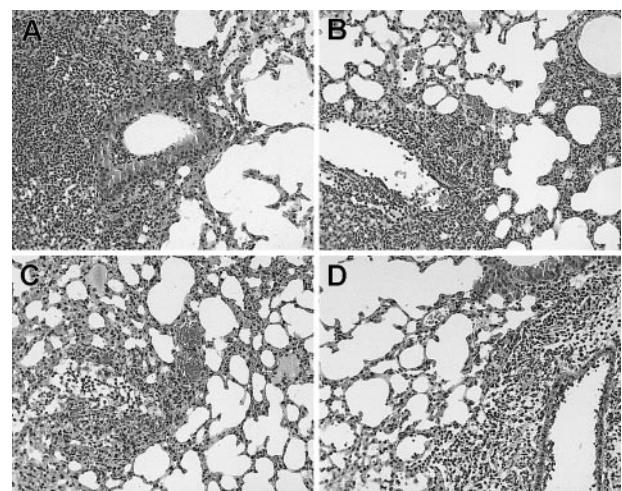
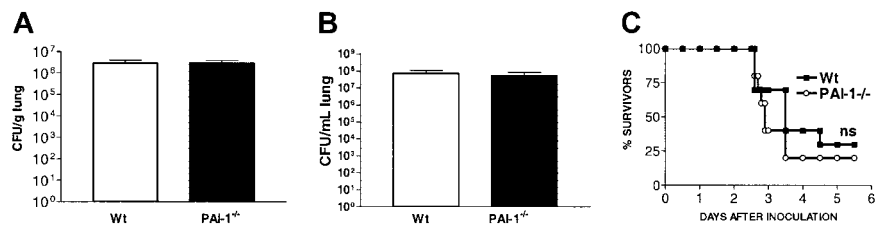


Figure 3. Histology of lungs. Lungs of Wt (A,C) and PAI-1^{-/-} mice (B,D) at 24 hours (panels A-B) and 48 hours (panels C-D) after infection. Hematoxylin and eosin (HE) staining; original magnification, $\times 20$. No difference was seen in the composition of inflammation between Wt and PAI-1^{-/-} mice.

Figure 4. Bacterial outgrowth and mortality. (A-B) *S pneumoniae* CFUs in the lungs of Wt (□) and PAI-1^{-/-} (■) mice at 24 (A) and 48 (B) hours after inoculation. Data are mean ± SE; n = 8 per group per time point. (C) Survival after *S pneumoniae* inoculation in Wt (□) and PAI-1^{-/-} (○) mice. Mortality was assessed twice daily for 10 days; n = 10 per group. ns indicates nonsignificant.



increased the concentrations of PAI-1 in lung homogenates obtained 48 hours after inoculation ($P < .05$ versus control; Figure 2A); mice with pneumonia also showed an increase of plasma PAI-1 antigen compared with control mice (PAI-1: 2.8 ± 0.9 ng/mL in controls; at 48 hours, 19.8 ± 18.6 ng/mL; $P < .09$).

To obtain insight into the cellular source of PAI-1 in the normal and infected lung, we performed in situ hybridization. In normal lung, a faint signal for PAI-1 mRNA transcripts was detected in endothelial cells as well as in bronchiolar and alveolar epithelium (Figure 2B). At 48 hours after infection, a strong expression of PAI-1 mRNA was observed, predominantly in vessels showing endothelial injury, in inflamed bronchi, in areas of inflammatory infiltrates, and in areas of pleuritis.

Inflammatory cell influx in BALF

To obtain insight into the role of PAI-1 in cell migration, we compared leukocyte counts in BALF of PAI-1^{-/-} and Wt mice 48 hours after inoculation with *S pneumoniae*. PAI-1^{-/-} mice had a similar influx of neutrophils in their BALF when compared with Wt mice (Table 2). In accordance, MPO activity in lung homogenates at 48 hours after inoculation was similar in both genotypes (Table 2).

Histopathology

At 24 and 48 hours after inoculation, the lungs showed patchy and sometimes dense inflammatory infiltrates, which were similar in both groups (Figure 3). Formation of thrombi was not observed in the blood vessels of either group.

Bacterial outgrowth

To investigate the potential role of PAI-1 in the clearance of *S pneumoniae* from the lungs, we determined the number of bacteria in lungs at 24 and 48 hours after inoculation. At both time points, PAI-1^{-/-} mice had numbers of CFUs in their lungs similar to those of Wt mice (Figure 4A-B). Furthermore, blood cultures were positive in 62.5% of mice of both strains at 24 hours, and in 90% and 75% of Wt and PAI-1^{-/-} mice, respectively, at 48 hours (nonsignificant).

Survival

To investigate whether PAI-1 influences mortality, we assessed survival twice daily in Wt and PAI-1^{-/-} mice after intranasal inoculation with *S pneumoniae* up to 10 days after inoculation (Figure 4C). No difference was found between the 2 groups.

Cytokines

Cytokines play an important role in the regulation of host defense against pneumonia,³⁸ and the PA system can influence the production of these mediators.³⁹ Therefore, we measured the concentrations of TNF, IL-6, and IL-1 β in lung homogenates. All cytokine levels measured were similar in PAI-1^{-/-} and Wt mice (data not shown).

Host response to pneumonia in Plg^{-/-} mice

The studies with PAI-1^{-/-} mice suggested that PAI-1 does not influence host defense against pneumococcal pneumonia. In addition, these experiments did not reveal a role for PAI-1 in the migration of leukocytes to the site of infection. PAI-1^{-/-} mice display a basal state of enhanced fibrinolytic activity, which is the result of the loss of the important inhibitory function of PAI-1 toward PAs.^{31,33} To evaluate whether deficiency to generate plasmin, the active end product of the fibrinolytic system, influences the innate immune response to pneumonia, we next infected Plg^{-/-} and Wt mice with *S pneumoniae*. Similarly to PAI-1^{-/-} mice, Plg^{-/-} mice displayed no difference in leukocyte recruitment into BALF (data not shown) and had equal numbers of *S pneumoniae* CFUs in their lungs at 48 hours after inoculation when compared with Wt mice (Figure 5A). In addition, Plg^{-/-} and Wt mice demonstrated similar mortality rates (Figure 5B).

Discussion

S pneumoniae is the most predominant micro-organism in CAP, responsible for more than 500 000 cases of lower respiratory tract infection in the United States each year.^{40,41} We recently found that endogenous uPA activity within the lung impaired neutrophil recruitment and antibacterial defense during murine pneumococcal pneumonia, most likely by occupying uPAR.¹³ These findings led us to hypothesize that PAI-1, as the main inhibitor of uPA, would be an important player in the regulation of neutrophil trafficking and host defense in *S pneumoniae* pneumonia. First, we show here that in patients with unilateral CAP, PAI-1 antigen levels are increased in BALF of the infected lung compared with the uninfected lung or with healthy controls and that this is associated with a decrease in alveolar PA activity in the infected BALF. We also demonstrate that PAI-1 is produced in the lungs in mice during pneumonia. However, PAI-1 deficiency appeared not to influence leukocyte influx, the clearance of pneumococci from the lungs, or mortality.

In earlier investigations, changes in the alveolar hemostatic balance have in particular been studied in patients with ARDS.^{11,42,43} In these patients, elevated PAI-1 concentrations have been documented in BALF, together with a profoundly decreased fibrinolytic

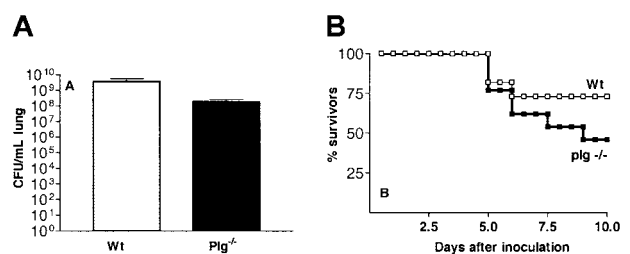


Figure 5. Host defense in Plg^{-/-} mice. (A) *S pneumoniae* CFUs in lungs of Wt (□) and Plg^{-/-} (■) mice at 48 hours after inoculation. Data are mean ± SEM; n = 8 per group per time point. (B) Survival after *S pneumoniae* inoculation in Wt (□) and Plg^{-/-} (■) mice. Mortality was assessed twice daily for 10 days; n = 12 per group.

activity. Recently, similar changes were reported in patients with severe pneumonia requiring mechanical ventilation.¹¹ Patients with less severe pneumonia, however, who breathed spontaneously, did not display elevated PAI-1 levels in their BALF.¹¹ These data contrast with our present findings of increased PAI-1 levels and suppressed PA activity at the site of the infection in patients with CAP. It is conceivable that the timing of the BAL procedure contributed to this discrepancy: that is, in the earlier investigation BAL was performed following a strictly time-matched protocol, whereas in our study BAL was conducted within 12 hours after admission. In addition, we lavaged the segment that showed infiltrative changes on the chest x-ray, whereas in the earlier study the lung with the predominant infection was examined.¹¹ Although our study involved only 4 patients, overlap with data obtained from the uninfected site or from healthy lungs did not occur. Thus, our human data, together with the demonstration of PAI-1 mRNA in mouse lungs during pneumococcal pneumonia, strongly indicate that PAI-1 is produced locally in the lung during lower respiratory tract infection.

Increased PAI-1 activity has been shown to predict lethality in patients with sepsis in a very sensitive way.⁴⁴⁻⁴⁸ Recently, it was shown that a (4G/5G) promoter deletion/insertion polymorphism in the PAI-1 gene influences the risk of the development of septic shock.^{49,50} In sepsis, 40% of the infections are caused by Gram-positive bacteria. Further, the most common site of infection in patients with sepsis is the respiratory tract.^{51,52} We here report, however, that the complete absence of PAI-1 does not influence the outcome of pneumococcal pneumonia. Our data further indicate that plasmin generation in general has no role in host defense against pneumonia, if one considers that *Plg*^{-/-} mice were indistinguishable from Wt mice in this model. Therefore, further studies are warranted to examine how an altered function (or absence) of PAI-1 can influence the host response to infection and sepsis and/or whether PAI-1 plays a role only in certain types of infection/sepsis (eg, meningococcal disease).^{49,50}

Mice lacking uPA and uPAR have a deficient migration of various cell types and are sensitive to infections.^{12,53,54} We recently found that mice deficient in uPAR are more sensitive to pneumococcal pneumonia owing to an inhibition of neutrophil recruitment into the inflamed lung.¹³ On the other hand, uPA-deficient mice showed enhanced cell recruitment and less bacterial outgrowth in their

lungs, resulting in the hypothesis that uPA negatively influences the function of uPAR as a chemotactic receptor. PAI-1 can affect cell migration by inhibiting uPA-mediated pericellular plasmin generation, or by inhibiting uPAR-mediated cell adhesion and migration through interaction with VN. However, PAI-1 deficiency did not influence either cell migration or host defense during pneumococcal pneumonia in mice. Accordingly, in a bleomycin-induced lung injury model, overexpression of PAI-1 did not prevent accumulation of leukocytes in the lung.⁵⁵ On the contrary, recent research showed a role for PAI-1 in the recruitment of interstitial macrophages and myofibroblasts to the kidney in unilateral ureteral obstruction.⁵⁶ It seems that although uPA and uPAR are both important for cell recruitment, PAI-1 does not show an interaction with uPA or uPAR related to neutrophil migration during pneumococcal pneumonia. Furthermore, *Plg*^{-/-} mice did not show a difference from Wt mice in host defense during pneumococcal pneumonia. Together, these data strongly suggest that the role of uPA and uPAR in inflammatory cell migration is not dependent on the fibrinolytic function.

PAI-1 has been found to be important for tumor cell invasion and also serves a role as a prognostic marker in patients with sepsis. To our knowledge, this study is the first to show that alveolar PAI-1 levels are increased in BALF obtained from the infected site in unilateral CAP and that this results in a diminished PA activity at the same site. However, mice deficient in PAI-1 or *Plg* have an unaltered inflammatory cell migration and host defense during pneumococcal pneumonia. Functional studies of the potential role of PAI-1 or other mediators of the fibrinolytic system in pneumonia in humans are lacking, and therefore our data obtained in mice need to be interpreted with caution. With this reservation in mind, current murine data suggest that the fibrinolytic system seems not important for host defense against respiratory tract infection, with an exception for uPA and uPAR, whose function is probably related not to their role in fibrinolysis, but to their involvement in inflammatory cell migration.

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