Mechanical ventilation affects alveolar fibrinolysis in LPS-induced lung injury

P. Dahlem^{*,#}, A.P. Bos[#], J.J. Haitsma^{*,†}, M.J. Schultz^{†,‡}, E.K. Wolthuis⁺, J.C.M. Meijers[§] and B. Lachmann^{*}

ABSTRACT: The aim of the present study was to determine the effects of mechanical ventilation on alveolar fibrin turnover in lipopolysaccharide (LPS)-induced lung injury.

In a randomised controlled trial, Sprague–Dawley rats (n=61) were allocated to three ventilation groups after intratracheal LPS (*Salmonella enteritidis*) instillations. Group I animals were subjected to 16 cmH₂O positive inspiratory pressure (PIP) and 5 cmH₂O positive end-expiratory pressure (PEEP); group II animals to 26 cmH₂O PIP and 5 cmH₂O PEEP; and group III animals to 35 cmH₂O PIP and 5 cmH₂O PEEP. Control rats (not mechanically ventilated) received LPS. Healthy rats served as a reference group. Levels of thrombin–antithrombin complex (TATc), D-dimer, plasminogen activator inhibitor (PAI) activity and PAI-1 antigen in bronchoalveolar lavage fluid were measured.

LPS-induced lung injury increased TATc, D-dimer and PAI activity and PAI-1 antigen levels versus healthy animals. High pressure-amplitude ventilation increased TATc concentrations. D-dimer concentrations were not significantly raised. Instead, PAI activity increased with the amplitude of the pressure, from 0.7 U·mL⁻¹ in group I to 3.4 U·mL⁻¹ in group II and 5.0 U·mL⁻¹ in group III. There was no change in PAI-1 antigen levels.

In conclusion, mechanical ventilation creates an alveolar/pulmonary anti-fibrinolytic milieu in endotoxin-induced lung injury which, at least in part, might be due to an increase in plasminogen activator inhibitor activity.

KEYWORDS: Adult respiratory distress syndrome, animal experimentation, endotoxin, fibrinolysis, mechanical ventilation

Intra-alveolar fibrin depositions are the pathognomonic hallmark of acute lung injury (ALI) on lung microscopy [1–3]. The fibrin matrix inactivates and incorporates surfactant, leading to severe hypoxaemic respiratory failure [4, 5]. Alveolar fibrin is part of the inflammatory response seen in ALI and may initiate fibrotic repair with long-term compromised pulmonary function [6, 7]. Intra-alveolar fibrin formation in ALI occurs after capillary alveolar leakage of plasma fibrinogen, activation of coagulation and suppression of local fibrinolysis [7–10]. Alveolar macrophages and alveolar epithelial cells are directly stimulated by bacterial endotoxins or indirectly by pro-inflammatory mediators (e.g. tumour necrosis factor (TNF)- α) to produce pro-coagulant and anti-fibrinolytic proteins. Activated factor VII and tissue factor on the pro-coagulant side, and plasminogen activator inhibitor (PAI)-1 on the anti-fibrinolytic side, are the main mediators of disturbed fibrin turnover. The degree of alveolar fibrin formation and the persistence of fibrin depend mainly on

suppressed fibrinolytic capacity due to increased local production of PAI-1 [3, 7–9, 11–15]. Under normal circumstances, intra-alveolar fibrin is resolved within minutes by plasmin [16] and intact surfactant is released, restoring pulmonary function [17]. During ALI, however, alveolar fibrin turnover is disturbed and aggravated by additional insults (e.g. haemorrhagic shock, infections, ventilator-associated pneumonia) [18, 19]. The present authors recently demonstrated that injurious mechanical ventilation can depress alveolar fibrinolytic capacity in healthy rats after iatrogenic fibrin formation [20]. In the present study, the effects of different ventilation strategies on alveolar fibrinolysis were examined in rats with “pre-injured” lungs due to endotoxin-induced lung injury.

MATERIALS AND METHODS

The present study was approved by the Animal Committee of the Erasmus University Rotterdam (Rotterdam, The Netherlands). Care and handling of the animals were in accordance with

AFFILIATIONS

^{*}Dept of Anaesthesiology, Erasmus MC-Faculty Rotterdam, Rotterdam, and

[#]Emma Children's Hospital, Paediatric Intensive Care Unit

⁺Adult Intensive Care Unit, and

[§]Dept of Vascular Medicine, Academic Medical Centre of the University of Amsterdam, Amsterdam, The Netherlands.

[†]Interdepartmental Division of Critical Care, University of Toronto, Toronto, ON, Canada.

CORRESPONDENCE

P. Dahlem

Coburg Medical Centre

Children's Hospital

Ketschendorferstr. 33

96450 Coburg

Germany

Fax: 49 9561225552

E-mail: PDahlem@hotmail.com

Received:

November 21 2004

Accepted after revision:

June 30 2006

European Respiratory Journal

Print ISSN 0903-1936

Online ISSN 1399-3003

the European Community guidelines. The experiments were performed at the Dept of Anaesthesiology, Erasmus MC-Faculty Rotterdam in male Sprague-Dawley rats (IFFA Credo, Someren, The Netherlands; $n=61$) with a mean $\pm$ SEM body weight of 283.4 ± 2.6 g.

Induction of intra-alveolar fibrin formation by local lung inflammation

Lipopolysaccharide (LPS)-induced lung inflammation, adapted from the model originally described by WHEELDON *et al.* [21] and VAN HELDEN *et al.* [22], was brought about in 51 animals by intratracheal instillation of $16 \text{ mg} \cdot \text{kg}^{-1}$ LPS, derived from *Salmonella enteritidis* (L6761; Sigma-Aldrich, St Louis, MO, USA). The procedure was performed after orotracheal intubation under gaseous anaesthesia (65% nitrous oxide:33% oxygen:2% isoflurane; Pharmchemie, Haarlem, The Netherlands), using a miniature nebuliser (Penn-Century, Philadelphia, PA, USA). After the procedure, rats were extubated.

Experimental protocol

Rats were anaesthetised 24 h after LPS instillation as detailed earlier and tracheotomised. A catheter was inserted into a carotid artery. Anaesthesia was maintained with hourly *i.p.* injections of pentobarbital sodium ($60 \text{ mg} \cdot \text{kg}^{-1}$, Nembutal; Algin BV, Maassluis, The Netherlands). Muscle relaxation was attained with hourly *i.m.* injections of pancuronium bromide ($2 \text{ mg} \cdot \text{kg}^{-1}$, Pavulon; Organon Technika, Boxtel, The Netherlands). After muscle relaxation, all animals were connected to a ventilator (Servo Ventilator 300; Siemens-Eléma, Solna, Sweden) set in a pressure-controlled mode with positive inspiratory pressure (PIP) of $12 \text{ cmH}_2\text{O}$, positive end-expiratory pressure (PEEP) of $2 \text{ cmH}_2\text{O}$, frequency of 30 breaths $\cdot \text{min}^{-1}$, inspiratory/expiratory time ratio of 1:2 and fractional inspired oxygen tension ($F_{\text{I}}\text{O}_2$) of 1.0. Body temperature was kept within normal range by means of a heating pad. After 15 min of stabilisation, arterial blood gases were taken using a carotid artery catheter and PIP was adjusted according to the ventilation group to which the animals had been allocated. Arterial blood gases were sampled every 30 min thereafter using conventional methods (ABL555; Radiometer, Copenhagen, Denmark). Mean arterial blood pressure (MAP) was monitored using the intra-arterial carotid artery catheter every 30 min for 3 h.

Study groups

Animals were allocated to one of three ventilation groups, each with different pressure amplitude. A PEEP of $5 \text{ cmH}_2\text{O}$ was selected for all groups, but PIP was different in each group. Group I ($n=12$) was ventilated with a PIP of $16 \text{ cmH}_2\text{O}$; group II ($n=13$) with a PIP of $26 \text{ cmH}_2\text{O}$; and group III ($n=13$) with a PIP of $35 \text{ cmH}_2\text{O}$. The ventilation period was designed to last 3 h, after which final measurements were made. $F_{\text{I}}\text{O}_2$ was kept at 1.0 throughout the study period to prevent hypoxaemia developing in any of the groups.

Control group animals ($n=13$) received LPS instillations as described previously ("LPS controls"), but were not mechanically ventilated. To create reference values, a group of healthy rats ($n=10$) did not receive LPS and were not ventilated ("healthy" animals). At the end of the ventilation period, all rats ($n=61$) were killed with an intra-arterial overdose of

pentobarbital sodium ($600 \text{ mg} \cdot \text{kg}^{-1}$) and all measurements were then made.

Bronchoalveolar lavage

After the rats were killed, the thorax and diaphragm were opened and lungs removed. As a parameter of lung injury, subsequently lung weight/body weight ratio was calculated. Bronchoalveolar lavage (BAL) was performed with normal saline ($30 \text{ mL} \cdot \text{kg}^{-1}$, heated to 37°C) and re-aspirated three times. BAL fluid (BALF) was centrifuged ($400 \times g$ for 10 min at 4°C) and the recovered supernatant fluid was snap-frozen and stored at -80°C until further processing. Measurements were not completed in two rats of group II and in three rats of group III, owing to air leakage during the test.

Measurements

Coagulation activation, as assessed by thrombin-antithrombin complexes (TATc), was measured in BALF with an ELISA-based method. Briefly, rabbits were immunised with mouse thrombin or rat antithrombin. Antithrombin antibodies were used as a capture antibody; digoxigenin-conjugated anti-antithrombin antibodies were used as detection antibodies; horseradish peroxidase-labelled sheep anti-digoxigenin antigen-binding fragments (Boehringer Mannheim, Mannheim, Germany) were used as staining enzyme, and o-phenylenediamine dihydrochloride (Sigma-Aldrich) was used as substrate. Dilutions of mouse serum (Sigma-Aldrich) were used for the standard curve, yielding a lower detection limit of $0.3 \text{ ng} \cdot \text{mL}^{-1}$ [23].

Fibrin breakdown of LPS-induced alveolar fibrin formation was determined by measurements of D-dimers (cross-linked fibrin degradation products) in BALF [24]. D-dimers were quantitated by a sandwich-type ELISA (Asserachrom D-dimer; Diagnostica Stago, Asnières-sur-Seine, France). This assay shows cross-reactivity with rat D-dimers.

PAI activity in BALF was determined using an automated coagulation analyser (Behring Coagulation System; Dade Behring, Marburg, Germany) with reagents and protocols from the manufacturer. This assay determines the urokinase-inhibiting activity of the sample. The remaining urokinase is then assayed by activating plasminogen to plasmin and subsequently determining plasmin chromogenic activity. The assay is independent of variable concentrations of plasminogen, α -2-antiplasmin and fibrinogen in the sample. The upper detection limit of this test is set at $6.9 \text{ U} \cdot \text{mL}^{-1}$. Protein concentration in BALF was measured using the Bradford method (Bio-Rad protein assay; Bio-Rad Laboratories, Munich, Germany [25].

To determine the levels of PAI-1 antigen in BALF, a rat PAI-1 ELISA was developed using a rabbit polyclonal antibody (Abcam Ltd, Cambridge, UK) as coating antibody and a biotinylated rabbit immunoglobulin G antibody (Molecular Innovations Inc., Southfield, MI, USA) as developing antibody. Rat PAI-1 (Calbiochem, La Jolla, CA, USA) was used as a standard.

To illustrate alveolar fibrin depositions, samples were taken from all lung lobes and 30 fields were analysed. The analysing pathologist was not informed about the purpose of the study and was asked to prepare rat lungs for illustration of alveolar

fibrin deposition. Histological analysis was performed as previously described [20]. Briefly, lungs were fixed at 10 cmH₂O MAP, and slices were stained for fibrinogen. During the washing procedure, fibrinogen would have been washed out leaving solely fibrin attached to the alveolar wall. Slides of lung tissue were deparaffinised and endogenous peroxidase activity was quenched by a solution of methanol/0.03% H₂O₂ (Merck, Darmstadt, Germany). After digestion with a 0.25% weight/volume solution of pepsin (Sigma-Aldrich) in 0.01 M HCl, the sections were incubated in 10% normal goat serum (Dako, Glostrup, Denmark) and then exposed to biotin-labelled goat anti-human fibrinogen antibody (Accurate Chemical & Scientific Corporation, Westbury, NY, USA). After washes, slides were then incubated in a streptavidin-ABC solution (Dako) and developed using 1% H₂O₂ and 3.3'-diaminobenzidin-tetra-hydrochloride (Sigma-Aldrich) in Tris-HCl. The sections were mounted in glycerine gelatin and counterstained with haematoxylin.

Statistical analysis

Data are presented as mean ± SEM. Group differences were analysed with ANOVA, while differences between the three ventilated groups were analysed using a Kruskal–Wallis test. Differences between the healthy and LPS control group, and between the ventilated groups and the LPS control group, were analysed with a Mann–Whitney U-test. A p-value <0.05 was considered significant.

In table 1, all values for TATc, D-dimer, PAI activity and PAI-1 antigen were corrected for their individual corresponding protein levels. Analysis was performed as described previously.

RESULTS

All animals survived the study period. LPS instillation caused severe respiratory distress with tachypnoea and significant weight loss, from 283.4 ± 2.6 g before LPS instillation to a mean of 260.3 ± 2.6 g (p<0.001) 24 h after LPS instillation.

Mechanical ventilation with high pressure amplitudes caused lung injury with compromised partial pressure of oxygen in

arterial blood (Pa,O₂); group III showed lower Pa,O₂ values compared with group I at 120, 150 and 180 min (fig. 1; p<0.05). Furthermore, animals in ventilation group II (pre-study MAP 124 ± 6 mmHg *versus* end-of-study MAP 105 ± 6 mmHg; p<0.05) and ventilation group III (pre-study MAP 126 ± 4 mmHg *versus* end-of-study MAP 73 ± 5 mmHg, p<0.001), ventilated with high pressure amplitudes, showed lower MAP values at the end of the study period. At the end of the experiment, the extent of lung injury was also evidenced by a significant increase in median lung weight/body weight in group II of 9.2 ± 0.4 g·kg⁻¹ and in group III of 13.3 ± 0.8 g·kg⁻¹, *versus* 7.8 ± 0.6 g·kg⁻¹ in animals from group I (table 1).

Coagulation activation

LPS instillation resulted in activation of coagulation. TATc levels increased from 3.3 ± 1.0 ng·mL⁻¹ in healthy animals to 78.6 ± 15.2 ng·mL⁻¹ in LPS-treated animals (fig. 2). Increased pressure amplitude resulted in a significant increase in TATc

FIGURE 1. The effect of mechanical ventilation on partial pressure of oxygen in arterial blood (Pa,O₂) over time in the three ventilated groups. ●: group I; ■: group II; ▲: group III. *: p<0.05 *versus* group I. 1 mmHg=0.133 kPa.

TABLE 1	Lung weight, total bronchoalveolar lavage fluid (BALF) protein levels and values for thrombin–antithrombin complex (TATc), D-dimer, plasminogen activator inhibitor (PAI) activity and PAI-1 antigen adjusted for protein levels in BALF					
	Healthy	LPS controls	Group I	Group II	Group III	p-value between ventilated groups
Animals n	8	11	11	12	12	
Lung weight/body weight g·kg ⁻¹	6.0±0.4	8.1±0.3*	7.8±0.6	9.2±0.4	13.3±0.8 [#]	0.01
Total protein mg·mL ⁻¹	0.09±0.01	0.7±0.11*	0.54±0.06	0.67±0.07	1.02±0.12	0.01
TATc/total protein ng·mg ⁻¹	42.6±13.7	116±15.8*	85.1±6.6	74.7±6.9	67.6±9.7	0.23
D-dimer/total protein µg·mg ⁻¹	21.8±7.2	22.2±4.2	88.6±35.2 [#]	62.7±7.9 [#]	56.3±13.4	0.76
PAI activity/total protein U·mg ⁻¹	0.0±0.0	0.9±0.6*	1.1±0.7	4.4±0.9 [#]	5.3±0.7 [#]	0.005
PAI-1 antigen/total protein ng·mg ⁻¹	0.3±0.2	19±2.3*	25.3±4.2	24.9±2.6	18.3±3.1	0.23

Data are presented as mean±SEM, unless otherwise stated. Adjustments were made for protein content in BALF per individual sample. LPS: lipopolysaccharide. *: p<0.05 *versus* healthy controls; #: p<0.05 *versus* LPS controls.

levels between the three ventilated groups (fig. 2). Only TATc levels in group I animals were significantly lower compared with LPS controls (fig. 2).

Fibrinolysis

In LPS controls, absolute D-dimer levels in BALF were $13.4 \pm 2.0 \mu\text{g}\cdot\text{L}^{-1}$, compared with $1.8 \pm 0.6 \mu\text{g}\cdot\text{L}^{-1}$ in healthy rats ($p < 0.001$), indicating alveolar fibrin formation caused by endotoxin (fig. 2). Mechanical ventilation increased D-dimer levels in BALF compared with LPS control animals. Different ventilation pressure amplitudes did not significantly alter D-dimer levels between groups I–III; group I D-dimer levels were $36.1 \pm 8.3 \mu\text{g}\cdot\text{L}^{-1}$, versus $65.8 \pm 16.9 \mu\text{g}\cdot\text{L}^{-1}$ in group III.

Anti-fibrinolysis

PAI activity was undetectable in BALF of healthy rats and was $1.24 \pm 0.8 \text{ U}\cdot\text{mL}^{-1}$ in LPS controls (fig. 2). Between the ventilated groups, PAI activity levels were significantly increased; PAI activity concentrations in BALF were $0.7 \pm 0.5 \text{ U}\cdot\text{mL}^{-1}$, $3.4 \pm 0.8 \text{ U}\cdot\text{mL}^{-1}$ and $5.0 \pm 0.7 \text{ U}\cdot\text{mL}^{-1}$ in groups I, II and III, respectively (fig. 2). PAI activity in group III was also significantly different from that in LPS control animals. Minimal levels of PAI-1 antigen were detected in healthy animals ($0.03 \pm 0.02 \text{ ng}\cdot\text{mL}^{-1}$), but instillation of LPS led to a

significant rise in PAI-1 antigen ($13.9 \pm 3.6 \text{ ng}\cdot\text{mL}^{-1}$; $p < 0.05$; fig. 2). There was no difference in PAI-1 antigen levels between the three ventilated groups, or between the ventilated groups and the LPS control animals (fig. 2).

Histology

In figure 3, microscopy of representative rat lung tissue shows lung inflammation caused by LPS. In all ventilated animals, unresolved fibrin depositions (fig. 3c and d) were observed. More fibrin deposits were observed in animals ventilated with the highest pressure amplitude, although there were no differences in levels of lung inflammation. Atelectasis was also more pronounced in animals of group II and especially group III.

DISCUSSION

The present study investigated the effect of mechanical ventilation on alveolar fibrinolysis in LPS-induced lung injury. Intratracheal LPS instillation caused local activation of coagulation, with an increase in TATc levels with fibrin formation (as documented by the appearance of D-dimer in BALF of animals of the LPS control group). The occurrence of alveolar fibrin formation after LPS-induced lung inflammation has been documented previously in a comparable experimental model [24].

FIGURE 2. Effect of mechanical ventilation on thrombin–antithrombin complex (TATc), D-dimer, plasminogen activator inhibitor (PAI) activity and PAI-1 antigen levels in bronchoalveolar lavage fluid in lipopolysaccharide (LPS)-pre-treated animals. *: $p < 0.05$ versus healthy controls; #: $p < 0.05$ versus LPS controls; ¶: $p < 0.05$ between ventilated groups.

FIGURE 3. Microscopy of representative fibrin-stained paraffin sections of rat lung tissue of lipopolysacchride (LPS)-treated rats. a) Normal lung tissue; b) lung tissue of a representative sample of the LPS control group (24 h after LPS instillation) predominantly showing interstitial infiltrates with neutrophils; c) representative lung tissue of group III, showing interstitial infiltrates with neutrophils and unresolved fibrin/fibrinogen depositions (counterstained with haematoxylin) after 3-h mechanical ventilation; and d) a close-up of c), showing unresolved fibrin/fibrinogen depositions (arrows). Scale bars=0.1 mm.

Mechanical ventilation aggravates this endotoxin-induced lung inflammation and might therefore influence alveolar fibrin turnover [26]. Therefore, the experimental model of the present study may be considered an appropriate model for the study of the effects of mechanical ventilation on alveolar fibrinolysis.

Acute lung injury is characterised by alveolar flooding. Ventilator-induced lung injury aggravates or may even be the cause of this flooding [27]. In the present study, pulmonary oedema formation was also observed, as characterised by the BALF protein levels and increased lung weights of the animals ventilated with higher pressure amplitudes.

D-dimer concentrations in the BALF of the ventilated animals were higher than in LPS controls. However, there were no significant differences between the ventilation groups. In contrast, PAI activity increased with the size of the pressure amplitude, without a change in PAI-1 antigen levels. The present results link injurious ventilation settings in inflamed lungs with depressed fibrinolysis for the first time. Furthermore, independent of protein leakage into the lung, injurious mechanical ventilation increased the PAI activity in BALF.

The current authors observed an increase in PAI activity but did not see an increase in PAI antigen levels. Conversion of PAI-1 between its active and latent forms is regulated by vitronectin, which circulates in plasma but is also a major constituent of the extracellular matrix [28, 29]. In the data adjusted for total protein levels, an increase in activity without a change in antigen levels was still observed, suggesting that PAI activity is dependent on protein/vitronectin influx and subsequent stabilisation of PAI activity during high pressure-amplitude ventilation.

Surprisingly, increased PAI activity did not result in lower amounts of D-dimer in BALF. There might be two possible explanations. First, 3-h mechanical ventilation might not be enough to demonstrate a larger effect on the downregulation of alveolar fibrin breakdown due to PAI production. Secondly,

the high D-dimer levels in the BALF of group III animals (although not significantly different compared with the other ventilated groups, despite high PAI activity) might be explained by additional alveolar fibrin formation, triggered by traumatic mechanical ventilation and an influx of plasma proteins. It is impossible in the current experiment to distinguish whether the increased levels of D-dimers reflect increased fibrinolysis only, or whether they also reflect a higher level of pro-coagulation, resulting in increased formation of fibrin, which will translate into increased D-dimer levels.

The idea of extra fibrin formation in a second-hit model of lung injury (endotoxin plus mechanical ventilation) is plausible. Under both circumstances, similar inflammatory mediators are expressed (*e.g.* TNF- α), which may activate the intra-alveolar coagulation system and fibrin formation [18, 30]. LPS induces coagulation activation as indicated by increased TATc levels, whereas mechanical ventilation with low pressure amplitudes significantly reduced this. A possible explanation for this phenomenon is that, with higher pressure amplitudes, resolution of coagulation is impaired.

Despite these questions concerning the levels of D-dimer in BALF, the interpretation of the PAI values is striking. Aggressive mechanical ventilation can upregulate PAI, the strongest antifibrinolytic mediator, which might contribute to the persistence of alveolar fibrin and aggravate lung injury (*e.g.* lung fibrosis). The clinical importance of PAI-1 upregulation in mechanically ventilated patients has only recently been reported [31]. In adult ALI patients, high levels of PAI-1 in pulmonary oedema fluid have been associated with an increase in mortality [31].

The fact that mechanical ventilation may affect the alveolar fibrinolytic milieu has been demonstrated previously [20]. However, in that study, alveolar fibrin formation was generated by instillations of fibrinogen/thrombin in healthy rats, whereas in the present study the amount of alveolar fibrin formation was dependent on the endogenous capability of

each animal to build alveolar fibrin after the LPS challenge. A recent report on early stress-response genes demonstrated that coagulation genes are upregulated during ventilation-induced lung injury [32].

The major limitation of the present study is that it was not possible to determine the origin of the fibrin deposits, or more accurately the breakdown product D-dimers, in the BALF. The immunohistochemistry data do not allow the present authors to distinguish between fibrin or fibrinogen in the alveolar, capillary or interstitial space. Despite this, the D-dimer values in BALF clearly demonstrate increased breakdown of intra-alveolar fibrin deposits during mechanical ventilation, suggesting increased fibrin deposits in the alveolar space.

In summary, the present study provides new information showing that mechanical ventilation influences alveolar plasminogen activator inhibitor activity after endotoxin exposure and can influence alveolar fibrinolysis. Future experimental studies are needed to elucidate the underlying mechanisms.

ACKNOWLEDGEMENTS

The authors would like to thank S. Krabbendam for expert technical assistance (Dept of Anaesthesiology, Erasmus MC-Faculty Rotterdam, Rotterdam, The Netherlands), M. Weijne for laboratory assistance (Dept of Vascular Medicine), H. Thygesen for statistical advice (Dept of Biostatistics and Epidemiology), L.A. Noorduyt for histological expert preparation (Dept of Pathology; all University of Amsterdam, Amsterdam, The Netherlands) and L. Visser-Isles for English-language corrections (Erasmus Medical Faculty).

REFERENCES

- Bachofen M, Weibel ER. Structural alterations of lung parenchyma in the adult respiratory distress syndrome. *Clin Chest Med* 1982; 3: 35–56.
- Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. *Lancet* 1967; 2: 319–323.
- Idell S. Extravascular coagulation and fibrin deposition in acute lung injury. *New Horiz* 1994; 2: 566–574.
- Seeger W, Elssner A, Gunther A, Kramer HJ, Kalinowski HO. Lung surfactant phospholipids associate with polymerizing fibrin: loss of surface activity. *Am J Respir Cell Mol Biol* 1993; 9: 213–220.
- Seeger W, Stohr G, Wolf HR, Neuhof H. Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. *J Appl Physiol* 1985; 58: 326–338.
- Fukuda Y, Ishizaki M, Masuda Y, Kimura G, Kawanami O, Masugi Y. The role of intraalveolar fibrosis in the process of pulmonary structural remodeling in patients with diffuse alveolar damage. *Am J Pathol* 1987; 126: 171–182.
- Idell S. Coagulation, fibrinolysis, and fibrin deposition in acute lung injury. *Crit Care Med* 2003; 31: Suppl. 4, S213–S220.
- Levi M, Schultz MJ, Rijneveld AW, van der Poll T. Bronchoalveolar coagulation and fibrinolysis in endotoxemia and pneumonia. *Crit Care Med* 2003; 31: Suppl. 4, S238–S242.
- Welty-Wolf KE, Carraway MS, Ortel TL, Piantadosi CA. Coagulation and inflammation in acute lung injury. *Thromb Haemost* 2002; 88: 17–25.
- Schultz MJ, Haitsma JJ, Zhang H, Slutsky AS. Pulmonary coagulopathy as a new target in therapeutic studies of acute lung injury or pneumonia – a review. *Crit Care Med* 2006; 34: 871–877.
- Idell S. Endothelium and disordered fibrin turnover in the injured lung: newly recognized pathways. *Crit Care Med* 2002; 30: Suppl. 5, S274–S280.
- van der Poll T, de Jonge E, Levi M. Regulatory role of cytokines in disseminated intravascular coagulation. *Semin Thromb Hemost* 2001; 27: 639–651.
- Barazzone C, Belin D, Piguet PF, Vassalli JD, Sappino AP. Plasminogen activator inhibitor-1 in acute hyperoxic mouse lung injury. *J Clin Invest* 1996; 98: 2666–2673.
- Bertozzi P, Astedt B, Zenzius L, et al. Depressed bronchoalveolar urokinase activity in patients with adult respiratory distress syndrome. *N Engl J Med* 1990; 322: 890–897.
- Idell S, James KK, Levin EG, et al. Local abnormalities in coagulation and fibrinolytic pathways predispose to alveolar fibrin deposition in the adult respiratory distress syndrome. *J Clin Invest* 1989; 84: 695–705.
- Chung SI, Lee SY, Uchino R, Carmassi F. Factors that control extravascular fibrinolysis. *Semin Thromb Hemost* 1996; 22: 479–488.
- Gunther A, Kalinowski M, Elssner A, Seeger W. Clot-embedded natural surfactant: kinetics of fibrinolysis and surface activity. *Am J Physiol* 1994; 267: L618–L624.
- Schultz MJ, Millo J, Levi M, et al. Local activation of coagulation and inhibition of fibrinolysis in the lung during ventilator associated pneumonia. *Thorax* 2004; 59: 130–135.
- Fan J, Kapus A, Li YH, Rizoli S, Marshall JC, Rotstein OD. Priming for enhanced alveolar fibrin deposition after hemorrhagic shock: role of tumor necrosis factor. *Am J Respir Cell Mol Biol* 2000; 22: 412–421.
- Dahlem P, Bos AP, Haitsma JJ, Schultz MJ, Meijers JC, Lachmann B. Alveolar fibrinolytic capacity suppressed by injurious mechanical ventilation. *Intensive Care Med* 2005; 31: 724–732.
- Wheeldon EB, Walker ME, Murphy DJ, Turner CR. Intratracheal aerosolization of endotoxin in the rat: a model of the adult respiratory distress syndrome (ARDS). *Lab Anim* 1992; 26: 29–37.
- van Helden HP, Kuijpers WC, Steenvoorden D, et al. Intratracheal aerosolization of endotoxin (LPS) in the rat: a comprehensive animal model to study adult (acute) respiratory distress syndrome. *Exp Lung Res* 1997; 23: 297–316.
- Rijneveld AW, Weijer S, Florquin S, et al. Thrombomodulin mutant mice with a strongly reduced capacity to generate activated protein C have an unaltered pulmonary immune response to respiratory pathogens and lipopolysaccharide. *Blood* 2004; 103: 1702–1709.
- Sato N, Takahashi H, Shibata A. Fibrinogen/fibrin degradation products and D-dimer in clinical practice: interpretation of discrepant results. *Am J Hematol* 1995; 48: 168–174.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing

- the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248–254.
- 26** Vreugdenhil HA, Haitisma JJ, Jansen KJ, *et al.* Ventilator-induced heat shock protein 70 and cytokine mRNA expression in a model of lipopolysaccharide-induced lung inflammation. *Intensive Care Med* 2003; 29: 915–922.
- 27** Verbrugge SJ, Vazquez de Anda G, Gommers D, *et al.* Exogenous surfactant preserves lung function and reduces alveolar Evans blue dye influx in a rat model of ventilation-induced lung injury. *Anesthesiology* 1998; 89: 467–474.
- 28** Lazar MH, Christensen PJ, Du M, *et al.* Plasminogen activator inhibitor-1 impairs alveolar epithelial repair by binding to vitronectin. *Am J Respir Cell Mol Biol* 2004; 31: 672–678.
- 29** Ngo TH, Hoylaerts MF, Knockaert I, Brouwers E, Declerck PJ. Identification of a target site in plasminogen activator inhibitor-1 that allows neutralization of its inhibitor properties concomitant with an allosteric up-regulation of its antiadhesive properties. *J Biol Chem* 2001; 276: 26243–26248.
- 30** Gunther A, Mosavi P, Heinemann S, *et al.* Alveolar fibrin formation caused by enhanced procoagulant and depressed fibrinolytic capacities in severe pneumonia. Comparison with the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2000; 161: 454–462.
- 31** Prabhakaran P, Ware LB, White KE, Cross MT, Matthay MA, Olman MA. Elevated levels of plasminogen activator inhibitor-1 in pulmonary edema fluid are associated with mortality in acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2003; 285: L20–L28.
- 32** Ma SF, Grigoryev DN, Taylor AD, *et al.* Bioinformatic identification of novel early stress response genes in rodent models of lung injury. *Am J Physiol Lung Cell Mol Physiol* 2005; 289: L468–L477.