Protein C and thrombomodulin in human acute lung injury

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¹Division of Allergy, Pulmonary, and Critical Care Medicine, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-2650; ²Cardiovascular Research Institute and ³Departments of Medicine and Anesthesia, University of California, San Francisco, California 94143-2650

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Ware, Lorraine B., Xiaohui Fang, and Michael A. Matthay. Protein C and thrombomodulin in human acute lung injury. Am J Physiol Lung Cell Mol Physiol 285: L514–L521, 2003. First published May 16, 2003; 10.1152/ajplung.00442.2002.—Decreased circulating protein C and increased circulating thrombomodulin are markers of the prothrombotic, antifibrinolytic state associated with poor outcomes in sepsis but have not been measured in patients with ALI (acute lung injury)/ARDS (acute respiratory distress syndrome). We measured circulating and intra-alveolar protein C and thrombomodulin in 45 patients with ALI/ARDS from septic and nonseptic causes and correlated the levels with clinical outcomes. Plasma protein C levels were lower in ALI/ARDS compared with normal. Lower levels of protein C were associated with worse clinical outcomes, including death, fewer ventilator-free days, and more nonpulmonary organ failures, even when only patients without sepsis were analyzed. Levels of thrombomodulin in pulmonary edema fluid from ALI/ARDS patients were >10-fold higher than normal plasma and 2-fold higher than ALI/ARDS plasma. Higher edema fluid thrombomodulin levels were associated with worse clinical outcomes. The higher levels in edema fluid compared with plasma suggest local release of soluble thrombomodulin in the lung, possibly from a lung epithelial source. To determine whether lung epithelial cells can release thrombomodulin, A549 cells and primary isolates of human alveolar type II cells were exposed to H₂O₂ or inflammatory cytokines. Both epithelial cell types released thrombomodulin into the media. In summary, the protein C system may be a potential therapeutic target in patients with ALI/ARDS.

acut respiratory distress syndrome; sepsis; endothelium; inflammation; coagulation

Protein C circulates as an inactive zymogen. Activation requires binding to two receptors on the endothelium, the endothelial protein C receptor and the thrombomodulin-thrombin complex. In the setting of sepsis, plasma levels of soluble thrombomodulin are increased, a finding that probably reflects cleavage from the cell surface of the endothelium (22, 31), leading to reduced availability for activation of protein C on the endothelial surface. For example, patients with meningococcal sepsis have decreased dermal endothelial staining for thrombomodulin, along with increased plasma levels of soluble thrombomodulin and decreased plasma levels of activated protein C (15). Thus decreased circulating protein C and increased circulating thrombomodulin are both biological markers of the prothrombotic, antifibrinolytic state associated with poor outcomes in sepsis.

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) share several common features with sepsis, including the frequent occurrence of multiple organ system dysfunction and apparent hypercoagulability with widespread microvascular thrombus formation (1, 62). In addition to microvascular thrombosis, there is also intra-alveolar activation of the coagulation cascade with the deposition of fibrin along the injured alveolar surface (hyaline membranes) (3). Plasma levels of circulating protein C and thrombomodulin in patients with ALI/ARDS have not been systematically investigated. Furthermore, the intra-alveolar concentrations of these proteins in the injured lung have never been measured. Given the recent report of the therapeutic effect of activated protein C in patients with severe sepsis (5), it is important to determine whether the protein C pathway is similarly affected in ALI/ARDS. Also, Idell (23) has recently emphasized the scientific basis for evaluating the potential therapeutic value of anticoagulants as a therapeutic strategy in patients with ALI/ARDS.

To measure the levels of circulating and intra-alveolar protein C and thrombomodulin in patients with ALI/ARDS, we studied 45 patients with early ALI/ARDS and a comparison group of 22 patients with hydrostatic pulmonary edema. The primary objective was to determine whether the concentrations of protein C and thrombomodulin in plasma or pulmonary

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edema fluid were associated with clinical outcomes. This analysis was undertaken in patients with both nonseptic and septic ALI/ARDS. Because intra-alveolar levels of thrombomodulin were twofold higher than simultaneous plasma levels, an intra-alveolar source of thrombomodulin was suggested. We, therefore, hypothesized that alveolar epithelial cells can release thrombomodulin into the air spaces in the setting of lung injury, and this hypothesis was investigated in vitro using a human alveolar epithelial type II cell line (A549 cells) and primary isolates of human alveolar epithelial type II cells.

**METHODS**

**Patients.** Patients were randomly selected from Moffitt-Long and San Francisco General Hospital intensive care unit (ICU) patients who had pulmonary edema fluid and plasma samples obtained between 1986 and 1998. Inclusion criteria included acute respiratory failure requiring mechanical ventilation and aspirable pulmonary edema fluid within 1 h of endotracheal intubation. ALI and ARDS were defined by American European Consensus Conference definitions (6). The initial ratio of edema fluid-to-plasma protein, a measure of alveolar-capillary barrier permeability, was required to be >0.65, consistent with increased permeability pulmonary edema (16). A comparison population of 22 patients with hydrostatic pulmonary edema due to acute myocardial infarction, congestive heart failure, or volume overload was also studied. These patients had no evidence of ALI/ARDS and were required to have an initial edema fluid-to-plasma protein ratio of <0.65 (60). The Committee for Human Research at the University of California, San Francisco (UCSF), approved this study.

**Collection of plasma and pulmonary edema fluid.** All samples were collected within 1 h of intubation (40). Briefly, a 14-Fr tracheal suction catheter was advanced through the endotracheal tube into a wedged position in a distal bronchus. Undiluted pulmonary edema fluid was aspirated by gentle suction. A simultaneous plasma sample was obtained. Plasma samples were obtained from 10 healthy volunteers for comparison. Pulmonary edema fluid and plasma samples were centrifuged (3,000 × g, 10 min). Supernatants were stored at −70°C until use.

**Measurement of protein concentration.** The total protein concentration was measured by the biuret method (40) or by refractometry if the sample volume was insufficient (<1% of samples).

**Measurement of protein C and thrombomodulin.** Protein C (Helena Laboratories, Beaumont, TX) and thrombomodulin (Diagnostica Stago, Asnières, France) were measured in duplicate in patient samples or conditioned media using commercially available ELISA assays. Protein C results are reported as relative percent compared with pooled normal plasma. Thrombomodulin results are reported in nanograms per milliliter.

**Clinical data collection.** The etiology of ALI/ARDS was determined from the clinical history. Sepsis was defined using published criteria (13). Pneumonia was defined as new radiographic infiltrates and positive cultures of endotracheal tube aspirates for pathogenic bacteria. Patients who met criteria for both sepsis and pneumonia were classified as having sepsis as the etiology of ALI/ARDS. Aspiration of gastric contents was defined as a witnessed aspiration event. Organ system failures were defined as previously described (13). Disseminated intravascular coagulation was defined as positive D-dimers in conjunction with a drop in platelet count or fibrinogen level and a rise in the protime. Demographics, physiological data, respiratory parameters, medications, and multiorgan system function were recorded. The Simplified Acute Physiology Score II (SAPS II) (33) and Lung Injury Score (LIS) (44) were calculated for 24 h after initiation of mechanical ventilation. Major outcome variables included death before hospital discharge and ventilator-free days during a 28-day period, a measure of the duration of mechanical ventilation (6).

**Isolation of human alveolar epithelial cells.** Alveolar epithelial type II cells were isolated using a modification of previously described methods (26–29). Briefly, human lungs were resected from organ donors whose lungs were not used for transplantation by the California Transplant Donor Network. Recent studies indicate that these lungs are in good physiological and pathological condition (63). After being resected, the lungs were preserved for 4–8 h at 4°C. The pulmonary artery was then perfused with PBS at 37°C, and the distal air spaces were lavaged 10 times with warmed Ca2+- and Mg2+-free PBS solution containing 0.5 mM EGTA and EDTA. Then, 12.9 U/ml of porcine elastase (grade II; Roche Diagnostics, Indianapolis, IN) in Ca2+- and Mg2+-free Hanks' balanced solution was instilled into the distal air spaces through segmental intubation. The lungs were minced finely in the presence of PBS and 500 μg/ml of DNase (Sigma-Aldrich, St. Louis, MO) and then sequentially filtered through one and two layers of gauze, 150-μm and 30-μm nylon mesh. The resultant cell pellet was resuspended in DMEM containing 10% FBS, and then the cell suspension was incubated in tissue culture treated plastic petri dishes in a humidified incubator (5% CO2, 37°C) for 90 min. The nonadherent cell solution was then layered onto a discontinuous Percoll (Sigma) density gradient 1.04–1.09 g/ml solution and centrifuged at 1,500 rpm for 20 min. The recovered top band contained a mixture of alveolar type II cells and alveolar macrophages. These cells were centrifuged at 800 rpm for 10 min, and the resultant pellet was resuspended in DMEM containing 10% FBS. To remove remaining macrophages, cells were incubated with magnetic beads coated with anti-CD14 antibodies (Dynabeads M-450 CD14; Dynal, Canada) and then sequentially passed through a 10-μm cell filter. The purity of isolated human alveolar type II cells was assayed by Papanicolaou staining or by anti-human type II epithelial antibodies (a gift from Dr. Leland Dobbs, UCSF) and was >90%. Freshly isolated alveolar type II cells were resuspended in cell preservation fluid and maintained in liquid nitrogen until they were thawed and used for the cell culture studies described later. All reagents for the human type II cell isolation and for cell culture were obtained from the UCSF Cell Culture Facility unless otherwise noted.

**Cell culture.** Human alveolar epithelial-like cells (A549 cells; ATCC, Manassas, VA) were grown in MEM with 10% FBS in 5% CO2. Primary isolates of human type II cells were grown in DMEM with 10% FBS. For assay of thrombomodulin levels in conditioned media, cells were plated in 24-well plates at a density of 5 × 10^4 cells/well for A549 cells and 1 × 10^6 cells/well for human type II cells. When cells reached confluence (48–72 h), the medium was replaced with serum-free MEM (A549 cells) or DMEM (human type II cells) containing varying concentrations of H2O2 or a mixture of proinflammatory cytokines (TNF-α, IL-1β, interferon-γ; R&D Systems, Minneapolis, MN), as we have done before (50). Each condition was assayed in triplicate. After 6 h, conditioned media was removed and stored at −20°C until later use.
Statistical analysis. Normally distributed variables were compared using the unpaired t-test or ANOVA with Tukey’s test for post hoc comparisons where appropriate (SPSS 10.0 for Macintosh). Categorical variables were compared using Chi square analysis. The Pearson correlation coefficient was calculated for bivariate correlations. Statistical significance was defined as $P \leq 0.05$.

RESULTS

Patients. There were 45 patients with ALI/ARDS in this study. Patient characteristics are shown in Table 1.

Protein C levels in patient samples. Plasma protein C was significantly lower in patients with ALI/ARDS compared with normal controls (Fig. 1). Lower levels of plasma protein C were associated with worse clinical outcomes, including higher hospital mortality and a shorter duration of unassisted ventilation (Fig. 2A). Significantly lower plasma levels of protein C were also measured in patients with shock or two or more non-pulmonary organ system failures (Fig. 2A). As the number of nonpulmonary organ failures increased, plasma protein C decreased (Fig. 3).

To determine whether low levels of plasma protein C in ALI/ARDS were simply a manifestation of sepsis, we compared levels in patients with and without sepsis. Plasma protein C levels were significantly lower in patients with sepsis compared with those without sepsis ($32 \pm 13\%$ vs. $41 \pm 15\%, P < 0.05$). However, when only patients with ALI/ARDS from nonsepsis causes ($n = 25$) were considered, lower plasma protein C levels were still associated with a higher mortality, a shorter duration of unassisted ventilation, two or more nonpulmonary organ system failures, and circulatory shock (Fig. 2B).

Plasma protein C levels were not correlated with the presence or absence of disseminated intravascular coagulation or the platelet count. There was a modest inverse correlation between the plasma protein C level and the protime international normalized ratio ($r = -0.49, P = 0.001$).

In patients with ALI/ARDS, pulmonary edema fluid levels of protein C were consistently lower than simultaneous plasma levels: edema fluid levels were $29 \pm 14\%$ compared with plasma levels of $37 \pm 14\%, P = 0.01$. Pulmonary edema fluid levels of protein C were

![Fig. 1. Boxplot summary of plasma protein C levels in 2 groups, 10 normal healthy controls and 45 patients with acute lung injury (ALI) or acute respiratory distress syndrome (ARDS). Box encompasses 25th to 75th percentile, error bars encompass 10th to 90th percentile, and horizontal bar shows the median. $^*P < 0.001$.](http://ajplung.physiology.org/)

![Fig. 2. Plasma protein C levels in 45 patients with ALI or ARDS (A) or the subset of 25 patients with ALI or ARDS from nonseptic causes (B) compared with clinical outcomes, including hospital mortality, <14 days of unassisted ventilation over a 28-day period, >2 organ failures, or the presence of circulatory shock. Data are means ± SD; $^*P < 0.05$ vs. the more favorable clinical outcome in each instance, $^{**}P = 0.06$ vs. survivors.](http://ajplung.physiology.org/)
correlated with outcome measures. Edema fluid protein C levels were lower in patients who died (26 ± 15% vs. 33 ± 12%, P = 0.07), patients with <14 days of unassisted ventilation (27 ± 14% vs. 34 ± 13%, P = 0.05), and patients with two or more nonpulmonary organ failures (25 ± 13% vs. 36 ± 13%, P = 0.01). Pulmonary edema fluid (but not plasma) levels of protein C were also associated with the degree of physiological respiratory impairment as measured by the LIS. ALI/ARDS patients with higher LIS (≥2.5 on a scale of 0–4) had lower edema fluid protein C levels (27 ± 15% vs. 35 ± 9%, P = 0.056).

Thrombomodulin levels in patient samples. Pulmonary edema fluid levels of thrombomodulin were >10-fold higher in patients with ALI/ARDS compared with normal controls (Fig. 4). In patients with ALI/ARDS, pulmonary edema fluid levels of thrombomodulin were also more than twofold higher than simultaneous plasma levels, suggesting local production in the lung (Fig. 4). Plasma levels were higher in patients with sepsis (412 ± 302 vs. 223 ± 144 ng/ml, P < 0.01), higher in patients with three or more nonpulmonary organ system failures (396 ± 270 vs. 157 ± 66 ng/ml, P = 0.001), and were associated with the severity of illness as measured by the SAPS II score (r = 0.51, P < 0.001). Pulmonary edema fluid levels of thrombomodulin were associated with clinical outcomes. Edema fluid levels tended to be higher in patients who died (880 ± 768 vs. 476 ± 470 ng/ml, P = 0.06). There was also a trend toward higher edema fluid levels in patients with <14 days of unassisted ventilation (810 ± 704 vs. 434 ± 545 ng/ml, P = 0.07).

Comparison with a critically ill control group. To test whether the alterations in protein C and thrombomodulin levels were specific for ALI/ARDS, a comparison population of 22 patients who were mechanically ventilated with hydrostatic pulmonary edema was studied. This group of patients was critically ill with a mean SAPS II score of 42 ± 15 and a mean LIS of 2.5 ± 0.7. In addition, 21 of the 22 patients had at least one nonpulmonary organ failure. Plasma protein C levels were significantly higher in this group of patients (48 ± 18% compared with 37 ± 14% in ALI/ARDS, P = 0.024). Edema fluid levels of thrombomodulin were lower in patients with hydrostatic pulmonary edema compared with ALI/ARDS (224 ± 402 vs. 704 ± 678 ng/ml, P = 0.004) and were not higher than simultaneous plasma measurements (242 ± 279 ng/ml, P = not significant).

Thrombomodulin levels in conditioned media from human alveolar epithelial cells. Because the levels of thrombomodulin were twofold higher in pulmonary edema fluid than in simultaneous samples of plasma from the same patients, an intra-alveolar source of soluble thrombomodulin was suggested. To test the hypothesis that alveolar epithelial cells can release thrombomodulin into the air space in response to an injurious stimulus, human alveolar epithelial-like cells (A549 cells) were exposed to increasing concentrations of H2O2 or inflammatory cytokines, and concentrations of thrombomodulin in the conditioned media were assayed. After 6 h, significant levels of thrombomodulin were released into the media in the presence of either stimulus in a dose-dependent fashion (Fig. 5). Because A549 cells are an immortal cell line derived from a neoplasm, we sought to confirm our findings in primary isolates of human alveolar epithelial type II cells. Because only a small number of these cells could be isolated, assays were done only in the presence or absence of inflammatory cytokines. Similar to the findings in A549 cells, when primary isolates of human type II cells were exposed to 50 ng/ml of cytomix for 6 h, there was a significant increase in release of thrombomodulin into the media (control 2.2 ± 1.4 ng/ml vs. 8.0 ± 3.5 ng/ml, P = 0.008).
Alveolar epithelial cells release soluble thrombomodulin in response to injurious stimuli. Confluent monolayers of human alveolar epithelial type II-like cells (A549 cells) were exposed for 6 h to varying concentrations of H$_2$O$_2$ or cytomix (equimolar amounts of TNF-α, IL-1β, and interferon-γ), and conditioned media was collected for assay of thrombomodulin by ELISA. *P < 0.01 compared with 0.01% H$_2$O$_2$ or control. **P < 0.01 compared with 10 ng/ml cytomix or control.

**DISCUSSION**

The protein C pathway is central to outcome from severe sepsis and has been shown to be a viable therapeutic target. Unlike sepsis, circulating and alveolar concentrations of protein C and its activator, thrombomodulin, have not been systematically investigated in patients with ALI/ARDS. The major findings of this study can be summarized as follows. First, plasma levels of protein C are reduced in patients with early ALI/ARDS, even in the absence of sepsis. Lower levels of plasma protein C are associated with worse clinical outcomes and a higher severity of illness. Second, intra-alveolar levels of protein C in pulmonary edema fluid are lower than simultaneous plasma levels, and the lower levels are associated with more severe physiological respiratory impairment and worse clinical outcomes. Third, levels of thrombomodulin are increased in the alveolar compartment in ALI/ARDS compared with simultaneous plasma levels or to normal control patients. These high intra-alveolar levels suggest that alveolar epithelial cells might be a source of soluble intra-alveolar thrombomodulin. Our in vitro studies confirm that human alveolar epithelial cells can release thrombomodulin in response to an injurious stimulus. Higher edema fluid levels of thrombomodulin are associated with worse clinical outcomes, whereas higher plasma levels are associated with greater severity of illness. In total, these findings indicate that the protein C pathway is significantly altered in ALI/ARDS.

Our findings can be compared with previous reports. There have been very few prior measurements of protein C and thrombomodulin in patients with ALI/ARDS; both proteins have predominantly been studied as biological markers in sepsis. In a small study of 18 patients with ALI, both protein C antigen and protein C function were decreased but the levels did not correlate with outcome (54). In another study of 20 patients with ARDS, plasma protein C levels were also decreased but no attempt was made to correlate levels with outcome (37). Decreased circulating levels of protein C have also been reported in 13 patients with pneumonia (52). In patients with sepsis, larger studies have been undertaken with fairly consistent findings (18). In the largest study to date, Yan et al. (65) reported that plasma protein C levels were reduced in 70 patients with severe sepsis, with 90% of patients meeting criteria for acquired protein C deficiency. Severe sepsis was defined as known or suspected site of serious infection with temperature (T) ≥ 38.3 or <35.5, heart rate (HR) ≥ 90, respiratory rate (RR) ≥ 20, and at least one organ system dysfunction. Lower levels of protein C were associated with poorer clinical outcomes, including lower survival rate, higher incidence of shock, and fewer ICU-free and ventilator-free days. Plasma levels of thrombomodulin are elevated in adults (22) and children (31) with sepsis, and high levels correlate with the development of multiple organ failure (22, 31). Elevated levels of serum thrombomodulin predicted the development of sepsis and multiorgan dysfunction in patients with severe trauma (28). In two small studies (9 and 16 patients), plasma levels were also elevated in patients with ARDS (35, 56), but no correlation was made with outcome. To our knowledge, neither protein C nor thrombomodulin has been measured previously in the alveolar compartment in patients with ALI/ARDS.

Comparison of our findings with the prior studies in sepsis patients is complicated somewhat by the use of different definitions for sepsis, a common problem in sepsis research (2). In the current study, the definition of sepsis was identical to that used in a large study of the epidemiology of ARDS (13). This definition included fever (or hypothermia), known or suspected infection and hypotension, and was an independent predictor of mortality in ARDS patients. Although this definition is similar to the severe sepsis definition used by Yan et al. (65) in the large study of protein C levels of sepsis [also used in the clinical trial of activated protein C for severe sepsis (5)], our definition may have been more restrictive because of the requirement for hypotension. Thus one could argue that some of our nonseptic patients may have had sepsis, thus explaining the low protein C levels. To ensure that our findings were consistent, even when a very liberal definition of sepsis was applied, we regrouped our patients into septic and nonseptic ALI/ARDS using a very liberal definition of sepsis, the 1992 American College of Chest Physicians/Society of Critical Care Medicine Consensus definition (10) (known or suspected infection with 2 of the following: T > 38 or <36, HR > 90, RR > 20 or PCO$_2$ < 32, white blood cell count > 12 or <4). Patients without sepsis by this definition (n = 16) still had far lower than normal protein C levels (43% ± 16%).
The finding of increased thrombomodulin in the alveolar compartment compared with the circulation is intriguing and strongly suggests local production in the lung. The cellular source of soluble thrombomodulin in the alveolar edema is uncertain, but the alveolar epithelium is a possible source. Although thrombomodulin is generally thought to be expressed predominantly by endothelial cells (38, 61), there is a growing number of reports of epithelial expression in a variety of tissues, including the skin (12, 29, 32, 66), the gingival epithelium (39), and the urinary bladder (46). Gingival keratinocytes can facilitate protein C activation by thrombin (39). Strong expression has also been reported in lung epithelial cell cancers, particularly squamous cell carcinomas (47, 58). These findings suggest that epithelial expression of thrombomodulin might also occur in the lung and could account for the very high levels seen in the setting of alveolar epithelial injury. Indeed, in in vitro experiments, both A549 cells and primary isolates of human alveolar epithelial type II cells released soluble thrombomodulin into the media in response to an injurious stimulus, confirming that the alveolar epithelium expresses thrombomodulin. Alternatively, the high levels of intra-alveolar thrombomodulin could simply reflect release from the lung microvascular endothelium into the alveolar compartment in the setting of endothelial injury since the human lung is rich in thrombomodulin (4). Leukocyte, platelet, and megakaryocyte production of thrombomodulin has also been reported (30, 48, 55).

The low protein C levels and high thrombomodulin levels in the alveolar compartment provide further support to the growing body of evidence that the alveolus is a procoagulant, antifibrinolytic environment in ALI/ARDS (23). Widespread alveolar fibrin deposition is a histological hallmark of ALI/ARDS (3, 25, 41). Procoagulant activity is enhanced in bronchoalveolar lavage from patients at risk of (53) and with established ARDS (20) peaking in the first 3 days after onset of lung injury (27). Simultaneously, anticoagulant and fibrinolytic activity is decreased. Levels of the antifibrinolytic plasminogen activator inhibitor-1 (PAI-1) are increased in bronchoalveolar lavage from patients with ARDS (8, 27). We have also recently reported that levels of PAI-1 are increased in the pulmonary edema fluid from patients with ALI/ARDS, and high levels are associated with adverse outcomes (51).

Idell (23) has recently explained that evidence is needed to determine whether procoagulant responses in clinical lung injury are similar in septic and nonseptic ALI/ARDS. The results of this clinical study support Idell’s published experimental studies of ALI, indicating that procoagulant activity develops in the lung injured by hyperoxia, oleic acid, or infection (24, 26). In the current study, the low plasma levels of protein C correlated with worse clinical outcomes in both septic and nonseptic-related ALI/ARDS. Thus regardless of the underlying etiology, alterations in the protein C pathway seem to be an important determinant of outcome in ALI/ARDS.

It is interesting to note that the comparison group of patients with severe hydrostatic pulmonary edema also had lower than normal levels of circulating protein C and higher than normal levels of circulating and intra-alveolar thrombomodulin. This group of patients was severely ill with high SAPS II scores and a high incidence of nonpulmonary organ failure. It is possible that critical illness, in and of itself, may lead to alterations in circulating protein C and thrombomodulin levels, a finding that has previously been reported (9). Alternatively, there is a growing body of evidence that the underlying causes of hydrostatic pulmonary edema (congestive heart failure or acute myocardial infarction) are associated with upregulation of proinflammatory cytokines that could lead to alterations in the coagulation cascade (36, 43, 59, 67). In support of this hypothesis, we have previously reported altered levels of markers of reactive oxygen-nitrogen species (68) and increased levels of the antifibrinolytic mediator PAI-1 (51) in patients with hydrostatic pulmonary edema compared with normal controls.

There are some limitations of this study. First, protein C antigen was measured instead of activated protein C. Activated protein C could not be measured in this retrospective study since samples must be collected with an activated protein C inhibitor, benzamidine (64). However, activated protein C has been measured in sepsis in only a handful of studies (7, 15, 42). In the largest study, the phase II study of drotrecogin alfa (activated), a recombinant human activated protein C, activated protein C was undetectable in the majority (80%) of placebo-treated patients, and levels were sporadic with no discernible pattern over time in the remainder (7). Thus even if activated protein C could have been measured, it is not clear that it would have had any predictive value. Second, we studied only soluble protein C and soluble thrombomodulin. The study was not designed to examine tissue expression of these proteins. It seems likely that the increased levels of circulating plasma and pulmonary edema fluid thrombomodulin that we measured reflect loss of thrombomodulin from the endothelial or lung epithelial surface, although there may be other sources of thrombomodulin in the lung in ALI/ARDS. Furthermore, protein C and thrombomodulin represent only a few of the many proteins involved in modulation of coagulation. We chose to focus only on these proteins because they have been shown to be of prognostic significance in septic patients (18, 22, 31, 65), and in the case of protein C, there is an opportunity for therapeutic intervention (5). Finally, in this retrospective study, it is not possible to determine whether restoration of normal protein C levels or infusion of activated protein C would be of therapeutic value in patients with ALI/ARDS. Given the similarity in the abnormalities in both protein C and thrombomodulin to the findings in sepsis, the protein C pathway could be a therapeutic target in ALI/ARDS. Our study provides evidence for the biological plausibility of this approach. However, randomized controlled clinical trials are necessary.
In conclusion, disruption of the protein C pathway occurs in both septic and nonseptic patients with ALI/ARDS and may contribute to the pathogenesis of ALI/ARDS. Although there are several potential reasons to explain why the pulmonary dead space fraction is markedly elevated early in ALI/ARDS (45), decreased pulmonary blood flow to some ventilated lung units because of microvascular injury and thrombosis could be one important mechanism. The results of this study support this possibility since there is evidence of both circulatory and intra-alveolar derangements in the protein C pathway in ALI/ARDS. The data also suggest that the protein C pathway may be a reasonable therapeutic target in patients with both septic and nonseptic etiologies of ALI/ARDS.

DISCLOSURES

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