Fibroproliferation Occurs Early in the Acute Respiratory Distress Syndrome and Impacts on Outcome

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The fibroproliferative phase of acute respiratory distress syndrome (ARDS) has traditionally been regarded as a late event but recent studies that suggest increased lung collagen turnover within 24 h of diagnosis challenge this view. We hypothesized that fibroproliferation is initiated early in ARDS, characterized by the presence of fibroblast growth factor activity in the lung and would relate to clinical outcome. Patients fulfilling American/European Consensus Committee criteria for ARDS and control patients ventilated for non-ARDS respiratory failure underwent bronchoalveolar lavage (BAL) and serum sampling within 24 h of diagnosis and again at 7 d. The ability of BAL fluid (BALF) to stimulate human lung fibroblast proliferation *in vitro* was examined in relation to concentrations of N-terminal peptide for type III procollagen (N-PCP-III) in BALF/serum and clinical indices. At 24 h, ARDS lavage fluid demonstrated potent mitogenic activity with a median value equivalent to 70% (range 31–164) of the response to serum, and was significantly higher than control lavage (32% of serum response, range 11–42; $p < 0.05$). At 24 h, serum N-PCP-III concentrations were elevated in the ARDS group compared with control patients (2.8 U/ml; range 0.6–14.8 versus 1.1 U/ml; range 0.4–3.7, $p < 0.0001$) as were BALF N-PCP-III concentrations (2.9 U/ml; range 0.6–11.4 versus 0.46 U/ml; range 0.00–1.63, $p < 0.01$). In addition, BALF N-PCP-III concentrations at 24 h were significantly elevated in nonsurvivors of ARDS compared with survivors ($p < 0.05$). At 7 d, the mitogenic activity remained elevated in the ARDS group compared with control ($p < 0.05$) and was also significantly higher in ARDS nonsurvivors compared with survivors (67%; range 45–120 versus 31%; range 16–64, $p < 0.05$). These data are consistent with the hypothesis that fibroproliferation is an early response to lung injury and an important therapeutic target.

Acute respiratory distress syndrome (ARDS) represents a severe and rapid form of microvascular lung injury. Traditionally, ARDS is divided into three stages (1, 2) in which an initial inflammatory phase is followed by fibroproliferation, during which mesenchymal cells, in particular interstitial fibroblasts, migrate, replicate, and secrete extracellular matrix proteins such as collagen (3, 4). Unabated, this process can lead to established interstitial and intraalveolar fibrosis, the final phase. Although mortality from ARDS is improving, approximately 60% of patients with ARDS fail to improve or are deteriorating after 1 wk of ventilation. Almost all of these patients demonstrate mechanical, biochemical, and histological evidence of fibrosis with a doubling of lung collagen in patients surviving more than 2 wk (3, 5). Progressive hypoxia and multiorgan failure result in up to an 80% mortality in this group (6) in the absence of corticosteroid therapy (7, 8).

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A number of observations have challenged the linear concept of ARDS pathogenesis. N-terminal procollagen peptide-III (N-PCP-III), a marker of collagen turnover, is elevated in bronchoalveolar lavage fluid (BALF) (9, 10) and tracheal aspirate (11) from ARDS patients within 24 h of diagnosis. This suggests an early up-regulation of fibrogenic pathways. Tracheal aspirate N-PCP-III above 1.75 U/ml at 24 h was also associated with a 2-fold increased risk of death. Furthermore, immunosuppressive therapy with corticosteroids has proven largely ineffective when administered early in ARDS (12, 13), perhaps suggesting parallel, noninflammatory processes that influence progression. The potential benefit of corticosteroids in patients not improving after 7 d of ARDS may be the result of their antifibrotic activity (7, 10).

The fibroblast is considered to be the major cell responsible for collagen synthesis in the lung and there is now considerable evidence to suggest that soluble mediators play a key role in fibroblast activation (14). These include classical growth factors such as transforming growth factor-β1, platelet-derived growth factor, and insulin-like growth factor-1 (14), but also coagulation cascade proteins (15, 16) and proinflammatory cytokines (17). A number of such factors, capable of stimulating fibroblast proliferation *in vitro*, have been detected in BALF from patients with ARDS including transforming growth factor-α (18, 19), tumor necrosis factor-α, and interleukin-1 (14, 17); however, their role in modulating the fibrotic response is unknown. Lavage fluid from patients with ARDS is potentially mitogenic for lung fibroblast *in vitro* (20), however, the relationship between this activity and the stage of ARDS, clinical progression, or additional markers of fibroproliferation has not previously been studied.

Establishing the temporal sequence of events in ARDS is critical to both our understanding of the key pathological processes involved and to the optimal timing and targeting of therapies. To test the hypothesis that fibroproliferative pathways are activated early in ARDS, we have determined the ability of BALF taken at 1 and 7 d after diagnosis to stimulate lung fibroblast proliferation as an index of soluble growth factor activity and the potential for fibroproliferation in the lung. We have also examined the potency of this proliferative activity in relation to serum/BALF N-PCP-III concentrations, clinical indices of disease severity, and survival.

METHODS

Patient Recruitment

This study was reviewed and approved by the Ethics Committee of University College London Hospitals. All patients over 18 yr of age fulfilling the joint American/European Consensus Committee (AECC) criteria for ARDS (21) admitted to the Intensive Care Units (ICU) of the Middlesex and University College London Hospitals were considered eligible for the study. Patients were identified prospectively by a research fellow within 24 h of diagnosis and were excluded from the study if there was a previous history of ARDS/fibrotic lung disease, or if they were currently on systemic corticosteroid therapy. Control patients were those admitted to the ICU over the same time period requiring positive pressure ventilation for a primary respiratory cause (hydrostatic pulmo-

nary edema and pneumonia) who did not fulfill the AECC criteria for ARDS at any stage of their illness. Patients or their next of kin gave informed consent to enter the study for both BAL and blood sampling or for blood sampling alone (as stipulated by the Ethical Committee).

To identify any potential selection bias, baseline clinical and demographic data were recorded for all eligible patients on admission to the ICU, regardless of their eventual entry into the study. In particular, lung injury score (not including compliance) (22), the Acute Physiology and Chronic Health Evaluation score (APACHE II) (23), and Simplified Acute Physiology Score (SAPS II) (24) were calculated. In addition, a more detailed clinical data set was obtained from patients with ARDS and control patients enrolled into the study on the day of serum or BALF sampling. This allowed laboratory measurements to be related to physiological status.

Patients were classified as survivors if they were discharged alive from the ICU and did not require mechanical ventilation.

Bronchoalveolar Lavage and Serum Sampling

BAL was performed within 24 h of the diagnosis of ARDS (or respiratory failure for control patients) and was performed by one of only two designated clinicians to ensure consistency of technique. Patients were preoxygenated with an $F_{I_{O_2}}$ of 1.0 for 10 min. A fiberoptic bronchoscope (Olympus) was placed via the endotracheal tube into the upper airways. The right middle lobe was identified and the tip of the bronchoscope wedged. Sterile saline was instilled into the right middle lobe in aliquots of 20 ml and after one respiratory cycle, 20–30 cm H_2O suction was applied. This was repeated until either 50 ml of instillate had been recovered or a total of 100 ml had been instilled. The recovered lavage fluid was pooled into a polypropylene tube (Falcon, Rockfalls, NJ), filtered, and placed on ice.

BALF was centrifuged ($200 \times g$ for 5 min at 4° C) to remove cellular material and the supernatant stored at −80° C for subsequent analysis. The time between the end of the BAL procedure and freezing was not allowed to exceed 20 min and samples were kept at 4° C until this time. In addition, 10 ml of supernatant was concentrated 10-fold using an ultrafiltration cell (Amicon, Beverley, MA) through filters with a molecular weight cut-off of 1000. This allowed the dilution of BALF to a suitable concentration in tissue culture media rather than saline (used in the BAL) for cell proliferation experiments.

Ten milliliters of blood specimens was obtained at 1 and 7 d (if appropriate, at the time of BAL) into a vacuumed bottle containing EDTA. Following centrifugation at $340 \times g$ for 5 min, plasma was removed and frozen at −80° C.

Procollagen Peptide Measurement

N-PCP-III concentration was determined using a double antibody-coated tube radioimmunoassay system (CIS-Behring-Werke, Cedex, France). BALF samples, concentrated as described above, were incubated in duplicate tubes coated with monoclonal mouse anti-N-PCP-III for 2 h. Each tube was then washed in phosphate-buffered saline supplemented with mouse immunoglobulin G (IgG). ^{125}I-labeled mouse anti-N-PCP-III was then added and following a further 3-h incubation, specific bound radioactivity was counted for 1 min using a gamma counter (Packard Canberra, Meriden, CT). The assay was linear over the range 0.4 to 11.5 U/ml. Samples in which the concentration was outside this range were diluted 1:5 in normal saline and reassayed. The normal range for serum N-PCP-III is 0.4–0.8 U/ml.

Total protein concentrations in BALF and serum were determined using a biocinchoninic acid (BCA) protein assay (Pierce, Rockford, IL). Serum samples were diluted 1:500 in normal saline. Briefly, serum or BALF sample and working reagent were added to 96-microwell plates and thoroughly mixed for 20 min. The plate was then incubated at 37° C for 30 min and left to cool at room temperature. The corrected absorbance of each well at 562 nm was determined by microplate photometer (Titertek Multiskan, Huntsville, AL). The assay was linear over the range 25 to 2,000 μg/ml. Samples in which the protein concentration remained outside this range were diluted 1:5 in normal saline and reassayed. All N-PCP-III and protein assays were performed in duplicate and a mean value was obtained.

Determination of BALF Mitogenic Activity

Cell culture. Human fetal lung fibroblasts (HFL-1) were obtained from the American Type Culture Collection (Rockville, MD). HFL-1 cells were subcultured in Dulbeccos's modified Eagle medium (DMEM) supplemented with penicillin (200 U/ml) and streptomycin (200 U/ml), and containing 10% newborn calf serum (NCS). Subconfluent cells at passages 15 to 21 were detached using a trypsin (0.05% wt/vol)/EDTA (0.02% wt/vol) solution, centrifuged ($300 \times g$ for 7 min at 4° C), and the cell pellet resuspended in DMEM. Fibroblasts were then seeded onto 96-microwell plates at a density of 5×10^3/well in serum-free DMEM and allowed to attach for 24 h.

[^{3}H]Thymidine incorporation assay. The effect of BALF on DNA synthesis was assessed by the incorporation of [^{3}H]thymidine. The optimum timing and length of the [^{3}H]thymidine labeling was established in preliminary experiments. Subsequently, fibroblasts seeded onto 96-well plates were exposed to concentrated BALF at a range of dilutions in DMEM from 1:16 to 1:256 for 24 h (giving an estimated dilution of the original lavage fluid of approximately 1:1.6 to 1:25.6). Control cultures were exposed to an equal volume of 0.9% saline diluted in DMEM in the presence or absence of 10% NCS. [^{3}H]Thymidine (0.5 μCi) was added for the final 4 h of culture in 10 μl DMEM. Cells were then lysed with 10 μl 0.1 M NaOH solution and frozen at −40° C. DNA was harvested onto glass-fiber filters using a 96-well plate cell harvester (Skatron). Filters representing individual wells were inserted into 6-ml scintillation vials and 5 ml of scintillant (EcoscintA; National Diagnostics, Atlanta, GA) was added. To determine the incorporated radioactivity, vials were counted for 2 min using a Minaxiβ Tri-Carb 4380 series β counter (Packard Instruments, Menden, CT). Any changes in cell number were also confirmed by direct cell counting.

Expression of Results

Proliferation in individual experiments was initially calculated as percentage stimulation above control cells exposed to media alone. However, the proliferative rates of cells may vary significantly between experiments as a result of passage and the precise degree of confluence. To allow comparisons of lavage mitogenic activity between experiments performed at different times, results are expressed as a percentage of the response to 10% NCS observed on the same microwell plate.

Statistical Analysis

Categorical patient variables were analyzed using chi-squared analysis (Fisher's exact test) and continuous patient variables by unpaired Student's *t* test. Comparison of proliferation and N-PCP-III between patient groups was made by Mann–Whitney U test. Pearson's rank correlation coefficient was used to determine correlation between continuous variables. $p < 0.05$ was considered to be significant for all tests.

RESULTS

Patient Recruitment and Characteristics

A total of 77 patients fulfilling the AECC criteria for ARDS were identified during the study period, of which 44 were enrolled. The main reason for nonentry into the study was failure to obtain consent within a 24-h period. No significant differences in age/sex or severity indices were observed between the study and total ARDS groups (Table 1). The most common diagnoses in the study ARDS group were pneumonia (28%), sepsis (25%), and trauma (11%).

A serum sample was obtained within 24 h of diagnosis in all patients. In addition, bronchoalveolar lavage was performed in 18 individuals. In the remaining 26 patients, a BAL was not possible either because specific consent for BAL was refused or a nominated bronchoscopist was not available. Severity scores and demographics were not different in the patients undergoing BAL compared with those in the study or total ARDS groups (data not shown). A second BAL sample was available in 11 of the 18 ARDS patients who had undergone BAL on Day 1. Of the remaining seven patients, two had died and five had recovered.

Sixteen control patients were recruited, 10 with cardiogenic pulmonary edema and 6 with pneumonia. BAL was performed in 10 patients within 24 h. A second BAL sample was available for 5 patients at the 7-d time point. Patient demo-

TABLE 1
CHARACTERISTICS OF ARDS AND CONTROL PATIENTS

Characteristic	Study ARDS (*n* = 44)	Control (*n* = 16)	All ARDS (*n* = 77)
Age*	50.1 (18–77)	54.8 (22–78)	48.7 (21–80)
Male/female	24/20	8/7	45/32
APACHE II score†	19.54 ± 7	21.4 ± 6	20.24 ± 7
SAPS II score†	34.93 ± 15	36.24 ± 9	35.48 ± 6
Pa_{O_2}/FI_{O_2}†	118 ± 30	195 ± 35†	125 ± 37
Lung injury score†	3.08 ± 0.72	2.45 ± 0.47	2.86 ± 0.34
Mortality	36.36%	18.75%‡	37.66%

Definition of abbreviations: ARDS = acute respiratory distress syndrome; APACHE II = Acute Physiology and Chronic Health Evaluation score; SAPS II = Simplified Acute Physiology Score; FI_{O_2} = fraction of inspired oxygen.

* Median values (range).

† Mean values ± standard deviation.

‡ $p < 0.001$ compared with the Study ARDS Group.

graphics in the ARDS and control groups were not statistically different (Table 1). APACHE II, SAPS II, and lung injury scores were not different; however, the arterial oxygen tension/pressure/fraction of inspired oxygen (Pa_{O_2}/FI_{O_2}) ratio was significantly lower in the study ARDS group on admission ($p < 0.001$). Mortality was also significantly higher in the study ARDS group (36.36% versus 18.75% in the control group, $p < 0.001$).

Mitogenic Activity in BALF

Potent mitogenic activity was detected in BALF obtained on Day 1 of ARDS. A typical dilution curve is shown in Figure 1. The highest responses were generally observed at a 1 in 16 dilution and results obtained at this dilution are used for comparison. At 24 h, the proliferation induced by ARDS lavage was approximately double that of control lavage (median 70% of the serum response; range 31–164 versus 32%; range 11–42, $p < 0.05$; Figure 2).

By Day 7 the median proliferative capacity of lavage from both groups was essentially unchanged from 24-h values remaining higher in the ARDS patients compared with control (74%; range 16–120% versus 31%; range 14–41%, $p < 0.05$).

Procollagen Peptide Levels

Figure 3 shows BALF and serum N-PCP-III concentrations obtained within 24 h of diagnosis. N-PCP-III concentrations in BALF were higher in the ARDS group (2.9 U/ml; range 0.6–11.4) compared with control (0.46 U/ml; range 0.00–1.63, $p < 0.01$). Serum values were similar to those in BALF and were also higher in the patients with ARDS (2.8 U/ml; range 0.6–14.8 versus 1.1 U/ml; range 0.4–3.7, $p < 0.0001$).

By 7 d, N-PCP-III in lavage from the ARDS group had increased significantly from 24-h values ($p < 0.05$) and also remained elevated compared with control levels (4.9 U/ml; range 0.3–10.4 versus 0.7 U/ml; range 0.0–1.2, $p < 0.001$). A nonsignificant trend toward an increase in serum N-PCP-III concentrations was observed in the ARDS group at 7 d compared to 24-h values ($p = 0.67$). Serum N-PCP in ARDS was also elevated compared to control patients at 7 d (3.9 U/ml; range 1.3–12.2 versus 1.9 U/ml; range 0.5–2.7, $p < 0.001$).

The mitogenic activity of lavage fluid from ARDS patients obtained within 24 h correlated positively with lavage N-PCP-III concentrations (Table 2). No correlation was seen between BALF mitogenic activity and serum N-PCP-III, BALF protein, or the BALF/serum protein ratio at either time point.

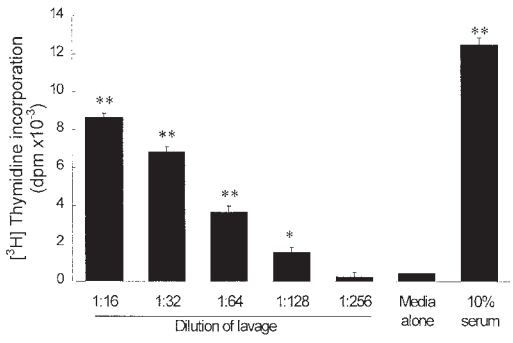

Figure 1. Effect of BALF on DNA synthesis in lung fibroblasts. Human fetal lung fibroblasts were exposed to BALF (1:16–1:256 dilution), media alone, or 10% neonatal calf serum for 20 h. [^{3}H]Thymidine (0.5 μCi/well) was added for the final 4 h of the incubation period. Control cells were exposed to media alone. Values are representative results from a single experiment of six determinations, and are shown as mean ± SEM disintegrations per minute ($\times 10^{-3}$). *$p \leq 0.05$; **$p \leq 0.01$.

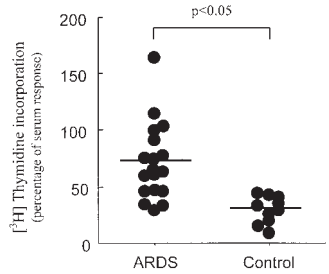

Figure 2. Proliferation of human lung fibroblasts in response to bronchoalveolar lavage fluid. [^{3}H]Thymidine incorporation was determined for human fetal lung fibroblasts exposed to bronchoalveolar lavage fluid obtained within 24 h of diagnosis from ARDS and control patients (1:16 dilution). Each point represents results from an individual patient and is the average of two separate experiments each of six determinations. Values show the percentage increase in proliferation compared with control cells exposed to media alone expressed as a percentage of the response to 10% neonatal calf serum under the same conditions. Bars represent median values.

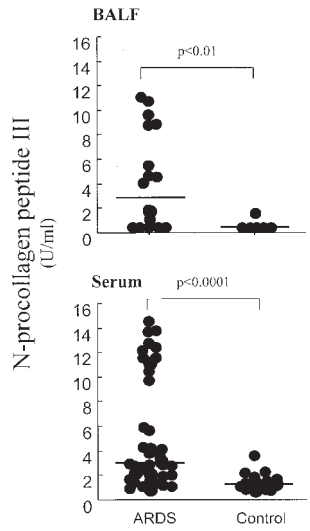

Figure 3. Bronchoalveolar lavage fluid and serum N-PCP-III concentrations at 24 h. N-PCP-III concentrations were determined by radioimmunoassay in BALF and serum samples obtained within 24 h of diagnosis. Each point represents the average of duplicate determinations for an individual patient. Bars represent median values.

TABLE 2

CORRELATION BETWEEN THE MITOGENIC ACTIVITY OF ARDS LAVAGE AND BOTH CLINICAL AND BIOCHEMICAL PARAMETERS*

	BALF Mitogenic Activity (Day 1)		BALF Mitogenic Activity (Day 7)	
Parameter	p Value	(CI)	p Value	(CI)
BALF/serum protein ratio	0.752	(−0.58–0.48)	0.929	(−0.89–0.87)
BALF N-PCP-III	0.017	(0.12–0.85)	0.663	(−0.88–0.71)
Serum N-PCP-III	0.580	(−0.36–0.64)	0.313	(−0.35–0.81)
APACHE II score	0.239	(−0.28–0.83)	N/A	N/A
SAPS II score	0.1468	(−0.19–0.86)	N/A	N/A
Pa_{O_2}/FI_{O_2}	0.339	(−0.83–0.39)	0.3391	(−0.83–0.38)
Lung injury score	0.881	(−0.78–0.34)	0.645	(−0.39–0.76)

Definition of abbreviations: BALF = bronchoalveolar lavage fluid; N-PCP-III = N-terminal procollagen peptide-III; APACHE II = Acute Physiology and Chronic Health Evaluation score; SAPS = Simplified Acute Physiology Score; FI_{O_2} = fraction of inspired oxygen; CI = confidence interval.

* p Value and 95% CI for Pearson's rank correlation coefficient are shown for each parameter.

TABLE 3

CHARACTERISTICS OF ARDS SURVIVORS AND NONSURVIVORS WITHIN 24 h OF DIAGNOSIS

	Survivors (*n* = 28)	Nonsurvivors (*n* = 16)	p Value
Mitogenic activity, %*	60 (45.8–99.5)	77 (44.6–164.3)	0.084
BALF protein, g/L*	1.1 (0.2–3.7)	1.7 (0.4–5.0)	0.056
BALF/serum protein ratio	0.1 (0.2–0.3)	0.2 (0.3–0.6)	0.048
BALF N-PCP-III, U/ml*	1.24 (0.60–3.42)	3.1 (1.8–11.4)	0.017
Serum N-PCP-III, U/ml*	2.5 (0.8–13.8)	3.4 (0.9–14.9)	0.097
APACHE II score†	17.5 ± 7.1	22.4 ± 8.3	0.0419
SAPS II score†	32.7 ± 17.0	39.9 ± 17.0	0.128
Pa_{O_2}/FI_{O_2}†	13.6 ± 3.3	14.13 ± 3.4	0.183
Lung injury score†	3.1 ± 0.6	3.55 ± 0.6	0.426

Definition of abbreviations: BALF = bronchoalveolar lavage fluid; N-PCP-III = N-terminal procollagen peptide-III; APACHE II = Acute Physiology and Chronic Health Evaluation score; SAPS = Simplified Acute Physiology Score; FI_{O_2} = fraction of inspired oxygen.

* Median values (range).

† Mean values ± standard deviation.

Similarly no relationship was observed with severity scores or Pa_{O_2}/FI_{O_2} ratio.

Mitogenic Activity and Outcome

The proliferative capacity of lavage, N-PCP-III concentrations, protein ratios, and a number of clinical indices measured at 24 h was compared between survivors and nonsurvivors of ARDS (Table 3). A trend toward higher mitogenic activity in nonsurvivors compared with survivors was observed on Day 1 ($p = 0.084$). There were insufficient matched Day 1/Day 7 lavages to determine the relative change in proliferative capacity for individual patients, often because patients had either died or had been extubated by Day 7. However, proliferation induced by ARDS lavage fluid from Day 7 was significantly higher in nonsurvivors (67%; range 45–120) compared with survivors (31%; range 16–64, $p < 0.05$).

Lavage N-PCP-III concentration ($p < 0.05$), BALF/serum protein ratio ($p < 0.05$), and APACHE II score ($p < 0.05$) on Day 1 of ARDS were all higher in nonsurvivors compared with survivors. No correlation was seen with serum N-PCP-III, lung injury score, SAPS II score, or Pa_{O_2}/FI_{O_2} ratio at either time point.

DISCUSSION

This study clearly demonstrates the potential for pulmonary fibroproliferation at the early stages of ARDS. The potent mitogenic activity of BALF and the elevation in N-PCP-III concentrations observed at 24 h support the hypothesis that two key mechanisms driving the deposition of lung collagen—fibroblast proliferation and procollagen synthesis—are rapidly up-regulated in this syndrome. These observations are also consistent with previous reports of increased N-PCP-III concentrations in the lung at this time (9–11). The mitogenic activity approached and occasionally exceeded that of serum and was abrogated by sequential dilution, implying the presence of significant soluble growth factor activity. Furthermore, a persistently high mitogenic activity after 1 wk of ARDS was associated with nonsurvival, suggesting pulmonary fibroproliferation is an important determinant of outcome.

Patients entered into the study were representative of the ARDS population seen in our hospitals and were closely matched to control patients in a number of important characteristics including age, sex, APACHE II/SAP II scores, and lung injury scores. The increased mitogenic activity present in ARDS lavage fluid may, therefore, represent the activation of fibroproliferative pathways that are, at least in part, independent of the presence of interstitial and alveolar edema (cardiac failure), or an acute inflammatory response (pneumonia). There is, however, likely to be significant cross-talk between immune and fibrotic pathways with inflammatory cells playing an important role in driving the repair response (25). Alveolar macrophages, for example, are a potent source of fibroblast growth factors (26). Conversely, interstitial fibroblasts also express key inflammatory cell chemoattractants (IL-8) (27) and growth factors (IL [interleukin]-6, IL-1) (28, 29).

Lavage fluid from a number of control patients also demonstrated significant mitogenic activity, which was greater than what we have previously observed in normal volunteers (30, 31). This suggests the presence of active inflammatory/repair mechanisms in other forms of severe pulmonary disease. It is also possible that the institution of mechanical ventilation in these patients is itself injurious to the lung (32–34).

The correlation between the mitogenic activity at Day 1 and N-PCP-III concentrations in lavage is biologically plausible and of great interest. The specificity of this relationship is implied by the absence of a significant correlation between mitogenic activity and either serum N-PCP-III or BALF protein concentrations at this time and suggests mitogenic activity observed *in vitro* is related to fibroblast activity *in vivo.* Against this is the lack of correlation between mitogenic activity and lavage N-PCP-III concentration at 7 d although insufficient patient number or sampling variability may account for this discrepancy in our study. No correlation was demonstrated between mitogenic activity and a number of clinical severity scores at either time point. Although this is perhaps not surprising in relation to general ICU scores, one might have expected a correlation with more specific indices of pulmonary disease. However, neither Pa_{O_2}/FI_{O_2} ratio or lung injury score on Day 1 correlated with outcome in our series. This is at variance with some previous studies (10), but similar observations have been noted by others (35). This may result from the limitations of such scoring systems when applied to smaller groups of patients. It is also possible that changes in these parameters over time might correlate better with outcome.

The elevation in lavage N-PCP-III in nonsurvivors of ARDS at Day 1 is in agreement with previous reports (9–11) and with an increase in collagen mRNA observed immediately after lung damage (36). Although there was a trend toward increased mitogenic activity at this time, the increased activity

and N-PCP-III concentration at Day 7 in nonsurvivors suggest that the persistence of the fibroproliferative response may be of importance in determining outcome. Reduced patient numbers at Day 7 may have limited the power of this study to detect differences between biochemical and clinical indices at this time point.

Our observations agree with the elevation in N-PCP-III in nonsurvivors of ARDS noted by others (9, 10). Collagen deposition in the lung represents a balance between procollagen synthesis and breakdown (37); it is thus also possible that the activity of degradative pathways may overshadow profibrotic pathways in determining net collagen deposition (38) at the later stages of ARDS (39). For example, matrix metalloproteinases, capable of degrading collagens, are present in lavage fluid from patients with ARDS, but their relationship to collagen turnover is as yet unclear (40, 41).

There is substantial evidence to support a key role for soluble growth factors in more chronic forms of interstitial lung disease (14, 39, 42). In patients with ARDS, a protein of similar molecular weight to platelet-derived growth factor (18) and transforming growth factor-α (19) have both been identified in BALF, but the functional role of these and other profibrotic mediators in ARDS is not known. Identifying the key profibrotic factors present in ARDS is an important future goal and we and others are currently employing a number of approaches to characterize growth factors present in the lavage fluid.

The ability to time the onset of injury in many patients with ARDS provides a unique opportunity to study the temporal relationship between pathophysiological processes following diffuse lung injury. This study, together with previous observations, suggests an alternative to traditional models of the injury response, whereby inflammatory and repair mechanisms occur in parallel rather than in series. It is not clear whether this represents a paradigm for acute injury responses in other settings (25), but it has important implications both for the study of repair mechanisms and the timing of therapies, which will need to be pluripotent if they are to be effective.

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