The Interdependence of Lung Antioxidants and Antiprotease Defense in ARDS*

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As in hereditary α1-antitrypsin deficiency, protease-antiprotease and oxidant-antioxidant balances play a significant role in the pathogenesis of ARDS. However, the disease processes and possibilities for therapeutic intervention differ markedly. (CHEST 1996; 110:2735-277S)

Key words: adult respiratory distress syndrome; antioxidant; antiprotease; α1-antitrypsin; neutrophil elastase; oxidant; protease; surfactant

ARDS is a catastrophic complication of several common medical and surgical disorders. Characterized by severe hypoxemia, diffuse pulmonary infiltrates, and decreased respiratory compliance, upwards of 100,000 individuals in the United States develop ARDS annually.1 While the clinical scenarios that place patients at risk for ARDS are as diverse as sepsis and the aspiration of gastric contents, the mortality rate for this form of respiratory failure is uniformly grim.2,3 Most published series currently report that only 50 to 60% of patients with this severe acute lung injury will survive.4

THE LOSS OF REGULATION OF LUNG HOST DEFENSE

A common theme unifying the various risk factors for ARDS is the development of an inflammatory injury within most of the alveolar structures. In fact, Weiland and colleagues5 demonstrated in 1986 that most cells recovered from the lungs of patients with ARDS were neutrophils. There is little to distinguish the inflammatory events operative in ARDS from the lungs’ response to lobar pneumococcal pneumonia except for the extent of lung involved and the clinical context. That is, the lung inflammation in ARDS usually occurs “out of context” of normal host defense. Although the neutrophil influx in the lung injury consequent to bacterial pneumonia is the necessary and appropriate response to an invasive pathogen, this context of lung defense is most often absent in ARDS. The neutrophil accumulation in the ARDS lung is unfocused (diffuse), nonspecific, and unregulated. Current concepts suggest that the neutrophil-mediated lung injury of ARDS results from a failure to regulate the inflammatory cascades that participate in host defense, although this is likely to prove to be an oversimplified view of the pathogenesis of ARDS. The cost of this loss of inflammatory regulation, however, is the diffuse lung injury and noncardiogenic pulmonary edema that characterize this severe form of respiratory failure.

In the context of a massive and persistent traffic of blood neutrophils into the ARDS lung, it is reasonable to hypothesize that the outcome of this conflagration, as it relates to lung structure and function, may be determined by the processes that defend the lung against neutrophil-mediated tissue injury. Although the most effective modulation of neutrophil-mediated lung injury is likely to be invested in the mechanisms that regulate cell recruitment (mediators of activation, chemokines, cytokines, peptides and lipids, adherence systems on cell surfaces), in ARDS it may be said that “the die is cast.” The blood neutrophils that are assiduously excluded from the extravascular spaces in ordinary times have already effected a massive invasion of the gas-exchange structures by the time the patient requires mechanical ventilation. The purpose of this discourse is rather to focus on the systems that modulate inflammatory tissue injury once it has been established.

NEUTROPHIL-MEDIATED LUNG INJURY

The events that act to contain the collateral tissue injury attendant to neutrophil accumulation are as relevant to host defense as they are to lung injury and repair. The physiologic role of the neutrophil in host defense is contingent on the interaction of this cell’s proteolytic and oxidative effector systems. Although some overlap may exist, the neutrophil’s proteases are largely responsible for damage to the lungs’ connective tissue matrix, while the free radicals produced by this cell are the primary means for injury to the cells of the lungs. While this two-pronged repertoire of the neu-

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trophil is operative in the physiologic (eg, antimicrobial function) and the pathologic (eg, lung injury in ARDS) context, the agenda is clearly distinct. In the context of host defense, the generation of connective tissue proteases (collagenase, elastase, and proteinase 3) provides a means for penetration of the extracellular space so as to access the pathogen that is dispatched by reactive oxygen species (O$_2^-$, H$_2$O$_2$, OH$_-$, etc). This effective host defense system is precisely focused by the lungs’ antioxidants and antiproteases, so as to facilitate directed migration, phagocytosis, and microbicidal activity, without undue injury occurring to the lung structure and function. The clinical outcome would appear to depend as much on the interdependence of the antiprotease/antioxidant defense systems as it does on the microbicidal efficiency of the neutrophil. Without the capacity to limit the collateral damage attendant to the neutrophil’s role in host defense, the resultant damage to the lung parenchyma can render any victory over a pathogen “Pyrrhic.” So much more so is the outcome in ARDS contingent on the effectiveness of this interaction between antioxidant and the antiprotease defense of the lung. The evidence increasingly speaks to the premise that clinical outcome in ARDS is determined by the early containment of injury and initiation of lung repair.

In the scenario of pathologic neutrophil recruitment to all areas of the lung, ARDS can be envisioned as a context in which all lung injury is “collateral” (ie, of no net benefit to the host). In this setting, only the effective interdiction of neutrophil proteases and free radicals offers the hope of lung defense and repair. Herein may be the determinant of survival in ARDS.

**Table 1: Inhibitor Profile of Elastase Activity**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Percent Inhibition</th>
<th>Neutrophil Elastase Standard</th>
<th>Neutrophil Elastase Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EDTA, 50 mmol/L</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PMSF, 5 mmol/L</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>CMK, 0.2 mmol/L</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>$\alpha$-AT, 0.2 µmol/L</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*Elastase activity as measured with the extended MEOSAAPNVA assay.

1 Peak fraction of elastase activity from G-75 chromatography (10 picomoles).

**HIGH-PERMEABILITY PULMONARY EDEMA: THE GOOD NEWS**

Typically in patients with ARDS, the chest radiograph shows lungs that are filled with noncardiogenic pulmonary edema fluid as a consequence of diffuse alveolar damage. These patients generally require mechanical ventilation and are at risk for barotrauma such as pneumothorax and pneumomediastinum.

In the early 1980s, evidence began to point to the importance of neutrophils in the pathogenesis of ARDS. The normal lung restricts neutrophil influx, but when the lung’s defenses are lowered and the neutrophils are able to enter, the consequences are severe. The neutrophils release their proteases along with oxidants—a two-pronged attack on the gas exchange structures. The proteases cleave the matrix, allowing the neutrophil and its oxidants to penetrate the alveolus and cause parenchymal cell death. This, in turn, leads to a massive loss in the selective permeability of both the endothelium and the epithelium. The lung then becomes filled with a filtrate of plasma that looks more like serum than alveolar fluid.

Several investigators have measured levels of free elastase in the lungs of patients with ARDS. Lee and his colleagues showed free neutrophil elastase in the lungs of most patients with ARDS, giving rise to a hypothesis that $\alpha$-antitrypsin ($\alpha$-AT) (Prolastin) therapy could be applied to ARDS, a lung disease that is much more prevalent than the inherited form of emphysema. $\alpha$-AT, currently used as a therapeutic agent against hereditary emphysema, is known to be a potent inhibitor of neutrophil elastase, an elastin-degrading protease released by neutrophils. When alveolar structures are unprotected from exposure to elastase, progressive destruction of elastin tissues occurs, resulting in permeability defects. Of note, this influx of pulmonary edema fluid in ARDS allows for the quenching of a large amount of neutrophil elastase activity.

However, in a clinical investigation of the effect of protease inhibitors on ARDS lavage conducted in our laboratory in 1987, we were surprised to find no free elastase such as other groups had reported previously. We concluded that the difference between earlier
studies and ours was that other studies had used low molecular weight substrates for detecting elastase. We, therefore, began to examine the catalytic properties of this elastase-like activity that cleaved only the low molecular weight substrate for neutrophil elastase. Figure 1 shows neutrophil elastase standard and the ARDS BAL fluid that contained this activity against low molecular weight elastase substrates. As can be seen, the ARDS BAL fluid elastase had no activity against elastin, the natural substrate for elastase, indicating that the BAL elastase activity did not have the capacity to induce direct lung injury. Table 1 compares the inhibitory profile of known protease inhibitors against both purified neutrophil elastase and the neutrophil elastase activity in ARDS BAL fluid. Importantly, low molecular weight inhibitors like phenylmethyl sulfenyl fluoride (PMSF) and chloromethyl ketone (CMK) can inhibit the elastase in both preparations, but the high molecular weight inhibitor of natural elastase (α1-AT) inhibits only the purified neutrophil elastase. This supports the presence of “authentic” neutrophil elastase.

The catalytic profile of this elastase against the small molecular weight substrate, and the inaccessibility of this activity to α1-AT, provided important clues as to the mechanism of interaction of the elastase and its inhibitors in the ARDS lung. When comparing the proteins present in ARDS lavage with those in the normal fluid, we found approximately a 100-fold increase in the molar concentrations of the very high molecular weight antiprotease α2-macroglobulin in the ARDS lung. This relatively high molar concentration of α2-macroglobulin approximates the levels seen in serum.

The elution profile (Sephadex G-75) of ARDS BAL elastase (Fig 2) shows the distribution of functional and antigenic elastase activity recovered from the lung. Huge amounts of neutrophil elastase activity are released into the lungs of individuals with ARDS and the functional activity is demonstrated in the first peak, a large molecular weight complex. Therefore, the structure and function of the ARDS elastase measured in the ARDS BAL duplicate that of elastase bound to α2-macroglobulin.

From a clinical perspective, this result implies that there may be no advantage to intervening therapeutically by enhancing the antineutrophil elastase activity in ARDS. In addition to allowing α2-macroglobulin access to the alveolar space, the lung fluid in patients with ARDS contains more than 20 times the concentration of α1-AT and antineutrophil elastase activity of normal lavage fluid. Therefore, if the patient were to receive the usual dose of α1-AT, incremental activity could not be expected.
Although this experiment did not support our original hypothesis that α1-AT would serve as a therapeuti
c agent in ARDS, it did increase our understanding of the mechanism by which the lung attempts to regu
late inflammation—that is, through an increase in the permeability of the lung to large molecular species
with potent antiprotease effect (ie, both α1-AT and α2-macroglobulin).

This brings us to the consideration of the second component of the neutrophil’s attack on the lung, the
generation and release of potent-free radicals of oxygen. As in hereditary α1-AT deficiency, there seems to be
an interaction between the oxidant and protease injury that occurs consequent to the neutrophil influx
into the lung in ARDS patients. In ARDS, however, the biochemical milieu is much different insofar as the
lungs are filled with large amounts of a protein-rich fluid that contains both antiproteases and antioxidants.
Although we expected that the influx of neutrophils would quickly exhaust the total antioxidant capacity of
the lung, in fact we found a marked increase in the total capacity of the lung fluid to inhibit lipid peroxidation.9
Figure 3 shows the dilution titration of BAL fluid ob
tained from patients with ARDS contrasted to that of normal individuals and the effect that it has on the in
hibition of lipid peroxidation. Once again, the permeability change associated with diffuse lung inflamma
tion acts as a stopgap measure to try to regulate the process. Given this marked increase in the amount of
total antioxidant activity, we set out to dissect the indi
vidual components of antioxidant activity in the lung and examine the hierarchy of antioxidant function. In
contrast to the earlier results relevant to total antioxi
dant activity, glutathione-specific antioxidant activity
was reduced significantly (Fig 4).10

In the ARDS lung, it appears that glutathione is
singly out for impairment by conversion to the
oxidized species. Thus, there is a marked glutathione
deficiency in the lungs of ARDS patients, a situation
that suggests a specific reduction in this important
oxidant protection of the lung. In addition, there is a
marked increase in the portion of the total alveolar
fluid glutathione that is in the oxidized form in patients
with ARDS.10

While the role of the neutrophil in ARDS differs
from that in α1-AT deficiency associated with emphy
sma, it is similar in the sense that it provides a mecha
nism to explore the coordinated attack of neutrophil
oxidants and proteases. When the structure of the lung
is impaired sufficiently to allow for changes in perme
ability, the regulation of neutrophil function can occur
as a result of access of anti-inflammatory plasma pro
teins into the compartments of the lung. Herein it is
apparent that the high permeability pulmonary edema
confers an advantage in the context of lung defense and
repair. Regrettably, this advantage is countered by the
considerable “inconvenience” attendant to ventilating
this edematous lung.

The challenge in the next 10 years or so will be to
consider regulation of neutrophil function at various
levels, α1-AT being only one of them, specifically to
interdict in a selective fashion neutrophil interactions
with the endothelium. In our laboratories, St. John et
al11 demonstrated the marked increase in the perme
ability of the intestinal wall; noncardiogenic edema
occurs in organs other than the lung in ARDS.

In a related study, St. John and his coworkers12
demonstrated that this marked increase in permeability
was a result of the neutrophil adherence that occurs
as a consequence of massive activation in the intravas
cular space. In using an anti-CD18 antibody, these

Figure 3. BAL fluid from ARDS patients has increased antioxi
dant activity compared with equal volumes of BAL fluid from nor
mal subjects. At volumes above 15 mL, lipid peroxidation is com
pletely inhibited in both groups (reprinted from Lykens et al.9 with
permission).

Figure 4. Total glutathione concentration in the epithelial lining
fluid of patients with ARDS compared with patients with cardio
genic pulmonary edema and normal volunteers. There was a sig
ificant deficiency of glutathione in patients with ARDS com
pared with normal subjects (p < 0.0001) and compared with patients
with cardiogenic pulmonary edema (p = 0.001). Nls = normal vol
unteers; CPE = cardiogenic pulmonary edema (reprinted from Bunnell
and Pacht,10 with permission).
researchers were able to interdict this permeability change. Therefore, by preventing neutrophil adhesion in the intravascular space, they were successful in preventing alteration of permeability in the extrapulmonary organs.

Another promising future direction related to proteolytic activity in the lungs of patients with ARDS is the prospect of lung surfactant replacement. One of the consequences of the proteolytic activity in the lungs is a marked reduction of surfactant activity. ARDS patients demonstrate severe abnormalities of surfactant function and quantity, which is likely the result, in part, of proteolytic destruction of the surfactant-associated proteins. In an important pilot study, replacement of surfactant was shown to be a useful treatment for patients with ARDS, which resulted in improved gas exchange and survival.13

It must be hoped that enhanced understanding of the pathogenic mechanisms operative in ARDS will eventually result in improved opportunities for effective therapy. Thus far, the transition from understanding to specific therapy has been painfully slow.

REFERENCES
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