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Acute Respiratory Distress Syndrome and Oxidative Stress

Mechanisms of Disease Development and Opportunities for Antioxidant Prevention

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I. Introduction

Acute lung injury (ALI) and the severe form of ALI—acute respiratory distress syndrome (ARDS)—are multifaceted pathophysiological entities that are characterized by severe respiratory failure, generalized lung inflammation, increased oxidative stress, and diffuse lung edema (1). For unknown reasons, ARDS is associated with, and appears to be a consequence of, a large number of diverse disorders. Numerous direct and indirect injuries to the lungs and systemic precipitating disorders have been implicated in the pathogenesis of ARDS. These include sepsis, toxins, smoke, oxygen toxicity, multiple transfusions, chemicals, drugs, radiation, viral/bacterial infections, aspiration of gastric contents, chronic ethanol ingestion, pancreatitis, uremia, disruption of iron metabolism, trauma, and oxidative stress. Sepsis and trauma are frequently associated with ARDS, and they are the major risk factors for increased mortality in most hospitals. Clinically, the disease is characterized by severe respiratory insufficiency associated with cyanosis and arterial hypoxemia, leading to a multisystem (multiple) organ failure. Additional diseases occurring

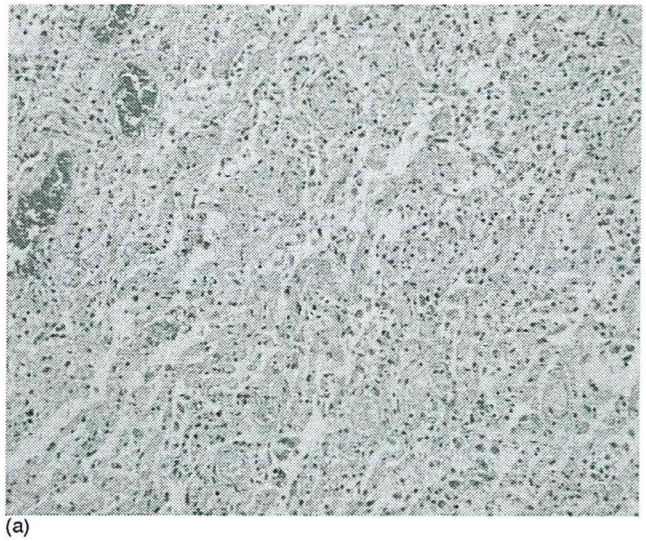
with features that resemble ARDS include diffuse alveolar damage, traumatic wet lung, shock lung, chemical pneumonia, and progressive fibrosing alveolitis.

A precise, accepted estimate of the incidence of ARDS has been difficult to establish in part because of the varying defining criteria associated with the disease. An early estimate by the National Institutes of Health in 1977 indicated an annual incidence rate of 75 deaths per 100,000 population in the United States (2). According to a recent estimate by the American Lung Association, approximately 150,000 Americans are affected each year (3). However, an epidemiological study using the 1994 American–European Consensus Conference Committee definition of ARDS reported higher incidences and rates of death in Scandinavian countries (4,5). Historically, ARDS was initially observed in battlefield casualities during World War I, but was most clearly described in 12 adults in 1967 as a clinical syndrome "remarkably similar to infantile respiratory distress syndrome," exhibiting diffuse bilateral pulmonary infiltrates, edema, hyaline membrane deposition, resistance to treatments, severe respiratory failure, and high mortality rate (6). Ashbaugh et al. first designated the entity as the *adult* respiratory distress syndrome in 1971 (7), but it is now known as the *acute* respiratory distress syndrome.

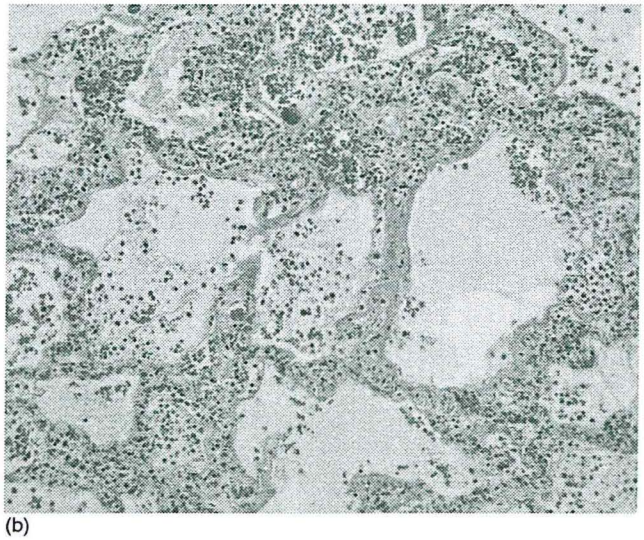
This chapter will focus on an overview of the contribution of oxidative stress in ARDS and mechanisms of disease development. Progress and therapeutic attempts using antioxidants to minimize morbidity and mortality for ARDS are discussed briefly. Medical progress and routinely used clinical protocols have been discussed previously in other reviews (1,8,9).

II. Pathology and Pathogenesis

The hallmarks most commonly present in the end-stage pathology of ARDS cases include diffuse alveolar damage associated with interstitial and alveolar edema, dense proteinaceous debris, desquamated epithelial cells, organized hyaline membranes, proliferation of fibroblasts, and granulation tissue along with inflammatory infiltrates predominated by neutrophils, alveolar macrophages, and red blood cells (Fig. 1). However, the disease is progressive and the early morphological manifestations are often described to be mostly exudative, caused by the diffuse alveolar capillary wall abnormalities, followed in some instances by a proliferative stage and then manifested by a chronic, desquamative, fibrotic stage. Depending on the situation, in the acute stages of lung injury usually during the first 2 or 3 days, alveoli are filled with a proteinaceous exudate containing a large



(a)



(b)

Figure 1 (a) Photomicrograph showing acute lung damage in an ARDS patient's lung. Diffuse alveolar damage, extensive proteinaceous exudates, desquamated cells, hyaline membrane deposition, inflammatory infiltrate, and blood cells are present; (b) Photomicrograph of an organizing ARDS lung tissue from an ARDS patient. The walls of the air spaces are thickened by some granulation tissue.

number of red blood cells, neutrophils, and macrophages. Swelling of endothelial cells and other cellular abnormalities, including apoptotic lesions in type I epithelial cells, are also apparent. Increased numbers of neutrophils are often visible adhering to the capillary endothelium and in air spaces. Although unproven, activated neutrophils appear to play an important role in the genesis of ARDS. Support for the pivotal role of neutrophils comes largely from experimental studies of acute lung injury. In general, when neutrophils are eliminated, lung injury severity decreases in animal models whereas adding activated neutrophils damages lung endothelial cells in vitro and isolated perfused lungs (10). In more advanced acute injury usually after a few days, diffuse alveolar damage and increased number of neutrophils, macrophages, plasma cells, and lymphocytes are detected in the interstitium and alveoli along with the appearance of fibroblasts, fibrous tissue, fibrin, and hyaline membranes on the basement membrane.

Loss of integrity of the alveolar-capillary membrane, leading to increased leakage of plasma into the alveolar space, is considered to be an important underlying problem of ARDS (1,11). The progressive pulmonary edema resulting from endothelial leakage of plasma into the lung is of noncardiogenic origin. The proteinaceous edematous fluid filling the alveolus may lead to inactivation of surfactant. It is postulated that the capillary endothelial defect is followed by alveolar epithelial injury, interstitial and intra-alveolar edema, deposition of fibrin, and formation of hyaline membranes. The persistent endothelial injury and progressive pulmonary edema that characterize ARDS can cause chronic pulmonary fibrotic scarring.

It is well documented that direct insults through pulmonary route, indirect insults through the systemic circulation, and their interactions can provoke alveolar capillary injury and the complex cellular disruption that causes ARDS (Fig. 2). Vascular permeability is associated with the interaction of neutrophils, macrophages, and cellular mediators that produce proteases, reactive oxygen species (ROS), reactive nitrogen species (RNS), cytokines, chemokines, complement and other activating factors—each of which appears capable in some ways of triggering detrimental lung injury and the pathophysiological outcomes that occur in ARDS (1,8–13). A schematic representation of the major cellular mediators involved putatively in this syndrome is presented in Fig. 3. Persistent generation of several cytokines, leukotriene E_4, platelet-activating factor, and ROS have been correlated with abnormalities of gas exchange, edema, and lung compliance (11,12,14). It is believed that the neutrophilic inflammation is enhanced and driven by macrophage-derived mediators that can also stimulate the production of extracellular matrix by fibroblasts.

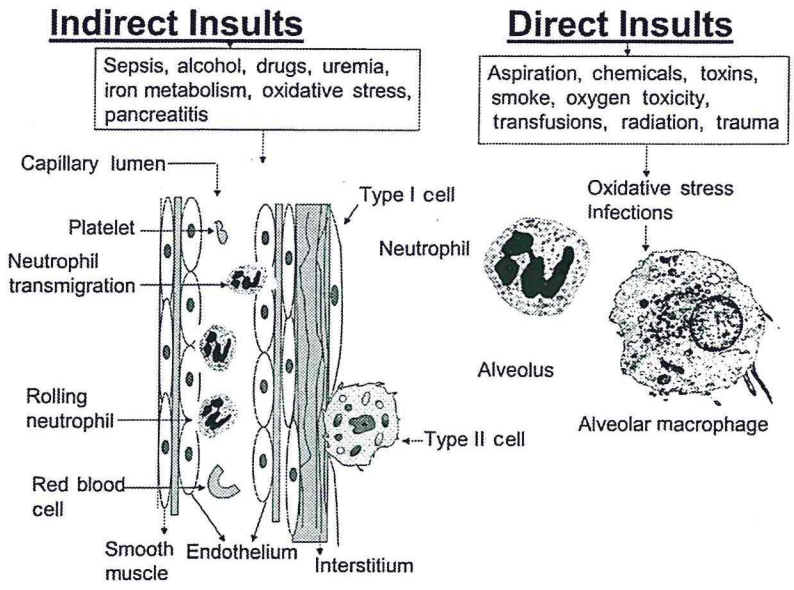


Figure 2 Schematic representation of major factors involved in provoking cellular reactions in ARDS through a direct pulmonary route and an indirect systemic route.

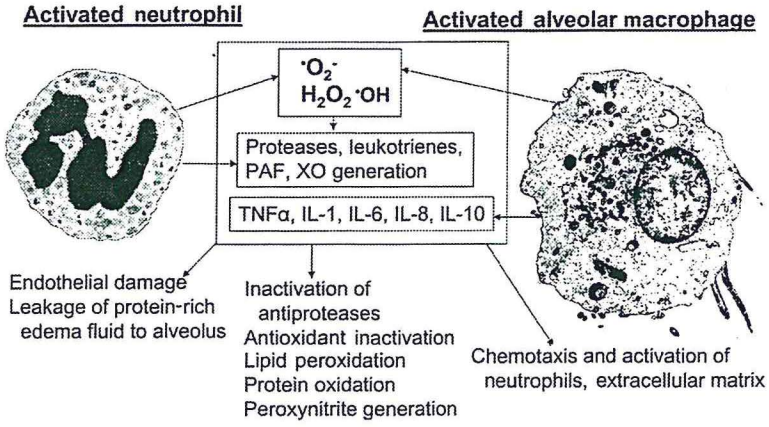


Figure 3 Postulated cellular mediators secreted by activated neutrophils and macrophages involved in the pathogenesis of ARDS.

III. Important Factors in the Pathogenesis of ARDS

A. Reactive Oxygen Species

Cochrane and his associates were the first to provide clear evidence of the suspected increased oxidant activity in bronchoalveolar lavage fluid (BALF) from patients with ARDS (15). He and his colleagues showed that α_1-proteinase inhibitor (α_1-PI) was inactivated in the BALF of patients with ARDS. Although α_1-PI was not inactivated in the blood of these same patients, these studies implied that oxidative stress was increased in the lungs of ARDS patients. Furthermore, support for the α_1-PI inactivation premise was reported in a study showing a site-specific targeted inactivation of methionine at the reactive center of α_1-PI by oxidative damage (16). Thereafter, several clinical and experimental studies provided additional observations showing that endothelial and epithelial injury in ARDS could be mediated through neutrophil-derived oxidants and that macrophages, or their products, play a pivotal role in the neutrophilic influx and progression of injury (1,17–20). More specifically, neutrophils and alveolar macrophages can release ROS, such as superoxide ($^{\bullet}O_2^-$) and hydrogen peroxide (H_2O_2), which in the presence of uncomplexed ("free") catalytic transition metals, such as iron and copper, can generate a toxic hydroxyl ($^{\bullet}OH$) radical. In addition, in the presence of the neutrophil enzyme myeloperoxidase (MPO), these species may form hypochlorous acid (HOCl). The enhanced oxidant generation by neutrophils may increase endothelial cell permeability and promote adhesion and extravasation of leukocytes (21). Interestingly, Kokura et al. have shown that a redox imbalance in endothelial cells causes a transcription-independent and transcription-dependent surface expression of different endothelial adhesion molecules that may lead to a two-phase neutrophil–endothelial adhesion response (22). In support of this redox imbalance, Baldwin et al. (23) reported that there was a fivefold increase in H_2O_2, in the breath condensate of ARDS patients who recently developed ARDS compared to more critically ill ARDS patients. These studies also demonstrated that patients with ARDS had higher levels of H_2O_2 in their expired breath condensates than other mechanically ventilated patients in intensive care with other pulmonary diseases. Additional studies of 55 ARDS patients with increased levels of H_2O_2 and 13 patients with normal lungs undergoing elective surgery who had no detectable levels of H_2O_2 confirmed the increased levels of H_2O_2 in expired breath condensates of ARDS patients (24). Although the source of these increased levels of expired H_2O_2 are unclear, other pulmonary conditions involving phagocytic activity, such as pneumonias, are also associated with increased expired H_2O_2 levels. These studies also support the notion that enhanced oxidative activity is involved in the pathogenesis not

only of ARDS but also of brain ischemia, sepsis, and other forms of organ injury.

Xanthine oxidoreductase (XOR), also called xanthine oxidase (XO), is an enzyme that generates $O_2^{\bullet -}$ and H_2O_2 during the conversion of hypoxanthine or xanthine to uric acid. There are substantial findings that XO and hypoxanthine levels are increased in the plasma of patients with ARDS. XO has also been implicated in oxidative injury following ischemia/reperfusion injury (27). XO is localized on the endothelial cell surface where it binds to polysaccharide chains of proteoglycans. In a study of 29 ARDS patients and control subjects using the consensus American–European guidelines for ARDS clinical diagnosis, ARDS nonsurvivors had a 2.5-fold higher level of hypoxanthine in their blood plasma than ARDS survivors and a 26-fold higher level than healthy controls (26). In addition, BALF hypoxanthine levels were 21-fold higher in ARDS patients ($n = 11$) than in normal control subjects. Implications of the enhanced oxidative stress seen in ARDS patients were also provided in studies of protein thiol levels. Briefly, Quinlan et al. (28) found that the plasma protein thiol levels were lower in patients with ARDS who do not survive than in ARDS survivors. These protein oxidations had been previously used as an indirect marker of oxidative damage in experimental animal models and human clinical studies (28). In a recent study of protein modification by tyrosine nitration in the plasma of patients with ARDS, the ferroxidase activity of ceruloplasmin and the elastase inhibitory activity of α_1-PI were reduced by 81% and 44% compared to controls, respectively, whereas α_1-chymotrypsin activity was not reduced (29).

ARDS patients also have increased lipid peroxidation. Eighteen ARDS patients requiring mechanical ventilation and 10 normal, healthy control individuals were evaluated for plasma concentrations of fatty acids and 4-hydroxy-2-noneal (4-HNE), a specific peroxidation product of linoleic acid (30). In nonsurvival patients, there was a 2.6-fold increase in plasma 4-HNE compared to control subjects. In patients with ARDS, 4-HNE increases in plasma were associated with a concomitant decrease in plasma linoleic acid. The potential for generating ROS by surviving and nonsurviving ARDS patients was also assessed, albeit indirectly, by measuring oxygen consumption in the presence of exogenous XO. A significant increase in oxygen consumption generated by plasma of nonsurvivors ($58.8 \pm 6.6\%$ vs. $17.5 \pm 7.2\%$) compared with survivors was reported (30). Isoprostane, a biomarker of lipid peroxidation, is formed nonenzymatically during oxidative damage to cellular lipids. In a study of 22 patients with ARDS or ALI and 10 patients without lung disease, breath condensate levels of isoprostane were 12.4-fold greater in patients with ARDS indicating increased oxidative stress (31).

Iron is present in lung epithelial lining fluids and is postulated to have an important role in oxidative stress. There are no studies measuring "free" iron levels in the BALF or lungs of patients with ARDS. However, and somewhat inexplicably, nonsurvivors of ARDS have increased levels of transferrin compared to survivors of ARDS (32). Serum ferritin was investigated in ARDS-afflicted men and women and healthy controls. It was reported that serum ferritin levels were increased in men and women with ARDS and could be valuable with moderate sensitivity for predicting ARDS (33). Ghio et al. also noted recently that disruption of normal iron metabolism was evident in ARDS patients compared to healthy volunteers (34). They demonstrated that there were increased concentrations of iron present in BALF from ARDS patients compared to control subjects.

In addition to the lipid peroxidation, damage to proteins often occurs in oxidative stress. In one investigation, oxidative damage to proteins in the BALF of patients with ARDS ($n=28$), ventilated patients without ARDS ($n=6$), and normal healthy control individuals ($n=11$) was assessed by measuring orthotyrosine, chlorotyrosine, and nitrotyrosine. All three amino acid residues were elevated in the ARDS group compared to ventilated patients without ARDS and controls (35). Furthermore, chlorotyrosine and nitrotyrosine correlated with BALF MPO levels (35). A report on oxidatively modified carbonyl proteins in BALF of ARDS patients and patients at risk for ARDS also suggests the occurrence of oxidative damage to proteins in ARDS. In clinically established ARDS patients, carbonyl proteins increased 3.8-fold compared to patients at risk for ARDS and 6.3-fold compared to healthy controls (36).

B. Reactive Nitrogen Species

Animal models, in vitro systems, and human clinical studies are beginning to support the view that RNS may also be involved in the pathogenesis of ARDS. An up-regulation of inducible nitric oxide synthase (iNOS) and excessive generation of nitric oxide (NO) occurs in animals with sepsis. Increased levels of NO were found in patients after trauma and during sepsis, and these measurements showed a good correlation with the severity of disease (37). In a similar study, 8 patients with ARDS, 8 patients with ARDS on mechanical ventilation, and 8 controls with normal lung function were evaluated for iNOS and inflammatory cytokines (38). Immunofluorescent staining depicted significant elevations of interleukin-6 (IL-6), IL-8, and iNOS in sepsis induced ARDS. In another study of patients with ARDS, NO-dependent pathways were altered before and after the onset of ARDS (39). In this study, 19 patients with risk for ARDS and 41 patients with ARDS were evaluated by BAL on days 1, 3, 7, 14, and 21 after the

onset of symptoms, and total nitrite and nitrotyrosine were monitored in BALF. Alveolar macrophages from patients with ARDS were positive for iNOS and nitrotyrosine from the onset of disease (39). Total nitrite in BALF was significantly elevated at days 3 and 7 in ARDS patients who did not survive. Two studies reported elevated nitrotyrosine in ARDS patients vs. healthy individuals (35,39). Zhu et al. provided in vivo evidence for the nitration of SP-A protein in edematous fluid of patients with ALI (40). They showed that edematous fluid from patients with ALI had appreciably higher levels of NO with correspondingly increased levels in plasma. Nitration of SP-A protein is speculated to have a role in increased susceptibility of ARDS patients to nosocomial infections (40). Nitration of tyrosine residues in proteins and oxidation of other critical amino acids, such as methionine, cysteine, and tryptophan, may also contribute to the biochemical mechanisms involved in ARDS. Nitration of fibrinogen may be responsible for the increased clot formation in ARDS (29).

C. Deficiency of Antioxidants in ARDS

Enhanced oxidant generation and inadequate antioxidant up-regulation may initiate and impact the progression of ARDS (1,41–47). In a study of 25 patients with ongoing ARDS and 16 healthy controls, Cross et al. measured ascorbate, ubiquinol-10, α-tocopherol, and lipid hydroperoxides in the plasma (42). They reported that plasma antioxidants were decreased in ARDS patients who had higher levels of lipid hydroperoxides compared to controls (42). However, α-tocopherol levels were not decreased in ARDS patients compared to control subjects. In a subsequent study of the BALF from ARDS patients, it was reported that GSH, an important enzyme involved in the neutralization of H_2O_2 and related intermediates, was significantly lower in ARDS patients than controls (21.7 ± 7.8 μmol vs. 91.8 ± 14.5 μmol) (43). Further studies in 8 patients with ARDS compared with 17 healthy volunteers as control subjects confirmed the possibility that antioxidant status may be severely compromised in ARDS patients (44). They measured plasma levels of ascorbate, α-tocopherol, retinol, β-carotene, selenium, lipid peroxidation, catalase, superoxide dismutases, and glutathione peroxidase in patients with ARDS at different stages of disease. They observed that plasma levels of ascorbate, α-tocopherol, β-carotene, and selenium were decreased at the onset of illness, whereas MDA levels were increased throughout illness. These observations suggest that the antioxidant system is severely compromised in ARDS patients (44). Despite the well-documented increased generation of H_2O_2 in patients with ARDS, the H_2O_2-catabolizing enzyme catalase was higher in serum of patients with sepsis and/or ARDS (45). This paradoxical observation does not yet have

a satisfactory explanation. One conclusion may be that a "mismatch" exists with respect to the generation of specific free radical species and the sites of effective antioxidants against these particular species.

D. Role of Cytokines in ARDS

Several experimental animal models of inflammatory disease have demonstrated that a complex network of cytokines and proinflammatory mediators may participate in the initiation and amplification of ARDS development and evolution. Although several proinflammatory cytokines are up-regulated in ARDS, their precise role in the initiation and progression of the condition is not yet well understood. Proinflammatory cytokines may be produced by activated macrophages, epithelial cell, or fibroblasts. Regulation and stimulation of cytokine production depends on several local and extrapulmonary factors. Leukocyte–endothelial adhesion molecules function like a "key and lock" mechanism and modulate chemical mediators, such as chemokines and cytokines, by the surface expression of adhesion molecules (48). Adhesion receptors involved in these reactions are E-selectin present on endothelial cells, P-selectin present on endothelial cells and platelets, and L-selectin residing on leukocytes. Two endothelial adhesion molecules, ICAM-1 (intracellular) and VCAM-1 (vascular), interact with integrins present in leukocytes. P-selectin, which is normally present in intracytoplasmic endothelial granules, is redistributed to cell surface upon stimulation and binds to leukocytes (49). Inflammatory cytokines, such as IL-1 and tumor necrosis factor-α (TNF-α), induce synthesis and expression of endothelial adhesion molecules within a few hours of cytokine release. E-selectin normally is not present in endothelial cells, but it is induced by IL-1 and TNF-α (50). When activated by chemotactic agents, the integrin receptor LFA-1 of neutrophils is converted to a high-affinity state for binding with ICAM-1. It is widely believed that cytokines set the stage for this increased affinity and perhaps activation of phagocytes and other processes.

Several studies have suggested that cytokines, such as IL-1β, TNF-α, and IL-6, have a pivotal role in the pathogenesis of ARDS (51). Siler et al. reported a significant elevation of IL-1 in the BALF of high-risk patients and patients with ARDS compared to normal controls (52). In addition, in immunohistochemical studies alveolar macrophages from ARDS patients have greater amounts of immunoreactive IL-β than alveolar macrophages from patients with severe pneumonia or those from normal controls (53). Similarly, in high-risk patients with ARDS, the BALF contained elevated levels of IL-1 compared to control subjects (52,53). TNF-α was also reported to be increased in the blood of patients with septic shock and those

who are likely to develop septic shock (54). TNF-α was also elevated in the bronchopulmonary secretions, BALF, and serum of patients with ARDS (55–58). In addition, it was noted that a regulatory cytokine, macrophage migration inhibitory factor (MIF), was present in high concentrations in the BALF of patients with ARDS (59). MIF augments proinflammatory cytokines, such as TNF-α and IL-8, and is suggested to have a role in sustaining the pulmonary inflammatory response in ARDS. This cytokine is thought to be involved in the production of proinflammatory cytokines, such as TNF-α, and IL-8. TNF-α was elevated in the plasma of patients with ARDS and in individuals at risk for ARDS (60). Marked increases in the expression of IL-6 and IL-8 in the alveolar macrophages and BALF of patients with ARDS and of patients on long-term ventilatory support suggest the involvement of macrophages and cytokines in lung injury.

Other cytokines, such as IL-2 and IL-15, have also been investigated in an effort to elucidate their potential role in the ARDS process (61). Serum and BALF levels of IL-2 and IL-15 were measured in 8 patients with ARDS and 26 patients at high risk for ARDS who never developed disease. Serum IL-2 and IL-15 levels were higher in the ARDS patients who did not survive (61). On the other hand, BALF IL-15 was higher in patients who never developed disease compared to nonsurvivors.

Based on the rapid procollagen deposition and progressive development of fibrosis in ARDS, it has been proposed that transforming growth factor-β (TGF-β) may play a major role in the stimulation of fibrosis. TGF-β is considered a major regulator of acute lung injury. In animal studies, TGF-β-inducible genes increased within 2 days after acute lung injury. In ARDS patients, TGF-β increased as early as 1 day after initiation of disease (62). Using immunohistochemistry and reverse transcriptase to polymerase chain reaction in 13 ARDS and 7 normal control subjects, Fahy et al. evaluated TGF-β_1 mRNA expression in lung tissue and BALF (63). ARDS cases were positive for protein expression as well as TGF-β levels in BALF, suggesting that activation of this cytokine may be a factor in the rapid fibroproliferative component of the disease.

E. Proteases

Activated neutrophils may promote lung injury by releasing several proteases, such as elastase, collagenase, and cathepsin G. These proteases can degrade several cellular components, including elastin, collagen, basement membrane, fibrin, and cartilage. They can also cleave C3 and C5, producing anaphytotoxins or kinin-like peptides. Neutrophil elastase is a particularly destructive enzyme, and the up-regulated activity of this enzyme may increase permeability and release of proinflammatory cytokines

that participate in acute lung injury (64). Intratracheal instillation of neutrophil elastase in experimental animal models causes hemorrhage and lung injury (1). In ARDS, neutrophil elastase could damage capillary endothelial cells and lead to lung edema. A macrophage peptide that causes the release of neutrophil proteases was elevated in the BALF from ARDS patients compared to controls (65). The potential of neutrophil elastase to induce degradation of pulmonary tissue would likely be held in check by an active α_1-PI in the lung. However, since inactivation of α_1-PI is probably an early event in ARDS, due to the oxidation of methionine residues, this protective mechanism may not be functional.

F. Transcription Factors

Endothelial and epithelial cells injured by activated neutrophils and macrophages may subsequently elaborate ROS/RNS, adhesion molecules, cytokines, chemokines, and several other factors that amplify inflammation and injury. The molecular regulation of these biological responses is most likely modulated through the expression of several genes that are modulated by the activation of transcription factors. In this respect, nuclear factor κB (NF-κB) emerges as a prominent transcription factor known to be sensitive to oxidative stress and cytokines. NF-κB is also critical in the expression of many cytokines, such as TNF-α. Anti-inflammatory cytokines, such as IL-10 and IL-13, can inhibit and regulate NF-κB activation. In experimental animal models, convincing evidence suggests that activation of NF-κB and the resulting production of TNF-α and other cytokines are critically important in the pathogenesis of ALI. However, the relative importance of cross-talk between cytokines and NF-κB in the pathogenesis of ARDS in human disease is not understood. In one report, patients with ARDS had increased activation of NF-κB in their alveolar macrophages compared to patients without the ARDS syndrome (66). The role of NF-κB activation needs more investigation in ARDS.

G. Surfactant

Pulmonary surfactant is composed of phospholipids, neutral lipids, and four different surfactant proteins. Surfactant is critical for the maintenance of low surface tension in the alveoli, which prevents collapse. Surfactant proteins may have other properties as well. In contrast to the surfactant deficiency in newborn infants with respiratory distress syndrome, surfactant deficiency in ARDS may be of secondary importance in the etiology of disease. However, several animal experimental and human studies have demonstrated that alterations of the surfactant system caused by oxidative inactivation and/or edema-related surfactant dilution may contribute to the lung injury

associated with ARDS. ROS and peroxynitrite can impair the surface tension–lowering properties of surfactant (67). Likewise, MPO released by the activated neutrophils during inflammation may facilitate the formation of nitrated surfactant proteins producing toxic peroxynitrite (68). This has led to several studies using exogenous surfactant as a therapy for ARDS. Unfortunately, aerosolized surfactant administration had no beneficial effect on survival or the effectiveness of other treatment modalities in sepsis-induced ARDS (69). Lewis and Brackenbury (70) and Lewis and Veldhuizen (71) recently reviewed all of the published reports on exogenous surfactant treatment and concluded that administration of surfactant in ARDS has shown only an inconsistent therapeutic value. These unimpressive therapeutic responses were attributed to clinical variability in the surfactant mixture and a number of other factors, such as the nature of injury, time of treatment, surfactant preparation, dose, delivery, and mode of ventilation.

IV. Antioxidant Therapy

Oxidants released by highly reactive neutrophils, macrophages, and other sources seemingly play a major role in the vascular endothelial injury and the subsequent alveolar epithelial damage that characterizes ARDS. Direct evidence for increased pulmonary oxidative stress comes from several studies that show the increased generation of oxidants, inactivation of α_1-PI, increased lipid peroxidation, inactivation of surfactant proteins, activation of proinflammatory cytokines, and antioxidant imbalances in plasma and BALF. These studies indicate that antioxidant therapy which targets and prevents oxidant injury may be valuable in arresting the progress of ARDS and, hopefully, decreasing morbidity and mortality. Several studies attempted to use *N*-acetylcysteine (NAC) as a therapeutic modality in ARDS (72). NAC is a precursor of glutathione, which has an ability to detoxify oxidants and nullify their damaging effects. In a prospective, randomized, placebo-controlled study of 66 intensive care patients with ARDS, NAC therapy did not improve the outcome nor did it change the Pao_2/Fio_2 ratio in the study group compared to controls. Moreover, in NAC-treated patients, pulmonary complaints were higher compared to controls (73). In another study, 61 adult patients presenting mild to moderate ALI and various predisposing factors for ARDS were administered 40 mg/kg/day NAC intravenously for 3 days in a randomized, double-blind, placebo-controlled study (74). It was reported that the NAC-treated group exhibited improved systemic oxygenation and a reduced need for ventilatory support. However, development of ARDS and mortality were not diminished by the NAC therapy. A clinical trial of the antioxidants,

NAC and procysteine in ARDS patients was conducted in five centers in the United States and Canada as a prospective, randomized, double-blind, placebo-controlled study (75). NAC or procysteine was administered through intravenous solution in dextrose. Both of the antioxidants corrected the glutathione deficiency of patients with ARDS. However, progression of ARDS and morbidity and mortality was not changed by these interventions (75). Other recent studies have attempted to test the antioxidant treatment hypothesis. In one, the effect of enteral feeding with eicosapentaenoic acid, γ-linolenic acid, and antioxidants in 146 patients with ARDS was done prospectively in a randomized, double-blind, controlled trial in five centers in the United States (76). Patients were tube fed for 4–7 days with eicosapentaenoic acid, γ-linolenic acid, and antioxidants, and various clinical outcomes and lavage measurements of ARDS indices were made. Beneficial effects of treatments were observed and indicated by improvements in neutrophil recruitment, gas exchange, the requirement of mechanical ventilation, and a reduction in organ failures. In a more recent study, the value of early, prophylactic antioxidant supplementation with α-tocopherol and ascorbic acid in reducing the rate of pulmonary morbidity and organ dysfunction in 595 critically ill surgical patients was investigated (77). The relative risk of pulmonary morbidity was 0.81 in patients receiving supplements; the multiple organ failure risk was 0.43 with a 95% confidence level. These studies begin to show that early administration of antioxidant supplementation has the potential of reducing organ failure and the length of intensive care unit stay of critically ill patients. The key seems to be in identifying and treating at-risk patients early enough and being able to give the best antioxidant or best combination of antioxidants to each individual.

V. Conclusions

ARDS is obviously a complicated disease with a high mortality rate. Although substantial progress has been made in the understanding of the pathophysiology of ARDS, very little attention has been paid to deciphering the fundamental molecular events that trigger the syndrome and its complications. Some of these triggering events are produced and regulated by the balance between oxidants and antioxidants. Because early events initiating the disease are difficult to investigate in humans, experimental models producing appropriate "ARDS-like" oxidant–antioxidant imbalances, complement and cytokine activation, up-regulation of adhesion molecules, recruitment and activation of neutrophils and macrophages, proteases, cytokines, and other recognized mediators of ARDS inflammation and injury will be valuable. In the therapeutic arena, numerous

therapeutic regimes targeting the molecular pathways and consequences of oxidant–antioxidant imbalances are needed. The roles of oxidant and antioxidant balance in signaling processes and the effect of genetic and environmental predispositions that affect the oxidant and antioxidant balance are the frontiers in the investigation of ARDS (79). They probably hold new answers to the questions that still confront us about the basic mechanisms responsible for ARDS as well as the persistent clinical puzzles related to why some at-risk individuals develop ARDS while other comparably at-risk patients do not. The parallel question of why some ARDS patients succumb while other seemingly comparable patients do not is also an unsolved mystery at this time.

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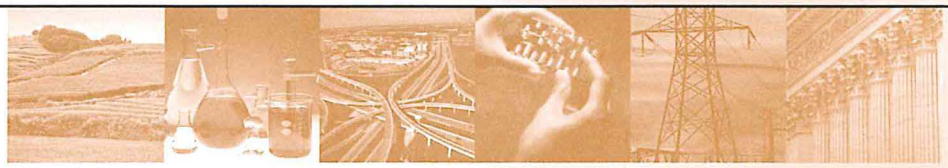

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