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Forum Review

Roles of Oxidants and Redox Signaling in the Pathogenesis of Acute Respiratory Distress Syndrome

SADATOMO TASAKA,[1] FUMIMASA AMAYA,[2] SATORU HASHIMOTO,[2] and AKITOSHI ISHIZAKA[1]

ABSTRACT

The acute respiratory distress syndrome (ARDS) is a disease process that is characterized by diffuse inflammation in the lung parenchyma and resultant permeability edema. The involvement of inflammatory mediators in ARDS has been the subject of intense investigation, and oxidant-mediated tissue injury is likely to be important in the pathogenesis of ARDS. In response to various inflammatory stimuli, lung endothelial cells, alveolar cells, and airway epithelial cells, as well as alveolar macrophages, produce reactive oxygen species (ROS) and reactive nitrogen species (RNS). In addition, the therapeutic administration of oxygen can enhance the production of these toxic species. As the antioxidant defense system, various enzymes and low-molecular weight scavengers are present in the lung tissue and epithelial lining fluid. In addition to their contribution to tissue damage, ROS and RNS serve as signaling molecules for the evolution and perpetuation of the inflammatory process, which involves genetic regulation. The pattern of gene expression mediated by oxidant-sensitive transcription factors is a crucial component of the machinery that determines cellular responses to oxidative stress. This review summarizes the recent progress concerning how redox status can be modulated and how it regulates gene transcription during the development of ARDS, as well as the therapeutic implications. *Antioxid. Redox Signal.* 10, 739–753.

INTRODUCTION

The acute respiratory distress syndrome (ARDS) is a process of acute inflammatory lung injury resulting from a variety of predisposing conditions including severe pneumonia, sepsis, massive transfusion, and trauma (6, 114). Since first being reported by Ashbaugh as a clinical syndrome in 1967 (6), the definition of ARDS has been modified and become more clinical (14, 75). The current definition and clinical criteria for ARDS were announced at the American–European Consensus Conference on ARDS in 1994 (14). In the report, ARDS was defined as a severe form of acute lung injury (ALI) and a syndrome of acute pulmonary inflammation. ALI/ARDS is characterized by sudden onset, impaired gas exchange, decreased static compliance, and by a nonhydrostatic pulmonary edema (14). The hallmark in the pathophysiology of the acute event is an increase in pulmonary capillary permeability (114). It was also stated that ARDS is characterized by a constellation of clinical, radiological, and physiological abnormalities that cannot be explained by but may coexist with left atrial or pulmonary capillary hypertension (14). Although the mechanisms of the disorder have not yet been fully elucidated, there is evidence for the implication of oxidative damage by reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are increasingly regarded as key substances modulating the pulmonary vascular endothelial damage that characterizes ARDS (9, 63, 65, 104). The resulting endothelial dysfunction and disruption is responsible for the principal clinical manifestations of the syndrome. This hypothesis is supported by several findings: hydrogen peroxide (H_2O_2) was detected in the exhaled breath of ARDS patients (9), and myeloperoxidase and oxidized α_1-antitrypsin have been found in bronchoalveolar lavage (BAL) fluid (65). Moreover, increased plasma lipid peroxidation has previously been reported in critically ill patients (104)

[1]Division of Pulmonary Medicine, Keio University School of Medicine, Tokyo, Japan.
[2]Department of Intensive Care and Anesthesiology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

and in patients with sepsis and at risk of developing ARDS (63). In this review, we discuss the pathophysiology of ARDS, the biological origins of ROS, the mechanisms of their damaging effects, the oxidant–antioxidant balance, and the roles of redox signaling system during ALI/ARDS, as well as possible therapeutic perspectives.

PATHOPHYSIOLOGY OF ARDS

ALI/ARDS can occur associated with various clinical disorders that can be divided into those associated with direct injury to the lung and those causing indirect injury in the setting of a systemic process (114). The former disorders include pneumonia and aspiration of gastric contents, and sepsis is associated with the highest risk of indirect injury (101). Higher mortality and ventilatory requirements were shown in ARDS after direct injury, which is predominantly characterized by pulmonary consolidation. In contrast, interstitial edema and alveolar collapse are major clinical manifestations of ARDS subsequent to indirect injury (101).

Like many pathophysiologic states of the lung, ARDS is characterized by the upregulation of inflammatory systems and involves enzyme systems to assist in the eradication of local in-

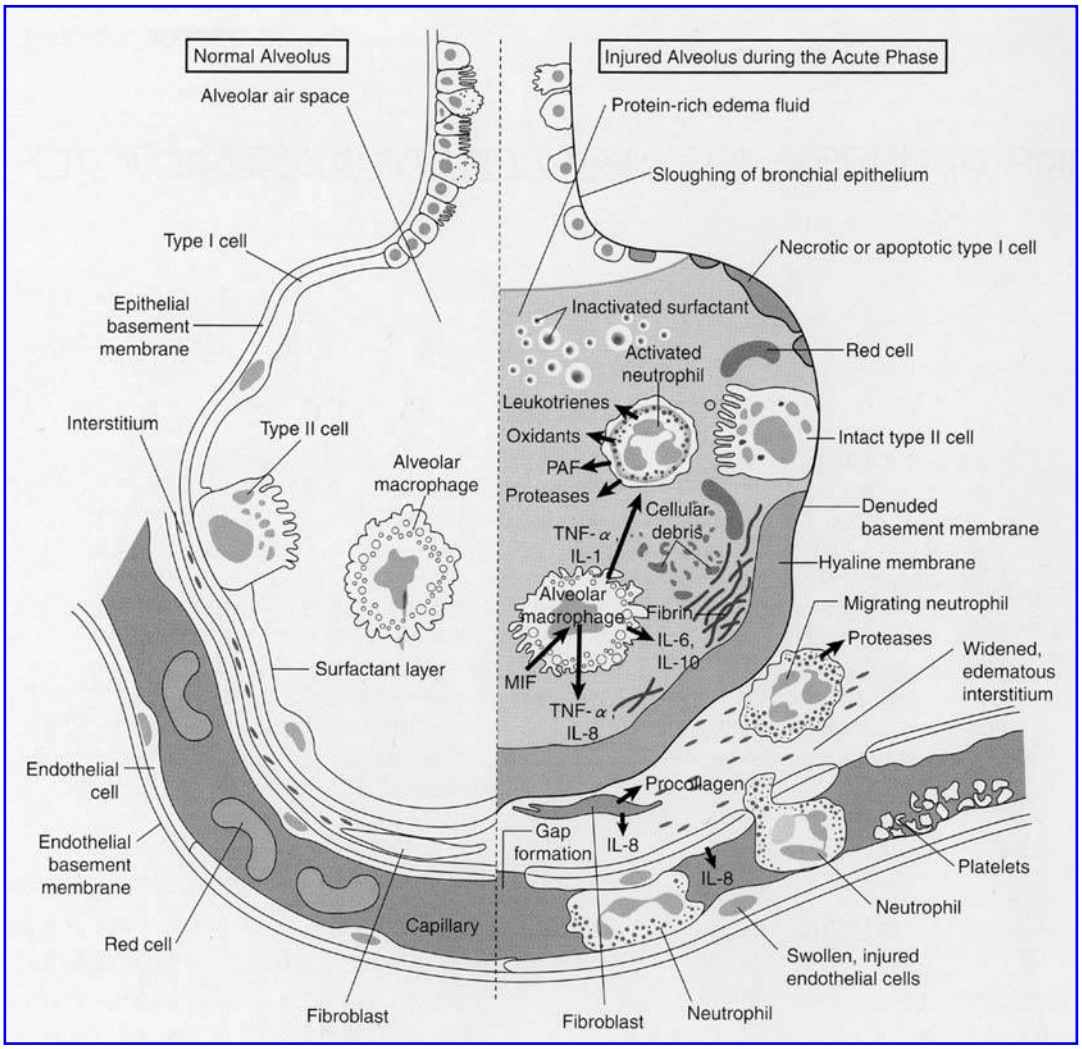

FIG. 1. The normal alveolus (*left-hand side*) and the injured alveolus in the acute phase of ALI/ARDS (*right-hand side*). In the acute phase of the syndrome (*right-hand side*), there is sloughing of both the bronchial and alveolar epithelial cells, with the formation of protein-rich hyaline membranes on the denuded basement membrane. Neutrophils are shown adhering to the injured capillary endothelium and marginating through the interstitium into the air space, which is filled with protein-rich edema fluid. In the air space, an alveolar macrophage is secreting cytokines, which act locally to stimulate chemotaxis and activate neutrophils. IL-1 can also stimulate the production of extracellular matrix by fibroblasts. Neutrophils can release oxidants, proteases, and other proinflammatory molecules. A number of anti-inflammatory mediators are also present in the alveolar milieu, including IL-1-receptor antagonist, soluble TNF receptor, and anti-inflammatory cytokines such as IL-10. The influx of protein-rich edema fluid into the alveolus has led to the inactivation of surfactant. From (114) with permission.

flammatory processes or to promote injury repair mechanisms (114). The failure to control inflammation locally can lead to the loss of control of these responses, with the end result being systemic inflammation, organ dysfunction progressing to organ failure, and, in the most extreme circumstances, death. Indeed, although the severity of hypoxemia has some influence on outcome, it is not in itself an adverse prognostic sign and the majority of patients with ARDS die a nonrespiratory death, principally from multiple organ failure (MOF) (114). It therefore seems likely that ARDS represents only the pulmonary manifestation of a panendothelial insult, which may result in interstitial edema formation in most organ systems, leading to impaired tissue oxygenation, partly through a direct effect on diffusion, but also through adverse effects on microvascular control within the regional microcirculation.

Early studies examining the characteristics of pulmonary edema fluid, and more recent isotopic studies, have suggested that increased alveolar capillary permeability is ubiquitous in ARDS and lesser degrees in ALI (21, 97). Increased pulmonary microvascular permeability, which is important to the formation of pulmonary edema, is a reflection of damage to the pulmonary endothelium (Fig. 1). Histologic studies of lung specimens obtained early in the course of ALI/ARDS show a marked accumulation of neutrophils (7). Neutrophils predominate in the pulmonary edema fluid and BAL fluid obtained from affected patients (82). In addition, experimental lung injury is known to be neutrophil dependent (99). Although ALI/ARDS has been described in neutropenic patients, activation and transmigration of circulating neutrophils are thought to be critical steps in the development of ALI/ARDS (Fig. 1) (28, 114).

In the systemic microcirculation, neutrophil recruitment from the blood stream into tissue at sites of inflammation usually occurs in postcapillary venules and requires capture, rolling, and firm adhesion on activated endothelial cells, which is mediated with various adhesion molecules (Fig. 2) (28, 48). Selectin, integrin, and immunoglobulin superfamilies of adhesion molecules, cytokines, chemokines, and other chemoattractants participate in this sequential process in a variety of vascular beds. In contrast, the principal site of leukocyte migration in the lung is the capillary bed (48). In pulmonary circulation, the transit time of neutrophils depends upon their deformability to travel through the highly anastomosing network of capillary segments (48). Inflammatory mediators, including interleukin-8 (IL-8), complement 5a (C5a), platelet-activating factor (PAF), leukotriene B_4 (LTB_4), and tumor necrosis factor-α (TNF-α), cause neutrophils to become less deformable and trapped in capillaries (48). Neutrophils and other mononuclear cells are activated through the interaction with various adhesion molecules to fully exert the inflammatory effect (Fig. 2). The expression of the molecules allows for the adhesion and extravasation (emigration) of the neutrophils that may induce local injury and participate in the orchestration of systemic inflammation and all of its consequences.

PRODUCTION OF ROS DURING ARDS

The presence of various altered proteins in the BAL fluid of patients with ARDS supports the production of large amounts of ROS that may alter protein function, especially in the surfactant system (59). In addition, substances indicating a relationship between the neutrophil influx into the lungs and the formation of ROS/oxidative stress, such as xanthine oxidase (XOD) and nicotinamide adenine dinucleotide phosphate, have been observed (86, 92).

There exist several potential causes of intracellular and extracellular oxidant stress in ARDS patients: the high inspiratory concentrations of oxygen required to achieve adequate arterial oxygenation, infection or extrapulmonary inflammation can all promote ROS accumulation and the consumption of antioxidative factors (34, 77). In addition, there are some drugs with prooxidative properties, although they are rarely used in patients with ARDS. Prooxidant drugs include anticancer drugs, such as anthracyclines, bleomycin, and platinum derivatives, 5-aminosalicylic acid for ulcerative colitis, and nitrofurantoin for urinary tract infections (90). Drug-induced oxidant lung injury is thought to be caused by the generation of superoxide, leading to the generation of hydroxyl radical ($OH^{\bullet}$) during metabolism of the drug (47).

Nitric oxide (NO) plays a multifaceted role in mediating inflammatory processes (11). Potential sources of NO in the lungs

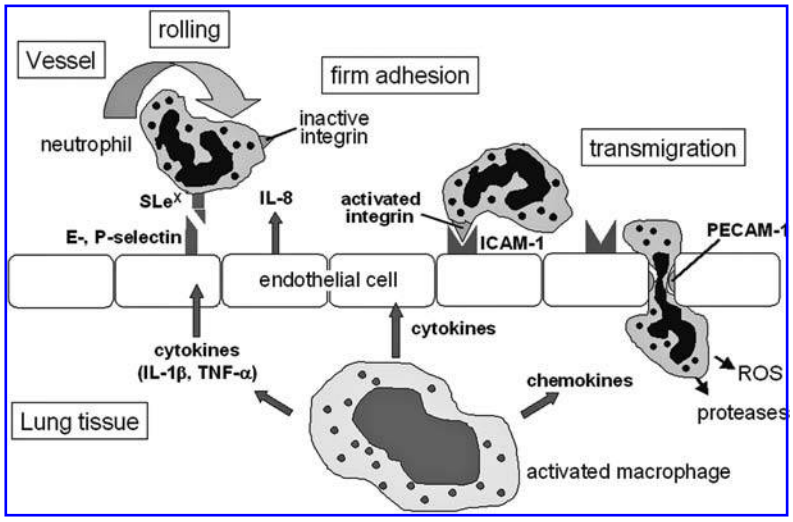

FIG. 2. Mechanism of the migration of leukocytes out of the pulmonary vascular space. In pulmonary capillary or venules, selectin-dependent rolling can occur. In response to inflammatory stimuli, neutrophils adhere to the capillary endothelium mainly by β_2 integrin/ICAM-1 interactions. Chemokines produced by alveolar macrophages and type II pneumocytes attract neutrophils to migrate through the endothelium, interstitial space, and epithelium to reach the alveolar space.

include activated alveolar macrophages, neutrophils, alveolar type II cells, endothelial cells, and airway cells. NO biosynthesis is enhanced during pulmonary inflammation via inducible NO synthase (iNOS or NOS2) induction. Alveolar macrophages isolated from the lungs of patients with ARDS following sepsis have been shown to express iNOS (53). Increased amounts of NO may be released during lung inflammation into the epithelial lining fluid, where it may have both beneficial (*i.e.*, antimicrobial) and detrimental (*i.e.*, tissue-damaging) effects.

α_1-Proteinase inhibitor (α_1-PI), an elastase inhibitor that is commonly found in BAL fluid, is inactivated during ARDS, whereas plasma α_1-PI retains >90% activity (60). This observation, coupled with the ability to restore the activity of α_1-PI with the reducing agent dithiothreitol, implicated the lung as the source of oxidation. The levels of H_2O_2 in expired breath condensates (EBC) were significantly greater in patients with acute hypoxemic respiratory failure and focal pulmonary infiltrates than in those without pulmonary infiltrates, indirectly implicating increased oxidation (9). Interestingly, H_2O_2 concentrations were greatest in patients with head injuries and sepsis, regardless of whether pulmonary infiltrates were present. This finding suggests the participation of oxidants in sepsis and other forms of vital organ injury, such as trauma to the brain.

Production of ROS by inflammatory cells

During the development of ALI/ARDS, a majority of oxidants are generated as a result of the inflammatory response by phagocytic cells, such as mononuclear cells. When phagocytes are exposed to appropriate stimuli, they form large quantities of superoxide ($O^{\bullet}{}_2^-$), an important precursor of other more reactive species (Fig. 3). Stimuli involved in the activation of the phagocytes include bacteria, cytokines such as TNF-α, platelet activating factor (PAF), and endotoxin (lipopolysaccharide; LPS). H_2O_2 is produced by adherent granulocytes when intact rat lung is treated with LPS (72). ROS production by neutrophils can be enhanced by prior exposure to inflammatory stimuli (12). Activation of neutrophils with the release of ROS in endothelial cell monolayers, isolated perfused lung, and in whole animals, causes increased pulmonary vascular permeability (44, 96).

In the presence of molecular oxygen and nicotinamide adenine dinucleotide phosphate oxidase, $O^{\bullet}{}_2^-$ is produced via a one-electron reduction, with nicotinamide adenine dinucleotide phosphate donating the electron (Fig. 3). The majority of $O^{\bullet}{}_2^-$ will be dismutated by superoxide dismutase (SOD) to form H_2O_2, which is central for bacterial eradication (34).

High levels of xanthine dehydrogenase (XDH), an enzyme involved in the production of $O^{\bullet}{}_2^-$ and H_2O_2, have been revealed in the lung vascular and epithelial cells after experimental lung injury (92). XDH can be readily converted to XOD by reversible sulfhydryl oxidation or by irreversible proteolytic modification. XOD uses molecular oxygen as its electron carrier and produces reactive oxygen intermediates, either $O^{\bullet}{}_2^-$or H_2O_2 (Fig. 3).

Peroxynitrite ($ONOO^-$), formed by the reaction of NO with superoxide radical in a reaction whose rate is nearly diffusion limited, causes both nitrosation and nitration of several amino acid residues within proteins including tyrosine (23). Since such reactions significantly alter protein function and may lead to tissue damage, peroxynitrite plays an important role in the pathogenesis of ARDS (23). Markers of peroxynitrite, which is formed on reaction of superoxide with nitric oxide, formation are present in the lungs of patients who died of ARDS (92).

ROS derived from oxygen therapy

The production of ROS can be greatly increased by the therapeutic administration of oxygen (thus supplying extra oxygen for oxidation/production of ROS) (23). Hyperoxic lung toxicity results when production of ROS exceeds the antioxidant capacity of the cells. The metabolism of oxygen by lung cells increases as the arterial pressure of oxygen increases. Therapeutic use of high O_2 concentrations in ARDS patients may be implicated in exacerbating the primary injury (23).

ROLES OF ROS AND RNS IN THE DEVELOPMENT OF ALI/ARDS

Oxidants that are generated in excess of antioxidant defenses or that are lacking in antioxidant defenses can result in severe pulmonary inflammation leading to ALI/ARDS, as has been previously defined (23, 114). A number of reports suggest that increased ROS production combined with decreased antioxidant capacity of pulmonary vascular tissue contributes to the pathogenesis of ARDS through cellular damage and loss of vasomotor tone control (59, 67, 69). There is evidence to support the presence of severe oxidative stress with ROS mediation of

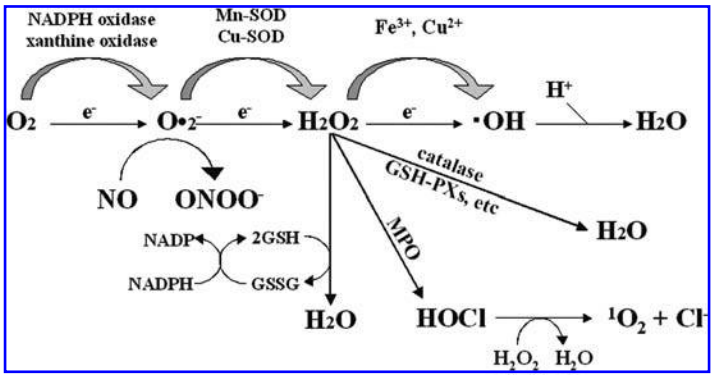

FIG. 3. The derivation of reactive oxygen species. Cu^{2+}, copper; e^-, unpaired electron; Fe^{2+}, iron; GSH,: glutathione; GSH-PX, glutathione peroxidase; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; MPO, myeloperoxidase; NO, nitric oxide; $O^{\bullet}{}_2^-$, superoxide anion; $^1O_2$, singlet oxygen; $^{\bullet}OH$, hydroxyl radical; $ONOO^-$, peroxynitrite; SOD, superoxide dismutase.

endothelial injury (23, 69) and diaphragmatic dysfunction (76) in patients with ARDS (Fig. 4). In addition, the expression of adhesion molecules and the generation of cytokines can be mediated by ROS (67).

Each human organ and each human cell is influenced by oxidative stress, which is separated into internal conditions (inflammation, autoimmune reactions, dysregulation of metabolism, ischemia) and external conditions (microbiological organism, electromagnetic radiation, mechanical stress, thermal-induced stress, and chemical-induced stress) (5). Damage to the alveolar surfactant system and the ability of the cells to actively transport sodium across the epithelium can occur from ROS. This is important in that both the surfactant system and active sodium transport contribute to alveolar fluid (and thus, edema) removal (67). As mentioned earlier, sepsis with endotoxic shock is often a precipitant of ARDS and may produce respiratory failure through oxidative mechanisms, namely lipid peroxidation of cellular membranes (47).

Oxidants almost certainly contribute to the vascular damage seen in sepsis, or after the ischemia/reperfusion inherent in the processes underlying ARDS, surgery necessitating cardiopulmonary bypass and lung transplantation procedures (23).

Lipid peroxidation, which produces many other highly cytotoxic byproducts, is thought to be an early event in ARDS and results in the destruction of membrane integrity, leading to a nonselective increase in membrane permeability (Fig. 4) (23). ARDS patients have increased levels of lipid peroxidation products in their plasma, which is evidence of increased lipid peroxidation during the disease development (23, 69, 67, 69).

There is substantial evidence indicating that ROS and RNS may be involved in pulmonary epithelial injury in a variety of pathologic situations, including ALI/ARDS. Intratracheal instillation of N(G)-monomethyl-L-arginine (L-NMMA), an NO synthase (NOS) inhibitor, ameliorates NO production and alveolar epithelial injury (36). Similarly, paraquat-induced diffuse alveolar damage (11) and ischemia/reperfusion-induced lung injury (50) both are associated with the stimulation of NO synthesis and are abrogated by NOS inhibitors. Likewise, influenza virus-induced lung injury results from the increased expression of iNOS and the increased generation of NO (4). The administration of NOS inhibitors significantly improves the survival of influenza-infected animals. Additional evidence that RNS play a role in pulmonary inflammation is derived from studies utilizing transgenic *Nos2*$^{-/-}$ mice. Lung damage induced by LPS administration (56) or hemorrhage and resuscitation (103) is markedly reduced in these mutant mice. Similarly, in an experimental murine model of allergic airway disease, deletion of the *Nos2* gene results in a significant decrease in eosinophil infiltration into the lungs (115).

REDOX IMBALANCE IN ARDS

The antioxidant defense system is a complex system that includes reduction–oxidation (redox) enzymatic systems, nonenzymatic scavengers, and dietary components (Table 1) (23). Oxidative stress occurs when there is a marked imbalance between the production and removal of ROS and RNS. This imbalance arises when antioxidant defenses are depleted or when free radicals are overproduced. The measurements of antioxidant concentrations and/or oxidant–antioxidant balance in patients with ARDS have revealed redox imbalance in patients with ARDS, which has two major implications (23, 69). First, production of toxic levels of ROS, RNS, and reactive iron species (RIS) leads to molecular damage of key molecules in cells. Second, subtoxic increases in ROS, RIS, and RNS can signal changes in cellular responses such as proliferation, apoptosis, and necrosis (23, 69).

Primary antioxidants

Compared with healthy subjects, patients with ARDS show decreased levels of plasma iron-binding antioxidant activity,

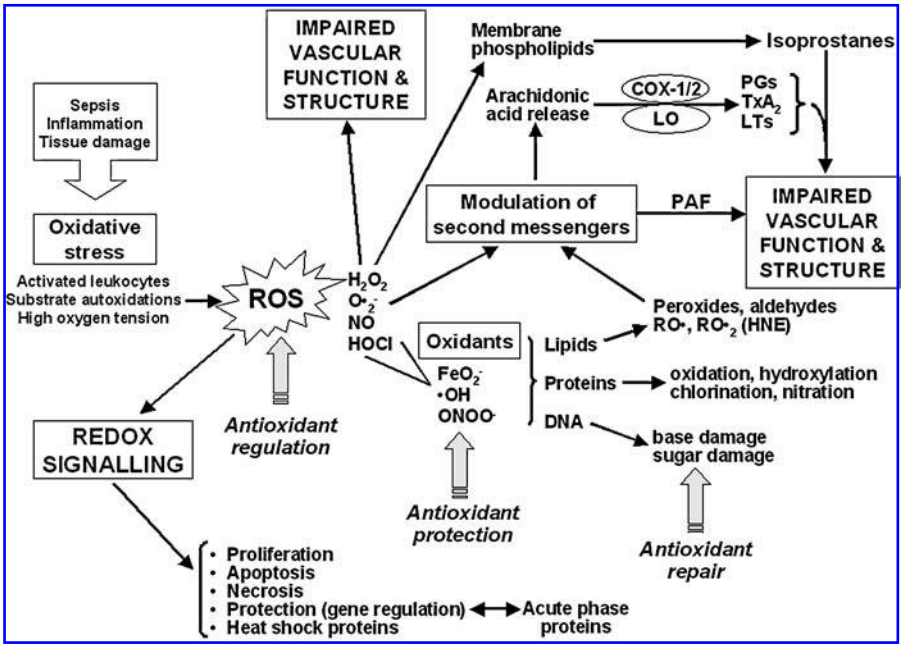

FIG. 4. The possible role of reactive oxygen species (ROS) in modulating the effects of inflammation on vascular structure and function. COX, cyclo-oxygenase; FeO_2^+, iron oxide; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; LO, lipoxigenase; LTs, leukotrienes; $O^{\bullet}{}_2^-$, superoxide anion; NO, nitric oxide; $^{\bullet}OH$, hydroxyl radical; $ONOO^-$, peroxynitrite; PAF, platelet activating factor; PGs, prostaglandins; $RO^{\bullet}$, alkoxyl; $RO^{\bullet}{}_2$ (HNE), peroxyl (4-hydroxy-2-nonenol); TxA_2, thromboxane A_2.

Table 1. List of Antioxidants

Dietary	
Albumin	β-Carotene
Glucose	Lactoferrin
Vitamin C (ascorbate)	Vitamin E (α-tocopherol)
Enzymatic	
Catalase	Glutathione peroxidase
Superoxide dismutases	
Nonenzymatic	
Apotransferrin	Bilirubin
Caeruloplasmin	Haptoglobin
Hemopexin	Red blood cell anion channels
Selenium	Transferrin
Uric acid	

and therefore a decreased ability to prevent iron-dependent ROS formation (39). In plasma of ARDS patients, iron-binding proteins, such as transferrin, are completely saturated, which leaves free iron available for reactions producing ROS (38). In the mice treated with bleomycin, the intentional depletion of iron, either by diet change or iron chelator administration, can reduce the risk of lung injury (90). A small group of patients with ARDS, showing impaired liver function as part of MOF, had low molecular mass iron present in their plasma, and a high iron saturation of their transferrin. In such cases, antioxidant protection is greatly decreased, or even lost (52).

The primary plasma antioxidant, caeruloplasmin, is an acute phase protein, and, as such, concentrations increase in the plasma after tissue trauma. In animal experiments, plasma caeruloplasmin increases after exposure to hyperoxia by a mechanism independent of the acute phase response (73). There have been two reports describing plasma caeruloplasmin levels in patients with ARDS, although the results were different. One study revealed normal levels of caeruloplasmin in plasma, which is surprising in view of the known response of the protein to trauma and hyperoxia (55). In contrast, the other report showed raised level of caeruloplasmin compared to healthy controls, although the iron-oxidizing (ferroxidase) antioxidant activity of plasma from ARDS patients was similar to that of control plasma, in spite of the measured higher caeruloplasmin protein levels present (39). The reason for this discrepancy remains unclear, but may suggest that some caeruloplasmin is not fully functional as a ferroxidase. Since caeruloplasmin is susceptible to proteolytic damage, increased proteolytic activity in plasma or lung tissue may contribute to the apparent loss of activity in patients with ARDS (33).

Secondary antioxidants

Numerous nonspecific low molecular mass molecules present in plasma have been ascribed biological antioxidant properties. These include the nutritive antioxidants ascorbate (vitamin C), α-tocopherol (vitamin E), β-carotene (provitamin A), and retinol (vitamin A), as well as thiols (34). In addition, plasma also contains low concentrations of specific high molecular mass scavengers, such as the antioxidative enzyme systems catalase, SOD, and glutathione peroxidase (GSH-PX), for which selenium acts as a cofactor.

In patients with ARDS, plasma levels of ascorbate, a major plasma antioxidant, are extremely low, ranging from <1 to 5 μmol/L, compared with 49 ± 1 μmol/L in healthy subjects (25). This finding is interpreted as evidence of increased oxidative stress, and suggests that the low plasma ascorbate levels reflect increased neutrophil activity. It was also shown that, after plasma from a healthy donor was incubated with activated neutrophils, rapid oxidation of ascorbate was observed (25). In addition, ubiquinol, a key lipid-soluble antioxidant residing in the membranes of the mitochondria, was significantly decreased in patients with ARDS as well (25).

α-Tocopherol is carried in plasma by lipoproteins, for example, in LDL there are about six molecules of α-tocopherol for each LDL particle. α-Tocopherol is a fat-soluble antioxidant that offers little protection to the surrounding aqueous milieu (98). Plasma α-tocopherol levels are not significantly lower in patients with ARDS when standardized to the total plasma cholesterol present (25).

Another group of important antioxidants are thiols, such as glutathione (GSH). Plasma from healthy individuals contains about 500 μmol/L of thiols mainly associated with the sulphydryl groups of plasma proteins. Plasma thiol values are, however, as low as $\sim$300 nmol/L in patients with ARDS, and even lower than this when calculated for nonsurviving patients (84). Interestingly, nonsurvivors have higher levels of plasma proteins compared with the survivors. The lung is a primary target for oxidant injury, and damage to lung tissue leads to a loss of sulphydryl groups of both protein and nonprotein molecules (81). Thiol groups are particularly susceptible to oxidation and can be destroyed by biological oxidants such as H_2O_2, $ONOO^-$, iron salts, and peroxyl and hydroxyl radicals.

GSH is the most abundant nonprotein thiol, especially for reducing H_2O_2 and HOCl, which are produced by activated neutrophils. Lung epithelial lining fluid contains high concentrations of GSH compared with plasma from the same individual. Most of the GSH (96%) is in the reduced form, and at levels some 140-fold higher than those of plasma. Normal human plasma GSH values reported in the literature are variable, probably due to the labile nature of the molecule and the nonspecific methods used to measure it. Patients with ARDS have depleted levels of GSH in plasma and red blood cells (16). The levels of GSH in BAL fluid and epithelial lining fluid were also found to be decreased in patients with ARDS, when compared to samples from healthy control subjects (80).

The levels of H_2O_2 are increased in EBC of patients with ARDS undergoing mechanical ventilation, whereas only low or undetectable amounts of H_2O_2 are found in their plasma (9). When H_2O_2-destroying enzymes, such as catalase and GSH-PX, were measured in the serum of patients with ARDS and control subjects, the former group had higher catalase activity, which may explain the undetectable levels of H_2O_2 in plasma (62). Interestingly, there was no evidence to support red blood cell hemolysis as the origin of the increased serum catalase. The same group of investigators assessed the serum levels of manganese-containing SOD (Mn-SOD) and other protein markers and found that of 26 patients with sepsis, six who developed ARDS had higher serum levels of Mn-SOD and catalase ac-

tivity (63). The elevated levels of catalase and Mn-SOD may indicate the body's efforts to neutralize ROS (23, 67, 69). Of interest was that GSH-PX activity was unchanged when compared to control subjects, septic patients without ARDS, and septic patients with ARDS. Endothelial injury, as measured by ^{51}Cr release, was greatest in the control group and was the least in patients with sepsis and ARDS (62). Additional studies have confirmed that, in the pro-oxidant pulmonary milieu observed in patients with sepsis and lung injury, antioxidant responses are significantly elevated when compared to those of control patients (63). The antioxidant properties of the drug *N*-acetylcysteine (NAC), together with the observed depletion of thiols in patients with ARDS, have led to clinical trials designed to replace or protect vital thiol groups using this drug (106). However, to date, these have not demonstrated that thiol supplementation is of great benefit to patients with ARDS (17, 30, 102).

Pro-oxidant changes

Patients with ARDS show elevated levels of iron saturation of transferrin, indicating the presence of RIS in their plasma (38). Unlike plasma from normal healthy controls, however, BAL fluids from the same controls contain RIS. This may in part explain why the lungs are so sensitive to oxidative damage from hypoxia, hyperoxia, inhaled oxidants, and 'activated' phagocytic cells. Patients who survive ARDS have more chelatable RIS in their lavage than controls, but patients who do not have none (37). This latter finding is compatible with increased alveolar capillary permeability, leading to the transfer of plasma proteins to the lung, which induces the interaction between transferrin and the chelatable iron. Since survival appears to depend on the severity of lung leak, it may not be true that the leak of plasma antioxidant proteins into the lung is beneficial in ARDS (80). Polyunsaturated fatty acids (PUFAs) are highly susceptible to oxidation mediated by free radicals, and many of the peroxidic and aldehydic products formed have profound pharmacological effects on cells. In an intensive care stay, patients with ARDS exhibited a decrease in their plasma concentration (%) of total linoleic acid, but an increase in their concentrations of oleic and palmitoleic acids (85). Such changes are highly characteristic of essential fatty acid deficiency disease and may be common to many conditions characterized by oxidative stress, whereby ROS act as signal molecules to alter desaturase enzymes (40). If such changes are an adaptive response, it is difficult to understand why levels of oleic acid are so increased in ARDS when this mono-unsaturated fatty acid is used pharmacologically in animal models to induce ARDS.

XDH is a widely distributed enzyme, which, upon oxidation of its thiol groups or following limited proteolysis, is converted to the oxidase (XOD) form. The oxidase enzyme utilizes O_2 as a co-factor in the oxidation of hypoxanthine and xanthine, forming the products $O^{\bullet}{}_2^-$, H_2O_2, and uric acid. Increased levels of XOD or its substrates, therefore, increase the formation of ROS. Increased levels of XOD, xanthine, and hypoxanthine have been reported in the plasma of patients with ARDS (86). Plasma hypoxanthine levels in nonsurviving patients were significantly greater than those in survivors, although both were markedly increased compared to normal healthy controls (86).

Heme oxygenase-1 and oxidative stress

Heme oxygenase (HO)-1, a ubiquitous heme-degrading enzyme, has generated much interest as a novel stress protein that is highly induced by and protects against oxidative stress. HO catalyzes the initial and rate-limiting step in the oxidative degradation of heme to biliverdin with the release of the catalytic byproducts carbon monoxide (CO) and iron (107). HO exists in three isoforms: whereas HO-2 and HO-3 are primarily constitutive, HO-1 is highly inducible (68, 71). HO-1 induction in models of oxidative stress has been shown to protect against noxious stimuli, including hyperoxia and LPS (61). The increased susceptibility of HO-1 knockout mice to oxidative stress (83) and a similar pattern in the one case of human HO-1 deficiency (116) have been reported. Reporter gene analyses revealed an increase in HO-1 gene expression after anoxia–reoxygenation in pulmonary endothelial cells, which is transcriptionally regulated and dependent upon the HO-1 promoter and a 5′-distal enhancer region, E1 (119). These findings indicate the importance of HO-1 in the host defense against oxidant injury.

REDOX-DEPENDENT TRANSCRIPTIONAL REGULATION

Redox reactions of biomolecules, mostly proteins, which used to be considered as 'oxidative stress', are now considered as 'signals', and they contain biological information that is necessary for maintaining cellular homeostasis (64). The response of a cell to an ROS-rich environment often involves the activation of numerous intracellular signaling pathways, which can cause transcriptional changes and allow the cell to respond appropriately to the perceived oxidative stress. In addition to the regulation achieved by classical cytosolic signaling pathways, such as the family of mitogen-activated protein kinases (MAPKs), the evidence suggests that certain transcription factors can directly or indirectly alter their activity, depending on cellular redox conditions (64). In the nucleus, oxidative stress can change the histone acetylation and deacetylation status, which, at least in part, regulates inflammatory gene expression by activation of the redox sensitive transcription factors. The histone acetylation and deacetylation status during ALI/ARDS and its role in the pathogenesis of the disease remains to be determined.

The role of nuclear factor-κB in oxidant-mediated lung injury

The expression of genes in response to oxidative stress-related transducing signals from surface receptors is predominantly determined by the conditions of the cell microenvironment. Nuclear factor-κB (NF-κB) is one of the most important transcription factors shown to respond directly to oxidative stress conditions (Fig. 5) (5, 43). Although NF-κB was originally recognized in regulating gene expression in B-cell lymphocytes, subsequent investigations have demonstrated that it is one member of a ubiquitously expressed family of Rel-related transcription factors that serve as critical regulators of inflammatory-related genes such as TNF-α and IL-1β (8).

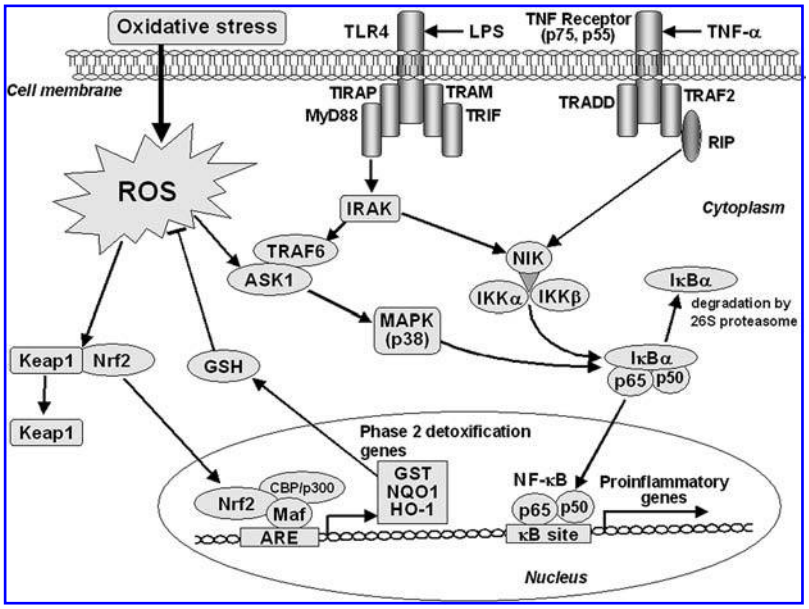

FIG. 5. Activation of signal transduction pathways and modulation by oxidative stress during ALI/ARDS. A variety of signals, including inflammatory mediators and oxidative stress, converge on activation of the inhibitory-κB kinase (IKK) complex via the upstream NF-κB inducing kinase (NIK). The IKK-α/IKK-β complex (signalsome) then phosphorylates inhibitory-κB (IκB) at two N-terminal serines, which signals it for ubiquitination and phosphorylation by the 26S proteasome system. Freed NF-κB (p50–p65 complex) enters the nucleus, binds specific κB moieties, and activates gene expression. ROS activates p38 MAPK via ASK1 in coordination with TRAF6, leading to activation of IκB. As intracellular ROS levels increase, critical cysteine residues in Keap1 become oxidized and Nrf2 dissociates from Keap1. Nrf2 becomes stabilized with transcription factors such as Maf, binds to antioxidant response elements (ARE) located in the proximal promoters of the phase II detoxification genes. ASK1, apoptosis signal-regulating kinase 1; ARE, antioxidant responsive element; CBP, CREB-binding protein; GST, glutathione S-trasferase; HO-1, heme oxygenase-1; IRAK, IL-1 receptor-associated kinase; Keap1, Kelch-like ECH-associated protein 1; MAPK, mitogen-activated protein kinase; Nrf2, NF-E2-related factor 2; NQO1, NAD(P)H:quinone oxidoreductase 1; RIP, receptor-regulated intramembrane proteolysis; TIRAP, Toll-interleukin 1 receptor (TIR) domain-containing adapter protein; TRADD, TNF receptor-associated death domain; TRAF, TNF receptor-associated factor; TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain-containing adaptor inducing IFN-β.

The Rel/NF-κB transcription factors are a family of structurally related eukaryotic transcription factors that are involved in the control of a vast array of processes, such as immune and inflammatory responses, developmental processes, cellular growth, and apoptosis. In addition, these factors are active in a number of disease states, including cancer, arthritis, inflammation, asthma, and cardiovascular abnormalities (8). The immunoregulatory approach aimed at targeting the NF-κB signaling pathway therefore remains of particular interest, and selective modulation of this transcription factor may bear a typical therapeutic approach for the control and regulation of inflammatory diseases, including ALI/ARDS. It was reported that inhibition of cytokine-induced NF-κB activation and IL-8 expression in human bronchial epithelial cells was associated with reduced IκBα degradation (112). Since IκBα kinase (IKK) is a redox-sensitive regulator of NF-κB activation, oxidative stress may alter intracellular signaling via modulation of the NF-κB pathway (Fig. 5). ROS enhance the signal transduction pathways for NF-κB activation in the cytoplasm and translocation into the nucleus. In contrast, the DNA binding activity of oxidized NF-κB is significantly diminished, and that activity is restored by reducing enzymes, such as thioredoxin or redox factor 1 (52).

The role of AP-1 in oxidant-mediated lung injury

The *c-fos* and *c-jun* genes encode Fos and Jun proteins that can dimerize to form homodimeric (Jun/Jun) and heterodimeric (Fos-Jun) complexes of the activator protein (AP)-1 family, which have been shown to play an important role in oxidant signaling, immune responses, and apoptosis (51). Oxidative stress can enhance AP-1-mediated gene expression, leading to upregulation of various inflammatory mediators (5, 64). NO· and H_2O_2 cause increases in *c-fos* and *c-jun* mRNA levels, nuclear proteins, and complexes binding the AP-1 recognition sequence in alveolar type II cells, leading to increased permeability (51).

Redox-dependent activation of JAK/STAT pathway

The signal transducers and activators of transcription (STAT) family of transcription factors, which are phosphorylated on tyrosine residues via Janus kinases (JAKs), activate critical mediators of cytokine responses. Among STAT family members, STAT3 is a redox-regulated transcription factor both *in vivo* and *in vitro* (95). Since LPS-induced STAT3 activation in the lung was significantly inhibited by overexpression of catalase, LPS-mediated STAT3 activation in the lung may be redox dependent (95).

Nrf2-Keap1 pathway in the regulation of oxidant-mediated lung injury

To protect against toxic compounds and oxidative stress, animals possess several defense systems. Antioxidant responsive element (ARE) has been shown to regulate the expression of a group of phase 2 detoxification enzymes and antioxidant enzymes (54). ARE-mediated induction of these enzymes cooperatively serves to reduce inflammation and injury by lowering xenobiotic and oxidative stress. NF-E2-related factor 2 (Nrf2)

is a member of the "cap 'n collar" basic leucine zipper transcription factor family and has been identified as a pivotal factor in the ARE-mediated enzyme induction (54). In mouse models, Nrf2 plays essential roles in the protection against acute lung inflammation and damage induced by oxidative stress, such as hyperoxia (24) and cigarette smoke (88). Nrf2 is a transcription factor that is known for providing cellular protection against oxidative insults and for regulating the expression of peroxiredoxin I and HO-1 (54). In a homeostatic condition, Kelch-like ECH-associated protein 1 (Keap1), a cytosolic actin-binding protein, constitutively represses the Nrf2 activity (54). Keap1 sequesters Nrf2 in the cytoplasm and enhances proteasomal degradation of Nrf2 (Fig. 5). When cells encounter oxidative or xenobiotic stress, Nrf2 is released from Keap1 and the actin cytoskeleton and rapidly accumulates in the nucleus.

Cyclopentenone prostaglandins, including 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), activate Nrf2 by directly binding to Keap1 (74). It is well known that cyclooxygenases (COXs), especially COX-2, are upregulated at sites of acute inflammation. In a rat carrageenin-induced pleurisy model, COX-2 is induced at early (2 h) and late (48 h) phases of pleurisy, and the late-phase induction of COX-2 may contribute to the resolution of inflammation by producing cyclopentenone prostaglandins, including 15d-PGJ_2 (74). It has been demonstrated that 15d-PGJ_2 exerts its anti-inflammatory activity through the activation of peroxisome proliferator-activated receptor-γ (PPAR-γ), and also via the inactivation of NF-κB (89).

Role of hypoxia-inducible factor-1 in oxidant-mediated lung injury

Hypoxia-inducible factor-1 (HIF-1) is a crucial transcription factor that is a master regulatory element in sensing hypoxic conditions and in integrating an adapted response via gene expression of oxygen-sensitive and redox-sensitive enzymes and cofactors (94). The stability and activity of HIF-1α, first identified as a DNA-binding activity expressed under hypoxic conditions, increase exponentially when oxygen tension is lowered (Fig. 6). Whereas HIF-1β is constitutively expressed under normoxic conditions, HIF-1α is rapidly degraded by the ubiquitin-proteasome system. Under hypoxic conditions, HIF-1α protein stabilizes and accumulates, thus allowing the heterodimer to translocate to the nucleus and to bind hypoxia response element (HRE), which regulates the expression of erythropoietin (EPO), vascular endothelial growth factor (VEGF), glycolytic enzymes, and glucose transporters, as well as cytokines and other inflammatory mediators (Fig. 6) (41).

Although the role of HIF-1α in oxidant-induced lung injury is less clear, or less prominent, than in hypoxia, it has been revealed that ROS signaling mediates cytokine-dependent regulation of HIF-1α (42). In the airway epithelium, recombinant human IL-1β and murine TNF-α induced, in a time-dependent manner, the nuclear translocation of HIF-1α, which is an effect associated with upregulating the activity of this transcription factor under normoxic conditions. In addition, analysis of the mode of action of IL-1β and TNF-α revealed a novel induction of intracellular ROS, including H_2O_2, O_2-$^{\bullet}$, and $^{\bullet}$OH (42). The antioxidants dimethyl sulfoxide and 1,3-dimethyl-2-thiourea, purported to be prototypical scavengers of H_2O_2 and $^{\bullet}$OH, attenuated cytokine-induced HIF-1α nuclear translocation and activation in a dose-dependent manner (10). The NADPH-oxidase inhibitor 4'-hydroxy-3'-methoxy-acetophenone, which may affect mitochondrial ROS production, attenuated cytokine-mediated nuclear translocation and activation of HIF-1α. Furthermore, inhibition of the mitochondrion complex I nicotinamide ADP-dependent oxidase by diphenylene iodonium abrogated IL-1β- and TNF-α-dependent nuclear translocation and activation of HIF-1α. Similarly, interrupting the respiratory

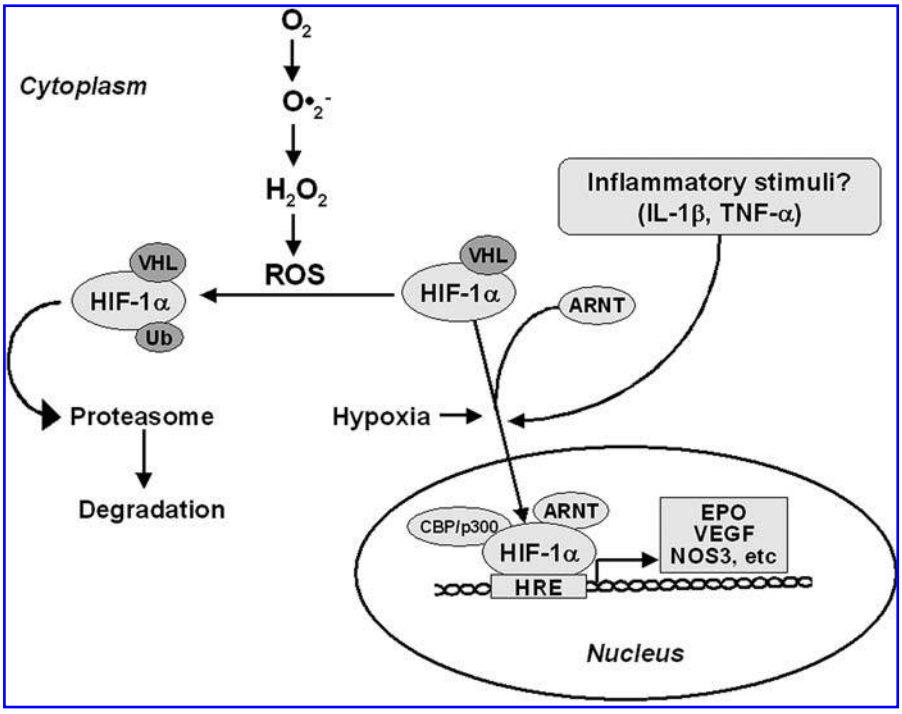

FIG. 6. Oxygen-sensing mechanisms for the regulation of gene transcription and the involvement of hypoxia-inducible factor-1 (HIF-1). Under normoxic conditions, HIF-1α is rapidly degraded by the ubiquitin–proteasome system. Under hypoxic conditions or inflammatory stress, however, HIF-1α protein is stabilized and translocated to the nucleus and binds to specific promoter moieties of selective genes encoding erythropoietin (EPO), vascular endothelial growth factor (VEGF), glycolytic enzymes and glucose transporters, as well as cytokines and other inflammatory mediators. ARNT, aryl receptor hydrocarbon nuclear translocator; CBP, CREB-binding protein; HRE: hypoxia response element; Ub: ubiquitin; VHL: von Hippel–Lindau tumor suppressor protein.

chain with potassium cyanide reversed the excitatory effect of cytokines on HIF-1α nuclear translocation and activation (42). These results indicate that a nonhypoxic pathway mediates cytokine-dependent regulation of HIF-1α translocation and activation in an ROS-sensitive mechanism.

Direct evidence implicating HIF-1 in lung injury emerged with VEGF, which has been recognized as a potent mediator of endothelial barrier dysfunction and is upregulated during ischemia in many organs (94). In a model of ventilated pulmonary ischemia that causes a marked increase in pulmonary vascular permeability, VEGF mRNA was increased by ventilated ischemia, independent of oxygen tension (10). VEGF protein increased in parallel to VEGF mRNA and appeared along alveolar septae (10). HIF-1α mRNA increased during both hyperoxic and hypoxic ischemia, but HIF-1α protein increased only during hypoxic ischemia. These results implicate VEGF as a potential mediator of increased pulmonary vascular permeability in this model of oxidant-induced lung injury.

Mitogen-activated protein kinase pathway

The MAPKs are a group of protein kinases that mediate the nuclear response of cells to a wide variety of extracellular stresses such as inflammatory cytokines, growth factors, and osmotic stress (Fig. 5) (58). Although three distinct subfamilies have been described, extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), and p38, there is significant crosstalk between the pathways as well as common downstream targets (22, 111).

Exposure of cultured endothelial cells to H_2O_2 increased the permeability and activated ERK and p38 MAPK in a dose-dependent manner (111). SB203580, a specific inhibitor of p38 MAPK, blocked H_2O_2-induced endothelial permeability. In addition, the H_2O_2-induced endothelial permeability was attenuated by NAC, which prevents H_2O_2-induced p38 MAPK, but not ERK activation (111). These results suggest a role for redox regulation of p38 MAPK in ROS-dependent endothelial barrier dysfunction. Furthermore, the MAPK pathways play an important role in HO-1 gene induction after anoxia-reoxygenation in pulmonary endothelial cells (119).

In alveolar type II cells, H_2O_2, at concentrations that cause significant DNA fragmentation and increases in the number of apoptotic cells, induced rapid activation of all three pathways of MAPK signaling: JNK1/2, ERK1/2, and p38 (22). Blockade of MAPK activation using specific inhibitors or antisense oligonucleotides showed that activation of the JNK pathway may be required to trigger the apoptotic program. In contrast, chemical inhibition of the ERK and p38 pathways led to an increase in apoptosis, suggesting they are activated in order to inhibit the cytotoxic effects of H_2O_2 (22). It was suggested that the balance between activation of the JNK pathway, on one side, and the ERK and p38 pathways, on the other, may influence cell fate.

THERAPEUTIC IMPLICATIONS

Various kinds of oxidants and antioxidants are involved in the pathophysiology of lung injury. Drugs aimed at antioxidant supply appear to be a reasonable approach to treat lung injury because levels of intrinsic antioxidative factors have been shown to decrease due to over consumption (18). Many studies have reported that antioxidant supplementation has beneficial effects in animal models of lung injury. Unfortunately, no drug has been proven to improve the clinical outcome of lung injury in humans (106).

Discrepancies between the results of animal research and human studies might be attributed, in part, to the diverse pathophysiology of lung injury in clinical settings (106). According to the current definition, ALI/ARDS is a heterogeneous syndrome with wide variety of the causes and clinical courses (14). In the following sections, we will describe findings of animal and human studies using drugs developed to improve the intracellular oxidative state. It should be understood that most antioxidative drugs have shown therapeutic potency in specific pathological conditions (*e.g.*, hyperoxic stress) and not universally to various lung injury conditions. Understanding the precise pathophysiology in each patient might be important for selecting better treatment options.

N-Acetylcysteine and procystein

Patients with ALI show reduced total glutathione levels and increased amounts of oxidized glutathione (GSSG) (19). GSH is a superoxide scavenger synthesized from glutamic acid and cysteine in the liver. Converted to L-cysteine *in vivo*, NAC and procystein can increase the intracellular GSH level. In an animal model of experimental lung injury, NAC administration effectively diminished endotoxin-induced alteration of pulmonary circulation, lung compliance, and production of inflammatory cytokines (15). Clinically, NAC administration successfully increased the intracellular GSH level in patients with ARDS (13). Accordingly, several prospective studies have been conducted to investigate NAC efficacy for human lung injury. Although each trial is unique in terms of patient selection (ARDS vs. mild ALI) and total dose of NAC, none of them showed a mortality benefit (17, 30, 102). A systematic review held by Cochrane library concluded that pooled analysis (a total of 235 patients were involved in five studies) showed no statistically significant effect on early mortality (3).

Lisofylline

Intracellular phospholipid signaling is crucial to the inflammatory response against cytokine stimulation. Inflammatory mediators, such as IL-1β, activate lysophosphophatidic acyl transferase (LPAAT) to produce phosphatidic acid (PA). PA is rapidly converted to 1,2 diacylglycerol (DAG) and both PA and DAG act as intracellular second messengers to promote inflammatory cell damage. Lisofylline is a specific LPAAT inhibitor and diminishes proinflammatory cytokine expression and acute lung injury following hemorrhage in mice (1). Delayed administration of lisofylline has been found to inhibit sepsis-induced acute lung injury in pigs (45). Critically ill patients who developed ARDS were associated with activated phospholipid signaling pathways, as evidenced by an increased plasma unsaturated free fatty acid ratio. Lisofylline administration successfully reduced the unsaturated free fatty acid ratio in patients who developed ARDS (20). Given these promis-

ing results, a large scale multicenter trial was conducted by the ARDS network group (2). This trial, however, was terminated prematurely at the first scheduled interim analysis due to poor results. The lisofylline group did not have any clinical benefits in terms of 28-day mortality, ventilator free days, or organ failure, compared with the placebo group. There are differing opinions concerning some aspects of this trial. First, since the serum unsaturated free fatty acid ratio did not change between the two arms, it may be possible that the dose (3 mg/kg) and administration schedule of lisofylline were inadequate. Second, patients with severe conditions (*i.e.*, who had developed full-blown ALI/ARDS) were selected. Finally, since this study was conducted at the same time as the ARDS lower tidal ventilation trial, two different ventilation strategies (tidal volume of 6 *vs*. 12 ml/kg) were included in both arms. Nevertheless, there is no strong evidence demonstrating a clinical benefit of lisofylline usage in patients with ALI/ARDS.

NO and iNOS

Whereas NO mediates adequate pulmonary microcirculation and alveolar gas exchange, it also promotes inflammatory injury as RNS. Animal studies have shown that nonselective inhibition of NOS with L-NMMA has beneficial effects on paraquat-induced tissue injury in isolated guinea pig (11) or ischemia–reperfusion injury in rat lung (50). Excessive production of NO during lung injury is accompanied by induction of NOS2 (iNOS) gene expression in inflammatory cells (53). NOS2 knockout mice showed reduced lung injury following LPS injection or hemorrhagic shock (56, 103). In contrast, overexpression of NOS3 (eNOS) attenuates LPS- and ventilator-induced lung injury (105, 117). Inhaled NO has been tested as a selective vasodilator in lungs in patients with ALI/ARDS and was found to have an effect on oxygenation but did not improve the mortality rate (27, 106). Since NOS2 involves an explosive increase in NO during lung injury, one can assume that an inadequate increase in NO by NOS2 induction might act as a free radical, while baseline level of NO from NOS3 is required for normal alveolar function. In this context, selective inhibition of iNOS seems to be a more attractive therapeutic approach compared to global inhibition of NOS. A recently developed selective iNOS inhibitor, ONO-1714, has been shown to be beneficial in animal models of endotoxin-induced lung injury (70) and hyperoxic lung injury (118). The effectiveness of ONO-1714 for clinical usage still needs to be established by future investigations.

CO and heme oxygenase

Multiple factors underlie the protective role of HO in lung injury. HO mediates the degradation of heme, which promotes membrane lipid peroxidation and ROS generation. Moreover, two primary end products of HO reaction, bilirubin and biliverdin, have antioxidative properties (100). The inducible isoform of HO, HO-1, is upregulated in response to various kinds of cell stress. In rodents, intratracheally administered recombinant adenovirus containing the HO-1 gene induced transient overexpression of HO-1 expression in the lung. Animals with HO-1 overexpression had a reduced lung injury score and increased survival rate against hyperoxia- and LPS-induced acute lung injury (49, 78). Since adenovirus mediated HO-1 gene delivery is an established method with which to increase HO-1 expression *in vivo*, it may be a promising candidate for future gene therapy in this field. Systemic administration of biliverdin has a similar protective effect on LPS-induced acute lung injury (93). Treatment with biliverdin improved survival after injection of a lethal dose of LPS, reduced cytokine expression, and blocked accumulation of inflammatory cells in the lung. Although the therapeutic efficacy in human lung injury has not yet been established, administration of exogenous biliverdin/bilirubin may be an alternative therapeutic approach.

CO, a catalytic byproduct of HO reaction, has important anti-inflammatory properties. Exposure to low concentrations (up to 250 ppm) of CO has been shown to have an anti-inflammatory effect in an animal model of ventilator-induced lung injury (VILI). CO inhalation was accompanied by a reduced TNF-α level and total cell count in alveolar lavage fluid (29). Since CO is toxic at higher concentrations, its concentration should be carefully controlled in clinical settings.

Albumin

Albumin is the most abundant protein in the plasma. Administration of fluid containing albumin is a common medical procedure, although its beneficial efficacy during fluid resuscitation for critically ill patients was not found in a multicenter trial (SAFE study) (31). Albumin has other biological properties in addition to its role in maintaining the intravascular colloid osmotic pressure. Albumin is believed to have antioxidant properties through its thiol residue. Albumin administration to septic patients or patients with lung injury induces an increase in plasma thiol levels and total antioxidant capacity (87). A small, single centered randomized study showed the beneficial efficacy of albumin infusion on gas exchange in lung injury patients with hypoproteinemia (66). Interestingly, subgroup analysis of the SAFE study demonstrated that albumin administration decreased mortality risk in patients diagnosed with sepsis or lung injury, although the differences were not statistically significant, presumably because of the small sample size (31). Further studies are necessary to establish the effectiveness of albumin administration on mortality in ALI/ARDS.

Ethyl pyruvate

While pyruvate is important for glucose metabolism as a start substrate in the TCA cycle, it also reacts with $O^{\bullet}{}_2^-$ and $^{\bullet}OH$ (26). H_2O_2 induces rapid nonenzymatic decarboxylation of pyruvate. Pyruvate is exported from mammalian cells and acts as an antioxidant extracellularly (26). Since pyruvate is unstable in solution and its degenerative products may be toxic, a stable derivative, ethyl pyruvate, was developed (32). Ethyl pyruvate attenuated intestinal mucosa damage following mesenteric ischemia–reperfusion injury in animal models (108). Even when administrated after inflammation has been established, ethyl pyruvate prevented sepsis-induced lethality in mice (110) and improved lung function in a porcine model of endotoxemia (46). Successful treatment with delayed administration of ethyl pyruvate is accompanied by the inhibition of high mobility group box-1 (HMGB-1) secretion from inflammatory cells (110). HMGB-1 is a proinflammatory molecule and acts

as a late mediator of experimental and clinical lung injury (109). Ethyl pyruvate inhibited LPS-induced TNF-α and MIP-1 production in human monocytes (113). It is still unknown whether ethyl pyruvate has any therapeutic impact in patients with ALI/ARDS.

Enteral nutrition and vitamins

In ARDS patients with standardized total parenteral nutrition, plasma levels of antioxidants including α-tocopherol, ascorbate, β-carotene, and selenium were reduced when compared to those of control subjects (25). In addition, malondialdehyde (MDA), a lipid peroxidation product, was steadily increased, which suggests a severely compromised antioxidative system and progressive oxidant stress during the development of ARDS (57, 84). Ascorbate, α-tocopherol, and carotenoids are nutrients that have antioxidant properties (98). Oral supplementation of ascorbate, α-tocopherol, and carotenoids prevented ozone-induced alteration of respiratory function and lung inflammation in healthy human subjects (91). Enteral nutrition supplemented with eicosapentaenoic acid (EPA), γ-linolenic acid (GLA), and antioxidant reduced pulmonary inflammation and improved oxygenation in patients with ALI/ARDS (35, 79). Consequently, patients with EPA, GLA and antioxidant feeding showed significantly reduced ventilator duration, ICU stay, and additional organ failure (35, 79). A decreased neutrophil count in the BAL fluid was also observed in the patients with specialized enteral formulation (79). Because EPA and GLA both have favorable effects on the immune system, it is unclear whether the improved outcome was attributed primarily to the antioxidant supplementation. Nonetheless, antioxidant nutrition might be a promising therapeutic option for patients who have an indication of enteral feeding.

CONCLUSIONS

The role of oxidants in ALI/ARDS is complex. Oxidants are not only produced as a result of severe inflammation during ALI/ARDS but are also involved in the development of the disease. The molecular response to oxidative stress is regulated, in part, by redox-sensitive transcription factors. Recognition of reactive species and redox-mediated protein modifications as potential signals may open up a new field of cell regulation via specific and targeted genetic control of transcription factors, and thus could provide us with a novel way of controlling the disease processes.

ABBREVIATIONS

ALI, acute lung injury; AP-1, activator protein-1; ARDS, acute respiratory distress syndrome; ARE, antioxidant responsive element; BAL, bronchoalveolar lavage; CO, carbon monoxide; COX, cyclooxygenase; DAG, 1,2 diacylglycerol; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; EBC, expired breath condensates; EPA, eicosapentaenoic acid; EPO, erythropoietin; ERK, extracellular signal-regulated kinase; GLA, γ-linolenic acid; GSH, glutathione; GSH-PX, glutathione peroxidase; GSSG, oxidized glutathione; HIF-1, hypoxia-inducible factor-1; HMGB-1, high mobility group box-1; H_2O_2, hydrogen peroxide; HO, heme oxygenase; HRE, hypoxia response element; IKK, IκBα kinase; iNOS, inducible nitric oxide synthase; JAK, Janus kinase; JNK, c-Jun NH2-terminal kinase; Keap1, Kelch-like ECH-associated protein 1; L-NMMA, N(G)-monomethyl-L-arginine; LPAAT, lysophosphatidic acyl transferase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; Mn-SOD, manganese-containing superoxide dismutase; MOF, multiple organ failure; NAC, *N*-acetylcysteine; NF-κB, nuclear factor-κB; NO, nitric oxide; NOS, nitric oxide synthase; Nrf2, NF-E2-related factor 2; $O^{\bullet}{}_2^-$, superoxide; $OH^{\bullet}$, hydroxyl radical; $ONOO^-$, peroxynitrite; PA, phosphatidic acid; PAF, platelet activating factor; α_1-PI, α_1-proteinase inhibitor; PPAR-γ, peroxisome proliferator-activated receptor-γ; PUFA, polyunsaturated fatty acid; RIS, reactive iron species; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; STAT, signal transducers and activators of transcription; VEGF, vascular endothelial growth factor; VILI, ventilator induced lung injury; XDH, xanthine dehydrogenase; XOD, xanthine oxidase.

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Address reprint requests to:
Sadatomo Tasaka, M.D.
Division of Pulmonary Medicine
Keio University School of Medicine
35 Shinanomachi, Shinjuku-ku
Tokyo 160-8582, Japan

E-mail: tasaka@cpnet.med.keio.ac.jp

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