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# Forum Review

# Redox Imbalance and Ventilator-Induced Lung Injury

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## ABSTRACT

**Mechanical ventilation (MV) is an indispensable therapy in the care of critically ill patients with acute lung injury and the acute respiratory distress syndrome; however, it is also known to further lung injury in certain conditions of mechanical stress, leading to ventilator-induced lung injury (VILI). The mechanisms by which conventional MV exacerbates lung injury and inflammation are of considerable clinical significance. Redox imbalance has been postulated, among other mechanisms, to enhance/perpetuate susceptibility to VILI. A better understanding of these pathologic mechanisms will help not only in alleviating the side effects of mechanical forces but also in the development of new therapeutic strategies. Here, we review the relevance of oxidative stress in VILI from human studies as well as cellular and mouse models of mechanical stress. Potential therapeutic avenues for the treatment of VILI with exogenous administration of antioxidants also are discussed.** *Antioxid. Redox Signal.* 9, 2003–2012.

## INTRODUCTION

Acute lung injury (ALI) is a life-threatening disorder in which extensive inflammation and edema in the lungs develop quickly, within hours to a few days (5, 12, 92). Diseases that incite ALI include pneumonia and other severe infections, severe trauma, and hemorrhagic shock. Acute respiratory distress syndrome (ARDS) is a subset of ALI in which intrapulmonary gas exchange is more severely impaired (12). The incidence of ALI/ARDS is ~190,000 patients per year in the United States (78). Mortality from ALI/ARDS has decreased substantially over the past 30 years but remains high at ~30–40% (59). Effective medical treatment usually requires weeks of hospitalization and rehabilitation. Survivors frequently have significant weakness, depression, and diminished physical function.

Most ALI/ARDS patients receive mechanical ventilation (MV) for several days and sometimes weeks. Without MV, most ALI/ARDS patients would die within hours to days of severe hypoxemia and hypercarbia. With MV, survival can usually be ensured for days to weeks. This provides time to administer therapies that are specific to the cause of ALI/ARDS, such as antibiotics for infections, and for a patient's immune system to fight infections and for natural healing processes to occur. However, some techniques of MV can also cause ALI (ventilator-induced lung injury, VILI), which may exacerbate or perpetuate the lung injury from treatable causes such as pneumonia (27). Thus, our primary means of life support may actually prevent some patients from recovering.

Two aspects of the traditional MV techniques for ALI/ARDS patients may cause VILI. First, traditional MV techniques used large tidal volumes ($V_T$s), ~10–15 ml/kg body weight (5). These large $V_T$s were helpful for maintaining adequate arterial oxygenation and carbon dioxide clearance, despite inefficient intrapulmonary gas exchange, with elevated intrapulmonary shunt and physiologic dead space. However, ventilation with large $V_T$s contributed to VILI by causing injury from overdistention (26, 89, 95). Use of smaller $V_T$s may reduce overdistention-induced VILI, but the gas-exchange advantages of the large $V_T$ approach is lost with this technique. A multicenter clinical trial demonstrated lower mortality and more days free of respiratory failure when ALI/ARDS patients received MV with a small $V_T$ technique (6 ml/kg lean body weight) compared with a traditional $V_T$ approach (12 ml/kg lean body

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weight) (4). Therefore, contemporary MV approaches use smaller $V_T$s than were used in the past.

A second aspect of the traditional MV technique that may cause VILI was the use of low to moderate levels of positive end-expiratory pressure (PEEP) (6). These PEEP levels are sufficient to maintain arterial oxygenation in most patients, but they allow repeated opening and closing of small bronchioles and alveoli with each breath, which can be mechanically injurious (64). Moreover, large portions of the lung may remain collapsed or fluid filled at the traditional levels of PEEP. When this occurs, $V_T$s are delivered to a relatively restricted aerated lung region, which may promote VILI from overdistention (41). Substantial evidence indicates that MV at low PEEP levels may cause inflammation and VILI, but recent clinical trials have not demonstrated improved clinical outcomes in ALI/ARDS patients who received higher PEEP levels (15). The beneficial effects of higher PEEP may have been counteracted by a deleterious effect of raising pressures, volumes, and distending forces during inspiration.

## PATHOPHYSIOLOGY OF VILI

One of the hallmarks of ALI/ARDS is loss of airway epithelial and endothelial integrity, resulting in pulmonary edema (92). Conventional MV at high $V_T$ causes excessive alveolar distention, resulting in lung injury and increased pulmonary vascular permeability with an influx of proteinaceous fluid in the alveolar space (92). Furthermore, a systemic inflammatory response may be elicited by MV by cyclic recruitment and derecruitment of unstable lung units and when alveolar regions are overdistended (74), which can ultimately lead to a multisystem organ dysfunction and death. Cytokines and chemokines are potential effector molecules that modulate and regulate VILI [see recent review (9)]. The accumulation of neutrophils in the lung tissue can also lead to enhanced levels of inflammatory cytokines, which play fundamental roles in the development of lung pathogenesis, including VILI (32, 36). KC (human homologue of CXCL1) and MIP-2 chemokines are potent neutrophil chemoattractants and play a critical role in lung inflammatory responses induced by MV (8, 9). Elevated levels of IL-6 have been detected in various experimental models of VILI (77, 82) and in bronchoalveolar lavage (BAL) fluid of ARDS patients on MV (4, 74). Recent expression profiling using injurious and noninjurious MV in the presence and absence of inflammatory stimuli revealed the involvement of a complex network of genes encoding for matrix-remodeling proteins, pro-inflammatory cytokines, and various transcription factors in VILI (3, 24, 31, 33, 53). The exact molecular mechanisms controlling the expression levels of these genes in response to MV and their contribution to the development of or susceptibility to VILI are unclear and remain the focus of research in various laboratories.

*In vitro* studies have used cyclic stretch to mimic the physical forces of MV. These studies demonstrate endothelial and epithelial cell deformation and physical disruption of plasma membrane integrity, leading to alteration in the structure and function of these essential components of the alveolar-capillary membrane, along with activation of proinflammatory and prooxidant pathways [see reviews (38, 88, 91, 93)].

Plasma concentrations of lipid peroxidation products are increased in ALI/ARDS patients. However, plasma levels of endogenous antioxidants are decreased (61). Concentrations of oxidized glutathione, a key endogenous antioxidant substance, are increased in the alveolar epithelial lining fluid of ALI/ARDS patients (16). Taken together, these studies suggest some pulmonary redox imbalance in favor of free radical production in the pathobiology of ALI/ARDS. Free radicals are known to activate phagocytic and nonphagocytic cells in the lung, which, in turn, generate high levels of reactive oxygen (ROS) and nitrogen species (RNS) and other toxic metabolites. The following sections focus on redox imbalance caused by ROS and RNS species and recent findings investigating the mechanisms involved in the generation and role of these free radicals in the development of VILI. We also review the strategy of antioxidant supplementation as a therapeutic intervention in ALI/VILI.

## REACTIVE OXYGEN AND NITROGEN SPECIES AND ALI/VILI

Under physiologic conditions, hydrogen peroxide ($H_2O_2$), superoxide anion ($O_2^{-\cdot}$), and hydroxyl radical ($^{\cdot}OH$), collectively known as reactive oxygen species (ROS), are generated as byproducts of metabolism of molecular oxygen. Nitric oxide (NO) is involved in many physiologic conditions such as blood vessel relaxation, neurotransmission, and host defense. However, RNS, such as nitrite ($NO_2^-$), nitrate ($NO_3^-$), and peroxynitrite ($ONOO^-$), are generated by the products of NO ($^{\cdot}NO$) metabolism. Although the basal levels of these species may function in cell-signaling processes and various biologic processes, when produced at high levels, ROS/RNS cause damage to cellular macromolecules (*i.e.*, DNA, lipids, proteins) and trigger generation of lipid peroxides and DNA adducts (14).

The endogenous cellular antioxidant defense system, which consists of both enzymatic and nonenzymatic antioxidant proteins, plays a central role in quenching the ROS/RNS levels to minimize the ROS/RNS-initiated damage to biologic molecules and in maintaining the "reducing" environment of the tissue/cells. Cellular and extracellular enzymatic proteins such as superoxide dismutases (SODs), catalase, and glutathione peroxidases (GPXs) and periredoxins act as classic antioxidant enzymes that are known to inactivate ROS/RNS and prevent ROS/RNS-initiated reactions (Fig. 1). Other antioxidant enzymes such as glutathione-*S*-transferases (GSTs), $\gamma$-glutamyl cysteine synthase [$\gamma$GCS; composed of glutamate cystenine ligase (GCL), catalytic (GCLC), and modifier (GCLM) subunits, glutathione reductase (GR/GSR), NAD(P)H:quinone oxidoreductase 1(NQO1), UDP-glucuronyl transferase (UGT), thioredoxin (TXN) reductase (TXNRD), and TXN peroxidase (or peroxiredoxin, PRX)] also play key roles in maintaining the redox status. These enzymes contribute to biosynthesis/recycling of thiols or facilitate excretion of oxidized, reactive secondary metabolites (*e.g.*, quinones, epoxides, aldehydes, and peroxides) through reduction/conjugation reactions during xenobiotic detoxification. In addition, small thiol-containing peptides/proteins, such as glutathione (GSH), thioredoxin (TXN), glutaredoxins (GRX), and periredoxins (PRX), also participate in antioxidant defenses by serving as substrates for antioxidant

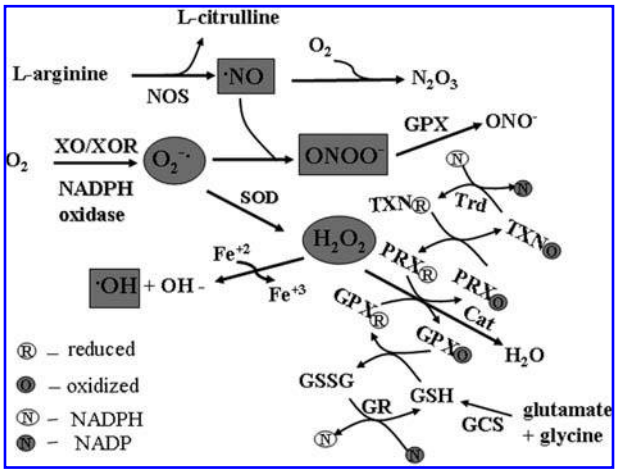


**FIG. 1. Schematic representing the production and detoxification of ROS and RNS by prooxidant and antioxidant systems.** Cat, catalase; GPX, glutathione peroxidase, GR, glutathione reductase; GCS, γ-glutamyl cysteine synthase; GSH, reduced glutathione; GSSG, oxidized GSH; NOS, nitric oxide synthase; TXN, thioredoxin, TXNR, thioredoxin reductase; PRDX, peroxiredoxin; SOD, superodixe dismutase; XO, xanthine oxidase; XOR, xanthine oxidoreductase.

enzymes, such as GPX and TXN peroxidase, in redox cycles. They are easily oxidized and rapidly regenerated by *de novo* synthesis or replaced through enzymatic reduction of their disulfide. In addition, stress-response proteins such as heme oxygenase-1 (HO-1) provide cellular protection against various oxidant or prooxidant insults (79).

*Generation of ROS by cyclic stretch.* The contribution of ROS generated by lung fibroblasts, epithelial cells, and endothelial cells (which are directly affected by cyclic strain elongation associated with MV) to the development or perpetuation of VILI is of increasing interest. Experimental evidence obtained from cell-culture studies indicates that cyclic strain enhances ROS production as early as 30 min after exposure of lung epithelial and endothelial cells (2, 17, 19, 72), suggesting that ROS might contribute to the onset of MV-induced lung injury. Importantly, ROS generation, as measured by the DCFDA fluorescence method, was markedly higher in cells exposed to pathologically relevant cyclic stretch (15–18%) than in those exposed to physiologically relevant cyclic stretch (5%), with levels of ROS production correlating with length of exposure. Cyclic stretch at higher magnitude reduces GSH levels (17, 43, 72) and enhances GSH oxidation (17, 72). These *in vitro* observations strongly suggest that redox imbalance caused by cyclic strain may be an important factor in perpetuating or contributing to the development of VILI.

Several studies demonstrated a redox imbalance in experimental models of VILI. Consistent with the *in vitro* studies are a decrease in antioxidant activity (SOD and GPX activity) in the lungs and enhanced levels of oxidative stress plasma markers, such as thiobarbituric-reactive substances and malondialdehyde, that are observed in response to MV *in vivo* (18, 23). We recently found that MV at high $V_T$ for 2 h causes no significant redox imbalance in the lungs of mice in a model of VILI (Papaiahgari *et al*., unpublished data). However, redox imbalance is observed in the lungs of mice with a disruption of the oxidative stress modifier gene *Nrf2* (see later). Moreover, these mice displayed enhanced levels of inflammatory and lung-injury responses. Thus, we speculate that a dysfunctional signaling that controls gene expression regulating cellular redox status may contribute to or perpetuate VILI in certain populations (*vide infra*). It is unclear whether the discrepancy observed between cell-culture studies and animal models is due to experimental artifacts or lack of sensitive methods to detect redox imbalance in the intact lung. Alternatively, it is possible that redox imbalance occurs only in selective lung regions undergoing enhanced stretch.

*Mechanisms of ROS generation by cyclic stretch.* Several studies using cyclic stretch associated with conventional MV at high $V_T$ have provided an insight into the generation of ROS production by lung epithelial and endothelial, fibroblasts, and vascular smooth muscle cells (2, 17, 19, 57, 58, 72). Exposure to cyclic stretch enhances NADPH oxidase activity, whereas pharmacologic inhibition of this enzyme greatly diminishes cyclic stretch-induced ROS production. The NADPH oxidase system consists of various oxidases that belong to NOX family members, which are transmembrane and cytosolic proteins. Both translocation and assembly of these oxidases at the plasma membrane are essential for transport of electrons across the membrane to the extracellular space (7). A prominent role for gp91$^{phox}$ (NOX2) and p47$^{phox}$ (NCF1) in various experimental models of ALI has been demonstrated. Deficiency of these NADPH oxidase subunits impairs ROS production and generally confers protection against oxidant- and toxin-induced lung injury (7). Moreover, cyclic stretch induced the recruitment of p47$^{phox}$ subunit to NADPH complex (34). Consistent with these studies is the observation that cyclic stretch failed to stimulate ROS production in pulmonary artery endothelial cells or vascular smooth muscle cells lacking the p47$^{phox}$ gene (34). Activation of MMP2 and adhesion molecules and cytokine expression are also impaired in p47$^{phox}$-deficient cells. Thus, it appears that the p47$^{phox}$ subunit plays a key role in ROS generation and in eliciting NADPH oxidase activity in lung epithelial cells in response to cyclic stretch. The expression, regulation, and role of several NOX isoforms, such as DUOX1 and DUOX2, in airway epithelial cells in response to external stimuli and inflammatory cytokines have been recently documented (96), but their role in VILI remains unclear. However, these studies and others performed in non–lung cell types collectively support a prominent role for the NOX family of NADPH oxidases in VILI.

Intriguingly, two different studies implied a role for mitochondria in ROS generation by cyclic stretch. A study performed by Ali *et al*. (2) demonstrated that the mitochondrial complex I inhibitor, rotenone, but not the NADPH oxidase inhibitor, completely blocks cyclic stretch-stimulated ROS generation in human umbilical vein endothelial cells (2). In a different study, a partial inhibition of ROS generation by rotenone was observed in human airway epithelial cells (17). It is unclear whether different experimental conditions (*e.g*., different cell types) could account for this discrepancy; these studies, however, suggest that both mitochondria and the NADPH ox-

idase system regulate cyclic stretch-induced ROS production at least in a context-dependent manner. The exact mechanisms by which cyclic stretch induces ROS generation *via* mitochondria remain unclear, but distention/deformation of mitochondria after cyclic stretch exposure and leading to alterations in mitochondrial $K^+$ ATP channel activity have been proposed (17). It remains to be seen whether any connection exists between the NADPH oxidase system and mitochondrial dysfunction in response to cyclic stretch.

These studies in lung cells, and others performed in other cell types, have clearly demonstrated that cyclic stretch induces ROS generation in isolated cell-culture systems. However, the exact relevance, especially the contribution of ROS generated by nonphagocytic lung cells (such as epithelial and endothelial cells, and fibroblasts) to the development or perpetuation of VILI *in vivo* remains to be investigated.

*Mechanisms of RNS generation by cyclic stretch in the lung.* Nitrosative stress *via* protein nitration by RNS generated by the products of NO has been recently postulated (49) in the pathogenesis of ALI on the basis of protein nitrotyrosine residue formation in lung tissue specimens from patients (35). RNS have also been found in BAL of patients with trauma or suspected sepsis (that either are at risk for or have ARDS) and have been related to increased mortality (81). Experimentally, the use of nitric oxide synthase (NOS) inhibitors have prevented MV-induced lung injury and inflammation, supporting a role for NO and its metabolites in VILI (73).

NO is produced by three isoforms (the neuronal, endothelial, and inducible forms) of nitric oxide synthase (NOS). Endothelial NOS (eNOS), which is calcium/calmodulin ($Ca^{2+}$/CaM)-dependent and activated by agonists (*e.g.*, acetylcholine), produces a low level of NO output, whereas inducible NOS (iNOS) is independent of $Ca^{2+}$/CaM and transcriptionally regulated by proinflammatory products (*e.g.*, LPS) and cytokines (IL-1$\beta$, TNF-$\alpha$, $\gamma$-IFN), and results in sustained and elevated release of NO (97). Overproduction of NO leads to nitrosative stress and tissue injury in conditions such as endotoxin-induced acute lung injury (48). We previously demonstrated that iNOS can be induced by cytokines and hypoxia in cultured pulmonary microvascular endothelial cells and postulated a role for this cellular source in endothelial damage (and potential capillary permeability) in inflammatory conditions such as sepsis and ARDS (100). Several investigators, including our group, demonstrated the activation of NOS in animal models of MV (20, 52, 60, 87). We recently demonstrated that MV with high (but not low) $V_T$ increases the expression of iNOS at mRNA and protein levels, specifically in endothelial and epithelial cells, leading to increased iNOS activity. Similarly, a study by Frank and colleagues (30) demonstrated increased lung iNOS protein expression and total nitrite levels in BAL fluid, and a decrease in air-space fluid clearance in rats ventilated with high $V_T$ as compared with low $V_T$. Inhibition of iNOS prevented the decrease in air-space fluid clearance, suggesting a role for RNS in the pathogenesis of VILI (30). Also in strong support of a role for iNOS in RNS-related stress to the pulmonary vascular barrier was the observation, in our studies, that therapeutic intervention with chemical iNOS inhibitors (*e.g.*, aminoguanidine) conferred protection against VILI and that iNOS-deficient animals were completely protected from such injury (73). In contrast, Choi *et al*. (22) were unable to detect any lung iNOS expression, but demonstrated an increase in eNOS protein expression, in a rat model of high $V_T$ (20 ml/kg for 2 h). Although these investigators did not measure any specific NOS activity in their model, they demonstrated that the nonspecific NOS inhibitor *N*-nitro-L-arginine methyl ester attenuated lung and kidney microvascular leakage (22) associated with this model.

Other potential sources of excess NO include macrophages, neutrophils, and smooth muscle cells, all known to express iNOS. Upregulation of iNOS in these cells could result directly from mechanical stress or indirectly after cytokine release. Along with an increase in iNOS expression, we demonstrated by immunohistologic examination an increase in nitrosative damage (*i.e.*, presence of nitrotyrosine residues) predominantly in endothelial, but also epithelial, cells in wild-type animals. In contrast, minimal formation of lung nitrotyrosine was found in iNOS-deficient mice in response to high $V_T$ (73). These studies suggest that MV alone (without any additional trigger of the inflammatory cascade) can upregulate iNOS, thus contributing to formation of NO end products and nitrosative stress.

*A link between ROS and RNS.* Xanthine oxidoreductase (XOR) is best known for its role in purine catabolism and as a target in gout therapy. XOR exists as two interconvertible forms, xanthine dehydrogenase (XDH) and xanthine oxidase (XO). The conversion of XDH to XO can be reversible, after treatment with sulfide reagents, or irreversible after proteolysis (83). In the process of oxidation of hypoxanthine to uric acid, $NAD^+$ and molecular oxygen are the preferential electron acceptors for XDH and XO, respectively. This relates to the inability of $NAD^+$ to bind to XO because of significant conformational changes of the enzyme. Therefore, conversion of XDH to XO by either proteolysis or posttranslational modification significantly increases the amount of ROS produced by XOR (83, 94). However, reduction of molecular oxygen by either form of the enzyme yields superoxide and hydrogen peroxide, and upregulation of overall XOR activity, irrespective of XDH/XO ratios, can lead to increased ROS levels (39). It is the capacity of XOR to generate such ROS that is of major interest in clinical syndromes.

With the recognition that MV can upregulate inflammatory cytokines (such as IL-8, TNF-$\alpha$, and $\gamma$-interferon) known to induce XOR (86), it is likely that oxidant injury related to formation of ROS and other potent oxidants such as peroxynitrite (from the reaction of XOR-derived superoxide with iNOS-derived NO) may promote or perpetuate VILI. In support of this notion, we recently demonstrated that MV induces lung XOR enzymatic activity in an experimental model of VILI (1). Consistent with this result, the XOR inhibitor allopurinol prevented the increase in capillary pulmonary permeability produced by MV with high $V_T$, strongly suggesting that oxidative or nitrosative stress or both, caused by XOR activation in response to mechanical stress, contributes to ventilator-induced alveolar barrier dysfunction (1).

Peroxynitrite, a powerful oxidant, results from the rapid reaction between NO and superoxide. As detailed earlier (section on RNS), by using fluorescent immunolocalization, we demonstrated that peroxynitirite formation (as evidenced by nitrotyrosine deposition, a footprint of peroxynitrite damage) occurs at the site of increased iNOS and XOR expression (*i.e.*,

essentially in endothelial cells). Because capillary permeability is prevented by iNOS deficiency or pharmacologic inhibition of either iNOS (73) or XOR, we postulate that damage to the endothelial barrier in response to mechanical stress could potentially be related to endothelial XOR-derived ROS reacting locally with iNOS-derived NO to form peroxynitrite in components of the alveolar–capillary membrane. The exact mechanisms of endothelial barrier dysfunction after ROS and RNS exposure have not been deciphered but are likely to involve cytoskeletal rearrangement and formation of endothelial gaps.

*Induction and dysregulation of antioxidant enzymes by cyclic stretch associated with MV.* The protective effects of several antioxidant enzymes/proteins/peptides in various experimental models of oxidant- and toxin-induced lung injury have been well documented (21), but their relevance in VILI is poorly understood. Recent expression profiling revealed an inducible expression of GPX2 and GCLC in the lungs of mice subjected to acute high $V_T$ of MV (24, 33, 53). Consistent with this finding, we recently demonstrated that cyclic stretch induces the expression of several antioxidant enzymes, such as GCLC, GCLM, NQO1, GPX2, and HMOX1, in murine type-II–like lung epithelial cells (72). Intriguingly, we also found that an increase in ROS production correlates with the induction of antioxidant enzymes, such as GCLC and HMOX1, but not that of GCLM (which is critical for biosynthesis of GSH) and NQO1 (which detoxifies quinones). Thus, it is likely that dysregulation of antioxidant enzyme expression in response to either a prolonged exposure or a higher magnitude of cyclic stretch may lead to redox imbalance (*i.e.*, an increased oxidation of GSH to GSSG), thereby resulting in MV-induced lung inflammation and injury (Fig. 2). In support of this notion, we and others demonstrated that exposure to a high magnitude of cyclic stretch causes a significant reduction in the detectable levels of the GSH/GSSG ratio in lung epithelial cells (17, 72). Several studies have shown that supplementation with the antioxidant *N*-acetyl-L-cysteine (NAC) attenuates cyclic stretch-induced cytokine gene expression in lung epithelial cells (43). In preliminary results, Syrika *et al.* (85) showed protective effects of NAC against MV-induced neutrophil influx in a rat model of VILI. We recently found that disruption of the Nrf2 transcription factor, a critical regulator of cellular redox status, exacerbates MV-induced lung permeability and inflammation (*i.e.*, increased neutrophil accumulation) and is accompanied by enhanced levels of several proinflammatory cytokines in lung tissue in a mouse model of VILI (Papaiahgari *et al.*, unpublished data). These changes were associated with a lack of induction of critical antioxidant enzymes essential for GSH biosynthesis and redox imbalance in Nrf2-deficient mice after MV, indicating that Nrf2-dependent antioxidant transcriptional responses may act in concert to counteract MV-induced oxidative stress, which otherwise contributes to the pathogenesis of VILI.

Dysregulation of expression of endogenous antioxidant defense system or elevated levels of ROS generated by stressful stimuli (such as MV) can overwhelm antioxidant capacity and perturb cellular redox homeostasis, eventually leading to oxidative stress in diseases such as sepsis, shock, and ALI/ARDS (37, 54, 55). Thus, understanding the mechanisms controlling the induction of cytoprotective antioxidant enzymes/proteins by injurious insults or stressful stimuli, and knowing the potential sources that contribute to elevated levels of ROS and RNS, is of high significance if the goal is to develop new effective therapeutic strategies aimed at restoring cellular redox homeostasis.

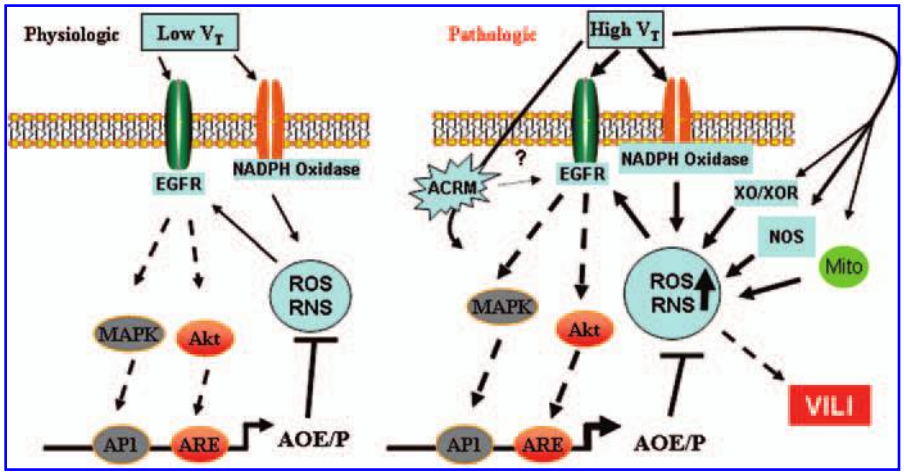


**FIG. 2. Molecular determinants of redox imbalance in VILI.** This model is based on data from studies summarized in this article, which provide a mechanistic framework for signaling pathways associated with mechanical stress and leading to VILI when MV with high (pathologic) $V_T$ is used. In this model, generation of basal levels of ROS by physiologically relevant cyclic stretch or low $V_T$ (left) seems to be suppressed by endogenous or basal-enhanced antioxidant defense systems, which consist of several enzymes/proteins or peptides (AOE/P) (see Fig. 1) induced by Nrf2–ARE transcriptional responses. In response to pathologically relevant mechanical stress associated with high $V_T$ (right), elevated levels of ROS/RNS are generated by NADPH oxidase, iNOS, XO/XOR activity, and mitochondria. These radicals may overwhelm cellular antioxidant capacity regulated by TRE (TPA response element) and ARE-mediated transcriptional programs, thereby leading to redox imbalance. We speculate that such redox imbalance is likely to alter various signal-transduction pathways (*e.g.*, EGFR-activated MAP kinase and Akt signaling) or directly modulate the thiol status of various transcription factors (*e.g.*, NF-$\kappa$B, Jun and Fos family of AP-1 proteins, and/or Nrf2). As a result of the dysregulated gene expression, lung inflammation and capillary leakage may contribute to the development/perpetuation of VILI. ACRM, actin and cytoskeletal remodeling. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

*Cyclic stretch–induced signaling and the antioxidant response element (ARE)-mediated transcriptional response.* Several *ex vivo* and *in vivo* studies have clearly shown that the rapid induction of antioxidant enzymes expression in response to oxidant and toxic insults is mediated mainly by the antioxidant/electrophilic response element (ARE or EpRE). This *cis* element is commonly found in the regulatory regions (promoter or enhancers or both) of detoxifying and antioxidant enzymes (46, 47). Evidence emerging strongly supports a pivotal role for Nrf2, a b-Zip transcription factor, in mediating the induction of pulmonary antioxidant enzymes/proteins in response to prooxidant stimuli, mainly through the ARE (21). As Nrf2 regulates redox status (46, 47), confers protection against prooxidant stimuli (21), and plays an important role in lung epithelial repair and proliferation (75), we briefly review the current status of signaling controlling the activation of Nrf2-dependent ARE-mediated transcriptional response in general and recent studies performed in pulmonary epithelial cells by hyperoxia and cyclic stretch associated with MV. Although the AP-1 transcription factor–dependent TPA response element (TRE)-mediated transcriptional response also plays a key role in regulating gene expression involved in oxidative stress (45) and regulates antioxidant gene expression (76), we do not discuss various signaling pathways that regulate AP-1 activation in this review because of space limitations.

In unstressed (quiescent) cells, Nrf2 is associated with Keap1 and predominantly localized in the cytoplasm. Various stressful and toxic stimuli disrupt sequestration of Nrf2 by Keap1, and permit Nrf2 translocation from the cytoplasm into the nucleus (42). Then Nrf2, on dimerization with small Maf or the AP-1 family of proteins, binds to the ARE (consensus sequence, 5′GTGACNNNGC-3′) and induces transcription of several genes involved in detoxification and cytoprotection (67). Because the Keap1/Nrf2 complex is considered a key cellular sensor of oxidative stress, the regulatory mechanisms of Keap1-dependent turnover and translocation of Nrf2 from the cytoplasm into the nucleus have been investigated with great interest. The signaling pathways converging at the ARE-mediated transcription by various prooxidants and toxins have been elucidated in great detail in several non–lung cell types (see recent reviews) (46, 47).

In pulmonary epithelial cells, we recently showed that hyperoxia through NADPH oxidase/EGFR-activated ERK signaling regulates Nrf2 nuclear translocation and the subsequent ARE-mediated transcriptional response (70). We recently demonstrated that cyclic stretch-activated Nrf2-dependent ARE-mediated gene expression is regulated by the PI3K-Akt pathway, and this appears to be regulated by ROS-dependent EGFR-activated signaling (71). PI3K inhibitor but not ERK inhibitor markedly reduced the binding of Nrf2 to the ARE, as well as ARE-mediated gene transcription and the expression of Gclc, a prototype target of Nrf2, in murine type-II–like alveolar epithelial cells (71). Recently, a role for the PI3K pathway in shear stress–induced ARE-regulated transcriptional responses was demonstrated (40). Thus, it appears that PI3K-Akt signaling plays a key role in regulating cyclic stretch–induced Nrf2-ARE–mediated transcription, thereby suppressing the effects of oxidative stress associated with MV. We also found that actin remodeling is critical for cyclic stretch–induced ARE-mediated gene expression (71), suggesting that EGFR-activated signaling and actin remodeling act in concert in transforming a mechanical signal into a biologic response leading to antioxidant gene expression. Intriguingly, inhibition of the PI3K and JNK1/2 kinase pathways confers protection against MV-induced lung injury and inflammation in mouse models of VILI (51, 52, 90). Future studies deciphering cellular signaling controlling the activation of Nrf2-ARE–mediated gene transcription by cyclic stretch and demonstrating how redox imbalance resulting from prolonged or high-magnitude cyclic stretch modulates the activation of various signal transduction pathways in more complex clinically relevant models of ALI/ARDS may help us to increase the understanding of the pathologic mechanisms underlying VILI/VALI.

*Gene polymorphisms in redox regulators and the risk of ALI/VILI.* ALI is a complex disorder, and the variable incidence of ALI in specific patient groups suggests that genetic factors may have a role in modulating development of this disorder. Genetic variations in a number of genes (*e.g.*, SP-B, pre–B-cell colony-enhancing factor, protein C, MLCK, and proinflammatory cytokines such as TNF-$\alpha$ and IL-10) that control lung injury and inflammation are thought to explain variability in the ALI phenotype [see recent review (62)]. Recent studies identified polymorphisms within genes, such as *GST* and *SOD1* (99), *HSP70* (80), and *HMOX-1* (98), that are known to regulate redox balance, suggesting that genetic variations in antioxidant enzymes/proteins may play a role in the development of ALI/VILI. More recently, we identified functional variants in the promoter region of NRF2 that were associated with an increased risk of ALI after trauma (56). Because NRF2 is a critical regulator of antioxidant gene expression, polymorphisms that affect this transcription-factor activity may have fundamental implications in ALI/VILI, and future investigations aimed at validating these observations related to NRF2 (and other genes that regulate redox balance) in additional populations at risk for ALI/VILI will be crucial.

## *Therapeutic interventions for ALI/VILI with antioxidant supplementation*

A substantial body of evidence demonstrates an important role of ROS/RNS in the pathogenesis of ALI/ARDS and VILI. Moreover, growing evidence in humans with ALI/ARDS suggests that endogenous enzymatic and nonenzymatic antioxidant systems are depleted. In animal models, administration of antioxidants such as *N*-acetyl-L-cysteine (NAC) reduces acute lung injury (10, 29, 50, 63, 65, 85), implicating oxidative stress in the pathogenesis of the injury. Therefore, great interest exists in the potential therapeutic effects of antioxidant substances. Clinical trials of these agents have yielded both promising and discouraging results (11, 13, 25, 28, 68, 69).

Glutathione, a key endogenous antioxidant agent, is decreased in the lungs and blood of ALI/ARDS patients. However, pharmacologic glutathione repletion is difficult because it must be metabolized extracellularly before it can be used intracellularly. NAC can replete intracellular glutathione because it is readily metabolized to cysteine, a glutathione precursor. NAC has been tested extensively in animal models in which oxidant stress is an important mechanism of pathogenesis, and

many of these studies demonstrated reduced oxidant injury and improved organ and system function (10, 29, 50, 63, 65, 85).

In a relatively small clinical trial, 66 patients with ALI/ARDS were randomized to receive intravenous NAC (150 mg/kg loading dose, and then 20 mg/kg/h) or placebo for 6 days (44). Pulmonary compliance (which is reduced by edema and inflammation) was higher in the group that received NAC, but no significant differences were found in the ratio of $Pao_2/Fio_2$ (which reflects the efficiency of gas exchange), chest radiographs, or in mortality. In another small clinical trial, 61 patients with mild to moderate acute respiratory failure from conditions that cause ALI/ARDS were randomized to receive intravenous NAC (40 mg/kg/day) or placebo for 3 days (84). The NAC study group experienced more rapid resolution of acute respiratory failure. Their requirements for supplemental oxygen and mechanical ventilation were significantly lower after 3 days. Moreover, their lung-injury scores decreased significantly, whereas those of the placebo group did not. As in the first clinical study of NAC, mortality at 1 month was not significantly different between the study groups, but a trend favored the NAC group (22 *vs*. 35%).

A third clinical trial assessed the potential therapeutic value of NAC and Procysteine *versus* placebo in 48 patients with ALI/ARDS (13). Like NAC, Procysteine (L-2–oxothiazolidine-4-carboxylate) is an antioxidant that increases intracellular cysteine, leading to increased intracellular glutathione levels. Patients in the NAC and Procysteine study groups had higher plasma cysteine and glutathione concentrations after receiving study drugs. Encouraging trends were observed in the occurrences of new organ failures (fewer in the combined NAC and Procysteine groups than in the placebo group). The number of acute lung injury–free days in the Procysteine group was significantly greater than in the placebo group. This study was the most encouraging of the earlier trials, but beneficial effects could be identified in one or more parameters in each of the studies, and adverse effects were not noted in any.

A larger randomized clinical trial of Procysteine versus placebo was conducted in more than 200 ALI/ARDS patients. Unfortunately, the mortality rate of the Procysteine group was higher than that of the placebo group. The study results are as yet unpublished, and the reasons behind this disappointing outcome are not understood. Despite the promising preclinical and earlier clinical studies, it is possible that unanticipated toxicities of the study drug occurred. Other explanations include the possibility that this drug was not sufficiently effective at reducing oxidant stress, and that failures of randomization caused the difference in mortality between the study groups, independent of any effect of the study drug.

In a large randomized and prospective study of critically ill surgical patients, Nathens *et al*. (66) reported that the early administration of antioxidant supplementation in the form of $\alpha$-tocopherol and ascorbic acid reduced the incidence of organ failure and shortened ICU length of stay. These results remain to be validated in critically ill medical patients with ARDS.

## SUMMARY AND FUTURE PERSPECTIVE

The data presented therein collectively suggest that maintenance of a proper redox balance may be essential for the integrity and function of different lung cellular components and the outcome of VILI. Dysregulation or variation in signaling pathway(s) that converge at the ARE, possibly regulated by an Nrf2–dependent transcriptional program, may enhance susceptibility to ARDS/VILI because of lack of a proper redox balance in the lungs. Of great clinical significance is the observation of functional polymorphisms in the human NRF2 gene, which were associated with an increased risk of ALI in a population of patients with major trauma. Analyzing the candidate target genes and signal-transduction pathways regulated by Nrf2 in response to MV and identifying the functional polymorphisms in other genes that regulate redox balance may provide additional insight into the factors that either promote or perpetuate VILI and help in developing novel therapies targeted at oxidative stress and inflammation in this syndrome. In addition, as the assembly of several subunits of the NADPH oxidase family of proteins is critical for elevated levels of ROS generation by cyclic strain, targeting a specific member(s) of NOX family of proteins represents a potential novel therapeutic avenue in the treatment of ALI/VILI that remains to be explored. This certainly requires the use of emerging tools such as specific SiRNA or pharmacologic inhibitor approaches or both targeting specific NOX family members required for elevated levels of ROS generation in lung cells.

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## ABBREVIATIONS

ALI, acute lung injury; ARDS, acute respiratory distress syndrome; AOE/P, antioxidant enzyme/protein; ARE, antioxidant response element; BAL, bronchoalveolar lavage; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; MAP kinase, mitogen-activated protein (MAP) kinase; MV, mechanical ventilation; NAC, *N*-acetyl-L-cysteine; NOS, nitric oxide synthase; Nrf2, nuclear factor erythroid 2–like 2; PI3K, phosphoinositide-3-kinase; ROS, reactive oxygen species; RNS, reactive nitrogen species; VILI, ventilator-induced lung injury; $V_T$, tidal volume; XO, xanthine oxidase; XOR, xanthine oxidoreductase.

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