Mechanisms of Sepsis-Induced Organ Dysfunction and Recovery

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Involvement of Reactive Oxygen and Nitrogen Species in the Pathogenesis of Acute Lung Injury

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Introduction

Lung injury can present with different signs and symptoms and emanate from a variety of etiologies. However, whether it is the acute respiratory distress syndrome (ARDS) or other forms of lung injury, inflammatory stimuli giving rise to the generation of reactive oxygen species (ROS) and reactive oxygen-nitrogen species (RNS) contribute to lung pathophysiology [1]. These species, generated by activated inflammatory cells, circulating enzymatic generators (such as xanthine oxidase) and multiple other sources, damage the alveolar and capillary endothelia, lung surfactant and connective tissue contributing to the formation of non-cardiogenic pulmonary edema, the development of the multiple organ dysfunction syndrome (MODS) and death.

Formation of Oxidative and Nitrosative Species

Reactive Oxygen Species

ROS implicated in pulmonary pathophysiology include superoxide anions ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$), and hypochlorous acid (HOCl) (Fig. 1). Superoxide anion generation has been demonstrated from a number of biological sources. An important enzymatic source of superoxide is nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) which catalyzes a one-electron reduction of molecular oxygen to form $\cdot O_2^-$. NADPH oxidase is vital for yielding ROS in phagocytic cells that inhabit the lung (e.g., macrophages and polymorphonuclear cells) where these species play a role in host defense mechanisms that target killing and removal of invading microorganisms. It is not surprising then that a variety of systems are present to prevent and/or limit oxidative tissue injury. Four types of superoxide dismutase (SOD) catalyze the conversion of two moles of $\cdot O_2^-$ to H_2O_2, which is then converted to water by catalase and glutathione peroxidase (Fig. 1). Copper (Cu) and zinc (Zn) SODs (CuZn) are present in the cytosol, while manganese (Mn) SOD is found in the mitochondria. An extracellular form of SOD (ECSOD) has also been identified and may play an important role in converting extracellular $\cdot O_2^-$ to H_2O_2 as well as in controlling blood pressure by modulating the reaction of $\cdot O_2^-$ with NO.

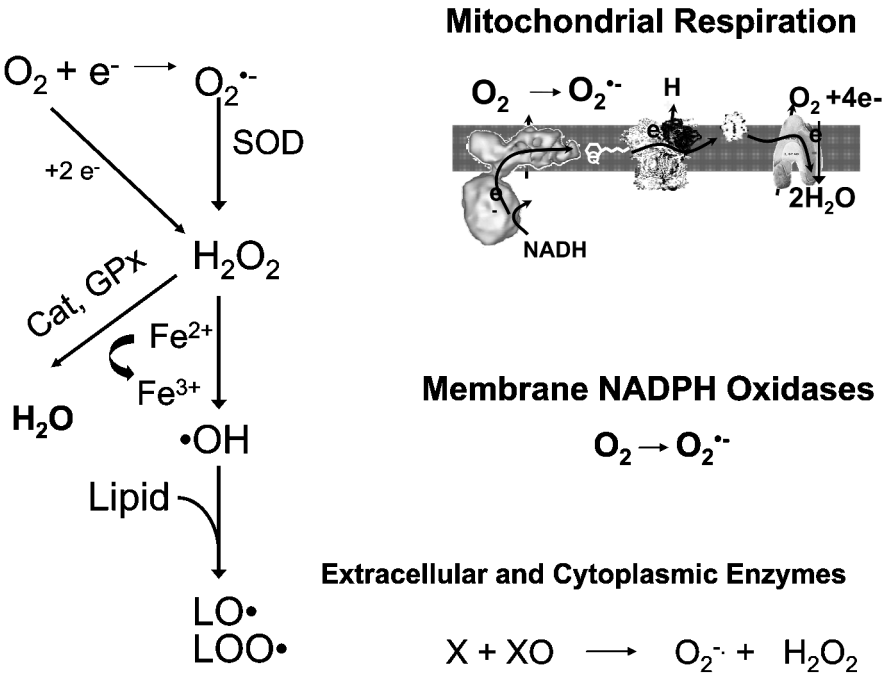

Fig. 1. Generation of reactive oxygen intermediates by the incomplete reduction of oxygen in the mitochondria, cytoplasm and cell membrane and extracellular space. O_2: oxygen; $\cdot O_2^-$: superoxide radical; H_2O_2: hydrogen peroxide; ·OH: hydroxyl radical; SOD: superoxide dismutase; Cat: catalase; GPx: glutathione peroxidase, LO·, LOO.: lipid peroxides; X: xanthine; XO: xanthine oxidase; NADH: nicotinamide adenine dinucleotide; NADPH: nicotinamide adenine dinucleotide phosphate. From [37] with permission

In newborns, ECSOD exists both intracellularly and extracellularly and plays an important role in intracellular antioxidant defenses.

Production of Nitric Oxide and Reactive Nitrogen Species

Nitric oxide (NO) synthases (NOS) catalyze the formation of NO and L-citrulline from L-arginine, and oxygen via a 5-electron redox reaction that also involves cofactors including NADPH, FAD and tetrahydrobiopterin. Various forms of NOS have been identified: NOS-1 or neuronal NOS (nNOS), NOS-2 or inducible NOS (iNOS), and NOS-3 or endothelial NOS (eNOS). nNOS and eNOS are expressed constitutively, and their activity is regulated largely by changes in intracellular Ca^{2+} concentration. Although previous studies claimed that iNOS was not constitutively expressed, more recent findings show expression of iNOS in inflammatory cells and lung tissue of humans and mice under baseline conditions (Fig. 2) [2] with significant upregulation of mRNA, protein, and activity following exposure to

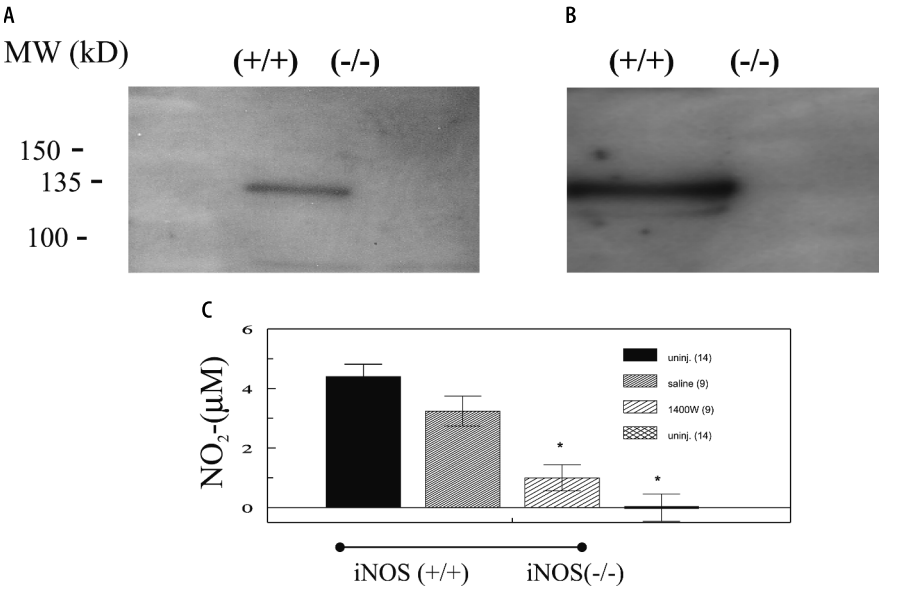

Fig. 2. iNOS is present and active under basal conditions in C57BL/6 mouse lungs. Representative western blots of (A) azygous lobes and (B) ATII cells isolated from iNOS(+/+) and iNOS(-/-) mice. Equal amounts of proteins were separated on a 7.5% SDS-PAGE, transferred to polyvinyldidene difluoride membranes, followed by probing with anti-mouse iNOS antibody, and then anti-rabbit horseradish peroxidase (HRP) conjugate as the secondary antibody, and finally developed by enhanced chemiluminescence (ECL) reagents. These measurements were repeated with proteins derived from five different mice with identical results. (C) Nitrite levels in the BAL of iNOS (+/+) and iNOS(-/-) mice. Some of the iNOS (+/+) mice were injected with either saline or 1400W. All mice were euthanized and their lungs were lavaged with sterile saline. NO_3^- was first converted to NO_2^- with *Escherichia coli* reductase and concentrations of NO_2^- were measured using fluorescence utilizing 2,3-diaminonaphthalene (DAN). Values are means ± SEM. The number of samples for each group is shown in parentheses. *$p < 0.01$ as compared to the uninjected iNOS (+/+) value. From [2] with permission

cytokines and LPS. A form of NOS also has been identified in the mitochondria and may play an important role in regulating mitochondrial function.

Reactive nitrogen species (RNS) are a variety of nitrogen containing molecules that are typically derived via nitric oxide (NO) reactions. Those implicated in pulmonary pathology include peroxynitrite ($ONOO^-$), nitrogen dioxide (NO_2), and nitroxyl (HNO) which can be formed via NO-reactions as discussed below but also through environmental exposure and inhalation (Fig. 2) [3]. Peroxynitrite is formed by the rapid reaction of NO with superoxide and when protonated (addition of H^+), will decompose into $NO_2^{\cdot}$ and ·OH, as well as nitrate (NO_3^-). These species may then interact with each other, as well as with O_2 or ROS, forming higher oxides of nitrogen which may oxidize thiols, nitrate aromatic amino acids, most notably tyrosines, nitrosate and glutathionylate cysteines and oxidize a variety of amino acids including methionine and cysteines (Table 1). Myeloperoxidase

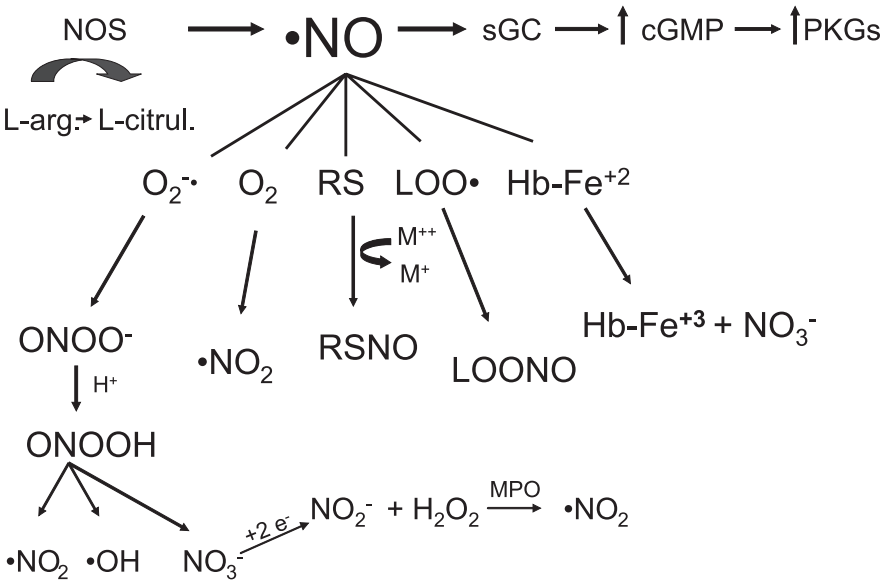

Fig. 3. Generation of reactive nitrogen species. Nitric oxide synthases (NOS) catalyze the formation of nitric oxide (NO) and L-citrulline from L-arginine. NO either binds to the heme center of soluble guanylate cyclase (sGC) leading to increased production of guanosine 3',5'-cyclic monospate (cGMP) and activation of cGMP-dependent protein kinases (PKGs), binds to oxygenated hemoglobin ($Hb\text{-}Fe^{+2}$) to form nitrate (NO_3^-) or interacts with superoxide ($\cdot O_2^-$), molecular oxygen (O_2), thiols (RS), or lipid peroxides ($LOO^\cdot$) to form various intermediates. ONOO-: peroxynitrite; ONOOH: peroxynitrous acid; $^\cdot NO_2$: nitrogen dioxide; RSNO: nitrosothiols; LOONO: nitrated unsaturated fatty acids; OH: hydroxyl radicals; NO_2^-: nitrite; MPO: myeloperoxidase; M: metal. From [37] with permission

(MPO), present in pulmonary neutrophils and secreted during their activation, catalyzes the production of nitrating, oxidizing and chlorinating species from H_2O_2, chloride and nitrite (Fig. 3).

Nitrite has also emerged as a key player in supporting NO-formation during hypoxemia and tissue ischemia, and in this context protects against reperfusion injury. Moreover, nitrite reactions *in vivo* also lead to diverse NO-dependent protein adducts including S-nitrosothiols and C-/N-nitrosamines, underscoring the rich biochemical interplay between distinct RNS and ROS. The therapeutic potential for this inorganic anion in replenishing NO during low oxygen states has also been demonstrated in the lung, with inhalation of nitrite reversing pulmonary hypertension in a manner analogous to inhaled NO. A key difference between nitrite and NO, however, was the lack of rebound hypertension upon withdrawing inhaled nitrite.

Table 1. Actions of reactive nitrogen species. From [37] with permission

Signal Transduction	
Activation of cGMP/PKG	Vessel relaxation Bronchodilation Modification of ion channel function Inhibition of platelet aggregation
cGMP-independent	Activation of NF-κb; MAPkinases
S-thiolation S-nitrosation	NMDA, PKC, adenylate cyclase, complex I, cardiac ryanodine receptor, L-type calcium channels, GPx + others, Caspase-3, p21ras, CFTR
Interactions/modifications	
Binding to heme protein metal centers	Inhibition of protein and DNA synthesis Inhibition of mitochondria respiration and ATP production Increased methemoglobin levels Deactivation of NOS Enzyme inhibition (lipooxygenase, cyclooxygenase; ribonucleotide reductase)
Post-translational modifications	
Nitration	Proteins: Cerulsoplasmin; SP-A; transferrin; albumin; α1-protease inhibitor; actin; α1-antichymotrypsin; MnSOD β-chain fibrinogen Lipids
Oxidation/deamination	Lipids, sulfhydryls, DNA base

Reactive Oxygen/Nitrogen Species as Signaling Molecules

Formation of RNS is related to the inflammatory environment within the lung at specific points in time, which has the potential to generate noxious concentrations of products detrimental to lung function. Production of NO in the lung serves as an important regulator of local functions, including airway tone, pulmonary vascular tone, mucin secretion, ciliary function, and ion channel activity. A number of studies have demonstrated that transcriptional factors (e. g., OxyR [4, 5]), ion channels (e. g., olfactory cyclic nucleotide-gated channel [6]) and enzymes can be activated or regulated by RNS via redox-based modifications of specific thiols within these proteins.

Thiols

NO-derived species, such as nitrosonium ion (NO^+), N_2O_3 and $ONOO^-$ may react with thiols to form nitroso-thiols (RS-NO) [7]. Micromolar concentrations of

S-nitrosoglutathione have been detected in the airway fluid of normal subjects and significantly higher levels were observed in the lungs of patients with pneumonia or during inhalation of 80 ppm NO [8]. Formation of RS-NO adducts stabilizes NO, decreasing its cytotoxic potential while maintaining its bioactive properties. ·NO can also be transported on cysteine residues of hemoglobin which may facilitate efficient delivery of oxygen to tissues [9]. Nitrosylation of the N-methyl-D-aspartate (NMDA) receptor in the brain leads to decreased calcium transport and neuroprotection [10]. On the other hand, ·NO-induced S-nitrosylation of glyceraldehyde-3-phosphate dehydrogenase stimulated the apparent auto-ADP ribosylation and inhibited enzymatic activity [11]. It is important to note that the direct reaction of ·NO with thiol groups is unbalanced and can only occur in the presence of a strong electron acceptor.

Activation of Protein Kinases

NO binds to the heme group of soluble guanylate cyclase (sGC) leading to an increase in cGMP levels. Many effects of cGMP are mediated by various isoforms of cGMP-dependent protein kinase which phosphorylate various substrate proteins, thereby reducing intracellular Ca^{+2} and causing smooth muscle relaxation. NO-mediated increases in cGMP levels also decrease platelet aggregation and adhesion of neutrophils to endothelial cells, thus reducing oxidant load [12]. At lower concentrations, RNS function as signaling molecules (Table 1) regulating fundamental cellular activities such as cell growth and adaptation responses; at higher concentrations they can induce significant cellular injury, apoptosis, and death.

Activation of Nuclear Factor-kappa B (NF-κB)

Among the most important transcription factors responsive to ROS during inflammation and oxidant stress is NF-κB, a transcriptional regulating protein. NF-κB is one member of a ubiquitously expressed family of *Rel*-related transcription factors. This is a family of structurally related eukaryotic transcription factors that are involved in the control of a vast array of processes, including immune and inflammatory responses, growth, development, and apoptosis. The production of ROS, cytokines, or other inflammatory stimuli can activate NF-κB and induce gene expression, eliciting a response generally observed to be pro-inflammatory in nature [13].

Intracellular Ca^{+2}, PKC and MAPK

Evidence also indicates that ROS lead to an increase in intracellular calcium concentrations which correlate with endothelial permeability [14]. Some observations suggest that Ca^{2+} influx occurs through membrane Ca^{2+} channels that are regulated by ·OH generation. Myosin light chain kinase phosphorylation also increases when

endothelial cells are treated with H_2O_2, suggesting that endothelial contraction may play an essential role in oxidant-induced endothelial barrier dysfunction. It appears that an important fundamental requirement for vascular endothelial permeability is the activation of endothelial contraction.

Additional signaling molecules, such as protein kinase C (PKC), mitogen-activated protein kinase (MAPK), tyrosine kinases and Rho GTPases appear vital in mediating endothelial barrier dysfunction. PKC (a family of serine/threonine protein kinases consisting of at least 12 isoforms) is activated in response to oxidants and increases endothelial permeability. In guinea pig lungs [15] pretreated with H-7 (a non-specific PKC inhibitor acting on the catalytic site of the enzyme), there was no increase in the pulmonary capillary filtration coefficient in response to perfusion of H_2O_2. Increases in pulmonary microvascular permeability were accompanied by reorganization of actin cytoskeleton, a process inhibited by PKC inhibitors. The exact mechanism(s) for the role PKC plays in endothelial barrier function is complex but appears due to activation of ROS and probably involves only a few select PKC isoforms. The MAPK pathway is activated by ROS and is an important mediator of cellular responses to oxidant stress. The ERK (extracellular signal-regulated kinases), JNK (c-JUN NH_2-terminal kinase), and p38 cascades all contain the same series of three kinases. A MEK kinase phosphorylates and activates a MAPK, and then MEK phosphorylates and activates a MAPK. Various ROS, most notably H_2O_2, have been demonstrated to mediate endothelial injury via stimulation of ERK pathways. This H_2O_2-mediated action was inhibited by PD-98059, an ERK kinase (MEK) inhibitor. Furthermore, both ROS and RNS induce a variety of actions that are potentially detrimental and include abnormal cell differentiation/proliferation, apoptosis, and DNA damage, with the ERK pathway implicated as playing the predominant role.

Adhesion Molecules

ROS have been shown to promote cellular and molecular events that result in enhanced aggregation and adhesion of leukocytes to endothelium. Prominent inflammatory participants emanating from these investigations include ICAM-1 (intercellular adhesion molecule-1) and selectins (a family of transmembrane molecules, expressed on the surface of leukocytes and activated endothelial cells involved in enhancing leukocyte-endothelial interactions). Investigations in diverse models using a variety of oxidant-generating systems (such as hypoxanthine/xanthine oxidase, H_2O_2, or prolonged hyperoxia) have demonstrated consistent increases in ICAM-1 and P-selectin expression in the vascular endothelium, which promote leukocyte adhesion. Interestingly, expression of these biomolecules is not uniform throughout the vasculature.

Functional Consequences of Protein Nitration *In Vitro*

Surfactant Protein-A (SP-A)

Protein nitration and oxidation by ROS and RNS *in vitro* have been associated with the diminished function of a variety of crucial proteins. Considerable levels of protein-associated nitrotyrosine ($\sim$ 400 – 500 pmol/mg protein), as well as nitrated SP-A were present in pulmonary edema fluid from patients with either acute lung injury (ALI)/ARDS or hydrostatic pulmonary edema, and in bronchoalveolar lavage (BAL) fluid of patients with ARDS [16]. *In vitro* studies have indicated that nitrated SP-A loses its ability to enhance the adherence of *Pneumocystis carinii* to rat alveolar macrophages. Thus, nitration of SP-A may be one factor responsible for the increased susceptibility of patients with ARDS to nosocomial infections. The use of inhaled NO in patients with ARDS was shown to increase both 3-nitrotyrosine and 3-chlorotyrosine (an index of neutrophil activation) concentrations compared to comparable patients who did not receive inhaled NO.

Current In Vivo Evidence Implicating RNS and ROS as Contributors to Lung Injury

Toxicity from oxygen-nitrogen metabolites released by stimulated neutrophils, macrophages and other cells has been proposed as one of the significant mechanisms of lung injury. One of the initial studies published described the effects of inflammation on alpha-1-proteinase inhibitor (α-1-PI), which was found to be inactivated in BAL fluid samples from patients with ARDS [17]. This contrasted to plasma samples from the same patients which retained > 90% α-1-PI activity. The activity of α1-PI IN BAL fluid could be restored by the reducing agent, dithiothreitol, implicating oxidants generated in BAL as being responsible for its loss of function. Shortly after this study, a different group measured expired fractions of H_2O_2, a more stable membrane permeable and volatile oxidant [18]. These samples were collected in patients with normal lungs undergoing elective surgery and critically ill patients suffering from acute hypoxemic respiratory failure. Expired breath condensates of H_2O_2were observed to be significantly greater in patients suffering from acute hypoxemic respiratory failure and focal pulmonary infiltrates than those without pulmonary infiltrates, indirectly implicating increased oxidation. Interestingly, H_2O_2 concentrations were greatest in patients with head injury and sepsis, whether pulmonary infiltrates were present or not. This unexpected finding suggested the participation of oxidants in sepsis and other forms of vital organ injury, such as in brain trauma.

Further studies have continued to create a solid foundation that implicates oxidant generation as a significant contributor to inflammatory-mediated lung injury. In fact, in one of the most recent studies, levels of plasma hypoxanthine, a key cofactor that accumulates during intervals of hypoxia leading to the production of $O_2^{\cdot-}$and H_2O_2, were found to be significantly elevated in patients with ARDS [19].

However, the highest concentrations occurred in patients who did not survive, implicating oxidative damage as an influential contributor to mortality. Higher levels of nitrate and nitrite were also noted in the BAL fluid of patients with ARDS as compared to those of normal volunteers, as well as in the edema fluid of patients with either ARDS or cardiogenic pulmonary edema (Fig. 4) [20, 21].

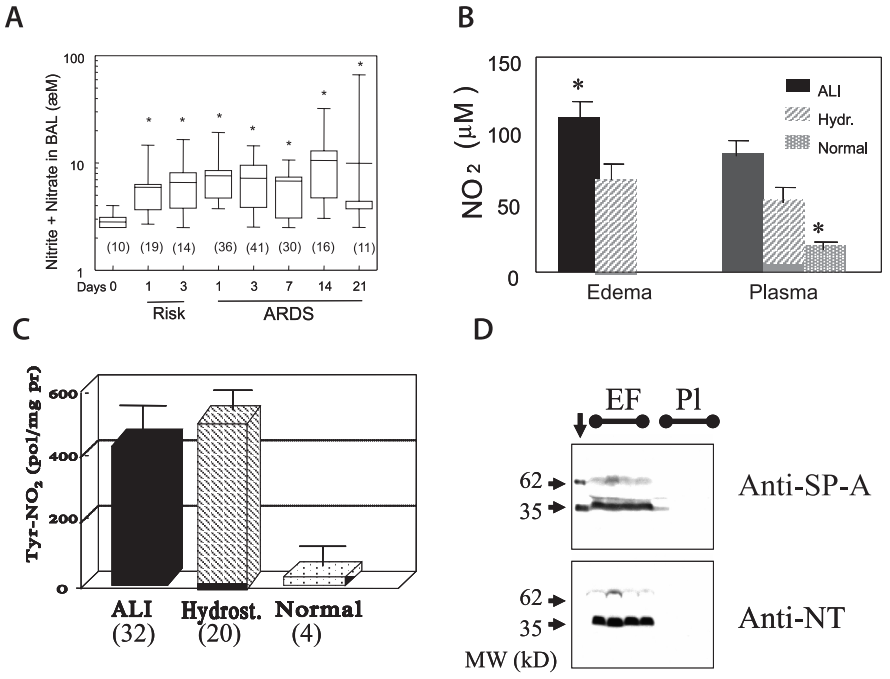

Fig. 4. Evidence for increased levels of reactive oxygen-nitrogen intermediates and nitrated proteins in the bronchoalveolar lavage (BAL), edema fluid (EF), and plasma (Pl) of patients with ARDS and hydrostatic pulmonary edema. (A) Nitrate and nitrite concentration in BAL from normal volunteers (NL), patients at-risk for ARDS (RISK), and patients with established ARDS (ARDS) studied at sequential times. The horizontal axis shows the patient group and the day on which the BAL was performed. (n) = number of subjects in each group. The data are presented as box plots showing the 10th, 25th, 75th, and 90th percentiles and the median. (*) $p < 0.005$ vs. normal subjects (From [20] with permission). (B) Nitrate and nitrite in pulmonary edema fluid and plasma samples from patients with acute lung injury (ALI), patients with hydrostatic edema (hydr.), and normal volunteers. Numbers in parenthesis are sample numbers. Values are means ± SEM (from [16] with permission). (C) Levels of nitrated proteins (measured by ELISA) in the plasma of patients with ALI, hydrostatic edema (hydrost) as well as normal volunteers (normal). Values are means ± SEM (n = number of patients or volunteers) (data adapted from [16] with permission). (D) Nitration of surfactant protein A (SP-A) in pulmonary edema fluid samples from ALI/ARDS patients. SP-A was immunoprecipitated from EF or Pl from four patients with ALI/ARDS. Immunoprecipitated SP-A was probed with polyclonal antibodies to SP-A (anti-SP-A) or nitrotyrosine (anti-NT). Nitrated SP-A was detected in the pulmonary edema fluid but not in the plasma of all patients. Vertical arrow shows purified human SP-A from a patient with alveolar proteinosis. Notice the lack of nitration in the control sample. From [16] with permission

Substantial evidence supports the notion that ROS and RNS are injurious to the pulmonary epithelium in a number of pathological conditions. Induction of immune complex alveolitis in rat lungs results in increased alveolar epithelial permeability, which is associated with the presence of NO decomposition products in the BAL fluid [22]. Moreover, alveolar instillation of the NOS inhibitor, N (G)-monomethyl-L-argnine, ameliorates NO production and alveolar epithelial injury [22]. Infection with pathogens such as *Bordetella pertussis* and influenza is associated with significant increases in NO production [23] and animals infected with *Bordetella pertussis* demonstrated a significant reduction in NO production with NOS inhibition.

The 'Good' Side of NO

Although formation of $ONOO^-$ can result in tissue damage, NO can ameliorate tissue injury by several mechanisms. As mentioned above, NO increases steady state levels of cGMP resulting in vasodilation, and decreased platelet and neutrophil adhesion to endothelium, thereby reducing cell-mediated inflammatory damage. Additional anti-inflammatory mechanisms include downregulation of the NF-κB pathway. The reaction of NO with $\cdot O_2^-$ reduces steady-state levels of O_2^- and limits H_2O_2 buildup, which may be especially important under conditions favoring O_2^--dependent hydroxyl radical formation. Finally, by scavenging lipid radical species, such as alkoxyl (LO·) and peroxyl (LOO·) radicals, NO can inhibit oxidant-induced membrane and lipoprotein oxidation and terminate chain radical propagation reactions. These reactions may be of particular importance, since NO concentrates in lipophilic cellular compartments. However, species resulting from the reaction of NO with lipid peroxides may themselves have biological activity which could be either pro- or anti-inflammatory.

Inhaled NO and ARDS: An ongoing debate

NO initially appeared to possess ideal properties for a selective pulmonary artery vasodilator in patients suffering from ALI/ARDS. In theory, selective pulmonary vasodilation would act on the endothelial surface of the lung to produce regional vasodilation in ventilated lung units, with the net effect being improved PaO_2/FiO_2 ratios and reduced pulmonary artery pressures. In a review of inhaled NO compared to placebo or no therapy administered to patients with acute hypoxemic respiratory failure, it was concluded that inhaled NO produced only moderate improvements in oxygenation and demonstrated no reduction in patient ventilator days or mortality [24]. However, there is agreement that oxygenation generally improves for 24–36 hours, which under certain clinical circumstances and combined with alternative treatment strategies, may lend itself to a multimodal approach to treatment in an individual patient with ALI/ARDS. Potential pitfalls of the recent clinical studies using inhaled NO in the treatment of patients suffering from inflammatory–mediated lung injury include: (1) Oxygenation may have

very little to do with survival in patients suffering from inflammatory-mediated lung injury (as very few patients die of refractory hypoxemia); (2) benefits may have been masked by the negative effects of ventilator-induced lung injury (VILI); (3) long-term inhalation of NO may damage the lung by increasing steady state concentrations of RNS/ROS and thus overshadow their acute physiologic benefit; (4) inhaled NO may have been applied too late after the onset of injury since most enrollment occurred up to 72 hrs after patients presented with ALI. Currently, the only recognized and FDA-approved application for inhaled NO is for the treatment of hypoxic respiratory failure of the term and near-term newborn.

Hypercapnia: An Example of a Radical Quandary?

The effect of carbon dioxide (CO_2) in excess (hypercapnia) and its impact on the generation of ROS/RNS is generating increased clinical interest. Due to the relatively higher concentration of CO_2 in plasma (1.2 mM), the majority of $ONOO^-$ generated in biological fluids will react with CO_2 to form the nitrosoperoxycarbonate anion ($O{=}N\text{-}OOCO_2^-$) [25, 26]. These species are more likely to nitrate and less likely to oxidize proteins. Thus, hypercapnia may either protect or enhance oxidant injury. For example, hypercapnia augmented LPS-induced injury across fetal alveolar epithelial cells *in vitro* [27] and rabbit lungs *in vivo* [28]. On the other hand, hypercapnia and acidosis decreased the inactivation of pulmonary surfactant by plasma proteins [29]. Thus, the precise mechanisms and consequences of hypercapnia are still unknown.

Therapies to Attenuate RNS/ROS-Mediated Lung Injury

While the direct measurement of oxidants poses problems, monitoring of antioxidant concentrations and/or oxidant-antioxidant balance can also be assessed. For instance, levels of selected antioxidants, including plasma ascorbate, a major plasma antioxidant, were significantly decreased in patients with ongoing ARDS when compared to healthy controls [30]. In addition, ubiquinol, a key lipid-soluble antioxidant residing in the membranes of the mitochondria, was significantly decreased in patients suffering from ARDS. Interestingly, α-tocopherol, another plasma antioxidant, was unchanged. In a series of separate experiments, after plasma from a healthy donor was incubated with activated polymorphonuclear cells (PMNs), rapid oxidation of ascorbate was observed. The ubiquinol concentration slowly and steadily decreased over time, whereas α-tocopherol levels remained virtually unchanged. Glutathione (GSH), which is the most abundant nonprotein thiol, is also an important antioxidant, especially for reducing $H_2O_2$and HOCl, which are produced by activated neutrophils. Recently, samples of BAL fluid and epithelial lining fluid were analyzed for GSH in ten patients with ARDS and found to be decreased when compared to healthy controls [31]. Administration of N-acetylcysteine to patients with ARDS significantly improved oxygenation, pulmonary mechanics, and increased total plasma GSH concentrations [31]. Catalase,

a scavenger of H_2O_2, was found to increase in patients with sepsis with and without the eventual progression to ARDS [32]. Interestingly, GSH peroxidase activity was unchanged when compared between control subjects, septic patients without ARDS, and septic patients with ARDS. Additional studies [33] have confirmed that in sepsis and lung injury, antioxidant responses are significantly elevated when compared to control patients. Recently, eight patients with ARDS receiving 'standardized' total parenteral nutrition were compared to 17 healthy individuals, on standard diets without vitamin or trace element supplementation, in an attempt to assess the influence of micronutrients on the oxidative system [34]. Plasma antioxidants and antioxidant enzyme systems were measured at baseline and on days 3 and 6. In addition, the lipid peroxidation product, malondiadehyde (MDA), superoxide anion, and H_2O_2 were measured over the same time points. Plasma levels of α-tocopherol, ascorbate, β-carotene, and selenium were reduced when compared to controls. MDA was significantly increased and was observed to increase significantly over the 6-day interval. The authors concluded that in patients with ARDS, the antioxidant systems are severely compromised, and there is evidence of progressive oxidant stress, as per the steady increase in MDA. Thus, administration of 'standardized' total parenteral nutrition seems inadequate to compensate for the increased requirement for antioxidants in ARDS.

In a contrasting study [35], when patients with ARDS were entered into a prospective, multicentered, double-blind, randomized controlled trial comparing a specialized enteral formulation (Oxepa®) containing fish oil (eicosapentanoic acid), borage seed oil (γ-linoleic acid), and elevated antioxidants (vitamin A, α-tocopherol, ascorbate, and β-carotene) versus an isonitrogenous, isocaloric standard diet, beneficial anti-inflammatory effects were observed, which translated into a reduction in mechanical ventilator days, a decreased length of stay in the ICU, and a reduction in new organ failure. When administered over a 4–7 day interval, the formulation significantly increased the PaO_2/FiO_2 ratio, decreased the production of neutrophils in BAL fluid, and decreased the total cell count in the BAL fluid. Oxidants and antioxidants *per se* were not directly measured, but a decrease in pulmonary inflammation with reduced neutrophil adhesion and oxidant production was observed. In a subsequent study conducted retrospectively by the same group [31], enteral feeding with the same formulation (Oxepa®) resulted in decreased BAL fluid interleukin (IL)-8 and leukotriene B_4 levels, together with a trend towards decreased BAL fluid total protein and neutrophils.

Albumin also has potential antioxidant ability, as a consequence of an exposed thiol group (Cys 34). Quinlan et al. [36] therefore, administered 25 g of albumin solution every 8 hours for a total of 9 doses to patients meeting criteria for ARDS and compared them to a placebo group. In this cohort of patients, supplementation with albumin increased total plasma albumin concentrations and decreased plasma protein carbonyls (a marker of protein oxidation). Positive correlations were found between albumin and plasma thiol concentrations, and thiols and antioxidant capacity. This result was not observed in the placebo group.

Conclusion

Reactive oxygen and nitrogen intermediates, produced by the interaction of NO with partially reduced oxygen species, affect lung function and homeostasis in a variety of different ways. They act as signaling agents and play an essential role in pathogen killing. On the other hand, they may contribute to tissue injury by upregulating genes responsible for the production of inflammatory mediators and by directly nitrating and oxidizing proteins, events known to adversely affect critical functions. A significant challenge to defining their role in lung injury results from their short biological half-lives, and lack of sensitive detection techniques, and the difficulty in deciphering the relevance of the various substrate concentrations to a particular measured response. Thus, many questions relating to the chemical, physiological, pathobiological, and clinical consequences of ROS and RNS generation remain unanswered. Therapeutic strategies, such as enhanced anti-inflammatory and antioxidant therapies are in their infancy in the clinical arena. Hence, this discussion of what is known leads one to realize how much is *not* known with regard to the role of RNS/ROS in lung injury.

References

1. Gow AJ, Farkouh CR, Munson DA, Posencheg MA, Ischiropoulos H (2004) Biological significance of nitric oxide-mediated protein modifications. Am J Physiol Lung Cell Mol Physiol 287:L262–L268
2. Hardiman KM, McNicholas-Bevensee CM, Fortenberry J, et al (2004) Regulation of amiloride-sensitive Na(+) transport by basal nitric oxide. Am J Respir Cell Mol Biol 30:720–728
3. Bruckdorfer R (2005) The basics about nitric oxide. Mol Aspects Med 26:3–31
4. Kim SO, Merchant K, Nudelman R, et al (2002) OxyR: a molecular code for redox-related signaling. Cell 109:383–396
5. Georgiou G (2002) How to flip the (redox) switch. Cell 111:607–610
6. Broillet MC (2000) A single intracellular cysteine residue is responsible for the activation of the olfactory cyclic nucleotide-gated channel by NO. J Biol Chem 275:15135–15141
7. Gaston B, Drazen JM, Loscalzo J, Stamler JS (1994) The biology of nitrogen oxides in the airways. Am J Respir Crit Care Med 149:538–551
8. Gaston B, Reilly J, Drazen JM, et al (1993) Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. Proc Natl Acad Sci USA 90:10957–10961
9. Jia L, Bonaventura J, Stamler JS (1996) S-nitrosohaemoglobin: a dynamic activity of blood involved in vascular control. Nature 380:221–226
10. Lipton SA, Choi YB, Sucher NJ, Pan ZH, Stamler JS (1996) Redox state, NMDA receptors and NO-related species. Trends Pharmacol Sci 17:186–187
11. Molina yVL, McDonald B, Reep B, et al (1992) Nitric oxide-induced S-nitrosylation of glyceraldehyde-3- phosphate dehydrogenase inhibits enzymatic activity and increases endogenous ADP-ribosylation. J Biol Chem 267:24929–24932.
12. Kubes P, Suzuki M, Granger DN (1991) Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 88:4651–4655
13. Janssen-Heininger YM, Persinger RL, Korn SH, et al (2002) Reactive nitrogen species and cell signaling: implications for death or survival of lung epithelium. Am J Respir Crit Care Med 166:S9–S16

14. Vepa S, Scribner WM, Parinandi NL, English D, Garcia JG, Natarajan V (1999) Hydrogen peroxide stimulates tyrosine phosphorylation of focal adhesion kinase in vascular endothelial cells. Am J Physiol 277:L150–L158
15. Johnson A, Phillips P, Hocking D, Tsan MF, Ferro T (1989) Protein kinase inhibitor prevents pulmonary edema in response to H2O2. Am J Physiol 256:H1012–H1022
16. Zhu S, Ware LB, Geiser T, Matthay MA, Matalon S (2001) Increased levels of nitrate and surfactant protein a nitration in the pulmonary edema fluid of patients with acute lung injury. Am J Respir Crit Care Med 163:166–172
17. Gole MD, Souza JM, Choi I, et al (2000) Plasma proteins modified by tyrosine nitration in acute respiratory distress syndrome. Am J Physiol Lung Cell Mol Physiol 278:L961–L967
18. Sznajder JI, Fraiman A, Hall JB, et al (1989) Increased hydrogen peroxide in the expired breath of patients with acute hypoxemic respiratory failure. Chest 96:606–612
19. Quinlan GJ, Lamb NJ, Tilley R, Evans TW, Gutteridge JM (1997) Plasma hypoxanthine levels in ARDS: implications for oxidative stress, morbidity, and mortality. Am J Respir Crit Care Med 155:479–484
20. Sittipunt C, Steinberg KP, Ruzinski JT, et al (2001) Nitric oxide and nitrotyrosine in the lungs of patients with acute respiratory distress syndrome. Am J Respir Crit Care Med 163:503–510
21. Zhu S, Basiouny KF, Crow JP, Matalon S (2000) Carbon dioxide enhances nitration of surfactant protein A by activated alveolar macrophages. Am J Physiol Lung Cell Mol Physiol 278:L1025–L1031
22. Mulligan MS, Hevel JM, Marletta MA, Ward PA (1991) Tissue injury caused by deposition of immune complexes is L-arginine dependent. Proc Natl Acad Sci USA 88:6338–6342
23. Heiss LN, Lancaster JR Jr, Corbett JA, Goldman WE (1994) Epithelial autotoxicity of nitric oxide: role in the respiratory cytopathology of pertussis. Proc Natl Acad Sci USA 91:267–270
24. Lundin S, Mang H, Smithies M, Stenqvist O, Frostell C (1999) Inhalation of nitric oxide in acute lung injury: results of a European multicentre study. The European Study Group of Inhaled Nitric Oxide. Intensive Care Med 25:911–919
25. Berlett BS, Levine RL, Stadtman ER (1998) Carbon dioxide stimulates peroxynitrite-mediated nitration of tyrosine residues and inhibits oxidation of methionine residues of glutamine synthetase: both modifications mimic effects of adenylylation. Proc Natl Acad Sci U S A 95:2784–2789
26. Gow A, Duran D, Thom SR, Ischiropoulos H (1996) Carbon dioxide enhancement of peroxynitrite-mediated protein tyrosine nitration. Arch Biochem Biophys 333:42–48
27. Lang JD Jr, Chumley P, Eiserich JP, et al (2000) Hypercapnia induces injury to alveolar epithelial cells via a nitric oxide-dependent pathway. Am J Physiol Lung Cell Mol Physiol 279:L994–1002
28. Lang JD, Figueroa M, Sanders KD, et al (2005) Hypercapnia via reduced rate and tidal volume contributes to lipopolysaccharide-induced lung injury. Am J Respir Crit Care Med 171:147–157
29. Haddad IY, Holm BA, Hlavaty L, Matalon S (1994) Dependence of surfactant function on extracellular pH: mechanisms and modifications. J Appl Physiol 76:657–662
30. Cross CE, Forte T, Stocker R, et al.(1990) Oxidative stress and abnormal cholesterol metabolism in patients with adult respiratory distress syndrome. J Lab Clin Med 115:396–404
31. Pacht ER, Timerman AP, Lykens MG, Merola AJ (1991) Deficiency of alveolar fluid glutathione in patients with sepsis and the adult respiratory distress syndrome. Chest 100:1397–1403
32. Leff JA, Parsons PE, Day CE, et al (1992) Increased serum catalase activity in septic patients with the adult respiratory distress syndrome. Am Rev Respir Dis 146:985–989
33. Leff JA, Parsons PE, Day CE, et al (1993) Serum antioxidants as predictors of adult respiratory distress syndrome in patients with sepsis. Lancet 341:777–780
34. Metnitz PG, Bartens C, Fischer M, Fridrich P, Steltzer H, Druml W (1999) Antioxidant status in patients with acute respiratory distress syndrome. Intensive Care Med 25:180–185

35. Gadek JE, DeMichele SJ, Karlstad MD, et al (1999) Effect of enteral feeding with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants in patients with acute respiratory distress syndrome. Enteral Nutrition in ARDS Study Group. Crit Care Med 27:1409–1420
36. Quinlan GJ, Mumby S, Martin GS, Bernard GR, Gutteridge JM, Evans TW (2004) Albumin influences total plasma antioxidant capacity favorably in patients with acute lung injury. Crit Care Med 32:755–759l
37. Lang JD Jr, Davis JR, Patel I, Matalon S (2006) Oxidative and nitrosative lung injury. In: Fishma AP, Fishman JA, Grippi MA, Kaiser LB, Senior RM (eds) Fishman's Pulmonary Diseases and Disorders, 4th ed. McGraw-Hill, Columbus (in press)