FORUM EDITORIAL

Neonatal Lung Function and Therapeutics

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Abstract

Respiratory diseases are increasingly recognized as having their origins during perinatal and early postnatal lung development, a time of significant adaptation to large changes in redox conditions as well as to mechanical forces. This Forum of the journal presents a Forum highlighting studies of the interplay between reactive oxygen/nitrogen species and the systems that have evolved to degrade them or exploit them, as well as the cellular repair processes which respond to early life redox stress in the lung. This group of authors suggests new understandings of these events that may point the way to improved therapeutic approaches. *Antioxid. Redox Signal.* 21, 1819–1822.

IN THIS FORUM, leading investigators review several important aspects of pulmonary alveolar development and repair, which are focused mainly on the community of cells that comprise the respiratory/gas exchange unit: vascular cells, alveolar epithelium, and alveolar fibroblasts, and their particular vulnerabilities around the time of birth. Indeed, respiratory diseases—which account for leading causes of death and disability across the human lifespan—are in many cases likely to have their origins during perinatal and early postnatal lung development, a time of significant adaptation to large changes in redox conditions as well as to mechanical forces. Increasing evidence implicates fetal origins of adult respiratory disease. Studies of the mechanisms common to development, injury, and repair suggest that these pathways may be durably affected by early life events, governed in many cases by redox stress.

In a sense, the adaptation from low oxygen concentrations to high concentrations parallels the adaptations seen in evolution with the rise in atmospheric oxygen, as outlined in detail by Rehan and Torday, in this Forum (6). They propose evolutionary pressures that likely drove lung development during major terrestrial environmental changes. A principal example featured in their review is the pulmonary lipofibroblast, which may serve as an oxidative stress sensing cell, although likely one of several. The lipofibroblast responds to the large redox shifts at birth by signaling pulmonary surfactant synthesis in adjacent type 2 pneumocytes. Rehan and Torday suggest the unifying concept that the lung would have

been the first line of exposure subjected to the selection pressure of rising atmospheric oxygen levels, and that pulmonary lipofibroblasts are first observed coincident with the appearance of land mammals. Under control of peroxisome proliferator-activated receptor gamma (PPAR γ), lipofibroblasts differentiate from the pulmonary mesenchymal myofibroblast. In turn, expression of PPAR γ is itself oxygen sensitive: it is upregulated by atmospheric oxygen concentrations, but its signaling effects that maintain lipofibroblast phenotype are downregulated by hyperoxia. Pulmonary adaptation to breathing is supported by lipofibroblast secretion of leptin, which stimulates the adjacent alveolar type 2 cells to produce pulmonary surfactant. Rosiglitazone, a PPAR γ agonist, is able to prevent alveolar simplification and upregulation of pro-fibrotic genes in both mouse and rat models of neonatal lung disease. Stimulation of the PPAR γ may represent a potential future pharmaceutical target in human infants born prematurely, subjected to elevated oxidant stress, but with a relatively impoverished repertoire of defenses.

These defenses, antioxidant enzyme (AOE) systems chief among them, are typically developmentally regulated. Dani and Poggi have described the normal gestational development of the key antioxidant systems, such as superoxide dismutases (SODs), catalase, heme oxygenase (HO), glutathione (GSH), thioredoxin (Trx), and their respective peroxidases and reductases as well as small-molecular-weight antioxidants, including vitamin C and E, which are involved

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in normal transition to extrauterine life (4). These systems are poised to achieve maximal antioxidant potential at the conclusion of a normal, term gestation, so prematurely born infants are inadequately prepared to deal with the oxidant stress during and after birth. Forces exerted by mechanical ventilation in supporting premature infants contribute to the oxidative stress. Berkelhamer and Farrow present a growing body of literature, suggesting that extrauterine oxidative stresses such as mechanical ventilation, hyperoxia, infection, and poor nutrition further impair the development of the AOE systems (1). This produces a feed-forward mechanism that propagates future oxidative injury when these systems have been compromised.

Activation of antioxidant defense responses involves redox-sensitive molecules interacting directly or indirectly with downstream transcription factors. Thiol residues in key positions on proteins often serve as these initial redox sensors: Oxidation or nitrosation exerts steric or allosteric effects on enzyme function. One example is the Keap1/Nrf2 system. When Nrf2 is de-coupled with its cytosolic repressor, Keap1 (via denitrosylation of Keap1) and translocates to the nucleus, where it activates AOE and HO-1 transcription. In her review, Dennery describes how HO-1 is both developmentally and spatially regulated, and has important effects on metabolic determinants of oxidative stress (5). Heme, a pro-oxidant, is degraded and CO, acting as an antioxidant in some cases, is released. In neonatal animals and isolated cells, hyperoxia induces HO-1, which, in turn, leads to cell proliferation of alveolar epithelial cells and pulmonary endothelial cells, but decreased proliferation of pulmonary artery smooth muscle cells. This pattern of cell-type-specific proliferation favors normal alveolarization and formation of capillaries, while preventing muscular remodeling of small pulmonary arteries. These adaptive effects require a narrow concentration, as high-dose HO-1 overexpression leads to epithelial hyperproliferation that interferes with gas exchange.

The thiol redox “sensors” that are susceptible to S-nitrosylation may, in turn, be regulated by Trx. Trx is a dithiol-disulfide protein that is maintained in a reduced state by thioredoxin reductase (TrxR). In his review, Tipple summarizes the role of Trx, and the controlling enzymes, Trx reductases, or peroxiredoxins, which play critical roles in the reduction of hydrogen peroxide, and the denitrosylation of S-nitrosothiols (SNO), both of which can contribute to cell signaling during lung development and repair (8). Genetic deletion of *Txn1* or *Txnrd1* is embryonically lethal in mice, suggesting that these enzymes are indispensable to multicellular postnatal development. Trx is heavily expressed in the airway epithelial cells, where it likely plays a key role in sensing and regulating oxidative stress. In various animal models, TrxR1 activity is reduced in hyperoxia-mediated lung disease, and recent data suggest that administration of recombinant human Trx1 is protective in a variety of animal models of oxidative stress. It is unclear whether the main benefits are attributable to reduction of hydrogen peroxide or to restoration of adaptive denitrosylation. These data suggest that modulation of this pathway may represent a potential therapy to prevent abnormal postnatal lung development subjected to oxidative stress.

Oxidative stress in the newborn lung has effects beyond the alveolus. The emergence of so-called “new BPD” that

develops after neonatal intensive care of very premature newborns which are often rescued with exogenous surfactant therapy has underscored the vulnerability of pulmonary vascular development. While it has been known for some time that *in utero* hypoxia contributes to pulmonary vascular remodeling which can produce pulmonary hypertension (PH) after full-term gestation, it is now being recognized that some premature newborns subjected to oxidative stress may also develop PH. As discussed in their review, Wedgwood and Steinhorn point out that reactive oxygen species (ROS) generation may deplete adaptive intrinsic nitric oxide (NO) signaling which is required for normal involution of pulmonary vascular smooth muscle as well as for normal alveolar development (9). They review their own studies and those of others who have used the therapeutic approach of combined antioxidant therapy with exogenous inhaled NO treatment, which effectively rescues an animal model of PH.

This successful combined approach is one possible answer to the questions raised by the failure of antioxidant “monotherapy” to protect the neonatal lung in clinical application. Clinical trials that have tested single agents—for example, vitamin A, vitamin E, and SOD—have demonstrated only modest effects, if any, as reviewed by Berkelhamer and Farrow and by Dani and Poggi (1, 4). Use of recombinant human superoxide dismutase 1 (rhSOD1) failed to prevent acute bronchopulmonary dysplasia (BPD) in preterm infants, but decreased the incidence of longer-term sequelae of prematurity: wheezing episodes, emergency room visits, and rehospitalizations in the first year of life. This would suggest that neonatal oxidant injury contributes to susceptibility to lung disease in later life. Treatment with retinoic acid, which has antioxidant properties as well as the capacity to signal epithelial proliferation *via* the retinoic acid receptor, was shown to have statistically significant, albeit relatively modest effects on the development of BPD. Clinical trial designs for the use of antioxidant treatments for prevention of lung injury and preservation of lung development have suffered from a paucity of dose-response data. Determining the proper amounts/ratios of antioxidants and the best stage of development/postnatal life at which to intervene remain serious obstacles to therapeutic development.

Since a proper balance between endogenous (or exogenous) NO signaling and endogenous ROS-based signaling would appear to be required for normal lung development, it followed that NO treatment in conditions of neonatal oxidative stress should preserve adaptive NO signaling, thereby promoting normal lung development, which has been demonstrated in several neonatal animal models—rodent, lamb, and baboon—that incorporate oxidative stress $\pm$ mechanical ventilation. However, the results from clinical trials have been mixed, possibly due to major differences in study designs and wide variations in NO doses administered. Another potential obstacle to inhaled NO therapy, described in the original science contribution by Brahmajothi *et al.*, may be the failure to deliver NO “activity” to the proper target (2). In addition to its effects on activation of soluble guanylyl cyclase (sGC), NO signaling is, in part, dependent on formation of SNO, particularly S-nitroso-L-cysteine, which enters alveolar epithelial cells *via* the system L-type amino-acid transporter (LAT) system. Brahmajothi, *et al.* show that SNO uptake and NO gas effects on sGC activation in alveolar

FIG. 1. ROS signaling in the respiratory unit. ROS that are endogenously generated and which are increased under conditions of oxidative stress contribute to signaling (*), or in some cases oppose signaling, as in the case of a reaction with NO, which itself contributes to vascular smooth muscle function (and in the case of neonatal pulmonary vasculature, apoptosis). This regulates blood flow from the PA through the alveolar capillary and PV. ROS signaling in some cases leads to differentiation (Δ) during development and repair. Examples are the differentiation of myofibroblasts (M ϕ) to lipofibroblasts (L ϕ) and type 1 (T1) alveolar epithelium to type 2 (T2). ROS may also oxidize Cys, preventing SNO formation, and possibly SNO uptake during NO treatment. While ROS-mediated injury stimulates recruitment of so-called SCs, to the lung, their role is most likely *via* paracrine signaling of resident progenitor (P) cells. NO, nitric oxide; PA, pulmonary arteriole; PV, pulmonary venule; ROS, reactive oxygen species; SC, stem cell; SNO, S-nitrosothiols. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

epithelial cells and SNO uptake by lung and brain during inhaled NO treatment are substantially dependent on the function of the LAT1 member (gene symbol *SLC7A5*) of the system-L transporter family. Exposure of alveolar epithelium to hyperoxia decreased LAT1 membrane expression and function in the alveolar type 1 and 2 cells *in vitro* and LAT1 expression in alveolar epithelia of newborn rat lung. These findings suggest a novel mechanism for a lack of response to NO acutely and for a failure of NO to uniformly protect lung development in premature infants subjected to oxidative stress. Defects in LAT1 function and/or lack of reduced alveolar cysteine may block SNO formation, uptake by alveolar epithelium, and extrapulmonary SNO transport during NO therapy. Further work is needed to determine whether augmentation of SNO production and/or uptake can enhance the effects of both endogenous and exogenous NO on lung development.

Indeed, the development, differentiation, homeostasis, and repair of the entire respiratory unit community of cells depends on the balance between ROS, RNS, and the systems designed to produce and degrade them, as illustrated in Figure 1. Mitigating the oxidant burden on extant cells may also protect the progenitor cells necessary for the repair processes that would be required to ensure successful lung development during oxidative stress typically encountered by newborns born very prematurely. The reviews by Sdrimas and Kourambanas (7) and by Collins and Thebaud (3) describe the use of technologies that exploit progenitor cells and their products. Many of the studies described in detail in these two reviews have attempted to define the best “stem cell” population to use to treat or prevent neonatal lung disease, which is typically aimed at BPD prevention. The two “front runners” so far are bone-marrow-derived mesenchymal stem

cells and human umbilical cord blood stem cells, and the reviews describe the challenges that remain for therapeutic application. Cells have been delivered both systemically and *via* an endotracheal tube in animal models of neonatal lung injury, and both routes appear effective in preventing/ameliorating abnormal lung development. As these two reviews emphasize, and as is the case with nearly all stem cell therapeutic approaches, cell therapy typically achieves low engraftment, suggesting that the benefits are mediated by paracrine effects on native progenitor cells. The transferred cells do not significantly contribute to the progeny. Conditioned media studies suggest that exosomes may convey the beneficial effects. Exosomes are microvesicles with adhesion molecules on their surface that can target fusion with particular cell populations. They have been shown to convey miRNA that can “instruct” the target progenitor cell. Important gaps that remain to be filled are the ways in which the instructions are determined and the nature of the cellular responses to these instructions. Exosomes may represent a cell-free, relatively well-targeted therapy that may promote endogenous repair.

In conclusion, through this special Forum, the authors have demonstrated the complexity of redox-mediated signaling events that should occur in a specific spatiotemporal pattern in order to ensure normal pulmonary alveolar and vascular development. Any disruption along this complex pathway leads to neonatal lung pathology that may have lasting effects through childhood and into adulthood. It is this complexity that proved to be a stumbling block to developing effective prevention of and treatment for neonatal lung disease. With the advent of stem cell therapy, it may be possible to harness nature to “fix” itself and improve outcomes for both pediatric and adult patients with lung injury.

Author Disclosure Statement

K.N.F. has nothing to disclose. R.L.A. is a co-inventor for U.S. Patent 7,943,667 “Potentiating the effect of compound comprising nitric oxide,” which is licensed to Duke University.

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Abbreviations Used

AOE = antioxidant enzyme
BPD = bronchopulmonary dysplasia
CO = carbon monoxide
HO-1 = heme oxygenase-1
Keap1 = Kelch-like erythroid-cell-derived protein with CNC homology-associated protein
LAT = L-type amino acid transporter
NO = nitric oxide
Nrf2 = nuclear factor (erythroid-derived 2)-like 2
PH = pulmonary hypertension
PPAR γ = proliferator-activated receptor gamma
RNS = reactive nitrogen species
ROS = reactive oxygen species
rhSOD = recombinant human superoxide dismutase
sGC = soluble guanylyl cyclase
SNO = S-nitrosothiols
SOD = superoxide dismutase
Trx = thioredoxin
TrxR = thioredoxin reductase