Neonatal oxygen adversely affects lung function in adult mice without altering surfactant composition or activity

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Yee M, Chess PR, McGrath-Morrow SA, Wang Z, Gelein R, Zhou R, Dean DA, Notter RH, O’Reilly MA. Neonatal oxygen adversely affects lung function in adult mice without altering surfactant composition or activity. Am J Physiol Lung Cell Mol Physiol 297: L641–L649, 2009. First published July 17, 2009; doi:10.1152/ajplung.00023.2009.—Despite its potentially adverse effects on lung development and function, supplemental oxygen is often used to treat premature infants in respiratory distress. To understand how neonatal hyperoxia can permanently disrupt lung development, we previously reported increased lung compliance, greater alveolar simplification, and disrupted epithelial development in adult mice exposed to 100% inspired oxygen fraction between postnatal days 1 and 4. Here, we investigate whether oxygen-induced changes in lung function are attributable to defects in surfactant composition and activity, structural changes in alveolar development, or both. Newborn mice were exposed to room air or 40%, 60%, 80%, or 100% oxygen between postnatal days 1 and 4 and allowed to recover in room air until 8 wk of age. Lung compliance and alveolar size increased, and airway resistance, airway elastance, tissue elastance, and tissue damping decreased, in mice exposed to 60–80% oxygen; changes were even greater in mice exposed to 100% oxygen. These alterations in lung function were not associated with changes in total protein content or surfactant phospholipid composition in bronchoalveolar lavage. Moreover, surface activity and total and hydrophobic protein content were unchanged in large surfactant aggregates centrifuged from bronchoalveolar lavage compared with control. Instead, the number of type II cells progressively declined in 60–100% oxygen, whereas levels of T1α, a protein expressed by type I cells, were comparably increased in mice exposed to 40–100% oxygen. Thickened bundles of elastin fibers were also detected in alveolar walls of mice exposed to ≥60% oxygen. These findings support the hypothesis that changes in lung development, rather than surfactant activity, are the primary causes of oxygen-altered lung function in children who were exposed to oxygen as neonates. Furthermore, the disruptive effects of oxygen on epithelial development and lung mechanics are not equivalently dose dependent.

BRONCHOPULMONARY DYSPLASIA (BPD) is a chronic lung disease often seen in premature infants with very low birth weight (21). At autopsy, lungs of infants who die from BPD are less vascularized, with fewer and larger alveoli (7). Although the pathophysiology of BPD is complex and related in part to gestational age, neonatal hyperoxia is recognized as an important contributing factor to this disease in many infants (see Refs. 3, 12, 17, 37 for review). Premature infants with BPD have low plasma levels of glutathione (59), and hyperoxia in the context of an immature antioxidant defense increases the potential for oxidative stress injury. The use of exogenous surfactant, antenatal steroids, and milder ventilation strategies has markedly increased survival and other improved outcomes for premature infants over the past two decades. However, many patients continue to show decreased lung capacity, even as young adolescents (19, 20, 55). Moreover, these children are often rehospitalized following respiratory infection and are at increased risk for asthma, infection, and other respiratory ailments (60, 67). They also are at higher risk for cerebral palsy, visual and hearing problems, and lower IQ, which are potentially caused by oxidant injury to developing neuronal cells.

A variety of changes at the level of the alveolar epithelium and capillary endothelium have been reported after hyperoxic exposure in animal models. For example, exposure of newborn mice to ≥85% oxygen for 10–14 days disrupts epithelial and endothelial cell proliferation, promotes inflammation, and leads to alveolar dysplasia, fibrosis, and pathological signs of BPD (10, 66). Similarly, premature baboons exposed to hyperoxia exhibit disrupted vascular development, with fewer and larger alveoli that eventually are lined with hyperplastic type II epithelial cells (39, 40). VEGF levels also decline during hyperoxia (33, 41). Since pharmacological inhibition of VEGF disrupts alveolar development (35, 47, 71) and recombinant VEGF partially protects the developing rat lung against hyperoxia (34, 62), the oxygen-dependent loss of VEGF likely contributes to the disrupted endothelial and epithelial development in BPD (1).

Studies in several species of newborn animals exposed to ≥65% oxygen and allowed to recover in room air reveal long-term changes in airway responsiveness and increased lung volumes (13, 16, 46, 58, 70). We previously showed that adult mice exposed as neonates to 100% oxygen for 4 days exhibit increased alveolar size in association with a depletion of type II epithelial cells and an increase of type I cells (50, 70). In contrast, significant changes in endothelium-specific genes were not observed in these mice, implying that vascular defects may be affected only after longer periods of hyperoxia. Since alveolar development occurs over the first 2 wk of life in mice, this short-term (4 day) neonatal oxygen exposure model encompasses the initial phases of alveolar development as relevant for premature infants receiving supplemental oxygen in the immediate postnatal period. The present study extends our prior work in this model (50, 70) to investigate the level of neonatal oxygen exposure sufficient to permanently disrupt lung development and function in adult mice. In addition, experiments directly address whether surfactant-related abnor-
malities, epithelial cell alterations, or both are present and contribute to deficits in pulmonary function/development in adult mice given neonatal oxygen.

**MATERIALS AND METHODS**

*Exposure of mice to oxygen.* Mice were housed in sterile microisolator cages in a specified pathogen-free environment according to a protocol approved by the University Committee on Animal Resources at the University of Rochester. Newborn C57BL/6J mice from several litters were mixed on the morning of birth and randomly separated into groups. Each group was then exposed to room air (21% oxygen) or 40%, 60%, 80%, or 100% oxygen until postnatal day 4, when the oxygen-exposed pups were returned to room air (70). Specific concentrations of oxygen were achieved with 100% oxygen mixed with medical-grade compressed air as needed, and oxygen levels were directly monitored with an oxygen sensor (model TED-60, Teledyne Analytical Instruments, City of Industry, CA). The mixed gas stream was humidified to 40–70% by passage through deionized water-jacketed Nafion membrane tubing (PermaPure) and delivered through a 0.22-μm filter before passage into a sealed Lexan polycarbonate chamber (~32 × 14 × 24 inches (30 liters)). The flow rate was set at 6 l/min, resulting in a complete exchange of gas every 5 min, or 12 changes per hour. Dams were cycled between litters exposed to room air and hyperoxia every 24 h to protect them from acute oxygen toxicity and to ensure that the nutrition provided to the pups exposed to hyperoxia was similar to that provided to the pups exposed to room air.

*Lang mechanics.* At 8 wk of age, five mice were anesthetized with pentobarbital sodium (40 ml/kg). The trachea was exposed and connected to a computer-controlled small animal mechanical ventilator (flexiVent, SCIREQ, Montreal, PQ, Canada) as previously described (48). Mice were injected with pancuronium (1 mg/kg ip) to paralyze the diaphragm and then ventilated with 8 ml/kg at a rate of 150 breaths/min with positive end-expiratory pressure of 2 cmH₂O. Estimated tissue damping and tissue elastance were obtained from the flexiVent by fitting a model to each impedance spectrum (29, 51). After assessment of lung mechanics, lungs were examined for histology and protein expression.

*Lang histology and morphometry.* The left lobe was tied off, and the right lobes were inflation fixed through the trachea for 10 min with 10% neutral-buffered formalin at 16 cmH₂O pressure. The trachea was tied off, the lungs were removed, and the right lobes were further fixed overnight at 4°C. The fixed lobe was dehydrated in graded alcohol and embedded in paraffin. Sections (5 μm) were prepared and stained with hematoxylin-eosin or Hart’s elastin stain (65). Fifteen randomly chosen areas were photographed with a ×10 objective of a microscope (Eclipse 80i, Nikon Instruments, Melville, NY). Mean linear intercepts and mean chord lengths of each image were measured using NIS-Elements AR software (Nikon Instruments). Large airways and vessels identified by the software were excluded from analysis.

*Immunohistochemistry.* Paraffin-embedded sections were rehydrated and incubated with primary antibodies against rabbit pro-surfactant protein C (pro-SP-C; 1:100 dilution; Santa Cruz Biotechnology) overnight at 4°C. Immune complexes were captured with fluorescently labeled secondary antibodies before sections were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Stained sections were visualized with a fluorescence microscope (model E800, Nikon Instruments), and images were captured with a digital camera (SPOT-RT, Diagnostic Instruments, Sterling Heights, MI). Five random images at ×20 magnification were captured from four separate mice, and the number of pro-SP-C-positive cells, normalized to the number of DAPI-positive nuclei per field, was quantified. Images were discarded if they contained >50% airway.

*Western blot analysis.* The left lobe was homogenized with a Polytron in ice-cold lysis buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 2 mM EDTA, 25 mM β-glycerophosphate, 25 mM NaF, 17.4 μg/ml PMSF, 2% Triton X-100, 3.0% Igepal CA-630, 9.5 μg/ml aprotinin, 10 μg/ml Igepal, 10 μg/ml pepstatin A, and 10 μg/ml leupeptin. The lysates were incubated on ice for 20 min and then centrifuged at 14,000 rpm for 10 min. Total protein was measured in supernatants using the BCA protein assay reagent kit (Pierce, Rockford, IL). Equivalent amounts of protein were resolved on Tris–HCl SDS-polyacrylamide gel and transferred to polyvinylidene difluoride membrane. After the membranes were blocked in 5% nonfat dry milk, they were incubated overnight with goat anti-platelet endothelial cell adhesion molecule (PECAM, 1:200 dilution; Santa Cruz Biotechnology), hamster anti-T1α (1:2,000 dilution; Iowa Hybridoma Bank), rabbit anti-pro-SP-C (1:500 dilution; Millipore), rabbit anti-Clara cell secretory protein (CCSP, 1:10,000 dilution; kindly provided by Dr. Barry Stripp, Duke University), or anti-β-actin (1:5,000 dilution; Sigma, St. Louis, MO) antibody and then with the appropriate horse-radish peroxidase-conjugated secondary antibody (Jackson Immuno-Research Laboratories, West Grove, PA). Immune complexes were detected with enhanced chemiluminescence (ECL kit, GE Lifesciences, Piscataway, NJ) and visualized on a Fluorchem gel documentation system (Alpha Innotech, San Leandro, CA) or by exposure to bluesensitive film (Laboratory Products Sales, Rochester, NY). Band intensities were quantified using Image J software.

*Lung surfactant composition and aggregate content.* Bronchoalveolar lavage (BAL) was collected from additional mice by instal-lation of 1 ml of ice-cold saline 10 times into the lung via the trachea. Recovered BAL fluid was immediately centrifuged at 150 g for 10 min to remove cells, and samples from 10 mice from each concentration of oxygen were pooled for analyses of composition and surface activity. Total phospholipid in cell-free BAL fluid was determined by the assay of Ames (2), and phospholipid classes were defined by thin-layer chromatography with a solvent system of chlorormethanol-2-propanol-trichlylamine-water (30:9:25:25:7, by volume) (63). The total protein content of whole cell-free BAL was measured by the Lowry assay (9) modified by the addition of 15% SDS to allow accurate quantitation in the presence of lipid. Large surfactant aggregates were obtained by centrifugation of cell-free BAL fluid at 12,500 g for 30 min and assessed for composition and surface activity. The content of large aggregates as a percentage of total BAL phospholipid was defined by phosphate assay (2), and large-aggregate protein content (total protein and hydrophobic protein following extraction by chlorormethanol) was measured by the SDS-modified Lowry assay (9).

*Surfactant activity studies.* The surface activity of resuspended large aggregates was assessed during cycling at a physiological rate of 20 cycles/min at 37 ± 0.5°C on a pulsating bubble surfactometer (General Transco, Largo, FL; formerly Electronetics, Buffalo, NY) (22). Briefly, an air bubble, communicating with ambient air, was formed in a 40-μl sample of the dispersed surfactant held in a sample chamber. The bubble was pulsed between maximum and minimum radii of 0.55 and 0.4 mm, and surface tension at minimum bubble radius (minimum surface tension, 50% area compression) was calculated as a function of time of pulsation from the measured pressure drop across the bubble interface with use of the Laplace equation for a spherical interface (22, 24). Surfactant samples were examined at a uniform phospholipid concentration of 1 mg/ml in 150 mM NaCl + 2 mM CaCl₂ (pH 7.0).

*Statistical analyses.* Values means ± SD. Group means were compared by ANOVA, and individual conditions were compared post hoc using Fisher’s procedure with Statview statistical software (Abacus Concepts, Piscataway, NJ). P < 0.05 was considered significant.

**RESULTS**

Neonatal oxygen alters lung development and function in adult mice. Newborn mice were exposed to room air (21% oxygen) or 40%, 60%, 80%, or 100% oxygen between post-
nontal days 1 and 4 and returned to room air until 8 wk of age, when changes in lung structure were studied. Alveolar simplification was clearly evident in adult mice exposed to 60% and 80% oxygen and was demonstrably greater in mice exposed to 100% oxygen (Fig. 1, A–E). Alveolar size was quantified by measurement of mean linear intercept and mean chord length of individual alveoli. Alveolar size increased by 15% in adult mice exposed to 60% or 80% oxygen and by 35% in mice exposed to 100% oxygen (Fig. 1F; *P* < 0.03–0.008). Consistent with these histological findings, lung compliance increased in mice exposed to 60% and 80% oxygen as neonates, with further increases in mice exposed to 100% oxygen (Fig. 2A). In contrast to alveolar size and compliance, neonatal oxygen exposure reduced tissue damping, tissue elastance, airway elastance, and airway resistance, but the effects were not equivalently dose dependent (Fig. 2, B–E). For example, tissue damping, a measure of energy dissipated into lung tissues, decreased in mice exposed to 60% oxygen and was not significantly suppressed further with 80% or 100% oxygen. On the other hand, tissue and airway elastance, a measure of energy conservation, was not significantly altered at 60% oxygen but was significantly reduced at 80% and 100% oxygen. Airway resistance was statistically unchanged in mice exposed to 60% or 80% oxygen but was significantly reduced in mice exposed to 100% oxygen.

*Neonatal oxygen disrupts epithelial development.* Our prior work showed fewer alveolar type II epithelial cells and more type I epithelial cells, as defined by reduced expression of pro-SP-C (type II cell marker) and increased expression of T1α protein (type I cell marker) (70), in adult mice exposed to 100% oxygen as neonates. To determine the amount of oxygen needed to alter alveolar epithelial cell development, the expression of cell-specific markers was investigated by Western blotting of lung homogenates prepared from adult mice exposed as neonates to different levels of hyperoxia. Oxygen exposure suppressed expression of the 15-kDa pro-SP-C precursor, with significant changes observed at ≥60% oxygen (Fig. 3, A and B). Quantitation of band intensities revealed that mean levels of pro-SP-C were reduced to the greatest extent in mice exposed to 100% oxygen, although differences were not statistically significant compared with 60% or 80% oxygen (Fig. 3B). A similar pattern of reduction was seen with the expression of CCSP, a protein expressed by airway Clara cells (Fig. 3, A and D). Exposure to ≥40% oxygen increased the expression of T1α in all groups compared with controls (Fig. 3, A and C), whereas expression of PECAM (or CD31) was not significantly altered by oxygen (Fig. 3, A and E).

Immunohistochemistry was used to further assess changes in the number of type II cells associated with reduced levels of pro-SP-C in hyperoxia. Pro-SP-C-positive cells were readily observed in adult mice exposed to room air at birth and were depleted in mice exposed to supplemental oxygen (Fig. 4, A–E). The numbers of pro-SP-C-positive cells were quantified in tissue sections and normalized to the number of DAPI-positive nuclei in the image. The proportion of type II cells in adult mice exposed to room air at birth was 22.1 ± 0.3% (Fig. 4F). This percentage progressively declined in an oxygen dose-dependent manner, reaching a nadir of 10.2 ± 0.1% in mice exposed to 100% oxygen as neonates (*P* < 0.0001). Although the total number of DAPI-positive cells counted in mice exposed to room air (261 ± 22) and 40% (240 ± 41.4) and 60% (249 ± 56.6) oxygen was not different (*P* = 0.3), it significantly declined in mice exposed to 80% (220 ± 46.5) and 100% (214.9 ± 70.6) oxygen (*P* < 0.03). However, the 15–20% reduction in total DAPI-positive alveolar cells in mice exposed to 80% and 100% oxygen did not account for the proportionally greater loss of type II cells in the lungs of these animals (Fig. 4F).

*Neonatal oxygen does not affect surfactant composition or biophysical activity.* The loss of type II cells in adult mice exposed to neonatal hyperoxia has the potential to cause surfactant deficiency and, hence, related deficits in lung me-

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**Fig. 1.** Neonatal oxygen promotes alveolar simplification in adult mice. A–E: representative images of hematoxylin-eosin sections of lungs obtained from adult mice exposed to 21% oxygen (room air) or 40%, 60%, 80%, or 100% oxygen between postnatal days 1 and 4. F: mean linear intercept (MLI) and mean chord length (MCL). Values are means ± SD of 4 animals per group. MLI and MCL were significantly elevated in mice exposed to 60% and 80% oxygen (*P* < 0.03) and 100% O₂ (*P* < 0.008). Bars with the same letters are not significantly different; bars with different letters are significantly different among each other.
mechanics or function. However, surfactant dysfunction would not be consistent with the increases in lung compliance observed in mice exposed to 60–100% oxygen as neonates noted earlier (Fig. 2A). To assess oxygen-induced surfactant dysfunction in our model more fully, experiments directly investigated the composition and surface activity of BAL from adult mice exposed to the maximum level of 100% oxygen for 4 days as neonates compared with room air. The amounts of total phospholipid and total protein in cell-free BAL did not differ between adult mice exposed as neonates to 100% oxygen and those exposed to room air (see supplemental Table 1 in the online version of this article). In addition, the phospholipid class composition of whole BAL was not altered by neonatal hyperoxic exposure (see supplemental Table 2). The percent content of centrifuged large surfactant aggregates in cell-free BAL also did not significantly differ between mice exposed as neonates to oxygen and those exposed to room air (see supplemental Table 1), and large aggregates from these animals had equivalent amounts of total protein and hydrophobic protein (see supplemental Table 3). Finally, direct measurements

Fig. 2. Neonatal oxygen disrupts normal pulmonary mechanics. A–E: lung compliance, tissue damping, tissue elastance, airway elastance, and airway resistance in adult mice exposed to 21% oxygen (room air) or 40%, 60%, 80%, or 100% oxygen [fraction of inspired oxygen (FiO₂)] between postnatal days 1 and 4. Oxygen significantly increased lung compliance (P < 0.002 and P < 0.007) and significantly reduced lung tissue damping (P < 0.05), tissue elastance activity (P < 0.05), airway elastance (P < 0.05), and airway resistance (P < 0.003 and P < 0.009). Values are means ± SD of 5 animals per group. Bars with the same letters are not significantly different; bars with different letters are significantly different among each other.

Fig. 3. Neonatal oxygen alters expression of epithelial cell-specific markers in adult mice. A: immunoblots of lungs of adult mice exposed to 21% oxygen (room air) or 40%, 60%, 80%, or 100% oxygen and treated with antibodies against pro-surfactant protein C (pro-SP-C), T1α, Clara cell secretory protein (CCSP), platelet endothelial cell adhesion molecule (PECAM), and actin (as a loading control). Each lane represents an individual animal, and a total of 5 animals per dose of oxygen were studied. B–E: band intensities for pro-SP-C, T1α, CCSP, and PECAM, normalized to β-actin. Oxygen significantly reduced expression of pro-SP-C (P < 0.04), increased expression of T1α (P < 0.05), and decreased expression of CCSP (P < 0.03 and P < 0.04) but had no effect on expression of PECAM (P > 0.10). Values are means ± SD; n = 5 animals per group. Bars with the same letters are not significantly different; bars with different letters are significantly different among each other.
of surface tension lowering for resuspended large aggregates on a pulsating bubble apparatus showed that surfactant biophysical activity was not different between adult mice exposed to neonatal hyperoxia and those exposed to room air (Fig. 5).

**Neonatal oxygen alters elastin deposition.** The observation that neonatal oxygen had not disrupted surfactant biophysical activity suggested that increased lung compliance might be attributed to changes in elastogenic molecules. To test this hypothesis, we stained lungs of adult mice exposed to varying levels of oxygen as neonates with Hart’s elastin stain. The lungs of mice exposed to room air or 40% oxygen displayed elastin bundles at tips of secondary crests (Fig. 6, A and B). Dense bundles of elastic fibers lining alveolar walls were evident in mice exposed to 60% and 80% oxygen and were widely observed in mice exposed to 100% oxygen (Fig. 6, C and D). These fibers were not observed in mice exposed to room air or 40% oxygen.

**DISCUSSION**

It is generally accepted that hyperoxia, ventilation, and immaturity of antioxidant defenses cause oxidative stress, which promotes BPD and disruption of normal lung development (3, 12, 17, 37). The view that oxidative stress detrimentally affects lung development is clinically supported by the observation that premature infants instilled with recombinant Cu,Zn-SOD had improved pulmonary function at 1 yr of corrected age (15). Consistent with this clinical finding, overexpression of extracellular SOD in respiratory epithelial cells of neonatal transgenic mice preserved type II cell proliferation during the first 3 days of exposure to 95% oxygen (5). However, despite its functional importance, little is known about specific levels of oxidative stress that permanently affect normal lung development and function. In the present study, we provide evidence that a 4-day exposure of neonates to >40% oxygen is sufficient to permanently alter alveolar epithelial development and lung function in adult mice. Neonatal exposure to 60% and 80% oxygen generally caused similar changes, whereas exposure to 100% oxygen was significantly more injurious. However, at this maximum exposure level, there were no apparent changes in surfactant phospholipid composition, large surfactant aggregate content in BAL, large surfactant aggregate protein (total or hydrophobic protein), or large surfactant aggregate biophysical activity. Although these studies do not directly address whether surfactant pool sizes were altered, increased lung compliance was associated with the presence of thick bundles of elastin fibers within alveolar walls.

In the present study, 4 days of neonatal exposure to 40% oxygen minimally affected lung structure and function in adult mice, whereas exposure to 60–80% oxygen was modestly
disruptive and exposure to 100% oxygen was the most severely disruptive. Some degree of pulmonary oxidative stress will always occur at birth, when the developing lungs are exposed to room air for the first time. However, our results indicate that normal (full-term) mouse lungs have the capacity to detoxify levels of stress produced by exposure to 40% oxygen in terms of the pulmonary variables investigated. When neonatal oxidant stress was increased by exposure to 60–80% oxygen, measurable increases in alveolar size and changes in lung mechanics were observed, as were changes in epithelial cell markers and reductions in the number of type II cells. Because 60% and 80% oxygen caused similar changes in lung structure and function, these levels may perturb lung development via a common pathway. Hypothetically, and as shown in HeLa cells, this range of oxygen tensions is sufficient to inhibit cell proliferation and synthesis of RNA and protein (56). Indeed, hyperoxia suppressed tritiated thymidine or bromodeoxyuridine incorporation in neonatal rodents (49, 66). This transient growth arrest of individual cell types may dysregulate the coordinated proliferation and differentiation of epithelial and mesenchymal cells required for alveolar development. Consistent with this concept, type II cell proliferation was temporally shifted in newborn mice exposed to 100% oxygen for 4 days and returned to room air (70). On the other hand, lung structure and function were most severely affected by 100% oxygen, implying involvement of additional mechanisms such as cell death and inflammation (4). We found that the numbers of alveolar cells were significantly reduced in adult mice exposed to 80% and 100% oxygen. Thus loss of progenitor cells critical for normal development may be occurring in mice exposed to high levels of oxygen.

Our results showing reduced pro-SP-C expression and decreased type II cell numbers in adult mice exposed as neonates to 100% oxygen agree with prior work examining pro-SP-C, pro-SP-B, and enhanced green fluorescent protein controlled by the SP-C promoter in this animal model (70). Since type II cells are progenitor cells for type I cells, short-term neonatal hyperoxia followed by long-term recovery in room air may have stimulated the differentiation of type II cells to type I cells. However, the increased expression of T1α did not inversely correlate across different levels of oxygen with the loss of pro-SP-C. This suggests that the increased expression of T1α may reflect an increase in the number of type I cells as well as an increase in the expression per cell. Despite these changes, surfactant composition and activity were unaltered in adult mice exposed to 100% oxygen as neonates. The various surfactant-related assessments used here have been shown to be able to document surfactant dysfunction in multiple animal models of acute lung injury (14, 25, 27, 44, 45, 54, 57, 69). Acute surfactant dysfunction directly following severe hyperoxic exposure in adult animals is well documented (27, 36, 44, 45), but this was not true for the recovered adult animals here that were exposed to 100% oxygen as neonates. Adult mice with neonatal exposure to 60–100% oxygen had increased, rather than decreased, lung compliance, again not consistent with surfactant dysfunction. Our experiments did not specifically measure the concentrations of specific surfactant apoproteins in BAL or large aggregates or whether surfactant levels were different between the groups of mice. However, the equivalent contents of hydrophobic protein in large aggregates from oxygen-exposed and control mice strongly suggest that SP-B/SP-C was not changed substantially in amount and imply that sufficient amounts of these proteins were produced, despite the loss of type II cells in this model. Similarly, the equivalent total protein contents of large aggregates from oxygen-exposed and control mice are consistent with no substantial alteration in the biophysically functional surfactant apoproteins (SP-A, SP-B, and SP-C). Taken together, these findings imply...
that the altered lung mechanics observed in the adult mice studied here are the result of structural changes associated with impaired development as opposed to surfactant dysfunction.

Consistent with this concept, neonatal oxygen altered the balance of alveolar type I and II cells and the deposition of elastin fibers. Hypothetically, an increase in the number of elongated type I cells at the expense of cuboidal type II cells could allow alveoli to be more expandable and, hence, exhibit increased lung compliance. At the same time, thick bundles of elastin fibers were observed within alveolar walls of mice exposed to ≥60% oxygen. Elastin fibers normally localize to tips of alveolar secondary crests, which are attenuated or lost in rodents exposed to neonatal hyperoxia and are replaced by more simplified alveolar structures lined with thick bundles of elastin fibers during recovery in room air (8, 65). These abnormal elastin bundles may contribute to increased expandability or compliance activity measured on the flexiVent (28, 43, 61). Although there are no animal models to confirm this hypothesis, it is supported by experimental silico modeling (6). The increased elastin deposition in the lungs following neonatal oxygen exposure shown in the present study provides a possible mechanism for the airway hyperresponsiveness noted in children born prematurely and treated therapeutically with oxygen as neonates, hence laying the groundwork for future studies in this area (32).

Increased compliance was associated with reduced tissue damping, a measure of tissue rigidity, and tissue elastance, a measure of energy conservation. On the other hand, exposure to ≥60% oxygen reduced airway elastance and airway resistance in adult mice (Fig. 2). Hypothetically, the terminal bronchioles have become less rigid and, hence, more likely to collapse upon end expiration as overdistended alveoli collapse. This would lead to gas trapping and wheezing, symptoms often seen in children who were born prematurely (60, 67). Although many models of emphysema demonstrate alveolar simplification leading to loss of alveolar support and resultant decreased airway stenting and increased airway resistance, our data are consistent with other models of emphysema that result in decreased resistance. Tissue inhibitor of metalloproteinase 3-null mice demonstrate alveolar simplification, as well as decreased resistance, compared with wild-type mice (42). Fetal lambs treated with betamethasone and thyroxine demonstrate alveolar simplification and decreased airway resistance (68). These observations may be a result of a decrease in the length of the airway tree or an increase in the airway radius. The alveolar simplification and increased compliance noted in our model also make it relevant to adult pulmonary emphysema (18, 23, 64). Although the present work supports altered alveolarization, rather than surfactant deficiency/dysfunction, as the primary mechanism of abnormal pulmonary mechanics following neonatal oxygen exposure, the loss of alveoli may dominate any effect observed with surfactant function (26, 38).

It is difficult to extrapolate the dose-dependent effects of oxygen in this study to outcomes in children born prematurely, because levels and duration of oxygen supplementation vary widely among premature infants depending on need. Because lung development was moderately and comparably affected by 60–80% oxygen and more severely by 100% oxygen in our study, interventions that target the reduction of oxidant stress in premature infants exposed to >80% oxygen may have greater potential for efficacy. However, there is clearly variability in available animal studies investigating the effects of neonatal hyperoxic exposure. For instance, altered lung compliance and interstitial thickening have been seen in newborn rats exposed to 40% oxygen for 6 days and allowed to recover in room air for 2 wk (11), whereas lung structure and function were minimally affected by 4 days of 40% oxygen in mice in the present study. Although the use of rats vs. mice may influence these differences, they also likely reflect the length of oxygen exposure. This hypothesis is consistent with an observation that 20 days of supplemental oxygen had little effect on forced expired volume in 1 s (FEV1) assessed in 11-yr-old children, but each additional week of exposure was associated with a progressive 3% loss in FEV1 (31). Thus, reducing the length of postnatal hyperoxic exposure for premature infants may be as beneficial as reducing absolute oxygen exposure levels.

Besides hyperoxia, there is growing evidence that pre- and postnatal environmental factors such as tobacco smoke and infection impact the normal structural development and programming of the lung and immune system (30, 52, 53). Reactive oxygen species are likely to play an important role in this process. The present findings using hyperoxia suggest that the developing lung is responsive to three levels of oxidative stress that lead to normal, modest, or severe deficits in lung structure and function. This may explain why children born prematurely or exposed to environmental pollutants display varying degrees of lung deficits. Understanding how different levels of oxidative stress reprogram normal lung development and, hence, immune functions could provide new opportunities for improving human health.

GRANTS

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