Functional and pathological effects of prolonged hyperoxia in neonatal mice

Barbara B. Warner, Lorie A. Stuart, Richard A. Papes, and Jonathan R. Wispe
Division of Neonatology and Pulmonary Biology, Children's Hospital Medical Center, Cincinnati, Ohio 45229-3039

Infants born prematurely often survive, only to face a lifetime of debilitating diseases that are the result of their prematurity and the therapies necessary to keep them alive. Despite surfactant replacement and technological improvements, chronic lung disease, "bronchopulmonary dysplasia" (BPD), is still the major morbidity of premature birth. In fact, the incidence of BPD has not changed in the past ten years (15).

BPD is caused by acute lung injury in a vulnerable host. Acute pulmonary oxygen injury is complex and multifactorial, but reactive oxygen species are felt to be a major cause of initial injury. Subsequent to the initial injury, there is an influx of inflammatory cells that increases lung damage. Neonatal responses to hyperoxia are unique, probably because injury occurs during the period of alveolar development. One important and uniquely neonatal response to hyperoxia is diminished alveolar development. Massaro et al. (20) and Frank and Groseclose (13) demonstrated that the neonatal period is critical for normal alveolar maturation. Delays or alterations in neonatal lung growth result in permanent architectural abnormalities in alveolar development. In humans, Northway (22) initially described and named BPD and observed that pulmonary immaturity was a consistent factor in its development. The factors regulating normal lung development are incompletely understood, and the effects of hyperoxia or lung injury on them are unknown.

Research into the prevention and treatment of BPD has been hampered by a lack of suitable animal models. In 1978, participants in a National Institutes of Health conference recommended that emphasis be placed on the development of appropriate models (14). Since then, animal models of immaturity have included the rat, rabbit, lamb, and primate. Large-animal models such as the primate offer many advantages and have been extensively studied. Postnatal lung development in primates parallels human lung development and can be subject to a pattern of injury identical to that seen in preterm infants, i.e., ventilation and hyperoxia. The primate model has provided important information regarding the effect of prematurity, ventilation, and hyperoxia on postnatal lung development, surfactant protein expression, and other developmentally regulated genes (7). However, the use of primates is not practical for many investigators, and small-animal models would have several advantages, including cost, ease of maintenance, capacity for genetic manipulation, and the ability to use multiple animals in one study. There is a body of literature that supports the use and validity of rodents for studying neonatal oxygen injury. Bonikos and colleagues (4) were the first to demonstrate that neonatal mice exposed to prolonged hyperoxia developed a chronic lung disease characterized by alveolar dysplasia and fibrosis. More recently, several groups of investigators have used the newborn rat to study the effects of chronic neonatal hyperoxia (13, 16).

The newborn mouse is particularly well suited for studies of neonatal oxygen injury. Murine alveolar development begins on postnatal day 3, and saccul formation is complete by the 14th postnatal day (2). This sequence, and the relative timing of alveolarization, resembles human lung development. In addition, the ability to manipulate the murine genome could prove useful for investigating the role of specific factors in lung injury or prevention. In this report, we use the "Northway protocol" (23) to characterize the morphometrical and physiological consequences of prolonged neonatal oxygen exposure. We demonstrate that neonatal hyperoxia causes alterations in lung alveolarization and function. These changes are closely associated with an early and profound effect on lung cell proliferation.

Methods

Animals and oxygen exposure protocol. The time-dated pregnant FVB/N mice were obtained from Charles River...
Laboratories (Wilmington, MA). Mice were maintained on standard laboratory food and water ad libitum and kept on a 12:12-h light-dark cycle in the accredited Children’s Hospital Animal Care Facilities. All portions of the experimental protocol were approved by the Children’s Hospital Medical Center Animal Care and Use Committee.

Exposure to hyperoxia. Within 12 h of birth, pups from four to eight litters were pooled before being randomly redistributed to the newly delivered mothers. One-half of the newborn pups were placed in 85% oxygen, and one-half remained in room air. Nursing mothers were rotated between oxygen-exposed and room air litters every 24 h to avoid oxygen toxicity in the mothers and to eliminate maternal effects between groups. Oxygen exposures were done in 3-ft³ Plexiglas chambers into which oxygen was continuously delivered at 3.5 l/min. Continuous flow achieved a constant level of 85% oxygen and prevented CO₂ accumulation. Oxygen levels were monitored with a Miniox II monitor (Catalyst Research, Owings Mills, MD). Survival was recorded daily, and mice were exposed to oxygen for up to 4 wk. Body weights were recorded at the time of death.

Tissue preparation. Animals were killed by an intraperitoneal injection of pentobarbital sodium and were exsanguinated by aortic transection. Lungs were fixed in situ with 4% paraformaldehyde in phosphate-buffered saline (pH 7.4). Tracheas were cannulated with a 25-gauge Silastic catheter, and paraformaldehyde was instilled at 25 cmH₂O pressure. After 5 min, lungs were removed and further fixed overnight in 4% paraformaldehyde. The next day lungs were washed in PBS and serially dehydrated in increasing concentrations of ethanol before embedding in paraffin. To standardize analysis, sections were taken from the right lower lobe and upper half of the left lung. Five-micrometer tissue sections were stained with hematoxylin and eosin (H&E), Masson’s trichrome for collagen, or Verhoeff-van Gieson’s stain for elastin.

Morphometrical analysis. Morphometrical measurements were done using Image 1/Metamorph Imaging System, version 2 for Microsoft Windows (Universal Imaging, West Chester, PA). For measurement of the average area of individual terminal air spaces, five-micrometer H&E sections were examined with a microscope that contained a square lattice composed of five intersection lines in the eyepiece. Fields for measurements of terminal air spaces were obtained from the points of intersection lines in the eyepiece. Calibrations for the images were made by acquiring standard micrometer images using the ×10 objective. These standards were then used to determine the number of pixels per micrometer. Air spaces were distinguished from tissue by image intensity and were converted to a red color, and solid tissue was converted to gray. Images were corrected for tears in air space wall extravasated debris, red blood cells, or inflammatory cells. To measure the area of individual terminal air spaces (17), the computer counted the number of pixels shaded in red per air space, and the number of pixels per air space was converted to square micrometers based on the calibrated standards. For measurement of average interstitial air space lengths, ×20 computer images with a superimposed square lattice composed of 10 intersecting lines was used. Measurements of terminal air spaces were obtained from the points of intersection lines. For each animal a minimum of 10 lung fields was used for the analysis. Measurement of each alveolar area and interstitial length were exported to Microsoft Excel (Microsoft, Redmond, WA).

Proliferative index. Rates of cell proliferation were determined using bromodeoxyuridine (BrdU) as a marker for dividing cells. BrdU is a thymidine analog that is incorporated into replicating DNA. Neonatal mice were injected subcutaneously if they were ≤1 wk of age and intraperitoneally if they were >1 wk of age. BrdU (ZYMED, San Francisco, CA) concentrated aqueous solution was used according to the manufacturer’s instructions (1 ml/100 g body wt). The concentrated aqueous solution was diluted 1:3 in sterile normal saline and warmed to 37°C before injection. Six hours later, mice were given a lethal injection of pentobarbital sodium, and lungs were fixed as described above. Incorporated BrdU was detected with a monoclonal anti-BrdU antibody and a streptavidin-biotin staining system. Tissue sections were counterstained with hematoxylin to identify unlabeled nuclei. Lung sections were examined using the ×40 objective, and BrdU-labeled cells and unlabeled cells were counted using the Metamorph Imaging System. A proliferative index was calculated with the equation (BrdU nuclei/total nuclei) × 100. For each animal a minimum of 10 lung fields was used for the analysis.

Lung lavage. Selected mice underwent lung lavage to recover alveolar cells. Mice were given a lethal dose of pentobarbital sodium and were placed in a supine position, and tracheas were cannulated as above. Room temperature normal saline was gently instilled by syringe until resistance was felt. After 1–2 min, the saline was withdrawn and replaced with 4 ml of saline. Each animal was lavaged five times, and lavage fluid was pooled and centrifuged at 800 rpm for 5 min. Lavage samples were not used unless 80% of instilled saline was recovered. Red blood cells present in the bronchoalveolar lavage (BAL) were lysed with a solution of 0.15 M potassium chloride, and the cell suspension was centrifuged at 800 rpm for 10 min. Cells were resuspended in 0.5 ml of PBS. Total cell counts were performed with a hemocytometer, and differential cell counts were performed on cytopsin preparations stained with Diff-Quick (Scientific Products, McGaw Park, IL). A blinded observer counting a minimum of 200 cells obtained the cell differentials.

PCR analysis of inflammatory cytokine mRNA. The expression of inflammatory cytokines in the lungs of neonatal mice exposed to oxygen or room air was analyzed by RT-PCR. Lungs were recovered, homogenized in 1 ml of 4 M guanidine-thiocyanate, 0.5% N-lauroylsarcosine, 20 mM sodium citrate, and 0.1 M β-mercaptoethanol. Total cellular RNA was isolated and purified using 5 M CsCl (23). The RNA pellet was dissolved in water, extracted with phenol and chloroform, and precipitated at –80°C with sodium acetate and absolute ethanol. Recovered RNA was quantitated spectrophotometrically, and 2 μg of RNA were used for individual RT reactions.

Reagents for the RT reactions were obtained from Gibco (Gaithersburg, MD) and used according to their protocol. Reaction mixtures were incubated at 42°C for 1 h. The reaction was stopped by boiling at 95°C for 5 min, and the product was stored at –20°C. Primers were designed using the software program Oligo (National Biosciences, Plymouth, MN). Total RNA was reverse transcribed into cDNA using the following downstream/upstream primer pairs: mouse IL-11 (5′-ACC ATG CTA CAG AC-3′/5′-GGC ACA TCA GGT ACG ATC CAG-3′), resulting in a 287-bp product; mouse macrophage inflammatory protein (MIP)-1α (5′-ACC ATG CTA CAG AC-3′/5′-GGG AAT CCA GGG GAA ACA CTG-3′), resulting in a 308-bp product; mouse tumor necrosis factor-α (TNF-α) (5′-GCC AGG TGG AAC TGG CAG AAG-3′/5′-GGT ACA ACC ATG CTA CAG AC-3′), resulting in a 277-bp product; mouse macrophage inflammatory protein (MIP)-1β (5′-TGG AAT CCA GGG GAA ACA CTG-3′/5′-ACC TGG CCA GAG GTT TTC-3′), resulting in a 287-bp product. Reagents and protocol for PCR reactions were obtained from Stratagene (La Jolla, CA). The PCR
EFFECTS OF PROLONGED HYPEROXIA IN NEONATAL MICE

RESULTS

Survival and growth of neonatal mice exposed to hyperoxia. Sixty percent of neonatal mice exposed to hyperoxia survive, with the majority of deaths occurring between 3 and 7 days of life. Prolonged neonatal exposure to hyperoxia adversely affected growth. Oxygen-exposed mice weighed an average of 8% less at 1 wk, 13% less at 2 wk, 15% less at 3 wk, and 26% less at 4 wk compared with room air controls. Activity level and neurological status were not obviously different between oxygen- and room-air-exposed mice.

Effects of neonatal hyperoxia on lung development. The murine lung matures postnatally, with alveolarization occurring in the first 2 wk of life (2). Continuous exposure of newborn mice to 85% oxygen for 4 wk resulted in marked changes in alveolar development and lung growth. This change was characterized by a simplification of acinar structure, with a decreased number of alveoli and greatly enlarged terminal airways (Fig. 1). Terminal air space areas after 3, 7, 14, 21, and 28 days of oxygen exposure are presented in Table 1. In room air mice, terminal air space size decreased with age, a pattern consistent with progressive alveolarization. In contrast, terminal air size increased over time in oxygen-exposed pups. After 1 wk, terminal air spaces were significantly larger in oxygen-exposed pups compared with room air controls, and the differences persisted throughout the 4-wk experimental period.

The change in terminal air space size is accompanied by increased collagen deposition. Increased collagen deposition was evident after 2 wk of oxygen exposure, increasing through 4 wk (Fig. 1). Collagen was diffusely distributed and increased progressively with continued exposure to oxygen. Morphometrical analysis of interstitial thickness demonstrated significant increases in mean interstitial lengths by 3 and 4 wk of oxygen exposure compared with room air controls (Table 2). Lung injury is often associated with changed elastin structure or distribution (5). Light level examination of elastin in oxygen-exposed animals over time did not show any differences from room air controls.

In summary, chronic neonatal exposure to 85% oxygen resulted in markedly decreased alveolarization, increased collagen deposition, and increased interstitial thickness.

Effect of neonatal hyperoxia on lung cell proliferation. It is crucial to identify the mechanisms involved in decreased alveolarization caused by neonatal hyperoxia. Alveolarization could be decreased if proliferation of epithelial or mesenchymal progenitor cells was decreased. To determine whether the decreased alveolarization was associated with decreased cell division, lung cell proliferation was measured. BrdU was used to identify dividing cells, and proliferation indexes were calculated in mice exposed to oxygen or room air (Table 3). In room air mice, proliferative indexes begin high (30 ± 10% on third day of life) and decrease significantly over time (ANOVA P < 0.05). In oxygen-exposed mice, proliferative indexes were significantly lower at 3
and 5 days (P < 0.05) compared with room air littermates. After 2 wk of hyperoxia, cell proliferation was slightly increased in oxygen-exposed mice, whereas proliferation was steadily decreasing in room air littermates. By 4 wk, there was no difference in cellular proliferation between oxygen-exposed pups and room air littermates. No statistically significant change in proliferation was found over time in oxygen-exposed animals. These results demonstrate that prolonged and early neonatal hyperoxia has profound effects on lung cellular proliferation.

Oxygen-induced lung inflammation. In adult rodents, hyperoxia is associated with lung inflammation, and it is presumed that these inflammatory cells exacerbate injury. Less is known about inflammation in neonatal pulmonary oxygen injury. We studied lung inflammation by 1) examining lung sections for inflammatory cells; 2) quantitating inflammatory cells in lung lavage; and 3) measuring the expression of proinflammatory cytokines during hyperoxia.

Histology. A mild inflammatory response was seen in histological sections from oxygen-exposed mice. The inflammatory cells were located mainly in the perivascular and peribronchiolar regions. The inflammation is maximal after 2 wk of hyperoxia and diminished thereafter.

Fig. 1. Architectural changes and collagen deposition in lungs from 4-wk-old mice exposed to 85% oxygen or room air from birth. Lung sections were stained for collagen with Masson's trichrome stain, which stains collagen blue. Lung sections are from the right lower lobe of oxygen-exposed mice (A) and room air littermates (B). Original print magnification was ×437.
Table 1. Median area of terminal airways

<table>
<thead>
<tr>
<th>DOL</th>
<th>Room Air</th>
<th>Oxygen</th>
<th>Room Air</th>
<th>Oxygen</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Median, µm²</td>
<td>Range, µm²</td>
<td>Median, µm²</td>
<td>Range, µm²</td>
</tr>
<tr>
<td>3</td>
<td>3 934 822–1,046</td>
<td>3 1,112 732–3,769</td>
<td>7 681 460–1,554</td>
<td>9 1,506 1,206–2,260*</td>
</tr>
<tr>
<td>7</td>
<td>7 412 399–580</td>
<td>11 2,789 1,882–4,420*</td>
<td>14 380 324–481</td>
<td>9 4,343 1,236–7,390*</td>
</tr>
<tr>
<td>21</td>
<td>21 170 207–240</td>
<td>11 4,762 1,260–10,000*</td>
<td>38 350 340–560</td>
<td>12 2,789 1,220–5,280*</td>
</tr>
</tbody>
</table>

DOL, day of life; n, no. of animals used per group; median, median individual air space area expressed in µm² (see METHODS). *P < 0.05 in comparison between oxygen-exposed and room air littersmates.

Lung lavage. To quantitate and more clearly identify the cellular components of the inflammatory response, BAL material from oxygen-exposed and room air mice was analyzed. Results from BAL paralleled what was evident on histological sections. The total number of inflammatory cells was increased in BAL material after 14 days of hyperoxia (Table 4). In room air mice, the majority of lavaged cells were alveolar macrophages or mononuclear white cells. After exposure to oxygen, the number of polymorphonuclear white cells was increased. This neutrophilic response peaked after 2 wk of hyperoxia, with neutrophils accounting for one-third of the cells.

Analysis of cytokine expression. The expression of neutrophil chemotactic cytokines was measured by RT-PCR to begin to identify the factors responsible for the inflammatory response. Expression of TNF-α, IL-1α, MIP-2, and MIP-1α was compared in lungs of hyperoxia or room air mice (Fig. 2A). IL-1α and MIP-1α mRNA were increased after 3 days of hyperoxia and remained elevated through 14 days. Densitometric analysis of PCR products demonstrated a modest increase of 50–80% in IL-1α over 5–14 days (Fig. 2B). More significantly, the change in MIP-1α increased an average of 300–400% over the 5- to 14-day time period (Fig. 2B). TNF-α and MIP-2 expression was not different between oxygen and room air mice.

In summary, histological sections and BAL from oxygen-exposed newborn pups demonstrate a polymorphonuclear inflammatory response after 2 wk of oxygen exposure. Increased expression of IL-1α and MIP-1α precedes the neutrophil influx, implicating a potential role for these neutrophil chemotactic cytokines in the inflammatory response.

Effect of neonatal hyperoxia on lung function. Lung function was tested to determine if there were functional correlates to the oxygen-induced changes in lung anatomy. Pulmonary function was studied in spontaneously breathing mice by whole body plethysmography (system model RSP 200E; Kent Scientific). For these studies, the same groups of mice were studied repeatedly to follow changes in lung function during continuous exposure to hyperoxia or room air. Noticeable changes in lung function developed by 2 wk of age, which progressed over time. In mice exposed to hyperoxia for 28 days, respiratory rates were decreased. The slowed rate of breathing was due to prolonged inspiratory and expiratory times, with decreased inspiratory and expiratory flow rates (Table 5).

To further evaluate the effects of neonatal hyperoxia, lung pressure-volume relationships and total lung volumes were compared between oxygen-exposed mice and room air controls. Figure 3 shows an inflation-deflation curve of one 4-wk-old mouse exposed to hyperoxia and one exposed to room air. Very similar pressure-volume curves were obtained from all other mice examined after a 28-day exposure to hyperoxia or room air. The lungs of oxygen-exposed mice are less compliant than those of room air mice. At any inflation pressure, lung volume is less in oxygen-exposed mice. Total lung volume, measured as the volume necessary to achieve a pressure of 30 cmH₂O, was significantly decreased after prolonged exposure to hyperoxia. V₃₀ was 0.65 ± 0.06 ml (n = 8) in oxygen-exposed mice and 1.0 ± 0.1 ml (n = 6, P < 0.05) in room air mice. These

Table 3. Cellular proliferation in lungs from neonatal mice exposed to O₂ or room air

<table>
<thead>
<tr>
<th>Days</th>
<th>n</th>
<th>RA, %</th>
<th>n</th>
<th>Oxygen, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>9</td>
<td>30±10</td>
<td>11</td>
<td>8±4*</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>19±3</td>
<td>8</td>
<td>6±2*</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>13±7</td>
<td>8</td>
<td>6±3</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>14±6</td>
<td>7</td>
<td>9±5</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>6±3</td>
<td>7</td>
<td>16±5*</td>
</tr>
<tr>
<td>21</td>
<td>3</td>
<td>3±1</td>
<td>5</td>
<td>10±4*</td>
</tr>
<tr>
<td>28</td>
<td>6</td>
<td>3±2</td>
<td>6</td>
<td>8±6</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of animals used for each group. Results are reported as the percent of cells labeled with bromodeoxyuridine (BrdU). Proliferative indexes were determined as described in METHODS, with BrdU used to label dividing cells. For each animal 10 lung fields were analyzed. *P < 0.05 in comparison between oxygen-exposed and room air (RA) littersmates.

Table 4. Bronchoalveolar lavage cell counts and differentials

<table>
<thead>
<tr>
<th>DOL</th>
<th>Group</th>
<th>n</th>
<th>Total Cells (x10⁴)</th>
<th>PMN</th>
<th>Mono</th>
<th>Lymph</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>RA</td>
<td>3</td>
<td>3±3</td>
<td>1±1</td>
<td>98±3</td>
<td>1±1</td>
</tr>
<tr>
<td></td>
<td>Oxygen</td>
<td>3</td>
<td>4±5</td>
<td>12±8*</td>
<td>86±8*</td>
<td>2±3</td>
</tr>
<tr>
<td>14</td>
<td>RA</td>
<td>6</td>
<td>19±18</td>
<td>2±1</td>
<td>98±2</td>
<td>2±2</td>
</tr>
<tr>
<td></td>
<td>Oxygen</td>
<td>6</td>
<td>21±16</td>
<td>30±3*</td>
<td>67±5*</td>
<td>2±1</td>
</tr>
<tr>
<td>28</td>
<td>RA</td>
<td>3</td>
<td>74±12</td>
<td>1±2</td>
<td>99±1</td>
<td>0±1</td>
</tr>
<tr>
<td></td>
<td>Oxygen</td>
<td>3</td>
<td>75±22</td>
<td>4±3</td>
<td>93±5</td>
<td>2±2</td>
</tr>
</tbody>
</table>

Results are means ± SD and are expressed as the total number of cells × 10⁴/ml, with differentials expressed as the percentage of the total count; n, no. of animals/group. PMN, polymorphonuclear neutrophils; Mono, monocytes; Lymph, lymphocytes. *P < 0.05 compared with room air.
studies demonstrate that prolonged exposure of mice to hyperoxia results in decreased lung compliance. These functional changes are very similar to those seen in infants with BPD.

DISCUSSION

In this study, we demonstrate that prolonged exposure of neonatal mice to hyperoxia resulted in dysplastic lung growth with decreased alveolarization, increased collagen deposition, and increased interstitial thickness. Decreased alveolarization began early and was preceded by decreased proliferation of lung cells during the critical period of alveolar septation. With continued exposure, a mild inflammatory response became evident, which resolved over time. Architectural and histological changes were associated with changes in lung function. Oxygen-exposed animals had decreased lung volume, decreased pulmonary compliance, and decreased inspiratory and expiratory flow rates. The anatomical and functional changes in neonatal mice exposed to chronic hyperoxia are very similar to those seen in infants with BPD.

The damaging effects of hyperoxia on the mature lung have been well described (8). Less is known about neonatal pulmonary oxygen injury, but it is clear that the neonatal lung responds to hyperoxia and lung injury in a unique manner. Presumably, this is because injury occurs on the template of rapid neonatal lung growth and maturation. It appears that there is a critical period during postnatal lung development when disruptions of the temporal or anatomical patterns of lung maturation result in permanent changes (13, 20). Infants with neonatal lung injury often never recover full lung function, and pathological examination of their lungs later in life shows persistent abnormalities in lung morphology. It is critical to develop an understanding of the mechanisms by which oxygen adversely impacts lung maturation. Our results suggest that the mouse can be used for such investigations.

The hallmark features of BPD are decreased number of alveoli, increased variability in alveolar size, and interstitial fibrosis (22, 24). Northway and others (16, 23, 24) have described the effects of continuous exposure of neonatal animals to hyperoxia. In mice exposed to 80% oxygen for 2 wk or longer, lung volume and alveolar surface area were decreased, as was alveolar surface area (23). We have extended this observation, demonstrating that changes in alveolarization occur very quickly. By 1 wk of age, oxygen-exposed mice had significant changes in alveolar size and number.

The most impressive effects of neonatal hyperoxia were on lung cell proliferation. These effects were early and profound, suggesting that the effect of hyperoxia on cell division is central to dysplastic alveolar growth. Exposure to 85% oxygen for 3 days markedly inhibited cell proliferation, which remained decreased through the 1st wk of life. This is precisely the period of maximal alveolarization in mouse lung. There was a slight increase in proliferation after 2 wk in hyperoxia. By 4 wk of age, proliferative indexes were the same in oxygen-exposed or room air mice. In contrast to our finding of a very early effect from hyperoxia, Han et al. (16) recently reported that in neonatal rat lung the major impact of 60% oxygen on lung DNA synthesis occurred during the 2nd wk of exposure. The differences between that study and ours may be explained by the difference in oxygen exposure (85 vs. 60%) or species (mouse vs. rat). Both studies demonstrated a biphasic effect of hyperoxia on cell proliferation, with
and MIP-1α increases early during oxygen exposure, suggesting that they might be important. Additionally, expression of IL-1α and MIP-1α increased early during oxygen exposure, before any obvious tissue injury. The timing of the increases for IL-1α and MIP-1α suggests that they may be important in modulating subsequent lung inflammation.

There is abundant literature about the histological changes found with pulmonary oxygen injury, but less is known about the functional changes of hyperoxia, particularly in small animals. Recent advances in small-animal physiology allow us to compare the functional effects of neonatal hyperoxia in mice and humans. The changes in lung volume and compliance are remarkably similar in mice after neonatal hyperoxia and human infants with BPD. Despite these similarities, the respiratory rates were decreased in hyperoxia-exposed mice, whereas infants with BPD are usually tachypnic. This may be due to differential effects of hyperoxia on the small-resistance airways or reflect the additive injuries of hyperoxia and barotrauma in ventilated infants.

Recent progress has been limited in clarifying mechanisms of neonatal lung dysplasia. A major factor in this slow progress has been debate over suitable models (9). The preterm infant is biologically unique, and models must be relevant for neonatal adaptation and postnatal lung growth and development. Immature nonhuman primates are very valuable in the study of neonatal lung injury because of their developmental similarities and because they can be mechanically ventilated. Lung injury and fibrosis in the oxygen-exposed primate are similar to that found in preterm neonates. The pattern of injury found in our mouse model is homogeneous, lacking the alternating areas of compression and over-expansion seen in ventilated infants with BPD. However, large animals are expensive and require high-technology intensive care facilities. They are also not suited for studies in which genetic manipulations can be used to study injury or to test therapeutic strategies. The mouse has a long history in the study of pulmonary oxygen injury. Pappas et al. (23), after initially describing the architectural changes in humans with BPD, demonstrated that similar changes were produced in neonatal mice by prolonged exposure to hyperoxia. This report extends these findings. We report that the decrease in alveolarization common to many models, and a prominent feature of BPD, is associated with a decrease in cell proliferation that occurs in neonatal mice exposed to hyperoxia. The architectural changes are associated with functional changes that parallel those found in BPD. In contrast to other models of oxygen injury in which there is an intense inflammatory response, the inflammatory response in oxygen-

![Fig. 3. Effect of hyperoxia on lung compliance. Four-week-old mice continuously exposed to 85% oxygen or room air were killed, and lung compliance was evaluated by pressure-volume curves as described in METHODS. Figure is a representative pressure-volume curve, with closed circles representing an oxygen-exposed mouse and closed squares representing a room air mouse.](http://ajplung.physiology.org/)
exposed mice is mild, resolving over a period of 4 wk. This difference may arise from the level of oxygen used, developmental age, and species of animals used.

We demonstrate that prolonged exposure of neonatal mice to hyperoxia created a lesion that is anatomically and functionally similar to the human disease. We will use this model in the future to explore the altered cell division and to determine the role of cytokines and growth factors in lung injury and repair.

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REFERENCES


