The Effect of Neonatal Hyperoxia on the Lung of p21Waf1/Cip1/Sdi1-Deficient Mice

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Hyperoxia is an important factor in the development of bronchopulmonary dysplasia and is associated with growth arrest and impaired alveolar septal development in the neonatal lung. p21Waf1/Cip1/Sdi1 (p21), a cyclin-dependent kinase inhibitor, acts as a checkpoint regulator in the cell cycle during periods of stress and is induced in neonatal lung during hyperoxia exposure. To determine if p21 protects against lung injury during neonatal lung development, we placed newborn p21 knockout (p21−/−) and p21 wild-type (p21+/+) mice in 85–90% O2 for 4 d. We found that newborn p21−/− mice exposed to O2 had decreased survival in hyperoxia compared with p21+/+ mice (P < 0.01). At 2 and 6 wk after exposure to neonatal hyperoxia, p21−/− O2 lung had significantly larger alveoli than p21+/− control lung, as assessed by mean alveolar size and mean linear intercept. Pulmonary function tests at 6 wk demonstrated increased lung volume in both p21−/− and p21+/+ O2 mice consistent with altered lung growth from neonatal exposure to hyperoxia. Antibodies to nitrotyrosine, a marker for oxidative stress revealed with altered lung growth from neonatal exposure to hyperoxia. p21Waf1/Cip1/Sdi1-Deficient Mice

The development of chronic lung disease in infancy remains a significant health problem, particularly in the premature infant. It has been known for many years that hyperoxia, when given to premature infants, is associated with the development of bronchopulmonary dysplasia (BPD). The lungs of infants with BPD are frequently characterized by enlarged simplified alveoli and overall growth arrest (1, 2). Fortunately, because the human lung has the potential for postnatal lung growth, many infants with BPD clinically recover. However, a significant minority of infants with BPD go on to develop chronic respiratory symptoms that are manifested by decreased pulmonary reserve and increased airway reactivity (3). Some of these children have even required lung transplantsations later in life (4).

Few models have been developed to study the impact of neonatal lung injury on adult lung function, particularly because most injury models are performed in adult animals. The neonatal lung, however, may respond quite differently than the adult lung to injury, with the consequence of neonatal injury resulting in impaired alveolar growth. In the human infant the majority of postnatal lung growth occurs in the first 2 yr of life (5). Therefore, the outcome of lung function in the adult may be dependent on the postnatal time period in which the injury occurs.

In this study we propose that p21Waf1/Cip1/Sdi1 (p21) protein protects postnatal lung during hyperoxic injury. p21, in addition to its role as a checkpoint regulator in the cell cycle, is also involved in cell differentiation and DNA repair (6–9). Recently it has been shown that adult p21 knockout (p21−/−) mice are more sensitive to hyperoxia than wild-type mice. At 10 d after recovery their lungs demonstrate evidence of pulmonary remodeling and increased cell proliferation (10, 11). We and others have shown that p21 is induced in the neonatal lung during hyperoxia (10, 12–14). p21 induction and its role in neonatal lung injury, however, has not been thoroughly investigated. We therefore hypothesize that induction of p21 during hyperoxia is important for attenuating neonatal lung injury and that p21−/− mice exposed to neonatal hyperoxia develop lung injury that is manifested by increased oxidative stress. Induction of p21 during hyperoxia may attenuate lung injury by causing growth arrest, which in turn allows the cell to undergo either DNA repair or apoptosis resulting in a lower oxidative stress burden to the lung.

To test our hypothesis we exposed neonatal p21+/+ and p21−/− mice to hyperoxia for 4 d, measured the effect of hyperoxia on overall survival, assessed lung growth and function, and quantified oxidative stress at different ages after the acute lung injury.

Materials and Methods

Mice

All animal experiments performed were done according to animal protocols approved by the Animal Care Use Committee of the Johns Hopkins University School of Medicine. Wild-type (p21+/+) and p21−/− mice of similar genetic background 129/Sv × C57BL/6p21−/− (obtained from Jackson Labs, Bar Harbor, ME) were bred and pups were used for the hyperoxia experiments. Some studies used heterozygote (p21+/−) mice bred from p21−/− and p21+/+ crosses. On day of life (DOL) #1 neonatal pups and mothers were placed in a hyperoxic chamber. The oxygen concentration was maintained between 85 and 90%. Mothers were rotated every 24 h to prevent injury from acute oxygen toxicity. At the end of a 4-d exposure period mice were removed from hyperoxia and allowed to recover in room air. Mice were killed at 2 and 6 wk of age.

Immunocytochemistry

Tissue processing. Lungs were infused through the trachea with 0.5% agarose at 50°F under 25 cm of H2O pressure and then cooled on ice. Lungs were then placed in either formalin or 4% parafomaldehyde overnight and then paraffin-embedded. Five-micron histologic sections were deparaffinized using xylene. Antigen retrieval was performed using citrate buffer for 30 min and slides were washed. Slides were blocked with 3% H2O2 for 15 min. The following antibodies were used: Ki67 antibody, at a concentration of 1:300 (cat# M7249; Dako, Carpenteria, CA); active caspase 3 rabbit polyclonal antibody (10 μg/ml) (ab2302; Abcam, Cambridge, MA); and nitrotyrosine rabbit IgG (5 μg/ml; Upstate, Lake Placid, NY). Slides were then incubated with a secondary antibody for 10 min at room temperature, washed, processed as per the Vectastain Universal Quick Kit (PK-8800), and developed with diaminobenzidine.
was accepted at P
cepted the alveoli (intercepts). The MLI was calculated by multiplying
deflation maneuvers. The lower limit of deflation was –10 cm H2O. The
peroxia (see circle and arrow, Figure 2a). Mean alveolar size (MAS) was significantly increased in both p21–/– and p21+/+ oxygen-exposed mice (Figure 2b). At 6 wk of age the p21–/– and p21+/+ mice exposed to neonatal hyperoxia had increased alveolar size compared with room air mice (Figure 2c). The increase in alveolar size, in both the p21–/– O2 and p21+/+ O2 lung, suggest that early administration of hyperoxia causes lung damage and emphysematous changes that persist into adulthood. Unlike the 2-wk-old p21–/– O2 lung, the 6-wk-old p21–/– O2 lung did not have areas of cellular hyperplasia. The p21–/– O2 mice, however, had significantly larger MAS and MLI when compared with all other groups including the p21+/+ O2 lung (Figure 2d).

The significant increase in alveolar size in 6-wk-old p21–/– O2 adult lung suggests that p21–/– mice exposed to neonatal hyperoxia may be more susceptible to lung injury and may be less likely to undergo repair of their lung injury during the recovery period.

Cell Proliferation and Apoptosis in Lung Exposed to Neonatal Hyperoxia

To determine if differences in cell proliferation existed between p21–/– and p21+/+ lung, after exposure to neonatal hyperoxia, we used the Ki67 antibody and examined lungs at 2 and 6 wk of age. At 2 wk of age both p21+/+ and p21–/– room air lungs had greater numbers of cells undergoing proliferation than 2-wk-old, p21+/+ and p21–/– lung previously exposed to neonatal hyperoxia (Figure 3a). There were no differences, however, in cell proliferation between p21+/+ and p21–/– RA mice or between p21+/+ and p21–/– O2 mice.

At 6 wk of age there were no statistical differences in cell proliferation in the lungs of any mice, regardless of genotype or previous exposure to neonatal hyperoxia (Figure 3c).

Next, we were interested in finding out whether apoptosis was increased in the lungs of 2- and 6-wk-old p21–/– mice exposed to neonatal hyperoxia. Cells were considered apoptotic if they were positive by caspase 3 staining. We found that at both 2 wk and 6 wk there were no significant differences in the number of cells undergoing apoptosis in the lungs regardless of genotype or previous exposure to neonatal hyperoxia (Figures 3b–3d).

Oxidative Stress in p21–/– Lung Exposed to Neonatal Hyperoxia

Because chronic oxidative stress may be an important factor in the development of emphysema and has been shown to be increased in diseases such as Alzheimer’s, we were interested

Lung Morphometry of Mice Exposed to Neonatal Hyperoxia

After exposure to neonatal hyperoxia, mice were placed in room air and allowed to recover. At 2 and 6 wk of age, mice were killed and lungs were equally inflated. At 2 wk of age the lungs of both p21–/– and p21+/+ mice formerly exposed to neonatal hyperoxia had enlarged and simplified alveoli compared with p21–/– and p21+/+ mice kept in room air (Figure 2a). Furthermore, in 2 wk, p21–/– O2 lung there were some areas of cellular hyperplasia in the alveolar septum. This was not seen in the p21+/+ O2 lung (Figure 2a).

Survival of Mice Exposed to Neonatal Hyperoxia

Neonatal mice were placed in hyperoxia at DOL#1 and kept in 85–90% O2 for 4 d. p21–/– neonatal mice had an increased mortality while in hyperoxia compared with p21+/+ mice in hyperoxia (P < 0.01) (Figure 1). The increase in mortality in p21–/– O2 mice was only evident in the first week of life. Both p21+/+ and p21–/– mice that survived hyperoxia and lived to 5 d of life had similar survival up to 6 wk of age.

Results

Survival of Mice Exposed to Neonatal Hyperoxia

Neonatal mice were placed in hyperoxia at DOL#1 and kept in 85–90% O2 for 4 d. p21–/– neonatal mice had an increased mortality while in hyperoxia compared with p21+/+ mice in hyperoxia (P < 0.01) (Figure 1). The increase in mortality in p21–/– O2 mice was only evident in the first week of life. Both p21+/+ and p21–/– mice that survived hyperoxia and lived to 5 d of life had similar survival up to 6 wk of age.

Pulmonary Function Testing

Quasi-static pressure–volume curves. Each animal was weighed and anesthetized with sodium pentobarbital intraperitoneally at a dose of 75 mg/kg body weight. After anesthesia, the trachea was cannulated and connected to a ventilator. The animal was ventilated with 100% O2 for 5 min before the cannula was sealed with a stopcock for 5 min to degas the lungs. Quasi-static pressure–volume curves were immediately performed in situ with a system detailed in previous studies (15). Briefly, the system consists of a water-filled syringe mounted on a dual infusion–withdrawal syringe pump (model 900–610; Harvard Apparatus, Dover, MA). A small air-filled vertical column was connected to the syringe and the tracheal cannula. A differential-pressure transducer (model 8510B-2; Endevco, San Juan Capistrano, CA) was used to measure airway pressure. The lungs were inflated by pumping water into the vertical column, thereby displacing air into the lungs. Water volume changes were determined by measuring the displacement of the syringe plunger with a linear displacement transformer (model 244-000; Transstek, Ellington, CT) and lung air volume was determined by correction for gas compression. Paw and volume were recorded by PowerLab digital data acquisition system running Chart v4.1.1 software (ADInstruments, Castle Hill, Australia). The initial inflation rate was low (0.009 ml/s) to ensure that initial lung recruitment did not occur at excessive pressures. Once a pressure of 30 cm H2O was reached, the flow rate was increased to 0.035 ml/s for the remaining inflation and deflation maneuvers. The lower limit of deflation was 10 cm H2O. The upper limit of inflation was 30 cm H2O.

Statistics

Statistical calculations were performed using the SPSS 8.0 statistical package for Windows (Chicago, IL). Differences in measured variables between experimental and control groups were determined using comparison of the means using a one-way ANOVA (Bonferroni and Tukey) and Student’s t test (two-tailed, equal variance). Statistical difference was accepted at P < 0.05.

Figure 1. Survival curve of p21–/– (diamonds) and p21+/+ (squares) mice in hyperoxia. p21–/– and p21+/+ mice were placed in 85–90% hyperoxia at DOL 1–4. The p21–/– mice had an increased mortality by Day 5 compared with p21+/+ mice (*P < 0.01). Each time point represents four separate litters. Error bars represent SEM.

Figure 2. Quasi-static pressure–volume curves. The figure demonstrates that at 2 wk of age both p21+/+ (A) and p21–/– (B) room air lungs had greater numbers of cells undergoing proliferation than 2-wk-old, p21+/+ (C) and p21–/– (D) lung previously exposed to neonatal hyperoxia (Figure 3a). There were no differences, however, in cell proliferation between p21+/+ and p21–/– RA mice or between p21+/+ and p21–/– O2 mice.

Figure 3. Lung Morphometry of Mice Exposed to Neonatal Hyperoxia. After exposure to neonatal hyperoxia, mice were placed in room air and allowed to recover. At 2 wk of age, mice were killed and lungs were equally inflated. At 2 wk of age the lungs of both p21+/+ and p21–/– mice formerly exposed to neonatal hyperoxia had enlarged and simplified alveoli compared with p21+/+ and p21–/– mice kept in room air (Figure 2a). Furthermore, in 2 wk, p21–/– O2 lung there were some areas of cellular hyperplasia in the alveolar septum. This was not seen in the p21+/+ O2 lung (Figure 2a). Mean alveolar size (MAS) was significantly increased in both p21–/– and p21+/+ oxygen-exposed mice (Figure 2b). At 6 wk of age the p21–/– and p21+/+ mice exposed to neonatal hyperoxia had increased alveolar size compared with room air mice (Figure 2c). The increase in alveolar size, in both the p21–/– O2 and p21+/+ O2 lung, suggest that early administration of hyperoxia causes lung damage and emphysematous changes that persist into adulthood. Unlike the 2-wk-old p21–/– O2 lung, the 6-wk-old p21–/– O2 lung did not have areas of cellular hyperplasia. The p21–/– O2 mice, however, had significantly larger MAS and MLI when compared with all other groups including the p21+/+ O2 lung (Figure 2d).

The significant increase in alveolar size in 6-wk-old p21–/– O2 adult lung suggests that p21–/– mice exposed to neonatal hyperoxia may be more susceptible to lung injury and may be less likely to undergo repair of their lung injury during the recovery period.
in determining whether the lungs of p21−/− mice had evidence of increased oxidative stress as compared with the lungs of p21+/+ mice (16). To this end, we used a nitrotyrosine antibody as a marker of oxidative stress.

In 2-wk-old mice, the lungs of p21−/− O2 mice had significantly greater staining of nitrotyrosine than any of the other groups of mice (Figures 4a and 4b). At 6 wk of age p21−/− O2 mice also had significantly greater staining of nitrotyrosine compared with the other groups of mice, but to a lesser extent (Figures 4c and 4d). 8-hydroxy-2′-deoxyguanosine (8-OHdG), a major oxidative DNA base product, was also significantly increased in 6-wk p21−/− O2 lung (data not shown) (17). Although nitrotyrosine staining was greater in p21+/+ O2 mice compared with room air mice, it did not reach statistical significance.

Pulmonary Function in Mice Exposed to Neonatal Hyperoxia

Pulmonary function tests (PFTs) were performed on 6-wk-old, p21−/− and p21+/+ mice. This was done to determine if morphologic findings were associated with functional changes on PFTs. The average weights of the p21−/− O2 and RA mice were less than the average weights of the p21+/+ O2 and RA mice (Table 1). Lung volumes at 30 cm H2O were measured from each group. Volumes from p21−/− O2 mice were significantly larger than lung volumes from p21−/− RA mice (P < 0.02). Similarly, lung volumes of p21+/+ O2 mice were significantly larger than p21+/+ RA mice (P < 0.003). There were no significant differences in lung volumes between the p21−/− O2 mice and p21−/− RA mice. Heterozygote (p21+/−) mice had PFTs similar to those of the p21+/+ mice (data not shown).

Because the lung volumes of p21−/− O2 mice are similar in size to the p21+/+ O2 mice, this may suggest that the p21−/− O2 mice have a mixed restrictive/obstructive pattern. A previous study from Staversky et al. found that adult p21−/− mice recovering from hyperoxia had increased areas of hyperplasia consisting of myofibroblasts (11). We did not, however, find areas of hyperplasia or increased collagen deposition in our 6-wk-old, p21−/− O2 mice (data not shown). Alternatively, p21−/− O2 mice may have lung volumes similar to those of the p21+/+ O2 mice, even though p21−/− O2 mice have larger alveolar spaces, if the p21−/− O2 mice have fewer alveoli and smaller lung sizes than the p21+/+ O2 mice.
Figure 3. Cell proliferation and apoptosis in lungs of 2- and 6-wk-old p21<sup>−/−</sup> and p21<sup>+/+</sup> (Wt) mice exposed to neonatal hyperoxia. Ki67 antibody staining was used as a marker for cell proliferation. Caspase 3 staining was used as a marker of apoptosis. Ten random sections of lung were quantified from each animal. (a) At 2 wk of age, Ki67 staining was significantly increased in Wt RA compared with Wt O<sub>2</sub> and p21<sup>−/−</sup> O<sub>2</sub> mice (P < 0.003 and P < 0.0001, respectively). p21<sup>−/−</sup> RA mice had significantly more staining than Wt O<sub>2</sub> and p21<sup>−/−</sup> O<sub>2</sub> mice (P < 0.02 and P < 0.001, respectively; n = 3). (b) There was no significant difference in caspase 3 staining at 2 wk of age. (c) At 6 wk of age no significant differences in cell proliferation were detected between p21<sup>+/+</sup> and p21<sup>−/−</sup> O<sub>2</sub> or p21<sup>+/+</sup> and p21<sup>−/−</sup> RA lung. (d) At 6 wk of age, no significant differences in apoptosis were detected between p21<sup>+/+</sup> and p21<sup>−/−</sup> O<sub>2</sub> or p21<sup>+/+</sup> and p21<sup>−/−</sup> RA lung (n = 3–7 for each group). Error bars represent SEM. Comparisons of the means were done by one-way ANOVA. Quantification was performed by measuring the intensity of DAB staining and normalizing it to lung perimeter. Solid bars, Wt RA; open bars, p21-RA; lightly shaded bars, Wt O<sub>2</sub>; darkly shaded bars, p21-O<sub>2</sub>.

Discussion

Exposure to high oxygen tension is a significant risk factor in the development of chronic lung disease. Our studies demonstrate that transgenic mice with a null p21 gene have an increase in mortality during acute exposure to hyperoxia but not in the subsequent 6 wk of follow-up in room air. After exposure to hyperoxia in the neonatal period, p21<sup>−/−</sup> mice go on to develop alveolar enlargement and increases in lung volume as compared with room air controls.

Why neonatal p21<sup>−/−</sup> mice succumb to acute hyperoxia more frequently then p21<sup>+/+</sup> mice is not clear. Staversky and coworkers found that adult p21<sup>−/−</sup> mice exposed to acute hyperoxia had a higher mortality rate than p21<sup>+/+</sup> (10). During recovery from hyperoxia, p21<sup>−/−</sup> lung had increased DNA damage by DNA laddering and fibroblast hyperplasia, suggesting that p21<sup>−/−</sup> mice may have an impaired response to acute lung injury. We found that the lungs of p21<sup>−/−</sup> mice, exposed to neonatal hyperoxia, exhibited impaired alveolar growth during recovery from hyperoxia. This was demonstrated by increases in MAS and MLI at 2 wk of age that did not resolve by 6 wk. The p21<sup>+/+</sup> O<sub>2</sub> mice also had evidence of impaired alveolar growth at 2 wk of age; however, by 6 wk of age, alveolar enlargement was not as signifi-

Figure 4. Quantification of oxidative stress by nitrotyrosine antibody staining in 2-wk and 6-wk p21<sup>−/−</sup> and p21<sup>+/+</sup> (Wt) lung exposed to neonatal hyperoxia. (a) Representative example of 2-wk-old p21<sup>−/−</sup> and Wt lung stained with nitrotyrosine as a marker of oxidative stress. (b) At 2 wk p21<sup>−/−</sup> O<sub>2</sub> mice (lightly shaded bars) had greater staining than Wt RA (solid bars), p21<sup>−/−</sup> RA (open bars), and Wt O<sub>2</sub> (darkly shaded bars) lung (P < 0.0001, P < 0.0001, and P < 0.001, respectively; n = 3). (c) Representative example of 6-wk-old p21<sup>−/−</sup> and Wt lung stained with nitrotyrosine. (d) At 6 wk p21<sup>−/−</sup> O<sub>2</sub> mice had greater staining (arrow) than Wt RA, p21<sup>−/−</sup> RA, and Wt O<sub>2</sub> lung (P < 0.001, P < 0.001, and P < 0.01, respectively). Error bars represent SD (n = 3). Comparisons of the means were done by one-way ANOVA.
TABLE 1. Lung volumes in p21−/− and p21+/+ mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Weight (g)</th>
<th>Volume at 30 cm H2O (ml)</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>p21−/− O2</td>
<td>16.6 ± 2.0</td>
<td>1.1 ± 0.14</td>
<td>5</td>
</tr>
<tr>
<td>p21−/− RA</td>
<td>16.3 ± 0.29</td>
<td>0.8 ± 0.08</td>
<td>4</td>
</tr>
<tr>
<td>p21+/+ O2</td>
<td>20.6 ± 2.7</td>
<td>1.1 ± 0.23</td>
<td>7</td>
</tr>
<tr>
<td>p21+/+ RA</td>
<td>21.2 ± 1.9</td>
<td>0.7 ± 0.13</td>
<td>6</td>
</tr>
</tbody>
</table>

p21−/− O2 mice were significantly smaller than p21+/+ O2 mice. p21−/− RA mice were significantly smaller than p21+/+ RA mice. p21−/− O2 mice had significantly larger lung volumes than p21+/+ RA mice. p21+/+ O2 mice had significantly larger lung volumes than p21+/+ RA mice.

P < 0.005.

1 P < 0.02.

2 P < 0.005.

3 P < 0.003.

cant as in the p21−/− O2 mice. These findings suggest that p21−/− lung exposed to neonatal hyperoxia may also have a decreased ability to undergo lung injury repair following acute hyperoxia, therefore resulting in impairment of lung development beyond that found in wild-type mice exposed to neonatal hyperoxia.

The phenotypic and functional changes found in our 6-wk-old, p21−/− O2 lung may be secondary to abnormalities of cell growth or increased cell death during a critical period of postnatal lung growth. Alveolar endothelial and epithelial cells are particularly sensitive to the effects of hyperoxia. Recent studies suggest that endothelial cell growth may be critical for normal alveolar growth in the postnatal lung (18). Staversky and colleagues showed that p21−/− lung exposed to hyperoxia had delayed expression of endothelial genes and myofibroblast hyperplasia. These findings suggest that p21−/− endothelial cells in the lung recover slowly after exposure to hyperoxia, whereas myofibroblasts have increased proliferation (at least transiently). We also found that 2-wk-old p21−/− O2 lung had areas of interstitial hyperplasia in the alveolar spaces. These areas of interstitial hyperplasia, however, resolved by 6 wk of age.

We found that at 2 wk of age, p21−/− O2 lung had significantly less cell proliferation than p21−/− and p21+/+ room air mice. Cell proliferation was also significantly decreased in the p21+/+ O2 mice, but not to as great an extent. Interestingly, we did not find an increase in apoptosis by caspase 3 staining in either p21−/− or p21+/+ oxygen exposed or room air lung at 2 or 6 wk. It is possible that we missed an increase in apoptosis in the oxygen exposed lung at an earlier time point, however, at 2 wk of age it appears that impaired lung growth from hyperoxia is driven by a decrease in cell proliferation and not an increase in cell death. At 6 wk of age there was no difference in cell proliferation between any of the groups. Therefore, because the majority of postnatal alveolar growth in the mouse occurs primarily in the first month of life, decreases in cell proliferation at 2 wk of age may profoundly effect ultimate lung growth (19). This effect appears to be augmented in the p21−/− mice.

At 2 wk of age the lungs of p21−/− mice exposed to neonatal hyperoxia had increased nitrotyrosine staining consistent with increased oxidative stress. This was also present at 6 wk of age, but to a lesser extent. The increase in oxidative stress at 2 wk was associated with decreased cell proliferation and impaired alveolar growth. oxidative stress appears to play an important role in the development of many chronic illnesses. The genes that protect against oxidative stress include intracellular and mitochondrial antioxidant enzymes. Alterations in specific gene pathways can also affect the cell’s ability to respond to oxidative stress. Mutations in the ataxia telangiectasia (AT) gene render the organism more susceptible to oxidative stress (20). The increase in oxidative stress in individuals with AT may occur from direct DNA damage or an inability to respond to a specific stress such as radiation. p21 is downstream of p53 and the AT gene. Our finding that neonatal hyperoxia causes increased oxidative stress in the p21−/− lung suggests that p21 protects against oxidative stress, and that in the absence of p21, lung cells are more sensitive to specific oxidative stresses with a decreased ability to handle high levels of free oxygen radicals. How this occurs is not clear. Previous studies indicate that p21 may limit the number of macrophages that are activated in response to hyperoxia (11, 21). Other studies have reported that p21 is induced during chronic oxidative stress and may have a role in decreasing oxidative stress during injury. In two separate studies involving cigarette smoke exposure, p21 induction was found. This was shown in macrophages obtained from cigarette smokers, and A549 cells exposed to cigarette smoke (22, 23). Another study found that overexpression of p21, using an adenovirus vector, decreased oxidative stress during injury. They used isolated vessel lesions and overexpression of p21 resulted in decreased macrophage migration and activation at the site of injury (21).

In our study, nitrotyrosine, a marker of oxidative stress was markedly increased in 2-wk p21−/− O2 lung. This increase in oxidative stress was associated with a decrease in cell proliferation during a critical period of postnatal lung development.

Exposure to hyperoxia in the neonatal period has been shown to be associated with increased lung volumes and impaired lung growth in adult mouse lung (24). In our study, the p21−/− O2 and p21+/+ O2 mice had significantly larger lung volumes than the room air control mice. The lung volumes of the p21−/− O2 and p21+/+ O2 mice, however, were similar. This was unexpected, because the MAS and MLI of the 6-wk p21−/− O2 mice were larger. This suggests that the p21−/− O2 mice may have a mixed restrictive/obstructive pattern, in contrast to the obstructive pattern of the p21+/+ O2 mice. We were, however, unable to demonstrate increased collagen staining to support this (data not shown). Alternatively, the p21−/− O2 mice may have fewer alveoli and smaller lungs, which could account for their lung volumes being similar to the p21−/− O2 mice, even though the p21−/− O2 mice have larger MAS and MLI.

In summary, neonatal mice deficient in p21 have an increased mortality during exposure to acute hyperoxia. Furthermore, p21 appears to be important during injury repair following hyperoxia by reducing oxidative stress and by partially attenuating the adverse effects of hyperoxia on postnatal alveolar growth.

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