ANTIOXIDANTS & REDOX SIGNALING
Volume 6, Number 1, 2004

Forum Original Research Communication

Increased Apoptosis and Expression of p21 and p53 in Premature Infant Baboon Model of Bronchopulmonary Dysplasia

KUMUDA C. DAS, DASHNAMOORTHY RAVI, and WILLIAM HOLLAND

ABSTRACT

Bronchopulmonary dysplasia (BPD) is a major complication of premature infants who receive prolonged ventilatory support. The pathophysiology of BPD involves oxidant injury, baro/volutrauma, and disordered lung repair. Exposure of premature lung that is poorly adapted for air breathing (>3% oxygen in fetal lung) to a higher concentration of oxygen can cause significant oxidant injury. Cell growth and differentiation of the developing lung require selective and ordered cell division. As hyperoxia can increase the expression of cell-cycle checkpoints that can cause growth arrest of lung cells, in this report we examined the expression of checkpoint proteins p53 and p21 in a premature infant the baboon model of BPD. Additionally, we also determined whether enhanced apoptosis occurs in baboon BPD model. We have shown that p53 and p21 expression are increased in 125-day as well as 140-day premature baboons with BPD. We also demonstrate increased apoptosis in lung tissue of premature baboons with BPD. These results demonstrate that cell growth inhibition is a likely factor in the evolution of BPD. Additionally, lung cells may undergo increased apoptosis that can impair the repair process in the postventilatory recovery period. *Antioxid. Redox Signal.* 6, 109–116.

INTRODUCTION

BRONCHOPULMONARY DYSPLASIA (BPD) is a chronic lung disease of premature infants that is characterized by prolonged hospitalization and substantial morbidity (8–12). Despite advances toward prevention of respiratory distress syndrome, BPD remains a major complication for premature infants who require prolonged ventilatory support. The pathophysiology of BPD involves oxidant injury, baro/volutrauma, and disordered lung repair (8, 9). Oxidative insult likely affects both growth and differentiation of the developing lung, and high levels of oxygen can inhibit lung cell division in cell culture models (1, 2, 24). As normal lung development and differentiation require a highly ordered proliferation of epithelial and mesenchymal cells, disruption of this process by oxidative injury would have disastrous consequences for lung architecture and function (3, 22). In addition to a generalized decrease in cell proliferation, BPD is also associated with loss of functional lung cells, including alveolar type I, bronchial epithelial, and other cell types (14, 36).

Studies in experimental animals show that hyperoxia decreases the lung cell population (36), suggesting either altered cell-cycle dynamics or increased cell death (apoptosis). Although potentially important to our understanding of BPD, little is known about the role of cell-cycle checkpoints or apoptosis in this disorder. Regulation of the cell cycle relies on the integrity of genetic information and requires a balance between growth and division. This balance is maintained by different feedback controls, or checkpoints, that respond to various cellular conditions (33, 37). Checkpoint pathways that mediate cell-cycle arrest in G1 or G2 are thought to operate through inhibition of cyclin-dependent kinases (cdks); these proteins are required for major cell-cycle transitions at the onset of S phase or mitosis (33, 37).

Department of Molecular Biology, University of Texas Health Center at Tyler, Tyler, TX 75708.

One checkpoint protein activated in response to DNA-damaging agents is p53. Hyperoxia has been shown to activate p53 in adult mice (27). Increased production of reactive oxygen species in hyperoxia causes DNA damage (4, 38), which, in turn, results in an accumulation of p53 protein in lung alveolar epithelial and endothelial cells. Activation of p53 results in increased transcription of the cdk inhibitor p21 protein, which then inactivates cdks and arrests the cell cycle (35). Recent studies also indicate that hyperoxia increases expression of p21 in lungs of mice (23, 24, 29, 30) and in cultured lung cells. p53 is a sequence-specific transcription factor that can either induce or suppress proapoptotic and antiapoptotic genes in response to DNA damage or irreparable cell-cycle arrest (21). Phosphorylation of p53 at Ser 15 residue dissociates MDM2 (mitotic arrest-deficient yeast homologue) and activates p53 as a transcription factor; binding of this activated factor to various p53-dependent genes results in activation or repression (21). Induction of p53 by hyperoxia has been observed in mouse lung epithelium (27). Expression of p53 also increases in human bronchial smooth muscle cells (32), but reports of p53 expression in premature infant baboons with BPD are lacking.

As a universal inhibitor of cdks, p21 functions as a checkpoint in the cell cycle (15). Overexpression of p21 results in growth arrest of cultured cells, and p21 is reported to be induced by hyperoxia in cultured cells and in murine neonatal lung (23). High concentrations of inhaled oxygen are frequently used in the treatment of acutely ill neonates or people with adult respiratory distress syndrome. Overexpression of p21 in hyperoxic treatment is a potential cause of growth arrest of lung cells, and failure of cell proliferation could exacerbate the original lung injury. Studies of p21 protein in neonatal lungs exposed to hyperoxia showed that the distribution was predominantly peripheral, suggesting that p21 induction may inhibit alveolar growth and impair healing (23).

In this study, we investigated whether the checkpoint proteins are induced in BPD, and whether apoptosis also occurs in BPD. We report here that p53 and p21 expression is increased in BPD. Additionally, lung cells undergo increased apoptosis in premature infant baboons with BPD.

MATERIALS AND METHODS

Animal studies

All animal care procedures were performed according to the National Research Council's Guide for the Care and Use of Laboratory Animals. Protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Southwest Foundation for Biomedical Research, San Antonio, Texas. Fetal baboons of varying gestational ages (±2 days) were delivered by hysterotomy. Gestational ages were determined by timed matings as previously described (13, 25) with confirmation by ultrasound at intervals during pregnancy. The control gestational age-matched premature infant baboons were not exposed to supplemental oxygen, and were killed before first breath.

In other studies pertinent to the effect of variable oxygen tension *in vivo* and to the development of BPD following hyaline membrane disease, treatment groups were delivered at either 140 ± 2 days or 125 ± 2 days of gestation and immediately placed on positive pressure ventilation. Animals of 140-day gestation were given either continuous 100% oxygen or an inspired oxygen tension as needed [pro re nata (PRN)] to maintain paO_2 at 40–50 Torr. Within 10 days, those 140-day animals given 100% oxygen develop lung histopathologic lesions that closely resemble human BPD, whereas the PRN animals do not develop these lesions, allowing near-normal lung development (17, 34). Most premature animals of 125-day gestation received immediate resuscitation with artificial surfactant, positive pressure ventilation, and PRN oxygen. These animals also develop BPD despite receiving lesser (PRN) inspired concentrations of oxygen (34). In either case, all animals received state-of-the-art care in a neonatal intensive care unit for up to 17 days. Following treatment, animals were killed by administration of intravenous pentobarbital. The lungs were perfused via the pulmonary artery with phosphate-buffered saline (PBS) (37°C), and distal lung tissue was dissected free from major airways and central structures and processed immediately as for fetal tissue.

Terminal deoxynucleotidyltransferase dUTP nick end-labeling (TUNEL) assay

Tissue sections were obtained from the baboon resources center (San Antonio) and were deparaffinized, rehydrated, and then treated with 20 mg/ml proteinase K in 10 m*M* Tris-HCl (pH 8.0). The sections were blocked for endogenous peroxidase activity by incubation in 3% hydrogen peroxide in methanol for 10 min at room temperature. The slides were then rinsed with PBS and incubated with labeling solution containing terminal deoxynucleotidyl transferase along with all four nucleotides and fluorescein-dUTP at 37°C for 1 h. All sections were rinsed three times with PBS, and the reaction was detected with converter POD (anti–fluorescein–peroxidase conjugate). Immunoreactivity developed with diaminobenzidine appeared as a dark brown precipitate in the nucleus. The sections were counterstained with hematoxylin and digitally photographed through an Olympus microscope.

Western blotting

Premature infant baboons were exposed to oxygen as described earlier (13). Lung tissue was homogenized in Tris-HCl buffer (pH 7.4). The homogenate was mixed with an equal volume of RIPA (radioimmunoprecipitation buffer; 1× PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1 m*M* phenylmethylsulfonyl fluoride, 30 μl/ml aprotinin (Sigma, St. Louis, MO, U.S.A.), and 1 m*M* Na_3VO_4] buffer, incubated on ice for 30 min, and then centrifuged. The supernatant contained the total lung lysate. Fifty micrograms of protein was resolved on sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and western blotted with an anti-p21 or anti-p53 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.).

Immunohistochemistry

Tissue sections were deparaffinized in xylene and rehydrated through graded mixtures of ethanol and deionized water. Im-

munostaining was done with either a Santa Cruz immunoperoxidase staining kit or a Dako universal ABC. Endogenous peroxidase was quenched with the peroxidase quench solution provided with the kit. All sections were blocked for nonspecific binding with serum and then incubated with primary antibody overnight at 4°C. The slides were then rinsed in PBS and incubated with a biotinylated secondary antibody, followed by incubation with streptavidin conjugated with horseradish peroxidase. Immunoreactivity was detected with an aminoethylcarbazole kit (Sigma), which develops the reaction as a reddish or brown color. The sections were then counterstained with hematoxylin and mounted in aqueous media, and the slides were observed and digitally photographed with an Olympus microscope.

RESULTS

Increased expression of p21 protein in BPD

To establish the role of p21 in BPD, we exposed premature infant baboons (125- or 140-day gestational age) to oxygen PRN or to 100% O_2. These studies were done at the baboon resources center at San Antonio (12). Western blotting detected p21 protein in the lung tissue homogenates. Figure 1A shows that untreated lungs from 125-day gestational control (GC) infants did not express p21, but ventilation with PRN oxygen for 6 days or 14 days resulted in significant expression of this protein. Similarly, lungs from 140-day GC animals did not express p21, but those exposed to PRN oxygen expressed it (Fig. 1B) Premature infant baboons exposed to 100% oxygen had similar p21 expression. Our data indicate that p21 could, indeed, contribute to the initiation and progression of BPD by inhibiting progression of the cell cycle and thereby compromising lung repair. We used immunohistochemistry to detect and localize p21-positive cells in tissues from 125-day animals that were treated for either 6 or 14 days with PRN O_2 and their GC. The 125-day animals exposed to 6 or 14 days of PRN oxygen demonstrated an increase in the expression of p21 protein (Fig. 2, upper panels). p21 appears to be localized within alveolar epithelial, bronchiolar epithelial, and interstitial cells. Additionally, 140-day animals also showed increased p21 expression after 6 days of PRN or 6 days of 100% oxygen exposure (Fig. 2, lower panels). Exposure to 100% oxygen caused increased p21 expression compared with 6 days of PRN oxygen exposure.

Increased expression of p53 protein in BPD

p53 is a sequence-specific transcription factor that can either induce or suppress proapoptotic and antiapoptotic genes in response to DNA damage or irreparable cell-cycle arrest (26). Induction of p53 by hyperoxia has been observed in mouse lung epithelium (27). Expression of p53 also increases in human bronchial smooth muscle cells (32), but reports of p53 expression in premature infant baboons with BPD has not been previously demonstrated. Furthermore, the p53 response in extremely premature baboons was unknown. Accordingly, we analyzed p53 protein expression in baboons in BPD. As shown in Fig. 3, lung tissue from 140-day premature baboons exposed to PRN oxygen or to 100% oxygen for 6 days had higher levels of p53 compared with age-matched GC animals. Histochemical examination of lungs from 140-day infants that had been exposed to 100% oxygen revealed increased p53 expression in bronchiolar epithelial cells as well as alveolar epithelial cells, (Fig. 4). Interstitial cells also contained p53. In addition, we found p53-positive cells in tissues from extremely premature baboons (125 days; Fig. 4).

Increased apoptosis in BPD

We noted increased expression of p53 in lung tissue from 125-day and 140-day baboons who had been exposed to oxy-

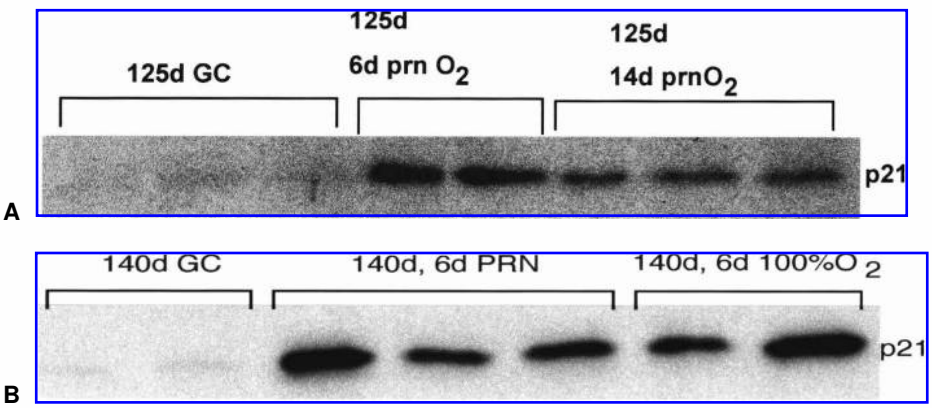

FIG. 1. Increased expression of p21 in premature infant baboons with BPD. Premature infant baboons were delivered at 125-day or 140-day gestational age and were exposed to PRN or 100% oxygen at the baboon resources center at San Antonio as described in Materials and Methods. Frozen lung tissue was prepared and western blotting was performed using p21 antibody raised in rabbit as described in Materials and Methods. **(A)** Lanes 1–3, 125-day GC baboons; lanes 4 and 5, infant baboons exposed to 6 days of PRN oxygen; lanes 6–8, infant baboons exposed to 14 days of PRN oxygen. **(B)** Lanes 1 and 2, 140-day GC baboons; lanes 3–5, 140-day baboons exposed to PRN oxygen for 6 days; lanes 6 and 7, 140-day baboons exposed to 100% oxygen for 6 days.

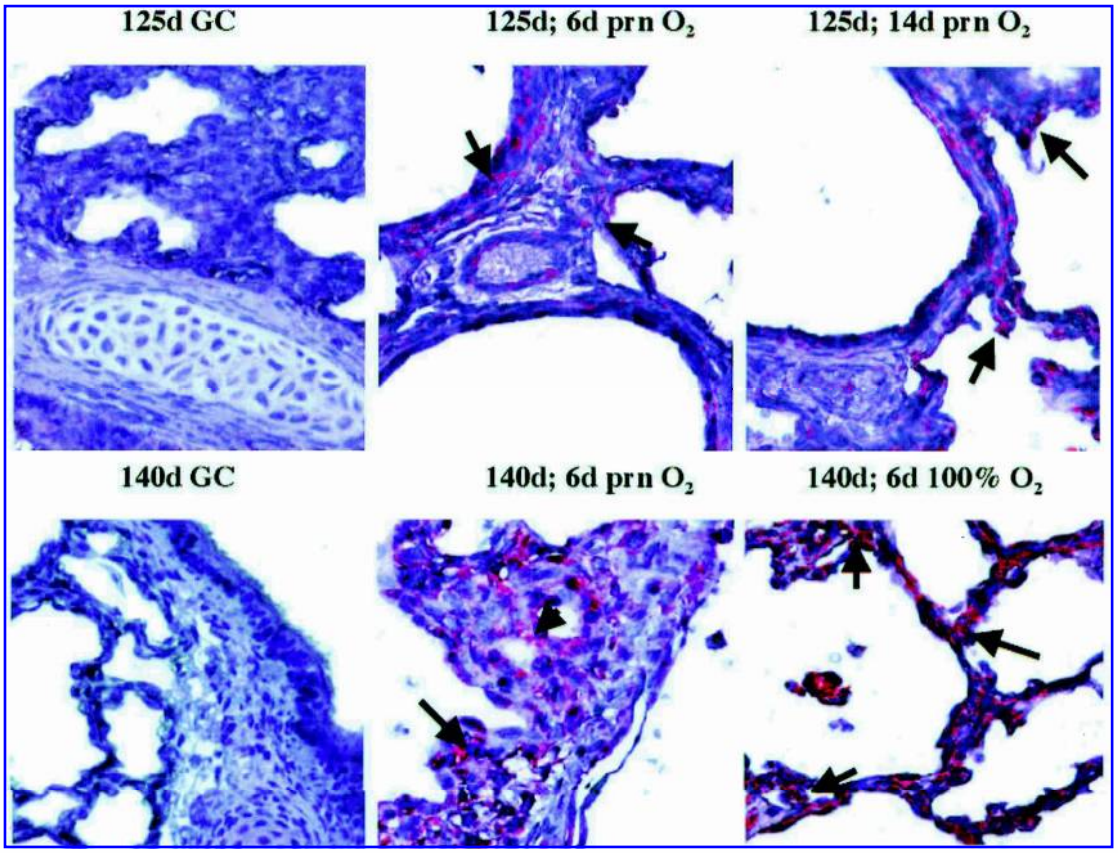

FIG. 2. Increased p21 localization in lungs of infant baboons with BPD. Paraffin-embedded baboon lung tissue sections were processed for p21 immunohistochemistry as described in Materials and Methods. Red or brown color indicates localization of p21. **(Upper panels)** 125-day GC and 125-day animals exposed to 6 days and 14 days of PRN oxygen. **(Lower panels)** 140-day GC and 140-day animals exposed to 6 days of PRN oxygen and 6 days of 100% oxygen.

gen (Figs. 3 and 4). Expression of p53 can either arrest the cell cycle for repair of damaged DNA or initiate apoptotic cell death where there is irreparable damage in order to maintain genome integrity. Because p53 expression increased in the lungs of 125-day or 140-day baboons exposed to oxygen, we sought to determine whether this increase was associated with increased apoptosis. Figure 5 shows that 125-day baboons exposed to oxygen for either 6 or 14 days PRN had increased apoptosis (Fig. 5, upper panels). We also recorded an increased number of apoptotic cells in the 140-day animals exposed to oxygen for 6 days PRN or to 100% oxygen (Fig. 5, lower panels).

DISCUSSION

The data presented in this study demonstrate that the expression of cell-cycle checkpoint proteins p21 and p53 are increased in 125-day or 140-day baboons exposed to PRN or 100% oxygen. Histochemical examination revealed that these

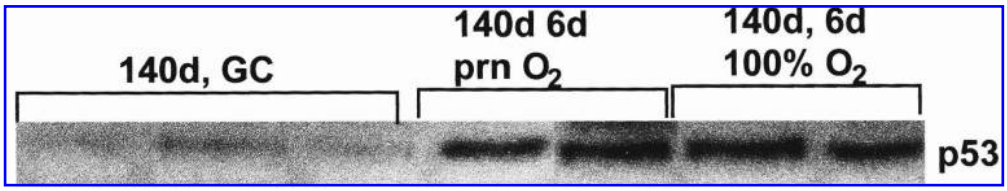

FIG. 3. Increased p53 expression in premature infant baboons in BPD. Premature infant baboons were delivered at 140-day gestational age and were exposed to PRN or 100% oxygen at the baboon resources center at San Antonio as described in Materials and Methods. Frozen lung tissue was was prepared and western blotting was performed using p53 antibody raised in rabbit as described in Materials and Methods. Lanes 1–3, 140-day GC baboons; lanes 4 and 5, 140-day baboons exposed to PRN oxygen for 6 days; lanes 6 and 7, 140-day baboons exposed to 100% oxygen for 6 days.

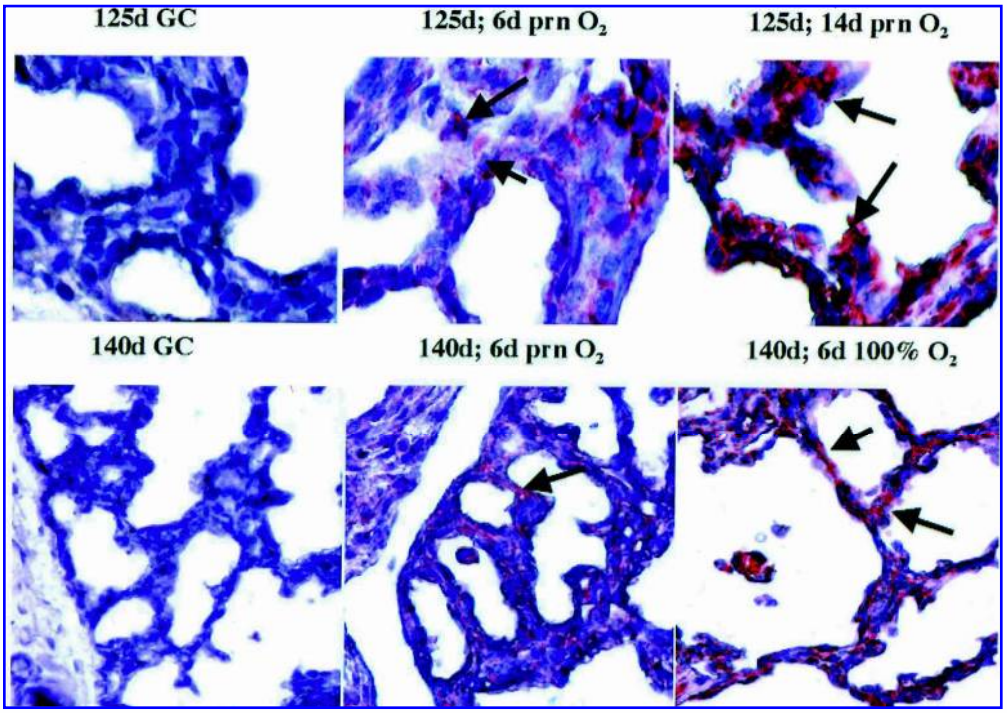

FIG. 4. Increased p53 localization in lungs of infant baboons with BPD. Paraffin-embedded baboon lung tissue sections were processed for p53 immunohistochemistry as described in Materials and Methods. Red or brown color indicates localization of p53. **(Upper panels)** 125-day GC and 125-day animals exposed to 6 days and 14 days of PRN oxygen. **(Lower panels)** 140-day GC and 140-day animals exposed to 6 days of PRN oxygen, and 6 days of 100% oxygen.

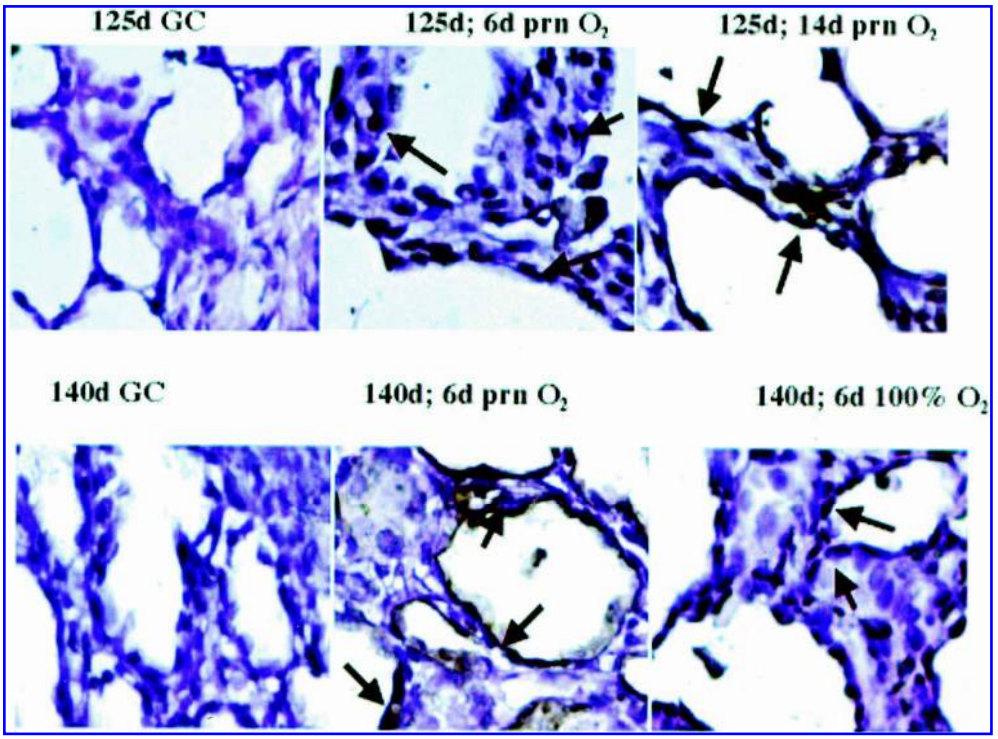

FIG. 5. Increased apoptosis in 125-day or 140-day premature infants with BPD. Paraffin-embedded baboon lung tissue sections were processed for TUNEL assay as described in Materials and Methods. Dark brown color indicates TUNEL-positive nuclei. **(Upper panels)** 125-day GC and 125-day animals exposed to 6 days and 14 days of PRN oxygen. **(Lower panels)** 140-day GC and 140-day animals exposed to 6 days of PRN oxygen and 6 days of 100% oxygen.

checkpoint proteins are expressed in bronchiolar epithelial as well as alveolar epithelial cells. Additionally, interstitial cells also demonstrate increased expression of these proteins. Because elevation of p53 is associated with apoptosis we determined apoptosis, in 125-day or 140-day baboons exposed to PRN or 100% oxygen and found that apoptosis increased in infant baboons exposed to PRN or 100% oxygen. Although hyperoxia (>95% O_2) has been shown to induce p53 and p21 expression in mouse lung epithelium and lung cells in culture, their increase in conditions of BPD in premature lung has not been previously reported. The observation that p21 expression increases in 125-day or 140-day premature infant baboons exposed to 6 or 14 days of PRN oxygen or 100% oxygen ventilation is significant because p21 plays a central role in the arrest of the cell cycle by inactivating critical cell-cycle regulatory proteins. We observed stronger p21 expression in 125-day animals exposed to 6 days of PRN oxygen than that in animals exposed to 14 days of PRN oxygen. The reason for this subtle difference may be explained by the fact that the initial inspired oxygen fraction (FiO_2) is higher in 125-day animals, which rapidly weaned over the 14-day ventilation period (25).

The 125-day premature baboons (68% full-term) exposed to PRN oxygen (25–40% FiO_2) more closely replicate the lung disease that is now seen in 24–26-week gestation human infants who go on to develop BPD (12). In contrast to *in utero* development from 125 to 140 days of gestation, there is no significant progression of alveolization in these animals. Enlarged simpified terminal airspaces, diffusely focal intersaccular fibroplasias, and a lack of extensive airway epithelial hyperplasia/metaplasia are the histologic feature (7, 8). On the other hand, 140-day animals exposed to 6 days of PRN oxygen or 6 days of 100% oxygen demonstrate epithelial loss, hyaline membrane/saccular edema, interstitial edema, endothelial edema, and necrosis (7, 8). These pathologic features suggest that lung development is severely compromised in oxygen ventilation. Oxygen was found to cause DNA damage and growth arrest of lung cells in various cell culture models, and oxidative insult to the developing lung may disrupt the orderly pattern of proliferation. Few studies examined the pattern of cell proliferation under conditions of increased oxygen tension, but exposure to high concentrations of oxygen is generally associated with decreased proliferation of endothelial, interstitial, type I and type II pneumocytes (36). However, when several species of adult animals were exposed to 100% oxygen and allowed to recover for 7 days, the cell numbers increased (36). Increased type II cell proliferation was noted during the recovery phase following exposure to hyperoxia (36). In addition to an inhibited proliferative response, monkeys exposed to 95% oxygen for 14 days had complete destruction of type I cells (14). A rapid proliferation of type II alveolar cells is crucial for proper healing after injury, because delayed reepithelialization may lead to the development of pulmonary fibrosis (5, 6).

A recent study of BPD in the baboon model noted increased proliferation of pro-surfactant protein B secreting cells (22) using nuclear antigen Ki67 as a proliferation marker. However, it is possible that many other cell types of lung could have been arrested due to the increase in p21 expression. Thus, further studies are needed to identify the cell types that are arrested in BPD. We observed increased p21 and p53 expression in 125-day or 140-day baboons with BPD, which suggests that growth arrest occurs in these affected infants. Although it is known that oxygen stimulates expression of p21 and p53 (28, 29), and these proteins can be expressed in cultured lung cells and mouse lung epithelium, their expression has not been previously documented in premature infant baboons with BPD. The fetal lung develops as a fluid-filled organ and is continuously situated in an environment that is relatively hypoxic (<3% oxygen), which is the potential oxygen-carrying capacity of umbilical veins (18). The transition from placental to lung-based respiration is perceived as normal in fully mature babies; in contrast, preterm infants may suffer as the lungs may be insufficiently developed and may be incapable of sustained breathing (18). Thus, in this development stage, a lower level of oxygen ventilation (25–40% in the 125-day PRN model) can pose an acute oxidative stress to the developing lung. The expression of p21 or p53 suggests that the lung cells are undergoing DNA damage and/or cell-cycle arrest. As cell division is a fundamental process in the lung development and differentiation, inhibition of lung cell division can cause inadequate development of lung that is characteristics of BPD. Additionally, because type II cells repopulate the type I cells of the alveoli that are destroyed in oxygen, inhibition of type II cell proliferation can impair repair of epithelium. Furthermore, a delay in reepithelialization following lung injury can cause fibrosis. Therefore, expression of p21 or p53 is a significant molecular event that can be a causal factor in the evolution of BPD.

However, p21 expression in hyperoxia can be induced independently from induction of p53 (29), and regulation of p21 appears to be redox-mediated (16). For example, depleting cellular free thiols with diethyl maleate induced p21 in Hela cells (16). Treatment of cells with *N*-acetylcysteine suppressed p21 expression by diethyl maleate (16), suggesting that p21 regulation involves a thiol–disulfide exchange reaction. This exchange could be directly modulated by thioredoxin, a protein involved in maintaining the redox state of the cells (19, 20). We observed that thioredoxin shifts the balance to an oxidized state in 140-day baboons with BPD (13). Therefore, it is possible that p21 is up-regulated in response to a redox shift. However, definitive studies are warranted to establish this fact.

ACKNOWLEDGMENTS

The authors acknowledge the superb and dedicated assistance of numerous physicians, scientists, nurses, technicians, and other personnel at the BPD Resource Center in San Antonio. Dr. Das was supported by a Scientist Development Award from the American Heart Association National Center and a research project grant from the American Cancer Society.

ABBREVIATIONS

BPD, bronchopulmonary dysplasia; cdk, cyclin-dependent kinase; FiO_2, inspired oxygen fraction; GC, gestational control; p21, Waf1–cyclin kinase inhibitor 1A; p53, tumor protein p53; paO_2, partial pressure of oxygen; PBS, phosphate-

buffered saline; PRN, pro re nata (as occasion requires); TUNEL, terminal deoxynucleotidyltransferase dUTP nick end-labeling.

REFERENCES

1. Bilodeau JF, Faure R, Piedboeuf B, and Mirault ME. Hyperoxia induces S-phase cell-cycle arrest and p21(Cip1/Waf1)-independent Cdk2 inhibition in human carcinoma T47D-H3 cells. *Exp Cell Res* 256: 347–357, 2000.
2. Buckley S, Barsky L, Driscoll B, Weinberg K, Anderson KD, and Warburton D. Apoptosis and DNA damage in type 2 alveolar epithelial cells cultured from hyperoxic rats. *Am J Physiol* 274: L714–L720, 1998.
3. Bui KC, Buckley S, Wu F, Uhal B, Joshi I, Liu J, Hussain M, Makhoul I, and Warburton D. Induction of A- and D-type cyclins and cdc2 kinase activity during recovery from short-term hyperoxic lung injury. *Am J Physiol* 268: L625–L635, 1995.
4. Cacciuttolo MA, Trinh L, Lumpkin JA, and Rao G. Hyperoxia induces DNA damage in mammalian cells. *Free Radic Biol Med* 14: 267–276, 1993.
5. Cantin AM, North SL, Fells GA, Hubbard RC, and Crystal RG. Oxidant-mediated epithelial cell injury in idiopathic pulmonary fibrosis. *J Clin Invest* 79: 1665–1673, 1987.
6. Clement A, Henrion-Caude A, Besnard V, and Corroyer S. Role of cyclins in epithelial response to oxidants. *Am J Respir Crit Care Med* 164: S81–S84, 2001.
7. Coalson JJ. Experimental models of bronchopulmonary dysplasia. *Biol Neonate* 71 Suppl 1: 35–38, 1997.
8. Coalson JJ, Kuehl TJ, Escobedo MB, Hilliard JL, Smith F, Meredith K, Null DM Jr, Walsh W, Johnson D, and Robotham JL. A baboon model of bronchopulmonary dysplasia. II. Pathologic features. *Exp Mol Pathol* 37: 335–350, 1982.
9. Coalson JJ, Kuehl TJ, Prihoda TJ, and deLemos RA. Diffuse alveolar damage in the evolution of bronchopulmonary dysplasia in the baboon. *Pediatr Res* 24: 357–366, 1988.
10. Coalson JJ, Winter VT, Gerstmann DR, Idell S, King RJ, and Delemos RA. Pathophysiologic, morphometric, and biochemical studies of the premature baboon with bronchopulmonary dysplasia. *Am Rev Respir Dis* 145: 872–881, 1992.
11. Coalson JJ, Winter V, and deLemos RA. Decreased alveolarization in baboon survivors with bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 152: 640–646, 1995.
12. Coalson JJ, Winter VT, Siler-Khodr T, and Yoder BA. Neonatal chronic lung disease in extremely immature baboons. *Am J Respir Crit Care Med* 160: 1333–1346, 1999.
13. Das KC, Guo XL, and White CW. Induction of thioredoxin and thioredoxin reductase gene expression in lungs of newborn primates by oxygen. *Am J Physiol* 276: L530–L539, 1999.
14. de los Santos R, Seidenfeld JJ, Anzueto A, Collins JF, Coalson JJ, Johanson WG Jr, and Peters JI. One hundred percent oxygen lung injury in adult baboons. *Am Rev Respir Dis* 136: 657–661, 1987.
15. el-Deiry WS, Harper JW, O'Connor PM, Velculescu VE, Canman CE, Jackman J, Pietenpol JA, Burrell M, Hill DE, Wang Y, *et al.* WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. *Cancer Res* 54: 1169–1174, 1994.
16. Esposito F, Cuccovillo F, Vanoni M, Cimino F, Anderson CW, Appella E, and Russo T. Redox-mediated regulation of p21(waf1/cip1) expression involves a post-transcriptional mechanism and activation of the mitogen-activated protein kinase pathway. *Eur J Biochem* 245: 730–737, 1997.
17. Frank L and Sosenko IR. Prenatal development of lung antioxidant enzymes in four species. *J Pediatr* 110: 106–110, 1987.
18. Haddad JJ. Oxygen-sensing mechanisms and the regulation of redox-responsive transcription factors in development and pathophysiology. *Respir Res* 3: 26, 2002.
19. Holmgren A. Thioredoxin. *Annu Rev Biochem* 54: 237–271, 1985.
20. Holmgren A and Bjornstedt M. Thioredoxin and thioredoxin reductase. *Methods Enzymol* 252: 199–208, 1995.
21. Lakin ND and Jackson SP. Regulation of p53 in response to DNA damage. *Oncogene* 18: 7644–7655, 1999.
22. Maniscalco WM, Watkins RH, O'Reilly MA, and Shea CP. Increased epithelial cell proliferation in very premature baboons with chronic lung disease. *Am J Physiol Lung Cell Mol Physiol* 283: L991–L1001, 2002.
23. McGrath SA. Induction of p21WAF/CIP1 during hyperoxia. *Am J Respir Cell Mol Biol* 18: 179–187, 1998.
24. McGrath-Morrow SA and Stahl J. Growth arrest in A549 cells during hyperoxic stress is associated with decreased cyclin B1 and increased p21(Waf1/Cip1/Sdi1) levels. *Biochim Biophys Acta* 1538: 90–97, 2001.
25. Morton RL, Das KC, Guo XL, Ikle DN, and White CW. Effect of oxygen on lung superoxide dismutase activities in premature baboons with bronchopulmonary dysplasia. *Am J Physiol* 276: L64–L74, 1999.
26. O'Reilly MA. DNA damage and cell cycle checkpoints in hyperoxic lung injury: braking to facilitate repair. *Am J Physiol Lung Cell Mol Physiol* 281: L291–L305, 2001.
27. O'Reilly MA, Staversky RJ, Stripp BR, and Finkelstein JN. Exposure to hyperoxia induces p53 expression in mouse lung epithelium. *Am J Respir Cell Mol Biol* 18: 43–50, 1998.
28. O'Reilly MA, Staversky RJ, Watkins RH, Maniscalco WM, and Keng PC. p53-independent induction of GADD45 and GADD153 in mouse lungs exposed to hyperoxia. *Am J Physiol Lung Cell Mol Physiol* 278: L552–L559, 2000.
29. O'Reilly MA, Staversky RJ, Watkins RH, Reed CK, de Mesy Jensen KL, Finkelstein JN, and Keng PC. The cyclin-dependent kinase inhibitor p21 protects the lung from oxidative stress. *Am J Respir Cell Mol Biol* 24: 703–710, 2001.
30. Rancourt RC, Keng PC, Helt CE, and O'Reilly MA. The role of p21(CIP1/WAF1) in growth of epithelial cells exposed to hyperoxia. *Am J Physiol Lung Cell Mol Physiol* 280: L617–L626, 2001.
31. Scholzen T and Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol* 182: 311–322, 2000.
32. Shenberger JS and Dixon PS. Oxygen induces S-phase growth arrest and increases p53 and p21(WAF1/CIP1) expression in human bronchial smooth-muscle cells. *Am J Respir Cell Mol Biol* 21: 395–402, 1999.
33. Sherr CJ. G1 phase progression: cycling on cue. *Cell* 79: 551–555, 1994.
34. Sosenko IR, Chen Y, Price LT, and Frank L. Failure of premature rabbits to increase lung antioxidant enzyme activi-

ties after hyperoxic exposure: antioxidant enzyme gene expression and pharmacologic intervention with endotoxin and dexamethasone. *Pediatr Res* 37: 469–475, 1995.
35. Taylor WR and Stark GR. Regulation of the G2/M transition by p53. *Oncogene* 20: 1803–1815, 2001.
36. Tryka AF, Witschi H, Gosslee DG, McArthur AH, and Clapp NK. Patterns of cell proliferation during recovery from oxygen injury. Species differences. *Am Rev Respir Dis* 133: 1055–1059, 1986.
37. Walworth NC. Cell-cycle checkpoint kinases: checking in on the cell cycle. *Curr Opin Cell Biol* 12: 697–704, 2000.
38. Zweier JL, Duke SS, Kuppusamy P, Sylvester JT, and Gabrielson EW. Electron paramagnetic resonance evidence that cellular oxygen toxicity is caused by the generation of superoxide and hydroxyl free radicals. *FEBS Lett* 252: 12–16, 1989.

Address reprint requests to:
Kumuda C. Das, Ph.D.
Department of Molecular Biology
University of Texas Health Center at Tyler
11937 U.S. Hwy. 271
Tyler, TX 75708

E-mail: kumuda.das@uthct.edu

Received for publication September 28, 2003; accepted October 20, 2003.

This article has been cited by:

1. Jie Dong, William A Carey, Stuart Abel, Christopher Collura, Guoqian Jiang, Sandra Tomaszek, Shari Sutor, Anja C Roden, Yan W Asmann, Y S Prakash, Dennis A Wigle. 2012. MicroRNA-mRNA interactions in a murine model of hyperoxia-induced bronchopulmonary dysplasia. *BMC Genomics* **13**:1, 204. [CrossRef]
2. Anita Zádori, Viktor Antal Ágoston, Kornél Demeter, Nóra Hádinger, Linda Várady, Tímea K#hídi, Anna G#bl, Zoltán Nagy, Emília Madarász. 2011. Survival and differentiation of neuroectodermal cells with stem cell properties at different oxygen levels. *Experimental Neurology* **227**:1, 136-148. [CrossRef]
3. CHRISTIAN GILLE, FRANZISKA STEFFEN, KIRSTEN LAUBER, HILDEGARD KEPPELER, ANJA LEIBER, BÄARBEL SPRING, CHRISTIAN F. POETS, THORSTEN W. ORLIKOWSKY. 2009. Clearance of Apoptotic Neutrophils Is Diminished in Cord Blood Monocytes and Does Not Lead to Reduced IL-8 Production. *Pediatric Research* **66**:5, 507-512. [CrossRef]
4. CLYDE J. WRIGHT, PHYLLIS A. DENNERY. 2009. Manipulation of Gene Expression by Oxygen: A Primer From Bedside to Bench. *Pediatric Research* **66**:1, 3-10. [CrossRef]
5. Kumuda C. Das . 2004. Redox Control of Premature Birth and Newborn Biology. *Antioxidants & Redox Signaling* **6**:1, 105-107. [Citation] [Full Text PDF] [Full Text PDF with Links]