Neonatal Chronic Lung Disease in the Post-Surfactant Era
Lessons Learned from Authentic Animal Models

Richard D. Bland
Stanford University School of Medicine, Stanford, Calif., USA

Key Words
Bronchopulmonary dysplasia • Lung development • Mechanical ventilation • Failed formation, alveoli/pulmonary capillaries • Respiratory failure • Elastin • Prematurity • Pulmonary inflammation/edema • Inhaled nitric oxide • Continuous positive airway pressure, nasal application

Abstract
This is a brief review of neonatal chronic lung disease, sometimes called the ‘new bronchopulmonary dysplasia (BPD)’. The clinical, radiographic and pathological features of this condition have changed considerably in recent years because of major advances in perinatal care, including widespread use of antenatal glucocorticoid therapy, postnatal surfactant replacement and improved respiratory and nutritional support. Authentic animal models, featuring lengthy mechanical ventilation of surfactant-treated, premature neonatal baboons and lambs, have provided important insights on the pathophysiology and treatment of this disease. Lung histopathology after 2–4 weeks of positive-pressure ventilation with oxygen-rich gas results in failed formation of alveoli and lung capillaries, excess disordered elastin accumulation, smooth muscle overgrowth in small pulmonary arteries and airways, chronic inflammation and interstitial edema. Treatment interventions that have been tested in these animal models include nasal application of continuous positive airway pressure, high-frequency mechanical ventilation, inhaled nitric oxide and retinol. The challenge now is to improve understanding of the molecular mechanisms that regulate normal lung growth and development, and to clarify the dysregulation of lung structure and function that occurs with injury and subsequent repair so that effective treatment or prevention strategies can be devised and implemented.

Introduction
Infants born at a very early stage of development commonly suffer respiratory failure because of their immature lungs, primitive respiratory drive and vulnerability to infection. Survival of such infants has improved considerably in recent years because of major advances in perinatal care, including widespread use of antenatal glucocorticoid therapy, postnatal surfactant replacement, and improved respiratory and nutritional support. Yet the need for prolonged assisted ventilation in such infants often leads to a chronic form of lung disease that was first described by Northway et al. [1] as bronchopulmonary dysplasia (BPD).
The typical clinical, radiographic, and pathological features of this condition have changed during the past two decades, perhaps because of the substantial increase in survival of the tiniest premature infants with very immature lungs, as well as changes in the application of assisted ventilation and various supportive measures used to manage these infants. This paradigm of what is now described as the ‘new BPD’ is the most common cause of chronic lung disease (CLD) in early infancy [2].

### Changing Pattern of BPD

In the years before surfactant replacement and antenatal glucocorticoid therapy became the standard of care, BPD was a condition that occurred primarily in babies who were of sufficient size and maturity to survive the ravages of prolonged exposure to high oxygen and positive-pressure ventilation. These were mainly babies who were born between 28 and 32 weeks’ gestation and who weighed between 1,000 and 1,500 g at birth. Their clinical course and lung pathology reflected the consequences of severe pulmonary oxygen toxicity and lung overexpansion, which typically manifest as extrapulmonary air leaks, protein-rich lung edema, extensive airway epithelial metaplasia, peribronchial fibrosis and airway and pulmonary vascular smooth muscle hypertrophy, sometimes leading to right heart failure [3–8]. Mortality among these infants was high (>50% in some series), and long-term ventilator-dependent respiratory failure was common among survivors.

With the advent of surfactant therapy to combat acute respiratory failure, coupled with widespread use of antenatal steroids to accelerate lung maturation before anticipated premature delivery, the epidemiological, clinical and pathological picture of BPD changed considerably [8–13]. Recent reports indicate that almost two-thirds of infants who acquire BPD weigh <1,000 g and are <28 weeks of gestation at birth. Most cases of BPD now evolve without a prior history of severe respiratory distress syndrome [14, 15]. In contrast to past experience, when pulmonary oxygen toxicity and lung overexpansion were considered major contributors to the development of chronic lung injury, these extremely small, immature infants often require little supplemental oxygen during their initial postnatal course, and it is uncommon for such infants to have received mechanical ventilation with high inflation pressures or large tidal volumes. In many instances, respiratory support for these infants is provided mainly because of apnea, weak inspiratory effort and a compliant chest wall, all of which contribute to CO₂ retention. Their respiratory status sometimes worsens because of pulmonary edema associated with a patent ductus arteriosus, or from pneumonia and sepsis [9], which may increase the need for oxygen and positive-pressure ventilation. Such infants tend to have a milder form of chronic respiratory failure than was described in the years that preceded surfactant replacement and antenatal glucocorticoid therapy.

Several reports indicate that the lung pathology of these extremely immature infants with BPD differs from the ‘classic form’ of BPD. The predominant pathological features of the ‘new BPD’ are alveolar hypoplasia, arrest of acinar and associated vascular development, abundant smooth muscle in both the lung circulation and small airways, variable degrees of interstitial proliferation of extracellular matrix components, including elastin and collagen, as well as interstitial fluid accumulation. Compared to BPD in the era preceding surfactant treatment, there is less evidence of fibroproliferative airway damage and parenchymal fibrosis [7, 8, 10, 16–21].

Although the pathogenesis of the ‘new BPD’ remains unclear, much evidence suggests that this form of chronic respiratory failure reflects abnormal growth and repair of the immature lung exposed to the continuous stress of repetitive inflation with O₂-rich gas in a setting of chronic inflammation, often aggravated by recurrent infection [22, 23]. Several studies have shown an increase in the number of neutrophils, macrophages and neutrophil-derived elastase activity in liquid suctioned from the airways of infants with acute respiratory distress syndrome who later acquire BPD [24–27]. These studies also showed that elastase inhibitory capacity and α₁-protease inhibitor activity are reduced, as is secretory leukocyte protease inhibitor [28], in infants with evolving BPD compared to infants without BPD. Other reports indicate that antioxidant enzymes are deficient in the immature lung [29, 30], which may increase vulnerability to the oxidant stress associated with postnatal lung inflammation. The reported association of maternal chorioamnionitis and early lung inflammation in infants with subsequent BPD [31] led to the notion that BPD sometimes may have a prenatal inflammatory origin [11, 32, 33]. The pivotal role of lung inflammation in the development of BPD has yet to be established, but remains a major thrust of many ongoing investigations into the pathogenesis of BPD and the pursuit of effective treatment or prevention.
Insight from Animal Models on the Pathophysiology of the ‘New BPD’

Because of declining mortality from BPD and fewer neonatal autopsies in recent years, defining the pathophysiology of BPD in the post-surfactant era has been a formidable challenge and has relied to a large extent on the detailed observations made on authentic animal models of this condition, namely the chronic ventilation experiments conducted with prematurely delivered baboons at the Southwest Foundation in San Antonio, Texas [34–41], and similar studies done with premature lambs that were mechanically ventilated for 3–4 weeks by the group in Utah [42–47]. Both of these experimental models of BPD use animals that are delivered at a very immature stage of development, such that prolonged assisted ventilation with O₂-rich gas is essential to allow survival of sufficient duration for chronic lung injury to develop. The clinical condition and early postnatal management of these premature animals closely resembles the clinical history of infants with BPD [34, 44]: (1) surfactant replacement is given at birth to reduce the need for supplemental O₂ and high inflation pressures; (2) complications associated with a patent ductus arteriosus are prevented with either pharmacological or surgical closure during the first few days after birth; (3) cardiovascular instability is common, often requiring treatment with plasma expanders and drugs to support the circulation; (4) recurrent infections develop that require protracted antimicrobial therapy; (5) intravenous nutrition is provided very early, later supplemented with enteral feedings, and (6) mechanical ventilation is applied using modest peak-inflation pressure and positive end-expiratory pressure, with sufficient supplemental O₂ and ventilatory support to maintain normal PaO₂ and PaCO₂ values.

Postsurfactant BPD in Extremely Premature Baboons

The most authentic animal homolog of the ‘new BPD’ is the premature primate model created by Coalson and her colleagues [48–54], whose pioneering efforts with prolonged mechanical ventilation and hyperoxia of baby baboons began over two decades ago in the pre-surfactant era. In recent years, this model has been modified to replicate almost all of the conditions that prevail in the development of the ‘new BPD’, including extreme prematurity, antenatal exposure to maternal glucocorticoid treatment, postnatal surfactant treatment and assisted ventilation with modest inflation pressures and appropriate concentrations of inspired O₂ [34]. The only missing feature of this ‘new BPD’ model is premature labor.

As described in the initial report, baboons were delivered by cesarean section at about two thirds of term gestation and then mechanically ventilated for at least 1–2 months. Airway secretions obtained from these animals showed evidence of lung inflammation, and at autopsy their lungs had fewer alveoli and capillaries than did control term lungs. The respiratory units of the chronically ventilated preterm baboons were described as large ‘simplified’ distal saccules, with walls that contained increased numbers of mesenchymal and mononuclear cells, and focal deposition of elastin and collagen fibers (fig. 1).
Subsequent studies showed decreased pulmonary expression of vascular endothelial growth factor (VEGF) and one of its receptors, fms-like tyrosine kinase receptor (Flt-1, also called VEGF-R1) in the baboons with BPD, which may help to explain their reduced capillary volume density [37]. Another study demonstrated reduced abundance of both endothelial nitric oxide synthase and inducible nitric oxide synthase, coupled with decreased NOS activity in the lungs of these baboons [38], findings that might contribute to pulmonary vascular and airway dysfunction in BPD.

The association of perinatal infection, lung inflammation and increased risk of BPD led the San Antonio group to study the impact of intra-amniotic infusion of live bacteria, Ureaplasma urealyticum (Uu), into pregnant sheep 2–3 days before cesarean section delivery at 125 days’ gestation [39]. All of the infected preterm baboons had significant numbers of Uu organisms in tracheal fluid samples taken 24 h after birth, and 4 of 10 Uu-exposed animals remained heavily colonized at necropsy performed after 2 weeks of mechanical ventilation. Compared to control animals that were mechanically ventilated for the same duration without antenatal infection, Uu animals required more O2 and ventilatory support between 2 and 10 days after birth, had greater concentrations of interleukins 6 and 8 in their tracheal fluid aspirates, and showed more severe airway and lung parenchymal inflammation at necropsy. Lung function was better
and O<sub>2</sub> needs were less in preterm baboons whose colonization with *Uu* was short-lived compared to those in which *Uu* persisted in the lungs throughout the 2-week course of mechanical ventilation, suggesting that the immune system response may have an important role in determining ultimate outcome after perinatal infection with *Uu*.

**Postsurfactant BPD in Premature Lambs**

An ovine model of neonatal CLD has provided further insight regarding the pathophysiology of the ‘new BPD’. Lambs that were delivered by cesarean section at ~80% of term gestation and then mechanically ventilated for 3–4 weeks had persistent elevation of lung vascular and respiratory tract resistances when compared to control lambs born at term. These physiological abnormalities were associated with increased abundance of smooth muscle and elastin in pulmonary arteries and airways [43, 44] (fig. 2). Studies of lung fluid balance showed a progressive increase in lung lymph flow and a consistent decrease in the lymph/plasma protein ratio, indicative of increased lung microvascular pressure rather than increased permeability, and postmortem histopathology revealed varying degrees of interstitial pulmonary edema [44]. Subsequent studies showed evidence of lung vascular dysfunction, with loss of the pulmonary vasodilator response to inhaled nitric oxide (iNO) that was attributed to diminished abundance of endothelial nitric oxide synthase [45] and soluble guanylate cyclase in the pulmonary circulation [46].

These lambs also had fewer alveoli and lung microvessels than did control lambs that were born at term. There was a striking increase in tropoelastin (TE) gene expression in the lungs of the preterm lambs with CLD, and this was associated with excessive and disordered deposition of elastic fibers throughout the lung parenchyma [42] (fig. 3). This abnormal abundance and distribution of elastin was especially notable in blunted secondary crests, where focal deposits of distally situated elastin normally define loci of future alveoli during lung development. The role that abnormal regulation of elastin plays in the pathogenesis of impaired alveolar and vascular development in BPD is unclear, but there is much evidence that cyclic stretch may induce TE expression in the developing lung [55–57], which in turn may disrupt normal elastin deposition and alveolar formation [17, 24, 25, 28, 58]. Understanding the mechanisms that regulate elastin distribution and abundance in the developing lung and its dysregulation in neonatal lung injury and repair is likely to provide important clues regarding the pathogenesis of impaired alveolar and lung vascular development in BPD.

**Insight from Animal Models on Treatment of Postsurfactant BPD**

Improved outcome of very premature infants in recent years has been attributed largely to the beneficial effects on lung structure and function of antenatal glucocorticoid administration and postnatal surfactant therapy. It is likely, however, that many other changes in newborn intensive care practices during the last two decades have contributed to the evolution of clinical and pathological features that are now described as the ‘new BPD’. Among
the most heralded of these changes in patient care has been the emphasis on gentler approaches to assisted breathing, advocating early nasal application of continuous positive airway pressure (nCPAP), and limited use of positive-pressure mechanical ventilation, delivering much smaller tidal volumes than were used in the past. Rationale for this strategy sprang from a survey that compared the incidence of BPD at different institutions, wherein the lowest incidence of the disease occurred in a neonatal unit that emphasized the use of nCPAP without endotracheal intubation [59].

**Nasal CPAP**

The San Antonio primate model of BPD has been used to test the possible benefit of several therapeutic interventions designed to reduce lung injury. Perhaps the most celebrated of these clinical trials tested the feasibility and possible benefit of nCPAP as a means of minimizing need for mechanical ventilation and thereby reducing or preventing chronic lung injury in very premature baboons [40]. This study used a modified version of the previously described experimental protocol [34], whereby the baby baboons, exposed to 2 doses of antenatal betamethasone 48 and 24 h before birth, were delivered by cesarean section at 125 days (term = 185 days) and given 2 doses of porcine surfactant (Curosurf), in addition to daily caffeine citrate to stimulate spontaneous breathing. Weaning from low-volume positive-pressure ventilation to nCPAP 24 h after birth was successful in 6 of these animals, 5 of which survived to 28 days. Their routine care included ‘permissive hypercapnia’, avoidance of supine positioning, immediate intravenous and early enteral nutrition (usually at 48–72 h), minimal handling and exposure to light and noise, 10 days of antibiotic treatment and 28 days of antifungal treatment. These supportive measures are notable because they differed somewhat from earlier studies conducted with the premature baboons, and because this study did not include contemporaneous control infants treated with conventional mechanical ventilation. Fetuses taken at 125 and 156 days’ gestation without breathing served as controls for lung histopathology and morphometric measurements. When compared with lungs of 156-day gestational controls, lungs from animals treated with nCPAP showed enlarged, thin-walled air spaces, with measurements of internal surface area and vascular volume density that were similar to those of gestational controls. These interesting observations suggest that postnatal support of very premature primates with nCPAP may help to facilitate near-normal formation of alveoli and lung blood vessels. Confirmation of this notion awaits completion of a carefully controlled, randomized trial comparing nCPAP and conventional mechanical ventilation of very premature infants or non-human primates using identical supportive measures and definitive endpoints.

**High-Frequency Mechanical Ventilation**

Another study from this group examined the effects of high-frequency oscillatory ventilation (HFOV), compared to conventional mechanical ventilation, in managing extremely premature baboons delivered at 125 days’ gestation [36]. HFOV appeared to reduce markers of lung inflammation and improved measurements of lung mechanics. Dynamic lung compliance was consistently greater and airways resistance during expiration was significantly less in preterm baboons that were treated for 28 days with HFOV compared to control animals that were treated with conventional mechanical ventilation.
HFOV, however, did not prevent alveolar hypoplasia in the immature baboon model of neonatal CLD.

A similar study using preterm lambs compared the effects on lung histopathology of high-frequency jet ventilation at 420 cycles/min versus conventional mechanical ventilation at 20 breaths/min. Lambs that received high-frequency jet ventilation for 3 weeks, compared to control lambs that received conventional mechanical ventilation, had twice the number of radial alveolar counts and significantly less smooth muscle and elastin around small pulmonary arteries and airways (fig. 4).

Inhaled Nitric Oxide

The San Antonio group recently reported better lung function, compared to controls, in chronically ventilated preterm baboons that received iNO continuously at 5 ppm for 2 weeks [41]. In this study, iNO begun 1 h after birth was associated with increased dynamic lung compliance and reduced airways resistance during the first week. Postmortem lung histology after 2 weeks of mechanical ventilation showed no significant differences in the number of alveolar septae or in lung volume measurements between iNO-treated and untreated baboons, though lung weight and DNA content were greater in the animals that received iNO compared to controls. In addition, excess accumulation of lung elastin, which is characteristic of CLD in human infants and preterm lambs [19, 42], was absent in the baboons that received iNO.

In studies done with preterm lambs that were mechanically ventilated for 3 weeks, continuous low-dose iNO beginning at birth led to a significant reduction in lung resistance during expiration, with less smooth muscle around terminal bronchioles in iNO-treated lambs compared to preterm control lambs [47]. Lambs that received iNO had more than twice the number of radial alveolar counts than chronically ventilated control lambs had. These findings indicate that iNO preserves structure and function of airway smooth muscle and also enhances alveolar development in preterm lambs with CLD.

Retinol

The use of retinol (vitamin A) to treat extremely premature infants at risk for BPD is supported by data from animal, as well as human, studies dating back more than a quarter of a century. Retinoids have been shown to have a profound influence on lung development and repair from lung injury in experimental animals [60–68]. Because plasma concentrations of retinol are low in very premature infants compared to infants born at term, especially low in infants who acquire BPD [69, 70], clinical trials of retinol treatment have been conducted in very premature infants with low plasma concentrations of retinol to determine the effects of retinol supplementation on the incidence of BPD [71, 72]. These randomized, controlled studies have shown that retinol treatment beginning soon after birth and continuing for 4 weeks thereafter leads to a modest, but statistically significant reduction in the incidence of BPD, without causing apparent toxicity.

Previous reports showed a beneficial effect of treatment with all trans-retinoic acid in attenuating both steroid-induced alveolar hypoplasia and oxygen-induced inhibition of lung septation in newborn rats [65, 68]. These observations, coupled with the aforementioned clinical
trials of retinol treatment for premature infants, provided the basis for studies that examined the effects of daily intramuscular retinol treatment (5,000 IU/day) in lambs that were delivered prematurely and mechanically ventilated for 3 weeks, compared to lambs that were managed in an identical manner except that they did not receive retinol [73, 74]. Lambs that received retinol had more alveoli, greater capillary surface density, and less elastin in their lungs than control lambs had (fig. 5). Immunoblot analysis of lung tissue harvested from these lambs showed greater expression of VEGF and its receptor, VEGF-R2 (also called fetal liver kinase 1), in the lambs that received retinol, and Northern analysis of peripheral lung tissue showed less expression of TE mRNA in the lungs of the retinol-treated lambs compared to controls.

Disordered Elastin in Neonatal CLD

These new findings may provide important clues regarding the pathogenesis of BPD, namely the association between excessive and disordered elastin accumulation and impaired development of alveoli and microvessels in the lung, as described in the premature lamb model of chronic lung injury [42–44] and in premature infants who have died with BPD [10, 19, 75, 76]. Elastin is known to have a pivotal role in normal mammalian lung development: deletion of the elastin gene in mice leads to neonatal death from cardiorespiratory failure associated with reduced terminal airway branching and impaired vasculogenesis in the lungs [77, 78]. The relationship between lung elastin and retinoic acid or retinol is less well established, but the observation that mice bearing a deletion of retinoic acid receptors had reduced numbers of both alveoli and elastic fibers in their lungs raises the possibility that elastin’s role in alveolar formation may be regulated, at least in part, through retinoic acid signaling pathways [67]. Disordered pulmonary elastin deposition in BPD could be triggered by early postnatal elastolytic activity in the lung, which is known to occur in respiratory failure that is managed with assisted ventilation after premature birth [24, 25], or it could result from prolonged, excessive lung stretch, which has been shown to increase TE gene expression in the developing lung of fetal sheep [57] and in cultured lung cells from fetal rats [55]. The relationship between abnormal elastin accumulation and impaired alveolar and vascular development in the lungs of infants with BPD is an intriguing set of observations that merits further exploration.

Mechanical Ventilation of Newborn Mice: Impact on Lung Development Genes

As the aforementioned studies indicate, failed formation of alveoli and lung capillaries, and excess, disordered elastin are key histological features of neonatal CLD, which typically afflicts premature lungs exposed to lengthy mechanical ventilation with O2-rich gas. As lung septation and angiogenesis occur after term birth in mice, notably between postnatal day 3 and 14 [79], we recently did studies to see if mechanical ventilation of newborn mice with either 40% O2 or air would alter lung expression of genes that regulate formation of alveoli, lung capillaries and elastin [80, 81]. We studied 6 groups of pups that were 2–4 days old and weighed 2–4 g. Four groups (n = 8–10/group) had sham surgery and then breathed spontaneously for 8 h in either air or 40% O2 at either 60 breaths/min (peak-inflation pressure 16 ± 1 cm H2O, positive end-expiratory pressure 1 cm H2O, mean airway pressure 4 cm H2O, tidal volume 22 ± 1 µl) or 180 breaths/min (peak inflation pressure 10 ± 1 cm H2O, positive end-expiratory pressure 1 cm H2O, mean airway pressure 4 cm H2O, tidal volume 13 ± 1 µl). Two control groups (n = 8–10/group) had sham surgery and then breathed spontaneously for 8 h in either air or 40% O2. Lungs were harvested for histology, microarray analysis and quantitative real-time PCR to measure mRNA for genes considered important in alveolar and vascular development (VEGF-A, its receptor VEGF-R2, and tenascin C) and for genes that are important in elastin synthesis and assembly (TE, and lysyl oxidase). Histology showed no evidence of lung injury from O2 or mechanical ventilation, though air spaces were larger in lungs of mice that received mechanical ventilation compared to lungs of control pups. Gene microarrays and qRT-PCR showed less lung mRNA for VEGF, VEGF-R2 and tenascin C in both groups of pups that were ventilated with 40% O2 (at 60 and 180 breaths/min) compared to controls. Mechanical ventilation with air had little or no effect on lung development genes. Mechanical ventilation with either air or 40% O2 increased lung expression of elastin-related genes. Recent 24-hour studies of mechanical ventilation with 40% O2 (and relevant controls) showed significant reductions in lung abundance of proteins that affect the formation of alveoli and lung capillaries. These results indicate that mechanical ventilation with O2-rich gas at a critical stage of development reduces lung expression of genes that regulate alveolar septation and angiogenesis. Mechanical ventilation with either air or 40% O2 increases pulmonary expression of genes that play a key role...
in synthesis and assembly of elastic fibers, which can affect the structural stability and distensibility of the lung and its vasculature.

**Paving the Way to Prevention**

During the past quarter-century, widespread use of surfactant treatment after premature birth has greatly facilitated management of even the tiniest infants, reducing their needs for extra O₂ and aggressive respiratory support. Increased survival of these ‘micro-premies’, whose respiratory drive is frequently faulty and whose host defenses are modest at best, often results in a protracted clinical course complicated by severe apnea and recurrent infection that calls for prolonged treatment with O₂ and assisted ventilation, culminating in the ‘new BPD’. The disease sometimes starts before birth, triggered by fetal inflammation associated with maternal chorioamnionitis. Thus, the clinical, radiographic and pathological features of this condition are considerably different than the well-defined progression of lung disease described by Northway et al. [1] almost four decades ago.

The challenge now is to improve understanding of the molecular mechanisms that regulate normal lung growth and development, and to clarify the dysregulation of lung structure and function that occurs with injury and subsequent repair. Such studies will provide new insights regarding the pathogenesis of BPD and help to formulate effective strategies to treat or prevent what is more aptly described now as neonatal CLD.

**Acknowledgments**

R.D.B. gratefully acknowledges the support of the National Heart, Lung and Blood Institute (Grant HL-62512 and HL-56401) and the March of Dimes Foundation that provided funds for much of the research on newborn sheep and mice that is discussed in this review. In addition, this work could not have been accomplished without the extraordinary efforts of many co-investigators, including Kurt Albertine, David Carlton, Soo-Chul Cho, Ronald Day, Beyong Kim, Amy MacRitchie and Richard Pierce, as well as the many University of Utah medical students, research associates and respiratory therapists who assisted in the daily management of the lambs. For the recent studies conducted on newborn mice at Stanford University, much credit goes to two major contributors to this project, Berit Jacobson and Robert Ertsey.

**References**
