Pathology of Bronchopulmonary Dysplasia

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Over the past three decades, advances in prenatal and neonatal intensive care have contributed to marked improvements in survival rates for extremely immature infants born during the canalicular phase of lung development at 24 to 26 weeks, a time when alveolar and distal vascular development is rapidly occurring. The histopathological lesions of severe airway injury and alternating sites of overinflation and fibrosis in “old” BPD have been replaced in “new” BPD with the pathologic changes of large, simplified alveolar structures, a dysmorphic capillary configuration, and variable interstitial cellularity and/or fibroproliferation. Airway and vascular lesions, when present, tend to be present in infants, who over time develop more severe disease. The concept that “new” BPD results in an arrest in alveolization should be modified to that of an impairment in alveolization as evidence shows that short ventilatory times and/or the use of nCPAP allow continued alveolar formation.

Bronchopulmonary dysplasia (BPD) is a chronic lung disease that now occurs primarily in preterm newborns that weigh <1000 g, are born at 24 to 26 weeks of gestation, and receive respiratory support with mechanical ventilation and/or prolonged oxygenation. Prenatal intrauterine infections may predispose the fetal lung to subsequent oxidant and volutrauma injuries. Advances in critical care management and technology followed by more widespread use of exogenous surfactant and prenatal steroids have resulted in the present pattern of injury: an extremely immature lung due to interruption of normal gestational growth and development, and altered by subsequent reparative processes of impaired alveolization, dysmorphic vasculogenesis, and usually minimal alveolar wall fibroproliferation. So the pathogenesis of “new” BPD involves extreme lung immaturity, treatment-induced oxygen and volutrauma injury, and prenatal and/or postnatal inflammatory responses.

A review of later stages of human lung development defines the immaturity aspect of current BPD in low-birth weight infants. The lung at 24 to 26 weeks is in the late canalicular stage of development; and at 30 to 32 weeks, the lung is in the saccular stage. The extent of lung development between 24 and 26 weeks gestation and 30 and 32 weeks gestation is substantive; extensive vasculogenesis within the developing terminal sacculles that then form secondary crests occurs along with interstitial extracellular matrix loss and remodeling. Although alveoli are present in some infants at 32 weeks gestation, they are not uniformly present until 36 weeks during the alveolar stage of development. Thus, premature birth and initiation of pulmonary gas exchange interrupts normal alveolar and distal vascular development.

“Old” BPD pathology, evoked by elevated oxygen and ventilator-induced injury on a relatively immature and surfactant deficient lung, yielded histopathological changes of severe airway epithelial lesions and smooth muscle hyperplasia, alternating sites of overinflation and atelectasis, extensive fibrosis, and severe vascular hypertensive lesions. Even before exogenous surfactant and prenatal steroids were used as treatment modalities in infants, these severe pathologic changes were disappearing, largely due to technological advances, improved ventilatory strategies, and better nursing techniques in neonatal intensive care units. However, these same advances in care led to the survival of smaller and more immature infants. In these infants with BPD born just before the era of treatment with exogenous surfactant, lung findings included variable, but overall less airway epithelial disease, less airway associated muscular hyperplasia, less severe vascular disease, varying degrees of interstitial fibrosis, increased elastic fiber deposition in alveolar walls, and an abundance of large, simplified air spaces yielding reduced alveolar numbers and decreased internal surface area measurements. Hussein and coworkers examined autopsy lung specimens from 14 surfactant-treated infants with BPD, 8 nonsurfactant-treated BPD patients, and 15 age-matched controls who were
autopsied from 1988 through 1994. The infants were treated with three doses of exogenous surfactant (either Survanta or Exosurf), given by intratracheal aerosolization during the first 24 hours. No history of prenatal steroid treatment was given. Gestational ages of the surfactant-treated BPD patients ranged from 24 to 32 weeks; birthweights were not stated. Gestational ages of the nonsurfactant-treated BPD group ranged from 27 to 29 weeks. Eight of the 14 surfactant-treated infants lived for 1 to 6 weeks, whereas the other six survived for 12 to 413 weeks. Length of life in the nonsurfactant-treated BPD group ranged from 2 to 71 weeks. Mild to moderate alveolar septal fibrosis was evident in 5 of the 14 surfactant-treated infants, whereas 7 of the 8 nonsurfactant-treated infants had moderate to severe alveolar septal fibrosis. Necrotizing bronchiolitis was not evident in the surfactant-treated group, and in most cases, a normal-appearing capillary bed was noted. When compared with control lungs from patients without BPD, all (surfactant-treated or not) BPD patient specimens with a postconceptional age greater than 40 weeks showed an inhibition of acinar development (defined as acinar arrest, ie, ratio of degree of alveolization and alveolar size).

A small series of lung biopsy specimens from low birth weight infants on ventilatory support at postnatal ages that ranged from 2 weeks to 7 months have been studied. The gestational ages at birth ranged from 24 to 28 weeks (mean = 26 weeks), and birthweights ranged from 570 to 1100 g (mean = 809 g). All had severe, but not necessarily lethal, disease, one had been treated with exogenous surfactant, and 50% of the infants went on to survive. The consistent findings in all of the biopsy specimens were negligible airway epithelial changes and a simplified distal lung acinus with large sacular/alveolar structures and a decrease in secondary crest formation/alveolar complexity. The most variable finding was the degree of cellularity and fibrosis in the simplified saccular structures. A dysmorphic pattern of vascular organization was seen in the lungs; there were prominent “corner” vessels, plus adjacent dilated vessels, and the capillaries were sparse in some alveolar walls, and dilated and more abundant in other sites. More recently, biopsy and autopsy lung specimens from infants who had received both prenatal steroids and surfactant treatment have been examined and the findings remain consistent; enlarged air spaces with minimal sacular/alveolar structures and a decrease in secondary crest formation/alveolar complexity. The most variable finding was the degree of cellularity and fibrosis in the simplified sacular structures. A dysmorphic pattern of vascular organization was seen in the lungs; there were prominent “corner” vessels, plus adjacent dilated vessels, and the capillaries were sparse in some alveolar walls, and dilated and more abundant in other sites. More recently, biopsy and autopsy lung specimens from infants who had received both prenatal steroids and surfactant treatment have been examined and the findings remain consistent; enlarged air spaces with minimal alveolization, dysmorphic capillary configuration by PECAM immunostaining and variable alveolar wall cellularity or fibrosis (Fig. 1).

Figure 1 Lung biopsy from a human infant treated with prenatal steroids and postnatal surfactant, born at 28 weeks gestation and had lung biopsy at 8 months. Enlarged, simplified alveoli are seen. The alveolar walls show increased interstitial thickening. Hematoxylin and eosin; 10×.

As noted above, many of the histopathological features of BPD described over the last 20 years are similar whether the infants received no prenatal steroid or exogenous surfactant treatments, surfactant treatment only, or both. The “variable” descriptor reflects a level of severity of the histopathological lesion being reported, and is likely influenced by the amount of time spent on ventilatory support, whether postnatal lung infections occurred and if nutritional needs were met. The consistent lesion seen in BPD is alveolar simplification and enlargement; it results from an impairment, not an arrest, in postnatal alveolization in an extremely immature lung following preterm birth.

Developmental arrest has been used to describe the lung findings in new BPD. Medically, the term arrest usually denotes to render inactive. Its use in describing BPD may be misleading to some in that a sustained or permanent stoppage of alveolization might be assumed. As reported and discussed below, our experience with the baboon model of BPD has convinced us that the immature lung can continue to alveolarize, especially if mechanical ventilation is avoided or is used as little as possible for respiratory support. Recall the study of Hislop and coworkers, in which children who had received mechanical ventilation, either with or without a history of RDS, had reduced alveolar number and decreased internal surface area measurements, whereas lungs of control premature infants, who did not receive assisted ventilation, had normal alveolar counts and internal surface areas at death. Also, in Cherukupalli and coworkers’s study, one of their group IV human infants (a group ventilated for only 25% of life; subjected to high oxygen levels for only 4% of life) had “lungs that were almost entirely normal and could scarcely be distinguished from controls of the same age.” Whether there is an arrest or impairment in the formation of the distal lung vasculature is not settled. Coordination of distal lung vasculogenesis and alveolization has been shown in several experimental and clinical studies. Treatment of rats
with antiangiogenic agents decreased alveolization and lung growth yielding lung findings similar to those of “new” BPD,15 treatment of newborn rats with a vascular endothelial growth factor (VEGF) receptor inhibitor also decreased alveolization and vascular growth,16 and treatment with VEGF in hyperoxic-treated rats enhanced alveolarization.17 In the human lung biopsy specimens, a striking finding was the abnormal capillary configuration termed dysmorphic; enlarged corner vessels with adjacent dilated vessels and in other sites microvasculature was sparse and narrowed.5 In the long-term 1- to 2-month baboon model ventilated with a low tidal volume positive pressure ventilatory strategy (LV-PPV), we reported a decrease in internal surface area and decreased platelet endothelial cell adhesion molecule (PECAM) staining of the dysmorphic microvasculature when compared with intrauterine developmental controls.11 VEGF levels of mRNA and protein have been examined with variable results in human infant studies and in the baboon model. In lungs of human infants who died with BPD, Bhatt and coworkers demonstrated a decrease in PECAM staining, an abnormal distribution of capillaries, decreased VEGF immunostaining, and reductions in mRNA for VEGF and angiogenic receptors Flt-1 and TIE-2 when compared with infants without BPD.18 Tracheal aspires from preterm infants who developed more severe respiratory distress had lower levels of VEGF during the first 10 postnatal days, but the lungs of both preterm and BPD infants showed VEGF staining.19 Maniscalco and coworkers also found decreased VEGF mRNA and protein in the 125-day baboon ventilated for 6 or 14 days when compared with 140-day gestational controls.20 Conversely, Asi-kainen et al. found increased VEGF protein in the lungs of preterm baboons that received either PRN (appropriate oxygen as needed) or 100% oxygen for 10 to 21 days.21 Using a stereologic volumetric method, De Paeppe and coworkers reported that PECAM-stained lung microvasculature of ventilated preterm human infants displayed marked angiogenesis, nearly proportionate to the growth of the air-exchanging lung parenchyma, but PECAM protein determinations were found to be lower in short-term ventilated infants and higher in long-term ventilated infants when compared with their control groups.22 Thibeault and coworkers found that infants with severe BPD had both stunted secondary septation and microvascular development, but over time, primary septal walls thinned and increased their volume density of vessels.23 At this time, the only consistent vascular finding in new BPD pathology is that the structural configuration of the distal microvasculature is abnormal, ie, either dysmorphic,5 shows an abnormal distribution of alveolar capillaries,18 has vessels more distant from the air surface,23 or appears immature, retaining a saccular architectural pattern.22

**Similarities/Differences in the Pathology of Animal Models of BPD in Relation to Human BPD**

Bronchopulmonary dysplasia is the end result of lung injury in a developmentally immature host, so developmental similarities should exist between the animal model and the human disease. The immature infant will breathe some level of oxygen, and even room air represents a hyperoxic exposure. Because ventilator-induced lung injury plays a significant role in the pathogenesis of BPD, studies would require that volutrauma or biotrauma be mechanistically examined. The presence of a predelivery stimulus to the fetus to induce lung inflammation comparable to the usual scenario of preterm labor, sometimes with chorioamnionitis or ruptured membranes, which often precedes premature birth in humans, is desirable in the animal model. And finally, since BPD is defined clinically and is related to a time factor, the ability to study both acute and chronic events in the lung and to test whether developed therapies would effect improved endpoints would also be wanted. The ideal animal model with all these attributes does not exist at this time, but the lamb and baboon models can be ventilated for weeks to months and offer opportunities to probe unanswered problems concerning chronic lung injury and repair.

The long-term 3- to 4-week lamb model developed by Bland and his coworkers has contributed greatly to our understanding of certain aspects of the pathogenesis of BPD, especially those related to the pulmonary circulation and lung fluid balance,24 interstitial connective tissue elements,25 and treatments using nitric oxide.26,27 The normal lung maturation of fetal lambs results in a fully alveolarized lung with a predominantly single capillary system at term (147 days), which differs from the human, baboon, rat, and mouse in which postnatal alveolization continues. Most of the studies use lambs delivered at 124 ± 3 days, about 84% of term gestation, development between the saccular and alveolar stages, so the degree of lung maturity is more than that of a human infant of 24 to 26 weeks of gestation (60-65% of gestation). In the original paper describing the chronic lung injury model, 2 groups of lambs received mechanical ventilation for 3 to 4 weeks at a respirator rate of either 20 breaths/min (tidal volume, 15 mL/kg) or 60 breaths/min (tidal volume, 5 mL/kg). PaCO2 was maintained at 35-45 mm Hg, with sufficient O2 to keep PaO2 at 60-90 mm Hg (generally FiO2 of 0.4-0.6).28 Pathology showed an altered inflation pattern, impaired alveolar formation, abnormal abundance of elastin, increased muscularization of bronchioles, inflammation, and edema. These features reflected some “old” BPD changes, ie, altered inflation pattern and airway muscularization, and “new” BPD pathology, ie, dilated, large alveoli and large fibroproliferation. The investigators noted that the histopathologic changes were noted to be similar to those of the 140-day gestation baboon model.

The 140-day gestation baboon model (term = 185 days, 76% of gestation, lung development saccular to early alveolar) was developed to mimic “old” BPD.29,30 Following delivery, postnatal surfactant treatment was not used and 100% oxygen was administered for 10 days, resulting in severe airway and distal parenchymal changes consistent with those described in human “old” BPD, except for the severe arterial changes.31-33 The control group for this model was delivered at 140 days gestational age, did not receive exogenous surfactant, was treated with clinically appropriate levels of oxy-
gen (PRN), and showed minimal airway and distal parenchymal changes. The 125-day gestation baboon model currently used (67% of term, lung development between canalicular and saccular stages) was developed to mimic the changed clinical scenario; the treatment of extremely immature infants of borderline viability. In contrast to the 140-day gestational age baboons, those delivered at 125 days required early exogenous surfactant instillation for survival. A low tidal volume ventilator strategy was used, and the infants received appropriate oxygenation levels, only 25% to 40% O2 during 14 days of mechanical ventilation. They subsequently acquired clinical and radiographic features consistent with BPD, and in contrast to intrauterine development from 125 to 140 days gestational age, 14-day ventilated survivors showed no significant progression of secondary crest formation and alveolization. Enlargement of the distal alveolar structures with minimal alveolar formation or complexity and varying degrees of fibroproliferation were the histopathologic changes in prenatal steroid and exogenous surfactant-treated baboon infants treated with LV-PPV and survived for 1 month or more34 (Fig. 2). The distal microvasculature was dysmorphic with admixtures of dilated vascular segments and other sites of sparse, narrowed vessels centered in the walls of the alveolar structures. The findings are similar to those described in more recent human biopsy and autopsy reports. In a recent 28-day study, the use of nasal continuous positive airway pressure (nCPAP) commencing at 24 hours postdelivery along with more aggressive nutritional support in the 125-day baboon model, resulted in alveolar development commensurate with that of 156-day developmental controls11 (Fig. 3). One of these nCPAP-treated animals was survived and underwent a right lower lobe lobectomy at 43 weeks, the equivalent to a 2-year-old human infant in whom alveolization should be complete. Despite a clinical course marked by seizures and apneic episodes, the internal surface area of this survivor was threefold greater than that of comparably aged term-delivered controls, a finding that strengthens our belief that mechanical ventilation plays a major role in BPD development and should be avoided and/or limited in neonatal care.

Jobe and his associates have used intraamniotic administration of E. coli endotoxin to induce a prenatal inflammatory stimulus, and in a series of studies have shown a rapid development of fetal lung inflammation,35 alterations in lung structure, and increases in lung surfactant,36 which resulted in functional improvements in the lung.35-37 We used a similar protocol in 125-day delivered baboons that were ventilated for 6 days following an intraamniotic injection of E. coli...
endotoxin 48 hours before delivery. The treated animals had several significantly improved physiologic parameters and showed no histopathological changes when compared with controls. Despite the desirable functional responses, a bothersome finding was that the proinflammatory cytokine levels of IL-6 and IL-8 peaked very early and then dropped below control levels by 72 hours; profiles that did not reflect those reported in human studies. Ureaplasma urealyticum-induced intrauterine infection was then used in the 125-day baboon model. Ureaplasma urealyticum (Uu) organisms were injected into the amniotic fluid of dams 2 to 3 days before delivery, and over a 14-day study period, two groups of responders were identified on the basis of high or low/no Uu CFUs in tracheal aspirates at necropsy. Compared with unexposed control animals, high Uu responders exhibited worse physiological parameters and higher IL-6 and IL-8 levels, whereas low Uu animals had better oxygenation and ventilation efficiency indices. Lung histopathology showed rare sites of a bronchiolitis and subjacent pneumonitis, but much of the lung parenchyma was similar to that of unexposed controls. Recently, the use of Ureaplasma organisms has been further explored using the lamb model. Moss and coworkers gave intraamniotic Ureaplasma parvum at 1, 3, 6, or 10 weeks before delivery of the preterm lambs at 124 to 125 days. The animals were then euthanized for evaluation of inflammation and fetal lung maturation parameters. No histopathological changes were noted in the lungs of any of the groups, but increases in mRNA for several proinflammatory cytokines were persistently elevated in lung tissue. Increases in pulmonary surfactant correlated with the improved pressure volume curves of the 3-, 6-, and 10-week treated lungs measured at necropsy. Intrauterine growth restriction was seen in fetuses exposed to Ureaplasma organisms for 10 weeks. Additional animal models are needed to better dissect the contributions of both antenatal and postnatal infection/inflammation to the pathogenesis and evolution of BPD.

Postnatal alveolization occurs in rat and mice lungs over a 2-week period following birth. More severe or “old” BPD pathology can be produced in mice lungs using 80% to 100% oxygen, and “new” BPD lung pathology has been described in newborn rats exposed to 60% oxygen for 14 days. Mechanical ventilators are now available to do studies in newborn rats and mice, and the Toronto group has published several interesting experiments of 1 to 3 hours duration in newborn rats concerning developmental susceptibility to ventilator-induced injury, effects of high tidal volume ventilation on lung inflammatory responses, and surfactant content and functional parameters. The compressed period of postnatal lung development in rodents has facilitated recent studies in which a triple transgenic construct of bioactive TGF-β1 was used in the newborn mouse and an adenoviral transfer of active TGF-β1 was done in the newborn rat. Lung lesions of large air spaces with widened, hypercellular septae, and poor alveolar and vascular development were evident in the mouse model, whereas focal fibrotic sites and large saccular structures were seen in the rat model. These types of models will help elucidate the molecular mechanisms that are operative in chronic lung injury.

In summary, whether infants with BPD have been treated with prenatal steroids and/or surfactant or not, they still show impaired alveologization, an abnormal capillary morphology, and an interstitium with variable cellularity/fibroproliferation. Continued improvements in neonatal care, eg, “gentler” ventilation, will likely abrogate some of this lung injury. However, to develop effective therapies for BPD, the control mechanisms of alveolar and capillary development that are coordinated by multiple structural, signaling, and remodeling molecules require elucidation, as well as studies in animal models to identify injury mechanisms.

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References