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Progress in Understanding the Pathogenesis of BPD Using the Sheep and Baboon Models

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Introduction

About half a million infants are born preterm in the US each year. Of these preterm infants, about 30,000 will develop respiratory failure (respiratory distress syndrome, RDS) that requires endotracheal intubation and mechanical ventilation with oxygen-rich gas. Respiratory failure is most frequently related to structural and functional immaturity of the lung that interferes with efficient gas exchange. Consequently, endotracheal intubation and mechanical ventilation with oxygen-rich gas are necessary for life. About half of preterm infants with RDS will require prolonged mechanical ventilation support and go on to develop bronchopulmonary dysplasia (BPD; also called neonatal chronic lung disease). Similar to RDS, risk for BPD is related to gestational age.¹ While BPD is a multifactorial disease, the common antecedents are prematurity and mechanical ventilation with oxygen-rich gas.

BPD, defined by oxygen requirement at 36 weeks corrected gestational age is among the most common *chronic* lung diseases in infants in the US. Over the past 2 decades, use of antenatal steroids to accelerate lung maturation, postnatal surfactant to reduce surface tension and therefore increase lung recruitment, and gentler ventilator support to reduce ventilator-induced lung injury has shifted BPD to more and more immature preterm infants.^{2–9} Nonetheless, high rates of BPD persist.^{10–12}

Respiratory failure secondary to BPD is mainly related to lung immaturity.¹³ Lung immaturity leads to persistently poor respiratory gas exchange. In addition, BPD leads to increased abundance of smooth muscle in the wall of pulmonary arterioles and bronchioles, as well as increased tone and reactivity.^{14–17} These structural and functional modifications of arterioles and bronchioles in the lung lead to pulmonary hypertension and excess airway

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expiratory resistance, respectively^{18–22} The causes of these pathological and pathophysiological features of BPD are incompletely understood. Effective prevention or therapy is unavailable. Ominously, the consequences of BPD may be life-long.²³

This review is organized into five sections. The first three sections deal with the pathogenesis of poor respiratory gas exchange, pulmonary hypertension, and excess airway expiratory resistance, respectively. Each section starts with the primary result from a recently published clinical study. Structural and functional insights are summarized from studies using the experimental large animal models of evolving neonatal chronic lung disease: chronically ventilated preterm baboons and preterm lambs. Both models recapitulate the clinical setting of preterm birth and respiratory failure that require prolonged ventilation support with oxygen-rich gas. The fourth section focuses on a gentler, non-invasive ventilation strategy (nasal continuous positive airway pressure; nasal CPAP). The final section presents our epigenetic hypothesis for the pathogenesis of BPD.

Pathogenesis of Poor Respiratory Gas Exchange

A recently published human clinical study shows that carbon monoxide diffusing capacity is lower among former preterm infants with BPD than control infants who were born at term gestation and were healthy.²⁴ This is a worrisome outcome.^{25–27} How does it come about? Part of the answer comes from understanding the process by which the normal diffusion (alveolar wall) barrier is formed.

In the fully developed human lung, the diffusion barrier encountered by oxygen and carbon dioxide is thin (~1.5 μm).^{28–30} The essential structural elements are alveolar epithelium, composite elements making up the alveolar wall, and the alveolar endothelium. These elements are encountered by oxygen and carbon dioxide as they diffuse along their respective concentration gradients. By comparison, during the second half of gestation, the wall barrier is many micrometers in thickness during the canalicular stage of lung development (weeks 16 to 26 of gestation) and saccular stage of lung development (weeks 24 to 38 of gestation).³¹ The predominant contributor to wall thickness is mesenchyme. Added thickness is contributed by cuboidal epithelial cells that line the immature airspaces during the canalicular and saccular stages of lung development. Moreover, during both stages of lung development, capillaries are distant from the immature airspaces. The combined thickness of the mesenchyme and distant location of capillaries in the preterm infant's immature lung parenchyma make a substantial anatomic barrier to diffusion of oxygen and carbon dioxide. A functional impediment to diffusion of oxygen and carbon dioxide is water because solubility of gas is much lower in water than air. Thus, the thicker and wetter parenchyma, and wetter airspaces in the lung of preterm infants reduces diffusion of oxygen and carbon dioxide compared to the thinner and drier environment of the mature lung.

An approach to identify underlying mechanisms that lead to BPD is to reproduce BPD in animal models.³² Only two animal models use chronically ventilated preterm neonates: the preterm baboon model^{33–39} and the preterm lamb model.^{40–47} The preterm baboon program ended almost a decade ago, however, leaving the preterm lamb model as the only large-animal, physiological model of neonatal chronic lung disease. Advantage of both models is that they reproduce the clinical setting of preterm birth, respiratory failure, and prolonged ventilation support with oxygen-rich gas for days or weeks. Advantages of the preterm baboon model were the close phylogeny of baboons (primates) to humans and the preterm baboons were more immature (~67% of gestation) than preterm lambs (~75% gestation). An advantage of the preterm lamb model is that the fetal lambs are larger (~3 Kg body wt; ~10-fold the size of fetal baboons) and therefore more amenable to physiological studies and

repeated blood sampling. Disadvantages of both models are that they are expensive and require 24-hour care.

The lung histopathology of evolving neonatal chronic lung disease in the preterm baboon and preterm lamb models recapitulates that which occurs in preterm infants with BPD.^{13,19,22,48} Specifically, histopathological characteristics in chronically ventilated preterm baboons and preterm lambs include alveolar simplification, persistent muscularization of pulmonary arterioles, and increased muscularization of bronchioles.^{33–36,40–44,49}

Alveolar simplification results from failure of alveolar secondary septa to sprout or from stunted growth from the walls of saccules.⁵⁰ Without outgrowth of secondary septa, the saccules are not divided into anatomic alveoli.³¹ Without division, airspace surface area for gas exchange is small. Stunted growth also reduces gas exchange surface area, a point that will be returned to later in this section.

An important extracellular matrix player in alveolar secondary septal formation is elastin. This is revealed through use of knock-out (null) mice constructs. For example, simplified distal airspaces typify the histological appearance of the lungs of mice that lack platelet-derived growth factor-A.⁵¹ Some of the mice survive through the early postnatal period but their lungs lack both elastin and myofibroblasts. Further evidence is provided by elastin knock-out (null) mice.⁵² Elastin-null mice survive through the first several days of postnatal life, but they are cyanotic. Their lungs, which lack elastin, have distal airspaces that are arrested at the saccular stage of lung development. Interestingly, their lungs also have fewer vascular and airway generations, suggesting that elastin also is associated with vascular and airway growth in the developing lung.

The lungs of chronically ventilated preterm baboons and preterm lambs with evolving neonatal chronic lung disease have aberrant and continuously upregulated synthesis and secretion of elastin.⁴⁰ Upregulated synthesis occurs quickly, by the third day of continuous mechanical ventilation. Increased secretion of elastin results in increased abundance of elastin fibers that accumulate within the walls lining the immature airspaces.^{40,41} The accumulated elastin fibers form dense bundles in the walls. Also, and particularly conspicuously, the excessive and aberrant accumulation of elastin fibers occurs across the width and height of stunted secondary septa.

Another important player in alveolar secondary septal formation is capillary growth. Normally, growth increases the number and surface area of capillaries in the mesenchyme.^{53,54} However, the growing capillaries are distant from the cuboidal epithelial cells that line the airspaces during the canalicular stage of lung development. During the saccular and alveolar stages of lung development, capillary growth continues in the walls that line the saccules and forming alveoli. In addition, capillaries grow into the mesenchymal core of alveolar secondary septa as the latter form and lengthen. Preterm birth and prolonged mechanical ventilation reduce capillary growth in the lung of preterm baboons⁵⁵ and preterm lambs.^{41,43,47} Studies using preterm lambs also show that the stunted alveolar secondary septa lack capillaries⁴⁷. Therefore, the secondary septa cannot contribute to the surface area of the air:blood barrier that participates in respiratory gas exchange. Another consequence of prolonged mechanical ventilation of preterm lambs is fewer pre- and post-capillary arterioles and venules.^{41,43} Thus, growth of the pulmonary microvascular bed is reduced. This contributes to the respiratory failure seen in these animal models.

The histopathology of neonatal chronic lung disease also includes persistently thick, cellular distal airspace walls. Normally, remodeling occurs during lung development such that loss

of mesenchymal cells contributes to thinning of the future alveolar walls. We showed, using preterm lambs, that mechanical ventilation for 3 days leads to less apoptosis of mesenchymal cells.⁴⁶ Not only is apoptosis lower, concomitantly, proliferation of mesenchymal cells is higher. Both effects on mesenchymal cells continue in the lung of preterm lambs that are mechanically ventilated for 3 weeks (unpublished data). Consequently, the distal airspace walls remain thick and more cellular, which also contribute to alveolar simplification.

A treatment that improves outcomes among some chronically ventilated preterm infants is vitamin A supplementation. The rationale for vitamin A supplementation is preterm infants is that they are deficient in retinol.^{56–59} Large multi-center studies show that vitamin A supplementation reduces the need for oxygen at 36 weeks postmenstrual age in subgroups of preterm infants at risk of BPD.^{60–62} An important note, however, is that not all human clinical studies have shown benefit of vitamin A supplementation.

Results from preterm baboons and preterm lambs given supplemental vitamin A daily also are inconsistent. Supplemental vitamin A (Aquasol; 5,000 U/Kg/d) given to mechanically ventilated preterm baboons for 14 days did not improve ventilation or oxygenation, did not promote alveolar formation or alveolar capillary growth, and did not increase expression of vascular growth factors.⁶³ That study calls into question the hypothesis that vitamin A supplementation is beneficial. However, the experimental design was short (14 days) and therefore may have been inadequate to allow the growth-promoting effects of vitamin A. Supporting the latter explanation are results of vitamin A supplementation (Aquasol; 5,000 U/Kg/d) to mechanically ventilated preterm lambs for 21 days.⁴⁷ Alveolar formation, alveolar capillary growth, and expression of vascular growth factors were greater in the treated group of preterm lambs compared to untreated preterm lambs. In contrast, expression and accumulation of elastin was less in the lung of the vitamin A-treated group. These structural and molecular improvements are associated with better respiratory gas exchange, although the improvement was not statistically significant.

A question remains as to whether the lung parenchyma eventually forms normal surface area for gas exchange in survivors of BPD who are clinically stable. Studies using recovered preterm baboons and preterm lambs show that disruptions in alveolar secondary septation, capillary and microvessel growth, and elastin synthesis and secretion persist later in life. Studies of the lung of preterm baboons showed that mechanical ventilation for 14 days and recovery for 33 weeks led to persistently enlarged airspaces and less parenchyma than control baboons.⁶⁴ Thirty-three weeks of life for baboons is equivalent to ~2 years of postnatal age for humans. New studies in progress in our laboratory suggest that mechanical ventilation for 3 days and recovery for 12 weeks or 24 weeks leads to persistence of alveolar simplification compared to control lambs born at term that live for 2 months or 5 months, respectively (unpublished results). Twelve weeks of life for sheep is equivalent to ~2 years postnatal age for humans, whereas 24 weeks of life for sheep is equivalent to ~6 years postnatal age for humans. These persistent structural manifestations of preterm birth and mechanical ventilation on alveolar formation provide a structural correlate for lower diffusing capacity among survivors of BPD.²⁴ Long term pulmonary outcomes in human neonates with BPD have been covered in the chapter by Bhandari and McGrath-Morrow in this issue.

Pathogenesis of Pulmonary Hypertension

Pulmonary hypertension also contributes to morbidity and mortality among preterm infants who develop BPD.^{14,65,66} For example, a recent clinical study shows that ~18% of 145 preterm infants (gestational age 26 weeks) had pulmonary hypertension at any time during

hospitalization.⁶⁷ Pulmonary hypertension in human neonates with BPD has been covered in the chapter by Berkelhamer, Mestan and Steinhorn in this issue.

Common features of pulmonary vascular pathology in preterm infants who died with BPD are thicker smooth muscle walls of pulmonary arterioles, more elastin in the walls of the arterioles, and fewer pulmonary microvessels.^{18,48,68,69} These pathological features also are evident in the lung of chronically ventilated preterm baboons^{33–36,49} and preterm lambs.^{40–43}

A dysregulated molecular pathway that contributes to pulmonary hypertension is nitric oxide (NO) biosynthesis. Results from both preterm baboons⁷⁰ and preterm lambs⁴² indicate that the NO biosynthetic pathway becomes progressively downregulated during prolonged mechanical ventilation. The main findings are that prolonged mechanical ventilation leads to less endothelial NO synthase (eNOS) enzyme activity, less eNOS protein abundance, and less guanylate cyclase protein abundance.^{40–42,44,70,71} Soluble guanylate cyclase is an intermediary enzyme through which inhaled NO causes vascular smooth muscle cells to relax. Two cellular sources of eNOS in the lung are vascular endothelial cells and airway epithelial cells. Relevant to the topic of pulmonary hypertension, endothelial cells that line pulmonary arterioles are an important endogenous source of NO.^{42,44,72} Smooth muscle cells in the wall of pulmonary arterioles are an endogenous source of soluble guanylate cyclase. The topic of airway epithelial cells as a source of NO will be considered in the next section of this review.

Less eNOS enzyme activity, eNOS protein abundance, and soluble guanylate cyclase protein abundance in the lung of chronically ventilated preterm baboons and preterm lambs are associated with thicker smooth muscle walls around pulmonary arterioles.^{42–44,72} These associated changes are related to greater pulmonary vascular resistance. The relationship between NO and increased muscularization of pulmonary arterioles stems from the effect of NO on vascular smooth muscle cells. Nitric oxide inhibits growth of vascular smooth muscle cells, *in vitro*^{73,74} and *in vivo*.⁷⁵ Furthermore, providing exogenous NO, by inhalation or donor, inhibits vascular smooth muscle growth after injury.^{76–78}

Retrospective clinical studies show that inhaled NO improves oxygenation in preterm infants with severe BPD.⁷⁹ But what remains unclear is whether inhaled NO changes reactivity and structure of pulmonary arterioles in the lung of preterm infants with BPD.

Studies using preterm baboons show that 14 days of continuous inhalation of 5 parts per million (ppm) of NO during mechanical ventilation decreases pulmonary artery pressure in the first two days of life.⁸⁰ Echocardiographic analysis revealed that left ventricular function is not impaired, even though mean systemic arterial pressure is increased transiently on days of life 5 and 6. Studies using preterm lambs provide additional insights. One insight is that tachyphylaxis to inhaled NO develops during weeks of mechanical ventilation.⁴⁴ Specifically, at week-of-life 2, pulmonary vascular resistance decreases ~20% in response to 1 hour inhalation of 15 ppm of NO. However, a week later (week-of-life 3), 1 hour of 15 ppm inhaled NO has no effect on pulmonary vascular resistance.

Because relaxation requires intact endothelium and intact vascular smooth muscle, identification of whether one or both cell types become dysfunctional is necessary. Subsequent tests evaluated function of each cell type in the same preterm lambs on the same day that NO was inhaled for 1 hour.⁴⁴ Tests were separated by recovery periods. A test of vascular endothelial cell function is infusion of acetylcholine to cause endothelial-dependent vasodilation. Pulmonary vascular resistance did not decrease in response to infusion of acetylcholine, indicating dysfunction of pulmonary vascular endothelial cells. This physiologic evidence of pulmonary vascular endothelial cell dysfunction was associated

with less eNOS protein. A test for vascular smooth muscle function is infusion of 8-bromo-cyclic guanosine monophosphate (cGMP), which directly relaxes smooth muscle cells. Pulmonary vascular resistance did decrease in the same preterm lambs, indicating normal function of pulmonary vascular smooth muscle cells. Therefore, prolonged mechanical ventilation of preterm lambs leads to dysfunction of endothelial cells in pulmonary arterioles.

The aforementioned results following inhalation of 15 ppm NO for 1 hour by preterm lambs led to a study of continuously inhaled NO for 3 weeks in different group of chronically ventilated preterm lambs.⁷² The principal results are that continuous inhalation of 5 to 15 ppm NO did not prevent pulmonary hypertension or persistent muscularization of pulmonary arterioles.

An interesting, and unexpected, result occurred in the lung of preterm baboons and preterm lambs that inhaled NO continuously: more advanced alveolar formation.^{72,80} Among preterm baboons, continuously inhaled NO also improves lung growth relative to body weight by ~20%.⁸⁰ These effects of continuous inhalation of NO for weeks are likely related, in part, to inhibition of alveolar epithelial cell apoptosis by NO.^{81–83}

The results of the preterm baboon and preterm lamb studies provide insights into pathogenic mechanisms leading to pulmonary hypertension in preterm infants who develop BPD.⁶⁷

Pathogenesis of Excess Airway Expiratory Resistance

A third source for concern about life-long outcomes is that premature infants with BPD have fixed airway disease at 11 years of age compared to class mates.⁸⁴ Baseline lung function in the survivors is abnormal and bronchodilators are used more. These respiratory morbidities are evident in about 70% of the 11-year-olds who were born preterm and developed BPD. Mechanisms by which persistence occurs in these children remain to be discovered.

Bronchioles during the canalicular and saccular stages of normal human lung development have little smooth muscle in their wall, the epithelium is immature, and cell-cell adhesion is weak. Normally, airway smooth muscle accumulation around bronchioles increases postnatally.^{31,47} Pathologically, in BPD, bronchioles have accumulation of airway smooth muscle cells in their wall, making wall thickness greater.^{16,18,19,85}

The preterm baboon and preterm lamb models provide insights about the pathogenesis of airway outcomes. The results show that prolonged mechanical ventilation leads to higher peak inspiratory pressure, excess airway expiratory resistance, and lower lung compliance.^{38,42} Prolonged mechanical ventilation also leads to increased thickness of the smooth muscle wall around bronchioles of preterm lambs.⁴²

Excess airway expiratory resistance and greater thickness of smooth muscle walls around bronchioles are related to low levels of eNOS protein abundance and localization in bronchioles of chronically ventilated preterm lambs.⁴² Diminished eNOS protein in airway epithelial cells likely contributes to excess airway expiratory resistance because NO derived from airway epithelium regulates bronchomotor tone in the developing lung.^{86,87} A role for NO in regulation of muscularization of bronchioles is demonstrated by use of NO donors, which inhibit proliferation of human airway smooth muscle cells cultured *in vitro*.⁷⁴

Studies using preterm baboons and preterm lambs also reveal that continuous inhalation of NO reduces airway expiratory resistance.^{72,80} In addition, continuously inhaled NO reduces the thickness of the smooth muscle wall of bronchioles in preterm lambs.⁷² Thinner airway smooth muscle walls of bronchioles surprised us because the adjacent pulmonary arterioles

had persistently thick smooth muscle walls,⁷² as described in the previous section. We attributed improvement in airway function and structure to direct exposure of airway smooth muscle cells to inhaled NO. A potential explanation for why the adjacent pulmonary arterioles had persistently thick smooth muscle walls may be related to the longer diffusion distance from the lumen of bronchioles or airspaces that flank vascular smooth muscle cells in the wall of the adjacent pulmonary arterioles.

Our new studies using former mechanically ventilated preterm lambs that are recovered provide some physiological insights about long-term outcomes for the lung's airways. The insights are that airway hyperreactivity, induced by methacholine challenge, persists at 2 months or 5 months after preterm birth and 3 days of mechanical ventilation (unpublished data). The measured parameters were expiratory tidal volume and airway expiratory resistance. The former preterm lambs that recovered have lower expiratory tidal volume and higher airway expiratory resistance than normal lambs when challenged with methacholine. We have not measured smooth muscle accumulation in bronchioles so whether increased muscularization persists remains to be determined. Structural results will be important from a translational perspective to better understand the basis of abnormal baseline lung function and increased use of bronchodilators among 11-year-olds who were born preterm and developed BPD.⁸⁴

Gentler, non-invasive ventilation

An encouraging observation is that the incidence of BPD is lower in preterm infants who are managed by nasal continuous positive airway pressure (nasal CPAP), which is non-invasive because endotracheal intubation is not used.⁸⁸ The causes of this difference remain incompletely understood. Emerging differences between nasal CPAP and mechanical ventilation include inflammation, alveolar formation, and alveolar capillary growth. Inflammation is less with nasal CPAP because fewer neutrophils and less hydrogen peroxide are retrieved in alveolar fluid at 2 hours of bubble nasal CPAP compared to 2 hours of mechanical ventilation.⁸⁹ Alveolar formation and alveolar capillary growth are greater with nasal CPAP than mechanical ventilation. These desirable outcomes occur in preterm baboons at 28 days of nasal CPAP compared to mechanical ventilation.⁹⁰ They also occur in preterm lambs at 3 days⁴⁶ or 21 days (unpublished results) of nasal high-frequency ventilation (nasal HFV) compared to mechanical ventilation. We use nasal HFV for prolonged ventilation of preterm lambs because their anatomic dead space is large (long snout and neck compared to humans). We tried bubble nasal CPAP; however, arterial carbon dioxide in blood progressively increased, whereas pH progressively decreased, to unphysiological levels within 3 to 4 hours. Retention of carbon dioxide and respiratory acidosis did not occur when high-frequency flow interruption was used (Percussionaire ventilator). Another advantage of nasal HFV was upregulation of vascular growth factors in the lung. A novel finding is that thinning of the distal airspace walls occurs during nasal HFV compared to mechanical ventilation. Thinning is related to more apoptosis, and less proliferation, of mesenchymal cells in the lung. Together, these results from chronically ventilated preterm baboons and preterm lambs provide important mechanistic insights regarding the beneficial effects of gentler, non-invasive ventilation on pulmonary outcomes. Information about the use of nasal ventilation in human premature neonates at risk for BPD has been provided in the chapter by Bhandari in this issue.

Epigenetic Hypothesis for the Molecular Basis of BPD

We focus on epigenetic regulation of gene expression because epigenetics provides a molecular mechanism for adjustment of gene expression in response to environmental

changes.⁹¹ For BPD, initial environmental changes include preterm birth, mechanical ventilation, and exposure to oxygen-rich gas.

Epigenetic regulation of gene expression involves modifications to chromatin. The fundamental unit of chromatin is the nucleosome. A nucleosome has 146 base pairs of DNA that wrap around a core of histone proteins.⁹² The modifications to chromatin are a code for regulating gene expression. Regulation facilitates or interferes with interactions between transcription complexes and DNA. The regulatory interactions are dynamic and therefore adjust over time, including life-long.

Epigenetic regulating mechanisms include histone modifications, DNA methylation, microRNAs, and nucleosome positioning.^{93,94} Histone modifications participate in regulation of initiation, elongation, and/or termination of transcription along a gene locus, a topic that will be returned to later in this section. Methylation of DNA happens at sites referred to as CpG: cytosine, linked by phosphate, to guanine. DNA methylation has varied functions, including regulating transcription at transcriptional start sites, at regulatory elements, along a gene locus, and at repeat sequences.⁹⁵ Another epigenetic regulating mechanism is microRNAs, which are RNA molecules that do not code for a protein. MicroRNAs bind to the 3' untranslated region of target genes to silence transcription.⁹⁶ A fourth epigenetic regulating mechanism is nucleosome positioning. Nucleosome positioning is important for exposing or not exposing transcription start sites to transcription complexes and RNA polymerase. Understanding how these epigenetic mechanisms influence development of BPD remains to be done.

A research question that our group is addressing is why, at the molecular level, is the incidence of BPD apparently lower among preterm infants who are managed by nasal CPAP compared to mechanical ventilation.⁸⁸ Our preterm lamb model provides unique opportunities to test mechanistic hypotheses. One of our hypotheses tests genome-wide epigenetic characteristics in the lung of preterm lambs managed by nasal HFV compared to mechanical ventilation.⁴⁶ An intriguing new observation is that nasal HFV leads to genome-wide *hyper*acetylation of histones in the lung. Mechanical ventilation, by comparison, leads to genome-wide *hypo*acetylation of histones in the lung. These observations suggest that ventilation mode results in unique epigenetic modifications.

A limitation of our genome-wide epigenetic observations is that they do not identify epigenetic effects on a specific gene involved in alveolar formation, capillary growth, or elastin synthesis and secretion. Therefore, another of our hypotheses focuses on insulin-like growth factor -1 (IGF-1) because IGF-1 directs lung development normally,⁹⁷ IGF-1 expression in the lung is increased in RDS and BPD,⁹⁸ and IGF-1 expression is epigenetically regulated.^{91,99}

Our newest results using chronically ventilated preterm lambs suggest that IGF-1 expression is higher in the lung of mechanically ventilated preterm lambs compared to those supported by nasal HFV. We are beginning to examine why the two ventilation modes have different effects on IGF-1 expression in the lung. We chose to start with the histone code along the IGF-1 gene locus because the histone code contributes to regulation of gene transcription.^{91,99,100} The histone code is the pattern of histone modifications along the full length of a gene locus. Increased expression may occur because of increased initiation of transcription, altered elongation of transcription, and/or altered termination of transcription. Our initial results suggest that the histone code for IGF-1 is scrambled in the lung when preterm lambs are managed by mechanical ventilation compared to nasal HFV. At this time, we do not know the meaning of the scrambled histone code on increased IGF-1 expression in the lung. However, a hypothesis that we are testing is that ventilation leads to

promiscuous initiation of transcription. We are testing the possibility that ventilation leads to exposure of multiple transcription start sites for IGF-1. Interestingly, fetal lambs that are not allowed to breathe have only one transcription start site for IGF-1 that is accessible. These initial results are intriguing but explore only one of the potential epigenetic mechanisms by which the histone code regulates IGF-1 expression.

Summary

Improved survival of preterm infants who developed BPD is becoming increasingly important because of the high risk for persistent pulmonary morbidities such as poor respiratory gas exchange, pulmonary hypertension, and excess airway expiratory resistance later in life. These are ominous long-term outcomes that have important implications about quality of life. Secondly, the long-term outcomes also lead to life-long health care costs. This review focused on unique insights provided by the two large-animal, physiological models of neonatal chronic lung disease: preterm baboons and preterm lambs. Both models recapitulate the clinical setting of preterm birth and respiratory failure that require prolonged ventilation support. The models show pathologic and pathophysiologic manifestations of alveolar simplification, reduced capillary growth, and aberrant accumulation of elastin fibers during the evolution of neonatal chronic lung disease, as well as persistence of alveolar simplification later in life. The latter finding correlates with lower diffusing capacity among survivors of BPD.²⁴ Both models also show that pulmonary arterioles have thicker smooth muscle walls and more elastin in the thickened walls, as well as fewer microvessels. These pathologic features are associated with pulmonary hypertension, which is a life-long concern for preterm infants who develop BPD.⁶⁷ Another contribution of both models is that prolonged mechanical ventilation leads to increased abundance of smooth muscle cells in bronchioles, a finding that is associated with excess airway expiratory resistance that persists later in life. This association between pathology and pathophysiology in chronically ventilated preterm lambs correlates with persistent abnormal lung function and increased use of bronchodilators at 11 years of age among survivors of BPD.⁸⁴

The preterm baboon and preterm lamb models also reveal that the NO biosynthetic pathway becomes progressively downregulated during prolonged mechanical ventilation. Furthermore, both models show that physiological responsiveness to inhaled NO progressively declines because of endothelial cell dysfunction. Two surprising results surfaced along the way. Surprisingly, alveolar formation is better. This unexpected beneficial effect may be related to paracrine signaling by NO to upregulate expression of growth factors, such as vascular growth factors.¹⁰¹ Unexpectedly, continuous inhalation of NO decreases abundance of airway smooth muscle cells in the wall of bronchioles of preterm lambs. In the same treated preterm lambs, abundance of pulmonary vascular smooth muscle cells remained greater, suggesting that inhaled NO diffusion to vascular smooth muscle cells in the wall of pulmonary arterioles may be interrupted during prolonged mechanical ventilation.

Other insights on pulmonary outcomes provided by chronically ventilated preterm baboons and preterm lambs are related to the beneficial effects of gentler, non-invasive ventilation compared to mechanical ventilation. Nasal CPAP (preterm baboons) and nasal HFV (preterm lambs) lead to appropriate alveolar formation, alveolar capillary growth, and expression of elastin and growth factors. Finally, new observations using preterm lambs suggest that ventilation mode results in different epigenetic modifications to histones that are both genome-wide and gene-specific in the lung. As we refine the epigenetic observations, the new results may open doors to potential treatment approaches to minimize the consequences of BPD and its long-term effects.

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