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Animal models of bronchopulmonary dysplasia. The preterm baboon models

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1Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah; and 2Department of Pathology, University of Texas Health Science Center at San Antonio, San Antonio, Texas

Yoder BA, Coalson JJ. Animal models of bronchopulmonary dysplasia. V. The preterm baboon models. Am J Physiol Lung Cell Mol Physiol 307: L970–L977, 2014. First published October 3, 2014; doi:10.1152/ajplung.00171.2014.—Much of the progress in improved neonatal care, particularly management of underdeveloped preterm lungs, has been aided by investigations of multiple animal models, including the neonatal baboon (Papio species). In this article we will highlight how the preterm baboon model at both 140 and 125 days gestation (term equivalent 185 days) has advanced our understanding and management of the immature human infant with neonatal lung disease. Not only is the 125-day baboon model extremely relevant to the condition of bronchopulmonary dysplasia but there are also critical neurodevelopmental and other end-organ pathological features associated with this model not fully discussed in this limited forum. We also describe efforts to incorporate perinatal infection into these preterm models, both fetal and neonatal, and particularly associated with Ureaplasma/Mycoplasma organisms. Efforts to rekindle the preterm primate model for future evaluations of therapies such as stem cell replacement, early lung recruitment interventions coupled with noninvasive surfactant and high-frequency nasal ventilation, and surfactant therapy coupled with antioxidant or anti-inflammatory medications, to name a few, should be undertaken.

baboon; bronchopulmonary dysplasia; lung; preterm infant; ureaplasma

140-Day Model (“Old BPD”)

The first long-term animal model for neonatal BPD in the 140-day premature baboon was developed in the early 1980s (19, 26). At that time surfactant replacement therapy had not been approved for human use. Additionally, antenatal corticosteroids were provided to less than 50% of women with threatened preterm birth. BPD was a problem of relatively older, larger premature infants with moderate-to-severe hyaline membrane disease (HMD) that had been managed with high-pressure/tidal volume mechanical ventilation. In the 140-day baboon, development of BPD required exposure to high levels of inspired oxygen (95–100%) and mechanical ventilation for several days (26). Continued exposure to 100% oxygen and mechanical ventilation after resolution of the HMD led to clinical features of BPD by days 8 to 9 of life. Surviving animals manifested histological evidence for alternating areas of atelectasis/emphysema, bronchiolar necrosis, early alveolar wall and peribronchial fibrosis, and squamous metaplasia of the airways, all classic findings of old BPD (19). deLemos and colleagues (20, 24, 28) further refined this model. They showed that a pro re nata (PRN) approach to oxygen therapy at 140 days of gestation (a relatively more mature preterm infant) allowed for complete recovery from HMD and was not accompanied by clinical, radiographic, and pathological manifestations of BPD. Subsequent studies demonstrated that postnatal...
infection, related to endotracheal instillation of gram-negative bacteria, and/or abnormal surfactant protein A (SP-A) metabolism were important cofactors in BPD morbidity of the 140-day model (5, 21).

125-Day Model (“New” BPD)

Improvements in pre- and postnatal care leading to improved survival among extremely preterm infants have also been associated with a downward shift in the gestational age for BPD. Management changes included early surfactant replacement, low-volume ventilation, tolerance to moderate elevation in Pco2, and limiting exposure to high inspired O2 fraction levels. Thus the approaches used to create BPD in the 140-day model for BPD did not reflect current therapy, minimizing the clinical relevance of the 140-day model. Most infants now diagnosed with BPD are of much younger gestation, typically <28-wk gestation vs. 32- to 34-wk gestation as originally described (54, 55). The clinical and pathological features of this new BPD have also changed. Severe HMD is no longer a prerequisite to developing BPD. Early and sustained exposure to mechanical ventilation appears to be a key risk factor. In contrast to the original findings of severe airway lesions coupled with atelectasis/emphysema, the key pathological feature of new BPD is interrupted alveolarization. This includes decreased secondary crest formation, disrupted elastin-collagen deposition, and altered microvascular development (16, 37, 75). The features of interrupted alveolarization and vascularization are consistently found in the 125-day premature baboon managed with antenatal steroids, early surfactant replacement, low-tidal-volume ventilation, and PRN oxygen (22, 47).

A major advantage of the 125-day baboon model is the ability to apply interventions in an animal model with developmental and histopathological features similar to the immature human in a clinical setting that mirrors the human neonatal intensive care unit. Several different approaches to prevent BPD have been investigated in this model including upregulation of hypoxia inducible factor 1, superoxide dismutase mimetics, anti-bombesin antibodies, inhaled nitric oxide, retinoic acid, patent ductus arteriosus (PDA) closure, and high-frequency ventilation (3, 17, 18, 49, 50, 60, 72, 92). The most promising interventional results, however, were seen with early transition to nasal continuous positive air pressure. This was the first long-term model to demonstrate the ability to support a preterm animal with long-term noninvasive support and sustained early enteral feeding (Fig. 1) (77, 78). Important differences between the 140-day and 125-day BPD models are summarized in Table 1.

Not only is the 125-day baboon model extremely relevant to the human condition of BPD from the perspective of developmental ontogeny, size, clinical management, and lung pathology, but there are also neurodevelopmental and pathological features associated with this model that appear to closely mimic the very preterm human. Specifically, we have reported an ontogeny of cerebral development similar to the human that shows abnormalities in postnatal growth and evidence for white and gray matter injury associated with neonatal intensive care, but not specific interventional therapies (25, 44, 62, 64). More importantly, this model first demonstrated the neurological impact of mechanical ventilation on the preterm brain and the relative sparing effect of early non invasive respiratory support (43, 63, 85). Other studies have demonstrated that this model also mimics the preterm human condition related to the occurrence of functional adrenal insufficiency and to adverse end-organ effects on the kidney and liver (33, 40, 41, 73, 93). Additionally, the 125-day baboon model is relatively unique among most animal models in that persistent patency of the ductus arteriosus is a common finding, similar to very preterm humans. Studies designed to evaluate the pulmonary effect of ductal closure in the 125-day baboon are inconclusive but suggest that early medical closure may be beneficial, whereas operative closure may not, findings that mimic the preterm human condition (11, 18, 50).
Table 1. Preterm baboon models for BPD

<table>
<thead>
<tr>
<th>140-day</th>
<th>125-day</th>
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<tr>
<td>Age: mean ± SD (Range)</td>
<td>140 ± 1 (138–142)</td>
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<tr>
<td>Gestational equivalence</td>
<td>75% (~28 wk)</td>
</tr>
<tr>
<td>Birth weight</td>
<td>527 ± 58 g</td>
</tr>
<tr>
<td>Lung development stage</td>
<td>Saccular</td>
</tr>
<tr>
<td>Antenatal steroids</td>
<td>No</td>
</tr>
<tr>
<td>FIO2 exposure</td>
<td>PRN &amp; 100%</td>
</tr>
<tr>
<td>Surfactant therapy</td>
<td>No</td>
</tr>
<tr>
<td>Ventilator approach</td>
<td>High pressures</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td>Rare</td>
</tr>
<tr>
<td>Cardiovascular function</td>
<td>Minimal pressor use</td>
</tr>
<tr>
<td>Adrenal function</td>
<td>Normal</td>
</tr>
<tr>
<td>Nutrition</td>
<td>IV glucose/amino acids</td>
</tr>
<tr>
<td>Infection</td>
<td>Postnatal colonization</td>
</tr>
<tr>
<td>BPD pathology</td>
<td>Classic: New</td>
</tr>
<tr>
<td>Applied therapies</td>
<td>Emphysema/atelectasis</td>
</tr>
<tr>
<td></td>
<td>High-frequency ventilation</td>
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<tr>
<td></td>
<td>PDA ligation</td>
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<tr>
<td></td>
<td>Desferoxamine</td>
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<tr>
<td></td>
<td>Allopurinol</td>
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BPD, bronchopulmonary dysplasia; FIO2, inspired O2 fraction; PRN, pro re nata; IV, intravenous; HAL, hyperalimentation; PDA, patent ductus arteriosus; CPAP, continuous positive airway pressure; SOD, superoxide dismutase.

BPD, Inflammation, and Infection

At its core, BPD is an inflammatory disease (13, 70, 95). BPD is the end result of a disruption in alveolarization related to a variety of noxious processes that, in combination, contribute to a sustained proinflammatory state in the lungs of preterm infants. Factors frequently contributing to this proinflammatory state include fetal and postnatal nutritional deficiencies, oxygen toxicity, volutrauma associated with invasive mechanical ventilation, and fetal and postnatal infections. The importance of perinatal infection in the pathogenesis of BPD, both fetal and neonatal, and particularly associated with Ureaplasma/ Mycoplasma organisms, has been recently highlighted (2, 48, 67, 76, 84). The incorporation of this perinatal infection into the primate model of preterm birth and BPD has been perhaps the most important new addition to models of BPD over the past decade. The major role of perinatal infection in the very preterm human may be one of the key reasons that most singular therapeutic agents found to be useful for the prevention or treatment of BPD in animal models have had relatively little clinical effect in very preterm humans (9, 12). Incorporating infection as an element of animal modeling will more closely mirror the human phenotype and should further our understanding of the pathophysiology of BPD and lead to more effective treatment options.

Ureaplasma Model

Ureaplasma urealyticum and Mycoplasma hominis are the two most common organisms isolated from amniotic fluid and infected placentas (42). Transmission of U. urealyticum increases as gestational age decreases and with the duration of ruptured membranes (30, 38). In addition to their apparent role in preterm birth, U. urealyticum and M. hominis have been associated with increased adverse outcomes among in utero exposed preterm infants including mortality, severe intraventricular hemorrhage, and BPD and are the most commonly identified microbes in the blood of preterm infants with associated increased markers of inflammation (15, 23, 29, 38, 39, 67).

Given that nonhuman primates have endogenous genitourinary colonization with mycoplasma species, including U. urealyticum and M. hominis, we, and others, investigated the perinatal role of Ureaplasma infection in the nonhuman primate model to more closely mimic the human condition. Novy and colleagues (55a) developed a chronically catheterized pregnant Rhesus (Macaca mulatta) monkey model for this purpose, using U. parvum or M. hominis as sole pathogens. This primate model closely simulates human pregnancy in many ways, including the presence of a single fetus, the hormonal control of parturition, and hemochorial placentation with a discrete chorioamnion and amniotic cavity (69).

In contrast to the sheep model, amniotic fluid inoculation with Ureaplasma in the Rhesus model at ~75% gestation results in a progressive increase in uterine contractility culminating in preterm labor, suggesting a unique species difference in host response (52, 65). Nearly 80% of preterm births occurred within 1 wk of inoculation and all occurred within 15 days.

In contrast to the Group B Streptococcus model, intra-amniotic Ureaplasma results in a slower rate of replication; lower amniotic fluid colony counts; smaller increases in amniotic fluid leukocytes, cytokines, and prostaglandins; less rapid induction of labor; and a longer interval between inoculation and preterm delivery (32). Amniotic fluid colonization by genital mycoplasmas in the Rhesus model was similar to average colony counts (~10^5 CFU/ml) reported from women diagnosed with amnionitis at the Diagnostic Mycoplasma Laboratory, University of Alabama at Birmingham. Pathological studies on the delivered fetuses demonstrated that in utero infection with either U. parvum or M. hominis resulted in acute and subacute fetal bronchiolitis. When infection was prolonged and more indolent (>10 days), the acute inflammatory response was partially resolved but lymphoid tissue aggregates were conspicuous in the peribronchiolar areas, as were epithelial hyperplastic changes. Additionally, bacteremia was found in 20% of Ureaplasma- and 60% of Mycoplasma-exposed fetuses and all fetuses had evidence for lung colonization. These findings are consistent with recent evidence that 35% of umbilical cord blood specimens from human infants born at 23- to 28-wk gestation are culture positive for Ureaplasma species and/or M. hominis and are associated with a neonatal systemic inflammatory response (29). The importance of perinatal infection on lung development and injury are further supported by studies with Group B Streptococcus in pregnant, preterm Rhesus demonstrating fetal lung injury and disturbed morphogenesis/angiogenesis, even when fulminant infection is not present (1, 48).

Of clinical interest, follow-up studies from these investigators have demonstrated that antenatal treatment of the infected pregnant Rhesus with azithromycin, initiated 6–8 days after...
inoculation with *U. parvum* and in conjunction with indomethacin tocolysis and antenatal steroids, is effective in clearing *U. parvum* from the amniotic fluid and preventing fetal infection (32). Additionally, the fetal lung pathology was substantially reduced or completely absent. Studies are needed to determine whether these apparent benefits translate to long-term improvement in lung pathology and function.

We investigated the effects of fetal *Ureaplasma* in the 125-day preterm baboon model of BPD (94). Pregnant 125-day baboons (*Papio papio*) delivered 48–72 h after inoculation with $10^7$ CFU of *U. urealyticum*. Infants were immediately intubated after delivery, treated with surfactant, and placed on low-tidal-volume ventilation for 14-day. None of the dams were culture or PCR positive for *U. urealyticum* in the amniotic fluid before inoculation, and all exposed dams had positive amniotic fluid cultures at delivery (range $3.6 \times 10^4$ to $2.1 \times 10^7$). Signs of localized or systemic infection did not occur in any of the *U. urealyticum*-exposed dams and there was no evidence for premature labor.

At delivery there was a significant increase in amniotic fluid levels, but not maternal or fetal serum levels, for white blood cells, IL-6, and IL-8. Although only one placenta was available for microscopic examination, there was histological evidence of acute inflammation of the chorioamniotic membranes after intrauterine *U. urealyticum* exposure. Serial tracheal aspirate cultures revealed an interesting dichotomous pattern for *U. urealyticum*-exposed infants with two discrete groups of infants identified based on high (+*Uu*) vs. low/no tracheal CFUs at necropsy. Clinical, biochemical, and pathological differences based on persistence of *U. urealyticum* are shown in Table 2 and Fig. 2. Follow-on studies of these animals identified inflammatory changes including increase in α-SMA-expressing myofibroblasts in lung sections from +*Uu* animals, accompanied by altered concentrations of Smad2 and Smad3 (TGF-β1 signaling agonists) and Smad7 (TGF-β1 signaling antagonist) resulting in upregulation of the multifunctional peptide TGF-β1, a key regulatory protein involved in lung pathology and development (46, 83, 89, 90).

The ability of some animals to resolve *U. urealyticum* infection/colonization implies that variance in the maternal-fetal-neonatal immunological response to antenatal *U. urealyticum* may play a critical role in the postnatal phenotype. Individual risk for infection from the organisms may be related to differences in specific organism virulence, intrinsic pulmonary defense factors (such as the collectin surfactant proteins A and D), as well as other factors involved in the maternal-fetal-neonatal immune system response (61, 74). For example, variable host intrinsic immune responses related to genetic variance in specific surfactant protein A alleles is related to respiratory syncytial virus infection risk (45).

Toll-like receptors (TLRs) are “pattern recognition receptors” that are key components of the innate immune response. TLRs are present in fetal tissues, including the lung and chorionic membranes, and have been shown to be upregulated in response to noxious events such as LPS stimulation, chorioamnionitis, and mechanical ventilation (36, 51, 59, 79).

*Ureaplasma* infection activates the expression of proinflammatory mediators by macrophages, neutrophils, dendritic cells, B cells, endothelial cells, and epithelial cells through cell membrane-embedded TLR proteins. Specifically, *Ureaplasma* lipoproteins promote activation of the NF-κB pathway (68). TLR2 and TLR4 mRNA and protein expression are low in 125-day and 140-day gestation, nonventilated fetal baboon lung. Mechanical ventilation of the 125-day baboon leads to a further decrease in SP-A, but increased tissue expression of TLR4, promoting a lung environment that accentuates pulmonary infection (6–8). The developmental susceptibility of preterm infants to *Ureaplasma* infection may, in part, be explained by these findings. Decreased TLR2 and TLR4 expression in early gestation may increase fetal lung susceptibility to *Ureaplasma* infection. Following preterm birth, exposure to mechanical ventilation, high oxygen concentrations, impaired nutrition, and other infections may contribute to increased pulmonary TLR expression and an enhanced inflammatory response to *Ureaplasma*. Recent data have shown that genetic variation in TLRs can affect BPD risk. Specifically, an increased BPD risk has been associated with single nucleotide polymorphisms (SNPs) in TLR2, TLR4, and TLR6. Interestingly, such specific SNPs may also be associated with reduced variance in specific organism virulence, intrinsic pulmonary defense factors (such as the collectin surfactant proteins A and D), as well as other factors involved in the maternal-fetal-neonatal immune system response (61, 74). For example, variable host intrinsic immune responses related to genetic variance in specific surfactant protein A alleles is related to respiratory syncytial virus infection risk (45).

**Table 2. Clinical, biochemical, and pathological features of the 125-day preterm baboon with intrauterine amniotic fluid exposure to *Ureaplasma urealyticum***

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th><em>Uu</em> (-)</th>
<th><em>Uu</em> (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: mean ± SD, days</td>
<td>125 ± 1</td>
<td>125 ± 1</td>
<td>125 ± 1</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>408 ± 49</td>
<td>391 ± 28</td>
<td>391 ± 28</td>
</tr>
<tr>
<td>CFU at birth</td>
<td>na</td>
<td>2×10^5–2×10^6</td>
<td>4×10^5–2×10^6</td>
</tr>
<tr>
<td>CFU at necropsy</td>
<td>na</td>
<td>0–2×10^5</td>
<td>6×10^5–2×10^6</td>
</tr>
<tr>
<td>Compared with “Control”</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Amniotic cytokines</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>TA cytokines, birth</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>TA cytokines, necropsy</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Compared with “Control”</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>FIO2 ≥ 48 h</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Paw ≥ 48 h</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>OI ≥ 48 h</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Lung pathology</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hypoalveolization</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mild/focal fibrosis</td>
<td></td>
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<tr>
<td>Mild/no bronchiolitis</td>
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<tr>
<td>Mild/no pneumonia</td>
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*Uu*, *Ureaplasma urealyticum*; Paw, mean airway pressure; OI, oxygenation index.
risk for *Ureaplasma* colonization and BPD as found for the TLR6 SNP rs5743827 (91).

**Advantages of the Baboon and Nonhuman Primate Models**

The baboon continues to be a well-studied nonhuman primate that has been used in infectious disease, cardiovascular, obesity, hypertension, and numerous other disease models. Access to baboon colonies is limited because there are only eight National Primate Research Centers. At the Southwest Foundation for Biomedical Research (now renamed Texas Biomedical Research Institute) established staff expertise in using sex skin changes to time pregnancy and gestation dates were vital to the National Heart, Lung, and Blood Institute-funded BPD Baboon Resource facility. The stages of intrauterine development of lung and other visceral organs (e.g., brain, kidney, adrenal) in baboons were comparable to those in the human fetus. The baboon’s growth and development, approximately three times more rapid than the human, were key factors that allowed the development of the BPD models and their use in multiple study designs. Readily available human antibodies for immunological, immunocytochemical and flow cytometry studies were additional assets. The baboon lung has desirable structural features comparable to those in the human. The subgross lung morphological features of pleurals and bronchioles in several species was published by Tyler in 1983 (81). Human, dog, cat, monkey, and ferret have terminal and several generations of respiratory bronchioles, whereas rodents, ox, sheep, and pig have terminal bronchioles with absent or a single short-generation respiratory bronchiole. BPD is a disease that reflects the immature stage of lung development when gestation is interrupted and lung injury/inflammation ensues, i.e., arrested lung development and impaired alveolar formation. Much work is now directed to sorting out the molecular aspects of alveolarization, but there is a growing consensus that the bulk of rapid alveolar development occurs during the first 2 years of life, and a recent study concludes that “alveolar growth does continue during adolescence” but at a reduced rate after 2 years (35). This potential of new alveolar formation in lungs of young people before the second decade of life may be benefiting some of preterm survivors who experienced BPD. Long-term preterm animal models of BPD, such as the baboon, are essential to further understanding this process and the effect of therapeutic interventions, not only on the lung, but other organ systems as well. Like that of the human, the tracheobronchial tree in the baboon and other nonhuman primates has cartilage and submucosal glands to the level of the nonrespiratory/terminal bronchioles, whereas both of these structures are absent in the mouse and rat. Recently, two important nonhuman primate models of respiratory syncytial virus (RSV) and bronchial asthma and RSV have been developed in the baboon and Rhesus monkey, respectively. These models are again important in reflecting physiological, immunological, and morphological similarities to human neonatal/pediatric diseases (27, 58). Additionally, both are known to significantly impact lung function in affected children who survive preterm birth. Also, with pertussis rates rising steadily in the United States over the last 20 years, a pertussis-infected baboon model has been developed that replicates the airway pathology, immunological findings, and clinical course of the human disease (87). The unique comparison of the baboon to the human is noted by the fact these investigators were unable to demonstrate similar model success with the Rhesus monkey model (86). Although we believe the short- and long-term premature baboon model most closely replicates the human condition, it is important to recognize the need for other BPD animal models, such as the rodent and preterm lamb (4, 14, 46). These models are cost-effective approaches to further our understanding of the molecular pathways and genetic regulation of the
inflammatory processes in the affected developing lung, including key events during late lung development (46). Additionally, they are important models for initial testing of possible therapeutic approaches in an effort to prevent or ameliorate BPD.

**Future Studies**

Recent important advances in accurate, unbiased, stereological methods (53, 56, 82) combined with appropriate in vivo and/or ex vivo imaging techniques recently reported in rodents are needed and should prove extremely helpful in expanding our knowledge concerning the evolution and long-term outcomes of preterm lung injury following various therapeutic interventions (34, 57, 82).

Unfortunately, the expense of maintaining a BPD baboon or other nonhuman primate facility has limited current research on this model and may prevent future funding of short- and long-term studies. We believe this is short-sighted given that, as a minimum, societal costs associated with preterm birth have been estimated to exceed $26 billion per year, and a disproportionate share of that cost is related to the acute and chronic care of extremely preterm infants (10). The close anatomical, physiological, and genetic approximation of the preterm baboon to the preterm human, coupled with the ability to provide similar approaches to noninvasive respiratory for extended periods of time and the ability to apply standardized neurodevelopmental testing to juvenile baboons (97), are key reasons to provide more research funding of the preterm baboon as a translational model for preclinical trial testing of single and multiple therapeutic approaches not just toward the prevention and treatment of BPD, but toward the prevention of prematurity. Premature birth and the long-term morbidities of survivors remain critically important societal health care issues.

**Summary**

For ongoing studies related to the pathophysiology, prevention, and treatment of BPD, the preterm primate animal model has several advantages including 1) nearly identical developmental pulmonary ontology; 2) similarities in cardiovascular function including persistent PDA; 3) the ability to establish noninvasive respiratory support of extremely preterm infants; 4) a size that is comparable to most immature human infants; 5) immaturity and developmental progression of the brain that also includes intraventricular hemorrhage as a comorbidity; and 6) the ability to produce intratracheal infection resulting in preterm birth similar to the human condition.

Efforts to rekindle the preterm primate model for future evaluations of therapies such as stem cell replacement, early lung recruitment interventions coupled with noninvasive surfactant and high-frequency nasal ventilation, and surfactant therapy coupled with antioxidant or anti-inflammatory medications, to name a few, should be undertaken. Incorporation of the primate prenatal infection model should be included in such studies to more closely replicate the human condition. Additional research is needed to better understand the activity and development of inflammatory pathways and processes in the preterm infant. Identifying factors that may minimize or prevent host colonization by specific microorganisms could provide a valuable adjunct approach in our efforts to prevent BPD in this high-risk population.

**REFERENCES**


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No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

B.A.Y. and J.J.C. conception and design of research; B.A.Y. and J.J.C. performed experiments; B.A.Y. and J.J.C. analyzed data; B.A.Y. and J.J.C. interpreted results of experiments; B.A.Y. and J.J.C. prepared figures; B.A.Y. and J.J.C. drafted manuscript; B.A.Y. and J.J.C. edited and revised manuscript; B.A.Y. and J.J.C. approved final version of manuscript.


