Recent advances in late lung development and the pathogenesis of bronchopulmonary dysplasia

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Madurga A, Mižíková I, Ruiz-Camp J, Morty RE. Recent advances in late lung development and the pathogenesis of bronchopulmonary dysplasia. Am J Physiol Lung Cell Mol Physiol 305: L893–L905, 2013. First published November 8, 2013; doi:10.1152/ajplung.00267.2013.—In contrast to early lung development, a process exemplified by the shaping of the developing airways, the later development of the immature lung remains very poorly understood. A key event in late lung development is secondary septation, in which secondary septa arise from primary septa, creating a greater number of alveoli of a smaller size, which dramatically expands the surface area over which gas exchange can take place. Secondary septation, together with architectural changes to the vascular structure of the lung that minimize the distance between the inspired air and the blood, are the objectives of late lung development. The process of late lung development is disturbed in bronchopulmonary dysplasia (BPD), a disease of prematurely born infants in which the structural development of the alveoli is blunted as a consequence of inflammation, volutrauma, and oxygen toxicity. This review aims to highlight notable recent developments in our understanding of late lung development and the pathogenesis of BPD.

BPD: lung development; alveolarization; hypoxia; growth factor; oxidative stress

The primary function of the lung is to transport oxygen from the inspired air into the blood and to clear accumulated carbon dioxide from the blood. The alveolus is the site of this gas exchange in the lung, which takes place across the alveolocapillary barrier, a double barrier that consists of the alveolar epithelium and the capillary endothelium located in close proximity to one another, and supported by the extracellular matrix (ECM). To facilitate effective gas exchange across this barrier, it is desirable that this barrier is as thin as possible to give the gas molecules a short distance to traverse and also that the functional surface area of the barrier is as large as possible, to maximize the functional gas exchange capacity of the lung. It is the objective of late lung development, through the process of alveolarization, to deliver these two essential elements of a mature, functional lung (114).

This is achieved first by the establishment of the core lung structure, during airway branching in the embryonic (4–7 wk postconception) and pseudoglandular (5–17 wk postconception) stages of early lung development. Late lung development follows with the canalicular stage (16–24 wk postconception), which is marked by the thinning of the interstitial tissue and the “opening” of air spaces in the developing lung. This is followed by the formation of saccular units by the developing airways in the saccular stage (24–35 wk postconception), after which secondary septa divide the saccular units during the alveolar stage (36 wk postconception to several years postnatal), increasing the number and reducing the size of the alveoli. Alveolar septal formation, the key event in alveolarization, proceeds from ~34 wk postconception. This process remains very poorly understood and is thought to be regulated by the concerted action of gene expression programs, growth factor signaling, ECM production and maturation, and physical forces, such as those generated by breathing motions (112, 113, 178).

When late lung development is disturbed, the lung architecture is malformed. Depending on the severity of the architectural malformation, there may be serious consequences in terms of respiratory function, as well as long-term consequences in later life. This is exemplified by diseases such as bronchopulmonary dysplasia (BPD) (124), a common complication of preterm birth, which is characterized by perturbations to lung structure that include reduced alveolar number, thickened septa, and a malformed pulmonary circulation. These structural abnormalities lead to respiratory complications during infancy, which may persist into adulthood (14, 188, 189). Improved medical management of BPD has dramatically increased the survival of BPD patients and has changed the pathophysiological picture of BPD today. BPD is now characterized less by fibroproliferative airway damage and parenchymal fibrosis and more by alveolar hypoplasia and a dysmorphic pulmonary circulation, making BPD today largely a disease of arrested lung development (84, 85). This has underscored an urgent need to better understand the molecular basis of late lung development. Additionally, improved perinatal care has increased the survival of very premature infants (61), which has also led to an increase in the incidence of BPD, highlighting the need for a better understanding of the pathogenic mechanisms at play, which might be targeted in the medical management and treatment of BPD. This review, which complements a recent Update article in the American Journal of Respiratory and Critical Care Medicine (105), aims to provide a perspective on very recent advances in our understanding of late lung development and BPD.

Hyoxia and Oxidative Stress

Adult patients with compromised respiratory function are often ventilated with high oxygen concentrations for life support. Similarly, prematurely born infants have lungs that are insufficiently developed to allow for proper gas exchange, and oxygen supplementation thus also represents a life-saving intervention in the neonatal intensive care unit. However, high oxygen levels are also very toxic and damage the lung, either
creating or exacerbating lung injury or blunting the development of immature lungs. As such, hyperoxia is employed as an injurious stimulus to cause experimental lung injury in adult lungs and is also a well-characterized stimulus for the study of arrested late lung development associated with BPD. This forms the basis of the “hyperoxia model” for BPD, an animal model that has been employed extensively to explore the molecular pathways that are perturbed during aberrant septation, and to evaluate chemical compounds that may represent candidate drugs for the prevention, management, or treatment of arrested septation and vascular development associated with BPD. The role of hyperoxia in the pathogenesis of clinical (145) and experimental BPD (34) has recently been reviewed. Currently, how hyperoxia results in blunted lung development is largely attributed to perturbations to growth factor signaling (4–6, 8, 40, 83, 102, 118, 134, 135, 174, 175), alterations to ECM assembly (28, 29), cell proliferation, differentiation, and apoptosis (63), and disturbed vascular development (156, 162, 163), among other factors.

Several recent studies have attempted to further elucidate mechanisms by which hyperoxia exposure may blunt late lung development. One particular noteworthy recent observation was the ability of hyperoxia to modulate the mechanical properties of epithelial cells (140), where hyperoxia treatment of primary alveolar epithelial cells and the MLE-12 epithelial cell line promoted resistance to deformation of both cell types, as assessed by an increased elastic modulus measured by atomic force microscopy. Indeed, mechanical strain continues to be an area of intense interest in the context of lung damage and development, particularly in the alveolar compartment (139). In their report, Roan and Waters (139) revealed that hyperoxia may increase the susceptibility of alveolar epithelial cells to wounding caused by deforming stress that would be expected from, for example, mechanical ventilation. These data add to a growing debate about whether interventions that promote cell stiffness are harmful or cytoprotective (176). This study was thoughtfully evaluated in an editorial by Rolf Hubmayr (79), who cautioned that different ex vivo models of cell injury due to deformation may not always represent the regional micromechanics at play in intact lungs. Furthermore, the need to better understand (micro) strains subjected to cells in inflated, aerated alveoli vs. airways and air spaces partially filled with liquid was underscored, as was the pressing need for more information about (micro) stress distribution in the lung. These exciting areas linking cell and ECM “stiffness” with hyperoxia, and the resultant impact on lung development and cell phenotype are likely to receive much attention in the coming years.

The impact of hyperoxia on cellular stress responses in the lung has also been addressed, where exposure of neonatal rat pups to 95% O\textsubscript{2} in the inspired air activated the integrated stress response (92) in the alveolar compartment of the immature lung. This same response is also induced by misfolded proteins, where it is then termed the unfolded protein response (UPR). A second study (99) has also addressed the connection between the UPR and hyperoxia, using transgenic mice deficient in the CCAAT enhancer-binding protein homologous protein (CHOP), which revealed that hyperoxia increased CHOP expression through an endoplasmic reticulum stress-independent pathway, and where CHOP expression was believed to protect against hyperoxia-induced lung injury. Both studies have identified pathways operative in the alveolar epithelium that may be manipulated to limit oxygen-induced damage to the developing lung. In addition to the activation of these protective pathways, a direct impact of hyperoxia on mitochondrial metabolism has also been demonstrated (51), where hyperoxia impaired glycolytic and mitochondrial energy metabolism in the MLE-12 mouse lung epithelial cell line, as well as in mouse lungs, by selectively inactivating complex I and complex II of electron transport system. Several other recent reports have underscored the importance of oxidative stress in blocking alveolar development, including the observation that deficiency of Nrf2, which is an essential transcription factor that affords protection against oxidant damage, augmented lung injury, and arrest of alveolarization caused by hyperoxia in newborn rodents (46). In addition to oxidative stress, these studies complement other work demonstrating a role for peroxynitrite and nitrative stress as a key factor in hyperoxia-induced effects on alveolarization (104), for which evidence also exists in clinical BPD (13).

Physiological apoptosis is a key process in organogenesis, notably, the removal of excess cell in developing organs. Key earlier studies have described increased apoptosis of cells in the developing (58, 108) and adult (21) mouse lung when exposed to high (92–100%) oxygen levels. Apoptosis has been accredited with a key role in the developing lung, both prenatal and postnatal, where apoptosis drives the thinning of the alveolocapillary barrier in utero (146) and removes “excess” alveolar type II cells and fibroblasts in the lungs of air-breathing rodents (147). Along these lines, evidence exists that defective apoptosis may promote aberrant lung structure in mouse (179) and baboon (101) BPD models. In a recent study, the inhibition of apoptosis in neonatal rodent lungs by exposure to 60% O\textsubscript{2} was described (197), and it was suggested that this decreased apoptosis may contribute to the increased septal thickness observed in aberrantly developing lungs. Indeed, a marked increase in the number of epithelial cells has been observed in areas of interstitial thickening in a neonatal rat model of BPD that also employed a hyperoxia level of 60% O\textsubscript{2} (128).

The impact of oxygen toxicity on the developing airway smooth muscle has also been addressed recently, where oxygen levels above 60% caused increased apoptosis and blunted intracellular Ca\textsuperscript{2+} responses to acetylcholine (74). Thus, although oxygen toxicity in the developing lung has previously been considered to primarily impact the distal airways, this study reveals that the developing airway (and perhaps vascular) smooth muscle may also be susceptible.

Hyperoxia, used to model BPD in mouse pups, has also been used to evaluate strategies to promote proper lung development after hyperoxic injury. One notable recent finding was the observation that phosphodiesterase (PDE) 4 inhibition with piclamilast improved pulmonary vessel architecture and promoted alveolar development in mouse (179) and baboon (101) BPD models. In a recent study, the inhibition of apoptosis in neonatal rodent lungs by exposure to 60% O\textsubscript{2} was described (197), and it was suggested that this decreased apoptosis may contribute to the increased septal thickness observed in aberrantly developing lungs. Indeed, a marked increase in the number of epithelial cells has been observed in areas of interstitial thickening in a neonatal rat model of BPD that also employed a hyperoxia level of 60% O\textsubscript{2} (128).

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10 days, followed by recovery in room air for up to 42 days (56). Furthermore, different PDE4 inhibitors were employed, cilomilast (190) and picamilast (56), and the route of administration was either subcutaneous or undeclared. This is but one example of the pressing need to standardize hyperoxia treatment protocols of neonatal rodents to study the impact of pharmacological interventions on late lung development. These observations must also be seen against the background that, at birth, mouse lungs correspond to a human lung at 32 wk postconceptual age, which represents a group of infants that rarely develop lung disease, raising the interesting question of whether the newborn mouse models actually model perturbed late lung development, or, rather, lung injury and emphysema.

Despite the problems with model standardization, the hyperoxia model has proved valuable in terms of identifying candidate interventional strategies and identifying pathogenic pathways. Recent developments include the identification of interleukin (IL)-1α/β as a pivotal mediator of experimental BPD, where postnatal hyperoxia was combined with intratracheal inflammation. This study also confirmed the anti-inflammatory IL-1 receptor antagonist (IL-1Ra) as a promising treatment for BPD (123), an idea originally forwarded by the Tanswell group (86), and adds to growing interest in anti-inflammatory strategies to drive late lung development and manage BPD. Seminal studies have already suggested that blockade of neutrophil influx into the developing lung may attenuate lung injury and the arrested septation associated with BPD (11, 196). Building on the idea of limiting inflammation in the aberrantly developing lung, another study revealed that treatment of neonatal rat pups in the hyperoxia model with semaphorin 3C impeded neutrophil influx and preserved alveolar and lung vascular growth (168). Both studies are also supported by the recent report that caffeine administration also improves lung structure in hyperoxia-treated rodent pups, concomitant with blunting inflammatory infiltration in response to hyperoxia exposure (185).

Clearly, there remains much to be understood about how hyperoxia perturbs late lung development and how well the “hyperoxia model” really recapitulates aspects of arrested late lung development associated with BPD. It is clear that the degrees of hyperoxia currently employed vary widely (from an FIO2 of 0.40 to 1.00) between laboratories that implement this model. Additionally, the duration of exposure to hyperoxia is also relevant, as is the time frame during late lung development when lung is exposed to hyperoxia. Future work in this area should aim to identify not only which developmental pathways are perturbed by exposure to elevated levels of oxygen but also how these processes are perturbed.

Progenitor and Stem Cells

The role of progenitor cells in lung development, injury, and repair is an area of intense interest. The role of stem cells in lung development has recently been reviewed (17, 136, 141), as has the use of stem cells to treat lung disease in preterm infants (7). Probably the most significant study recently reported was the documentation that alveolar type II cells act as stem cells in the adult lung (15). More specifically in the context of alveolarization, the field remains in its infancy. However, some preliminary observations are setting the stage for understanding how progenitor and stem cells may help us understand alveolarization and treat BPD. Several signaling pathways are currently emerging as important regulators of progenitor cell differentiation, including the β-catenin pathway (153), which was demonstrated to be critically important for facultative basal cell progenitor function and differentiation into secretory cells in the trachea, namely club (Clara)-like and ciliated cells.

How progenitor and stem cells may function to promote or repair damaged developing lungs is currently an area of intense interest. Recent reports have demonstrated that the abundance of circulating vascular progenitor cells is decreased in cord blood of preterm infants with BPD, which suggests that prenatal factors may contribute to respiratory outcome (12).

Mesenchymal stromal cells (MSCs) applied via the intratracheal route have shown some promise in restoring proper lung structure in the hyperoxia BPD animal model (157). Similarly, intratracheal application of human amniotic fluid stem cells also improved lung structure in the same hyperoxia BPD animal model (70), while umbilical cord mononuclear cells applied via the intraperitoneal route in a two-hit model also exhibited a therapeutic effect (111). Encouraging data have also been obtained by using bone marrow mesenchymal stem cells applied via the intravenous route in the rodent hyperoxia model of BPD (198). Thus MSCs appear to have tremendous promise in the restoration of normal lung development in BPD.

Recent studies suggest that the MSCs may exert effects by driving the expansion of the bronchioalveolar stem cell pool (167). Additionally, there is some suggestion that bone marrow mesenchymal stem cells applied via the intravenous route in the rodent hyperoxia model of BPD may limit lung inflammation and hence promote proper lung development (198). In neonates with respiratory distress, and thus at risk for BPD, MSC populations have also been examined, and MSCs derived from these patients exhibited myofibroblast characteristics. In both experimental and clinical BPD, the transforming growth factor (TGF)-β/phospho-glycogen synthase kinase (GSK)-3β/β-catenin pathway has been identified that drives myofibroblastic differentiation of neonatal lung MSCs (133).

Work on MSCs continues to evolve in exciting directions. Originally, MSCs were thought to function by engrafing and repopulating damaged organs and thereby promoting repair directly. However, more recently, paracrine effects and soluble stem cell-derived factors have received much attention (65). Notable recent developments include the use of MSC-conditioned medium to reverse features of hyperoxia-induced damage to lung structure in neonatal rodents (73, 132), as well as attenuating acute lung injury induced by bacterial lipopolysaccharide (LPS) (80). Additionally, ex vivo preconditioning of MSCs not only may enhance the paracrine effects of MSCs that promote proper development of injured lungs but may also facilitate the identification of molecules secreted by MSCs that mediate the protective activity of MSCs in neonatal lung disease (180).

Imaging

An area of emerging interest is lung imaging, which has led to a recent call by the American Journal of Physiology-Lung Cellular and Molecular Physiology, specifically attracting papers related to real-time imaging of lung function (115). Substantial progress has been made in the imaging of postnatal growth trends of proximal pulmonary arteries by phase-con-
A new method $^3$H and hyperpolarized $^3$He diffusion MRI has been developed permitting the noninvasive serial assessment of lung growth in vivo, in mice (177). This methodology will find widespread use, for example, in monitoring the impact of pharmacological interventions in chronic lung disease models. The $^3$He diffusion MRI has also recently been used in humans, to noninvasively assess lung growth in subjects 7–21 yr of age. The exciting data that emerged revealed that lungs grow partly by nealveolarization, and that this occurs throughout childhood and adolescence (119), an idea for which evidence has already been forwarded by Schnittny and others (148). If validated, this idea demonstrates that damaged developing lungs can recover (regenerate). Clearly, these data also indicate that insults including drugs, diseases, or exposures to environmental stresses could also adversely impact alveolarization not only in neonates, but rather throughout childhood.

**Gene Regulation**

Currently, microRNAs are receiving much attention in the context of organogenesis and development. Although specific roles have been established for microRNAs in early lung development (36), namely, lung branching and tissue differentiation, currently little is known about the roles for microRNA in late lung development, namely, alveolarization. Several preliminary microRNA microarray studies (22, 60, 199) or associative studies (173) have been performed in whole lung homogenates on normally and aberrantly developing lungs and these studies have suggested that specific microRNAs may be relevant to late lung developmental processes. However, to date, no single microRNA species has been causally implicated in normal or aberrant late lung development. Current knowledge on microRNAs and lung development has been summarized in two recent reviews (90, 151). There is clearly vast scope for a tangible improvement in our understanding of how microRNAs contribute to late lung development. The ease of manipulating microRNA function in vivo with antagonirs underscores the urgent need for more work in this exciting area of developmental biology, since this represents real possibility for the genetic correction of aberrant late lung development. These studies will certainly benefit from data recently published on transcriptional regulation of lung maturation (192), as well as genomewide transcriptional profiling studies that have already identified mast cells (24), and possibly SOX5 (75), as candidate regulators of normal and aberrant late lung development. Advances in modeling disease processes that permit the construction of disease-relevant microRNA/mRNA networks will be of increasing importance in making sense of the tremendous mass of gene regulation information that we are currently accumulating (150).

**Environmental Impact**

The impact of the environment on postnatal lung growth (and disease) is another area of increasing interest, particularly concerning the role of environmental smoke exposure on lung growth, with ozone and ethanol exposure also being of interest. An interaction between intrauterine smoke (IUS) exposure and the transcription factor RUNX1 was recently documented (72), where single-nucleotide polymorphisms in the RUNX1 gene were both associated with airway responsiveness in asthmatic children, and that these associations were modified by IUS, where IUS was also able to modify RUNX1 expression directly. Beyond IUS exposure, exposure of mouse pups to sidestream tobacco smoke (as a surrogate for environmental tobacco smoke) increased airway responsiveness and sensory innervation in later life. The increased tracheal innervation and consequent changes in airway responsiveness caused by the tobacco smoke were dependent on neuropeptide Y (191). As an extension to this idea, the impact of thridhand tobacco smoke on lung development has also been explored. Fetal rat lung explants were exposed to nicotine, 1-(N-methyl-N-nitro-samino)-1-(3-pyridyl)-4-butanol, or 4-(methylisothioamino)-1-(3-pyridyl)-1-butaneone, which are the two primary tobacco-specific N-nitrosamine constituents of thirdhand smoke. These substances all disturbed ECM production and lipofibroblast-to-myofibroblast transdifferentiation in fetal lung explants (138).

The impact of prenatal ethanol exposure on lung structure has also received attention, where prenatal exposure of lambs to ethanol caused alterations in the composition of the amniotic fluid, but no persistent effect on the structure of the developing lung was evident 2 mo after birth (155). Ozone, which continues to emerge as an environmental hazard, has recently been demonstrated to exert sustained effects on lung and systemic innate immune responses in an infant baboon model, although the implication for the structural development of the lung have not yet been addressed (100).

**Mechanical Ventilation**

The issue of how the ventilated premature lung may repair itself has recently been addressed in a study in which fetal lambs were partially exposed by hysterectomy at 125 days gestational age, injuriously ventilated for 2 h, and then returned to the uterus (33). Although interesting, the immediate implication of these data is not clear, since fetal lungs were ventilated, but lambs were then returned to the uterus to continue development and were analyzed 1 or 15 days after mechanical ventilation. Nevertheless, it appears that in utero lungs of mechanically ventilated fetsuses are injured but are also able to repair.

Ventilation strategies are being continually modified in an effort to make mechanical ventilation “less injurious.” Work by Hillman et al. (78) in preterm sheep (128 days gestation) has recently revealed that the use of positive end-expiratory pressure (PEEP; 8 cmH2O) promoted improved lung function and decreased the production of early mediators of lung injury, compared with preterm sheep that were ventilated without PEEP. A combination of PEEP ventilation with surfactant treatment had an additional protective effect.

Mechanical ventilation is also the basis of a very clinically relevant BPD model, which has been successfully used to address the role of elastase, and the elastase-mediated activation of TGF-$\beta$ in experimental BPD (76, 77). This model has also been employed to demonstrate the potential usefulness of elafin, a proteinase inhibitor, as a therapeutic intervention in BPD, where both endogenous elafin administration as well as...
overexpression of the elafin gene in vivo could promote normal lung structure and normalize TGF-β signaling in experimental BPD (77).

Growth Factors

Peptide growth factors are accredited with a key role in late lung development, and disturbances to peptide growth factor signaling have been associated with arrested late lung development and BPD, as already highlighted in several places in this Perspective. Several recent studies have continued to highlight roles for vascular endothelial growth factor (VEGF) in arrested lung development associated with intrauterine growth restriction (IUGR) or preeclampsia. In an ovine IUGR model, fetuses were characterized by perturbed alveolar and vascular growth, and pulmonary artery endothelial cell migration and tube formation were disturbed. Furthermore, levels of VEGFA and VEGFR2 were reduced in these endothelial cells (109, 142). Additionally, levels of soluble VEGFR1 (sVEGFR1), an endogenous antagonist of VEGF, are elevated in the amniotic fluid and maternal blood in preeclampsia. Intra-amniotic administration of sVEGFR1 to pregnant rats prevented activation of VEGFR2 and increased apoptosis in endothelial and mesenchymal cells in the lungs of newborn rat pups, which also exhibited blunted alveolarization and reduced lung vessel density (159). These data provide an exciting link between preeclampsia and BPD. Advances have also recently been made in unraveling a podoplanin/Cdc42 pathway that regulates how VEGF drives lymphatic endothelial cell migration (120).

New roles for connective tissue growth factor (CTGF) have also been identified, with inducible overexpression of CTGF in alveolar type II cells causing a dramatic macrophage and neutrophil infiltration into the developing lung, accompanied by impaired alveolarization and lung vascularization, and increased pulmonary vascular remodeling accompanied by the development of pulmonary hypertension. These processes were demonstrated to be associated with activation of an integrin-linked kinase (ILK)/GSK-3β/β-catenin signaling cascade (43).

The question of fibroblast phenotype and fibroblast differentiation is a “hot topic” in late lung development today, and significant progress has been afforded by the recent elegant demonstration that epithelial-mesenchymal cross talk regulates fibroblast phenotypes during alveolar septation and emphasizes the role of fibroblast growth factor (FGF) signaling in this process (42). Platelet-derived growth factor (PDGF) has also received recent attention (96), where inhibition of the PDGF receptor kinase with imatinib over the first week of life resulted in a pronounced decrease in alveolarization that was evident already on the eighth day of life. The critical role of PDGF in alveolarization has been long established (31, 32, 97, 107, 130). These new data reveal that, even after a 58-day recovery period, pronounced differences in the number of alveoli in the (now) adult rats that had been treated with imatinib were observed, compared with control mock-treated mice. These observations are thus also relevant to the long-term consequences of disturbed alveolar development in infancy.

Other reports have revealed an essential role for epidermal growth factor (EGF) in regenerative alveolarization after unilateral pneumonectomy (59), as well as possible roles for FGF18 (64) in the alveolarization process.

Intrauterine Infection, Inflammation, and Steroids

Recent studies in this area have addressed the role of chorioamnionitis and intrauterine infection on lung development, the signaling pathways that mediate pulmonary responses to intrauterine infection, and the usefulness of anti-inflammatory strategies in intrauterine infection. Recent progress includes the identification of IL-1α as a powerful mediator of the fetal inflammatory response syndrome (88), and prostaglandins (186) and ErbB4 (149) have been demonstrated to mediate the fetal response to intrauterine inflammation.

Hyperoxia is known to provoke pronounced inflammation in the developing lung, and the inflammation occurs concomitantly with arrested late lung development. Several recent studies have focused on mediators of hyperoxia-induced inflammatory processes in the developing lung. One interesting mediator that has emerged is the heme oxygenase 1 (Hmox1)/carbon monoxide (CO) system (62). Constitutive overexpression of Hmox1 in the developing mouse lung dampened the inflammatory response elicited by hyperoxia and partially restored proper vascular growth that is normally perturbed by hyperoxia exposure. However, overexpression of Hmox1 did not prevent the arrested alveolarization associated with hyperoxia exposure. Several interesting questions are posed by this study. For example, what is the protective factor: is it CO that is generated by Hmox1 activity or is it another Hmox1 reaction product? Additionally, the question of why the effects of hyperoxia on vascular and alveolar growth were uncoupled in the Hmox1-overexpressing mouse remains, since these two processes are usually intimately associated, with vascular growth believed to precede septation (163). Like Hmox1, curcumin also possesses potent antioxidant and anti-inflammatory properties. Curcumin was demonstrated recently to accelerate lung maturation, purportedly through the stimulation of epithelial-mesenchymal interactions in the alveolus, and curcumin was also demonstrated to prevent arrested septation associated with hyperoxia exposure by blocking TGF-β activation (143), adding curcumin to the growing list of candidate interventional strategies for BPD.

Similar to what was observed with Hmox1 and curcumin, a role for NF-κB in late lung development has also been addressed. Pharmacological inhibition or genetic ablation of NF-κB disrupted angiogenesis and alveolarization of the developing lung (81). These data, which build on other studies on NF-κB and macrophage activation in BPD (26), point to an important role for NF-κB in promoting lung maturation, although how the NF-κB pathways may be impacted by intrauterine infection and other inflammatory signals present in immature developing lungs in which septation has been arrested remains to be clarified. Similarly, interference with NF-κB signaling is generally deleterious to organ development; thus it remains meaningful to assess which processes the NF-κB pathway impacts to promote proper development of the postnatal lung. Along these lines, exciting new data from Lance Prince have laid some foundations linking innate immune signaling and developmental programs, starting with the demonstration that inflammatory signaling inhibits FGF-10 pathways through NF-κB-dependent interactions between RELA,
SP3, and the FGF-10 promoter (37) and that NF-κB activation limits airway branching through inhibition of Sp1-mediated FGF-10 expression (18).

Although inflammation has been associated with arrested late lung development in the hyperoxia model, and some evidence exists to implicate M1-to-M2 macrophage differentiation during lung development (87), much work remains to be done to understand how inflammatory cells can contribute to or direct the course of normal and aberrant late lung development. This remains a gap in our knowledge that needs to be filled.

A connection between viral infections and exaggerated cell death and inflammatory pathways in the developing lung has also recently been revealed. Human respiratory syncytial virus (RSV) is the most common cause of severe lower respiratory tract illnesses in premature/newborn infants and young children. In a newborn lamb model, RSV infection was demonstrated to enhance the degree of apoptotic cell death and drive TGF-β inflammatory pathways in the lung and tracheobronchial lymph nodes, emphasizing the role of inflammation in RSV disease (154). In addition to an impact of elevated oxygen levels on lung development, oxygen exposure in preterm infants can also increase the risk for respiratory viral infections later in life. Buczynski and coworkers (35) revealed that the host response to respiratory viral infections in late life can indeed be disrupted by different doses of oxygen. Adult mice exposed to high oxygen levels (80 or 100% oxygen) at birth exhibited increased inflammatory responses to respiratory viral infection compared with littersmates exposed to room air at birth. There was no apparent impact of exposure to lower levels of oxygen (40 or 60% oxygen) at birth. This study represents an important advance in our knowledge about how oxygen supplementation in early life may impact responses to viral infection in late life.

Related to this theme, after inducible deletion of hypoxia inducible factor (HIF)-1α specifically in the alveolar type II cells and club cells of mice during the early postnatal period, mice later displayed exaggerated responses to ovulambum challenge in an asthma model, including increased cellular infiltrates, eosinophilia, and T\(_\text{H}\)2 cytokines. This exaggerated response was not observed when loss of HIF-1α was induced in early adulthood (69). These data are also complemented by another report demonstrating that HIF proteins promote proper alveolarization (169). Since early hyperoxic exposure might also inhibit HIF-1α signaling, the investigators proposed that preterm oxygen therapy might mimic the functional deletion of HIF-1α described in that study. These data, which build nicely on Carl White’s original studies on HIF in preterm birth (9, 10), also add to an emerging body of data that suggest that early postnatal events led to long-term consequences for respiratory function that persist into adulthood.

The impact of corticosteroids on lung development is complicated, since the positive anti-inflammatory and lung maturation impact of corticosteroids is counterbalanced by growth retardation and other effects on the lung. As such, corticosteroid use in BPD remains controversial, and current consensus is that early (<7 days) intravenous corticosteroid use is generally not beneficial, although late (>7 day) corticosteroid use may remain useful to promote ventilator weaning (52). Much work continues to be done to understand the complex interplay of corticosteroids and inflammation in the developing lung in animal models. In a sheep chorioamnionitis model, the intra-amniotic exposure to bacterial LPS downregulated sonic hedgehog (Shh) signaling and also disturbed elastin deposition and alveolar structure. These effects were reversed by betamethasone treatment (48). In the same model, it was demonstrated that the order of administration of betamethasone vs. that of intra-amniotic LPS was important, since betamethasone pretreatment suppressed LPS-induced lung inflammation and promoted lung maturation, whereas betamethasone treatment after LPS administration did not impact lung inflammation but did enhance lung maturation (94). In terms of molecular mechanisms, the combination of dexamethasone together with 8-bromo-3',5'-cyclic adenosine monophosphate and isobutylmethylxanthine was demonstrated to promote compensatory lung growth by increasing expression of thyroid transcription factor 1 (TTF-1), which may represent one mechanism by which corticosteroids promote alveolarization (158).

Hypoxia

Hypoxia represents a potent stimulus that negatively impacts late lung development. Indeed, like hyperoxia, hypoxia is often employed as a model of BPD, where exposure of newborn rodents to a hypoxic environment leads to alveolar simplification. The effects of hypoxia have largely been attributed to increased TGF-β signaling in response to hypoxia exposure, which arrests late lung development. Recent studies have furthered our understanding of the molecular pathways regulated by the hypoxia/TGF-β pathway (127). A complicated relationship between TGF-β and the nuclear receptor peroxisome proliferator-activated receptor (PPAR)-γ is also emerging, with reports that hypoxia-induced inhibition of lung development was attenuated by the PPAR-γ agonist rosiglitazone (121). Along these lines, hypoxia downregulated PPAR-γ in isolated pulmonary arterial smooth muscle cells and in rodent lungs via TGF-β (68).

Apart from TGF-β and PPAR-γ, the Ca\(^{2+}\)-dependent transcription factor, nuclear factor of activated T cells isoform c3 (NFATc3) (25), histone acetylation (194), ryanodine receptor-mediated Ca\(^{2+}\) signaling (71), and the osmomechanosensitive TRPV4 channel (195) have been implicated as mediators of hypoxia-induced pulmonary hypertension and vascular remodeling in neonatal (as well as adult) mice. It would be most interesting to know whether all of these candidate mediators of the effects of hypoxia on late lung development may be connected in a single pathway that is globally regulated and may be targeted to normalize alveolarization in the background of hypoxia exposure.

Vascular Growth and PPHN

The development of the pulmonary vasculature is impacted in clinical BPD and in experimental animal models of BPD. Additionally, persistent pulmonary hypertension of the newborn (PPHN) is a common complication seen in infants with perturbed lung development (164). Several key earlier reports have demonstrated important vascular changes associated with BPD in preterm infants, including reduced VEGF expression in the lungs of infants dying with BPD (23), and increased endoglin expression in the lungs of preterm infants who died after receiving supportive mechanical ventilation (55). Inter-
estingly, in preterm infants that received prolonged mechanical ventilation, marked angiogenesis and increased proliferation of lung endothelial cells were observed (54). Some studies have attempted to understand the molecular basis of these observations, where inflammation may play a role, since chorioamnionitis appears to stimulate angiogenesis in saccular stage fetal lungs (110). Furthermore, microarray studies have revealed that there is a shift in the balance of classical angiogenic growth factors to alternative (including antipsprouting) regulators in the lungs of preterm infants that received mechanical ventilation (53). Currently, the exact pattern of vascular changes observed in infants with BPD is difficult to delineate, since short periods of ventilation cause a paucity of arteries and fewer endothelial cells, whereas longer ventilation periods are characterized by decreased vessel branches but increased endothelial cell proliferation (1). Taken together, there appears to be a broad spectrum of pulmonary vascular disease present in preterm infants with BPD, irrespective of whether PPHN is present or not (116). Much remains to be investigated, including the degree to which animal models of BPD [characterized by a infant respiratory distress syndrome (IRDS) general loss of vessels (47, 163)], actually model the changes to the pulmonary vasculature that occur in preterm infants with BPD [who generally exhibit a dysmorphic pulmonary circulation (156, 162)]. Much recent work has addressed the molecular mechanisms, such as NADPH oxidase activity and autophagy, that may underlie the development of PPHN, and impaired angiogenesis related to PPHN (45, 57, 129, 160, 161, 181–183), as well as the possible beneficial impact of antioxidants, soluble guanylate cyclase activators, NADPH oxidase inhibitors, and modulating NO and serotonin levels as candidate interventional strategies to correct PPHN. However, an impact on lung growth and alveolarization was not addressed in any of these studies, and thus further discussion in this Perspective would be premature.

PPHN is also a common complication of congenital diaphragmatic hernia (CDH) (172), which like BPD is often associated with perturbed lung growth and alveolarization. In the well-established nitrofen animal model of CDH (20, 170), the tyrosine kinase inhibitor imatinib has been introduced as a possible therapeutic intervention to drive proper vascular growth (38), although alveolar development was not addressed in the study. Given that imatinib inhibits PDGF signaling and that this impacts alveolarization (see growth factor discussion above, and Refs. 31, 32, 97, 107, 130), how imatinib will be developed for antenatal therapy for PPHN in CDH will no doubt be the subject of future investigation.

Another recent study addressed, for the first time, pulmonary neural development in CDH and revealed profound disturbances to pulmonary innervation in the nitrofen animal model of CDH (95), with a less complex neural innervation being observed, and accompanied by autonomic imbalance. Thus defective embryonic pulmonary innervation is now introduced as a contributor to lung developmental defects in CDH. These studies, although not directly addressing alveolarization, lay important groundwork for the investigation of the contribution of pulmonary innervation to normal and aberrant late lung development.

Surfactant

Pulmonary surfactant is composed of a complex lipoprotein-like mixture and lines the inner surface of the lung thus preventing alveolar collapse at the end of expiration. Premature infants have immature lungs with surfactant deficiency that triggers vulnerability to injury from mechanical ventilation (14, 91) and IRDS. Surfactant replacement therapy has played a pivotal role in improving outcome in premature infants with or at risk for the development of BPD (27, 84). Following from discussion presented in the ventilation subsection of this Perspective, recent work has addressed combining surfactant replacement and high-PEEP ventilation (78). More specifically related to surfactant, a new surfactant-associated protein [carcinoembryonic cell adhesion molecule (CEACAM) 6] has been identified in the human lung (39), and an albumin-associated phospholipase A2-like activity that is secreted from fetal type II pneumocytes has been demonstrated to destabilize surfactant (49). Additionally, microbial exposure in infancy has been demonstrated to increase the abundance of immunity-relevant (19, 187) surfactant pools and inflammatory cells that may be involved in airway resistance to pathogens (89). Although surfactant metabolism is clearly directly related to normal and aberrant late lung development, none of these new findings mentioned above has yet been demonstrated to impact alveolarization. Intriguingly, a role has now been hinted at for surfactant in the development of extrapulmonary organs (122).

Among some novel regulators of surfactant production, leptin has been suggested as an enhancer of lung maturity, partly through the observation that respiratory problems in ob/ob mice can be alleviated by exogenous leptin treatment (125). However, whether leptin itself may regulate surfactant production remains controversial, since conflicting reports exist. One study has recently reported that neither exogenously administered leptin nor leptin deficiency has any impact on lung maturation or surfactant production in fetal mouse and lamb lungs (144), whereas another study recently reported that leptin may indeed have therapeutic potential for the treatment of fetal growth restriction, since those investigators observed that leptin could drive surfactant protein-A production and promoted fetal lung maturity in a TFF-1-dependent manner (41). Work remains to be done to clarify the impact of leptin on lung maturation and surfactant production.

Discussion and Concluding Remarks

In sum, this Perspective has updated the reader about very recent advances we have made in understanding late lung development and the pathogenesis of BPD. Preliminary data on the role of regulators of fibroblast proliferation and differentiation (106, 171), telomeres and telomerases (82), the TTF-1 (50) C/EBPα (193) transcription factors, and prenatal iodine deficiency (66) have not been individually dealt with. Although some progress has been made, our understanding of how the alveolar structure of the lung develops remains very poor. Thus we still have far to go.

Although widely used to model BPD in animals, a clear understanding how oxygen toxicity impacts the developing postnatal lung is lacking. Key questions remain, including what level of hyperoxia is toxic to the developing lung, what duration of exposure to hyperoxia is deleterious to lung development, and whether there are specific critical windows during
Lung development when the lung is particularly sensitive to hyperoxia. The interplay between the ECM and cell phenotype is also an area in which there are more questions than answers, particularly how the ECM defines cell phenotype and differentiation. Additionally, although the ECM structure and the expression of ECM remodeling systems are known to be disturbed in both clinical (44, 165, 166) and experimental (2, 3, 28–30, 93, 103, 131) BPD, to date, an experimental demonstration of a causal link between disturbed elastin and/or collagen structures and impeded lung development is lacking. Similarly, much work has linked inflammatory signaling and inflammatory cells to arrested lung development, but the molecular mechanisms at play that underlie the arrested lung development are only now being revealed. In terms of genetics, several microRNA screens have revealed changes in the expression of multiple microRNA species, but no microRNA has yet been causally implicated in normal or aberrant late lung development. The first reports on long noncoding (Inc) RNAs are no doubt just around the corner. It is also evident that much remains unclear about the development of the pulmonary circulation, particularly during aberrant late lung development, where the widely used animal models available do not appear to mimic the pathological changes leading to the dysmorphic pulmonary circulation that is characteristic of clinical BPD, making mechanistic studies in this area particularly difficult. On a technical note, two recent reports have also highlighted the importance of the stereological analysis of structural parameters in the lung (117, 126), which warrants consideration in future studies (184).

In terms of novel therapy and management strategy, many “firsts” highlighted in this Perspective have indicated novel agents that may correct or reverse arrested lung development. However, if our experiences with vitamin A and all-trans retinoic acid (ATRA) are anything to go by, there is a long road ahead to translate these findings to the bedside: since the original observations by Harry Goldblatt and Maria Benischek in 1927 that vitamin A deficiency causes metaplasia in the lung (67), followed by the pioneering work of Frank Chytíl on retinol in the lung in the 1970s (16), and studies on the vitamin A status of BPD patients in the 1980s (152), still today it remains unclear whether vitamin A/retinol/ATRA administration may be useful at all in patients with or at risk for BPD (98). Further advances in the use of MSCs to promote lung development are particularly exciting, and the next steps must include the identification of soluble factors produced by MSCs that drive late lung development and understanding how MSC administration results in the corrective and therapeutic effects that have been reported.

The increasing prevalence of perturbed late lung development encountered in the neonatal intensive care unit and the emerging evidence that damage to the immature lung leads to long-term respiratory consequences that persist into adulthood underscores the need to better understand the basic mechanisms underlying alveolarization. This is important not only because the therapeutic management of BPD, CDH, and other lung structures, such as emphysema and the acute respiratory distress syndrome. This Perspective has also highlighted a number of controversies that have emerged from recent reports that conflict in their conclusions and has attempted to illustrate serious gaps in our knowledge that persist and limit our understanding of how the lung develops. It is clear that much challenging but exciting work remains to be done.

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A.M., I.M., J.R.-C., and R.E.M. edited and revised manuscript; A.M., I.M., J.R.-C., and R.E.M. drafted manuscript; A.M., I.M., J.R.-C., and R.E.M. approved final version of manuscript.

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