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POSTNATAL INFLAMMATION IN THE PATHOGENESIS OF BRONCHOPULMONARY DYSPLASIA

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Abstract

Exposure to hyperoxia, invasive mechanical ventilation and systemic/local sepsis are important antecedents of postnatal inflammation in the pathogenesis of bronchopulmonary dysplasia (BPD). This review will summarize information obtained from animal (baboon, lamb/sheep, rat and mouse) models that pertain to the specific inflammatory agents and signaling molecules that predispose a premature infant to BPD.

Keywords

invasive ventilation; infection; hyperoxia; lung; newborn

Bronchopulmonary Dysplasia (BPD)

BPD is the most common chronic respiratory disease in infants and is a devastating condition that disrupts the developmental program of the lung secondary to preterm birth. BPD occurs as a result of interactions between genetic and environmental factors (invasive mechanical ventilation, sepsis, and hyperoxia) (Bhandari and Bhandari, 2009; Bhandari and Bhandari, 2011). Although the definition of BPD has evolved over the past decade, it is currently defined as the need for O₂ supplementation for 28 days of life and a “physiologic” assessment of the O₂ requirement at 36 weeks postmenstrual age (Bhandari and Bhandari, 2011; Trembath and Laughon, 2012). It is estimated that 10,000–15,000 new cases of BPD occur each year in the United States, and significantly, 97% of all BPD cases occur in infants with a birth weight <1250 grams (Bhandari and Bhandari, 2011). Despite many advances in neonatal ventilation techniques, widespread use of surfactant and antenatal corticosteroids, as well as aggressive fluid management, the incidence of BPD has remained the same (Smith et al., 2005) or even increased slightly (Bhandari and Bhandari, 2011; Trembath and Laughon, 2012). BPD is associated with significant pulmonary and neurodevelopmental sequelae that continue to have health ramifications into adulthood (Anderson and Doyle, 2006; Bhandari and Panitch, 2006; Bhandari and Bhandari, 2011).

Stages of Lung Development

Five distinct stages of lung development have been recognized: embryonic, pseudoglandular, canalicular, saccular and alveolar. These stages tend to be conserved across species but differences do exist with respect to the duration of each stage and its temporal relationship to gestational age (Table 1) (Thurlbeck, 1975; Kotecha, 2000; Hislop, 2002; Maeda et al., 2007; Schittny and Burri, 2008; Maniscalco and Bhandari, 2010; Meller and Bhandari, 2012). In the present context, the human preterm babies most predisposed to develop BPD tend to be born with their lungs in the late canalicular (characterized by formation of primitive alveoli, differentiation of Type I and Type II pneumocytes and formation of alveolar capillary barrier) and early saccular (initiation of surfactant production, enlargement of terminal airways, pulmonary vascularization) stages (Kotecha, 2000; Joshi and Kotecha, 2007; Kramer et al., 2007; Maeda et al., 2007). It is important to mention that for neonatal rodents, the saccular stage begins in the uterus and continues through post natal day 5 (PN5), while in sheep/baboons /humans, this occurs *in utero* (Kramer et al., 2007; Maeda et al., 2007). The distinction between the timing/*ex* or *in utero* location of developmental stages of the lung in animal models versus humans assumes critical importance when designing/assessing experimental BPD models.

Postnatal factors and Inflammation in the Pathogenesis of BPD

Inflammation is a cornerstone in the pathogenesis of BPD. While a variety of clinical factors have been associated with BPD (Akram Khan et al., 2006), the critical ones that have been linked to inflammation-induced cell signaling pathways include antenatal and postnatal factors (Bhandari and Bhandari, 2009; Bhandari and Bhandari, 2011). In the context of a genetic predisposition in an immature lung, a combination of pre- and post-natal factors initiate an inflammatory process that is mediated by a variety of molecular mediators, including cytokines. Damage to the developing lung by the activation of the cell death pathways is followed by resolution of injury to close to normal lung architecture, or repair. The latter state is characterized by the pulmonary phenotype of “new” BPD as evidenced by fewer and larger simplified alveoli, along with dysmorphic vasculature, leading to the description of impaired alveolarization and dysregulated vascularization (Bhandari and Bhandari, 2009; Bhandari, 2010; Bhandari and Bhandari, 2011).

The contributory role of the major prenatal factor – chorioamnionitis – has been covered by recent reviews (Gien and Kinsella, 2011; Viscardi, 2012; Thomas and Speer, 2013) and elsewhere in this issue. Among the postnatal factors, the 3 most important ones: invasive mechanical ventilation, postnatal local/systemic sepsis and hyperoxia, will be discussed in this review.

Invasive Ventilation: Animal Models

Early studies using a chronically-ventilated (3–4 weeks) preterm lamb model of BPD showed evidence of non-uniform inflation patterns and impaired alveolar formation with an abnormal abundance of elastin (ELN) (Albertine et al., 1999). Inflammation was evident by the presence of inflammatory cells, namely alveolar macrophages, neutrophils, and mononuclear cells and edema (Albertine et al., 1999). In this model, there was also reduced

lung expression of growth factors that regulate alveolarization and differential alteration of matrix proteins that regulate ELN assembly (Bland et al., 2007). Conversely, a non-invasive (nasal) ventilation approach preserved alveolar architecture (Reyburn et al., 2008) and had a positive effect on parathyroid hormone-related protein-peroxisome proliferator-activated receptor-gamma (PTHrP-PPAR γ)-driven alveolar homeostatic epithelial-mesenchymal signaling (Rehan et al., 2011).

More recently, even short-term stretch injury (15 minutes) secondary to invasive ventilation in preterm fetal sheep led to increased levels of proinflammatory cytokines interleukin-1 β (IL-1 β), IL-6, monocyte chemoattractant protein (MCP)-1, and MCP-2 mRNA by 1 hour (h) (Hillman et al., 2011). This was accompanied by increased presence of inflammatory cells in the bronchoalveolar lavage fluid (BALF) with initial increases in neutrophils and monocytes by 1h and a transition to macrophages by 24h (Hillman et al., 2011).

The preterm ventilated baboon model of BPD [delivered at 125 days (d) – at 68% of gestation] showed evidence of alveolar hypoplasia and dysmorphic vasculature, akin to that seen in human BPD (Coalson et al., 1999). Importantly, there were significant elevations of tumor necrosis factor-alpha (TNF- α), IL-6, IL-8 levels, but not of IL-1 β and IL-10, in tracheal aspirate fluids at various times during the period of ventilatory support, supporting a role for inflammation (Coalson et al., 1999). In addition, increased matrix metalloproteinase-9 (MMP-9) levels were associated with lung inflammation and edema seen in this invasive ventilation model (Tambunting et al., 2005). Alteration of vascular growth factors (vascular endothelial growth factor or VEGF) was also noted in the lungs of various baboon models (Maniscalco et al., 2002; Tambunting et al., 2005). Bombesin is a 14-amino acid peptide, initially detected in amphibian skin, but immunoreactive studies have shown the presence of bombesin-like peptides (BLP) in multiple organ systems in mammals (Ganter and Pittet, 2006). In the lung, BLP have been shown to be released by pulmonary neuroendocrine cells (Ganter and Pittet, 2006). BLP blockade improved alveolar septation and angiogenesis in the preterm baboon models (Sunday et al., 1998; Subramaniam et al., 2007).

In the 125d baboon model, treatment with early nasal continuous positive airway pressure (NCPAP) for 28d led to a pulmonary phenotype similar to 156d gestational control lungs, suggesting that this non-invasive approach could minimize lung injury (Thomson et al., 2004). In the same model, delayed extubation (till 5 days) versus early extubation to NCPAP at 24h, led to significantly increased BALF IL-6, IL-8, MCP-1, macrophage inflammatory protein-1 alpha (MIP-1 α), and growth regulated oncogene-alpha (GRO- α) in the delayed NCPAP group (Thomson et al., 2006).

In neonatal rats (7–14d old – in the alveolar phase of lung development), high tidal volume ventilation increased IL-6 mRNA and upregulation of the transforming growth factor-beta (TGF- β) signaling molecule, connective tissue growth factor (CTGF) mRNA and protein expression, compared to controls (Wu et al., 2008). In an 8d old rat ventilation model, high tidal volumes increased the neutrophilic and inflammatory cytokine mRNA and/or protein expression (IL-1 β , IL-6, CXCL-1 and 2) response (Kroon et al., 2010). In a 7d rat model,

exposure to mechanical ventilation for 24h in room air led to cell cycle arrest (Kroon et al., 2011), suggesting a possible prelude to impaired alveolarization, the hallmark of BPD.

In an invasive ventilation model in 2-week old mice (well into the alveolar phase of lung development) for 1h, IL-6 lung levels were increased in the high tidal volume ventilation group (Cannizzaro et al., 2008). Studies conducted in 2–6d old mice (late saccular to early alveolar phase of lung development) ventilated for 8–24h with room air or 40% O₂ revealed dysregulated elastin assembly, a 3–5 fold increase in cell death, TGF- β activation, and a decrease in VEGF-receptor 2 (VEGF-R2) expression (Bland et al., 2008; Mokres et al., 2010). Inhibiting lung elastase activity by using recombinant human elafin or genetically-modified mice that expressed elafin in the vascular endothelium was protective of the lung injury (Hilgendorff et al., 2011; Hilgendorff et al., 2012).

Interestingly, transcriptomic analyses of lung samples from ventilated premature baboons, mouse and rat models of BPD and humans resulted in a final “cross-species” data set which included 90 healthy and 24 ventilated samples of various ages, four species, and both sexes and identified several highly conserved genes in response to mechanical ventilation (Kompass et al., 2010). These included ELN, gastrin-releasing polypeptide (GRP, which is a BLP), and CTGF (Kompass et al., 2010).

To summarize, while the lamb/sheep/baboon ventilation models are in the saccular stage (akin to the human premature babies who are at most risk for BPD at birth), the rat/mouse ventilation models are in the alveolar phase of lung development. However, it is quite obvious that mechanical stretch injury generates an inflammatory response (mostly neutrophils, IL-1 β , IL-6, CXCL-1 / -2, TGF- β signaling), along with alterations in matrix proteins (ELN, MMP-9) and vascular growth factors (VEGF). In addition, there is increased cell death and cell cycle arrest. Thus, it appears that an initial inflammatory cascade triggers the signaling of additional molecular mediators that lead to dysregulated vascularization and impaired alveolarization. Interestingly, non-invasive (nasal) ventilation approaches were protective of these responses.

Postnatal Sepsis: Animal Models

In a preterm lamb model treated with CPAP or mechanical ventilation up to 3h, mRNA expression of inflammatory cytokines was increased, compared to unventilated controls (Polglase et al., 2009). Lipopolysaccharide (LPS) administration increased inflammatory cells and enhanced the responses of IL-1 β , IL-6 and IL-8 mRNA expression; however, cytokine markers of lung injury from intratracheal LPS were not decreased in the CPAP versus the ventilated group (Polglase et al., 2009).

In a systemic Gram-negative sepsis (*Salmonella* endotoxin) neonatal (10d) rat model, there was increased pulmonary neutrophil recruitment at 1h and expression of cytokine-induced neutrophil chemoattractant (CINC) at 2h, but with no overt lung injury (up to 16h) (Tillema et al., 2000). However, in a 6d neonatal rat model intratracheally exposed to LPS, there was increased neutrophil accumulation in the lungs at 24h, which changed to mostly macrophages at 15d (Franco et al., 2002). The latter time-point (postnatal day 21 or PN21) was also associated with enlarged peripheral air spaces and fewer alveoli and increased 92-

kDa gelatinase activity, compared to controls. Interestingly, doxycycline, a non-specific MMP-inhibitor, worsened LPS-induced alveolarization abnormalities despite partly inhibiting the gelatinase overactivity (Franco et al., 2002).

Neonatal mice injected with intraperitoneal LPS demonstrated reduced lung inflammation and apoptosis after 24h as compared to adults, and this was associated with activation of the transcription factor nuclear factor-kappa B (NF- κ B) (Alvira et al., 2007). Inhibition of NF- κ B resulted in increased cell death and alveolar simplification and disruption of angiogenesis via VEGF-R2 (Iosef et al., 2012). Independent work by others using a targeted deletion of NF- κ B signaling (using a lung epithelium-specific deletion of IKK β – which is a known activating kinase upstream of NF- κ B) in a mouse model revealed alveolar hypoplasia with decreased VEGF expression (Londhe et al., 2011).

Viral infections of epithelial cells are characterized by the generation of the pro-inflammatory molecule double-stranded RNA (dsRNA) during intracellular replication of viruses, and has been shown to induce activation of the neutrophil chemoattractant, IL-8. Hence, there is a possibility that inflammatory processes secondary to viral infections could also lead to a BPD pulmonary phenotype. This was tested by intratracheal delivery of dsRNA into a 10d-old mouse model, which resulted in neutrophil accumulation, increased lung injury and decreased alveolarization (Londhe et al., 2005). In addition, there was increased expression of CXCL-1, as well as its receptor CXCR2. Pre-treatment with CXCR2-neutralizing antibody was able to reverse the effects in the developing lung (Londhe et al., 2005).

Taken together, it would suggest that both bacterial and viral agents in the PN developing lung stimulate a strong inflammatory response, which leads to altered alveolar architecture, suggestive of the BPD pulmonary phenotype. Interestingly, infection-initiated inflammatory response in the developing lung appears not to be dampened by using a non-invasive ventilation approach.

Hyperoxia Exposure: Animal Models

Hyperoxia exposure to preterm rabbits led to impaired alveolarization (Mascaretti et al., 2009). In neonatal lambs, cimetidine (Hazinski et al., 1989) as well inhaled nitric oxide (iNO) (Cotton et al., 2006) have been reported to reduce hyperoxia-induced lung injury.

In hyperoxia-exposed neonatal rats, a variety of agents have been used as therapeutic agents to ameliorate hyperoxia-induced lung inflammation/injury and/or the BPD phenotype. These include keratinocyte growth factor (KGF) (Frank, 2003; Franco-Montoya et al., 2009), VEGF (Kunig et al., 2005; Thebaud et al., 2005; Kunig et al., 2006), iNO (ter Horst et al., 2007), inhaled ethyl nitrite (Auten et al., 2007), sildenafil (Ladha et al., 2005; Park et al., 2013), L-citrulline (Vadivel et al., 2010), leukotriene inhibition (Funk et al., 2007), azithromycin (Ballard et al., 2007), apelin – a potent vasodilator and angiogenic factor (Visser et al., 2010), phosphodiesterase-4 (PDE-4) inhibition (de Visser et al., 2008; de Visser et al., 2012), curcumin (Sakurai et al., 2011), colchicine (Ozdemir et al., 2012), pentoxifylline (Almario et al., 2012), Nigella sativa oil (Tayman et al., 2013), calcitonin-gene related peptide (Dang et al., 2012), caffeine (Weichelt et al., 2013), inhibition of Wnt-

signaling (Alapati et al., 2013; Hummler et al., 2013), and administration of cytidine 5'-diphosphocholine (Cetinkaya et al., 2013). A variety of stem cells delivered to hyperoxia-exposed neonatal rat lungs have improved alveolar and vascular growth (van Haaften et al., 2009; Zhang et al., 2012; Baker et al., 2013; Pierro et al., 2013).

Prolonged exposure to hyperoxia to the neonatal mouse, for 14d or longer (Warner et al., 1998; Woyda et al., 2009; Velten et al., 2010; Zhang et al., 2011; Zhang et al., 2012; Tibboel et al., 2013), does result in lung injury, but the phenotype is more reminiscent of “old” rather than “new” BPD (Bhandari and Bhandari, 2011) – the major differentiating feature being the increased fibrosis in the former condition.

To replicate human BPD, exposure to hyperoxia during the critical saccular stage of lung development is sufficient to create the pulmonary phenotype, with the effects being not only dose-dependent on the fraction of inspired oxygen (FiO₂) concentration, but lasting life-long and mimicking the increased susceptibility to respiratory tract infections (O'Reilly et al., 2008; Yee et al., 2009; Li et al., 2011 Dec. 15; Buczynski et al., 2012; Choo-Wing et al., 2013), which has also been well-documented in former premature human infants with BPD (Bhandari and Bhandari, 2003).

The specific role of individual inflammatory molecular mediators in the pathogenesis of BPD has been particularly well-illustrated by utilizing lung-targeted overexpressing transgenic models, in room air, resulting in pulmonary phenotypes reminiscent of human BPD. These include IL-1 β (Bry et al., 2007; Backstrom et al., 2011), and interferon-gamma (IFN- γ) (Harijith et al., 2011; Choo-Wing et al., 2013). In the case of IL-1 β transgenic mice, absence of the beta6 integrin subunit was protective of the BPD phenotype (Hogmalm et al., 2010). Interestingly, inhibition of cyclooxygenase-2 (Cox-2) ameliorated the BPD phenotype in the hyperoxia-induced as well as the IFN- γ lung overexpressing transgenic mouse model in room air. A recent paper has reported that increased Cox-2 activity may contribute to proinflammatory responses in hyperoxia-exposed developing mouse lungs (Britt et al., 2013).

Increased TGF- β signaling has been implicated in resulting in the BPD phenotype (Alejandro-Alcazar et al., 2007), with neutralizing antibodies against it improving hyperoxia-induced lung injury (Nakanishi et al., 2007). This has been corroborated by TGF- β 1 overexpression models (Gauldie et al., 2003; Vicencio et al., 2004; Li et al., 2011 Dec. 15). Another molecule that has been implicated as a downstream mediator of TGF- β 1 signaling in the newborn lung is CTGF (Li et al., 2011 Dec. 15). Targeted overexpression of CTGF in the developing mouse lung has been reported to have a BPD phenotype (Wu et al., 2010; Chen et al., 2011), with CTGF antibody treatment attenuating hyperoxia-induced lung injury in neonatal rats (Alapati et al., 2011). Periostin, a secreted extracellular matrix protein involved in TGF- β signaling, also appears to be playing a role, as periostin null mutant mice were protected from some features of hyperoxia-induced lung injury (Bozyk et al., 2012).

The importance of just the “right amount” of a molecular mediator (the “Goldilocks effect”) has also been made obvious with such a genetic approach in the case of macrophage migration inhibition factor (MIF) (Sun et al., 2013; Sun et al., 2013).

Besides inflammation, hyperoxia exposure has been well-recognized to cause cell death (McGrath-Morrow and Stahl, 2001; Bhandari, 2010) in mouse lungs, and not surprisingly, BPD models (hyperoxia-exposed or transgenic in room air) do reflect this (Choo-Wing et al., 2007; Harijith et al., 2011; Choo-Wing et al., 2013). Activation of the prosurvival factor, Akt, was protective of hyperoxia-induced lung injury and BPD phenotype in neonatal rats (Alphonse et al., 2011). Another important feature is cell cycle arrest and upregulation of the cyclin-dependent kinase inhibitor, p21 (Londhe et al., 2011). p21 null mutant neonatal mice exposed to hyperoxia had decreased survival and larger alveoli (McGrath-Morrow et al., 2004). Among matrix proteins, cathepsin-S deficiency (Hirakawa et al., 2007) and MMP-9 lack and/or deficiency (Chetty et al., 2008; Harijith et al., 2011) was protective of the BPD phenotype in mice models. Regarding anti-oxidants, the transcription factor Nrf2, appeared to have a role as lack of Nrf2 enhanced hyperoxia-induced lung injury, and was associated with increased IL-6 expression (McGrath-Morrow et al., 2009). While a blockade of neutrophil influx in hyperoxia-exposed rat lungs has been shown to be protective of the BPD phenotype (Auten et al., 2001), this effect was enhanced by combining with overexpression of extracellular superoxide dismutase in mice (Min et al., 2012).

Recently, newborn sphingosine kinase 1-deficient mice were reported to be protected from hyperoxia-induced lung injury, including decreased IL-6 BALF concentrations (Harijith et al., 2013). Another report noted that neonatal mice with dysfunctional aryl hydrocarbon receptor were more susceptible to hyperoxia-induced alveolar simplification and inflammation (Shivanna et al., 2013).

A variety of potential therapeutic agents have been used in hyperoxia-exposed mice models that have been shown to decrease inflammation and/or attenuate other parameters of lung injury/BPD phenotype. These include rosiglitazone (Dasgupta et al., 2009; Takeda et al., 2009), hepatocyte growth factor (HGF) (Ohki et al., 2009), B-naphthoflavone (Courouclis et al., 2011), arginyl-glutamine as well as docosahexaenoic acid (Ma et al., 2012), and a combination of vitamin A and retinoic acid (James et al., 2013). Treatment with human amnion epithelial cells attenuated some parameters of hyperoxia-induced inflammatory lung injury (mRNA expression of IL-1 α , IL-6, TGF- β , platelet-derived growth factor-beta or PDGF- β , mean linear intercept, and septal crest density), but not other aspects, for example, alveolar airspace volume, collagen content or leukocyte infiltration in neonatal mice (Vosdoganes et al., 2013).

To summarize, while variable initiation and duration of exposure to hyperoxia animal models have been reported as models of human BPD, exposure to hyperoxia for a relatively-short (PN1-4) duration in mice, which is at the critical saccular stage of lung development, can result in an inflammatory response sufficient to create the BPD pulmonary phenotype. This can be recapitulated using transgenic mice models of the inflammatory mediators, but kept in room air. Importantly, exposure to 0.4, 0.6, >0.8 FiO₂ can mimic mild, moderate and severe BPD, respectively. A vast array of therapeutic agents has been reported to be effective in improving alveolar and/or vascular architecture of the hyperoxia-exposed neonatal lung in lambs, rats and mice. While hyperoxia-exposure is a good starting point for testing the efficacy of potential therapeutic agents, it is important to be able to delineate the responsible molecule/signaling pathway in developmentally-appropriate room air models

and confirm the results in preventing/ameliorating the BPD phenotype. This would avoid the confounding variable of hyperoxia-induced alterations in multiple other molecular mediators, allowing delineation of targeted molecules in specific signaling pathways for maximal potential therapeutic relevance. Among the inflammatory mediators of hyperoxia-induced lung injury that can mimic the BPD phenotype in room air, the well-defined ones are IL-1 β , TGF- β 1, CTGF, IFN- γ , and MIF. In addition, there has been limited translation of therapeutic targeting of hyperoxia-exposure models to premature human neonates to prevent/improve BPD. In some cases, where this has been done, the results have been quite disappointing, for example, with iNO (Cole et al., 2011). Hence, it would be important to attempt to translate some of the newer targets in specific signaling pathways that have been recently reported, for example, inhibition of Cox-2 (Britt et al., 2013; Choo-Wing et al., 2013) as a potential therapeutic option for prevention/amelioration of BPD.

Combination Models

Short-term (6h) invasive ventilation in preterm lambs increased mRNA expression of the acute phase reactant serum amyloid A (which is known to be induced upon exposure to pro-inflammatory cytokines), which was further enhanced by concomitant exposure to intratracheal endotoxin (Wilson et al., 2005). In another short-term (4h) study, invasive ventilation combined with a recombinant Clara Cell secretory protein (CC10) decreased systemic and/or lung inflammation markers such as TNF- α , IL-6 and IL-8 (Shashikant et al., 2005).

There was increased TNF- α production from macrophages obtained from the BALF of neonatal rats exposed to hyperoxia and LPS *in vitro*, compared to room air animals and LPS-exposed cells (Lindsay et al., 2000).

In an interesting study design, neonatal rats (PN6-7) were administered LPS 24h prior, and then ventilated for 8h, with assessments being made soon after or delayed for 48h (Trummer-Menzi et al., 2012). Results revealed the inflammatory reaction was significant with mechanical ventilation plus O₂ exposure (FiO₂: 0.6) leading to increased pro-inflammatory gene expression for MIP-2 (CXCL2, the rodent homolog for IL-8) and a trend towards up-regulation of IL-1 β , IL-6, and TNF- α , mostly at the earlier time point. Lung histology was abnormal at both time points, but MMP-9 expression was significantly increased at the 48h time-point (Trummer-Menzi et al., 2012). In 8d old rats being ventilated, the inflammatory response was modified by pre-exposure to systemic LPS combined with low tidal volume ventilation, but enhanced by exposure to 50% O₂ (Kroon et al., 2010).

Intra-amniotic exposure to 2 different doses of LPS at embryonic day (E)20, followed by hyperoxia-exposure (FiO₂: 0.85) at birth for 1 or 2 weeks in neonatal rats, led to amplification of lung injury at 1 week (Choi et al., 2009). However, the authors noted that 2-weeks of hyperoxia caused extensive lung injury, perhaps, masking the effect of LPS (Choi et al., 2009). Similarly, enhancement of lung injury has been reported by other investigators, by postnatal hyperoxia exposure, with intra-amniotic priming to LPS (Kim et al., 2010; Lee

et al., 2010; Velten et al., 2010), with attenuation of these effects by antenatal betamethasone (Yoo et al., 2013).

Another animal model combined antenatal hypoxia-induced growth restriction (FiO₂ 0.1 from E14 to 18) with PN hyperoxia (FiO₂ 0.7 for 2 weeks) to generate a double-hit mouse model of BPD, which was associated with increased expression of IL-1 β (Gortner et al., 2013).

In a recent report, hyperoxia was noted to differentially contribute to macrophage polarization by enhancing LPS-induced M1 and inhibiting the IL-4-induced M2 phenotype (Syed and Bhandari, 2013). Breast regression protein -39 (BRP-39) absence led to further enhancement of the hyperoxia- and LPS-induced M1 phenotype. In addition, neonatal BRP-39 null mutant mice were significantly more sensitive to LPS plus hyperoxia-induced lung injury and mortality, compared to wild-type mice. This was evident by abnormal histology, abnormal lung morphometry, increased BALF total cell counts and increased BALF protein concentrations, compared to controls. IL-6 and IL-1 β were significantly increased in neonatal BRP-39 null mutant mouse lungs exposed to LPS plus hyperoxia, compared to controls (Syed and Bhandari, 2013).

Overall, experimental data from combination models suggests that exposure to endotoxin antenatally or after birth enhances the inflammatory response to PN invasive ventilation and/or hyperoxia. In a neonatal mouse model, lack of BRP-39 appears to be important for this response, possibly via alteration of the macrophage polarization status (Syed and Bhandari, 2013).

Relevant Human Studies

A large variety of biomarkers have been detected and associated with the development of BPD in tracheal aspirates, as well as blood and urine samples of premature infants (Bhandari and Bhandari, 2009; Bhandari and Bhandari, 2013). The ones that have been implicated in animal models (discussed above) include inflammatory cytokines (IL-1 β , IL-8, IL-10, IFN- γ , MCP-1, MIF, TGF- β , TNF- α), matrix proteins (MMP-9), growth (BLP, HGF, KGF, PTHrP) and vascular factors (VEGF, VEGF-R2). Some inflammatory markers or their downstream targets have been shown to be increased in BPD lung specimens. These include endoglin, CTGF and periostin (TGF- β signaling) (De Paepe et al., 2008; Alapati et al., 2011; Bozyk et al., 2012), IP-9 (CXCL11), Cox-2 and C/EBP homologous protein (CHOP) (IFN- γ signaling) (Harijith et al., 2011; Choo-Wing et al., 2013). VEGF immunostaining was reported to be either decreased (Bhatt et al., 2001) or increased (Lassus et al., 2001), probably reflecting the temporal sequence of VEGF release/production in the neonatal human lung (Meller and Bhandari, 2012). BLP – which is elevated in BPD – has been shown to increase mast cells in the lung (Subramaniam et al., 2003). This may have a correlation with a recent publication of increased mast cells in human lungs with BPD (Bhattacharya et al., 2012). Lower tracheal aspirate concentrations of YKL-40, the human homolog of BRP-39, have been reported with development of BPD in premature neonates (Sohn et al., 2010). Others, such as Angiopoietin 2, have been implicated in human studies (Bhandari et al., 2006; Aghai et al., 2008), but developmentally-appropriate animal

modeling data is lacking, to date. Table 2 lists selective mediators that have a role in the pathogenesis of BPD in animal models that have been associated with preterm human lungs (tracheal aspirates and/or tissue) with BPD.

Summary and Conclusions

While the significantly different responses between adult and neonatal lungs to the postnatal factors discussed here – invasive ventilation (Naik et al., 2001; Ikegami et al., 2003; Copland et al., 2004; Kornecki et al., 2005), local/systemic sepsis (Martin et al., 1995; Lee et al., 2000; Lee et al., 2001; Franco et al., 2002; Alvira et al., 2007), and hyperoxia (Bhandari, 2002; Bhandari and Elias, 2006; Choo-Wing et al., 2007; Bhandari et al., 2008) – are well established, it is also important to be cognizant of the stages of lung development, when comparing animal data for relevance to humans. After all, it is the preterm lung in the late canalicular/ saccular phase of development that is most predisposed to BPD, on exposure to the postnatal factors. This is best exemplified by studies that highlight the differential responses in the various stages of lung development (mostly, saccular vs. alveolar) in animal models (Backstrom et al., 2011).

Figure 1 shows a schema of the relative contribution of 3 major postnatal factors in the inflammation-induced effects that are involved in the pathogenesis of BPD. The animal data would suggest that avoiding postnatal local/systemic infection is more important than avoiding invasive mechanical ventilation to decrease the inflammatory response in developing lungs. In other words, one could speculate that controlling postnatal lung/systemic infection (and thus, inflammation) would be likely to result in a more dramatic decrease in BPD. Once infection rates are relatively low, non-invasive ventilation approaches would provide an additional boost to decrease BPD. Hyperoxia exposure (>40% O₂) appears to exacerbate both infection and invasive ventilation-induced inflammatory responses. Thus, among the environmental factors, modifying the O₂ concentration and/or duration of exposure, in the context of a non-invasive ventilation approach, in conjunction with effective control of PN sepsis would offer the ideal combination to decrease inflammation-induced lung injury and BPD.

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Figure 1. Postnatal inflammation in the pathogenesis of bronchopulmonary dysplasia (BPD)

The 3 major postnatal factors act on the immature lung initiating an inflammatory cascade involving specific cells and cytokines. Local/systemic sepsis is most prominent among the 3 to cause inflammation, followed by hyperoxia and invasive ventilation (as noted by the thickness of the arrows). These, in turn, instigate effects promoting cell death, cell cycle arrest, and abnormal production of growth factors, matrix proteins and vascular factors. Mediators that have been consistently reported to have the above noted effects have been listed in the figure. The net result is impaired alveolarization and dysregulated

vascularization culminating in the pulmonary phenotype of BPD. Ang-2: Angiopoietin 2.
For additional abbreviations and details, please see text.

Table 1

Stages of lung development in various animal models and humans.

Developmental Stage of Lung	Sheep (days)	Rat (days)	Mouse (days)	Baboon (days)	Human (weeks)
Embryonic	E17 – 30	E11 – 13	E9 – 11.5	<E42	3 – 7
Pseudoglandular	E30 – 85	E13 – 18.5	E11.5 – 16.5	E57 – 80	5 – 17
Canalicular	E85 – 110	E18.5 – 20	E16.5 – 17.5	E80 – 140	16 – 26
Saccular	E110 – 120	E20 – PN4	E17.5 – PN5	E140 – 168	24 – 38
Alveolar	E120 – 145	PN4 – 28	PN5 – 28	E168 – 185	32 weeks - PN 3 years*

E: embryonic; PN: postnatal.

* Some data suggest that this occurs up to 8 years of age.

Table 2

Selective mediators involved in the pathogenesis of BPD in animal models and associated with human preterm lungs with BPD.

Mediators	Major effects in relation to BPD	References
Bombesin-like peptide	Increased levels associated with BPD	(Sunday et al., 1998; Subramaniam et al., 2007)
BRP-39	Decreased expression associated with BPD	(Sohn et al., 2010; Syed and Bhandari, 2013)
CTGF	Increased expression associated with BPD	(Wu et al., 2008; Wu et al., 2010; Alapati et al., 2011; Chen et al., 2011)
Interferon gamma	Increased levels leads to the BPD pulmonary phenotype	(Harijith et al., 2011; Aghai et al., 2012; Choo-Wing et al., 2013)
IL-1 β	Increased expression leads to the BPD pulmonary phenotype	(Bry et al., 2007; Backstrom et al., 2011; Bhandari and Bhandari, 2013)
IL-6	Increased expression associated with BPD	(Choo-Wing et al., 2007; Bhandari and Bhandari, 2013)
Keratinocyte growth factor	Lower levels associated with BPD	(Frank, 2003; Franco-Montoya et al., 2009; Bhandari and Bhandari, 2013)
MIF	Lower levels associated with BPD	(Kevill et al., 2008; Sun et al., 2013)
MMP-9	Increased levels associated with BPD	(Tambunting et al., 2005; Chetty et al., 2008; Harijith et al., 2011)
Nitric oxide	Inhaled nitric oxide leads to improvement in pulmonary phenotype of BPD*	(ter Horst et al., 2007; Cole et al., 2011)
Nuclear factor kappa-B	Disruption of signaling pathway associated with BPD	(Alvira et al., 2007; Londhe et al., 2011; Iosef et al., 2012; Bhandari and Bhandari, 2013)
Periostin	Increased expression associated with BPD	(Bozyk et al., 2012)
PTHrP	Disruption of signaling pathway associated with BPD	(Rehan and Torday, 2006; Rehan et al., 2011; Bhandari and Bhandari, 2013)
TGF- β	Increased levels leads to the BPD pulmonary phenotype	(Gauldie et al., 2003; Vicencio et al., 2004; Alejandro-Alcazar et al., 2007; Nakanishi et al., 2007; Li et al., 2011; Bhandari and Bhandari, 2013)
VEGF	Altered ligand and VEGF receptor 2 levels associated with BPD	(Maniscalco et al., 2002; Kunig et al., 2005; Tambunting et al., 2005; Thebaud et al., 2005; Kunig et al., 2006; Bland et al., 2008; Mokres et al., 2010; Meller and Bhandari, 2012; Bhandari and Bhandari, 2013)

BRP: breast regression protein; CTGF: connective tissue growth factor; IL: interleukin; MIF: macrophage migration inhibitory factor; MMP: matrix metallo proteinase; PTHrP: parathyroid hormone related protein; TGF: transforming growth factor; VEGF: vascular endothelial growth factor.

* Not shown consistently in human trials.