Bronchopulmonary dysplasia: pathophysiology and potential anti-inflammatory therapies.

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1 Introduction

Globally, 15 million infants are born preterm each year, representing more than 1 in 10 live births.1 Extremely preterm infants (born before 28 weeks’ gestational age (GA)) are the most vulnerable, most commonly complicated by the chronic lung disease, bronchopulmonary dysplasia (BPD).2 BPD is the only complication of preterm birth for which the incidence is increasing at the same time that all other co-morbidities are decreasing².

There is no cure for BPD. The use of antenatal corticosteroids and exogenous surfactant administration has improved the acute respiratory complications of preterm birth dramatically, but these treatments do not prevent BPD.3, 4 The early application of non-invasive respiratory support is an alternative strategy aimed at preventing BPD but is unsuccessful at reducing its incidence.5

Although BPD is clearly a respiratory disease of prematurity, BPD may not simply be a consequence of lung immaturity. The immature immune system of preterm neonates coupled with the immune modulating effects of factors that commonly accompany preterm birth (e.g. antenatal corticosteroids, intrauterine infection/inflammation and postnatal sepsis),6 may be responsible for aberrant regulation of lung inflammation. Thus, targeting the chronic lung inflammation that underlies BPD with the use of new anti-inflammatory therapies offers the prospect of preventative and reparative treatment for infants with BPD.

2 Bronchopulmonary dysplasia

BPD was first described by Northway in the 1960s as a form of chronic lung disease in moderately preterm infants, characterised predominately by fibrosis.7 Northway attributed the injurious effects of high oxygen levels and airway pressures used in mechanical ventilation as the main causes of the disease7. However, changes in respiratory management strategies for preterm infants has transformed BPD into a disease characterised by more subtle lung abnormalities, including alveolar hypoplasia (fewer and larger simplified alveoli),8, 9 dysmorphic pulmonary vasculature and chronic pulmonary inflammation.10-12 The lungs of infants with severe BPD exhibit a lack of septation within the developing airspace, resulting in reduced surface area for gas exchange in the lungs, hyperconstrictive vasculature that further compromises gas exchange, and impaired surfactant synthesis,13 favouring atelectasis. The National Institute of Child Health and Human Development defines this “New BPD” as the requirement for at least 28 days of supplemental oxygen, with the severity of BPD indicated by the level of respiratory support required at 36 weeks postmenstrual age.14

3 The aetiology of BPD

3.1 Prenatal inflammation

Inflammation within the uterus during pregnancy is a common antecedent of preterm birth that manifests as chorioamnionitis; inflammation of the chorion and amnion. Chorioamnionitis is commonly classified as clinical or histological: clinical chorioamnionitis is diagnosed prior to labour, when women present with symptoms including fever, a tender uterus and preterm rupture of membranes (PROM) with purulent liquor; histological chorioamnionitis is an asymptomatic inflammation of the membranes.15 Histological chorioamnionitis is more common than clinical chorioamnionitis but may be undiagnosed because post-partum histological examination
of the placenta is required. Histological chorioamnionitis complicates between 30-70\% of preterm births with PROM and spontaneous labour, with incidence inversely related to GA. Thus, rates of histological chorioamnionitis exceed 70\% for infants at highest risk of BPD (23 weeks GA).}

Prenatal inflammation alters fetal lung development, with consequences that may be detrimental or beneficial for preterm newborns. Histological chorioamnionitis reduces the risk of respiratory distress syndrome (RDS), likely due to elevated surfactant production in the lungs. However, despite lower risk of RDS, infants exposed to chorioamnionitis may require longer term respiratory support and have higher rates of BPD and persistent pulmonary hypertension of the newborn (PPHN). Although increases or decreases in the incidence of BPD are reported after exposure to chorioamnionitis, the relationship is complicated by low-birth-weight and postnatal events, such as sepsis. The avoidance of prolonged mechanical ventilation in low-birth-weight newborns exposed to chorioamnionitis is associated with decreased incidence of BPD, compared to newborns exposed to chorioamnionitis who received prolonged ventilation. It is unclear whether a prenatal (chorioamnionitis) or postnatal (ventilator-induced) origin of lung inflammation is the greater contributor to the pathogenesis of BPD.

Fetal sheep exposed to inflammation induced by intra-amniotic (IA) injection of lipopolysaccharide (LPS) have lung abnormalities like those observed in infants who die of BPD: alveolar hypoplasia and decreased septation, impaired surfactant secretion (despite increased surfactant protein), and impaired pulmonary vascular development and function. The similarities in the lungs of fetal lambs exposed to inflammation and the pathological features of BPD support a prenatal origin of BPD. Although preterm infants exposed to chorioamnionitis have less RDS, experimental intrauterine inflammation does not reduce the postnatal respiratory support required by preterm baboons and lambs.

3.2 Respiratory Support

Mechanical ventilation initiates an influx of neutrophils and macrophages into the alveoli. These cells produce cytokines, which can disrupt lung development and may be used as biomarkers in serum and tracheal aspirates to identify infants at risk of BPD. Infants who develop BPD have persistently elevated pro-inflammatory cytokines (IL-1β, IL-6 and IL-8) in tracheal aspirates and blood, compared to infants who recover from initial RDS. Preterm infants may become increasingly dependent on respiratory support, which exacerbates pulmonary inflammation, inducing the production of reactive oxygen species (ROS). ROS promote inflammation and epithelial cell death in the lungs via the cleavage, and thus activation, of caspase-1 (Figure 1).

In preterm lambs, 2 hours of mechanical ventilation initiates inflammation within the lungs resulting in similar upregulation of IL-1β, IL-6 and IL-8, the same biomarkers as infants that are ventilated or were exposed to chorioamnionitis. Longer ventilation of preterm lambs (3-4 weeks) increases neutrophil and macrophage infiltration into the lungs and causes non-uniform inflation patterns and abnormal lung vascular development, similar to that observed in infants who die from BPD.

4 Long-term pulmonary consequences of BPD

The long-term sequelae of new BPD are described by very few studies. Autopsies reveal its major pathological features: abnormal alveolar architecture (alveolar hypoplasia) and impaired pulmonary vascular development,
where vessels are distant from airspaces. Pulmonary gas exchange is impaired in 2-year-old infants with BPD compared to non-BPD controls; however, alveolar volume was normal in both cohorts, suggesting a lower alveolar surface area, consistent with an alveolar hypoplasia lung phenotype. Persistent abnormalities in lung parenchyma have long-term functional consequences: 7-to-8-year-olds who had BPD and received surfactant during the perinatal period had lower forced expiratory volume and higher airway resistance, compared to age- and sex-matched controls without BPD, indicative of increased work of breathing.

5 Steroidal approaches to prevent pulmonary inflammation

5.1 Antenatal corticosteroids

Antenatal corticosteroids administered to women at risk of preterm delivery accelerate fetal lung maturation to prevent RDS but do not prevent BPD. Antenatal corticosteroids cause remodeling of the lung parenchyma, which improves gas exchange but results ultimately in fewer, larger alveoli, like the lungs of fetal sheep exposed to prenatal inflammation. Antenatal corticosteroids alter immune activity by suppressing lymphocytes but increasing neutrophils in preterm infants, and altering immune cell function, which may underlie an increased risk of early-onset sepsis.

Antenatal corticosteroid treatment can suppress lung inflammation (induced by IA injection of LPS) in fetal sheep but the reduction of LPS-induced inflammation is transient. Thus, the timing of antenatal corticosteroid administration in humans may influence the lung inflammation that accompanies chorioamnionitis. The optimal timing, dose, and frequency of administration of glucocorticoids to women at risk of preterm birth are unknown. While chorioamnionitis is not a contraindication to the use of antenatal corticosteroids, their interaction likely affects lung development differently to either in isolation; the impact of this interaction on rates of BPD is not known.

Few studies investigate any effect of antenatal glucocorticoids on postnatal ventilator requirements and lung inflammation. In adult animals, pretreatment with corticosteroids reduces ventilation-induced lung injury. Preterm lambs ventilated following exposure to antenatal glucocorticoids have less lung injury and inflammation in comparison to ventilated preterm lambs exposed to antenatal saline. The maturational and anti-inflammatory effects of antenatal glucocorticoids in the preterm lungs appear to be maintained for the initial ventilation period, consistent with lower RDS incidence. However, meta-analyses show that antenatal corticosteroids do not reduce BPD.

5.2 Postnatal glucocorticoids

Postnatal glucocorticoid use peaked in the late 1990s after observations of improved extubation rates in a small trial. Later meta-analyses revealed reduced BPD incidence in preterm infants who received postnatal glucocorticoids, but increased incidence of cerebral palsy and death. Thus, postnatal glucocorticoids are now used reluctantly in infants with intractable BPD: fewer than 10% of preterm infants receive them.

Ventilator management of preterm infants has evolved since the 1990s, aiming for respiratory management with lower airway pressures and inspired oxygen concentrations, and avoidance of prolonged periods of intubation. The practice of more 'gentle' ventilation coupled with significantly less postnatal steroid exposure may result
in infants receiving longer periods of respiratory support than may have been used prior to adoption of these changes in practice. It is unclear whether the increasing BPD incidence is attributable to longer periods of respiratory support and/or the decrease in steroid use. Typically, infants who receive postnatal steroids are those who require prolonged periods of invasive ventilation, indicating a stronger predisposition for BPD development, and complicating any assessment of the impact of postnatal steroids on BPD incidence.

Early studies of postnatal high-dose dexamethasone therapy (~1 mg/kg/day over 42 d)\textsuperscript{49} focused on immediate respiratory outcomes, at the expense of neurological follow-up. Optimal postnatal steroid dosing regimens are undefined, contributing to reluctance for their clinical use. Early administration of lower dexamethasone doses may be safe and effective in infants at high-risk of developing BPD.\textsuperscript{53}

Despite the key role of inflammation in the pathogenesis of BPD, few studies of postnatal steroids include inflammation as an outcome. Small studies describe a reduction in neutrophils\textsuperscript{55, 56} and IL-1 concentration in bronchoalveolar lavage (BAL) of ventilated preterm infants receiving dexamethasone.\textsuperscript{55, 57} Similarly, IL-1β expression is lower in the lungs of preterm lambs receiving a single dexamethasone dose (0.5 mg/kg) immediately before initiation of ventilation compared to placebo controls.\textsuperscript{47} No animal studies assess the ability of postnatal dexamethasone to treat established lung inflammation and injury.

Neurological impairment is associated with high dose postnatal steroids. The risk of cerebral palsy increases by 40\% for every 1 mg/kg increase in dexamethasone dose.\textsuperscript{58} The most premature infants are not the worst affected (despite presumably more immature organ systems); treatment after 33 weeks' postmenstrual age is associated with greatest neurological deficit at follow-up.\textsuperscript{58} Adverse clinical neurological outcomes associated with dexamethasone are consistent with animal studies.\textsuperscript{59-61} Other complications of high-dose postnatal steroid use include stunted growth\textsuperscript{62}, intraventricular haemorrhage (IVH),\textsuperscript{53, 63} gastrointestinal bleeding,\textsuperscript{52} sepsis and hypertension.\textsuperscript{51-53, 64} Combined use of dexamethasone and indomethacin (for closure of the ductus arteriosus) increases the likelihood of gastrointestinal perforation three-fold.\textsuperscript{62}

The DART trial aimed to investigate the ability of a low-dose 10-day tapered course of postnatal dexamethasone (0.89 mg/kg cumulative over 10 days) to prevent BPD in preterm infants born before 28 weeks GA.\textsuperscript{65} The trial was terminated because of low (10\%) recruitment,\textsuperscript{65} but infants who received dexamethasone spent less time intubated on mechanical ventilation; although this did not reach statistical significance. Major disability and cerebral palsy were not different between dexamethasone-treated and placebo-treated preterm infants at 2 years follow-up.\textsuperscript{66}

6 Non-steroidal approaches to prevent pulmonary inflammation

Inflammation associated with BPD involves the activation or over-expression of a number of inflammatory cytokines and pro-inflammatory mediators (Figure 1) providing opportunity for multiple therapeutic targets to prevent lung inflammation in newborn infants. Inhibition of cell signaling that exacerbates inflammation or inhibition of specific pro-inflammatory cytokines in the lungs may prevent BPD progression.
6.1 Suppressing inflammation at various sites: Pentoxifylline, NLRP3 inhibition, IL-1Ra and Adenosine Monophosphate Proteins

6.1.1 Pentoxifylline

Pentoxifylline is a synthetic theobromine derivative, structurally similar to caffeine.\(^{67}\) Pentoxifylline is an immunological agent sometimes used in septic shock due to its ability to lower blood viscosity and improve tissue perfusion.\(^{67}\) Pentoxifylline acts by inhibiting erythrocyte phosphodiesterase, which increases expression of the anti-inflammatory protein adenosine monophosphate protein kinase (AMPK), suppressing neutrophils and pro-inflammatory cytokines\(^{67,68}\) and likely preventing chronic inflammation.

Pentoxifylline is well tolerated by neonates, in whom it is predominately used during sepsis. Pentoxifylline infusion (5 mg/kg/hour for 6 hours) over six days lowers plasma IL-6 and TNF in preterm infants with sepsis, when compared to placebo.\(^{69}\) Infants receiving pentoxifylline had less hypotension and overall improved clinical course,\(^{69}\) highlighting its immuno- and vasculo-modulatory effects. However, comparison of pentoxifylline and dexamethasone in low-birth-weight infants with oxygen requirements >30 % at 72 hours of age revealed neither treatment impacted BPD incidence.\(^{70}\)

It is unclear if pentoxifylline is beneficial for BPD prevention. Current studies are limited by small sample size and poor design (e.g. no blinding is apparent in any of the studies). Hypotension, arrhythmia\(^{67}\) and more rarely, IVH, are noted in adult trials using pentoxifylline.\(^{71,72}\) It is unclear whether pentoxifylline may have particular side effects in infants with BPD. Preclinical studies using pentoxifylline for BPD are rare, and animal studies are warranted to ensure effective translation of pentoxifylline into larger randomised controlled trials (RCTs). One RCT is currently recruiting preterm infants to receive either pentoxifylline or placebo for preventing sepsis and necrotising enterocolitis (NEC), with BPD as a secondary outcome (ACTRN: 12616000405415). No RCTs using pentoxifylline assess BPD as a primary outcome.

In newborn rats exposed to hyperoxia, pentoxifylline administration increased survival and expression of lung vasculogenic markers compared to normoxic controls.\(^{73}\) However, hyperoxia produces a classic fibrotic BPD phenotype, not contemporary BPD. Adult rats subjected to intratracheal hydrochloric acid (HCl) have lung inflammation and develop acute RDS.\(^{74}\) Prophylactic, but not rescue, pentoxifylline reduces HCl-induced lung inflammation and normalises alveolar architecture,\(^{74}\) indicating the timing of pentoxifylline may be important when considering its application in preterm infants.

6.1.2 NLRP3 inflammasome

The NLRP3 inflammasome is part of the innate immune system, responsible for sensing pathogens and initiating inflammation.\(^{75}\) The NLRP3 inflammasome is activated by numerous stimuli,\(^{76}\) forming a complex with other molecules, including procaspase-1.\(^{75,77}\) Procaspase-1 is activated upon formation of the NLRP3 complex and induces IL-1β maturation.\(^{75,77}\) Expression of IL-1β is tightly regulated by activation of the NLRP3 complex.

NLRP3 is implicated in adult ventilator-induced lung injury\(^{78}\) and is upregulated in BAL after 5 hours of ventilation.\(^{78}\) There are no data suggesting activation of NLRP3 in neonatal ventilation, however NLRP3 is part of the innate immune system and should be present at birth.
The NLRP3 inflammasome can be activated by ROS (through injurious ventilation), or TLR activation (through LPS; Figure 1).79 Ventilation using low or high tidal volumes both stimulate NLRP3 and IL-1β expression in mice lungs.78 Overexpression of NLRP3 interrupts alveolar formation and leads to abnormal lung morphogenesis of mice.80 NLRP3-deficient mice are protected from ventilator-induced lung injury and have low IL-1β in their lungs.78, 81

NLRP3 activity is altered in the presence of glucocorticoids and LPS, which may compromise NLRP3 blockade for BPD prevention in preterm infants. Cultured human and mouse macrophages pre-treated with LPS have elevated NLRP3 and IL-1β following exposure to dexamethasone, despite glucocorticoids being anti-inflammatory (dexamethasone alone blocks IL-1β).79 The use of postnatal steroids may be less effective in reducing NLRP3-induced inflammation in preterm infants exposed to both chorioamnionitis and ventilation. Targeting inflammatory inhibition downstream of NLRP3 may be more appropriate in these infants.

Other avenues for NLRP3 suppression include administration of the antidiabetic drug glibenclamide and IL-1 inhibitors [see section 6.1.3]. In ventilated adult mice receiving glibenclamide, compared to placebo, NLRP3 and IL-1β in the lungs was reduced.79 Glibenclamide is used antenatally in mothers with gestational diabetes82 and in neonates with permanent neonatal diabetes mellitus,83 but not in neonatal lung inflammation.

6.1.3 IL-1 receptor antagonist

IL-1 plays a crucial role in inflammation.84 IL-1α and IL-1β enhance their own upregulation and recruit other pro-inflammatory cytokines, including IL-6 and IL-8, to aggravate inflammation.84, 85 Upregulation of IL-1 is apparent in tracheal aspirates of preterm infants who were exposed to chorioamnionitis86 or who have BPD.32 Elevated IL-1 in tracheal aspirates between days 1-3 of life may better predict BPD than GA alone for infants born <27 weeks GA.81

Imbalance between IL-1 and its endogenous IL-1 receptor antagonist (Ra) may be involved in the pathogenesis of BPD. Preterm infants <30 weeks GA have elevated IL-1 and lower IL-1Ra,87, 88 an imbalance that can persist for the first month of life.87 However, levels of IL-1 and IL-1Ra are both higher than non-BPD controls,88 indicating an inability to inhibit IL-1β by IL-1Ra in preterm infants at risk of BPD. Increases in IL-1:IL-1Ra, favouring inflammation, may contribute to prolonged pulmonary inflammation and BPD development. Elevated IL-1 in tracheal aspirates preceded increased macrophage activity between 7-10 days of life in preterm infants who develop BPD, indicating a hyperactive immune system.

The ratio of IL-1:IL-1Ra increases exponentially in tracheal aspirates of baboons delivered at 70 % of full gestation and ventilated for 2, 6 or 14 days,81 suggesting an ongoing inflammatory response. Synthetic IL-1Ra prevents BPD-like lung pathology in mice and rats by reducing pulmonary inflammation and normalising alveolar development.89,90 Synthetic IL-1Ra reduces lung inflammation and suppresses IL-1β in fetal lambs exposed to LPS.91

IL-1Ra has a well-established safety profile for therapeutic use in adults,52 but 100-fold levels of IL-1Ra to IL-1 are required for functional inhibition of IL-1.92 Synthetic IL-1Ra is used in neonatal-onset multisystem inflammatory disease to control relapsing inflammation.93, 94 However, clinical use for BPD prevention is not reported. There are no guidelines for IL-1Ra use in the neonate and only one IL-1Ra (Anakinra) has shown to be
safe in patients less than 2 years old. Other FDA-approved IL-1 inhibitors have not been tested in neonates.

### 6.1.4 Adenosine monophosphate proteins

AMPs are produced by macrophages, neutrophils and epithelial cells in response to inflammation, ROS or infection, and suppress the release of inflammatory mediators. Excess ROS activates AMP to AMPK, which suppress NLRP3. AMPs are elevated in tracheal aspirates of newborn ventilated infants with pulmonary infections compared to ventilated infants without infection but it is unclear if these AMPs are active.

Intratracheal LPS administration in mice increases lung endothelial cell permeability and white cell infiltration, in parallel with AMPK inhibition. Pretreatment, but not rescue treatment, of wild-type mice with an AMPK activator reduces LPS-induced inflammation and injury. Infants exposed to prenatal inflammation have similar lung morphology to that observed in mice exposed to intratracheal LPS; thus these infants may have reduced AMPK activity.

AMPK activity is enhanced by resveratrol in obese patients (still in clinical trials), consistent with inhibition of inflammation in LPS-exposed macrophages from mice in vitro. The use of resveratrol has not been investigated in lung inflammation, but stimulation of innate AMPK may inhibit inflammation and prevent BPD lung pathology in preterm infants.
7 Cell therapies for prevention of pulmonary inflammation

7.1 Mesenchymal stem cells

Mesenchymal stem cells (MSCs) with multi-lineage differentiability are usually derived from bone marrow. MSCs home to sites of injury and possess immunomodulatory functions.\textsuperscript{105} In culture, MSCs can differentiate into alveolar epithelium.\textsuperscript{105, 106} \textit{In vivo}, MSCs engraft in the lung and produce surfactant,\textsuperscript{106} but engraftment rates are low,\textsuperscript{107, 108} suggesting MSCs work via paracrine effects. Prophylactic administration of MSCs mitigate lung injury in mice but are ineffective in repairing established lung injury.\textsuperscript{108}

Meta-analysis of RCTs in adult diseases indicates MSCs are safe;\textsuperscript{109} they do not increase rates of infection, death or malignancy,\textsuperscript{109} despite previous concerns about tumourogenicity.\textsuperscript{110} Transient fever was noted in trials using MSCs\textsuperscript{109} but it is unclear whether this is the consequence of an immune reaction to MSCs.

Clinical studies of MSCs in preterm infants are limited. One trial counted MSCs in BAL and another administered MSCs before BPD diagnosis. The presence of endogenous MSCs within BAL of preterm infants was associated with increased risk of developing BPD.\textsuperscript{111} However, the source of MSCs in BAL was unclear, and may be a result of injured lung epithelium. A phase I dose-escalation trial examined administration of MSCs intratracheally to preterm infants at risk of developing BPD (23-29 weeks GA) who required continuous ventilator support.\textsuperscript{112} Pro-inflammatory cytokines in BAL were lower at day 7 compared to day 3 after MSC transplantation, highlighting the paracrine effects of the cells. Thirty-three percent of preterm infants enrolled developed BPD. This trial targeted infants before BPD developed, who may not have had established lung inflammation. Six patients in the trial developed serious adverse events up to 84 days after MSC transplantation, including pneumothorax, NEC and IVH (< grade 3). The majority of adverse events occurred in infants receiving the highest MSC dose (20 million cells/kg).

The origin or handling of MSCs may influence their therapeutic potential. The yield of cord-blood-derived MSCs is low and MSCs need to be expanded for adequate cell numbers for delivery to a patient, but culturing induces ageing.\textsuperscript{113} Cultured, MSCs have weaker immunomodulatory properties \textit{in vitro} compared to primary MSCs,\textsuperscript{113} potentially compromising the therapeutic effects of MSCs. Additionally, MSC expansion often requires growth factors (containing animal products),\textsuperscript{112} or plating onto a glycoprotein-rich fibronectin matrix.\textsuperscript{114}
media, growth factors or matrices used with MSCs, and whether these alter MSC function, must be considered when proposing MSC transplantation in preterm infants.

7.2 Human amnion epithelial cells

The amniotic membrane is the innermost placental membrane surrounding the fetus,\textsuperscript{115} made up of a single layer of cuboidal columnar epithelial cells.\textsuperscript{115} The amnion predominately provides the developing fetus with protection, but also produces growth factors, cytokines, prostaglandins and erythropoietin.\textsuperscript{116} The amniotic epithelium is formed before gastrulation, and thus is pluripotent even at term gestation.\textsuperscript{115}

Amniotic membranes were first used over a century ago as biological dressings for skin wounds.\textsuperscript{117} This practice continues, highlighting the safety of these cells as a therapeutic for human disease.\textsuperscript{118} Unlike MSCs, isolation of human amnion epithelial cells (hAECs) from a single placenta yields enough cells for administration to multiple patients. Primary hAECs may be used immediately after isolation, obviating concerns about cell manipulation.

Human AECs do not express telomerase,\textsuperscript{119} and have low tumourigenicity.\textsuperscript{120} Rejection does not occur when hAECs are administered to humans\textsuperscript{121} or other animals,\textsuperscript{119, 122} likely due to low HLA class II expression.\textsuperscript{122, 123} Thus, hAECs can be applied without concerns of tumorigenesis or rejection.

Human AECs reduce lung collagen and fibrosis in mice,\textsuperscript{124, 125} up to 14 days after bleomycin insult.\textsuperscript{125} Hyperoxia-exposed mice treated with hAECs have increased expression of vascular endothelial growth factor receptor and angiogenin1 in their lungs and this correlates with normalized pulmonary vasculature.\textsuperscript{126} Human AEC administration normalises alveolar structure in hyperoxia-exposed mice,\textsuperscript{126} LPS-exposed fetal sheep\textsuperscript{127} and in fetal and preterm sheep following injurious ventilation.\textsuperscript{128, 129} Thus, hAECs prevent BPD-like lung pathology, independent of the model, and this reduction in BPD-like lung pathology is consistently accompanied by reduced lung inflammation.

The immunomodulatory effects of hAECs are demonstrated by their ability to downregulate pro-inflammatory cytokines, IL-6,\textsuperscript{128, 130-132} IL-1α and IL-1β,\textsuperscript{131} and upregulate anti-inflammatory cytokine IL-10.\textsuperscript{129} However, hAECs increase total immune cell numbers in the lungs of LPS-exposed fetal sheep and mechanically ventilated preterm lambs,\textsuperscript{127, 129} demonstrating the ability of hAECs' to augment inflammatory cell recruitment but seemingly without proinflammatory effects. IL-10 has a role in activating M2 pro-reparative macrophages and opposing differentiation of pro-inflammatory M1 macrophages,\textsuperscript{133} and is upregulated following hAEC administration.\textsuperscript{129, 132}

Indeed, lung and liver macrophage activity shifts from a predominately M1 to M2 phenotype after hAEC administration in bleomycin-exposed mice.\textsuperscript{134, 135} Similar to MSCs, hAECs modulate the immune system and prevent BPD-like lung pathology, likely via paracrine effects.

The safety of hAECs has been explored in an unpublished phase I trial of hAECs in infants with intractable BPD (ACTRN: 12614000174684). Subsequent trials are required to determine the appropriate dose of hAECs and when to administer hAECs in preterm infants with BPD.
8 Final comments and future research

BPD remains a major cause of morbidity and mortality in preterm infants. Historically, therapies like postnatal steroids have been used without appropriate preclinical data regarding safety and long-term outcomes. Pharmacological inhibitors of inflammation, such as IL-1 inhibitors, pentoxifylline and AMP activators, are promising therapies for pulmonary inflammation but are in preclinical stages. Cellular therapies, arguably, have more preclinical evidence surrounding their use for BPD and are approaching RCTs. However, it is unlikely that a one-drug-fix-all approach to BPD will eventuate. Likely, the most beneficial outcomes for infants developing, or who have developed, BPD will be achieved with combined therapies. If an infant is unresponsive to a therapy (e.g. steroids or hAECs) within several days an alternative therapy should be considered. Future studies will need to consider interactions between therapies in preterm infants if tailoring therapies for neonates with BPD is necessary.

Educational aims

The reader will appreciate:

- The incidence of BPD is increasing, despite advancements in clinical care of preterm infants.
- Maturational agents to improve lung architecture, including antenatal glucocorticoids and surfactant therapy, have not improved BPD incidence.
- Anti-inflammatory therapies may be beneficial over maturational agents in preventing BPD incidence.
- Glucocorticoids reduce BPD incidence but have poor neurological outcomes. Glucocorticoid use in the newborn requires significant optimisation.
- Cellular therapies likely modulate lung inflammation associated with BPD, without adverse outcomes.

Future research directions

BPD is a complex disease involving aberrant regulation of lung inflammation. The use of preclinical, translational studies to identify or optimise pre-existing therapies, such as postnatal steroids or IL-1Ra, need to be conducted and compared to less conventional cellular therapies. Future studies should aim to encompass the inflammatory, structural and vascular complications of BPD for the best outcomes in preterm infants.

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