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REVIEW

The immunological link between neonatal lung and eye disease

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Abstract

Bronchopulmonary dysplasia (BPD) and retinopathy of prematurity (ROP) are two neonatal diseases of major clinical importance, arising in large part as a consequence of supplemental oxygen therapy used to promote the survival of preterm infants. The presence of coincident inflammation in the lungs and eyes of neonates receiving oxygen therapy indicates that a dysregulated immune response serves as a potential common pathogenic factor for both diseases. This review examines the current state of knowledge of immunological dysregulation in BPD and ROP, identifying similarities in the cellular subsets and inflammatory cytokines that are found in the alveoli and retina during the active phase of these diseases, indicating possible mechanistic overlap. In addition, we highlight gaps in the understanding of whether these responses emerge independently in the lung and retina as a consequence of oxygen exposure or arise because of inflammatory spill-over from the lung. As BPD and ROP are anatomically distinct, they are often considered discreet disease entities and are therefore treated separately. We propose that an improved understanding of the relationship between BPD and ROP is key to the identification of novel therapeutic targets to treat or prevent both conditions simultaneously.

Keywords: bronchopulmonary dysplasia, disease association, inflammation, neonatal immune system, retinopathy of prematurity, supplemental oxygen

INTRODUCTION

Globally, it is estimated that 15 million neonates are born preterm (< 37 weeks of gestation) each year.[1] Many preterm neonates are at risk of respiratory, immune, neurological and cardiovascular disorders, which are leading causes of mortality in children under the age of 5.[2] A primary life-saving intervention in the neonatal intensive care unit (NICU) is the use of supplemental oxygen therapy for respiratory support; however, prolonged exposure places preterm infants at risk of developing complications.[3] The lungs and retina, in particular, are vulnerable to oxygen exposure, and this can be compounded by a range of antenatal and postnatal insults, such as infections.[4–7] As a consequence, 75% of preterm neonates with a

birthweight of < 1000 g are at risk of developing the respiratory disorder bronchopulmonary dysplasia (BPD),[8] while 82% of preterm neonates born at a similar birthweight of < 1000 g are at risk of developing the eye disorder retinopathy of prematurity (ROP).[9] Importantly, it is estimated that 60–71% of those with BPD have comorbid ROP, suggesting a potential association between these two disorders.[10,11] BPD and ROP also share several other risk factors including low gestational age, prenatal and postnatal inflammation, maternal smoking, the use of mechanical ventilation, patent ductus arteriosus and necrotising enterocolitis.[12,13] Similarities in predisposing factors for the two disorders suggest that a mechanistic overlap may exist between BPD and ROP.

AN ASSOCIATION BETWEEN BPD AND ROP

In the present day, premature babies admitted to the NICU are given an ensemble of antenatal corticosteroids and postnatal surfactants, along with advanced respiratory support, to limit severe respiratory sequelae observed in infants from decades ago.[14,15] Despite these best efforts, supplemental oxygen therapy delivered in early neonatal life continues to interrupt normal lung development and gives rise to lung pathology referred to as BPD.[16] The disease is typified by alveolar simplification and enlargement as a result of interrupted secondary septation and abnormal microvascular development.[17] Parenchymal changes in combination with lung immaturity hinder gas diffusion.[18] Meanwhile, the oxygen insult also promotes the secretion of inflammatory cytokines from activated resident immune cells and structural cells in the alveoli which can exacerbate the structural deterioration of the lung.[19] Following the cessation of supplemental oxygen therapy, type II alveolar cells have been observed to transdifferentiate into type I alveolar cells to repair the scaffold of the alveoli; however, this process is typically incomplete and the lungs remain altered.[20]

Retinopathy of prematurity is also a disease associated with the interruption of normal development,[7] accompanied by local and systemic inflammatory changes.[21,22] ROP chiefly affects the vasculature of the inner retina. Normal blood vessel growth in the retina becomes arrested during supplemental oxygen exposure in a process known as 'vaso-obliteration'. The weaning from oxygen therapy results in inner retinal hypoxia because of the underdeveloped retinal circulation driving the 'vasoproliferative' phase of ROP.[7] The newly formed vascular networks are prone to rupture, causing vitreous haemorrhage, and predispose to fibrosis and retinal detachment.[23] Simultaneously, activated immune cells and pro-inflammatory cytokines damage the developing vasculature.[21,24] The compromised state of the retinal pigmented epithelium (outer blood–retinal barrier) and choroid may also allow for myeloid cells to traffic into the retina.[25,26] Unfortunately, the respiratory and ocular sequelae of these diseases are not isolated to the neonatal period but can persist throughout adolescence and into adulthood, with potentially severe consequences, including the development of chronic obstructive pulmonary disease[18] and blindness (Figure 1).[27]

Our group recently described a preclinical model of coincident BPD and ROP, which has, for the first time, enabled the investigation of these shared pathological processes.[28] We identified that inflammatory factors, in addition to angiogenic and oxidative processes, are essential to the pathogenesis of ROP and BPD.[28] In addition, our study showed that in the absence of an identifiable respiratory infection, inflammation increased in the lung following hyperoxia exposure in early infancy, as indicated by the upregulation of *interleukin (IL)-1*β, *tumor necrosis factor (TNF)-*α and *IL-6* transcripts as well as increased leukocyte numbers in bronchoalveolar lavage fluid (BAL) which persisted into adulthood, likely contributing to the long-term histological damage observed in the lung. The inflammatory pattern observed in the neonatal lung paralleled the response that has been reported in the ROP-affected retina.

ADDRESSING AN UNDERSTUDIED AREA OF NEONATAL RESEARCH

It is clear that the developing lung and retina is adversely affected by fluctuations in oxygen tension. However, the connection between the immune responses that occur in these organs remains underexplored. Given the invasive nature of current therapies to treat ROP, which include laser and cryotherapeutic ablation of ischaemic retina, and the limited efficacy of therapies to prevent BPD such as corticosteroids,[29] an improved understanding of underlying

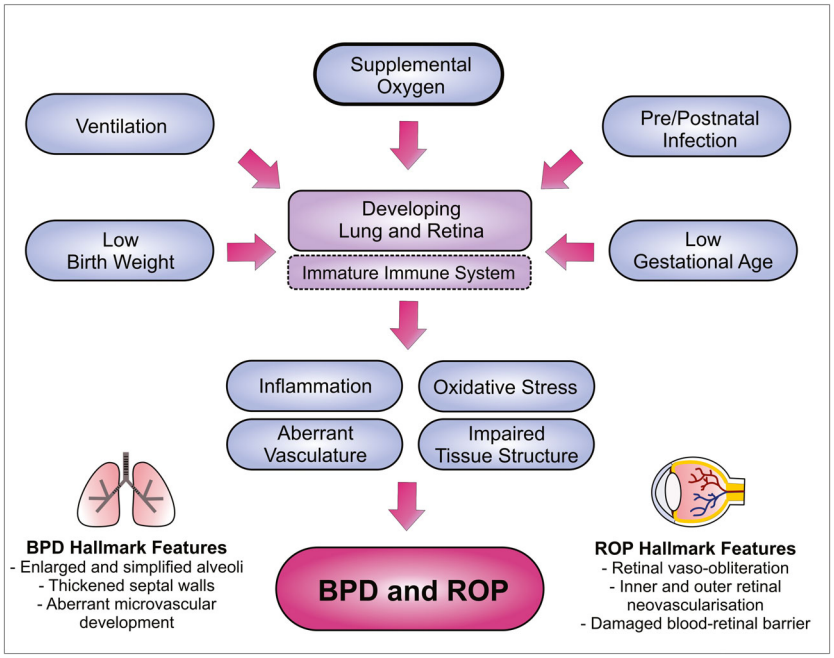

Figure 1. Clinical traits that impact on the developing lung and retina in bronchopulmonary dysplasia (BPD) and retinopathy of prematurity (ROP). Supplemental oxygen is the most potent initiating factor, followed by low birthweight, low gestational age, ventilation and pre/postnatal infection. An immature immune system may also contribute. Clinical traits in common with both conditions can induce inflammation and oxidative stress, impair the blood vasculature and damage the lung and retinal tissue structure leading to the development of pathological hallmarks of BPD and ROP.

pathological mechanisms may inform the development of novel therapies. Furthermore, the discovery of shared disease pathways in the eye and lung may provide a unique opportunity for the development of a unified treatment strategy for both diseases. As the involvement of specific angiogenic factors in vascular growth and maldevelopment in BPD and ROP has been recently reviewed in detail,[30] the goal of this review is to highlight the key cells and mediators of the innate immune system that are common to the pathological development of BPD and ROP, and indicate limitations in the current literature on the immune contributions to both conditions (Figure 2). In doing so, we shed light on opportune areas for future research.

IMPAIRED CELLULAR IMMUNITY IN PRETERM INFANTS

At birth, the neonatal immune system is in the early stages of development, maturing rapidly within the first 3 months of life.[31,32] During this period, neonates are at an increased risk of acquiring microbial infections. Infections were reported to account for approximately 30% of deaths in a cohort of 1109 preterm infants admitted to NICUs, only 15% less than the proportion of deaths because of respiratory distress, signifying the lethality of infection when acquired in the newborn period.[2] From 32 weeks of gestation to birth, maternal IgG antibodies transferred via the placenta provide passive protection to the foetus and, postnatally, maternal immunoglobulins in breastmilk provide similar protection to the newborn.[33] This maternal–foetal antibody transfer increases with foetal age[31,34]; however, the degree of protection conferred is insufficient against some pathogens, such as poliovirus and coxsackievirus.[35,36]

As passive immunity wanes, term infants rely on the innate immune system to provide the critical first line of defence against infection, as the adaptive immune system matures.[37,38] The cellular mediators of innate immunity include monocytes, macrophages, natural killer cells, neutrophils, eosinophils, innate lymphoid cells (ILCs) and dendritic cells (DCs). However, in the neonatal period, these innate immune cells are fewer in number and have reduced function compared to those of adults.[39] In newborns, humoral immunity is polarised towards a protective phenotype to

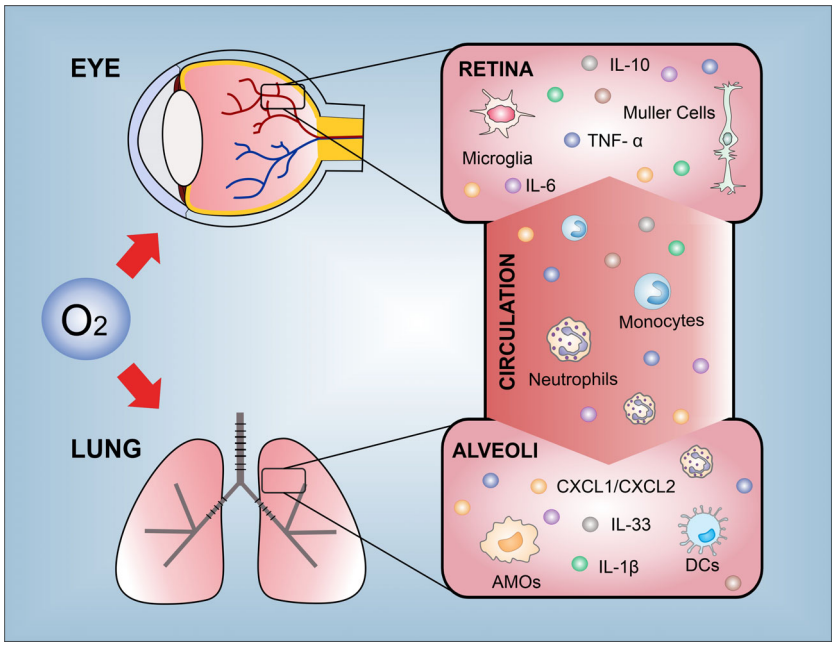

Figure 2. Potential disease crosstalk between the lung and eye in bronchopulmonary dysplasia (BPD) and retinopathy of prematurity (ROP). Supplemental oxygen (> 21% O_2) initiates the activation of tissue-resident cells and the release of mediators that recruit immune cells to the neonatal eye and lung, contributing to the development of ROP and BPD, respectively. Numerous cytokines and chemokines are upregulated in the eye and lung as well as in the neonatal circulation in response to a high oxygen environment. We propose that immune crosstalk between the lung and eye, via the movement of mediators through the circulation and potentially via spill-over from the lung, plays an important role in the shared pathogenesis of these diseases. It is not known whether inflammatory overflow from the eye has an influence on lung disease. The cytokines presented in the figure are the mediators that have been discussed in detail within the main body of the review. AMOs, alveolar macrophages; CXCL, chemokine (C-X-C motif) ligand; DCs, dendritic cells; IL, interleukin.

prevent tissue damage triggered by inert environmental antigens.[31,40] Preterm infants demonstrate even poorer immunological competence than their term counterparts, because of immaturity of both the innate and adaptive immune systems. All newborns have low numbers of lymphocytes, with maturation of adaptive immunity occurring in a pathogen-dependent fashion post-birth; however, premature infants have lower total numbers of lymphocytes than those born at term. Newborns are also protected by circulating maternal IgG, which is transported *in utero* across the placenta. As this transfer rises with foetal age, neonates who are born at less than 32 weeks' gestation have very low levels of maternal IgG.[41] In addition, premature infants have reduced numbers of innate leukocytes, with impaired phagocytic activity and a reduced ability to produce cytokines.[34] In the face of insults to the neonatal immune system brought on by birth and the exposure to the environment, it is plausible that immunological immaturity might predispose to the development of BPD and ROP and to the progression of both diseases.

INNATE IMMUNE CELLS

Macrophages as key perpetrators in BPD and ROP

The key cellular sources of inflammatory cytokines in the lung and eye are alveolar and retinal macrophages, respectively. There is substantial evidence to implicate activated macrophages as the primary leukocytes involved in the development of BPD and ROP.[22,42] In the lungs, alveolar macrophages (AMOs) are found at the airway epithelial and endothelial interface.[43] As a result, AMOs can directly sense insults such as pathogens, toxins or foreign matter that reach the airspaces, and they remove them by phagocytosis to maintain alveolar homeostasis.[43] Oxygen exposure causes oxidative damage and activation of these cells leading to the upregulation of pro-inflammatory cytokines and chemokines.[44] Some of the cytokines produced include IL-1β, TNF-α and IL-6, which are elevated in the BAL of preterm infants with BPD.[45,46] In the retina, microglia serve as central nervous

system-resident phagocytic cells akin to tissue-resident macrophages. These cells are derived from the yolk sac and are widely distributed in the retina,[47] where they perform a surveillance role. Microglia are activated by a range of injurious stimuli, including local changes in oxygen tension.[48,49] As these cells are located close to the blood vessels of the developing retina, microglia can respond immediately to stimuli activating an efficient immune response to promote the repair of damaged neurons or retinal vessels.[48,50,51] Typically, AMOs and microglia assist in the development of the vasculature in their resident organs during foetal development. Under homeostatic conditions, AMOs ordinarily suppress inflammation, and during foetal lung development, they contribute to tissue remodelling by clearing apoptotic cell debris and surfactants and participate in angiogenesis during the saccular and alveolarisation stage.[52,53] Similarly, within a healthy retina, microglia have a quiescent, immunosurveillant phenotype-secreting neurotrophic factors such as glial cell-derived neurotrophic factor and brain-derived neurotrophic factor, which protect and regulate the survival of retinal photoreceptors and ganglion cells.[48]

During infection, inflammation or tissue injury, tissue-resident macrophages and infiltrating blood monocytes can transition into pro-inflammatory macrophages.[52,54] In steady-state room air conditions, mice have AMOs that express a unique set of quiescent markers, whereas those exposed to hyperoxia have AMOs with a pro-inflammatory phenotype.[55,56] Evaluation of the AMOs extracted from preterm rabbits exposed to 95% oxygen overnight showed a significantly greater expression of *IL-1*β and *IL-8* mRNA, a hallmark of pro-inflammatory polarisation, than in AMOs from term rabbits.[56] Therefore, the transition of AMOs to a pro-inflammatory state during oxygen exposure could also be an important contributor to perturbed alveolar development. In the mouse retina, hypoxia induces pro-inflammatory cytokines such as interferon-γ and TNF-α, which trigger microglia to adopt a pro-inflammatory phenotype.[54,57] The consequence of microglial activation in the retina is the upregulation of TNF-α, IL-1β, IL-17 and nitric oxide, which directly activate Müller glial cells that ordinarily provide support for retinal neurons.[24,58] As a result, retinal cells, including Müller cells and retinal ganglion cells, upregulate vascular endothelial growth factor (VEGF) production, triggering retinal neovascularisation and hyperpermeability as seen in human ROP.[54,57,59] Müller cells and microglia enter into a positive feedback loop, induced by high levels of pro-inflammatory cytokines. The resulting amplification of the immune response within the retina can lead to the breakdown of the blood–retinal barrier.[7,57,60] The processes that mediate the transition of microglia from a quiescent into an activated state in ROP are incompletely understood. However, microglia are viewed as central players in exacerbating disease and are therefore important targets for ROP therapeutics.[61]

Myeloid cell involvement in neonatal disease

Neutrophils are rapid responders during acute inflammation caused by infection or exposure to harmful agents.[62] In BPD, neutrophils are often found together with activated AMOs in the BAL of preterm neonates with the disease.[63] In ROP, the transmigration of neutrophils into the retina is not well characterised, despite the upregulation of neutrophil-associated chemokines, such as CXCL1, in the retinas of ROP-affected neonatal mice.[64] Another cellular subset that links the innate and adaptive immune system is DCs. Limited research suggests that DCs are involved in prenatal lung development and have been found in the conducting airways and peripheral parenchyma from the late canalicular stage.[65] Lung tissue from at-risk preterm infants or those with BPD exhibits larger numbers of DCs compared to infants without lung disease, marked by expression of DC-SIGN (CD209).[65] A dramatic increase in DC numbers adjacent to pulmonary vessels has also been observed in infant lungs with evidence of antenatal infection, suggesting a role for these cells in the disease process.[65] Therefore, it is likely that these cells are competent to respond to environmental stimuli early in development, but whether early priming of DCs has long-term consequences for lung immune responses remains to be elucidated. It is conceivable that the increased susceptibility of preterm neonates to viral respiratory infections may be associated with changes in DC function.[66] In the steady-state retina, novel 33D1^{+} DC populations have been identified; however, the precise role of these DCs in ROP is currently undetermined.[67]

Adaptive immune cell subsets in the neonatal lung and retina

The adaptive immune system of the neonate is immature and little is known of its role in BPD and ROP. B cells in premature infants may be functionally distinct from those of term infants, as indicated by the augmented antibody responses to vaccination observed in preterm infants.[68,69] An innate subset of B cells, known as B1 cells, is found in greater numbers in the cord blood at birth and is a source of antibodies that are produced spontaneously.[70,71] It is currently unknown whether B1 cell proportions differ between preterm and term infants. However, another population of B cells, B regulatory cells, have recently been identified and have been shown to have immunoregulatory function in response to RSV infection.[72] In the circulating blood of term and preterm infants, these B-cell subsets were increased but diminished with age.[72] It would therefore be of interest to explore how these B-cell subsets are augmented in the lungs and retinas of preterm infants affected by BPD and ROP.

With respect to T-cell populations, infants that develop BPD show reduced numbers of $CD4^+$ T cells in blood compared to infants without the disease, but have a normal $CD8^+$ T-cell population.[73] Preterm babies have also been reported to have lower $CD31^+CD4^+$ T cells expressing IL-8 than their term counterparts, which may render preterm infants vulnerable to respiratory infections because of the key role of IL-8 as a neutrophil chemoattractant.[74] Incidentally, in an experimental model of ROP known as oxygen-induced retinopathy (OIR), immunosuppressive regulatory T cells have been shown to expand in peripheral lymphoid tissue and migrate to the ischaemic retina. Therapeutic expansion of this cell type using two approaches – either IL-2/anti-IL-2 delivered at PN5, PN6 and PN7 in the OIR model or adoptive transfer of purified regulatory T cells administered at PN7 and PN12 – led to a substantial reduction in vaso - obliteration, neovascularisation and haemorrhage.[21] While little is known about regulatory T cells in BPD, one study has shown that infants who develop moderate BPD had reduced numbers of $CD4^+$ T cells and regulatory T cells.[75] While there is mounting evidence to tie the immature adaptive immune system to BPD and ROP, further research is needed, especially pertaining to T- and B-cell involvement in ROP.

IMMUNE MEDIATORS ARE IMPORTANT IN BPD AND ROP PATHOGENESIS

Immune cells, as well as the epithelium and endothelium, release a plethora of cytokines which can initiate and modulate the immune responses to pathogens and injury. A number of these immune mediators have been directly implicated in the pathogenesis of ROP and BPD. In the following sections, we highlight several key cytokines that may facilitate the immunological link between these two diseases.

IL-1β is a pathogenic mediator of BPD and ROP

Cytokines such as IL-1β are integral to the initiation and progression of an inflammatory response and are secreted by innate immune cells including macrophages, monocytes, DCs and neutrophils. IL-1β has also been shown to play a key role in the pathogenesis of BPD and ROP.[46,76] Increased IL-1β levels have been found in the tracheal aspirates and serum of preterm infants with BPD.[45,77] Furthermore, animal studies have shown that transgenic overexpression of IL-1β led to decreased VEGF protein in the lung and demonstrated dysmorphic pulmonary capillary formation, a hallmark of BPD.[78] In addition, IL-1β overexpression was observed to result in disrupted deposition of α-smooth muscle actin and elastin in alveolar septal walls, both of which are integral to normal lung alveolarisation.[78] These results suggest that excess IL-1β is detrimental to postnatal lung morphogenesis. In keeping with this, the development of alveolar hypoplasia following 8 days of hyperoxia can be partially reversed by blocking IL-1β using a receptor antagonist.[79] It is possible that IL-1β expression in the lung could have adverse effects on AMOs, hindering their ability to resolve inflammation, which subsequently results in distended airspaces in BPD.[80] The deleterious effect of IL-1β lies in its ability to propagate and sustain an inflammatory cascade.

Although elevated cord blood levels of IL-1β in humans have been associated with an increased risk of developing BPD, a link with susceptibility to ROP has not been established.[81] Nonetheless, the importance of IL-1β in the pathogenesis of

ROP has been highlighted in several studies reporting its cytotoxic effects on the neonatal retina[25,57,58] and also its ability to trigger microvascular degeneration indirectly via the release of Sema3A, an inhibitor of angiogenesis.[58] Further to this, it has also been shown that IL-1β has a sustained negative impact on the choroidal endothelium leading to its involution.[25] Although ROP chiefly involves the inner retina, outer retinal dysfunction is being increasingly recognised as a consequence of ROP and this may account for reduced visual function.[82–85] For example, damage to the retinal pigment epithelium (RPE) and photoreceptor layers of the outer retina triggered by oxidative stress can lead to poor dark adaptation, abnormal cone function and retinal depigmentation.[25] In a rat model of OIR, intravitreal injection of IL-1R antagonist to inhibit IL-1β led to relative preservation of the RPE and choroid, reduced outer retinal hypoxia and improved retinal function.[25] In the lungs of mice exposed to high oxygen using the concurrent model of BPD and ROP, an increase in *IL-1β* mRNA expression was observed at a time point that corresponds with IL-1β expression in the retina in a rat model of ROP,[28] suggesting upregulation of a common inflammatory mediator in both conditions. Overall, these studies indicate that IL-1β expression in the lung and retina may serve as a mechanistic link between BPD and ROP.

Dysregulation of IL-6 and TNF-α is associated with BPD and ROP

The upregulation of IL-1β can promote the expression of a wide variety of pro-inflammatory cytokines in response to hyperoxia in the postnatal period, including TNF-α and IL-6, which are often produced sequentially. TNF-α and IL-6 are involved in the promotion of angiogenesis, the initiation of apoptosis, the activation of immune cells, including macrophages, DCs and T cells, and the induction of hepatic acute-phase response proteins.[86–88] In combination, these processes may also promote a cytokine-driven immunological link between the two diseases.

In the BPD lung, IL-6 and TNF-α are produced by macrophages, neutrophils, endothelial cells and type II pneumocytes.[88,89] Preterm infants at risk of developing BPD had elevated TNF-α and IL-6 as well as IL-8 in tracheobronchial aspirate fluid, which correlated with lower gestational age and duration of supplemental oxygen exposure.[90] In tracheal aspirates from infants who subsequently developed BPD, concentrations of IL-6 and TNF-α were higher on the second and third day of life than in a control infant group with respiratory distress syndrome that did not go on to develop BPD.[90] Another study assessing daily IL-6 and TNF-α cytokine levels in tracheobronchial fluid from premature infants for 4 weeks found that those that progressed to BPD had elevated levels of these pro-inflammatory cytokines for 14 days of a 28-day assessment period,[91] suggesting that TNF-α and IL-6 are involved in the initial immune response in the neonatal lung during respiratory support. TNF-α and IL-6 have been associated with endothelial cell damage in the lungs, which can lead to vascular leakage and pulmonary oedema.[90,92] TNF-α and IL-6 have similar roles in the eye, promoting vascular hyperpermeability and pathological angiogenesis.[60,93]

In the ROP-affected retina, IL-6 and TNF-α are produced by retinal microglia/macrophages, Müller cells and retinal ganglion cells.[94] Early increases in retinal TNF-α and IL-6 are associated with the development of severe ROP and, in combination with IL-1 and IL-8, can promote angiogenesis.[95] Cord blood samples demonstrated an increase in TNF-α concentration 24 h post-birth, while IL-6 serum levels were elevated immediately after birth and then at 5–12 weeks postnatal age in infants receiving treatment for ROP.[95] In addition, cultured Müller cells exposed to activated microglia exhibited elevated expression of *IL-6* and *IL-1β* mRNA. Supernatants from these co-cultures also contained elevated levels of *iNOS* and *CCL2*. These findings indicate that these pro-inflammatory cytokines drive a positive feedback loop between Müller cells and microglia, which likely contributes to the pathogenesis of ROP.[96–98] In addition, IL-6-deficient mice given subcutaneous infusion of angiotensin II over 2 weeks to induce retinal vascular inflammation showed a reduction in leukocyte adhesion, oxidative stress as well as retinal and choroidal vascular remodelling.[93] Similarly, the inhibition of TNF-α in a mouse model of ROP reduced the development of pathological retinal neovascularisation.[60,99] Therefore, local expression of IL-6 and TNF-α can exert damaging effects on the retina and lung. Interestingly, IL-6 has also been recognised to have anti-inflammatory effects and while its production by fibroblasts, endothelial cells and epithelial cells in the lung can be promoted by TNF-α, IL-6 in turn can also inhibit the synthesis of TNF-α,

ameliorating its pro-inflammatory activity.[100] However, whether this immunoregulatory capacity of IL-6 is impaired in the neonatal lung during an oxygen insult is not clear, and it is also unknown whether this mechanism occurs in the retina. What remains to be elucidated is whether systemic inflammatory mediators contribute to this local inflammatory milieu. In this case, it is plausible that inflammation originating in the lung as a consequence of high levels of inspired oxygen may act as a driver of ROP.

Neutrophil chemoattractants contribute to BPD and ROP

In humans, neutrophil recruitment to inflamed tissues is governed by the chemoattractant IL-8/CXCL8, mediating its actions via two receptors CXCR1 and CXCR2.[88,101] Rodents lack CXCL8; instead, KC/CINC-1/CXCL1 and MIP2/CXCL2/3 serve as the major chemokines responsible for neutrophil recruitment, and both bind to CXCR2. These chemokines are often used as surrogate measures of neutrophil-mediated inflammation. Lungkine/CXCL15 is another mouse neutrophil chemokine although it has no human homologue. It is expressed by lung fibroblasts and epithelial cells and functions as a chemotactic factor for neutrophils in the developing lung in response to inflammation.[102,103] Using a mouse hyperoxia model to simultaneously induce eye and lung disease reflective of ROP and BPD, we have confirmed the importance of this factor in lung development.[28] *CXCL15* mRNA expression was elevated following oxygen exposure during the alveolar stage of development, and this correlated with neutrophil influx.[28] In hyperoxia models of BPD, blockade of CXCL1 and CXCL2 has been shown to ameliorate alveolar septal wall thickening and reduce neutrophil trafficking into the lung.[19] CXCL1 and CXCL2 expression was predominantly increased in AMOs and in the alveolar epithelium of mice exposed to 95% oxygen for 8 days.[19] Similarly, in neonatal rabbit pups, exposure to high oxygen for 10 days promoted an increase in *CXCL1* mRNA and protein expression in the BAL, which correlated with increased numbers of neutrophils in the airspaces.[104] In another study, rats infused with anti-CXCL1 antibodies exhibited better lung function than control rats following exposure to 95% oxygen.[105] Alveolar volume and surface density were also conserved, suggesting an overall preservation of lung anatomy and function with CXCL1 inhibition.[105] Further research has demonstrated that mice deficient in CXCR2 chemoattractant receptors exhibited significantly reduced neutrophil recruitment, reduced lung injury and better survival outcomes in a BPD model.[106]

There is evidence to suggest that a systemic increase in CXCL8 in preterm infants receiving respiratory support contributes to a pro-inflammatory state that predisposes to both ROP and BPD.[107] Furthermore, in a mouse OIR model, increased *CXCL1* mRNA expression and protein levels were observed in the retina during the vasoproliferative stage of the disease.[64] CXCL1 was localised on nerve fibres and radial Müller cell processes.[64] In another study, retinas isolated 1 day after cessation of high oxygen exposure in an OIR model (PN13) showed an increase in protein levels of CXCL1, along with other granulocyte–macrophage lineage-specific chemoattractants such as CCL2/3/4 and CXCL12, compared to normoxic and hyperoxic postnatal day 12 retinas.[108] These studies suggest that the switch to lower concentrations of inspired oxygen, and thus blood oxygen levels, may be a key factor for the induction of neutrophil chemoattractants in the retina in ROP.

Just as CXCL1 has been shown to perturb lung development in BPD, the factor has also been shown to impair normal retinal development. Specifically, in a rat model of OIR, CXCL1 promoted rat retinal ganglion cell apoptosis, likely via reducing levels of Bcl-2 while enhancing Bax and caspase 3 levels.[109] Despite similar elevations in CXCL chemokines during disease progression in BPD and ROP, the extent to which these chemokines are interconnected in the pathogenesis of these diseases is not clear. Side-by-side comparisons of chemokine regulation in lung and retina in response to changes in oxygen exposure will inform the understanding of the dynamic control of these inflammatory factors in each tissue. Interestingly, neutrophil infiltration in the retina is not well described in ROP, despite the known induction of neutrophil chemoattractants and warrants further investigation.

IL-10, a contrasting role in BPD and ROP

Interleukin-10 is commonly thought of as a master regulator of inflammation, performing anti-inflammatory functions and protecting against various autoimmune and inflammatory diseases, such as inflammatory bowel disease and

diabetes.[110] IL-10 modulates inflammation by inhibiting the production of pro-inflammatory cytokines including IL-1β, IL-6 and TNF-α, as well as CC and CXC type chemokines and metalloproteinases from immune cells.[111] The expression of IL-10 was found to be less pronounced in the placenta of infants with BPD compared to placenta from age- and birthweight-matched infants who did not develop the disorder, while IL-6 was similarly expressed in both groups, suggesting that IL-10 is also an early response cytokine that provides protection against the development of BPD.[112] In contrast, BAL samples from ventilated neonates have shown IL-10 mRNA and protein to be variably detectable, with no major differences between BPD and non-BPD patient groups.[113] A strong association between *IL-10* gene expression and gestational age has also been observed, which may explain observed differences in the IL-10 levels in BAL taken at different ages.[114] Interestingly, in IL-10-deficient mice under steady-state conditions, no histological changes to the lung were apparent, despite the fact that these mice spontaneously develop inflammation at another mucosal site,[115] which may indicate that IL-10 is less important for pulmonary immune homeostasis during the early life period. However, in a model of hyperoxia-induced acute lung injury, IL-10 treatment administered for 3 days during a 95% oxygen exposure regime reduced mRNA expression of pro-inflammatory cytokines as well as the influx of immune cells into the lung and oxidative stress in the parenchyma, highlighting the anti-inflammatory properties of IL-10 in a hyperoxic environment.[116]

In the development of ROP, IL-10 has been shown to protect retinal ganglion cells from oxidative stress-induced death.[117] This protection is because of improved cellular survival compared to an increase in proliferation,[117] and the effect has also been observed in primary microglia.[118] In addition, IL-10 treatment has been shown to suppress the expression of various pro-inflammatory cytokines in explanted retinal microglia.[119] Contrasting findings come from a study of IL-10-deficient mice in the OIR model of ROP which exhibited reduced pathological neovascularisation in the retina.[120] This was attributed in part to altered polarisation states of the retinal macrophages.[120] In addition, macrophages harvested from IL-10-deficient mice showed reduced VEGF expression in both normoxic and hyperoxic conditions, which could contribute to reduced pathological angiogenesis in the eye.[120] Similarly, in the IL-10-deficient choroid, neovascularisation was down-modulated promoting the infiltration of anti-angiogenic macrophages, suggesting a key role of IL-10 in regulating macrophage activity in the eye.[121] While further studies are needed, it is possible that the environment generated in the lung and retina in high oxygen conditions modulates IL-10 activity in BPD and ROP. In BPD, IL-10 appears to be a general immunomodulator, whereas in ROP the effect of IL-10 appears to be cell-specific.

IL-33, an emerging marker of ROP and BPD

There is mounting interest in the role of IL-33 in early life lung injury. It is expressed by airway epithelium, endothelium and lung stromal cells and released in response to tissue damage, providing a danger signal to the immune system.[122] Its receptor, ST2/IL-1RL1, is highly expressed on type 2 immune cells such as eosinophils, mast cells and ILC2, which populate mucosal tissues.[123] Recent studies have shown that the release of IL-33 in the lung in response to various stimuli leads to the accumulation of type 2 immune cells during the alveolar phase of lung development, which can promote allergic asthma in later life.[124–126] Importantly, some neonates with BPD have been reported to develop wheeze, or difficulty breathing, and asthma as they age.[126] High serum levels of IL-33 have been suggested to be a novel predictive marker of BPD severity,[127] although some studies have shown no change in IL-33 levels in preterm neonates relative to levels measured in neonates from the non-BPD group.[128] Concordant with human data, an abnormal increase in IL-33 and ILC2 responses following neonatal hyperoxia in mice can result in increased airway hyperresponsiveness, mucus production and a type 2 inflammatory profile in the lung,[126] reminiscent of asthma.

In addition, following an allergen challenge, neonatal mice exposed to hyperoxia exhibited exacerbated allergic responses in the lung.[126] Therefore, these studies suggest that IL-33 and ILC2 contribute to lung inflammation, airway hyperactivity and long-term respiratory complications that often affect preterm infants diagnosed with BPD. At the same time, systemic and epithelial cell-specific overexpression of IL-33 has been associated with retarded lung

development in hyperoxia-induced BPD in mice.[129–131] These findings suggest that IL-33 is under tight regulation in the lung, and an imbalance in this pathway can result in alveolar damage. This is further evident in a study of BPD showing the capacity of IL-33 to induce neutrophil-associated extracellular traps that promote degradation of the extracellular matrix.[131] It has also been speculated that IL-33 may indirectly enhance TGF-β expression in the developing lung via IL-1β activation, thereby promoting neutrophil infiltration.[129]

While IL-33 has been well explored in mucosal immunity, its role in the retina and retinal diseases is only just emerging, with recent studies showing that IL-33 is expressed in both mouse and human retina and choroid.[132] Serum IL-33 was found to be elevated in infants with ROP before retinal laser photocoagulation, which reduced following treatment, suggesting IL-33 may serve as a blood biomarker of severe ROP.[133] In settings of neurodegeneration and retinal detachment, which occur in very advanced ROP, Müller cells are observed to express IL-33,[134] which can lead to the upregulation of pro-inflammatory cytokines and chemokines involved in macrophage recruitment to the retina. Similarly, activated cells within the RPE are able to upregulate IL-33 to enhance inflammation,[135,136] but also inhibit angiogenesis in the eye, which would be of benefit during pathological neovascularisation if IL-33 activity could be conditionally enhanced during this period.[132] That being said, these distinct effects of IL-33 in the retina begs the question of whether targeting of IL-33 would ameliorate or potentiate the pathogenesis of ROP. Further understanding of the mechanistic involvement of the IL-33/ILC2 axis in ROP will be informative for future target-based research. The contribution of IL-33 as a pathogenic mediator is evident in both BPD and ROP, which may form a shared pathway of inflammatory damage occurring in both organs. The crosstalk between IL-33 and key mediators of lung remodelling, such as TGF-β, suggests a potential mechanism for how IL-33 may contribute to the alveolar hypoplasia that occurs in BPD. As TGF-β is also involved in the pathogenic angiogenesis that occurs in ROP,[137] it is possible that IL-33 is also indirectly connected to BPD and ROP by modulating factors essential for growth and development of the lung and eye.

CURRENT TREATMENTS FOR BPD AND ROP THAT MODULATE INFLAMMATION

The contemporary treatments used for very low birthweight premature babies have had a notable impact on survival. However, they have been less effective at preventing the onset of severe clinical symptoms in the neonatal lung and eye. The invasive nature and adverse side effects associated with these interventions remain a major challenge.[138] Three treatments will be highlighted in this review: corticosteroids and caffeine, which are widely used in the NICU; and mesenchymal stem cells, which are emerging as an alternative regenerative medicine approach.

Corticosteroids

Corticosteroids act by improving the maturation of the foetal/neonatal lung and have been in routine clinical use for the past three decades, leading to a substantial reduction in neonatal mortality and morbidity.[139] Steroid delivery in the immediate post-birth period reduces the incidence of BPD, while treatment within the first week of life prevents the progression of lung injury.[140] Similarly, corticosteroids administered to mothers at high risk of preterm birth have been shown to mitigate the risk of developing of ROP.[141] While the intended target is the immature lung, the developing immune system may also be impacted. In clinical studies, neonates that were given antenatal glucocorticosteroids had reduced lymphocytes in their cord blood,[142] while in experimental studies, foetal offspring (E18.5) from pregnant dams given antenatal betamethasone showed a reduced thymus volume and a loss of immature $CD4^{+}CD8^{+}$ thymocytes, suggesting steroid treatment can have a direct impact on the developing thymus.[139] Corticosteroids have also been shown to polarise monocytes towards an anti-inflammatory phenotype in the tissue of preterm lambs to promote improved phagocytic capacity of apoptotic cells, which may be beneficial in the short term; however, the early skewing of monocytes in neonatal life may be detrimental in the face of infection.[143] What remains elusive is whether the use of steroids in preterm infants induces a transient or persistent change to the neonatal immune system which can adversely impact on immune responses in later life. Given the increased frequency of preterm infants being re-hospitalised because of

respiratory infections following discharge,[144] it is possible that corticosteroid use in prenatal and postnatal life is a major contributor to the increased vulnerability of preterm infants to respiratory complications as they age.

Caffeine

Caffeine is a treatment commonly given to preterm infants with apnoea of prematurity, which has concurrently improved oxygenation and thereby lessened the risk of BPD and ROP.[145] Pre-treatment of LPS-activated cord blood macrophages with a specific inhibitor against adenosine receptor 1, to mimic the receptors to which caffeine commonly binds, demonstrated a reduction in TNF-α production.[146] In BPD, caffeine administration just prior to hyperoxia exposure in a mouse model of BPD decreased the number of $CD11b^+$, myeloperoxidase-expressing cells in the lungs of rat pups.[147] Similarly, antenatal caffeine administration to nursing dams was sufficient to reduce protein levels of NLRP3 inflammasome and NF-kB pathway activity in the lungs of neonatal offspring after 14 days of 75% oxygen exposure.[148] This indicates that caffeine delivered in the antenatal and postnatal period is able to moderate inflammation within the lungs of neonatal mice following oxygen exposure. However, at higher plasma concentrations (10–20 μg/mL), caffeine has been shown to induce a pro-inflammatory response in preterm infants, by increasing levels of IL-1β, IL-6 and TNF-α, and a decrease in IL-10.[149] In this study, preterm infants with BPD were reported to have plasma caffeine levels at extreme values compared to non-BPD counterparts, suggesting that caffeine may be differently metabolised in infants with BPD. Therefore, correct dosing of caffeine is essential to ensure the immunomodulatory benefits of caffeine are not diminished or contribute to a pro-inflammatory milieu.

In experimental ROP in mice, caffeine administered to nursing dams between PN0 and PN17 was effective at reducing ischaemic areas in the retinas of pups.[150] In addition, treatment with another antagonist of the adenosine A2A receptor reduced both avascular areas and retinal neovascularisation at PN17.[150] Thus, caffeine appears to ameliorate pathological angiogenesis in ROP,[151] but whether this phenomenon is mediated by a dampening of local or systemic inflammation remains to be determined. Emerging evidence points to local effects, as the treatment of primary rat microglia with caffeine inhibited LPS-induced cyclooxygenase synthesis,[152] in a similar manner to cord blood-derived monocytes likely via antagonism of A2A receptors.[153] It is possible that caffeine may modulate immune cells other than microglia and macrophages, thereby contributing to a more favorable immune profile. Given that infants with BPD and ROP often possess inflammation from pre- and postnatal sources, which can influence disease severity, it would be of great clinical interest to investigate whether caffeine can also differentially regulate disease outcomes under diverse inflammatory settings.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are a potential regenerative therapy for BPD and ROP with potent anti-inflammatory and pro-angiogenic properties. MSCs modulate tissue microenvironments by paracrine signalling through the release of soluble bioactive mediators such as growth factors, immunomodulatory cytokines and exosomes.[154] Interactions between MSCs and lung cells have been shown to decrease local and systemic inflammation.[155] In phase I clinical trials, intratracheal delivery of human umbilical cord MSCs to preterm human infants with BPD appeared to ameliorate lung inflammation.[156] In a rat model of BPD, intratracheal instillation of MSCs during hyperoxia exhibited a potent decrease of inflammatory mediators, while hyperoxia-induced downregulation of an important driver of lung morphogenesis, thyroid transcription factor, was limited. Altogether, this was associated with a major improvement in alveolar structure that was sustained into the adult period.[157] Similarly, the intravenous administration of bone marrow-derived MSC-conditioned media was able to prevent alveolar and septal wall injury, and lung inflammation.[158] *In vitro* migration experiments revealed that bone marrow-derived MSCs preferentially migrated to oxygen-damaged lungs, adopting a phenotype reminiscent of type II alveolar cells indicated by the expression of surfactant protein C transcripts and the presence of lamellar bodies,[159] suggesting that MSCs could both sense the injury and differentiate into alveolar populations to repair the oxygen-mediated damage. Overall, MSCs appear to

counter the detrimental effects of an oxygen insult to the lung when delivered in early life.

In ROP, there has been limited research on the impact of MSCs on neonatal retinal inflammation. Recent studies have suggested that MSCs have the capacity to confer protection against pathological angiogenesis in the retina by dampening local inflammation. In OIR, MSCs stimulated the expression of semaphorin 3E in ganglion cells, leading to the downregulation of IL-17A expression in myeloid cells, which subsequently controlled the production of pro-inflammatory cytokines such as IL-1β, TNF-α and IL-6 from myeloid cells and also prevented retinal neovascularisation.[160] The regulation of IL-17A in myeloid cells is thought to be mediated by inhibition of nuclear receptor, RORγ.[24] Similarly, *in vitro* stimulation of the BV-2 microglial cell line with LPS in the presence of MSC-derived micro-vesicles induced upregulation of the anti-inflammatory microglial gene, *CCL22*, which was accompanied by reduced cell surface expression of activation markers CD45 and CD11b.[161] These results suggest MSC-secreted vesicles can modulate microglial activity by downregulating genes associated with inflammation, thereby reducing retinal pathology. Studies into the signals mediating MSC crosstalk with host stromal and immune cell populations are an important area for future research in both BPD and ROP.

Despite the availability of several therapies with immunomodulatory capacity, there are numerous uncertainties regarding the effects of current and experimental treatments for BPD and ROP on immune maturation. Furthermore, it will be important to ascertain whether resultant immune reprogramming can have detrimental influences on immune responses later in life.

FUTURE PERSPECTIVES

The majority of the inflammatory mediators discussed in this review show similar patterns of action in the neonatal eye and lung, suggesting that a dysregulated immune system connects BPD and ROP pathogenesis. Nonetheless, several questions remain unanswered. First, it is unclear whether these two diseases arise independently because of tissue site-specific effects of hyperoxia, or are linked via the circulation and spill-over of inflammatory factors from diseased tissue. A more thorough investigation of the timing, spectrum and activity of immune cells that are mobilised as well as their mediators in the peripheral blood during disease progression is needed to answer this question. In addition to the retina and lung, other tissues in the premature neonate may also be affected and contribute to the inflammatory milieu, such as the gut, with necrotising enterocolitis, a relatively common disorder of the premature infant known to increase the risk of developing BPD,[162] ROP[12] and brain injury.[163] Of particular interest, a recent study has shown that gut-derived effector CD4 T cells may underlie the development of severe brain damage associated with necrotising enterocolitis[164] and it would be of interest to assess their contribution to BPD. The gut to brain axis and the gut to lung axis may play key roles in these diseases and are avenues that demand further study. Second, the contribution of oxygen-induced haematopoietic stress is unknown. It would be valuable to examine how an oxygen insult affects the response of the bone marrow stem cell compartment, and other sites where extramedullary haematopoiesis can occur, to promote immune cell development and activation. Third, the synchrony of events that take place in the retina and lung from the time of the initial oxygen insult, to the activation of the immune response and finally in the transition to disease, warrants further evaluation. The use of the concurrent model of ROP and BPD that we have recently described will be useful to study these outstanding questions.[28] This model, paired with new optical clearing and live-imaging techniques, will enable the delineation of immune-related pathomechanisms of these diseases. Finally, as the therapies that are used to treat these diseases impinge on the developing immune system, with potential for long-term consequences, further insights into the mechanisms by which they act are essential. A key driver of future research in this domain is the unmet need for safer and more effective treatments to prevent the development and progression of BPD and ROP. The existence of an immunological link between the lung and eye presents a unique opportunity to identify novel disease biomarkers and potentially new therapeutic targets to simultaneously treat BPD and ROP in preterm infants.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Lakshanie C Wickramasinghe: Conceptualization; Investigation; Writing-original draft. **Peter van Wijngaarden:** Investigation; Writing-review & editing. **Evelyn Tsantikos:** Funding acquisition; Investigation; Writing-review & editing. **Margaret Hibbs:** Conceptualization; Funding acquisition; Investigation; Resources; Supervision; Writing-review & editing.

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