Early origins of lung disease: towards an interdisciplinary approach

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ABSTRACT The prenatal and perinatal environments can have profound effects on the development of chronic inflammatory diseases. However, mechanistic insight into how the early-life microenvironment can impact upon development of the lung and immune system and consequent initiation and progression of respiratory diseases is still emerging. Recent studies investigating the developmental origins of lung diseases have started to delineate the effects of early-life changes in the lung, environmental exposures and immune maturation on the development of childhood and adult lung diseases. While the influencing factors have been described and studied in mostly animal models, it remains challenging to pinpoint exactly which factors and at which time point are detrimental in lung development leading to respiratory disease later in life. To advance our understanding of early origins of chronic lung disease and to allow for proper dissemination and application of this knowledge, we propose four major focus areas: 1) policy and education; 2) clinical assessment; 3) basic and translational research; and 4) infrastructure and tools, and discuss future directions for advancement. This review is a follow-up of the discussions at the European Respiratory Society Research Seminar "Early origins of lung disease: towards an interdisciplinary approach" (Lisbon, Portugal, November 2019).

Introduction

Chronic respiratory diseases, such as asthma and COPD, place an enormous burden on society. A better understanding of how early-life exposures may predispose people to develop chronic respiratory disease is key to developing prevention and treatment strategies. The prenatal and perinatal environments can have profound effects on the development and progression of chronic inflammatory diseases. However, mechanistic insight into how the early-life microenvironment can impact the development of the lung and immune system, and the consequent initiation and progression of respiratory diseases, is still emerging. Recent studies investigating the developmental origins of lung diseases have started to delineate the effects of early-life changes in 1) lung development, 2) environmental exposures and 3) immune maturation on the development of childhood and adult respiratory diseases. Alterations in what is considered "healthy" lung development may prime the neonate for increased susceptibility to develop respiratory complications in later life. Although this viewpoint is widely accepted, the exact mechanisms underlying and timeframe in which these alterations take place remain to be unravelled (figure 1).

Prenatal and perinatal lung development
### *Premature delivery interrupts normal lung development*

The development of the lung architecture results from the coordinated activity of several cell-driven mechanisms of lung development, together with physical forces such as those that result from breathing movements [1]. These cellular mechanisms include, for example, extracellular matrix (ECM) deposition and remodelling, primarily by myofibroblasts in the developing septa; coordination of epithelial–mesenchymal interactions by growth factors; cell–ECM interactions; and the proliferation, migration, apoptosis and transdifferentiation of the constituent cells of the developing lung at the correct time and place during lung maturation [2]. Any disturbances to these pathways result in an aberrant lung structure, which in turn compromises lung gas-exchange function, and contributes to the development of lung disease. One major disturbance is preterm delivery, which accounts for 6–12% of the 5.9 million live births annually in Europe [3]. Prematurity is a leading cause of perinatal and childhood mortality [4]. With intensive care in countries with a more advanced healthcare system, many preterm infants survive, but a major complication is bronchopulmonary dysplasia (BPD). Stunted alveolar lung development has emerged as the histopathological hallmark of BPD, which arises from accelerated lung maturation (alveolar epithelial type II cell maturation, surfactant production and alveolar wall thinning) following exposure to corticosteroids and/or (intrauterine) inflammation during prematurity. This is further complicated by the deleterious effects of prenatal and postnatal exposures to inflammatory, oxidant, stretch and other injurious agents [5, 6]. The consequences of aberrant lung development are evident in adolescent and adult survivors of BPD, who exhibit disturbances in breathing mechanics [7], as well as predisposition to lung disease [8–11]. Recent pre-clinical studies in experimental animal models of BPD support these conclusions, where neonatal exposure to hyperoxia resulted in persistent disturbances to pulmonary vascular function and right-heart remodelling [12, 13], long-term effects on airway hyperreactivity [14–16] and increased sensitivity to bleomycin-induced lung fibrosis in adult mice [17]. Thus, a solid body of evidence suggests that injury to the lung in the immediate postnatal period predisposes affected lungs to disease in later life.

A relatively common pregnancy complication associated with pre-term labour and BPD is acute chorioamnionitis, which is characterised by neutrophilic infiltration and inflammation at the maternal–fetal interface [18]. A surprising consequence of chorioamnionitis is increased production of surfactant in the fetal lung [19]. However, despite the associated increased lung compliance, chorioamnionitis inhibits alveolar and pulmonary vascular development, causing changes similar to BPD [20]. These include decreased and aberrant expression of elastin which identifies sites of secondary septation demonstrated in animal models of chorioamnionitis [21, 22]. Although the mechanisms are not entirely clear, NF-κB activation in innate immune cells and subsequent interleukin (IL)-1 signalling [23–25] inhibit *FGF10* expression in mesenchymal cells. Inflammatory mediators induce transforming growth factor (TGF)-β1 and connective tissue growth factor signalling and decrease *Shh* mRNA levels and Gli1 protein expression in the distal lung [21, 26, 27].

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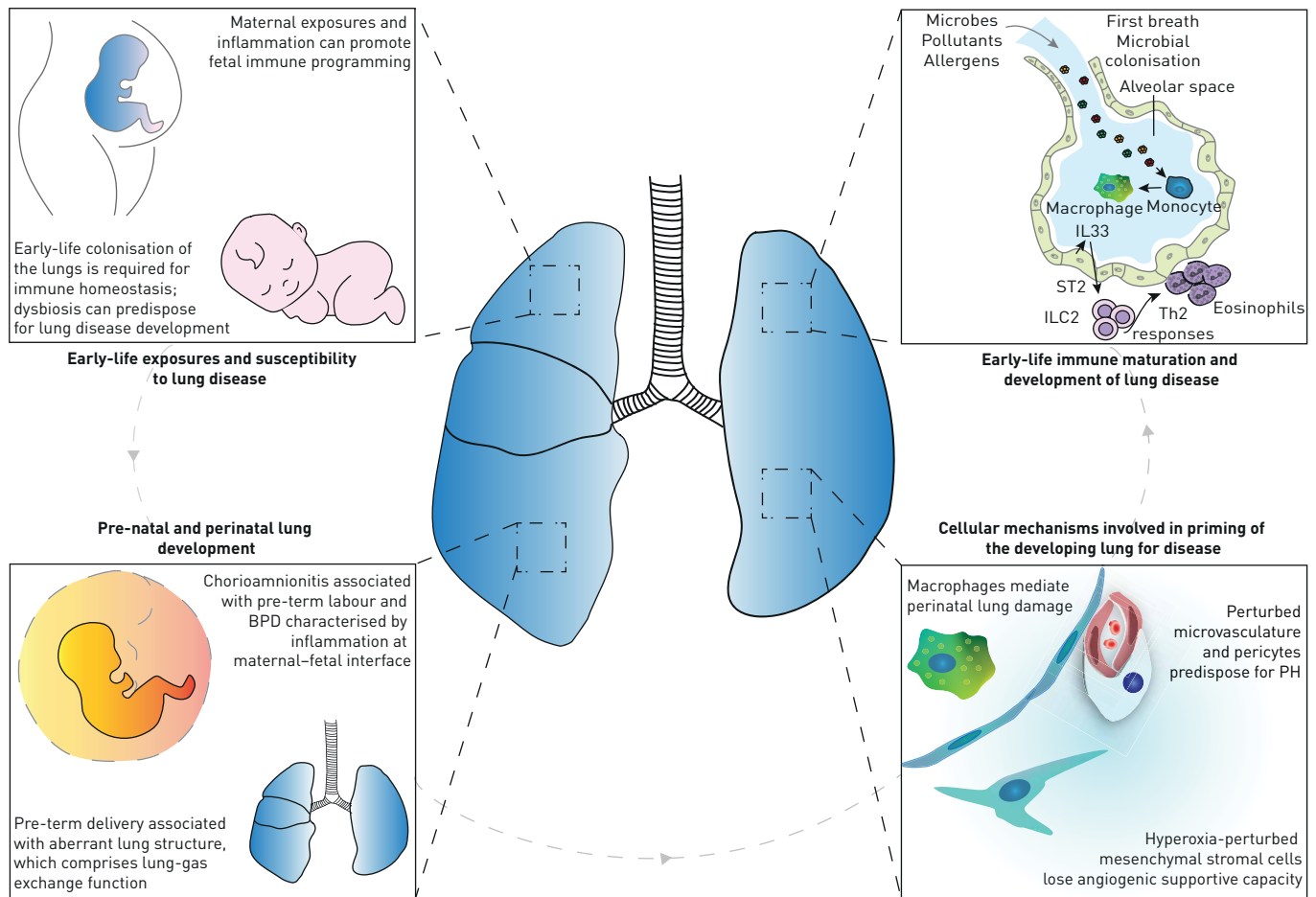
FIGURE 1 Early-life exposures, immune maturation and priming of the developing lung for disease. The prenatal and perinatal environments can have profound effects on the development and progression of respiratory diseases. Different maternal exposures (diet, smoking, medication usage) and maternal inflammation can promote fetal immune programming. Moreover, early-life colonisation of the lungs is imperative for shaping of the immune response. Early-life changes in lung development, specific environmental exposures and alterations in lung immune maturation following such changes and exposures can lead to the development of childhood and adult respiratory diseases. Alterations in what is considered "healthy" lung development, caused by, for example, chorioamnionitis-associated bronchopulmonary dysplasia (BPD), or aberrant lung structure associated with preterm birth may prime the neonate, *via* changes in immune maturation or cellular mechanisms in the lung, for increased susceptibility to develop respiratory complications in later life. IL: interleukin; ST: suppression of tumorigenicity; ILC: innate lymphoid cell; Th: T-helper; PH: pulmonary hypertension.

Early-life exposures and susceptibility to lung disease
Maternal exposures and transplacental immune modulation

The maternal immune system plays a fundamental role in protecting the *in utero* environment [28, 29], and can provide signals that influence developmental trajectories within the fetal immune system, shaping immune functions post-birth [30–33]. Although mechanisms remain poorly defined, maternal inflammation can promote fetal programming that manifests as enhanced susceptibility to development of asthma (and related diseases) [31, 33, 34]. A broad spectrum of environmental maternal exposures have been implicated, including diet, stress, environmental toxins and pathogens and pre-existing clinical syndromes including obesity and asthma [34–37]. Notably, maternal inflammatory responses can be exaggerated during pregnancy (such as asthma [36, 37] and influenza [38]). The mechanisms involved in transplacental transmission of maternal-derived inflammatory immune signals are not well understood, but probably involve direct transfer and cell receptor mediated signalling [32]. Of note, fetal stem cells responsive to these programming signals contribute to development of a range of cellular subtypes that interact to play crucial roles in lung homeostatic processes and inflammation, including local lung tissue resident immune cells (*e.g.* alveolar macrophages and type 2 innate lymphoid cells (ILC2)) and haematopoietic stem cells in bone marrow that seed myeloid populations within respiratory tissues, providing multiple pathways for dysregulated immune function.

The capacity of the fetal immune system to be trained *via* maternal environmental signals provides exciting possibilities for prevention of lung diseases after birth. A seminal example is the profound reduction in allergic asthma (and related diseases) in children born to mothers exposed during pregnancy to benign microbial products from traditional farming environments [39–41]. Mechanistic studies using cord blood mononuclear cells from farmers' children indicate that protection is associated with accelerated development of postnatal immunocompetence [42, 43]. These findings have been replicated in experimental animal models *via* exposure of pregnant mice to a variety of microbial extracts, and these processes were found to be Toll-like receptor-dependent [44–46]. The precise mechanisms that induce transplacental protection against disease are not well characterised, but recent evidence points to innate immune training *via* metabolic programming within the fetal bone marrow dendritic precursor populations [46–48]. Furthermore, exposure to microbial extracts during pregnancy may also provide potential benefits to support maternal immunity, as recently shown in pre-clinical animal models [49]. Harnessing the potential of immune training presents exciting opportunities to enhance control/regulation of maternal inflammatory responses for protection of pregnancy, fetal growth and development, in addition to training the fetal immune system for enhanced immunocompetence in the post-natal environment.

Neonatal and infant airway microbiota composition and development

Little is known about the crosstalk between immune cells and microbes (bacteria, viruses and fungi) in newborns, but evidence from mouse models suggests that immune priming in the lungs is essential for protection against infections and appropriate responses upon allergen exposure [33]. A study using lower-airways samples (tracheal aspirates) from children in the first year of life identified signs of bacterial colonisation as early as 1 day after birth [50]. The authors showed that the lower-airways microbiome developed within the first two postnatal months. During this period, three microbiome profiles were evident: two were dominated by either *Staphylococcus* or *Ureaplasma*, whereas the third was enriched with diverse bacterial genera such as *Streptococcus*, *Prevotella*, *Porphyromonas* and *Veillonella*, all known to be constituents of a healthy adult lung microbiome. Gestational age and delivery mode were important drivers of microbiome composition. The *Ureaplasma* profile was associated with pre-term vaginal delivery, whereas the *Staphylococcus* profile correlated with pre-term C-sections. The diverse microbiome profile was associated with term birth. Concomitant evaluation of the host gene expression revealed increased expression of IL33 and genes linked with IgA production pathway with increasing gestational age. This correlated with an increased predicted IgA1 protease function of the microbes. This concordance is suggestive of tightly regulated host-immune crosstalk, which may influence the development of the immune system and inform early-life interventions to prevent respiratory diseases.

In line with the lower respiratory tract microbiota, a birth cohort of 112 healthy infants indicated rapid development of the nasopharyngeal microbiota, characterised by early *Staphylococcus*-dominance, followed by enrichment of *Corynebacterium*/*Dolosigranulum* and a late introduction and predominance of *Moraxella* by the age of 3 months [51]. This microbial developmental trajectory is governed by birth mode, feeding type, antibiotics and crowding conditions, among others, and appears to be related to both bacterial and viral respiratory tract infection (RTI) susceptibility. Infants who suffered from more RTIs over the first year of life demonstrated reduced *Corynebacterium*/*Dolosigranulum* colonisation accompanied by an early *Moraxella* introduction at 1 month of age. These findings suggest a "window of opportunity", within which timely microbial cues may modulate host immunity and determine RTI susceptibility [52]. Given the theory that lower RTIs may develop following micro-aspiration of upper respiratory tract pathogens, it was postulated that oral microbiota would show a similar pattern of maturation and association with RTI susceptibility as the nasopharyngeal microbiota. However, the oral microbiota assembles even more rapidly into highly stable and niche-specific microbial communities, showing no differences in maturation patterns related to RTI susceptibility. Instead, it was found that niche differentiation within the upper respiratory tract (nasopharynx *versus* oral cavity) is less explicit in children developing more RTIs over the first year of life. This phenomenon was even more pronounced shortly prior to RTI episodes, and characterised by an influx of oral bacteria such as *Fusobacterium*, *Prevotella*, *Neisseria* and streptococci into the nasopharynx [53]. These findings indicate that a "collapse" of bacterial community structure may precede RTI symptoms. Moreover, outgrowth of the number one cause of bacterial RTIs, *Streptococcus pneumoniae*, is controlled by the local respiratory microbiota, contributing to mucosal homeostasis [54].

However, the respiratory microbiota appears to not only be involved in protection against infections, but also play a role in controlling the severity of acute infections. In a recent study, particularly respiratory syncytial virus (RSV), *Haemophilus influenzae* and *S. pneumoniae* were indicative of infection, regardless of phenotype (pneumonia, bronchiolitis or mixed), suggesting a joint contribution of viruses and bacteria as the driver of these infections, whereas the absence of protective commensals was associated with severity [55]. Interestingly, children born pre-term are affected by respiratory viruses, including RSV, at a

higher rate [56, 57]. The risk of readmission due to respiratory infections during the first year of life was shown to be 3.6 times higher in infants born in moderate or late pre-term stages [57]. One may speculate that the burden of RSV disease in these preterm infants may also be related to distorted microbial and/or immune maturation patterns.

Cellular mechanisms involved in priming of the developing lung for disease
Influence of vascular changes on lung development
The vasculature is important for branching morphogenesis of the future airways [58–61], and the pulmonary vessels are an integral part of the systemic circulation, and mainly expand through angiogenesis [62]. Angiogenesis is the process of the formation of new vessels through the expansion and sprouting of existing vessels. These new extended endothelial tubes are highly unstable and require perivascular cells, pericytes, to become fully functional vessels. The interaction between endothelial cells and pericytes is mediated through platelet-derived growth factor (PDGF)-β signalling, which induces stabilisation of endothelial cells and subsequent differentiation of pericytes into smooth muscle-like cells [63, 64].

Smooth muscle precursor cells (perivascular cells) are primed cells that express Kruppel-like factor-4. These cells respond to induced damage in adult mice, such as hyperoxia, by proliferation and migration, which eventually leads to pulmonary hypertension-like vascular abnormalities [65]. By studying paediatric congenital lung abnormalities, such as congenital diaphragmatic hernia (CDH), which is characterised by concomitant vascular problems (persistent pulmonary hypertension), it has become clear that vascular abnormalities associated with CDH develop *in utero*. Histological studies in premature and term CDH patients uncovered a thickening of the vascular smooth muscle layer in the mid-sized vessels as well as neo-muscularisation of capillaries. Additionally, smooth muscle cells expressed markers associated with a contractile phenotype [66]. Given the role of perivascular cells during angiogenesis, subsequent studies focused on these cells as a potential source of the vascular abnormalities observed in CDH. Clear vascular abnormalities already become apparent at the pseudoglandular and canalicular stage of development in a mouse model for CDH [67], including significant reductions in vessel length and number of branches from the main pulmonary vessels. Moreover, perivascular cells associated with vessels extended more distally compared to controls, indicative of a higher coverage of vessels by these cells, which was also shown for pulmonary hypertension in adults [68]. The perivascular cells in CDH subsequently expressed markers of advanced differentiation, as compared to controls.

Upon differentiation, perivascular cells lose the ability to support endothelial cells that form new branches during angiogenesis. As a result, lung development in CDH is characterised by a simplified microvascular development and subsequent hypoplasia, similar to BPD. In both diseases, retinoic acid signalling has been identified as a key pathway. Retinoic acid is required during the formation of the prospective lung field at the onset of lung development, and reduced retinoic acid signalling leads to increased TGF-β [69], which induces differentiation of pericytes [70]. Moreover, inhibiting retinoic acid signalling impaired vessel formation *in vitro* and caused a reduction in the production of collagen IV, which perivascular cells start to secrete upon interaction with endothelial cells, and has been observed in lungs of human CDH patients [67]. Considering the aberrant function of perivascular and endothelial cells in CDH and BPD patients, it will be important to create a better understanding of whether these patients are predisposed to chronic lung and vascular disease, and perhaps even infectious and inflammatory disease, in later life.

Mesenchymal cells in lung alveolar development and injury
The cellular mechanisms underlying the long-term effects of lung disease in infancy have not been clarified. However, recent studies have identified disease-relevant changes in an emerging spectrum of mesenchymal cells, namely fibroblast subsets, as well as in resident immune cells, particularly macrophages, as new cellular mechanisms on priming the developing lung for disease. Mesenchymal cells are important in orchestrating early lung development, by interacting with epithelial and endothelial cells, communicating back and forth to shape the branching structure that becomes the lung [2]. In saccular and alveolar lung development studying mesenchymal cells and their interplay with their niche has long been based on histological and *in vitro* studies. The mesenchymal compartment of the distal lung was long thought to consist of two types of fibroblasts based on structural properties: lipofibroblasts and myofibroblasts [71]. By studying lung regeneration after pneumonectomy in adult mice, it became clear that some lipofibroblasts function not only as progenitor cells for myofibroblasts, but also as orchestrators of new alveolar growth [72]. The notion that stromal stem cells can function as regulators of repair and regeneration, communicating with a variety of epithelial, endothelial, immune and even neuronal cell types, has been extensively studied in amphibians [73, 74]. The discovery of the potent regenerative and anti-inflammatory properties of bone marrow- and umbilical cord-derived mesenchymal stromal cells (MSCs) has led to a surge in pre-clinical and clinical studies showing the promise that these cells hold as a therapeutic agent [75]. In BPD, exogenous MSCs (derived from bone marrow or umbilical cord) have the

potential to prevent and repair lung injury and perturbed alveolar development [76–78] and are currently the subject of a number of ongoing clinical trials worldwide (Clinicaltrials.gov identifiers NCT04062136, NCT03873506, NCT03558334, NCT03645525, NCT03378063, NCT03631420, NCT03392467, NCT03601416, NCT04255147, NCT02443961). However, this also leaves us to question why resident mesenchymal cell types are unable to fulfil this same role and highlights how little we really understand about the dynamics of mesenchymal cells with the developing alveolar niche. Using tracheal aspirates of pre-term infants, it was found that the shedding of mesenchymal cells into the airway compartment predicted the development of BPD [79]. Moreover, mesenchymal cells derived from the lungs of BPD patients have a strong pro-fibrotic phenotype [79] and lower expression of PDGF receptor-α and -β [80], similar to histological findings in the lungs of patients dying with BPD, suggesting that mesenchymal cells potentially contribute to BPD pathogenesis. This hypothesis is further supported by studies reporting that CD146$^+$ lung MSCs exposed to hyperoxia *in vivo* [81] or *in vitro* [82] exhibited a diminished microvascular supportive capacity and an impaired secretion of factors that are important in orchestrating lung development. It has been suggested that exogenous MSC therapy restores the function of perturbed lung MSCs, but future studies will need to show whether this is indeed the case [83].

Mesenchymal cells are heterogeneous, making it hard to characterise and delineate mesenchymal cell interaction with the alveolar microenvironment. Lineage tracing studies and more recently single-cell RNA sequencing (scRNAseq) have allowed the identification of a variety of different mesenchymal subsets [84–88]. These mesenchymal subsets appear to be not only very heterogeneous, but also plastic in phenotype, as different subsets are found at different ages and after various exposures [88–90]. Defining how these expression-based subsets differ functionally, and what role they play in the cellular crosstalk during development, repair and susceptibility for chronic lung disease is a major challenge that remains to be addressed.

Macrophages in lung alveolarisation
Resident macrophages migrate into the lung in multiple waves during development, and have been hypothesised to contribute to lung development [26, 91]. Upon exposure to inflammation, fetal pulmonary macrophages play an active role in disrupting lung development by actively inhibiting expression of critical genes that are required for developmental processes [23]. While inflammation *per se* has long been considered a key pathological feature of BPD [92], a variety of inflammatory cell types have recently been implicated in perturbations to lung function, and the development of the lung structure associated with BPD. CD11b$^+$ mononuclear cells have been demonstrated (using diphtheria toxin mediated depletion) to mitigate hyperoxia-induced lung injury in newborn mice [93]. These studies were expanded to reveal that an imbalance in Ly6C^{hi}/Ly6C^{lo} monocyte populations mediated hyperoxia-induced lung injury in the developing lung, which could be rescued by interferon-γ treatment [94].

Beyond these monocyte populations, alveolar macrophages were documented to mediate the deleterious impact of hyperoxia on lung alveolarisation in newborn mice (using *Csf1r*-lineage depletion) [95], in a study where neutrophils were documented to be without any effect (through antibody-mediated neutrophil depletion studies with anti-Ly6G IgG). These reports highlight the possibility that oxygen toxicity may reprogramme immune cells in hyperoxia-exposed developing lungs. These studies are important, since early-life exposure of mouse pups to hyperoxia profoundly impacted the adult response to influenza virus infection [96]. This suggests a persistent reprogramming of the inflammatory response during early life, which was attributed to intrinsic changes in hematopoietic cells, and changes in the reparative *versus* cytotoxic nature of natural killer cells [97]. The mechanisms underlying these persistent changes in immune cell behaviour that are maintained from infancy to adulthood have not been clarified. However, eosinophil-associated RNAse 1 (Ear1) in type 2 alveolar epithelial cells (AEC2) was suggested to play a role in this phenomenon [98]. There is clearly tremendous scope for the further exploration of terminal inflammatory cell programming in the lung in the immediate postnatal period, with consequences for both lung development and innate (and perhaps adaptive) immunity that last into adulthood. This is currently considered a priority area for investigational studies [99], and is explored further in the following section.

Early-life immune maturation and development of lung disease
Early-life development of the pulmonary immune system
The perinatal/postnatal period represents a critical window in the pulmonary immune development with enduring effects on pulmonary physiology, susceptibility to lung disease and resistance to infections. Postnatal lung developmental changes, genetic influences and novel (micro-) environmental cues shape the migration, expansion and/or maturation of pulmonary immune cells. Macrophage precursors, mainly originating from the fetal liver, seed the lungs during embryogenesis [100, 101]. These macrophage precursors at first reside in the interstitial space, to migrate to the alveolar space after birth, where they undergo final differentiation and remain with the potential of local self-renewal [102]. In line with the substantial adaptations of lung tissue at birth, several groups discovered the sudden release of the alarmin

IL33 by AEC2 [103, 104]. By studying the impact of IL33 on post-natal immune development, it became clear that this phase is accompanied by an IL33-driven wave of type 2 immune cells, most prominently shown by the expansion and activation of ILC2s and eosinophils [103, 104]. The expansion of ILC2s shapes the functional performance of newly differentiating alveolar macrophages, by dampening their pro-inflammatory phenotype, at the cost of an increased susceptibility to bacterial pneumonia [103], and at the same time, skews scarcely present neonatal dendritic cells to drive T-helper type 2 (Th2) responses, thereby promoting asthma development at young age [104]. A scRNAseq study on mouse lungs before and after birth confirmed macrophage developmental trajectories and the IL33-driven ILC expansion [105]. Furthermore, examining the receptor–ligand interaction in lungs, a resident basophil population was revealed, which functions as an amplifier of the IL13 and colony-stimulating factor-2 driven maturation and expansion programme of alveolar macrophages after birth [105]. Another cell type shown to exert protective effects against bacterial pneumonia in neonates is pulmonary ILC3s, which develop postnatally upon the support of lung fibroblast derived insulin-like growth factor (IGF)1 [106]. Importantly, newborns with BPD exhibit reduced IGF1 levels and ILC3 numbers in their bronchoalveolar lavage fluid, as do neonatal mice with experimental BPD.

Allergic immunity

The relatively limited exposure to antigens *in utero* dictates that newborns are more reliant on innate immune pathways for protection against infections. However, the fetal immune system contains mature T- and B-cells that are actively suppressed by regulatory T-cells [107]. Moreover, nasal-associated lymphoid tissue is established before birth, while bronchus-associated lymphoid tissue expands rapidly postnatally [107]. This immature immune system is shaped following postnatal exposure to pathogens (bacteria, viruses and fungi), and inhaled particles such as dust, pollen and animal dander. Moreover, genetic makeup is also an important factor in shaping of the immune responses in early life and can consequently influence lung disease development, as evidenced by the high heritability of an allergic phenotype (and asthma). The timing and nature of the exposures has a significant impact on the developing immune system and may result in skewing towards health or disease. The majority of studies to date have used peripheral blood, and have shown that Th2-cell preference is required for a healthy pregnancy [108], but also that this preference is maintained during the neonatal period, reducing gradually during the first 2 years [109]. However, a deviation from this physiological Th2 skewing, with exaggerated Th2 responses in either pregnancy or the first 3 months of life has been associated with an increased risk of subsequent childhood asthma or wheeze [110]. Mouse models have shown that these Th2-cells are critical in the development of allergic inflammation [111]. Factors that may result in accentuated or prolonged Th2 skewing include maternal allergy [112], but environmental exposures are also critical, as has become apparent from farming exposure studies [113]. Similarly, diet has a profound influence on the systemic microbiome, which impacts upon the developing immune system and thus the trajectory towards disease or health [114]. Although it is apparent that the composition of inhaled exposures has a direct impact on the airway immune profile, mechanistic studies in the context of a developing immune system are scarce, while studies in children predominantly used peripheral blood rather than cells directly isolated from mucosal surfaces.

Therefore, it is apparent that early life represents a window of both vulnerability and opportunity that impact immune and tissue homeostasis, but it is not known whether pathology progresses because of an imbalance in regulatory cells *versus* effector cells. Peripheral blood isolated during the first postnatal year shows that the proportion of both resting naïve T-regulatory cells (Tregs; $CD4^+CD45RA^+FoxP3^+$) and activated Tregs ($CD4^+CD45RA^-FoxP3^{high}$) increased markedly from birth to 6 months of age [115]. In contrast, little is known regarding the phenotypes of cells within airways of neonates, although children who develop allergic disease in the first year of life have deficient Treg responses to microbial stimuli, but not allergens, from birth [116], highlighting the importance of skewed immunity in early life.

Inter-organ crosstalk

In recent years, the importance of inter-organ crosstalk and interactions in the initiation, development and progression of respiratory disease has become apparent, and the microbiome plays a central role in these processes [117, 118]. Alterations in the gut microbiome composition can influence early-life and adult immune development, and consequently affect the development of respiratory disease (gut–lung axis) [119–121]. Moreover, antibiotic use is strongly associated with the development of asthma, particularly when antibiotics were taken during the first 6 months of life [122]. In line with these observations, antibiotic treatment in the perinatal period enhances susceptibility to and severity of murine allergic airway inflammation [119, 123]. This effect was suggested to be linked to the loss of Tregs in the colon, which could enhance the development of allergic airway inflammation, probably mediated by the gut–lung axis. Dietary intake, in particular high fibre consumption, alters gut microbiome composition and

consequently increases local and circulating short-chain fatty acid (SCFA) levels [34, 124]. Maternal high-fibre diet consumption and consequent reduction in allergic airway inflammation in offspring was found to be mediated by maternal–fetal transfer of SCFAs leading to altered gene expression in the fetal lung and changes in immune regulation [34].

Recent evidence has indicated that microbe- and age-mediated immune maturation in the skin can determine the nature and severity of allergic skin inflammation using a neonatal skin sensitisation model [125]. Interestingly, the nature of the observed skin inflammation (Th2 mediated in neonates and Th2/Th17 mediated in adults) determined the inflammatory profile (eosinophilic *versus* neutrophilic) in the lung following allergen challenge, suggestive of skin–lung interactions. Moreover, there is now first evidence of inflammatory immune cell seeding in remote non-allergen exposed mucosal tissues [126]. These data indicate that allergen challenge on the skin, in the gut or in the airway induces not only a local allergic inflammatory response, characterised by local eosinophil influx, but can also initiate increased eosinophil frequencies in distant non-allergen exposed mucosal tissues. There is strong evidence of interactions between the skin, gut and the lung. However, future mechanistic studies focusing on this inter-organ crosstalk are needed to dissect the mechanisms underlying these responses and their implications for disease development in early life [127].

Future perspectives

Lifelong respiratory health starts before birth. If we can provide each child with a healthy start, the chances of attaining maximal lung capacity in adult life are markedly increased. To advance our understanding of the early origins of lung disease and to allow for proper dissemination and implication of this knowledge, we propose four major focus areas and future directions for advancement within these areas (figure 2).

Policy

The largest impact of and earliest time for prevention of lung diseases is prior to birth. Education of mothers, especially by midwives, with respect to exposures to avoid prior to and during pregnancy can be complemented with advice on healthy behaviour. Such advice will require targeted evidence-based information. Policy makers need to be provided with scientific evidence of specific risk factors associated with explicit diseases. This research and policy advice should be an ongoing and active effort, as exposure to risk factors might fluctuate and change over time and display regional differences. For interventions and/or protective measures that will prevent disease development, scientific evidence is delivered by randomised controlled trials.

In underdeveloped and low-income countries, prenatal disease prevention is yet to become part of public health policies and more effort should be put into raising awareness. In these settings, cascading teaching methods and getting individuals with a respected position in society involved is imperative to spread good practices (education). It can be expected that if mothers adopt healthier lifestyles during pregnancy, they will raise their children under healthier circumstances, and this next generation will therefore take this as normal behaviour. This will not only affect early life, but also impact upon the development of adult-onset diseases. Moreover, given the lack of identified biomarkers so far, the focus should be on higher risk groups, with a prominent role for midwives.

Clinical assessment

The worst-case scenario of a difficult start to life with respect to lung function is the development of BPD, which we will use here as a prototypic early-life lung disease. As survival of pre-term infants at lower gestational ages has increased due to improvements in care and treatment, BPD has taken on new dimensions. There is a widespread consensus that the current definition is clinically useful, but too broad [128]. The fact that BPD is not a single disease, but rather a syndrome, is not captured by this definition. Moreover, the pathological features underlying the disease are difficult to assess in the clinic. In addition, the long-term consequences of BPD, such as airflow limitation, need to be clearly defined and the question remains whether this should be termed as an early COPD phenotype. Better imaging techniques, including magnetic resonance imaging, are needed to obtain greater insight in for instance alveolar simplification [129], Moreover, this will allow us to discern consequences of BPD from early COPD development and treat patients accordingly.

Awareness of the need for respiratory follow-up after suffering from early-life lung disease is only just emerging and local efforts are putting specific clinics into place. As the prognosis and long-term consequences of early-life lung disease are highly variable and cannot be predicted, these initiatives are essential in order to obtain better insight. To achieve this, the transfer of clinical data is an important issue. Patients and their data undergo transitions between multiple healthcare providers: from the obstetricians,
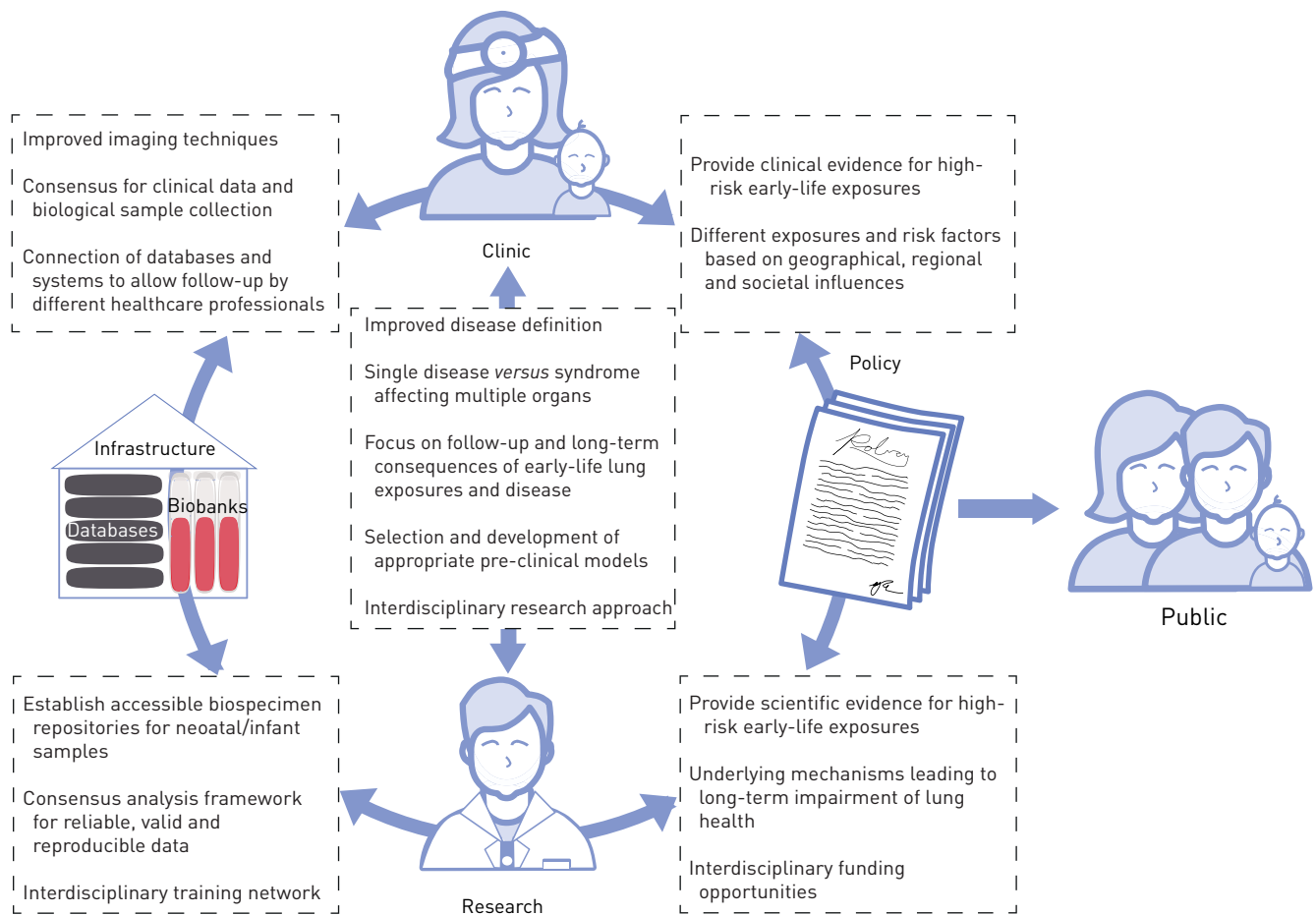
FIGURE 2 Focus areas for future interdisciplinary research into early origins of chronic lung disease. To advance our understanding of the early origins of chronic lung disease four main focus areas and future directions for advancement within these areas have been identified: 1) policy; 2) clinical assessment; 3) research; and 4) infrastructure and tools. Central to these focus areas is education of researchers and clinicians, but also education of and communication to the general public. Clinical observations and research findings are required to inform policy making which consequently serves as a basis to inform, guide and educate the public. In order to facilitate proper clinical assessment and both basic and translational research, adequate infrastructure is needed in the form of databases and biobanks. Moreover, interdisciplinary training of professionals (clinicians, midwives, basic and translational researchers, and bioinformaticians and biostatisticians) working across the focus areas is required.

the neonatologists and paediatricians to adult pulmonologists; and probably also between hospitals using separate databases and systems. Ethics and privacy regulations hinder the connection of these databases. For prospective monitoring, it will be essential to determine which clinical data and possibly biological samples should be collected and stored (infrastructure). Prior to establishing standardised prospective follow-up, adult pulmonologists should at least become aware of early-life events and exposures of their patients that might have significantly contributed to their current health problems (education). This insight into early-life events can offer extremely valuable information to help our understanding of the pathogenesis of adult lung diseases and the heterogeneous representation of each individual condition.

Research

A major challenge in a multidisciplinary research approach to early origins of lung disease is connecting basic and translational researchers to understand the clinical conditions and manifestations. Based on the clinical definition of the respective lung disease, various animal models are utilised for these studies. However, not a single model fully represents the complexity of the human situation. Each individual model simulates specific parts of the condition. These models are chosen based on their appropriateness to answer a specific research question and contribute to moving the field forward. However, we need to increase awareness of their limitations (such as phenotypic variability observed in patients) and search for alternatives to tackle them. Furthermore, better representative models for neonates should be established and coupled with *ex vivo* and *in vitro* models, including gut-on-a-chip or lung-on-a-chip.

Interdisciplinary approaches and collaborations will increase creativity and often the likelihood of major break-troughs. Here, "interdisciplinary" does not only relate to technologies, but also refers to the multisystemic dimensions of lung diseases and the use of multiple exposure models to reflect the complexity of the clinical situation, but also encompasses modelling and examination of the extrapulmonary effects and inter-organ crosstalk and their consequences for the disease. Furthermore, integration and connection of existing datasets from cohorts (infrastructure) should help to decipher sex-, ethnic-, age- and environment-specific effects as well as iatrogenic (for example antibiotics) causes linked to the onset of lung diseases.

To investigate how early-life exposures and alterations in the microbiome could interfere with developmental, cellular and immunological processes and ultimately contribute to development of lung disease, it is imperative that the composition of a physiologically "healthy" lung at different times in early life is defined on these different levels. This would allow for the recognition of altered processes that may predispose to the development of lung disease. Epidemiological and intervention studies (translational/clinical) are essential to define such physiological "healthy" conditions that lead to, for example, a health-promoting lung microbiota and absence of lung disease. These studies should highlight the functional role of these processes and the importance of investigating the underlying molecular mechanisms.

Finally, translational research should always aim for understanding, prevention and treatment of lung diseases. Endogenous and exogenous risk factors that negatively affect the development of the lung, pulmonary immune system and the lung microbiome and predispose to lung disease should be defined. Subsequently, new targets should be discovered to develop new treatment strategies, including maternal or post-natal nutritional interventions and antimicrobial treatments beyond antibiotics. Definition of nutritional aspects that promotes a healthy gut microbiota could enhance respiratory host defence mechanisms as part of immune homeostasis and decrease viral/bacterial infections/colonisations that may affect lung development, lung immune maturation and lung microbiome composition. Addressing these aims will not only provide new insights in the host–microbe interaction in the context of lung health and disease, but also offer new avenues for early intervention and prevention of respiratory disorders.

Infrastructure and tools

Without the proper infrastructure and tools, it is difficult to enhance and join our research efforts. Calls for interdisciplinary funding opportunities are scarce and it is imperative to join forces through collaborative networks. An important opportunity to move the field forward relies on the creation of a cohort of patients with a biospecimen repository, similar to the Global Alliance to Prevent Prematurity and Stillbirth repository (www.gapps.org/), where the focus has been placed on the sharing of samples. This will allow not only access to the samples, but will also forge a collaborative and potentially pluridisciplinary effort toward achieving common goals to understand the mechanisms underlying the early origins of lung diseases. Moreover, enhanced infrastructure is needed to facilitate the acquisition of cross-country ethical approvals to aid in these efforts.

To ensure the acquisition of more reliable, valid and reproducible data in this field between different research groups and countries, it is imperative to develop a consensus analysis framework with standard operating procedures for sample preparation and acquisition, methods, instruments and bioinformatics pipelines. This requires an interdisciplinary network including clinicians, microbiologists, immunologists, molecular and cell biologists, computational scientists and bioinformaticians.

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