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Lung development and emerging roles for type 2 immunity

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Abstract

Lung development is a complex process mediated through the interaction of multiple cell types, factors and mediators. In mice, it starts as early as embryonic day 9 and continues into early adulthood. The process can be separated into five different developmental stages: embryonic, pseudoglandular, canalicular, saccular and alveolar. Whilst lung bud formation and branching morphogenesis have been extensively studied, the mechanisms of alveolarization are incompletely understood. Aberrant lung development can lead to deleterious consequences for respiratory health such as bronchopulmonary dysplasia, a disease primarily affecting preterm neonates, which is characterized by increased pulmonary inflammation and disturbed alveolarization. While the deleterious effects of type 1-mediated inflammatory responses on lung development have been well established, the role of type 2 responses in postnatal lung development remains poorly understood. Recent studies indicate that type 2-associated immune cells, such as group 2 innate lymphoid cells and alveolar macrophages, are increased during postnatal alveolarization. Here, we present the current state of understanding of the postnatal stages of lung development and the key cell types and mediators known to be involved. We also provide an overview of how stem cells are involved in lung development and regeneration, and the negative influences of respiratory infections.

Keywords

Lung development, type 2 immunity, alveolarization, bronchopulmonary dysplasia, group 2 innate lymphoid cells, ILC2, postnatal development, stem cells, respiratory infection, macrophages

Overview of embryonic lung development

The development of the lung is a complex process mediated through the interaction of epithelial and mesenchymal cells. In humans/mice, it is subdivided into five stages: embryonic (embryonic day (E) 26-49/E9.5-12.5), pseudoglandular (E35-119/E12.5-16.5), canalicular (E112-182/E16.5-17.5), saccular (E168-E266/E17.5 – postnatal day (P) 5), and alveolar (E252-2 years/P5-P30) (figure 1) [1,2]. Thus, mechanistic insights defined in mice can be inferred to translate into huma lung development at the equivalent timepoint Briefly, the embryonic and pseudoglandular stages involve the formation of the lung buds and bronchial tree [1,3]. During the canalicular and saccular stages, cells start to differentiate, and the terminal branches narrow and form epithelial sacs, which develop into alveoli in the postnatal alveolar stage (figure 1) [1,3]. While rodents are born during the saccular stage, human lungs are in the early alveolar stage at full term (reviewed in [2]).

In mice, the formation of the lung begins around E9.5 when the trachea starts to bud off the endoderm of the anterior foregut [3,4]. This can be followed by the expression of NK2 homebox 1 (Nkx2.1), the earliest known marker of the respiratory epithelium [5]. Budding of the trachea from the anterior foregut endoderm is tightly regulated by fibroblast growth factors (FGFs) [6], sonic hedgehog (Shh) [4] and Wnt signalling pathways [7]. After budding at E9-9.5, further separation of the foregut is observed. The ventral part of the foregut separates into the trachea and two primary lung buds, and the dorsal part forms the oesophagus [3,7]. In subsequent days, the lung buds elongate and branch into the surrounding mesenchyme (figure 1) [8]. This branching program can be subdivided into three different patterns, domain branching, planar bifurcation and orthogonal bifurcation, specific for the scaffolding of the lobes, the formation of the edges, and the lobe's surface/interior, respectively [8].

Recently, it has been shown that in mice, mechanical forces are involved in organ development by controlling the ratio of fixed and rotating spindles needed for normal airway

tube morphogenesis [9]. Additionally, the transmural pressure, i.e. the difference between the pressure in the airway lumen and the pleural cavity, caused by the secretion of fluid from epithelium into the lumen, controls gene expression, timing and rate of airway branching [10]. While the lung buds expand in the surrounding mesenchyme, cell fates begin to be determined. Surfactant protein c (SP-C), a downstream target of Nkx2.1 and a marker of distal pulmonary epithelial cells, can be detected as early as E9.5 [6]. At E14.5, during the pseudoglandular stage, columnar cells prevail in the distal parts of the lung [11,12]. By E16.5, alveolar progenitor cells, Club (formerly known as Clara cells) and ciliated cells can be detected (figure 1) [11,12]. Around E17.5, during the saccular stage, cuboidal and flat squamous cells appear [11]. Transcriptomic analysis of distal epithelial cells at E18.5 revealed the presence of five different cell populations: Club cells (Uteroglobin^{+}), ciliated cells (forkhead box protein J1 (Foxj1^{+})), nascent alveolar type (AT)1 (podoplanin^{+}) and AT2 (SP-C^{+}) cells, and an intermediate between AT1 and AT2 cells, the bipotential alveolar progenitor (BP), found at the tips of the branching epithelium (figure 1) [12].

Mechanical stress has also been implicated in the differentiation of alveolar epithelial cells [13]. At E16.5, actin-dependent cell protrusions can be identified at the distal airway epithelium, mediated through FGF-10 and mesenchymal cells. When inhaled amniotic fluid reaches the distal airways around E17.5 and induces mechanical tension, it causes flattening of non-protruded cells which later differentiate into AT1 cells [13]. Protruded cuboidal cells show markers of AT2 cells [13].

Signalling pathways

Lung development is distinct from other organs as it is filled by fluid *in utero*, but following birth, has to rapidly adapt to facilitate breathing [13,14]. Consequently, the structure is not yet fully developed and keeps remodelling during the postnatal saccular and alveolar stages (figure

1) [1]. At birth, murine lungs are characterized by the presence of saccules whilst alveoli, the units of gas exchange, are not yet present. During the first two weeks after birth, the lung experiences drastic changes, including a decreased saccular/alveolar volume (until P10) and septal thickness (until P28) [15]. At the same time, alveolarization is accelerated until P10, and then continues at a slower pace until early adulthood [15]. There are two phases of alveolarization in rodents, classical and late alveolarization. During classical alveolarization, alveoli are formed from immature pre-existing septa (P4-21) [16]. In late alveolarization, they form from mature septa after maturation of the capillary network (microvascular maturation) (P14-P36 adulthood) [16]. In mice, approximately 5% of the adult septal surface is formed prenatally, 55% during classical and 40% during late alveolarization [17]. In humans, continued alveolarization after microvascular maturation starts around 2 years of age and continues into young adulthood [2,18].

The exact mechanisms and signalling pathways of alveolarization require further elucidation. Multiple mouse models have revealed important players in pulmonary development, including but not limited to FGF- [19-22] and hedgehog (Hh)-signalling [23], as well as miRNAs [24-29]. FGF-signalling has been shown to be relevant for pre- and postnatal pulmonary development. While a lack of FGF-10 leads to complete lung agenesis, overexpression of FGF-10 during E10.5-13.5 (pseudoglandular phase) prevents the differentiation of distal progenitors [19]. FGF-10 also prevents the differentiation of sex determining region Y-box 2 $(Sox2)^+$ epithelial cells into ciliated cells and instead promotes a basal cell (BC) phenotype [19] and the differentiation of BPs into AT2 rather than AT1 cells [20]. FGF receptors 3 and 4 in the mesenchyme are important for elastin fibre organization [21]. Deletion of the receptors in the mesenchyme leads to elastin disorganization and alveolar simplification at P3 [21]. It is suggested that the main ligand for these receptors is FGF-18, however FGF-18 deficient mice show abnormal lung structures at E18.5 and die shortly after

birth, indicating important roles in late embryonic lung development [22]. Collectively, these studies show the importance of FGF-signalling in lung development.

The expression of Shh and its transcriptional target glioma-associated oncogene 1 (Gli1) peak during the first two weeks of development, which corresponds with maximal alveolarization, whereas Shh-signalling decreases towards week 3 [23]. The function of Hh-signalling seems to be involved in myofibroblast differentiation and proliferation, and its inhibition in early life decreases the number of newly formed septa [23].

Mesenchymal cells and angiogenesis

In mice, lung development is mediated through the interaction of different cell types, including mesenchymal cells [30-32]. Alveolar myofibroblasts form the alveolar ring muscles around the openings of the alveoli and are responsible for extracellular matrix (ECM) deposition during alveologenesis [30,31]. They express the receptor for the platelet-derived growth factor-A (PDGF-A), which is also expressed by epithelial cells and mediates epithelial-mesenchymal crosstalk [30]. PDGF-A signalling plays an important role in alveologenesis as PDGF-A deficiency leads to loss of alveolar myofibroblasts and elastin fibres, disrupted alveologenesis, generalized emphysema and early death [30-32]. Deficiency in the ECM protein elastin similarly results in impaired lung development, airway branching and early death [33]. Moreover, mice deficient in latent transforming growth factor-β (TGF-β)-binding protein-4, an ECM protein essential for elastin fibre formation, show increased mortality, abnormal elastin deposition, altered lung structure, reduced angiogenesis and pro-fibrotic changes [34]. These studies show the importance of ECM proteins in alveolar development.

Recent *in vitro* studies showed that culture of mesenchymal lineages with AT2 cells results in the formation of organoids and promote AT2 self-renewal and differentiation into AT1 cells [35,36]. Crosstalk between the mesenchyme and epithelium may be regulated by

interleukin (IL)-6, FGF-7 and bone morphogenetic protein (Bmp) signalling. While IL-6 and FGF-7 promote AT2 self-renewal and differentiation, Bmp signalling inhibits these processes [35]. This implicates the mesenchyme in alveolar repair after injury which is further supported by the fact that intra-tracheal administration of mesenchymal stem cells attenuates the detrimental effects of hyperoxia on alveolarization and angiogenesis [37-40].

Lung vessel generation begins during early development and follows overall lung growth [41]. Though important for alveolarization, it is not a major focus of this review. Briefly, in mice, important mediators include vascular endothelial growth factor (VEGF) [42-44], nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) [45,46], insulin-like growth factors [47] and platelet endothelial cell adhesion molecule (PECAM-1) [48]. *Vegf-a* mRNA expression peaks during alveolarization [42]. Its deletion disrupts alveolarization [42], and inhibiting the VEGF receptor in rats reduces pulmonary arterial density and induces persistent alveolar simplification [43,44]. NFκB is constitutively active in neonatal developing endothelium and is needed for survival and proliferation of endothelial cells [45]. Inhibiting NFκB or its activating kinases leads to impaired angiogenesis *in vitro* [45,46], and loss of distal pulmonary vessels and alveolar simplification *in vivo* [45]. It has been suggested that disrupted alveolarization in PECAM-1 null mice may be caused by impaired endothelial cell motility [48].

In human preterm infants, mechanical ventilation-induced hyperoxia causes the development of abnormal alveolar microvessels [49], and differential expression of angiogenesis-related genes, including an upregulation of angiostatic and downregulation of pro-angiogenic genes [50]. In rats, hyperoxia-induced alveolar simplification may be caused by the suppression of VEGF signalling [51,52]. Indeed, VEGF has protective effects on alveolarization after hyperoxia-induced injury [40,52-54] and prenatal stress [55]. Angiogenesis is also needed for compensatory lung growth after pneumonectomy [56-58].

Stem cells in lung development and repair

The identification of stem and progenitor cells in the lung has proven difficult for several reasons. A limitation of animal studies is that they often involve severe injury models in adults as pulmonary cells have low turnover rates during homeostasis [59]. Consequently, most studies are performed *ex vivo* by culturing primary cells. Stimulation with different cytokines gives an indication of which lineages they give rise to, however, it is often unclear if this holds true *in vivo*. Finally, the identification of some stem or progenitor cells is controversial due to their plasticity and shifts in the expression of markers over time [60]. Additionally, some differentiated cells can transdifferentiate into another cell type during injury, making them progenitor cells in some circumstances [61-63]. As developmental studies of human stem cells prove even more complex, the following information is based on mouse studies, which has been clarified to a much greater degree.

Club cells

Club cells are exocrine cells situated in the tracheal and bronchiolar epithelium [64]. In 3D *in vitro* culture, uteroglobin$^+$ cells (containing club cells and putative bronchioalveolar stem cells (BASCs)) differentiate into AT1 and AT2 cells [59], and into secretory, ciliated and alveolar cells when co-cultured with mesenchymal cells [66]. This highlights the potential importance of stromal cells in lung development and regeneration. *In vivo*, bleomycin- or influenza virus-induced lung injury causes uteroglobin$^+$ cells to give rise to AT1 and AT2 cells to repair the alveolar epithelium [62,65,67]. While it has been shown that uteroglobin$^+$ cells self-renew and give rise to ciliated cells during postnatal lung development, it has been suggested that they do not significantly contribute to normal development of the alveolar epithelium [64]. Thus, these cells may be present largely to give rise to alveolar cells following a severe injury *in vivo* [65].

Basal cells

BCs can restore Uteroglobin$^+$ cell populations in the bronchiolar airways following naphthalene challenge [68]. Acting as multipotent progenitor cells, they proliferate and give rise to Club and ciliated cells [68]. After injury, distal airway stem cells (DASCs) have been shown to restore the pulmonary epithelium by giving rise to AT1 and AT2 cells, as well as bronchiolar secretory cells [69]. Recently, human SOX9$^+$ BCs, the counterpart of murine DASCs, were transplanted into injured mouse lungs [70]. The human cells incorporated into the murine lungs and expressed markers for AT1 but rarely AT2 cells in the distal areas, and Club and ciliated cells in the bronchiolar region. The SOX9$^+$ population was unable to incorporate into healthy lungs [70]. A trial with two human bronchiectasis patients showed positive outcomes including improved lung function and less exacerbations when their cultured SOX9$^+$ cells were transplanted into their lungs [70]. Collectively, these data show the importance of BCs in pulmonary regeneration.

BASCs

In healthy lungs at the bronchioalveolar duct junction, a columnar cell positive for Uteroglobin and SP-C has been identified [73]. These cells are relatively sparse (about 1-3 cells per terminal bronchiole; 0.2% of Club cells [59]) and are resistant to naphthalene-induced injury [73]. After naphthalene or bleomycin treatment, BASCs temporarily increase, and *in vitro* cultures they can differentiate into Club, AT1 and AT2 cells. Cell tracing studies after bleomycin-induced lung injury revealed that Uteroglobin$^+$ cells, most likely BASCs, are capable of reconstituting AT2 cells [74]. Even though no increase in BASCs was observed after hyperoxia-induced injury, treatment with mesenchymal-stromal cells or their conditioned media ameliorated alveolar simplification and increased BASC numbers, implicating potential roles for these stem

cells in tissue regeneration. After pneumonectomy, BASCs increase in numbers, further suggesting a regenerative function [56].

AT2 cells

After birth, mature AT2 cells can self-renew and differentiate into AT1 cells. This has been observed in homeostasis and after injury [36,61-64], as well as *in vitro* [75-77]. AT2 cell expansion is important during alveologenesis and regeneration, and is caused by increases in Wnt-responsive axin2^{+} AT2 cells [78,79]. This alveolar epithelial progenitor, comprising ~20% of adult AT2 cells, acts as a progenitor for both AT1 and AT2 cells after injury [78,79]. An expansion of AT2 cells also occurs after pneumonectomy [56] and AT1 cells have the capacity to regenerate AT2 cells after injury [77,80].

Inflammatory responses in early-life affect lung development

BPD is a chronic lung disease that occurs in preterm infants and is defined by the need for long-term supplemental oxygen after birth. Its pathogenesis is multifactorial, with the main risk factor being prematurity, hyperoxia and mechanical ventilation. Additional risk factors include genetic susceptibility, maternal smoking and sepsis. As a consequence of disrupted lung development and insufficient gas exchange, supplemental oxygen is provided. BPD is characterized by alveolar simplification, disrupted angiogenesis, airway remodelling, and lung impairment can be persistent [81-84].

In mice, BPD can be modelled by exposure to hyperoxia, leading to increased inflammation and decreased alveolarization [38,53,85]. These changes are likely mediated through colony stimulating factor 1 receptor (Csf1r)-positive monocytes/macrophages [86] and increases in the pro-inflammatory cytokine IL-1β [85].

Overexpression of IL-1β in pulmonary epithelial cells [87] and prenatal exposure to IL-1β [93] mimic the phenotype of BPD and cause pulmonary inflammation and emphysema [94]. The defects in alveolarization following early-life IL-1β-administration can persist into adulthood [95], and can be partly rescued by treatment with IL-1 receptor antagonist (IL-1Rα) [85,96]. Interestingly, IL-4/-13-activated macrophages are a natural source of IL-1Ra [97].

Similar effects on murine lung development were observed following lipopolysaccharide (LPS) administration [98-100]. Early postnatal exposure impaired alveolarization and angiogenesis [98-100], which could be alleviated by administration of vitamin D through reducing interferon (IFN)-γ [101]. Inhibiting NFκB-signalling worsened the LPS-induced phenotype, suggesting an anti-inflammatory function in the developing lung [98,102]. NFκB-signalling is also beneficial in hyperoxia-induced lung injury [103,104], compensatory lung growth after pneumonectomy [105], and has roles in alveolarization [45,106,107] and angiogenesis [45,46]. However, during the saccular stage, overexpressing NFκB in the airway epithelium induces inflammation and disrupts lung development [108], and its activation in macrophages causes aberrant lung morphogenesis upon LPS-exposure [109]. Such differences may be caused by NFκB-activation in different cell types and developmental stages, and through action of different pathways and NFκB-subunits [110].

Recently, it has been shown that intrauterine infection with *E. coli* causes lung developmental defects in rats [111]. These were accompanied by reduced alveolar vesicular structure, less alveoli, increased mRNA expression of inflammatory molecules and decreased levels of surfactant proteins [111]. Taken together, it appears that inflammatory stimuli and responses have detrimental effects on both pre- and postnatal lung development.

Type 2 immunity in murine lung development and regeneration

Several type 2 immune cells, including group 2 innate lymphoid cells (ILC2s), mast cells, eosinophils and basophils as well as alveolar macrophages (AMs; in this review AMs may include interstitial macrophages) occur low in numbers at birth but increase during alveolarization, peak around P7-P14, and then decrease in adulthood (figure 1) [112-114]. Thus, these cells peaking during the period of maximal pulmonary remodelling suggests possible roles for type 2 immunity in lung development. This peak is associated with increases in IL-33 levels after birth, a cytokine secreted from AT2 cells due to changes in pulmonary pressure and mechanical stress during parturition [112,113]. IL-33 skews type 2 responses by expanding [115] and activating ILC2s [112], stimulating macrophages [116] and by inhibiting the expression of IL-12p35 in dendritic cells (DCs) (figure 2) [112]. IL-33 may play roles in lung development by activating ILC2s, which in turn regulate homeostatic and repair processes in the lung [117]. However, IL-33-deficient mice did not show any abnormal lung structure [112,113] suggesting that other factors may compensate for a lack of IL-33. Mechanical tension-induced IL-33 secretion is seen in AT2 cells [112,113] and fibroblasts [118]. Considering that mechanical stress is involved in several aspects of lung development, including tube morphogenesis, branching and pneumocyte differentiation [9,10,13], it is intriguing to speculate that it may activate type 2 responses during development.

Recently, ILC2s were shown to only become responsive to IL-33 weeks after birth, however, *in vitro* the presence of IL-33 and other co-stimulatory factors such as IL-2, IL-7 or thymic stromal lymphopoietin (TSLP) activated ILC2s from E17, marked by IL-5 and IL-13 production [119]. Lineage commitment of ILCs occurs in foetal liver whereas terminal differentiation happens in peripheral tissues facilitated by PDGFRα/podoplanin^{+} mesenchymal cells [119]. These data suggest that mesenchymal cells help differentiating ILC2s and induce their secretion of IL- 5 and IL-13. Both cytokines are involved in airway remodelling [120,121]. Indeed, crosstalk between lung epithelial cells and ILC2s has been identified in

models of allergic airway inflammation [122]. In response to chitin or helminths, AT2 cells release IL-33 and TSLP, which then activate ILC2s and stimulate IL-5 and IL-13 production through autocrine IL-9 signalling [122]. In a murine influenza model, lung resident ILC2s were involved in tissue homeostasis and promoted airway epithelial integrity through the secretion of amphiregulin [117]. Crosstalk between ILC2s and epithelial tuft cells has recently been identified in remodelling of the small intestine [123]. Collectively, these data suggest that there is a bidirectional relationship between ILC2s and lung structural cells.

Further crosstalk occurs with endothelial cells as in sepsis, ILC2s protect these cells from pyroptosis [115]. Though ILC2s express VEGFA [124], it is unknown whether they actively participate in angiogenesis (figure 2). Similarly, it is unclear whether AMs stimulate angiogenesis, though IL-4/IL-10-activated macrophages are well known promoters of angiogenesis [125]. Angiogenesis, in turn, is needed for proper alveolarization (figure 2) [42-44].

Macrophages have important roles maintaining tissue homeostasis in various organs, including the lung [127]. In the developing lung, they are detected as early as E10, can be found throughout foetal pulmonary development (figure 1) [109], and increase in numbers during alveolarization [113,114]. AMs are a heterogeneous population that display functional plasticity and therefore, classification into M1 and M2 subsets is oversimplified and an obsolete nomenclature system [128-131]. AMs that express YM1, arginase-1, CCL17 and mannose receptor are present in early-life [85,113,114,132], and a similar subset increases after pneumonectomy in adult mice [133]. Expression of these markers has generally been associated with type 2-mediated macrophage activation, which is induced by IL-4,-10 or -13 following stimulation such as by an infection [134]. Macrophages activated in this way are generally thought to be anti-inflammatory and stimulate tissue repair and remodelling [125,130,135,136].

In the lung, AMs are plastic and may change phenotype depending on the conditions. This can be seen during development [85,114,132,137] and in disease [85,86,129], and may affect macrophage function. Type 2 cytokines such as ILC2-derived IL-13 or epithelial-derived IL-33 promote AMs to express increased levels of YM1, arginase-1, CCL17 and mannose receptor [113,116]. Recently, it has been identified that IL-33 induces a unique gene expression profile in lung basophils [137]. These cells are located near alveoli and macrophages, and polarize AMs to an anti-inflammatory phenotype [137]. These AMs are located at branch points during embryonic lung development and are present in increased numbers during postnatal alveolarization [114]. LPS-activated macrophages inhibit airway branching through NFκB signalling during the saccular stage (E14-18), suggesting a dual role of macrophages in lung development depending on their activation stimulus [109]. The fact that AM expression profiles change during development may further indicate roles for them in alveolarization [85,114,132]. AMs have also been implicated in compensatory lung growth after pneumonectomy [138]. While increased and in close proximity to AT2 cells after pneumonectomy, *in vitro* studies showed positive effects of these macrophages on AT2 survival and proliferation (figure 2) [133]. Additionally, in this setting, ILC2s were increased and a source of IL-13, which promotes pulmonary regeneration [133]. Interestingly, pneumonectomy studies also revealed a role for mechanical stress in regeneration and AT2 development [139]. Mechanical stress causes actin cytoskeleton remodelling, which in turn leads to the proliferation of AT2 cells and subsequent differentiation into AT1 cells. First breath-induced mechanical forces promote IL-33 release from AT2 cells [112,113], which stimulates ILC2s to release IL-13 and stimulate macrophages (figure 2) [113,116]. This raises the possibility that developmental and regeneration processes involve similar mechanisms.

Consequences of aberrant lung development in later life

Human aberrant lung development has negative consequences for both infants and adults. Neonates diagnosed with BPD have reduced lung function and respiratory symptoms that persist into adulthood. BPD survivors report increased wheeze, shortness of breath, and are more likely to develop asthma [140,141] and airway obstruction [142]. A similar phenotype is observed when comparing hyperoxia-exposed with control mice in adulthood. Neonatal hyperoxia leads to increased mortality and vascular differences indicative of pulmonary hypertension [143]. Other effects include changes in lung structure and function, airway hyperresponsiveness and asthma-like features [143-148]. Administration of IL-1β during the first postnatal days, mimicking the symptoms of BPD, results in permanent changes in lung structure, which are still detectable at 12 weeks of age [95].

Early-life respiratory infection may also cause abnormal lung development and increased susceptibility to chronic lung diseases [149-153]. Human respiratory infections in infancy reduce lung function and increase susceptibility to asthma in children and adults [154-156]. Early-life intranasal infection of mice with *Chlamydia muridarum* increases the severity of allergic airway disease in later life [149-151,153,157-159]. Even in the absence of allergen challenge, respiratory infections of neonatal and infant mice causes long term impairment of lung function [149,150]. The age of infection is critical in determining the impact on alveolar structure and lung function. Only neonatal (day 0) but not infant (3 weeks) or adult (6 weeks) infection causes emphysema-like alveolar enlargement. The infection-induced impairment of alveolar structure involved tumour necrosis factor-related apoptosis-inducing ligand-signalling [150]. Additionally, both IL-13 [151] and programmed death-ligand 1 [159] have been implicated in the development of early-life infection-induced persistent airway hyperresponsiveness. Furthermore, respiratory *C. muridarum* infection in infant (3 weeks) but not neonatal or adult mice altered hematopoietic cells to induce more severe allergic airways disease [153].

Maternal *in utero* smoke exposure, particularly when combined with early-onset asthma, reduces lung function in children [160] and adolescents [161-163], and increases the risk of wheeze and asthma [161,164]. A murine model of intrauterine smoke exposure showed changes in airway remodelling, increased responsiveness to methacholine and allergic stimulation in 10-week old offspring [165]. Neonatal smoke exposure modifies the immune response and increases oxidative stress in neonatal mice and lowers lung volume and number of alveoli in adult mice [166]. This highlights that both pre- and postnatal stimuli can influence lung development and susceptibility to subsequent allergic diseases.

In summary, early-life events such as mechanical ventilation or respiratory infection may change the alveolar structure due to aberrant postnatal alveolarization. These effects can persist into adulthood, further highlighting the importance of understanding lung developmental processes as a strategy to prevent the development of chronic respiratory diseases in later life.

Conclusions

Lung development involves complex interactions between different cell types, mediators and environmental factors. We propose that type 2 immunity may have an underappreciated role in lung development. Although there are findings implicating roles for type 2 immune cells in lung development, their exact contributions remain to be elucidated. It is important to fully understand the mechanisms behind pulmonary development and alveolarization to be able to intervene in aberrant processes. Future studies will be critical in determining whether promoting type 2 responses may be a novel intervention to promote normal postnatal alveolarization and healthy lung development. This may be critical in preventing chronic respiratory diseases.

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Statement of author contributions

SL drafted the manuscript and figures. GJMC assisted with literature review and editing of manuscript. MRS and PMH conceptualised, reviewed and edited the manuscript.

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Figure legends

Figure 1. The process of embryonic and postnatal lung development. The development of the lung can be divided into 5 different stages; embryonic, pseudoglandular, canalicular, saccular and alveolar. In the embryonic stage, the lung buds start to separate from the foregut, whereas the pseudoglandular stage is where the clear separation of the trachea and branching of the bronchi occurs. In the canalicular stage, distal airways are formed, and both columnar and ciliated cells can be found. During the saccular stage and the formation of saccules, further cell differentiation occurs and basal cells, alveolar type 1 (AT1) and type 2 (AT2) cells, and their bipotent progenitor can be identified. After sacculation, in the alveolarization stage, alveoli start to form from pre-existing septa. While lung macrophages can be detected as early as embryonic day (E) 10, increases in alveolar macrophages, group 2 innate lymphoid cells (ILC2) and the type 2 cytokine interleukin (IL)-33 occur during the first postnatal days, peaks during postnatal days (P) 7-10, the time of maximal alveolarization, and decreases in adulthood as the lung matures.

Figure 2. Interactions between type 2 immune cells and the pulmonary epithelium in lung development and repair. Mechanical stress, for instance initiated by first breath, causes the release of interleukin (IL)-33 from alveolar type 2 (AT2) cells and fibroblasts. IL-33 in turn inhibits type 1 responses by acting on dendritic cells (DCs) while inducing activation of type 2 immune cells such as group 2 innate lymphoid cells (ILC2) and macrophages. Due to their localization at branch points during lung development and a positive effect on AT2s survival and proliferation *in vitro*, M2 macrophages are thought to be involved in pulmonary regeneration and development. Both ILC2s and M2 macrophages are increased in the lung

during the time of maximal alveolarization. The exact role of ILC2s is unknown but they have been shown to produce vascular endothelial growth factor (VEGFA) and IL-13, both of which are involved in angiogenesis, which is required for optimal alveolarization. Black line: cytokine production, green line: positive effect/stimulation, yellow dashed line: potential interaction/effect, red line: inhibition.