Lung growth: implications for the newborn infant

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Introduction
Modern neonatal practice has improved the outcome of extremely preterm infants. However, why some infants require prolonged periods of respiratory support while others improve after a short period of mechanical ventilation, remains largely speculative. Many risk factors, including barotrauma or volutrauma due to mechanical ventilation, oxygen toxicity, and infection, have been identified for the development of chronic lung disease of prematurity (CLD). Attempts to minimise these with modern neonatal practice, including newer ventilatory techniques, have had minimal impact on its incidence. Factors other than barotrauma and oxygen toxicity are likely to be important in the development of CLD.

Although our understanding of normal fetal lung development has increased substantially over the past few years, it nevertheless remains rudimentary, especially in infants who have survived neonatal intensive care. Animal models have provided many clues to the effects of interventions in the neonatal unit on the lung growth of preterm infants. Normal lung growth and some of the abnormalities that may result from disordered growth or from medical interventions are reviewed in this article. There are a vast number of other factors which influence lung growth—some, such as fetal breathing and lung fluid dynamics, deserve reviews of their own.

Normal lung growth
Normal lung development, which occurs as a series of complex tightly regulated events, can be divided into a number of stages (table 1). During the earliest embryonic stage, the lung develops as an outgrowth of the ventral wall of the primitive foregut endoderm. Epithelial cells from the foregut endoderm invade the surrounding mesoderm to form the proximal structures of the respiratory tract. Following the formation of the trachea and the main bronchi, the five lobes are formed, and by the end of this stage, the 18 major lobules are recognisable. Current evidence suggests that the surrounding mesoderm regulates the branching of the tracheobronchial tree. At the end of this stage, the pulmonary arteries develop from the sixth aortic arches and accompany the branching airways.

The embryonic phase is followed by the pseudo-glandular stage—so-called because the epithelial tubules are surrounded by thick mesenchymal tissue. Branching of the airways and vessels continues, and by the end of this stage the conducting airways, terminal bronchioles, and primitive acinus, are completed. The pseudo-stratified columnar epithelium is progressively replaced by tall columnar cells in the proximal airways and cuboidal cells in the distal acinar structures.

During the canalicular stage, which occurs between 16 and 26 weeks in utero, further development of the distal airways into definitive primary acini occurs. The acinar structures consist of respiratory bronchioles, alveolar ducts, and rudimentary alveoli. Development of the intracinar capillaries, which are derived from the surrounding mesenchyme, accompanies the evolution of the acinus. Lamellar bodies containing surfactant proteins and phospholipid in type II pneumocytes can be observed lining the acinar tubules at this stage. Differentiation into type I pneumocytes occurs in conjunction with the formation of the alveolar–capillary barrier.

The saccular phase begins with marked enlargement of the peripheral airways as the acinar tubules dilate and the walls thin, resulting in increased gas exchanging surface area. Lamellar bodies in type II cells increase and further maturation into type I cells occurs. Capillaries are closely associated with type I cells, thus reducing the distance between the future air–blood interface.

The secondary alveolar septa are formed during the alveolar stage, which occurs from 36 weeks of gestation until at least 24 months postnatally. The secondary septa consist of projections of connective tissue and a double capillary loop. Alveolar formation and maturation occur, with thinning of the alveolar walls and remodelling of the double capillary loops by apoptosis to form a single capillary loop. During this stage marked proliferation of all cell types occurs. Mesenchymal cells proliferate and deposit the necessary extracellular matrix. Epithelial cells, especially type I and II pneumocytes, increase in numbers to line the alveolar walls, and endothelial cells undergo massive growth in the secondary septa with...
Table 2 Growth factors that may have a role in normal lung growth

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Regulation of lung growth

Most of our knowledge about lung growth is derived from the study of animals which often have very different timing of morphological lung growth compared with humans. For instance, in sheep most of the alveolar development occurs before birth. Rats and mice may be more useful models of human lung growth as most alveolar development occurs postnatally.

Despite widespread interest in this area, our understanding of the mechanisms involved in normal lung growth remains limited. Table 2 shows the increasing list of transcriptional and growth factors which are implicated in normal lung growth. Hepatic nuclear factor-β (HNF-β) seems to be required for the formation of the foregut from which the primitive lung bud is derived. Genetic disruption of HNF-β disrupts formation of the foregut endoderm and its derivatives, including the lung. In human neonatal lung, it is present in type II pneumocytes as well as ciliated and non-ciliated epithelial cells. HNF-β also influences expression of other nuclear factors including thyroid transcription factor 1 (TTF-1). TTF-1 mRNA is detected in rat primordial lung and the protein has been detected as early as 11 weeks of gestation in human lungs. TTF-1 seems to increase expression of the surfactant proteins, at least in vitro, and its ablation by genetic targeting impairs lung morphogenesis, resulting in hypoplastic lung with poorly differentiated epithelium and poor gas exchanging areas. Interactions between the transcription factors are likely to be more complex than described above and very tightly regulated.

As with transcription factors, our understanding of the role of growth factors remains in its infancy. The number of growth factors identified continues to increase (table 2), but their exact role in both normal lung development and in abnormal repair processes after acute or chronic lung disease, remains largely rudimentary. Proliferation of cells forming the respiratory airways seems to depend on several of these growth factors, including keratinocyte growth factor (KGF). KGF seems to promote epithelial cell proliferation and the resulting branching of the airways. Disruption of its receptor FGFR2 in epithelial cells of the airways results in blockage of dichotomous branching of the conducting airways. By contrast, transforming growth factor β (TGF-β) inhibits branching morphogenesis, epithelial cell growth, and differentiation of fetal lung explants. TGF-β decreases with increasing gestation, which removes the inhibitory effects of this growth factor and allows branching to proceed. Other growth factors which may be important are listed in table 2. A more comprehensive review of growth factors and their importance in normal lung growth is discussed elsewhere.

Many of these growth factors are produced by the mesenchyme surrounding the lung epithelial cells. Indeed, the mesenchyme directs the ultimate destiny of the epithelial cells. For instance, salivary epithelium grown on mammary mesenchyme results in mammary gland morphology, and transposition of the bronchial mesenchymal to the peripheral airways results in a bronchial-like morphology. Further weight to the importance of the mesenchyme in directing the epithelial development is given by the presence of the mRNA of growth factors in the mesenchyme and the corresponding protein in epithelial cells—for example, KGF and IGF. The mesenchymal–epithelium interactions may result from direct cell to cell contact by soluble molecules, including growth factors (paracrine) or by cell–extracellular matrix interactions.

Factors which may affect lung growth

DEVELOPMENTAL ABNORMALITIES

Lung growth may be affected by several factors. During development of the pulmonary tree, laryngeal, tracheal, or esophageal atresia; tracheal stenosis; tracheo-oesophageal atresia or fistula; pulmonary agenesis; arterio-venous malformations or congenital lung cysts (including bronchogenic cysts) may develop during the embryonic stage. Pulmonary sequestration, pulmonary hypoplasia or lymphangectasia, congenital cystic adenomatous malformations, and lung cysts may develop during the pseudo-glandular stage. Failure of the pleuro-peritoneal membranes to close at this stage may lead to the formation of a congenital diaphragmatic hernia (CDH). During the canalicular stage, pulmonary hypoplasia may be seen, often secondary to oligohydramnios or prolonged rupture of membranes. Preterm birth occurring during the canalicular stage is very likely to lead to severe respiratory distress because of poorly developed peripheral airways and poor maturity of cells important to lung maturation—for example, poor surfactant production by type II cells and inadequate antioxidant responses to increased ambient oxygen. Poor development of the alveolar–capillary interface during the saccular phase may result in alveolar–capillary dysplasia, and other abnormalities during this period include pulmonary hypoplasia, acinar dysplasia, and respiratory distress syndrome if the fetus is delivered after preterm labour.

subsequent remodelling to form a single capillary loop from a double one. The net result is a great increase in gas exchanging surface area and maturation of cells which will respond to the postnatal environment.

Due to the difficulties of estimating alveolar numbers at birth, numbers ranging from 20 million to 50 million have been quoted. A final number of around 300 million is reached by adulthood.

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The newborn term or near term infant will have respiratory distress if the underlying developmental abnormality is clinically significant and may be compromised further by postnatal disorders such as persistent pulmonary hypertension of the newborn, meconium aspiration, respiratory distress syndrome, etc. Development of the lung will also be affected by developmental abnormalities of other organs. Both anencephaly and renal hypoplasia cause pulmonary hypoplasia as do abnormalities of the thoracic cage—aplasia or hypoplasia of the sternum, rib cages, vertebral column, ribs, and diaphragm. It is of importance to note that these abnormalities are associated with a high incidence of lung hypoplasia. In general, any restriction to lung expansion reduces lung growth. Restriction to either results in lung hypoplasia, and overdistension of the lung by lung fluid results in overdistended lungs. In contrast, increased fluid in the fetal lung seems to promote or accelerate lung growth. Both fetal breathing and the volume of amniotic fluid are critical to the development and growth of the lungs. In general, any restriction to either results in lung hypoplasia, and overdistension of the lung by lung fluid improves lung growth.

Factors which may restrict normal fetal breathing include abnormalities of the chest wall; intrathoracic space occupying lesions such as congenital diaphragmatic hernia and decreased amniotic fluid result in lung hypoplasia. In a series of experiments Wigglesworth and Desai clearly showed that fetal breathing was critical to normal lung growth as ablation of the phrenic nerve substantially decreased lung growth. Similarly, decreased amniotic fluid as a result of decreased production—as in renal hypoplasia—or increased leak due to premature rupture of membranes, decreases lung growth. Whether this is due to restricting fetal breathing or to properties of the fluid itself is unclear. In contrast, increased fluid in the fetal lung seems to promote or accelerate lung growth: in laryngeal atresia the lungs are increased in volume, surface area, and alveolar numbers. Tracheal plugging has been used in both animal models and in humans to improve lung growth. The exact mechanisms underlying the influence of fetal breathing and amniotic fluid on lung growth are not entirely clear. Certainly, stretch is likely to be important as this release many growth factors which are important to lung growth.

CORTICOSTEROIDS

The effects of antenatal and postnatal administration of corticosteroids have recently been reviewed. Antenatal administration of corticosteroids accelerates lung growth by several mechanisms, including maturation of type II pneumocytes, thinning of the double capillary loops during the saccular and alveolar stages of lung development, and partial suppression of the formation of secondary septa. Although the normal thinning of the double capillary loops to form thin gas exchanging walls occurs rapidly, thus increasing alveolarisation, the final numbers of alveoli are decreased. Corticosteroids may also decrease somatic growth, so also affecting lung growth. Similarly, postnatal administration of corticosteroids to newborn animals with doses similar to those used in human infants seems to accelerate alveolarisation with decreased final numbers of alveoli. It is somewhat reassuring that antenatal treatment with corticosteroids in humans seems not to affect lung function at 7 years of age, although it should be noted that lung function is a very insensitive marker of lung growth.

NUTRITION

By increasing or decreasing the litter size, nutrition can be increased or decreased artificially. Enhanced nutrition increases surface area, but not septation, and decreased nutrition during fetal life seems to impair septation and surface area, but not the final alveolar size. However, prolonged deprivation of nutrition may increase both surface area and alveolar size. Restriction of protein intake during fetal development decreases somatic growth as well as reducing lung volumes. Specific lung volumes—that is, volume per body weight—were, however, increased which suggests that the lung is less affected by malnutrition than the rest of the body, or that the lack of elasticity results in overdistended lungs. Interestingly, re-feeding results in catchup growth, with normalisation of specific lung volume, suggesting that the potential for recovery is present despite severe fetal malnutrition. Fetal malnutrition therefore seems to decrease lung volumes but not the maturation of the pulmonary airways.

Postnatal food restriction may exacerbate lung injury, as has been shown by Langley and Kelly. 72 hour food restriction resulted in increased mortality in preterm guinea pigs exposed to hyperoxia whereas mortality was unaffected in animals exposed to air. Although acute lung injury was shown, this was not thought to be due to an alteration of the antioxidant enzymes because these were unaffected by starvation.

OXYGEN TOXICITY

Both hypoxia and hyperoxia disrupt septation and the ultimate gas exchanging surface area. Although newborn animals are more resistant to hyperoxia than adults, increased oxygen, via the formation of reactive oxygen species including superoxides and hydroxyl ions, severely disrupts alveolarisation in animal
models.\textsuperscript{27, 28} Even after recovery from hyperoxic exposure, persisting abnormalities remain in lung morphology. Recently, Warner \textit{et al} reported that mice exposed to 85% oxygen for 28 days had decreased pulmonary septation, increased terminal space diameter, and decreased surface area. These effects may be mediated through an increase in pulmonary inflammation as pro-inflammatory cytokines were greatly increased.\textsuperscript{29} In the baboon model, using preterm animals, oxygen toxicity produces a similar picture of decreased alveolisation, decreased surface area, increased alveolar size and increased pulmonary inflammation.\textsuperscript{30} Pulmonary inflammation is likely to be important in disrupting normal alveolisation but the exact sites and mechanisms whereby septation is disrupted by hyperoxia are not well understood.

**PULMONARY INFLAMMATION**
Margraf \textit{et al} have shown that lung growth, as estimated by alveolisation, surface area, and mean linear diameter (which reflects airway size), is substantially affected in infants who die from chronically lung disease of prematurity (CLD).\textsuperscript{31} Both pre- and postnatal factors are likely to decrease alveolisation. The baboon model of CLD, where the animals are delivered at a pre-determined time, suggests that postnatal factors can produce disease similar to CLD, including decreased alveolisation.\textsuperscript{32} Mechanical ventilation may be one important factor,\textsuperscript{33} but others such as oxygen toxicity and infection may all affect lung growth (fig 1). The common pathway whereby lung growth is affected is likely to be mediated by pulmonary inflammation. Pulmonary inflammation in infants has been reported by many groups.\textsuperscript{34–36} It is tempting to speculate that the risk factors for CLD result in pulmonary inflammation as a common pathway through which lung growth is compromised (fig 1). Ante- and perinatal factors, which have been poorly investigated in humans, may also be equally important in compromising lung growth in preterm infants. The uterine environment, especially if there is infection due to chorioamnionitis, is associated with the development of CLD,\textsuperscript{37} but other factors such as compromised lung growth of the fetus itself may contribute toward the development of CLD, especially if this requires a need for respiratory support at birth. There are few data on CLD in humans, but we have reported an increase at birth of the potent pro-fibrotic growth factor TGF-\(\beta\), in infants who develop CLD. In addition to promoting synthesis and deposition of the extracellular matrix, this also inhibits branching morphogenesis which may then explain the decreased alveolisation seen in CLD.\textsuperscript{38} These data suggest that perinatal or antenatal factors may be important in the development of CLD. Further work in this area is impeded by the lack of adequate methods to assess lung growth in the human fetus or newborn infant.

A model such as that shown in fig 1 may be useful in helping us to understand some of the underlying mechanisms that lead to the development of CLD. In the infant who develops respiratory distress syndrome, respiratory support with mechanical ventilation and oxygen treatment are likely to be required. As shown by the baboon model,\textsuperscript{39} lung growth is affected by these postnatal factors. The infant who is well at birth may sometimes progress to oxygen dependency (often called Wilson-Mikity syndrome). The newborn infant does not develop respiratory distress syndrome due to adequate surfactant and antioxidant systems. However, other risk factors for the development of CLD, such as infection, gastro-oesophageal reflux, or fluid overload may exacerbate abnormal perinatal fetal lung development. Although the infant can adapt at birth, continuing minimal insults may compromise the lung such that oxygen treatment is needed. Proving such an hypothesis is difficult because current methods are very poor at assessing lung growth—alveolisation, for example—at birth, and lung function tests do not have the sensitivity to detect subtle lung growth abnormalities.

**Strategies to mature the lung**
Antenatal corticosteroids can result in maturation of the surfactant and antioxidant systems, but may also accelerate lung maturation.\textsuperscript{40} Thyrotropin releasing hormone (TRH) in animal models seems to mature the surfactant system but not the antioxidant enzymes.\textsuperscript{41} In clinical practice, TRH does not seem to affect the incidence of neonatal respiratory distress syndrome, mortality, or CLD.\textsuperscript{42} Recently there have been several publications on tracheal ligation for congenital diaphragmatic hernia (CDH) to promote maturation of the lungs.\textsuperscript{43} The data are encouraging in animal models, but in humans the results have been uniformly poor. Tracheal ligation of the human fetus with CDH has been attempted in North America, but since the infant might be born with an occluded airway this also remains highly experimental (Albanese C, presentation at the European Respiratory Society, Geneva, 1998).\textsuperscript{44} The techniques available to promote lung growth postnatally remain poor. Extracorporeal membrane oxygenation remains a rescue treatment for infants with severe respiratory

\begin{figure}[h]
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\caption{Model of lung growth. Top half shows prenatal factors that may be important in normal lung growth; each may cause inflammation, so affecting lung growth. Bottom half shows postnatal factors that may affect lung growth.}
\end{figure}
distress, but can also buy time to stabilise the child with CDH before performing surgery. Corticosteroids can accelerate lung growth in newborn rats with decreased final numbers of alveoli. The implications of this observation on human newborn infants are difficult to assess as few objective means of assessing lung growth are available. Whether ventilatory methods such as continuous positive airway pressure (CPAP), given for prolonged periods to distend the airways, can improve lung growth postnatally is also difficult to assess in human babies. Zhang et al recently reported that CPAP at 6.0 cm H2O in ferrets given tracheotomies results in increased total lung capacity together with increased lung weight, total lung protein, and DNA, suggesting that this mode of treatment may increase lung volumes at a critical time in newborn animals. The data must, however, be interpreted with caution as an increase in total lung capacity may not necessarily be due to improved pulmonary function or lung growth. Similarly, perfluorocarbons have been used in animal models to distend the lung in the hope of improved lung growth. Improved distension may only increase lung volume, but both lung growth and its function must be improved if this technique is to be recommended.

Whether any of the techniques or methods described above have a future in promoting lung maturation in human infants remains to be seen. It must be emphasised that the risk/benefit ratio for any new intervention must be fully assessed because the interventions themselves may be associated with significant adverse effects, such as infection, bleeding, or need for intubation.

In summary, the factors which are important in lung growth are clearly complex, with interactions of various tissues occurring in the face of multiple regulatory elements. Fluid in the airways maintained at a minimal pressure seems to be important and any interference with the mechanism of amniotic fluid production will adversely affect lung growth postnatally is also difficult to assess in human babies. Zhang et al recently reported that CPAP at 6.0 cm H2O in ferrets given tracheotomies results in increased total lung capacity together with increased lung weight, total lung protein, and DNA, suggesting that this mode of treatment may increase lung volumes at a critical time in newborn animals. The data must, however, be interpreted with caution as an increase in total lung capacity may not necessarily be due to improved pulmonary function or lung growth. Similarly, perfluorocarbons have been used in animal models to distend the lung in the hope of improved lung growth. Improved distension may only increase lung volume, but both lung growth and its function must be improved if this technique is to be recommended.

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