American Thoracic Society Documents

Mechanisms and Limits of Induced Postnatal Lung Growth

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INTRODUCTION

The mature lung has traditionally been assumed to be nonmalleable; structural changes were considered irreversible once normal alveolar development was completed, and treatment for chronic lung disease typically focuses on minimizing symptoms and preventing further tissue destruction. This approach is being revised in light of recent findings. There is growing clinical evidence of accelerated or "catch-up" lung growth in youngsters whose lung disease is no longer active. Several experimental models have shown the possibility of accelerating the rate of lung growth during and beyond the period of postnatal maturation. There has been new insight into the signals, mediators, and anatomic sites of postnatal lung growth as well as their interactions and physiologic correlates. Although much remains unknown, this field is poised on the verge of practical application, with potential therapeutic implications for the management of chronic lung disease, lung volume reduction surgery, and transplantation. This workshop was conducted to summarize relevant recent advances with the objectives of (*1*) promoting an integrative understanding of the potential for postnatal lung growth that encompasses not just gene expression and cell division, but also the complexities of tissue remodeling, structural interaction, and physiologic function; (*2*) providing an update on the mechanisms and functional limits of induced lung growth; and (*3*) identifying key issues for further investigation.

POSTNATAL LUNG DEVELOPMENT

Normal Development

Alveolarization. Transformation of the immature saccular lung with a limited gas-exchange area to a mature lung with a large internal surface area entails thinning of alveolar walls, growth of capillary network, and extensive subdivision of gas exchange units. This period is marked by interstitial fibroblast proliferation while epithelial cells flatten and decrease in number, resulting in a net thinning of distal airspace walls. Concurrently, alveolar capillary network becomes more complex. Alveolar septation begins as secondary crests that extend from primary alveolar walls. Development of these crests or septae occurs through deposition of new basement membrane, outgrowth of epithelial cells and myofibroblasts at the tips of septae, and elastin deposition (1). In humans, the process begins at approximately 36 weeks of gestation (2). At birth, only approximately 15% of alveoli have formed, with the remaining subdivisions developing in the first few postnatal years (2–5).

Bronchovasculoalveolar interaction. Vascular development occurs via two processes: (*1*) vasculogenesis, the development of blood vessels from the differentiation of angioblasts in mesoderm, and (*2*) angiogenesis, classically described as sprouting of blood vessels from existing vessels (6–8), but can also occur by "intussusception" (9), formation and growth of a transcapillary tissue pillar that eventually divides an existing capillary segment into two. The bronchial (systemic) vessels develop with preacinar airways; development is complete by approximately 16-weeks gestation with further growth in size to match lung growth (3). Bronchial vessels generally are not found in the peripheral acinar region and thus do not normally participate in alveolar gas exchange. Preacinar pulmonary arteries, supplied by the right heart, grow with the airways into the intra-acinar region and fuse with peripheral microvasculature that has arisen from the mesenchyme by vasculogenesis (8). It is not clear whether capillary invasion stimulates alveolar septation or vice versa, but alveolar septation is always associated with capillary invasion. The importance of vascular supply to alveolarization is demonstrated by studies using antiangiogenic agents such as fumagillin, thalidomide, and Su-5416, an inhibitor of the vascular endothelial growth factor (VEGF) receptor; these agents disrupt vascular development, leading to reduced pulmonary arterial density and inhibition of alveolar growth (10).

Bronchial arteries are responsive to angiogenic stimuli throughout life. Unilateral pulmonary artery ligation augments growth of bronchial arteries in lambs and mice (11, 12). In chronic pulmonary hypertension, bronchial vessels grow even as peripheral pulmonary vessels are lost (13). In lung disease, angiogenesis of the bronchial circulation does not support normal gas exchange, and the high pressure of these systemic vessels can lead

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to life-threatening hemoptysis. In regions of bronchial angiogenesis, local pulmonary vessels do not show angiogenesis, suggesting that the two vascular systems respond independently (14). There is only limited evidence supporting induced postnatal growth of intra-acinar blood vessels, as after chronic pseudomonas infection in rats (15) and in patients with veno-occlusive disease (16).

Although the lung is extensively innervated from early fetal life, postnatal denervation by cervical vagotomy and sympathectomy does not significantly affect subsequent lung growth or mechanics in growing rats and rabbits (17–19); hence, pattern and extent of growth are not critically dependent on neural control or communication.

Abnormal Development

Clinical syndromes. Syndromes that alter expression of growth factors, cytokines, transcription factors, or extracellular matrix (ECM) proteins provide models to explore mechanisms that control alveolar development. Signals that regulate the saccular-to-alveolar morphologic transition are poorly understood, but disrupting lung septation during this transition impairs alveolar formation, resulting in a loss of alveolar surface area (20, 21). There are several syndromes of abnormal lung alveolarization in humans. Trisomy 21 (Down syndrome) is associated with reduced lung volumes, alveolar surface area, fewer alveoli, defective elastin deposition, and vascular abnormalities (22, 23). Leprechaunism, caused by a defect of the insulin/insulin-like growth factor-I receptor, is associated with reduced lung surface areas as well as fewer and larger alveoli (24). Oligohydramnios (25), congenital diaphragmatic hernia (CDH) (26), and intrathoracic mass lesions are all associated with pulmonary hypoplasia caused by the lack of interplay between thoracic expansion and stretch imposed on the lung. Lung hypoplasia in CDH is not just a consequence of mechanical lung compression caused by a hole in the diaphragm but also involves primary developmental defects (27). Prenatal tracheal occlusion markedly increases lung size in some fetuses with severe CDH; however, survival remains poor because of respiratory insufficiency and prematurity (28). After CDH repair, significant postnatal alveolar growth and vascular remodeling are seen (26); long-term survivors show lower lung volumes but normal airway function and exercise tolerance (29), suggesting the occurrence of "catch-up" lung growth once the underlying abnormalities are corrected.

By far the most common cause of abnormal postnatal human lung development is bronchopulmonary dysplasia (BPD). Classically, BPD occurs in the preterm infant lung exposed to hyperoxia and mechanical ventilation (30–32), resulting in extensive alveolar fibroproliferation, bronchovascular smooth muscle hyperplasia, and inhibition of distal lung formation leading to long-term pulmonary dysfunction persisting into adolescence and adulthood. The advent of antenatal steroids, postnatal surfactant replacement, and improved intensive care has ushered in the "new BPD," which lacks the severe bronchovascular lesions and interstitial fibrosis but is characterized by abnormal lung development with simplified acinar structure, poorly formed secondary crests, dysmorphic alveolar capillaries, and blunted expression of angiogenic growth factors and their receptors (33–36). There is little information on the pathology of "new BPD" in infants who survive; it remains to be seen whether long-term "catch-up" lung growth occurs in these survivors.

MODELS OF INDUCED POSTNATAL LUNG GROWTH

In general, larger species exhibit more mature lungs at birth. Alveolar size is similar among mammals at birth but increases more postnatally in larger species than in smaller ones, associated with a thicker septal interstitium, which contains more collagen and elastin fibers to bear the force of lung distension (37, 38). Rodents are born with the lung in the saccular stage; alveolarization occurs exclusively from Days 4–14 after birth, followed by alveolar wall thinning between Days 14–21 (39). Because rodent epiphyses never close (40), thoracic growth and alveolarization persist throughout life (41). In comparison, most of the alveoli in guinea pig lungs have already formed at birth (42, 43) containing more septal connective tissue (44) and less pulmonary vascular smooth muscle (45). Guinea pigs also undergo epiphyseal closure beginning at approximately 5 months of age (46), suggesting that older animals reach an upper limit of thoracic and hence lung size. In rabbits, alveoli continue to form after birth into adulthood (36 weeks), but septal thinning occurs mostly before birth (47). Their alveolar blood–gas barrier is thinner with a higher capillary wall stress compared with larger species (dog and horse) (48, 49); septal thickness and capillary wall strength increase with postnatal maturation (50).

There are no major differences between dogs and humans in the characteristics of lung structure or function during postnatal maturation and aging (51). Because they are highly aerobic and easily trained, dogs are used in exercise studies to define the functional correlates of lung growth. Sheep are born with well-formed alveoli; postnatal growth only modestly increases alveolar complexity and total alveolar number (52). Pigs are born with alveolar–capillary surface densities and air–blood tissue barrier thickness already at adult levels (53). Airspace expansion and septal thinning during the first postnatal week increases elastic recoil, followed by vigorous septal subdivision and thickening (54, 55). Lung development in primates closely mimics that in humans, although postnatal alveolar growth in monkeys has been attributed to an increase in alveolar size rather than an increase in number (56). Primates that assume an upright position (the great apes) show an elongated thoracic cross-section in the lateral direction and similar anatomic relationships among lungs, thorax, heart, and diaphragm as humans. Primates that are quadrupeds (monkeys and baboons) have an elongated thoracic cross-section in the anteroposterior direction and an additional infracardiac lung lobe separating the heart from the diaphragm; their mechanical interactions among intrathoracic structures are more similar to that of nonprimate quadrupeds (57). Across mammalian species, adult dimensions of alveolar surface area and diffusing capacity (D_L) are directly related to body mass and aerobic capacity (58), indicating an evolutionary match between lung growth and functional demands of the species.

One fundamental difference between prenatal and postnatal lung growth is that prenatal alveolar tissue arises from undifferentiated mesenchyme, whereas postnatal alveolar growth is constrained by an already highly differentiated scaffold. Hence, adaptive strategies may also differ. Strategies that have been attempted to induce postnatal lung growth experimentally are summarized later here and in Table 1.

Increased Metabolic Oxygen Demand

Early observations that champion swimmers possess greater lung volumes and lung D_L (59) inspired attempts to stimulate lung growth by increasing chronic O_2 flux across the lung via increased metabolic O_2 consumption (60–63), leading to conflicting results. Cold exposure modestly increases lung tissue volume, alveolar–capillary surface areas, and D_L in rats (62) but not guinea pigs (60). Exercise training does not increase D_L in rats (61) or guinea pigs (43, 60). The higher D_L in Japanese Waltzing mice (63) is complicated by species differences in lung metabolism, protein synthesis, and pneumocyte mitochondrial characteristics (64). Increasing O_2 uptake and D_L in hyperthyroidism may or may

TABLE 1. EXPERIMENTAL STRATEGIES TO INDUCE POSTNATAL LUNG GROWTH

Strategy	Example	Effect on Lung Growth (*Reference No.*)
Increase oxygen demand	Exercise	No effect in rats and guinea pigs (43, 60, 61)
	Cold exposure	Enhanced in rats but not guinea pigs (60, 62)
	Hyperthyroidism	No effect (61) or modest effect (65) in rats
Mechanical strain	Hypercapnia-induced hyperventilation	No effect in normoxic guinea pigs (138) May enhance hypoxia-induced lung growth in rats (488)
	Continuous positive airway pressure	Enhances lung cell proliferation in ferrets (71)
	Liquid lobar distension	Enhances alveolar growth in neonatal lambs (72)
	Major lung resection	Enhances growth and function of remaining lung in small and large species (77, 81, 84, 87, 95, 439, 464, 489, 490)
	Increase pulmonary perfusion	Enhances alveolar growth in young pigs (181); no effect on lobar DNA synthesis in pneumonectomized adult ferrets (186)
	Lobar lung transplant into a growing thorax	Enhances alveolar formation (104, 105) or causes simple alveolar dilation (103, 106) in graft
Hypoxia	Hypoxia +/− hypobaria (high altitude)	Accelerates alveolar growth in young rats, guinea pigs, and dogs (122, 124–126, 136, 137, 491)
Hormones	Growth hormone	Increases lung volume because of a larger thorax in rats (249)
	Adrenal corticosteroids	Enhance fetal lung maturation, inhibits postnatal alveolar growth (253, 258, 492), synchronizes compensatory lung growth in rats (265)
Growth factors	EGF	Enhances lung maturation in fetal monkeys and rabbits (280, 493) and after lung resection in adult rats (279)
	KGF	Causes transient hyperplasia of type II pneumocytes and Clara cells (285, 494–496) and attenuates induced lung injury in rats (497)
	HGF	Enhances lung angiogenesis in rats and postpneumonectomy DNA synthesis in mice (304, 306)
	Retinoic acid	Enhances alveolar septation in young rats (159, 256), emphysematous rats (381) and adult rats after pneumonectomy (382); no enhancement in adult emphysematous mice (383). Enhances alveolar capillary endothelial cell growth in adult dogs after pneumonectomy (385)

Definition of abbreviations: EGF = epidermal growth factor; HGF = hepatocyte growth factor; KGF = keratinocyte growth factor.

not alter lung structure in rats (61, 65). Although metabolic O_2 demand and mechanical stress of exercise cause hypertrophy of working muscles, diffusive gas exchange, a passive physical transport across the air–tissue–blood barrier that is primarily determined by the available alveolar–capillary–erythrocyte surface areas and thickness of the diffusion barrier, may not be stressed beyond existing structural capacity.

Increased Mechanical Lung Strain

Mechanical stretch *in vitro* stimulates fetal rat lung cell proliferation (66). Mechanical stresses of fetal breathing movements and fluid tension promote fetal lung development (67). Diminished alveolar wall tension imposed on developing lung is associated with lung hypoplasia (68). Expression of immediate early growth-associated genes *c-fos* and *jun*B is elevated acutely both *in situ* and in isolated lungs subjected to distending airway pressures or increased perfusion (69). Positive pressure lung inflation rapidly induces ECM remodeling in open-chest rabbits by selectively increasing mRNA levels for a number of procollagens, fibronectin, basic fibroblast growth factor (FGF), and transforming growth factor (TGF). (70). Application of continuous positive airway pressure increases lung protein and DNA content in weaning ferrets (71). Lobar liquid distention by instillation of perfluorocarbon accelerates lung growth in neonatal but not adult lambs (72). Extensive published data, reviewed by Rannels (73), are consistent with a major regulatory role for mechanical signals in both initiation and progression of normal as well as compensatory lung growth (*see* MECHANICAL SIGNALS).

A highly robust model of strain-induced lung growth is surgical resection of one or more lobes (lobectomy) or one lung (pneumonectomy), which initiates rapid growth of the remaining lung in mice, rats, rabbits, ferrets, and dogs (74–82) (Figure 1). In rodents after pneumonectomy, various transcription factors are transiently upregulated (83), followed by an accelerated increase in lung weight (84, 85) associated with proportional increases in all major alveolar septal cell populations (86–89), protein and DNA synthesis (78, 87), collagen, and elastin accumulation (90, 91), leading to normalization of alveolar architecture. Whether compensatory growth in rodents normalizes lung function is not clear.

There are important species differences in the postpneumonectomy response. Primitive organisms are more capable of tissue regeneration than higher order complex organisms (92). Among mammals, rodent lung architecture is simpler and physiologic reserves for gas exchange more limited than in larger animals. Somatic growth continues in rats throughout their lifespan (40), and there is no stable final thoracic size. Consequently, in the adult rat, alveolar tissue growth is easily reinitiated after the removal of only one or two lobes (74, 93), and compensatory growth advances more rapidly and completely (90) than in larger adult mammals (94, 95) where the lung and thorax attain a maximum size on epiphyseal closure.

There are also maturity-related differences in the postpneu-

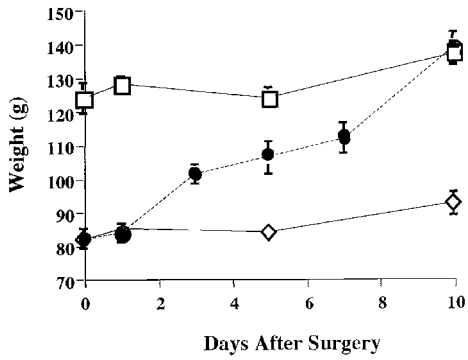

Figure 1. Rapid compensatory lung growth after left pneumonectomy (PNX) in mice. The weight of the remaining right lung increases within 10 days to reach that of two lungs in SHAM control animals. Sham two lungs, *squares*; sham right lung, *diamonds*; right lung after left PNX, *circles*. Adapted by permission from Brown and colleagues (84).

monectomy response. In immature dogs, accelerated postpneumonectomy growth of the remaining alveolar tissue normalizes total septal tissue volume and resting gas exchange within 8 weeks (96). In adult dogs, compensatory alveolar growth is reinitiated only when the amount of lung resected exceeds a threshold (approximately 50%) (94, 95). Subthreshold resection elicits compensation via recruitment of existing physiologic reserves and remodeling of existing septal tissue; these mechanisms can adequately maintain arterial O_2 saturation up to heavy exercise without the need for new lung tissue growth (94, 97, 98). Above the threshold, both physiologic recruitment and new alveolar tissue growth occur, but functional improvement progresses slowly and never completely normalizing oxygen transport (95, 99). Functional compensation generally lags behind cellular growth. Early after pneumonectomy, the septum thickens as a result of disproportional enlargement of the interstitial compartment (95) without a significant compensatory increase in D_L, and arterial hypoxemia develops early during exercise. Subsequently, tissue remodeling occurs with thinning of septa and progressive improvement in D_L as well as arterial O_2 saturation (99).

Reduced Size (Lobar) Lung Transplant

Reduced-size or lobar transplant, where one lobe instead of a whole lung is transplanted into the recipient's hemithorax, could significantly expand the donor organ pool for treating patients with end-stage lung disease. In children receiving living donor lobar transplants compared with those receiving cadaveric whole lung transplant, lung volume increased but volume-adjusted D_L progressively decreased over 6–12 months, suggesting that alveolar dilation, rather than alveolarization, was responsible for the increased lung volume after lobar transplant (100). Other factors such as immunosuppression and infection may influence the growth of the graft and complicate interpretation of the functional data.

In animal studies, mature lobar allografts gradually expand within the growing chest of an immature recipient (101–104); whether the mechanism responsible for this increase in size is alveolarization (lung growth) or simple alveolar dilation is still a matter of debate. In a study comparing lung growth after reduced-size lobar transplant and that after lobectomy (105), the left lung from immature swine was replaced with the left lower lobe from mature swine. Response in the allograft was compared with that in the left lower lobe of the remaining after left upper lobectomy in the mature swine. After lobectomy, the remaining left lower lobe undergoes rapid weight gain by 2 weeks. Weight gain of the transplanted mature lobe is more gradual over 3 months. Alveolar cell proliferation increases markedly 2 weeks after lobectomy, returning to baseline by 3 months, whereas cell proliferation in the allograft increases gradually, reaching significance at 3 months. The slower allograft growth may be related to the available thoracic space; the free space after lobectomy allows the remaining lobe to expand, whereas the allograft fits snugly within the hemithorax and its expansion is limited. The lack of mechanical strain and the presence of ischemia/reperfusion injury associated with transplantation may hinder allograft growth. Functional residual capacity and resting oxygenation did not differ after lobar transplantation or lobectomy (101, 102). Alveolar surface density and architecture are unaltered after lobectomy or transplant. Given the increased lung weight, alveolar number presumably increased in the lobar allograft as in the remaining lung after lobectomy. A study in sheep also concluded that alveolar multiplication occurs in the mature lobe transplanted into the growing thorax of an immature animal (104). However, other studies in rats (103) and minipigs (106) reported predominantly alveolar enlargement in the transplanted lobe.

Chronic Hypoxia or High-altitude Exposure

Chronic hypobaric hypoxia affects approximately 400-million people living above approximately 5,000 ft altitude. Native Tibetan newborns show higher arterial oxygen saturation than Han newborns whose mothers had resided at high altitude for approximately 2 years (107). Whereas somatic growth is slowed and perhaps prolonged at high altitude (108, 109), native highlanders show larger vital capacities and thoracic volumes than lowlanders regardless of ethnic origins (110–116). Where measured, D_L is also increased in native highlanders as well as persons born and raised at sea level who subsequently move to high altitude as adults (116, 117). The adaptive pattern is consistent with high-altitude–enhanced lung growth. A major problem of human population studies at high altitude is a lack of structural data to discern the basis of functional differences. It is unknown whether alveolar–capillary surface and volume have increased by growth or whether the thorax has simply become more compliant allowing the lung to expand to a higher volume. The higher D_L could also be due to reversible increases in microvascular pressures, blood volume, and hematocrit instead of induced lung growth.

In vertebrates, hypoxia uniformly causes hypertrophy of gas exchange organs independent of rhythmic stretch of the organ; examples include gills in fish and salamander (118), redundant skin folds in bullfrogs (119), and placental hypertrophy in mammals (120). Mice native to high altitude show a greater volume fraction of lung tissue and smaller alveolar volume than the same species living at sea level, attributed to hypertrophy of types I and II pneumocytes and endothelial cells (121); epithelial, endothelial, and erythrocyte surface areas per gram body weight are also greater. Chronic hypoxia initially accelerates lung growth and alveolarization in rats while variably retarding somatic growth (41, 122–127). Within 3 weeks of hypoxic exposure, lung volume becomes 20% larger than in normoxic control animals; then the rate of lung growth returns to normal, although the relative increase in volume is retained (128).

Growing rats show maturity-dependent responses to hypoxia (129, 130). Perinatally, even brief hypoxic stress blunts lung development at age 7 days; effects are sustained to 30 days (130). Between 2–14 days of age, hypoxia impairs alveolar septation. However, between 14–40 days, alveolar volume increases less and alveolar number increases more in hypoxic rats than in normoxic rats, suggesting stimulated alveolar growth. Older rats (23 days), raised in normoxia and then exposed to hypoxia, demonstrate greater alveolar surface area and volume than corresponding normoxic control animals, but the alveolar number is unchanged, suggesting alveolar enlargement (129). In addition to hypoxia, exposure to hypobaria (in normoxia) in growing rats may accelerate cellular proliferation and modulate structural lung growth in subtle ways (126, 127, 131, 132). Hypoxia also enhances postpneumonectomy lung growth in rats (133). Unlike humans, pulmonary gas exchange efficiency does not significantly limit O_2 transport in rats regardless of altitude (134). These studies are uniformly of short duration and do not address long-term effects. After rats exposed to high altitude return to sea level, their lungs stop growing, whereas the lungs of sea level control animals continue to grow until lung size in the two groups are similar; hence, an increased lung volume at high altitude may not be permanent (135).

Two studies have addressed long-term lung growth at high altitude and reached diverging conclusions (136, 137). In guinea pigs, bony epiphyses close beginning at approximately 20 weeks of age (46). Weanling guinea pigs raised from 2 to 16 weeks of age at simulated extreme altitude (5,100 m) (136) show initially accelerated increase in lung volume and alveolar surface area

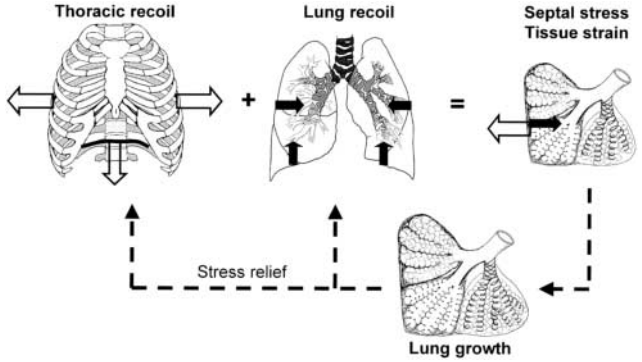

Figure 2. Mechanical interaction between the thorax and lung during growth. Recoil of a growing thorax (*A, open arrows*) exerts an outward force on the lung that is partly counterbalanced by the inward force of lung elastic recoil (*B, closed arrows*). Stress and strain imposed on the alveolar septa constitutes a potent signal for cellular proliferation and tissue remodeling (*C* and *D*). Lung growth in turn minimizes septal stress/strain and allows the thorax to expand further, creating a feedback loop that continues until somatic maturity is reached, when epiphyses close and the thorax and lungs reach their final stable dimensions. Adapted by permission from Hsia and colleagues (498).

compared with normoxic control animals in a manner independent of hyperventilation (138). However, the stimulatory effect progressively diminishes with duration of exposure, suggesting that severe hypoxia may not extend the upper limit of alveolar growth at maturity but merely accelerates the rate of its attainment, and the ultimate lung dimension may be determined predominantly by thoracic size. Because both somatic growth and the age of epiphyseal closure tend to be retarded at high altitude (108), lung growth of high-altitude guinea pigs may continue beyond the reported observation period. In contrast, dogs raised from age 2 to 14 months at 3,100 m (137) show significantly larger lung volumes, septal tissue volumes, and D_L than matched control animals raised to maturity at sea level; differences persist 9 months after returning to sea level. Results in dogs suggest that hypoxia enhances lung growth during maturation and elevates gas exchange capacity at maturity. The size of rib cage, rib length, and curvature are not altered in dogs raised at high altitude, but the dome of diaphragm moves to a more caudal position at a given transpulmonary pressure to accommodate the larger lungs. Pulmonary vascular reactivity to hypoxia returns to normal, but right ventricular hypertrophy persists 8 months after returning to sea level, suggesting permanent pulmonary vascular structural alterations (139). Differences between responses in guinea pigs and dogs may be related to differences in the severity of hypoxia and the length of observation.

Comparative data provide important clues regarding the interplay between lung and rib cage in determining the final lung size and support the hypothesis of two interacting growth stimuli at high altitude. During normal development, distending pressure generated by the outward recoil of the growing rib cage exerts mechanical traction on alveolar septa, and the resulting tissue tension provides a major signal for cellular lung growth. Growth of the lung relieves tissue tension and allows the rib cage to expand further in a feedback loop that continues until somatic maturity when epiphyseal union occurs (Figure 2). Ultimately, dimensions of the lung and thorax are matched. Hypoxia independently stimulates lung growth but if severe enough may retard rib cage growth. At a moderate altitude, stimulation of lung growth predominates. Space for the expanding lung is provided by passive rib cage expansion and caudal displacement of the diaphragm. At extreme altitudes, inhibition of rib cage growth may predominate; although lung growth is initially stimulated, the effect diminishes with time as lung size becomes limited by the smaller rib cage. At extreme altitude ($>$ 5,000 m), somatic growth is so retarded as to prevent an absolute increase in lung volume, even though volume with respect to body weight is larger than in sea level controls (135) (*see* Limits Imposed by Dysanaptic Lung Growth).

EXTRACELLULAR MATRIX AND ELASTIC FIBERS

Association of cells with their basement membrane, ECM proteins and fibers regulates cell survival, proliferation, and differentiation (140–142). Elastin is a chief component of septal interstitium (143, 144). Tropoelastin monomers are assembled and cross-linked on pre-existing fibrillin-containing microfibrillar scaffolds (145). First expressed near airway branch points during the pseudoglandular stage, elastin gene expression peaks during alveolarization when elastin fibers localize to the tips of alveolar secondary crests, forming rings that surround alveolar entrances and deposit in bundles within alveolar walls (144, 146, 147). As elastic fibers are the primary means by which the energy of mechanical stretch is stored and released in the septum, these fibers and the elastin-expressing cells fundamentally determine septal mechanical properties and strain-related signal transduction. The composition of microfibrils and the cellular events that control elastin gene expression, secretion, and assembly are actively under investigation, but little is known of how cells work together to assemble elastic fibers in diverse patterns required for maintaining mechanical integrity of elastic organs.

Alveolar walls have been likened to tents supported by ropes (elastic fibers) and poles, whose lengths and tension largely determine the shape of the tent (148). Mechanical stresses provide signals for the orientation and differentiation of alveolar myofibroblasts as well as directional organization of a complex elastic fiber network. The position, smooth muscle phenotype, and elastin-expressing capacity of alveolar myofibroblasts suggest that they construct, reinforce, and modify elastic fibers to accommodate changing stresses. Intermittent stretching of fetal lung cell in organotypic culture increases tropoelastin gene transcription (149). Elastin expression in alveolar myofibroblasts is upregulated by TGF-β and downregulated by basic FGF (150, 151). Preventing alveolar elastin deposition through inhibition of lysyl oxidase-mediated crosslinking (152, 153), exposure to hyperoxia (154, 155), dexamethasone (20), depletion of alveolar myofibroblasts (156, 157), or targeted ablation of elastin gene (158) all result in enlarged emphysema-like alveoli containing fewer septa. Moreover, there is a link between retinoids (159) and lung elastin (160, 161). A subset of lipid-laden alveolar myofibroblasts metabolizes retinoids (162), which upregulate elastin expression (160). Deletion of retinoic acid receptors leads to reduced lung elastin and alveolar numbers (161). Whether alveolar myofibroblasts are the primary responsive cells in retinoid-enhanced lung development is unclear.

Elastic Fibers in Alveolarization

During transition from the saccular to the alveolar stage, the walls of terminal respiratory units become thinner by apoptosis (163), and a single layer of capillaries replaces the double-capillary network found in rudimentary alveolar walls (164). Later, new septae arise at bends in alveolar walls, effectively increasing gas exchange surface. It is likely that paracrine signals among myofibroblasts, type II pneumocytes, and capillary endothelial cells are necessary for formation and extension of secondary septae. Individual alveolar walls can form free "ends" at the

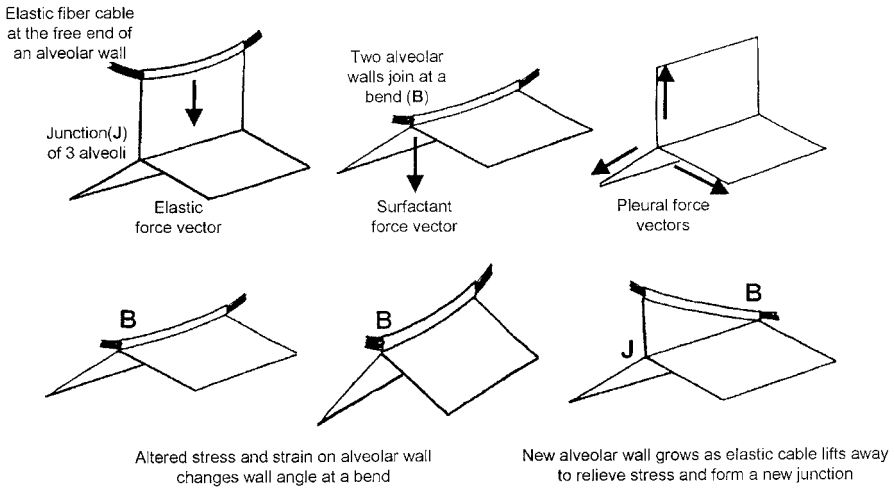

Figure 3. Proposed formation of new alveolar wall. *Top three panels* show the balance of forces acting on alveolar walls (represented as two-dimensional planes). Elastic fibers form a thick cable at the free ends of alveolar walls. *Arrows* represent various forces acting on the walls. Bends (B) are angular joints of two alveolar walls with an elastin cable at the angle. Junctions (J) are intersections of three alveolar walls, where tissue retractive forces and surface tension balance and no elastin fiber is necessary or present. *Bottom three panels* show altered mechanical stress, which changes the wall angle at a bend causing elastin to lift off leading to the outgrowth of septal tissue to create a new alveolar wall and a new junction. Adapted by permission from Wood and colleagues, Oldmixon and colleagues, and Butler and colleagues (148, 165, 166).

mouths of alveoli, join another alveolar wall at an angle forming "bends," or join with two alveolar walls at a "junction" (148, 165, 166) (Figure 3). Elastic fibers and alveolar myofibroblasts localize to ends and bends where retractive forces develop during inspiration, but not at junctions, which are reinforced with collagen fibers. Bends in alveolar walls have been proposed as sites where new alveolar septae arise and extend. Mechanical stress applied to an elastic fiber running along a bend in an alveolar wall may cause the fiber to pull away from the wall, initiating the formation of a new septum.

If elastic fibers transmit force at bends in alveolar walls during secondary septation, aberrant elastic fibers might contribute to failed septation in disease. In the classic BPD, the lung appears "fibrotic," with large consolidated regions and simple, enlarged terminal airspaces (167). In preterm lambs with BPD (168), thickened and tortuous elastic fibers were localized to stubby, malformed alveolar secondary crests. Elastin gene expression continues unchecked in the alveolar walls of BPD lungs, while declining markedly as alveolarization progresses in age-matched gestational control subjects. Human BPD is characterized by increased elastin turnover but a paucity of elastic fibers in alveolar walls (169, 170). However, blocking alveolarization in newborn rats by hyperoxia represses elastin gene expression (143). Divergent observations indicate that altered elastic fiber production and turnover are associated with failed alveolarization, but they do not establish a cause–effect relationship. Nonetheless, it remains likely that altered elastic fiber morphology may contribute to persistence of BPD by altering lung mechanics and posing a barrier to remodeling of the alveolar wall.

Remodeling of Elastic Fibers

Elastin is one of the most durable proteins, with a half-life that can exceed the lifespan of the organism (171). Elastic fibers must be remodeled, expanded, or extended during lung growth. As elastic fibers are insoluble polymers with desmosine crosslinks, remodeling cannot occur by redistributing monomeric tropoelastin. Rather, enzymatic digestion and new synthesis must occur. Elastases include the zinc-binding matrix metalloproteinases (MMP) and serine proteases such as pancreatic elastase and neutrophil elastase. Several of these, including MMP-9, MMP-12, and neutrophil elastase, are implicated in the pathogenesis of emphysema (172, 173). No clear role for these enzymes in lung development and growth has yet been demonstrated by gene targeting, perhaps because of functional redundancy of many MMPs. Specific or broad-based MMP inhibitors may provide another approach to assess the roles of MMPs in latter stages of lung development.

In adult rats after removal of the left lung and accessory lobe, expression of tropoelastin mRNA in the remaining lung was induced by 3 days, peaked at 7 days, and declined by 14 days, when the mass of the remaining lung had doubled (91). Insoluble elastin content measured by desmosine increased in parallel with lung growth. Postpneumonectomy expression of tropoelastin mRNA localizes to myofibroblasts in alveolar walls at sites similar to those found during normal postnatal alveolarization, leasing to suggestions that similar signals might drive both developmental and induced lung growth.

Other matrix proteins, such as fibronection, are also present throughout developing lung mesenchyme (174, 175) and at tips of secondary septae in the alveolar stage (176). Via binding to integrin receptors and formation of cell–matrix adhesions, fibronectin is essential for initiating cleft formation during epithelial branching (175) and for the migration, proliferation, differentiation, and apoptosis of various cells during organogenesis (174). The mechanisms and significance of its role during postnatal reinduction of lung growth remain poorly understood.

SIGNALS AND MEDIATORS OF INDUCED LUNG GROWTH

Mechanical Signals

Septal strain. After reaching somatic maturation, the thorax attains a maximum size, and lung strain stabilizes so that a larger increase in mechanical strain must be imposed before alveolar growth is reinitiated in the adult animal. After pneumonectomy when the resected lung is replaced by a space-occupying plombage or prosthesis to prevent mediastinal shift and mechanical lung strain in mice (87), rats (177), ferrets (81), rabbits (178), and dogs (179, 180), growth of the remaining lung is blunted, but compensatory increases in D_L, septal tissue volume (179), or DNA synthesis (87) are not totally eliminated. Instead of expanding laterally across the midline, the remaining lung changes shape and enlarges modestly in the caudal direction via expansion of the ipsilateral rib cage and displacement of the hemidiaphragm (178, 179). Hence, other signals, perhaps endothelial distention and shear or nonmechanical factors, are also implicated in growth reinitiation and progression. Delayed reinstitution of lung strain after deflation of the prosthesis weeks to months after pneumonectomy leads to progressive expansion of the remaining lung and a vigorous tissue response (81, 179, 180).

Capillary distension and shear. Ligation of one pulmonary artery increases perfusion to and augments alveolar growth of the contralateral lung in newborn pig (181). After pneumonectomy, pulmonary perfusion per unit of remaining lung tissue doubles. Typically, pulmonary arterial pressure is normal at rest but elevated on exercise (182–184). Pulmonary capillary blood volume per unit of remaining lung tissue is increased (185). Chronic capillary distention and increased shear forces could contribute to endothelial cell growth and septal remodeling but have not been thoroughly investigated. In postpneumonectomy ferrets, restricting blood flow to the remaining lung by banding one lobar pulmonary artery has no effect on the compensatory increase in DNA and protein content of the banded or unbanded lobe (186).

To understand how alveolar capillaries adapt to mechanical stress, we need to refer to models of sustained alveolar–capillary distension and congestion, including chronic heart failure (CHF) and pulmonary venous hypertension where alveolar–capillary pressure is elevated both at rest and during exercise (187, 188). Whereas CHF increases transendothelial water flux in lungs (189–191), interstitial or alveolar edema does not always develop (192–194). This adaptive response involves structural remodeling of the alveolar–capillary barrier, septal interstitium, extra-alveolar pulmonary vasculature, as well as functional remodeling of signaling pathways regulating endothelial barrier function. Thickening of the endothelium and epithelium of the alveolar–capillary barrier occurs within 1 month of pacing-induced CHF, whereas remodeling of the basement membrane takes longer (195, 196). Within 5 months of left ventricular failure caused by aortic banding, there is marked thickening of the alveolar septum as well as basement membrane (195). Although no report has specifically addressed the progression of septal remodeling in humans, thickening of the septal barrier and basement membrane has been observed in mitral stenosis (197). Septal remodeling in response to chronic capillary congestion serves a protective role by raising the pressure required to induce stress failure of the blood–gas barrier (48, 196, 198). Despite early thickening of the septal barrier and increased resistance to stress failure, basal endothelial permeability remains normal (192, 196, 199), and the endothelium is refractory to injury (200) after 1–2 months of pacing-induced CHF. The more extensive alveolar septal remodeling seen in the aortic banding model is actually associated with decreased endothelial permeability (195). On the other hand, remodeling of the blood–gas barrier can also be maladaptive by decreasing D_L and exercise tolerance as in patients with CHF (201, 202).

Remodeling of extra-alveolar resistance network lags temporally behind alveolar septal remodeling. In pacing-induced failure, there are no structural changes in small extra-alveolar arteries or veins up to approximately 500 μm in diameter (203). Within 4–5 months of CHF, medial hypertrophy develops in resistance microvessels (195) and pulmonary artery (204). In patients with CHF, the decrement in D_L correlates with the increase in pulmonary vascular resistance (202). Postmortem histology of lungs from patients with long-standing pulmonary venous hypertension also shows significant remodeling of extra-alveolar pulmonary vasculature (205).

Acute interstitial edema increases synthesis and deposition of collagen and glycosaminoglycans in the lung, which are known determinants of the interstitial pressure–fluid volume relationship (206–208). However, chronic pulmonary capillary congestion alters the interstitial pressure–fluid volume relationship in a way that maintains a normal interstitial fluid pressure in the face of lung edema and does not change the lung content of glycosaminoglycans, hyaluronan, or collagen relative to blood-free dry mass (203). However, because blood-free dry lung mass increases significantly in CHF (192, 199, 200, 209), matrix deposition likely also increases. In acute lung injury or pulmonary fibrosis, glycosaminoglycan deposition precedes deposition of mature collagen; myofibroblasts in regions of increased glycosaminoglycan deposition also contain type I procollagen (210). Dilated cardiomyopathy is associated with increased plasma levels of type III collagen propeptide, the 7S domain of type IV collagen, laminin, and type I collagen telopeptide (211, 212), which can be attributed to increased matrix turnover in the heart, lung, and liver.

Mechanical alveolar capillary strain stimulates matrix synthesis in lung fibroblasts (213), and acute pulmonary venous hypertension increases mRNA expression for types I and III procollagens, laminin, and fibronectin in lung parenchyma (214). Increased synthesis of matrix proteins in either septal interstitium or extra-alveolar vessel walls may not result in increased net deposition if degradative pathways are concomitantly stimulated. Acute hydrostatic pulmonary edema increases parenchymal activity of MMP-2 and MMP-9 (215), which is known to mediate pulmonary vascular remodeling (216); similar upregulation is seen in CHF. In experimental pulmonary arterial hypertension, endothelial injury stimulates rapidly progressing matrix synthesis and medial hypertrophy (217). The lack of early endothelial injury in CHF likely contributes to a relatively slow time course of vascular remodeling and pulmonary venous hypertension. These models can offer important clues as to how remodeling occurs within alveolar septa in response to capillary congestion. However, other effects of CHF such as neurohormonal alterations complicate the picture and preclude a clear separation of the mechanical signals from nonmechanical signals acting on the alveolar capillary bed.

Strain-induced signal transduction. Transduction of mechanical signals within and between cells is mediated via cytoskeletal proteins, integrins, and various cell–cell adhesion molecules (218–222). At focal adhesions sites, cell membranes and their underlying substratum are physically connected via integrins that bind specifically to the Arg-Gly-Asp amino acid sequence in ECM proteins (223). The cytoplasmic tails of integrins may bind to the actin cytoskeleton via several structural linking proteins, including talin, vinculin, and paxillin (224). Techniques such as magnetic twisting cytometry (225, 226), where ferromagnetic microbeads are bound to specific cell surface integrins, have been used to probe effects of mechanical deformation on individual cells. Stressing integrin receptors enhances tyrosine phosphorylation of cytoskeletally anchored proteins (227) and activates the cAMP cascade (228). Twisting Arg-Gly-Asp–coated beads bound to human endothelial cells more than doubles endothelin-1 gene expression, whereas twisting nonadhesion molecules does not alter endothelin-1 gene expression (229).

Intracellular mechanotransduction is mediated via the cytoskeleton, a discrete, tensed network that enables adherent cells to resist distortion and regulate cell shape, polarity, locomotion, growth, signal transduction, gene expression, as well as protein synthesis (225, 230–232). The concept of "tensegrity" (tensional integrity), exemplified by Fuller's geodesic dome (233), has been applied to tissue and cytoskeletal mechanics (234–236). Tensegrity structures are envisioned as a network of support elements that interact under continuous tension to resist mechanical deformation; after external loading, elements reorient themselves to achieve a new equilibrium to reduce distortion (Figure 4). The concept broadly applies to all size scales and provides one explanation of how localized mechanical stresses elicit distant biochemical responses. Tensegrity structures require prestress (preexisting tension before external loading) to maintain stability of a given shape; when prestress approaches zero, the structures collapse. Within the cell, prestress is generated actively by the

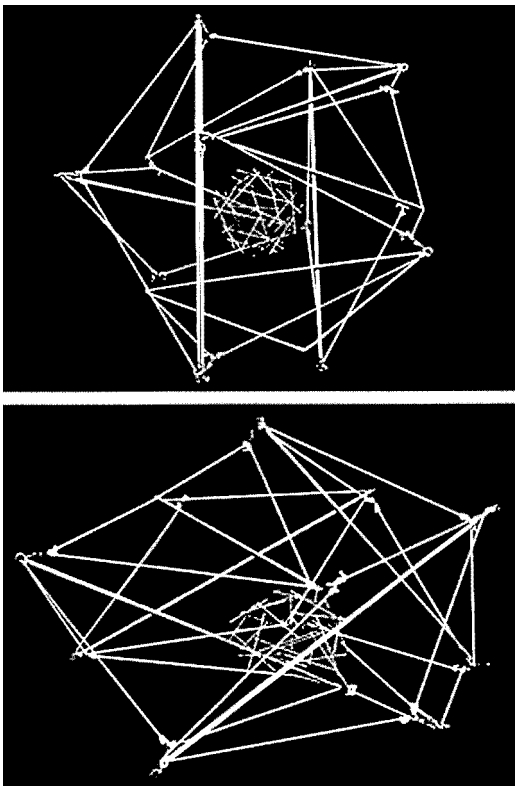

Figure 4. A tensegrity model of mechanotransduction. The cytoskeletal network of a nucleated cell is envisioned as composed of sticks and elastic string (compression elements that constantly redistribute cell tension to maintain cell shape) when the cell is unanchored and nucleus is round (*top*) or attached and spread on a rigid substrate (*bottom*), which is associated with shear stress at the cell surface causing a distorted shape. The nucleus is an independent tensegrity sphere (or geodesic dome) that is hardwired to the cell surface by elastic filaments (not shown). The tension at cell surface receptors is transmitted to the nucleus via rearrangement of cytoplasmic filaments, causing immediate physical alterations in molecules within the nucleus leading to activation of biochemical pathways. Tension at the cell surface can also be transmitted across adjacent cells via focal adhesion molecules in an analogous fashion. Tensegrity has been used to explain how localized mechanical perturbations translate into widespread cellular responses. Reprinted by permission from Ingber (234).

actomyosin apparatus and passively by traction at the cell–ECM interface; these forces are carried by actin microfilaments and intermediate filaments and stabilized via adhesions to the ECM. As prestress increases, cells become stiffer and resist further distortion. A measure of tissue or cell resistance to distortion is the shear stiffness (or elastic modulus, the ratio of shear stress to shear strain), which increases directly with prestress or distending force. Thus, stiffness of cultured cells increases with contractile agonists and decreases with relaxing agents in a dose-dependent manner (235, 237–239). Cell stiffness also increases with biaxial stretch (240), supporting the role of prestress in determining the stability of cell shape.

At a given level of growth factor stimulation, the stability of cell shape and the extent of cell spreading are the key factors controlling cell growth and apoptosis *in vitro* (241). Cell tension increases as cell spreading is promoted (242). Restricting the shape and spreading of cultured capillary endothelial cells suppress growth and induces apoptosis (241). In cultured fibroblasts, mechanical strain stimulates connective tissue synthesis, inhibits matrix degradation, and increases growth factor gene expression in a manner dependent on the type of strain as well as mechanical interaction with the substratum (243). Normal cells grown on flexible substrates show decreased DNA synthesis and increased apoptosis associated with lower cell spreading area and traction forces compared with cells cultured on identical but stiff substrates (244). In contrast, oncogenically transformed cells lose the normal mechanical feedback and maintain their growth and apoptosis rates regardless of substrate flexibility, highlighting the importance of mechanical cell–ECM interaction in regulating cell growth. Although the material properties of purified cytoskeletal filaments or reconstituted cytoskeletal gels are known, it remains unclear how the mechanical feedback response of intact cells emerges from collective interactions of individual components. Dramatic changes in tissue force balance as occurs after pneumonectomy or with continuous positive airway pressure must alter the distribution of, and response to, cell prestress; this pertubation has yet to be delineated but likely regulates subsequent biochemical and molecular events in important ways.

Nonmechanical Signals and Mediators

Hormones. Early interests were spurred by studies of patients with acromegaly who demonstrate partially reversible increases in lung volume and distensibility but normal D_L per unit lung volume (245–248); supranormal lung tissue volume is seen in some but not all patients (245). Functional measurements were done at rest, and thus, it is unclear whether D_L could increase to a greater extent during exercise in acromegalic patients. Administering excess growth hormone to young adult rats accelerates body growth with proportional increases in lung volume and alveolar surface area (249), suggesting that excess growth hormone primarily enhances growth of rib cage without specific stimulation of alveolar growth.

The most extensively investigated hormone, adrenal glucocorticosteroid, accelerates late-gestation fetal lung maturation in low doses (250–252) and is widely used in premature infants to improve perinatal survival and lung function. However, high or repeated doses can inhibit postnatal somatic and lung growth (253). During the period of active postnatal alveolarization in rats, glucocorticoids inhibit secondary crest formation, accelerate alveolar wall thinning, decrease alveolar number and surface area (20, 254–257), diminish replication of fibroblasts, impair the conversion of types II to I pneumocytes (255, 258, 259), and alter collagen and elastin deposition (149, 254, 260, 261), resulting in a morphologic appearance resembling emphysema. In ferrets, corticosteroid treatment reduces size-corrected airway conductance, suggesting that the central airways are more sensitive to its effect than lung parenchyma (262). On the other hand, glucocorticoids can enhance other signals of lung growth. For example, both the rate and extent of postpneumonectomy alveolar growth are enhanced in adrenalectomized rats (88, 263–265); the enhancement is prevented by adrenocorticosteroid hormone replacement (263). Clinically, the wide-ranging effects of perinatal corticosteroid treatment may be long lasting (266, 267); therefore, balancing the short-term gain versus long-term adverse effects of glucocorticoid therapy in premature infants remains a difficult issue.

Growth factors. In pregnant rats, pneumonectomy increases DNA content in the fetal lung (268). Assuming that the maternal blood is not hypoxemic, data suggest that soluble growth factors released into maternal circulation are capable of crossing the placenta to stimulate fetal lung growth. However, initial findings of elevated serum growth hormone and insulin-like growth factor-1 levels in postpneumonectomy rats were later attributed to nonspecific surgical manipulation (269, 270). More likely, the postpneumonectomy response is mediated locally. In the fetal

lamb, gene expression of insulin-like growth factor-I, a fibroblast mitogen, is reduced in lungs of experimental CDH and restored to normal by tracheal ligation (271). Postnatal insulin-like growth factor-I gene expression is also increased during accelerated lung growth after both liquid-based airway distension and pneumonectomy (271). A higher insulin-like growth factor-1 activity has been found in bronchoalveolar lavage fluid of postpneumonectomy rats (272); it is not clear whether the activity comes from parenchymal cells or the postoperative influx of circulatory neutrophils and macrophages.

Transgenic mice without epidermal growth factor receptor (EGFR −/−) show variably impaired alveolarization, branching morphogenesis, and type II pneumocyte differentiation (273, 274). Injection of EGF in rabbits accelerates lung maturation *in utero* (275) and induces widespread though transient epithelial mitogenic activities after birth (276). EGF and EGFR proteins are higher in 2-month-old growing dog lungs than in 1-year-old adult lungs, supporting a continuing regulatory role of the EGF axis during postnatal lung growth (277). In the swine after lobectomy, EGFR protein in the remaining lung is upregulated at 2 weeks, returning to baseline by 3 months (278). After lobar lung transplant, EGFR in the transplanted lobe is upregulated only after 3 months, coinciding with a slower increase in cell proliferation (105). Administration of EGF to rats augments postpneumonectomy lung growth and is associated with EGFR upregulation (279). Thus, the EGF axis could represent an avenue for modulating postnatal in addition to fetal lung growth (280). However, immature dogs studied 3 weeks after pneumonectomy do not show increased EGF or EGFR proteins above that in matched control animals despite active cell proliferation in the remaining lung (277), suggesting species-specific differences in the response to growth factors.

Keratinocyte growth factor. Keratinocyte growth factor (KGF) is a specific epithelial cell mitogen belonging to the FGF family (FGF7) and expressed in mesenchyme. Transgenic mice overexpressing KGF develop large cystic lungs resembling cystic adenomatoid malformation (281). Transgenic mice overexpressing a dominant-negative form of KGF receptor develop a trachea that branches only once (282). However, KGF (FGF7) −/− mice develop normal-looking lungs (283). Another ligand for the KGF receptor, FGF10 (KGF2), is also critical for lung development; FGF10 −/− mice have a trachea but no further branching (284).

In both adult and developing lungs, KGF induces alveolar type II pneumocyte proliferation *in vitro* and *in vivo* (285). KGF stimulates surfactant protein and phospholipid expression as well as transepithelial transport of fluid and electrolytes, minimizes injury and enhances repair of damaged epithelia, and may dampen the epithelial response to inflammatory mediators (286, 287). Exogenous KGF treatment is protective in injury models, including hyperoxia (288, 289), bleomycin (290, 291), radiation (291), and hydrochloric acid (292). Endogenous KGF mRNA is induced in neonatal rabbits exposed to hyperoxia (293), and KGF protein is increased in adult respiratory distress syndrome (294), suggesting a role in lung repair. In fetal sheep lung hypoplasia associated with CDH, KGF protein is reduced but increases after tracheal ligation concurrent with accelerated lung development (295). Overproduction of KGF in mice using a temporally inducible system (296) causes hyperplasia of type II pneumocytes and bronchial epithelial cells and increases inflammatory cells in the lung; effects disappear completely after cessation of KGF overproduction. Such conditional expression systems will be highly valuable for dissecting the effects of growth factors in models of lung growth.

Hepatocyte growth factor. Hepatocyte growth factor (HGF), derived from mesenchyme, is a heparin-binding growth factor implicated in organogenesis and compensatory growth of liver, kidney, and lung. HGF stimulates proliferation of airway and alveolar epithelial cell (297–299) and vascular endothelial cells (300) and enhances migration of type II pneumocytes (301). In fetal lung organ culture HGF, along with FGF family members, mediates branching morphogenesis (302). In postnatal lung, HGF facilitates lung repair after injury by minimizing collagen accumulation and fibrosis (303). Transpulmonary arterial transfer of human HGF gene into rat lung increases capillary density and blood flow (304). Serum HGF levels increase acutely in patients after pneumonectomy, although control data after thoracotomy alone are not available (305). In pneumonectomized mice, neutralization of endogenous HGF suppresses the compensatory increase of DNA synthesis in the remaining lung, whereas exogenous HGF administration stimulates DNA synthesis (306). Whether HGF-induced DNA synthesis translates into structural growth and functional augmentation remains to be seen.

TGF-α. TGF-α, produced as a precursor proprotein and existing in membrane anchored as well as secreted forms, is mitogenic for epithelial and mesenchymal cells and also mediates remodeling in lung injury (307). TGF-α −/− mice have apparently normal lungs (308); however, mice overexpressing TGF-α show a marked increase in type II pneumocyte and fibroblast proliferation, with increased collagen deposition and fibrosis (309). Overexpression also causes severe pulmonary vascular disease, mediated through EGF receptor signaling in distal epithelial cells and associated with reduced VEGF expression in lung (310). TGF-α protein increases in bronchoalveolar lavage fluid after hyperoxic exposure and remains increased during the fibrotic period (311). Because TGF-α induces KGF mRNA *in vitro* in fibroblasts and KGF induces TGF-α *in vitro* in keratinocytes, there is likely to be important "cross-talk" between these growth factors *in vivo* as well.

Platelet-derived growth factor. Platelet-derived growth factor (PDGF), consisting of four gene products (PDGF-A–PDGF-D) that act via two receptor tyrosine kinases (PDGFR-α and PDGFR-β), is strongly expressed in developing lung mesenchyme. In neonatal rats, PDGFR-α localizes to airway epithelium and PDGFR-β to subendothelial perivascular areas and to airway and alveolar epithelium; their expression is delayed by postnatal hyperoxic exposure (312). PDGF-A participates in recruiting smooth muscle cells to alveolar sacs during alveolarization, but not specifically in early branching morphogenesis (313). PDGF-A(−/−) mouse lungs lack alveolar smooth muscle cells and exhibit reduced tropoelastin expression as well as elastin fiber deposition in lung parenchyma, associated with abnormal alveolar formation (157). It is possible that cells bearing PDGFR-α are progenitors of the tropoelastin-producing alveolar myofibroblasts, and the presence and distal spreading of these cells are necessary for the normal development of alveolar septum. PDGF has been implicated in postpneumonectomy compensatory lung growth in rats (314).

VEGF. VEGF is highly expressed in the airway and alveolar septum, placing it in a position to mediate airway–vascular interactions (315, 316). Heterozygous VEGF knockout mice die in embryonic life (317, 318), and postnatal inactivation of VEGF increases mortality (319). VEGF is induced by hypoxia and stretch and the expression modulated by other growth factors (315, 320–322). The major isoforms (VEGF-120, VEGF-164, and VEGF-188) act through two tyrosine kinase receptors localized mainly to endothelial cells: VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1). VEGFR-1 is primarily responsible for endothelial cell maintenance and vascular organization (323), whereas VEGFR-2 regulates endothelial cell differentiation and migration, vascular permeability (324), and lung maturation (325). Members of the VEGF family, including placental growth factor, regulate

angiogenesis in placenta, the fetal gas exchange organ (326). Early gestational mouse lungs express abundant VEGF-120 and VEGF-164; VEGF-188 becomes predominant as gestation progresses (327). Mice that produce only VEGF-120 survive with pruned pulmonary vasculature that grows poorly (327), suggesting that VEGF-120 is sufficient for pulmonary vascular development, but VEGF-164 and/or VEGF-188 are required for vascular growth and maintenance. Aberrant VEGF expression in transgenic mice disrupts both vascular and airway development (328). Although dexamethasone blocks *in vitro* VEGF induction, treatment in postnatal mice does not affect cell-specific expression of VEGF or the proportions of VEGF mRNA splice variants (329).

VEGF is an important factor for the growth of nonvascular alveolar cells (10, 330). Airway epithelial cells express VEGF and its receptors (331–333); VEGF stimulates alveolar epithelial cell proliferation as well as surfactant production *in vitro* (334). Chronic VEGF receptor blockade in adult rats results not only in pulmonary arterial pruning but also apoptosis of alveolar septal cells, resulting in emphysema-like morphology (330, 335). Expression of VEGF is regulated via transcriptional activation and mRNA stabilization of hypoxia-inducible factor-1α (HIF-1α) in hypoxia and via HIF-2α in normoxia (325, 336, 337). The administration of VEGF improves survival of premature mice with respiratory distress syndrome (325), suggesting a possible clinical application.

The capillary bed is essential for the growth and maintenance of alveolar septa, and the loss of capillaries leads to loss of septa. Not surprisingly, the relatively quiescent adult pulmonary vasculature contains large amounts of VEGF, serving as a reservoir for vascular repair, maintenance of capillary permeability, or as a survival factor for endothelial cells (316, 338). The reported effect of retinoic acid in rescuing failed alveolar septation in rats (159) must be associated with normal septal vascularization to be functionally useful. Retinoic acid presumably acts on the epithelium, but because epithelial cells are the major source of VEGF in the lung, it is possible that retinoic acid may also modulate VEGF (339) and other vascular growth factors to support alveolar capillary as well as epithelial growth.

Another vascular growth factor, angiopoietin, works in concert with VEGF and is regulated by hypoxia (340) but acts more on vascular organization, maturation, and maintenance than cell proliferation (6). High angiopoietin levels are found in the developing mouse lung (341, 342) and in lungs of patients with pulmonary hypertension (343).

TGF-β. TGF-β in its isoforms (TGF-β1, TGF-β2, and TGF-β3) is highly expressed in lung (344), acting as a potent matrix inducer for wound repair and pulmonary vascular remodeling (345, 346) with complex effects on lung morphogenesis. Although TGF-β may act as a paracrine inducer of VEGF in cultured fibroblasts and epithelial cells (347), *in vivo* overexpression of TGF-β1 arrests fetal mouse lung development with thickened mesenchyme, abnormal vasculature, and decreased levels of VEGF (348). It is likely that the timing and mode of TGF-β signaling as well as interactions with other growth factor pathways determine its effect on lung development and growth. Bone morphogenetic protein receptor-2, a member of the TGF-β receptor family, has been identified as the gene responsible for familial pulmonary hypertension (349, 350), attesting to the role of the TGF-β family in maintaining normal lung vasculature. It is not clear whether vascular remodeling interferes with communication between pulmonary vasculature and the associated airways, possibly creating a physical barrier for delivery of growth factors from the airway.

Nitric oxide. Nitric oxide (NO) is synthesized from L-arginine via three NO synthase (NOS) isoenzymes: endothelial NOS (eNOS) and neuronal NOS are expressed in endothelial cells and neurons, respectively, and generate small amounts of NO on activation by Ca^{2+}, whereas inducible NOS (iNOS) is induced by various proinflammatory cytokines. NO mediates proliferation, migration, and differentiation of endothelial cells (351, 352) and interacts with multiple angiogenic growth factors. For example, the angiogenic action of VEGF requires NO production (351, 353). Gene deficiency of eNOS is associated with impaired fetal perfusion and growth restriction, impaired survival, limb reduction defects (354), and delayed wound healing (352). Mice deficient in eNOS exhibit elevated pulmonary vascular resistance in normoxia (355) as well as impaired alveolar and vascular formation when exposed to hypoxic stress during the period of alveolarization (356).

NO also mediates organ regeneration. Both flow and shear stress are determinants of endothelial NO release in vascular cells (357). In the liver after partial hepatectomy, increased blood flow and shear stress stimulate eNOS expression and elevated cytokine production triggers iNOS activity (358). Liver regeneration is impaired in iNOS gene knockout mice (359). In the remaining lung after pneumonectomy, the greater perfusion and shear stress increase intracellular Ca^{2+} and calmodulin content (360), which increases eNOS expression independent of iNOS expression (361). Both eNOS- and iNOS-knockout mice show severely impaired postpneumonectomy lung growth; treatment of wild-type mice with the NOS inhibitor N^G-nitro L-arginine methylester has the same effect (361), suggesting specific growth impairment caused by NO deficiency. Pneumonectomy in mice elevates serum levels of VEGF (361), which is known to interact variably with NO (353, 362). Although NOS gene knockout does not alter postpneumonectomy VEGF production, VEGF signaling may still be impaired at the receptor level. Mitogenic activity of VEGF requires activation of the mitogen-activated protein kinase cascade, a family of serine/threonine protein kinases involved in cell proliferation and migration known to be mediated by NO (362). Mitogen-activated protein kinase activation is also necessary for iNOS induction by interleukin-1 in endothelial cells (363). It remains possible that impaired postpneumonectomy lung growth in NO-deficient mice is at least partly mediated via blunting of VEGF signaling.

Retinoids. The retinoic acid–nuclear receptor complex regulates more than 300 target genes (364). Retinoids transcriptionally enhance *in vitro* synthesis of fibronectin, reduce the synthesis of collagenase and some keratins (365), and alter gene expression for surfactant-associated proteins (366). Retinoic acid regulates the expression of *hox* genes (367) during embryonic branching morphogenesis to favor growth of proximal airways and suppress distal epithelial buds (367, 368). Retinoic acid augments elastin gene expression in alveolar myofibroblasts (160, 161), alters the expression of receptors for various hormones and growth factors essential for maintaining a differentiated state of cultured alveolar epithelium (369), inhibits DNA biosynthesis, and enhances surfactant–phosphatidylcholine biosynthesis (370) consistent with accelerated epithelial maturation. Epithelial–mesenchymal actions of retinoic acid are partly mediated through induction of EGF receptors (371–373). EGF and retinoic acid synergistically increase collagen synthesis in fetal rat lung type II pneumocyte culture (374). Retinoic acid also exerts variable effects on VEGF production *in vitro* (339, 375).

The retinoic acid receptors subserve distinct functions. Retinoic acid receptor–α mediates alveolar growth after the perinatal period of alveolarization (376). Retinoic acid receptor–β inhibits septation (377), whereas retinoic acid receptor-γ is needed for normal lung elastin production and alveolarization (161). Administration of *all-trans* retinoic acid to normal neonatal rats and to older mice with a genetically failure of lung septation

enhances alveolar septal formation without increasing specific alveolar surface area, suggesting that lengthening of septal crest is regulated by other mechanisms (159, 256, 378). In neonatal rats, retinoic acid prevents glucocorticoid-induced inhibition of alveolar septation (256). Retinoic acid also enhances epithelial repair, improves survival, and attenuates the inhibition of alveolar formation in neonatal rats after acute hyperoxic lung injury (254, 379, 380). Retinoic acid protects against the pathologic increase of alveolar volume and the reduction of alveolar number/surface area in adult emphysematous rats (381) and enhances compensatory lung growth in adult rats after pneumonectomy (382). However, retinoic acid does not alter airspace size or elastin or collagen gene expression in adult mice with emphysema (383). Retinoic acid–enhanced septal cell growth has not improved morphologic or physiologic indices of alveolar function in rats with elastase-induced emphysema (384) or in adult dogs after pneumonectomy (385, 386). Findings indicate additional complexities in structure-function integration of the growing lung that are yet to be understood.

Hypoxia. Exposure of fetal lung explants to severe hypoxia reversibly suppresses biochemical and morphologic markers of cell differentiation (387). However, because lung cells are normally exposed to a higher local O_2 tension than cells of other organs and are capable of responding to a small decline in local O_2 tension, *in vitro* studies conducted at O_2 concentrations below approximately 5% may not mimic *in vivo* conditions at high altitude. In addition, the endothelium plays an essential role in the *in vivo* response to hypoxia. Cultured endothelial cells respond to small reductions of O_2 tension by upregulating PDGF-B gene expression (388). An endothelial cell-derived soluble factor(s) is necessary for the increased polyamine uptake and metabolism observed in lungs exposed to hypoxia (389, 390). Polyamines (putrescine, spermidine, and spermine) are low molecular weight organic cations that interact with DNA and RNA to effect signal transduction, cell growth, and differentiation as well as survival after lung injury. Hypoxia also upregulates a unique set of stress proteins in endothelial cells *in vitro*, possibly mediating their longer survival and metabolic adaptation (391).

The identity of the cellular oxygen sensor is not known, but a favin-heme protein residing in the plasma membrane has been proposed (392). This heme protein functions as an nicotinamide adenine dinucleotide phosphate reduced oxidase, transferring electrons through favin and heme to oxygen and generating superoxide. In the presence of iron, superoxide is converted to reactive oxygen species, which induces rapid degradation of the inducible HIF-1α subunit. In hypoxia, a more stable HIF-1α subunit forms a heterodimer with constitutive HIF-1β subunit, thereby activating HIF-1 and leading to its nuclear translocation to enhance the transcription of various responsive genes. Activation of HIF-1 leads to induction of VEGF (329) and VEGF receptor-1 but not VEGF receptor-2 expression in endothelial cells in the lung (393). On the other hand, HIF-2α is implicated during normal lung development. Deficiency of HIF-2-α in neonatal mice is associated with low VEGF levels in alveolar cells, surfactant deficiency, arrested alveolar development, and fatal respiratory distress syndrome (325). Thus, signaling through HIF-VEGF axis emerges as a key mechanism for alveolar growth in hypoxia as well as in normoxia.

Role of Pluripotent Stem Cells

Hematologic stem cells develop endothelial cell phenotype when cultured in the presence of appropriate growth factors and form new blood vessels *in vivo* when injected subcutaneously with lung cancer cells (394). Engrafted hematologic stem cells facilitate neovascularization and functional recovery after focal cerebral ischemia (395). Murine embryonic stem cells cultured in the appropriate media can develop the phenotype of type II pneumocytes (396). Cultured bone marrow cells injected intravenously into mice after bleomycin lung injury engraft to lung parenchyma and develop the phenotype of type I but not type II pneumocytes (397). These transformations underscore the broad differentiation potential of stem cells and suggest that type I pneumocytes need not arise solely from transdifferentiation of type II pneumocytes. Systemic administration of stem cells has been plagued by a low engraftment rate and lack of a sustained response. Detailed characterization of resident progenitor cell subpopulations within the growing lung offers another approach to identify and isolate pluripotent cells capable of sustaining alveolar tissue growth (398).

After lung injury, a specialized pneumocyte subpopulation shows the capacity to proliferate (399, 400) and express telomerase, an enzyme complex that stabilizes the telomeres of chromosomes in actively dividing cells (401, 402). The telomerase complex includes a highly conserved catalytic subunit (telomerase reverse transcriptase) and an RNA oligonucleotide that primes the ends of newly replicated chromosomes for repair (401–403). Differentiating pneumocytes exhibit downregulated telomerase expression and activity. In normal adult tissues, telomere length and activity correlate with the potential of a cell to reinitiate proliferation on appropriate stimulation (404–406). In early lung development, pneumocytes evolve from a relatively undifferentiated precursor population that co-expresses surfactant proteins C and A, the Clara cell marker CC10, and neuroendocrine marker cGRP (407). As development progresses, these markers are expressed by separate differentiated lineages, with type II pneumocytes expressing only the surfactant proteins (408, 409). Type II pneumocytes can transdifferentiate into type I pneumocytes, serving as progenitors of the alveolar epithelial cell pool (409, 410). In the mouse, late gestation pneumocytes strongly express active telomerase reverse transcriptase; expression decreases after birth, and very low levels are sustained into adulthood (411), similar to observations in mature human lung (401, 402, 411, 412). In human epithelial lung cancer, telomerase reverse transcriptase is highly expressed (413). In adults, the ability of pneumocytes to divide is coupled with both survival and repopulation of damaged alveolar epithelial surface (414, 415). Telomerase activity is reinitiated in adult pneumocytes during the proliferative repair phase after hyperoxic injury (411), consistent with the presence of a subpopulation of pneumocyte progenitor cells capable of responding to injury.

At least four subpopulations of pneumocytes have been isolated from hyperoxic rat lung: nonproliferative/apoptotic, nonproliferative/nonapoptotic, proliferative/apoptotic, and proliferative/nonapoptotic, in order of decreasing abundance (414). The proliferative/nonapoptotic compartment might harbor potential progenitor cells, but it has not been possible to isolate those cells physically. An alternative approach is to use specific surface markers differentially expressed between quiescent and proliferating cells to sort out specific cell subtypes. For example, E-cadherin, a zonula adherens protein that allows cell-to-cell attachment, is critical for epithelial morphogenesis (416); loss of its expression is a marker for cell proliferation, migration, and tumorigenic transition (417–420). Higher levels of E-cadherin, β-catenin, and β3 integrin are expressed in cells isolated from hyperoxic rats than from normoxic controls (421). Using these markers, type II pneumocytes have been fractionated into distinct subpopulations: the highly proliferative E-cadherin($-$) subpopulation and the less proliferative E-cadherin($+$) subpopulation (421).

The proliferative E-cadherin($-$) pneumocyte subpopulation is further characterized by its high telomerase activity and damage resistance. In cultured pneumocytes isolated from hyperoxic

animals, telomerase activity of the E-cadherin(−) subpopulation is threefold higher than in normoxic control cells (411), whereas telomerase activity of the hyperoxic E-cadherin(+) subpopulation is similar to that of normoxic control cells. Compared with the original pneumocyte population from hyperoxic animals, the E-cadherin(−) subpopulation show markedly less apoptosis, whereas the E-cadherin(+) subpopulation shows more apoptosis (421). The E-cadherin(−) pneumocyte subpopulation may represent a proliferating, nonapoptotic, and telomerase(+) compartment that can effectively resist or repair hyperoxic damage. This approach of using differential surface markers holds considerable promise for identifying progenitor cell subpopulations as targets for further manipulation.

FUNCTIONAL CORRELATES AND LIMITS OF INDUCED LUNG GROWTH

Compensatory Versus Reparative Lung Growth

Compensatory lung growth differs from reparative growth after lung injury in gene expression, biochemistry, morphology, and organ function. Postpneumonectomy induction of tropoelastin and type I procollagen mRNA expression localizes to alveolar walls and alveolar ducts in a pattern similar to that during postnatal development (91). Elastin and collagen content of the lung increases proportionally with the increase in dry lung weight (422, 423), and connective tissue morphology is largely preserved. In acute lung injury and fibrosis, there is initial collagen breakdown (424) followed by disproportionately (70- to 80-fold) increased lung elastin mRNA, localized preferentially to the muscularis of conducting airways and interstitial cells in fibrotic foci associated with distorted elastic fiber morphology (425). Hypertrophy of type II pneumocytes isolated from the remaining lung postpneumonectomy (426) is in the most active phase of the cell cycle but, in contrast to the response in acute lung injury, is not activated for surfactant lipid biosynthesis or storage (427). Although the remaining lung after pneumonectomy is not "inflamed" as in injury models, there is an initial influx of circulatory proteins and cells associated and an expanded extravascular albumin space (272). This influx is likely a vital process to provide growth factors for mitogenic activities. Pulmonary endothelial permeability is acutely elevated after major lung resection (428) and may precipitate pulmonary edema in patients with inadequate pulmonary vascular reserves. However, experimental pneumonectomy does not increase extravascular lung water formation at a given level of hemodynamic challenge (429). This is likely due to the high compliance and capacitance of the pulmonary vascular bed, as lung mass must be reduced by more than 60% before pulmonary vascular resistance and capillary pressure is increased in the isolated lung perfused at a constant flow (430). Neither vascular congestion nor extravasated protein and water contribute to the progressive weight gain in lungs following pneumonectomy (431).

Nonstructural Sources of Adaptation: Recruitment of Reserves

In any physiologic transport system, the capacity of the system exceeds normal loading by a factor of 2 to 7 (432), reflecting the existence of large physiologic reserves (or safety factor), which are essential for safeguarding against unexpected perturbation. Maintenance of physiologic reserves is a primary goal of adaptive regulation and a crucial factor in determining (*1*) the threshold for reinitiation of structural response and (*2*) the magnitude of functional compensation. Alveolar–capillary reserves for diffusive oxygen transport is reflected by the recruitment of D_L, which increase more than 100% from rest to exercise in a linear relationship with respect to pulmonary blood flow

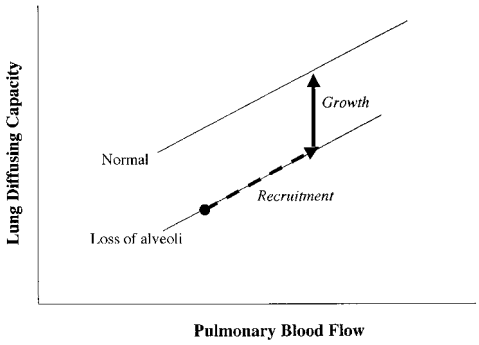

Figure 5. Evaluating the functional significance of compensatory lung growth from lung diffusing capacity (D_L). Normally D_L increases linearly with respect to pulmonary blood flow without reaching an upper limit even at peak exercise, indicating continued recruitment of large alveolar microvascular reserves. Diseases causing a loss of alveoli reduce D_L at a given blood flow. Ventilation and perfusion redistributed to the remaining functioning units cause a greater use of existing physiologic reserves and a compensatory increase in D_L along a normal slope (*dashed arrow* labeled "recruitment"), whereas compensatory growth of gas exchange tissue elevates D_L at any given pulmonary blood flow (*solid arrow* labeled "*growth*"). Adapted by permission from Hsia (442).

(433, 434) (Figure 5); D_L also increases approximately 25% with alveolar volume (435). At rest, only approximately 50% of alveoli and capillaries are sufficiently patent to participate in gas exchange, and capillary erythrocytes are nonuniformly distributed because partially collapsed capillaries particularly at lung apices allow plasma but not erythrocyte flow (436). On exercise, the number of perfused capillaries increases directly with perfusion pressure (437), and the number of distended alveoli increases with tidal volume, leading to (*1*) greater effective alveolar–capillary surface area, which increases D_L, and (*2*) greater traction on small airways, which increases airway diameter, reduces flow resistance, and facilitates uniform distribution of ventilation. Increased flow and pressure also allows more uniform regional erythrocyte flows, resulting in better matching of diffusion to perfusion that theoretically can account for 30–50% of the augmentation in D_L from rest to peak exercise (438).

Lung volume, alveolar–capillary surface area, barrier thickness for diffusion, and pulmonary capillary blood volume are major anatomical determinants of D_L. Estimates of D_L from these structural parameters in fixed lungs agree well with that measured physiologically at heavy exercise in the same animal (439). Thus, alveolar structure determines the upper limit of diffusive oxygen transport; this limit is normally not approached except at peak exercise when physiologic reserves are more fully used. In the event of loss of alveoli, ventilation and blood flow are diverted to the remaining units, causing alveolar–capillary distension and recruitment of reserves in those units. As a result, apparent D_L at a given workload is higher than that expected from the severity of anatomic destruction. Alveolar–capillary recruitment is a vital and ubiquitous mechanism of functional compensation. Because of recruitment, oxygen transport is relatively preserved until more than 50% of the alveolar–capillary surface is obliterated (440). For example, resection of 45% of lung in dogs results in no appreciable impairment of aerobic capacity and only 25% reduction of D_L at a given workload (97, 441). It is possible to separate the functional consequences of new alveolar tissue growth from the concurrent recruitment of physiologic reserves in the remaining alveoli (Figure 5). Based on experimental data from animals and clinical data from pa-

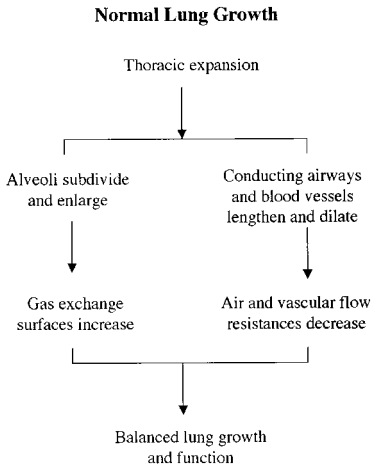

Figure 6. Normal balanced lung growth.

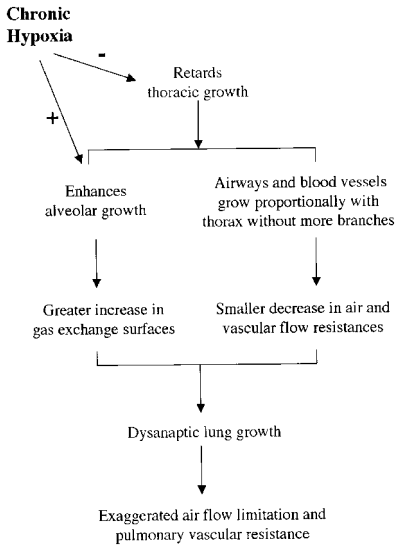

Figure 7. Dysanaptic lung growth exemplified by chronic exposure to hypoxia.

tients with various cardiopulmonary diseases, the slope of the D_L versus pulmonary blood flow relationship can provide an index of the functional integrity or "recruitability" of existing alveolar–capillary network. On the other hand, induced growth of new gas exchange tissue is evidenced by an increased D_L at any given cardiac output and an elevated intercept of the entire relationship (442).

Limits Imposed by Dysanaptic Lung Growth

Normal lung growth involves balanced expansions of all intra-acinar tissue components, conducting structures, as well as the bony and muscular thorax leading to proportional enhancement in gas exchange, mechanics, and hemodynamic function (Figure 6). Dysanaptic (i.e., unequal) lung growth refers to the observation that conducting airways and blood vessels, which primarily form in fetal life, demonstrate limited growth potential compared with acinar tissue during postnatal development (443–445) or during compensatory lung growth induced by high-altitude residence (111) or pneumonectomy (446, 447). The term was originally invoked to explain the large variation in maximal expiratory flow rate relative to lung volume among normal subjects (445); it was later used (111) to explain the small airway dimensions and low maximal flow rate with respect to lung volume observed in high altitude natives. Hypoxia-enhanced alveolar growth without corresponding enhancement in the growth of conducting structures creates an apparent airway limitation that diminishes the overall functional benefit of alveolar growth (Figure 7). The opposing effects of high-altitude exposure on lung and thoracic growth are other manifestations of dysanaptic growth where retardation of rib cage growth may effectively set the limit of functional compensation that can be achieved through stimulation of alveolar tissue growth alone.

Airway–parenchyma dissociation also occurs during postpneumonectomy lung growth, evidenced by a disproportionally low maximal expiratory flow rate, increased airways resistance, and elevated work of breathing in both animal models (184, 447–450) as well as in patients after lung resection (451–454). Abnormal airway function persists in spite of a doubling of lung volume and normalization of the static recoil of the remaining lung parenchyma. Anatomic studies show that volume and cross-sectional area of conducting airways in the remaining lung increase less than expected from the volume increase in lung parenchyma (93, 446, 455). Early after pneumonectomy, conducting airways elongate with little change in diameter, which markedly elevates airflow resistance and work of breathing because more than 50% of total airway cross-sectional area has been removed. Subsequently, airways slowly dilate, resulting in partial mitigation of airflow resistance and work of breathing (184, 456). In contrast to conducting airways, respiratory bronchioles proliferate (457) in proportion to the increase in alveolar air and tissue volume; that is, all intra-acinar structures grow equally. An increased number and volume of respiratory bronchioles might be expected to increase total airway cross-sectional area and improve diffusive gas mixing, but the anticipated benefit is offset by a longer total acinar airway length for gas diffusion.

Postpneumonectomy alveolar growth also does not normalize long-term pulmonary vascular resistance (184), suggesting a similar lag between structural adaptation of pulmonary blood vessels and that of the parenchyma. Limited compensation in airflow and vascular conductances regardless of somatic maturity or extent of septal tissue growth underscores the relative lack of plasticity in conducting structures. Because these structures cannot adapt by adding more branches, they effectively impose an upper limit of functional compensation achievable by septal tissue regrowth, as more lung units are lost.

Dysanaptic growth could potentially impose a functional limit after therapeutic intervention such as pediatric lobar lung transplant. In spite of normal alveolar growth, conducting airways in the transplanted lobe may not remodel sufficiently during subsequent somatic growth to maintain normal airflow conductance in the graft. Dysanaptic lung growth could also occur as a long-term sequelae of BPD or chronic lung disease of prematurity where small airway obstruction persists despite normalization of lung volume and mechanical function (458, 459). Yet another type of dysanaptic growth can result from nonuniform stimulation of alveolar septal cells. For example, selective stimulation of epithelial cell proliferation via exogenous growth factors could potentially outstrip capillary blood supply, resulting in more alveolar tissue but limited improvement in gas exchange. Tracheal occlusion accelerates lung organogenesis in fetal lambs with CDH, leading to increased mesenchyme tissue but disproportionately fewer type II cells, a surfactant deficit (460), and altered alveolar morphology incompatible with efficient gas exchange (461) consistent with the lack of clinical benefit (462). Exogenous retinoic acid given to adult dogs after right pneumonectomy selectively enhances alveolar capillary endothelial cell growth in the remaining lung without stimulating other alveolar cell compartments, leading to distortion of alveolar architecture and a lack of overall functional benefit (385, 386). Such distor-

tions cannot be predicted from molecular or cellular studies alone. Further investigation should clarify the extent and significance of such mismatches.

Limits Imposed by Heart–Lung–Thorax Interaction

Compensatory lung growth is thought to occur in children after pneumonectomy, inferred indirectly from long-term improvement in their pulmonary function (454, 463–465). However, more pronounced and persistent functional abnormalities in adult patients after pneumonectomy (466–469) suggest that compensatory lung growth in adults is likely minimal; instead, recruitment of existing physiologic reserves is the main source of functional compensation. Comparative responses between animals and patients have identified the abnormal mechanical interactions among heart, lung, and the thorax as the major factors limiting adaptation in adult patients. Unlike in animals, postpneumonectomy patients who have relatively normal remaining lungs and have not received any adjuvant therapy show limited expansion and reduced compliance of the remaining lung compared with age-matched control subjects (98, 452). Gas exchange impairment is generally mild until more than 67% of lung tissue is removed (468, 470). Long-term aerobic capacity is markedly limited because of concurrent cardiac and respiratory muscle dysfunction. Maximal stroke volume, ventilatory capacity, and respiratory muscle power are reduced more than 50% (452, 470, 471). Peak pulmonary arterial pressure during exercise is no higher than that expected in a normal subject (468, 472), suggesting impaired right ventricular response to an elevated afterload, that is, an inability to use the Starling mechanism of diastolic ventricular dilation to preserve stroke volume. Airflow resistance is increased, whereas effective respiratory muscle mass is reduced. Abnormalities cannot be attributed to respiratory muscle or cardiovascular deconditioning (470) but are compatible with consequences of anatomic restriction of the heart, rib cage, and diaphragm caused by pleuromediastinal serofibrous adhesions, which are present in patients but absent in pneumonectomized dogs. Fibrous adhesions distort and immobilize the thorax and diaphragm, reduce compliance of the cardiac fossa, irreversibly limit lung expansion, and attenuate strain-related signals for lung growth.

Reasons for persistent serofibrous accumulation in patients are not clear but are likely related to a large residual hemothorax, postoperative inactivity, poor pleural lymphatic clearance, and/or an intense pleuromediastinal inflammatory response. It remains possible that compensatory lung growth might be induced in adult patients as in quadrupeds if pleuromediastinal reactions could be minimized by strategies such as separating the pleural surfaces with an inert gas (473), instillation of a lubricant solution (474), or the use of antiadhesion barriers (475). Long-term lung function tends to be best in the most physically active patients; hence, aggressive postoperative rehabilitation and sustained exercise programs may help maintain the mobility of the diaphragm and mediastinum (476). These issues of mechanical interdependence among intrathoracic structures cannot be predicted from animal studies alone; they are broadly relevant to the adaptive response not only after pneumonectomy but also in other destructive lung diseases.

Growth Induction and Carcinogenesis

A newly recognized characteristic of induced lung growth is the potential for the growth-enhancing microenvironment to stimulate tumor cell proliferation. In mice exposed to a carcinogen, pneumonectomy acts as a tumor promoter and increases pulmonary adenoma multiplicity as much as sevenfold. The effect is observed whether pneumonectomy is performed before or after administration of carcinogen (84). In addition, pneumonectomy promotes metastasis of systemic tumor to the lung. In pneumonectomized mice injected with melanoma cells, there is up to threefold more pulmonary melanoma cell metastases than in sham control subjects (477). The greatest numbers of tumors are present when melanoma cells are injected during the initial rapid phase of compensatory lung growth. In contrast, pneumonectomy does not enhance subcutaneous growth of melanoma tumors, reflecting the selective local nature of metastatic enhancement. Observations support the premise that after pneumonectomy, the microenvironment of the remaining lung regulates the initiation, promotion, and progression of metastatic cancer. This response may involve VEGF; elevated serum VEGF levels in patients after surgical lung resection for cancer have been linked to the subsequent development of aggressive secondary lung metastases perhaps via an increased capillary permeability (478). These observations provide a framework for future studies to define conditions within the lung that differentially promote normal and abnormal cellular growth.

FUTURE DIRECTIONS

Current understanding of growth induction in postnatal lung highlights several key issues and caveats in need of further research:

1. At the molecular level, gene knockout models have been invaluable in elucidating the function of specific proteins during development, but interpretation is limited by the possibility of compensatory or redundant mechanisms. It remains uncertain whether the same gene products are implicated during development as during reinduction of growth in the mature lung and whether their molecular interactions are similar (479). The developing lung differs from the adult/nongrowing lung in mechanical stresses, hormonal/growth factor levels, responsiveness to a given signal and epithelial–mesenchymal–endothelial interactions. Although it is convenient to assume that morphogenetic signaling pathways controlling lung development become reactivated during reinduction of growth in the mature lung, interpretation of earlier studies, reviewed by Cagle and Thurlbeck (464), cautions against this assumption and is supported by recent comparisons showing divergent patterns of surfactant protein expression and EGF axis activation in dogs during normal and compensatory lung growth (277). More comparative studies are needed to define the similarities and differences between different types of lung growth.
2. At the cellular level, cell architecture and mechanical stress distribution are tightly coupled to metabolic processes, including cell growth, proliferation, differentiation, and turnover. The properties, organization, and distribution of stress-bearing cytoskeletal filaments and fibers critically determine the pattern of mechanotransduction. The central role of elastin in secondary crest formation requires further delineation. New culture systems that more precisely mimic the movements and stresses of developing elastic tissues and the use of "nanotechnology" may offer new insight into the cellular assembly that confers macroscopic mechanical properties of stress-bearing fibers. There is a need to develop specific models of mechanical loading on alveolar capillaries to study the mechanisms of capillary growth and remodeling in the lung. Another promising direction, arising from the rapidly evolving advances in stem cell biology, is to isolate and characterize progenitor cell subpopulations within the lung, and testing whether implantation of exogenous stem cells or stimula-

tion of endogenous stem cells can induce or accelerate alveolar cellular growth.

3. At the organ level, the structural basis of how new alveolar tissue is added postnatally remains incompletely understood. Current understanding of the feedback communication among growing structures, for example, between bronchial and alveolar epithelium or among alveolar epithelium, interstitium, and endothelium, is sketchy without a coherent integration of how the extensive array of hormones and paracrine growth factors known to act on various alveolar compartments are coordinated to bring about balanced three-dimensional structural growth. Secondary alveolar crest formation only partially account for the observed septal growth. Additional mechanisms for increasing alveolar complexity related to other anatomical compartments of the acinus, such as respiratory bronchioles and alveolar ducts, need to be delineated. Questions remain regarding how overall alveolar–capillary architecture is governed and how septal growth is ultimately limited.
4. At the translational level, mechanical strain and chronic hypoxia emerge as major signals for reinduction of alveolar growth in a threshold-dependent manner, whereas metabolic and hormonal mediators quantitatively and qualitatively modulate the response. Although this knowledge offers exciting possibilities for designing pharmacologic or physiologic manipulations to induce lung growth, it should be recognized that selective stimulation of one or a few alveolar cell populations is likely to be of limited utility. To achieve optimal function, epithelial growth must be matched to that of the matrix, capillary network, conducting blood vessels, and airways as well as the bony thorax. Dysanaptic responses among structural components, distortion of alveolar architecture, or mismatch among intrathoracic organs limit the effectiveness of induced alveolar tissue growth. Herein lies a caveat of the "magic pill" approach to growth induction because no single agent can possibly replicate the coordination seen in normal lung growth. Multiple and possibly all of the regulatory pathways must be activated synchronously to orchestrate a balanced and functionally useful enhancement of lung growth.

Several lines of basic research have approached clinical application, such as exogenous retinoic acid for patients with emphysema (480) and perfluorocarbon lung distention for neonates with CDH (481–483). Other applications yet to be explored include inducing compensatory lung growth in children who survive BPD beyond the injury–repair stage or those who survive repair of CDH but are left with small lungs and marginal gas exchange capacity. For example, nasal continuous positive airway pressure has been reported to mitigate the development and severity of BPD in preterm infants (484, 485). Chronic intermittent continuous positive airway pressure in survivors of BPD or CDH might provide sufficient mechanical signals to augment subsequent lung growth. Potential applications should be based on appropriate animal models that mimic the inciting injury, the organ response, as well as the long-term growth pattern seen in humans. The baboon model of mild-to-moderate BPD (486) and the neonatal lamb model of CDH (487) could be very valuable for testing these strategies. The pneumonectomy model is particularly robust because the remaining lung is not "injured" in the classic sense, and the anatomic loss of alveoli and lung function is quantifiable and reproducible. As the bulk of research thus far involves rodents, there is a need to define rigorously the structural basis and functional consequences of interspecies differences within and among models of compensatory lung growth.

Finally, reinitiating lung growth may increase susceptibility to carcinogenesis and metastasis. Implication of this tantalizing observation remains to be seen, but it is an important reminder that there are no free rides.

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Participants:
Connie C.W. Hsia, *Chair*
Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, TX.

Mary Anne Berberich
Division of Lung Diseases, National Heart, Lung and Blood Institute, Bethesda, MD.

Barbara Driscoll
Children's Hospital Los Angeles Research Institute, University of Southern California, Los Angeles, CA.

Victor E. Laubach
Thoracic & Cardiovascular Surgery, University of Virginia, Charlottesville, VA.

Craig W. Lillehei
Department of Surgery, Children's Hospital Harvard Medical School, Boston, MA.

Donald J. Massaro
Lung Biology Lab, Georgetown University School of Medicine, Washington, DC.

Elizabeth A. Perkett
Pediatric Pulmonary Medicine, University of New Mexico, Albuquerque, NM.

Richard A. Pierce
Department of Medicine, Washington University, St. Louis, MO.

D. Eugene Rannels
Cell/Molecular Physiology, Pennsylvania State University, Hershey, PA.

Rita M. Ryan
Division of Neonatology, SUNY Buffalo, Buffalo, NY.

Robert S. Tepper
Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN.

Mary I. Townsley
Department of Physiology, University of South Alabama, Mobile, AL.

Kathleen A. Veness-Meehan
Neonatal/Perinatal Medicine, University of North Carolina, Chapel Hill, NC.

Ning Wang
Physiology Program, Harvard School of Public Health, Boston, MA.

David Warburton
Children's Hospital Los Angeles Research Institute, University of Southern California, Los Angeles, CA.

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