Signals and mechanisms of compensatory lung growth

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Hsia, Connie C. W. Signals and mechanisms of compensatory lung growth. J Appl Physiol 97: 1992–1998, 2004; doi:10.1152/japplphysiol.00530.2004.—Growth of the lung involves unique structure-function interactions not seen in solid organs. Mechanical feedback between the lung and thorax constitutes a major signal that sustains developmental as well as compensatory lung growth. After the loss of lung units as by pneumonectomy (PNX), increased mechanical stress and strain on the remaining units induce adaptive responses to augment oxygen transport, including 1) recruitment of alveolar-capillary reserves, 2) remodeling of existing tissue, and 3) regenerative growth of acinar tissue when strain exceeds a critical threshold. Alveolar hypoxia, hormones, and growth factors may feed into the mechanical feedback system to modify an existing growth response but are unlikely to initiate compensatory growth in the absence of sufficient mechanical signals. Whereas endogenous post-PNX alveolar growth preserves normal structure-function relationships, experimental manipulation of selected metabolic pathways can distort these relationships. Finally, PNX widens the disparity between the rapidly adapting acini and slowly adapting conducting airways and blood vessels, leading to disproportionate airflow and hemodynamic dysfunction and secondary hypertrophy of the right ventricle and respiratory muscles that limits overall organ function despite regeneration of gas exchange tissue. These are key concepts to consider when formulating approaches to stimulate or augment compensatory growth in chronic lung disease.

pneumonectomy; alveolar regeneration; recruitment; remodeling; alveolar hypoxia; dysanaptic lung growth

COMPLEXITIES IN EVALUATING LUNG GROWTH

The term “regenerative growth” denotes the direct replacement of an organ or appendage that was lost, whereas “compensatory growth” denotes accelerated hyperplasia and/or hypertrophy of the remaining tissue in a damaged organ to restore normal function. Historically, regenerative and compensatory growth have been more difficult to define in the lung than in solid organs for several reasons. 1) The volume of gas-exchange tissue relative to overall lung volume is small and approximately equals pulmonary capillary blood volume (~8% of lung volume). 2) Anatomic relationships are complex among structural components derived from different embryonic origins that must conform to geometric constraints within a relatively rigid thoracic container. 3) Structure-function relationships are unusually intricate; the wide fluctuations in convective airflow and perfusion as well as gas flux that must be accommodated in the lung are not seen in any other organ. Nevertheless, general principles governing the evaluation of lung growth can be stated. These are outlined below.

1) Mammals cannot regenerate an entire gas-exchange organ as can amphibians; however, under the appropriate stimuli, they retain the ability to grow new acinar tissue and alveolar capillaries at an accelerated rate, forming new alveolar septa and respiratory bronchiolar branches to augment the effective surface area for gas exchange (i.e., regenerative growth). Other components such as conducting airways and blood vessels cannot regenerate new branches after birth; they grow only by elongation and dilatation of existing branches to reduce net flow resistance (i.e., compensatory growth). Useful lung growth involves activation of different mechanisms within different structures and proportional stimulation of each structure in the appropriate temporal and spatial sequence to preserve normal architectural organization and optimize organ function.

2) Growth of new alveolar tissue should be distinguished from remodeling of existing tissue components, such as altered size of intra-acinar airways and alveolar sacs, redistribution of surface folding, or rearrangement of matrix elements within alveolar septa. Remodeling can significantly modify gas-exchange efficiency with or without new tissue growth.

3) A large fraction of anatomic alveolar-capillary surfaces and capillary blood volume is not utilized for gas transport at rest but is recruited as ventilation and blood flow increase. The difference between basal and maximal oxygen flux across the alveolar blood-gas barrier at peak exercise is termed “safety
factor” or “physiological reserves” (83). The lung possesses unusually large physiological reserves; recruitment of reserves significantly augments oxygen transport in the face of anatomic loss of lung units. These reserves need to be taken into account when assessing compensation from lung growth in any model.

THE PNEUMONECTOMY MODEL

Given the above considerations, experimental pneumonectomy (PNX) has proven to be a robust model for characterizing the sources, mechanisms, and limits of adaptation to the loss of a known number of lung units. Experimental lung resection dates back to 1881 (69); it was realized early that the remaining lung expands to nearly the volume of two lungs associated with alveolar enlargement. Early animal studies established the basis for application of the procedure to humans, beginning in the 1930s as a treatment for chest trauma, bronchiectasis, or cancer. Cohn (15) in 1939 first established mechanical forces as a major signal for the compensatory increase in lung mass following lobectomy. Schilling et al. (70) in the 1950s detailed the well-preserved functional status in dogs that underwent removal in stages of up to nearly 70% of lung mass. Literature from the 1960s to 1990s centered on the use of small animals for defining hormonal, cellular, and molecular responses to PNX, summarized by Cagle and Thurlbeck (12). Significant strides were also made in understanding the cellular and molecular pathways of tissue regeneration in vertebrates, summarized by Gilbert and Rannels (24). In general, studies in small animals did not address the relative importance of non-structural sources of compensation or the consequences of cellular adaptation on organ function. Functional compensation to PNX has been described mainly in dogs at rest (2, 26, 85) and later during exercise (31, 32, 35, 36) to define limits of compensation.

COMPENSATION WITHOUT LUNG GROWTH

The amount of lung removed by PNX is reproducible, and, because the remaining lung is not injured, adaptive changes are readily quantified. The right and left lungs normally comprise 55–58% and 42–45%, respectively, of total volume and diffusing capacity and receive a similar fraction of ventilation and blood flow (44, 69). After PNX, if ventilation and cardiac output remain the same, airflow and perfusion through the remaining lung increase by a factor of one divided by the fraction of lung remaining, leading to recruitment of existing alveolar-capillary reserves in the remaining lung via unfolding of epithelial surfaces, opening and distension of alveolar microvessels, and redistribution of capillary erythrocyte flow, all of which increase the effective surfaces for gas exchange. The pattern of alveolar-capillary recruitment can be assessed from the relationship of diffusing capacity for carbon monoxide or oxygen with respect to pulmonary blood flow (30).

Persistent increases in ventilation and perfusion to the remaining lung initially causes preferential enlargement of alveolar duct relative to alveolar air space and later lengthening as well as redistribution of interalveolar septa that restores the original volume proportions between alveolar ducts and alveoli as well as increases gas-exchange surface (5, 10). In addition, the mean harmonic tissue-plasma barrier distance for diffusion is reduced to facilitate gas diffusion (33), and airways and blood vessels lengthen as well as dilate to mitigate airflow resistance (17). Enlargement of the alveolar-capillary network by recruitment of remaining alveolar-capillary reserves and remodeling of remaining structures account for the entire compensatory response after left PNX, sufficient to maintain a near-normal maximal oxygen uptake despite a lower arterial oxygen saturation with exercise (32). These mechanisms can increase post-PNX lung diffusing capacity at a given workload by ~30% above that expected from anatomic loss of lung units (13) without invoking additional growth of new alveolar tissue. The magnitude of compensation observed in dogs is consistent with that measured in post-PNX patients where the remaining lung is relatively normal (21, 39).

COMPENSATION BY ALVEOLAR GROWTH

With greater impairment of diffusion after 55–58% resection by right PNX, regeneration of alveolar tissue is also invoked (35), signifying an effective limit to the recruitment of remaining reserves and a critical stimulus threshold for initiation of additional cellular pathways of compensation. In rodents that underwent removal of one to two lobes, early response genes are activated (23, 48) and rapidly induce alveolar DNA synthesis, cellular proliferation, and tissue growth, restoring DNA and protein content, alveolar-capillary surface areas, and barrier thickness to that of two lungs and normalizing alveolar morphology (9, 71, 81). The intense post-PNX cellular activities appear highly susceptible to pharmacological manipulation (45–47, 66, 90) as well as to carcinogenesis (9). In large mammals, post-PNX responses are maturity dependent. When PNX is performed before somatic maturity, regrowth of alveolar tissue is vigorous, similar to that in rodents; alveolar morphology, tissue volume, surface areas, and gas exchange and aerobic capacities are normalized at maturity (77). On the other hand, when PNX is performed after maturity is reached, reinitiation of quiescent growth pathways not only requires a higher stimulus threshold but also progresses slowly, underscoring the innate difficulty in modifying a fully differentiated structural scaffold. Although intensity of post-PNX alveolar growth is directly related to the magnitude of lung resection, the compensatory response in adult animals ultimately remains incomplete.

MECHANICAL INTERACTIONS BETWEEN THORAX AND LUNG

What could explain the difference in response between rodents and large mammals? One critical consideration is reciprocal mechanical interactions between thorax and lung. There is extensive literature, detailed elsewhere (37, 65, 86), showing how mechanical forces alter cell function, organogenesis, and normal and compensatory lung development. Stretch of alveolar cells induces all the changes associated with cell growth and septal formation, including signal transduction (14, 53), ion flux (55, 88), protein turnover (7, 67, 75, 88), growth factor production (52, 82), proliferation (14, 54), and apoptosis (68). Normal lung growth is in part driven by thoracic growth; the rib cage exerts inspiratory recoil against the expiratory elastic recoil of the lung. The resulting negative intrathoracic pressure creates chronic lung stress and strain that initiates and sustains cellular activities of tissue growth. Growth of lung tissue in turn reduces the stress that generates tissue strain in a feedback loop (Fig. 1).
Reduction of mechanical stress/strain during development leads to lung hypoplasia, as in congenital diaphragmatic hernia or severe kyphoscoliosis. On the other hand, when mechanical stress/strain are accentuated during development, lung growth is enhanced, as seen after experimental application of continuous positive airway pressure (91) and perfluorocarbon distension (61) in growing animals. It seems that lung stress and strain generated post-PNX overlaid on a background of heightened developmental lung strain generated by the expanding thorax intensifies the compensatory response in growing animals. In rodents, the epiphyses do not close and somatic growth continues throughout much of their life span. Hence, developmental signals are never completely shut off, keeping these animals susceptible to even modest imposition of further lung strain.

In large mammals, mechanical interaction between thoracic and lung growth ceases when somatic maturity is reached and when the epiphysis closes, ending further rib cage enlargement. Thereafter, lung growth also ceases, and the relevant biochemical pathways are either inactivated or downregulated to levels required only for tissue maintenance. It is not surprising that much larger and sustained signals of mechanical stress and strain are required to reinitiate quiescent pathways of growth in an adult animal than to amplify already active pathways in a small strain are required to reinitiate quiescent pathways of growth in much larger and sustained signals of mechanical stress and strain of the remaining lung with space-occupying material blunts the compensatory increase in DNA synthesis (8), mitotic index (19), alveolar cell volumes and surfaces (40), maximal oxygen uptake and lung diffusing capacity (38, 87). However, the remaining lung still enlarges ~20% via caudal and outward displacement, respectively, of the ipsilateral hemidiaphragm and lower rib cage (62, 87); this modest residual response suggests the influence of other signals that compel the lung to make space for its expansion where space is not readily available. The residual signaling could still be mechanical in origin, such as a larger tidal volume or flow-induced capillary distension and shear, or it could be nonmechanical signaling via hormones, paracrine growth factors, or alveolar hypoxia.

THE ROLE OF HORMONES AND GROWTH FACTORS

There is good evidence that hormones and biochemical growth factors act primarily as modulators rather than instigators of post-PNX lung growth. The production of many of these substances is sensitive to mechanical strain; each substance modulates different aspects of cellular growth but any one substance cannot recapitulate the entire post-PNX growth response and there is much functional overlap among substances. A concrete example is provided by all-trans-retinoic acid (RA), which is known to exert wide-ranging biochemical effects and promote alveolar septation in young rats (56, 57). In adult dogs, RA enhances alveolar capillary formation only in the presence of on-going compensatory lung growth, as subsequent to right PNX (89). In the absence of compensatory lung growth after the less-extensive left PNX in adult dogs, RA treatment has no effect on lung function, volume, morphology, or ultrastructure (unpublished observations). It seems that, when endogenous signals for lung growth remain active, alveolar growth is highly susceptible to pharmacological manipulation. When endogenous signals are quiescent, as in the adult large animal, alveolar growth is much less susceptible to pharmacological manipulation. This makes teleological sense because it would not be metabolically efficient to turn growth on and off in response to minor changes in stress and strain. Although biochemical factors significantly modify a variety of cellular activities and synchronize the organization of actively growing tissue, they are unable to reinitiate post-PNX alveolar growth in vivo in the absence of on-going growth activated by another signal.

Most studies that manipulated growth factors post-PNX have dramatically augmented one or a few factors or pathways over a few weeks in rodents, leading to increased alveolar tissue or surface area (45–47); these studies have not examined alveolar ultrastructure or defined the functional consequences of growth enhancement. The significance of characterizing...
ultrastructure and organ function is also illustrated by studies of RA action in adult dogs during active post-PNX lung growth (89). In these animals, RA treatment further enhances the compensatory increase in alveolar capillary formation and endothelial cell volumes compared with placebo controls but does not alter volumes of epithelium, interstitium, alveolar surface area, or the harmonic mean diffusion barrier length. Alveolar surface-to-volume ratio actually declines in RA-treated animals, suggesting loss of surface folding or altered three-dimensional alveolar geometry. Membrane diffusing capacity, whether estimated by physiological or morphometric methods, is not significantly altered (18, 89). Because septal cells are not stimulated uniformly by RA, alveolar septal architecture is distorted and overall gas-exchange efficiency is not improved. RA can also exert additional effects on airway structure and ventilatory distribution (18, 60) that counterbalance its effect on alveolar structure and physiology. This is an example of mismatched alveolar septal response due to selective manipulation of a few components within a coordinated endogenous compensatory response. Selective manipulation of cells or pathways may be inherently an unrealistic approach because it can distort anatomic and physiological relationships and negate the expected functional benefit. Dramatic short-term activation of a few pathways may be equally unrealistic because a sustained mild to modest enhancement of multiple interacting pathways is the more likely scenario, especially during the extended course of compensation in large animals (20).

Balanced growth is also an important consideration in the induction or implantation of pluripotent stem cells to regenerate lung tissue. For this approach to be effective, the microenvironment and macroenvironment must not only provide discriminating signals to direct these cells into multiple lineages in the appropriate temporal and spatial order but also perpetuate their subsequent proliferation, differentiation, and organization. At present, type 2 pneumocytes and certain Clara cell secretory protein-expressing cells located at the bronchoalveolar duct junction (22) are believed to contribute to alveolar epithelial repair and repopulation. Hematopoietic stem cells may also participate. Female recipients of bone marrow stem cell transplant from male donors show significant chimerism of epithelial and endothelial cells in the lung (76), indicating uptake and persistence of transplanted cells. It is not known whether transplanted stem cells retain the ability to differentiate into multiple lineages or the ability to recapitulate normal alveolar organization. One report in mice indicates that transplanted marrow-derived vascular progenitor cells do not significantly contribute to post-PNX vascular growth or remodeling (80), even though mechanical strain is known to alter the function of human mesenchymal stem cells in culture (74). It remains to be seen whether mechanical signals post-PNX activate resident progenitor cells to proliferate and differentiate.

**THE IMPORTANCE OF BALANCED LUNG GROWTH**

Optimal lung growth also requires functional balance among interdependent lung regions. Balance is not always achievable when interdependent structures develop at different rates and exhibit variable capacity for growth. The classic example of mismatched (or dysanaptic) growth is between airways and blood vessels and the gas-exchange units (acini). After birth, conducting airways and blood vessels enlarge without multiplication, and their growth lags behind that of acini (25, 28, 29); disparity is accentuated in the highly stratified lungs of large mammals and further exaggerated after PNX (26, 59, 84), as the conducting structure adapts less vigorously than the acinar structure irrespective of age or species. This dissociation results in disproportional and persistent elevation of airflow resistance as well as pulmonary hemodynamic dysfunction after PNX (78), which in turn evokes secondary compensation via hypertrophy of right ventricle and respiratory muscles, respectively (35). Dysanaptic lung growth is well documented in children after major lung resection (59, 84). As more lung units are removed, alveolar growth intensifies, whereas airway-vascular adaptation remains slow and incomplete, further widening the functional disparity, accentuating right ventricular and respiratory muscle hypertrophy, and potentially restricting how much functional improvement can be derived from alveolar growth alone.

**THE ROLE OF ALVEOLAR HYPOXIA**

Hypoxic preconditioning of human endothelial progenitor cells in vitro enhances neovascularization when cells are transplanted into ischemic limbs (1). Brief hypoxic conditioning of microvascular endothelial cells grown on three-dimensional matrix accelerates spontaneous tubular (capillary) morphogenesis in a process that involves reactive oxygen species and nuclear transcription factor NF-κB (51). In vertebrates, hypoxia stimulates hypertrophy of gas-exchange organs such as skin folds and gills. Rodents native to high altitude possess higher lung tissue and alveolar cell volumes than their counterparts native to sea level (63). Alveolar growth rate is transiently accelerated in rats exposed to hypoxia (3, 4, 11, 16, 72, 73, 79), but then this rate returns to normal with sustained hypoxia (41); the increase in lung volume is reversible once the hypoxic stimulus is withdrawn (64). These observations suggest that hypoxia stimulates alveolar growth either directly or via interaction with other signals and that its structural effects may not be permanent.

Morphological features of hypoxia-enhanced alveolar growth are age dependent in rats (6, 58), suggesting interaction
between hypoxia and other developmental signals. Response in nonrodent species (guinea pigs and dogs) has demonstrated how hypoxia-enhanced alveolar growth is constrained by signals of somatic growth. Epiphyseal union occurs in guinea pigs in a definite order beginning at ~20 wk of age (92); eventually, the rib cage stops growing as in large animals. When weanling guinea pigs are raised at 5,100-m altitude, alveolar volume and surface area initially increase rapidly independent of hyperventilation (49, 50); however, with longer exposure, alveolar growth rate progressively declines and the projected lung size at 20 wk of age is no larger than that in control animals raised at sea level. Data suggest that hypoxia accelerates alveolar growth only up to the limit determined by thoracic size. A similar conclusion was reached in a long-term study of beagle dogs (27, 43) born and raised at sea level and either exposed to moderate high altitude (3,100 m) as adults or exposed as puppies and raised to adulthood at 3,100 m. Animals were studied after deacclimatization when reversible changes of high-altitude exposure had subsided. Compared with their respective controls kept at sea level, pups raised at high altitude show higher lung air and tissue volumes, alveolar surface area, and diffusing capacity; a depressed diaphragm rather than expanded rib cage accommodates the added lung volume. In contrast, none of the markers of alveolar growth is enhanced in adult animals residing at high altitude (42). A reasonable interpretation of these findings is that alveolar hypoxia amplifies in vivo alveolar growth initiated by other signals such as mechanical strain during maturation. The effect of hypoxia diminishes as maturation proceeds; after adulthood is reached, hypoxia alone cannot reinitiate alveolar growth in the absence of concurrently increased mechanical lung strain.

Can hypoxic conditioning facilitate compensatory alveolar growth? After PNX, alveolar oxygen tension is lower and the alveolar-arterial oxygen tension gradient is higher; changes are further accentuated during exercise so a hypoxic stimulus is already presented to the alveolar septal cells. Further imposition of ambient hypoxia in pneumonecrotomized rats accelerates compensatory alveolar growth, whereas imposition of hyperoxia has the opposite effect (71). It is not known whether hypoxia-enhanced alveolar growth early post-PNX is sustained or whether the effect is maturity dependent in large animals. If so, we should expect hypoxia to further enhance strain-induced alveolar growth that normally occurs in adult animals after right PNX, but hypoxia may not be able to independently initiate alveolar growth in adult animals after left PNX, where lung strain is normally not sufficient to initiate compensatory alveolar growth.

SUMMARY AND FUTURE DIRECTIONS

On the basis of studies from many laboratories spanning over 100 years, a general framework can be formulated for understanding the mechanisms of compensatory lung growth and for developing rational approaches to growth enhancement. The key concepts are outlined below.

1) Mechanical stress and strain emerge as the predominant in vivo signals initiating and sustaining compensatory remodeling of lung tissue; if great enough, they also stimulate new growth of gas-exchange units. Both remodeling and new growth feedback to reduce stress to the point that a steady state is reached (Fig. 2). A much stronger mechanical signal is required to reactivate quiescent pathways of lung growth in large mature lungs than to enhance pathways already activated in small growing lungs.

2) Alveolar hypoxia and endocrine or paracrine growth factors may feed into the mechanical feedback system and accentuate a growth response that already exists, but these factors are unlikely to initiate alveolar growth de novo in the absence of sufficient preexisting mechanical stress and strain.

3) Endogenous stimuli and modulators of alveolar growth are synergistic; therefore, it should be possible to augment compensatory alveolar growth by combining signals and mediators, for example, mechanical tissue strain plus ambient hypoxia after PNX.

4) Maintenance of balanced cellular growth and integrated structure-function relationships is essential when pharmacological agents are used to alter cellular pathways. Selective manipulation of a few pathways could distort anatomic and physiological relationships at a microscopic or macroscopic level and offset the expected benefit of cell hyperplasia and hypertrophy.

5) Dysanaptic lung growth caused by the slow and restricted adaptability of conducting airways and blood vessels limits how much functional benefit can be derived from alveolar growth alone. Future investigation should include exploration of novel approaches to initiate and/or augment compensatory airway and vascular growth in concert with alveolar growth.

There is as yet a paucity of studies that utilize the variety of transgenic animals and conditional knockout models now available to examine the significance of specific genes and their products in compensatory lung growth and function. In particular, it would be informative to know how disrupting the signaling of mechanotransduction alters compensatory growth and how independent signals might converge on common downstream pathways. These issues are immediately relevant to the human subject, not just after PNX but also in other types of lung disease, for example, when formulating approaches to enhance remodeling or stimulate growth of the remaining lung units in pulmonary fibrosis or to reconstitute normal lung stress-strain relationships in emphysema.

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REFERENCES


73. Sekhon HS and Thurlbeck WM. Time course of lung growth following exposure to hypoxia and/or hyperoxia in rats. Respir Physiol 105: 241–252, 1996.


