Editorial

Stem Cell INVESTIGATION

The puzzling mechanism of compensatory lung growth

Steven J. Mentzer

Laboratory of Adaptive and Regenerative Biology, Brigham & Women's Hospital, Harvard Medical School, Boston, MA, USA

Correspondence to: Dr. Steven J. Mentzer, MD. Brigham &Women's Hospital, Room 259, 75 Francis Street, Boston, MA 02115, USA.
Email: smentzer@bwh.harvard.edu.

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In a recent publication, Lechner *et al.* ("Recruited monocytes and type 2 immunity promote lung regeneration following pneumonectomy", *Cell Stem Cell* 2017;21:120-34.e7) have contributed an important missing piece to the enigmatic puzzle of compensatory lung growth (1).

In most adult mammals, the surgical removal of one lung (pneumonectomy) results in the rapid growth of the remaining lung (2). Compensatory lung growth has been observed in mice (3), rats (4), cats (5), dogs (6), rabbits (7) and ferrets (8). In adult humans, recent evidence suggests that compensatory growth can occur—but the time course is years rather than days to weeks (9).

Lung regeneration has been historically underappreciated (5) because of the difficulty in measuring lung growth. In normal circumstances, lung volume varies widely. Lung volumes vary with tidal ventilation from a few hundred cm^3 at rest to a liter or more with exercise (10). In small animals after pneumonectomy, experiments using controlled inflation pressures indicate that the remaining lung grows to approximately the same volume as the original two lungs within weeks of pneumonectomy (11). Associated with the increase in volume is an increase in lung weight and cell number (2).

In mice, the strongest evidence for growth is morphometry of the lung microarchitecture. Fehrenbach and colleagues (12) have used advanced stereological techniques to demonstrate an increase in the number of alveoli. After left pneumonectomy, the number of alveoli in the right lung increased from 643,000 to 925,000 within 3 weeks of surgery (12). There appears to be some increased variability in alveolar dimensions (12), but a mean alveolar diameter that is relatively constant (13). Furthermore, the construction of the alveoli is surprisingly rapid; 74% of the new alveoli are detectable within 6 days of pneumonectomy (12). This increase in alveolar number is associated with a complete restoration of the gas exchange surface area (14).

In considering the mechanism of regeneration, these empirical observations suggest several intriguing features of lung growth. First, the clinical consequences of post-pneumonectomy lung growth are virtually undetectable. After a transient increase in ventilation tidal volume post-pneumonectomy, there is no detectable change in lung mechanics, oxygenation or activity level (15).

Second, regrowth of the post-pneumonectomy lung occurs after tissue loss, but without detectable parenchymal lung injury. The return of alveolar number to near-baseline levels without apparent injury suggests mechanisms for "sensing" the loss of lung tissue and initiating compensatory growth.

Third, lung regeneration is not orthotopic, but rather new growth occurs remotely in the remaining lung. The supranormal growth of the remaining lung indicates that lung size is not limited by animal size, but is responsive to physiologic demands.

Fourth, the post-pneumonectomy lung growth involves more than the simple addition of new cells through a straightforward process of cellular proliferation and maturation. The alveolar regrowth involves the construction of complex alveolar microarchitecture (12,14) with stringent performance demands (16).

Fifth, post-pneumonectomy lung growth occurs in the

absence of obvious histologic evidence of regeneration. Histologic examination of the subpleural regions of the remaining lung demonstrates few areas of cellular aggregation or evidence of a localized "proliferative phase" of wound healing (13).

These observations suggest important distinctions between developmental morphogenesis and compensatory lung growth. In regenerative processes, in which stem cell proliferation precedes maturation, undifferentiated cell proliferation is followed by a succession of cell-cell interactions. Through these inductive interactions, groups of cells signal the differentiation of their neighbors (17). In the case of lung regeneration, the temporal sequence of growth and patterning is less clear. Growth and patterning may not be distinctly sequential, but rather closely linked in both time and space—analogous to the remodeling regeneration observed in organisms such as Hydra (18,19). The functional consequence of a tightly coupled process is a growing lung that can maintain the performance demands of gas exchange.

Several recent reports suggest practical mechanisms that might help explain post-pneumonectomy lung growth. Ysasi *et al.* have shown that post-pneumonectomy lung deformation or "stretch" leads to pleural mesothelial-mesenchymal transition (MMT) (20). MMT is a process characterized by the loss of microvilli, disruption of intercellular junctions, and changes in cell shape. The transitional cells acquire a fusiform shape and a migratory phenotype. Ysasi *et al.* (20) have shown that post-pneumonectomy MMT is associated with myofibroblast transition and the migration of these cells into subpleural septa. By analogy with septal lifting in development (21,22), it is plausible that these migratory cells participate in the repartitioning of the alveolar duct.

MMT in the pleura has several features uniquely suited for lung regeneration and repair. Pleural transition produces cells spatially positioned to contribute to growth where lung volume expansion is the greatest. In addition, transitional cells are positioned to facilitate rapid repair in a region of the body (pleura) where prolonged injury can be life threatening (e.g., pneumothorax). Further, pleural transition facilitates the centripetal migration of regenerative cells, likely via interstitial fluid flows (23), to subpleural parenchymal tissues (24).

A complementary mechanism of lung growth has recently been proposed. Using genetic loss of function and adoptive transfer experiments, Lechner *et al.* demonstrated the dependence of compensatory lung growth in $CCR2^+$ monocytes and CCL2 signaling (1). The primary source of the CCL2 signaling appears to be the alveolar type II (ATII) cell. ATII cells are an intriguing cell with regulatory and stem cell functions. ATII cells are traditionally characterized by their cuboidal morphology and ultrastructural lamellar bodies. The lamellar bodies, subcellular structures containing the lipid-protein complex of the surfactant system (25), provide a mechanism for reducing alveolar surface tension. ATII cells are physically located within the alveolar airspace and positioned at the corners of the alveolus (26)—an optimal position for detecting changes in alveolar surface tension (27) and post-pneumonectomy deformation of the alveolar ducts (28).

The ATII-mediated recruitment of blood-borne monocytes into the lung (1,29) insures that local signaling regulates the distributed recruitment of monocytes. Post-pneumonectomy bronchoalveolar lavage suggests that $CD11b^+$ monocytoid cells are selectively recruited into the air space (29). The spatial location of these cells within the alveolar microcompartment is important because of the unique structural features of the lung. Signaling of soluble mediators and diffusible morphogens is limited by an air filled alveolar cavity that spans 5–10 cell diameters.

The recruitment of blood-borne monocytes is coincident with the proliferative increase in ATII cells and the presumed progressive transdifferentiation of ATII cells into alveolar type I (ATI) cells (1,29). The colocalization of these transcriptionally active cell types suggests the potential for multiple ATII/monocyte interactions. Lechner *et al.* suggest that these interactions result in functional maturation of the blood-borne monocytes into M2 macrophages (1); that is, macrophages associated with anti-inflammatory and stem cell accessory functions (30,31). Supporting the concept of M2 macrophage involvement, Lechner *et al.* implicate IL4RA and IL-13 signaling in post-pneumonectomy lung growth (1). The complexity of these potential interactions, and the evolving phenotypes of monocytes/macrophages in vivo, suggests that this is an area to be explored in future work.

The findings of Lechner *et al.* (1) contribute an important insight into the potential mechanism of neoalveolarization. An emerging concept is that neoalveolarization involves spatially distinct processes, such as septal partitioning, epithelial proliferation and capillary angiogenesis, linked by the common influence of post-pneumonectomy deformation. The post-pneumonectomy deformation or stretch of the pleura results in MMT and the migration of transcriptionally active myofibroblasts into subpleural alveoli. By analogy with developmental processes (21,22),

these migratory myofibroblasts, along with resident myofibroblasts, likely participate in the septal repartitioning in compensatory lung growth.

Also linked to post-pneumonectomy changes, Lechner *et al.* (1) have shown that ATII cells recruit blood-borne monocytes into the air spaces where they likely influence ATII cell proliferation. ATII cell proliferation and transdifferentiation into ATI cells provides a mechanism for epithelialization of the alveolar surface area required for new alveoli.

Although myofibroblasts, ATII cells and blood-borne monocytes contribute to the growth and patterning of the alveolar septa and epithelium, it is less clear how these cells might influence the construction of new capillaries within the alveolar duct partitions. Intussusceptive, or nonsprouting, angiogenesis provides a mechanism for rapidly expanding the vascular network without commensurate endothelial proliferation (32,33). The intraluminal intussusceptive pillars also provide a mechanism for localizing $CD34^+$ endothelial progenitor cells and expanding the capillary gas exchange surface area in growing regions of the lung (34,35).

Interestingly, single-cell transcriptional profiling of subpleural "hotspots" of alveolar growth after pneumonectomy has shown that myofibroblasts, ATII cells and $CD34^+$ endothelial progenitor cells are the most transcriptionally active cells in the regenerative alveolar duct (Ysasi *et al.*, submitted). Future work will determine if the intersection of these processes can explain the puzzling phenomenon of post-pneumonectomy compensatory lung growth.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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