Resident Cellular Components of the Human Lung

Current Knowledge and Goals for Research on Cell Phenotyping and Function

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The purpose of the workshop was to identify still obscure or novel cellular components of the lung, to determine cell function in lung development and in health that impacts on disease, and to decide promising avenues for future research to extract and phenotype these cells. Since robust technologies are now available to identify, sort, purify, culture, and phenotype cells, progress is now within sight to unravel the origins and functional capabilities of lung cells in developmental stages and in disease. The Workshop's agenda was to first discuss the lung's embryologic development, including progenitor and stem cells, and then assess the functional and structural cells in three main compartments of the lung: (*1*) airway cells in bronchial and bronchiolar epithelium and bronchial glands (basal, secretory, ciliated, Clara, and neuroendocrine cells); (*2*) alveolar unit cells (Type 1 cells, Type 2 cells, and fibroblasts in the interstitium); and (*3*) pulmonary vascular cells (endothelial cells from different vascular structures, smooth muscle cells, and adventitial fibroblasts). The main recommendations were to: (*1*) characterize with better cell markers, both surface and nonsurface, the various cells within the lung, including progenitor cells and stem cells; (*2*) obtain more knowledge about gene expression in specific cell types in health and disease, which will provide insights into biological and pathologic processes; (*3*) develop more methodologies for cell culture, isolation, sorting, co-culture, and immortalization; and (*4*) promote tissue banks to facilitate the procurement of tissue from normal and from diseased lung for analysis at all levels.

Keywords: novel cells; cell markers; culture methods; progenitor or stem cells

Cellular activity within the structure of viable tissue permits physiologic functioning of mammalian organ systems that are incredibly complex, responsive, interactive, and adaptable; collectively, multiple systems support a complex organism's life. The respiratory tract, from the point of air entry at the nares and/or mouth, conditions and moves ambient air down conducting airways to alveolar units for oxygen–CO_2 exchange. To accomplish successful respiration, the respiratory tract uses approximately 40 different resident cell types (Table 1) and their products to make this occur. Unquestionably, much is known about normal respiratory function, and what goes awry when disease develops, thanks to investigative pulmonary research to date. But the current era of nanotechnology, genomic and other "-omic" approaches favors continued and auspicious research success in this field.

Yet what still remains unknown in biological science is humbling. To dramatize this, the July 1, 2005 issue of *Science* (1), its 125th anniversary of publication, dedicated a special section to the question, "what don't we know?", exploring 125 questions that pointed to gaps in our basic scientific knowledge. This stimulated reflection. While a lot is known about the 40 resident cell types that compose the human respiratory tract, including more information about some functions than others, should an assessment be made to determine if there are cells that are still not recognized, remain obscure, or may have a limited lifespan or function during the spectrum of the lung's "life": from embryonic beginning to maturation to healthy adult function to aging? Using the current technologies and "-omic" methods, investigators might be challenged to study these as yet still obscure cells to isolate them, determine biomarkers to conclusively identify them, seek their function and biologic products, and track their origins. Finally, how might they contribute to normal lung development and then to lung disease? This desire to learn more about the ingredients of the respiratory tract is compatible with the recently formulated NHLBI Strategic Plan: Shaping the Future of Research (2, 3), that is designed to be a "working blueprint for our scientific directions over the next 5 to 10 years." A challenge

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TABLE 1. RESIDENT CELLS OF THE RESPIRATORY TRACT

- Airway Epithelial Cells
  - Goblet
  - Ciliated
  - Clara cells
  - Neuroendocrine (neuroendocrine bodies)
  - Basal
  - Intermediate (or parabasal) cells
  - Serous cell (like Clara cells)—major secretory cell in rat. Also present in human fetal conducting airway mucosa; some reports in adult humans
  - Brush cell
  - Special type cells with numerous intracytoplasmic membrane-bound inclusions
  - Oncocytes
  - Nonciliated columnar cells—unclassifiable columnar cells
  - Metaplastic cells—squamous cells and Clara-mucous cells, bronchiolar metaplasia
- Alveolar cells
  - Type 1 and type 2 pneumocytes
  - Transition between 1 and 2 consisting of cuboidal nonciliated cells
- Salivary gland cells (in bronchi)
  - Serous cells
  - Mucous cells
  - Ductal cells
- Interstitial connective tissue
  - Smooth muscle
  - Cartilage
  - Fibroblasts
  - Myofibroblasts
  - Meningothelioid cells of minute meningothelioid nodules
  - Adipose tissue
  - Neural cells (intrapulmonary nerves)
- Blood vessels
  - Arteries/veins
    - Endothelial cells (differences in arterioles versus veins versus capillaries)
    - Smooth muscle cells
    - Fibroblasts/myofibroblasts
    - Pericytes
  - Lymphatics
    - Endothelial cells
    - Smooth muscle cells
    - Fibroblasts/myofibroblasts
- Hematopoietic and lymphoid tissue
  - Lymphocytes
  - Plasma cells
  - Bronchial mucosal associated lymphoid tissue
  - Megakaryocytes
  - Macrophages
  - Langerhans cells
  - Mast cells
  - Eosinophils
  - Neutrophils
  - Basophils
- Pleura
  - Mesothelial cell layer
  - Pleuripotent submesothelial fibroblasts
  - Adipose cells—intrapleural fat
  - Lymphatics (*see above*)
- Poorly defined cells
  - Stem cells
  - Perivascular epithelioid cells (PEC)—precursor for LAM cells
  - Pluripotent epithelial stem cell—precursor for lung cancer—especially small cell carcinoma—where no precursor is yet identified
  - Meningothelioid cells
  - Endothelial progenitor cells
  - Mucinous cells in certain pediatric conditions

outlined is to "develop a detailed understanding of the molecular, cellular, and physiological mechanisms that maintain health from embryonic development to the end of the human lifespan."

Who better to point out what they see in lung tissue, wonder where an influx of cells originates, or what influences cellular changes (metaplasia) that occur in disease than a group of clinical and research lung pathologists? So a small group was invited to be an organizing team for a Workshop that was held on July 9 and 10, 2007 in Bethesda, Maryland by the Division of Lung Diseases, National Heart, Lung, and Blood Institute, of the National Institutes of Health.

Current knowledge of resident lung cells has been largely established through light and electron microscopy, but methodologic advances in the past 2 to 3 decades have allowed for refinement of characterization of lung cells, and these methods should provide a pathway to investigate novel cell lines. Thus, the overarching concept driving the organization of this Workshop was to learn more about common/known cells, as well as identify novel and still obscure cells in the human lung that may be important in lung development, homeostasis, injury, repair, and disease utilizing the most promising new technologies to create a "molecular toolbox" for isolating, phenotyping, and discerning the functions of all lung cells.

The primary goals of the workshop were to:

1. Determine cell function of known cells in health and its impact on disease
2. Identify novel cellular or still obscure components of the lung
3. Suggest the most promising avenues for future research to extract and phenotype known and novel cells.

After a pathologist's perspective of why better characterization of resident lung cells would provide better understanding of normal lung structure and alterations in disease, major segments of the respiratory tract were explored. The Workshop's outline was fashioned to discuss first the lung's embryonic development, including airway and vascular development, and then three major sites and structural tissues within lung: (*1*) airway cells in bronchial and bronchiolar mucosa and epithelium (basal, secretory, ciliated, and neuroendocrine cells); (*2*) alveolar unit cells, including Type 1 cells, Type 2 cells, and fibroblasts in the interstitium; and (*3*) pulmonary vascular cells (endothelial cells from different size vascular structures, smooth muscle cells, and adventitial fibroblasts). In addition, the possible lung site and activity of progenitor and/or stem cells were discussed, as well as the potential contribution of the bone marrow to supply such cells in the circulation.

Obviously, many well-known cells and their functions could not be considered with a constraint on the amount of material to cover. As a considerable amount is known about many cells that participate in host defense and innate/adaptive immunity, including alveolar macrophages, dendritic cells, lymphocytes, and inflammatory cells, these cells were not discussed. Many products made by cells that affect inflammation and fibrosis have recently been reviewed (4, 5) and were not emphasized, nor were special respiratory diseases receiving active research discussed specifically (6, 7). Pulmonary brush cells were the subject of a recent workshop summary (8).

WHAT IS THE POTENTIAL CLINICAL AND DIAGNOSTIC IMPACT OF BETTER CHARACTERIZATION OF RESIDENT AND NOVEL CELLS IN PULMONARY DISEASE? (A PATHOLOGIST PERSPECTIVE)

As mentioned, the lung is composed of over 40 types of cells including cells of the epithelium, interstitial connective tissue, blood vessels, hematopoietic and lymphoid tissue, and the pleura (9, 10). These are summarized in Table 1. In addition, some poorly defined cells still exist.

Resident lung cells are involved with pathologic conditions in a variety of ways.

1. There are single types of cells linked to specific lung diseases. For example, glandular cells of the lung give rise to lung adenocarcinomas (11). Sixty to eighty percent of lung adenocarcinomas express thyroid transcription factor-1 (TTF-1), making it a valuable marker (12, 13). Langerhans' cell histiocytosis also represents a pulmonary disorder caused by a specific cell, the Langerhans' cell (14).
2. There are cells that lead to certain differential diagnoses such as granulomas that raise the consideration of sarcoidosis, infection, or Wegener's granulomatosis (10). Eosinophils are another example, and their accumulation in the lung forming the lesion of eosinophilic pneumonia raises the consideration of infection, drug toxicity, collagen vascular disease, allergic bronchopulmonary fungal disease, Churg-Strauss syndrome, and hypereosinophilic syndrome.
3. There are some disorders in which morphologic cellular changes may not be visible but lung findings reflect local or systemic cell dysregulation, resulting in accumulation of material such as amyloid, lipids from storage disorders, or alveolar proteinosis (10).
4. There are a few disorders in which specific poorly characterized lung cells appear to be involved. In lymphangioleiomyomatosis (LAM), "perivascular epithelioid cells (PECs)" have been postulated to be the cell of origin (15); however, these have never been identified in the lung. Minute meningothelioid nodules are a common incidental histologic finding in lung biopsies (16, 17). The function of the cells forming the nodules has not been determined. However, as these lesions are so commonly encountered in lung tissue, this suggests that these cells may play some as yet undiscovered physiologic role in the lung biology.
5. Most commonly multiple resident cells participate in lung disease, particularly in inflammatory conditions in chronic bronchitis, emphysema, asthma, and cystic fibrosis. For example, desquamative interstitial pneumonia is defined by a massive increase in airspace macrophages, although a metaplastic proliferation of type 2 cells lining the alveoli and a mild proliferation of mesenchymal cells within the alveolar walls are also present (10). Pathologic diagnoses for inflammatory diseases of the lung frequently hinge on patterns of lung reaction that involve multiple cell types, and the factors that mediate these changes and govern their proportions are important in understanding basic disease processes.

Many inflammatory conditions in the lung result in metaplasia. Bronchiolar metaplasia refers to the presence of bronchiolar type epithelium where it is not normally found, for example, the abnormal honeycomb spaces in scarred lung and scarring of bronchioles accompanied by peribronchiolar metaplasia involving the peribronchiolar alveoli (10, 18). Understanding why bronchiolar metaplasia occurs in these regions (as opposed to type 2 cell metaplasia or squamous metaplasia) is important in understanding the pathogenesis of these changes.

Squamous metaplasia of the large airways is well known and considered part of the metaplasia, dysplasia, carcinoma sequence in the bronchi (19). Squamous metaplasia also occurs around small airways in resolving acute lung injury (20), and it is not known whether this reaction has similar implications for carcinoma in the large airways. Anecdotal experience would suggest that it does not.

Type 2 cell hyperplasia refers to lining of airspaces by type 2 cells as opposed to type 1 cells, which normally involve alveoli (9). It is generally thought that this represents a repair phenomenon, and pathologically type 2 cell hyperplasia is used as a common nonspecific marker for injury of the alveolar type 1 cells. The precise characterization of the events inciting type 2 cell hyperplasia as well as the factors that govern the return of type 1 cells lining alveoli during the healing process have important implications in terms of lung function and repair after injury.

Thus, better characterization of resident and novel cells in the lung parenchyma will provide pathologists with a better understanding of the pathologic conditions of the lung and the cells involved, as well as provide insight into the pathogenesis of these conditions based on known functions and interactions of the cells involved. Characterization of the various forms of metaplasia in the lung represents a logical extension of study related to normal resident cells in the lung. It is possible that certain forms of metaplasia have prognostic significance. For example, squamous metaplasia in acute lung injury is likely a reversible change, whereas anecdotal experience suggests that bronchiolar metaplasia in scarred lung is irreversible.

FURTHER DISCUSSION

As mentioned, the Workshop participants considered four major topics, beginning with the embryonic and developmental aspects of the respiratory tract that establish the cell lineages and initial structures. But in this discussion, a pertinent theme was stressed that in later life, in later stages of disease, or during injury repair of the lung, there may be changes in gene expression and cell behavior that can be reminiscent of those described in some of the specific developmental processes in the lung. Thus, some of the developmental signals and cell constituents persisting from the embryonic stage, perhaps as progenitor cells, might be reactivated later during lung repair and regeneration after injury or compensating for disease processes.

Discussion followed about three structural regions in the lung: the airway epithelium and submucosal structures, the alveolar unit, and the pulmonary vasculature. Emphasis was given to the various major cell lineages and their function (s) in the mature lung in both normal and disease states. Material is presented about these topics in accompanying workshop reports, with recommendations for future research concerning what still needs to be learned and discovered about lung cells and their functions. Hopefully, this will help to elucidate further the complexity of the mature lung, what goes awry in disease, and how research can lead to better therapies for respiratory illnesses.

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