Cell-based Therapy for Chronic Obstructive Pulmonary Disease

Rebuilding the Lung

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Abstract

As the prevalence and impact of lung diseases continue to increase worldwide, new therapeutic strategies are desperately needed. Advances in lung-regenerative medicine, a broad field encompassing stem cells, cell-based therapies, and a range of bioengineering approaches, offer new insights into and new techniques for studying lung physiology and pathophysiology. This

provides a platform for the development of novel therapeutic approaches. Applicability to chronic obstructive pulmonary disease of recent advances and applications in cell-based therapies, predominantly those with mesenchymal stromal cell-based approaches, and bioengineering approaches for lung diseases are reviewed.

Keywords: bioengineering; cell therapy; mesenchymal stromal cell

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One of the earliest references to the concept of regenerative medicine is found in a 1992 article on hospital administration, in a series of short paragraphs on future technologies that would impact hospitals and medical care: “A new branch of medicine will develop that attempts to change the course of chronic disease and in many instances will regenerate tired and failing organ systems” (1). As this initial concept has evolved, regenerative medicine can be thought of as encompassing several related disciplines. One of these is stimulating the body’s own repair mechanisms to heal previously irreparable tissues or organs. This is particularly applicable to complex tissues such as lung, in which limited endogenous reparative mechanisms can be overcome by either the severity of illness or the chronicity of the insult; that is, cigarette smoking. Arguably, a means to stimulate controlled alveolar and airway regeneration *in situ* in patients with chronic obstructive pulmonary disease (COPD) and other destructive lung diseases such as idiopathic pulmonary fibrosis would be a significant

advance. Use of pharmacologic means to stimulate reparative postnatal lung growth, for example, administration of retinoic acid, although promising in mice, did not have beneficial effects in clinical trials (2). More recently, and as further discussed below, cell-based therapies, utilizing systemic or intratracheal administration of a variety of cell types including endogenous lung progenitor cells, induced pluripotent cell-derived lung epithelial cells, endothelial progenitor cells, and allogeneic mesenchymal stromal cells (MSCs), have been postulated to promote structural and functional regeneration of gas exchange (3, 4). A broader field of antiinflammatory actions of MSCs, and a primary focus of this review, has also been explored with the primary goal of decreasing inflammation and injury without necessarily promoting structural repair in both preclinical lung disease models and in clinical trials in lung diseases (5, 6). A second broad regenerative medicine approach is that of growing tissues and organs in the laboratory for implantation when the body cannot heal

itself. Although there has been success with tissues such as skin, muscle, and bone, this has been a challenge for lung (5). Several developing strategies such as “organ on a chip,” organoid culturing, three-dimensional (3D) bioprinting, and de- and recellularization have provided novel approaches, particularly with respect to lung (7–11). A third broad area of regenerative medicine is the development of adjunct devices to replace or augment defective tissue or organ function. Left ventricular assist, portable dialysis devices, and extracorporeal membrane oxygenation (ECMO) devices are good examples of advanced biomedical engineering approaches that can augment defective organ functions for both short and sometimes more prolonged time periods (12). As will be discussed, there is significant unmet need and thus opportunity for new types of lung assist devices, particularly in ambulatory patients with end-stage lung disease on transplant waiting lists or who will never qualify for lung transplant.

COPD as a paradigmatic lung disease is both a challenge and opportunity for regenerative medicine. Better understanding of the role of endogenous progenitor cells, particularly basal airway epithelial cells, and their potential dysfunction in the etiology of COPD and also of lung cancer, are covered in the other State of the Art reviews from this conference. Investigations utilizing MSCs for COPD have encompassed both preclinical studies as well as clinical investigations that reflect the potential, but also the realistic practicalities, of attempting MSC-based therapies in a chronic destructive lung disease. Organoid and lung-on-a-chip strategies have revealed new insights into lung cell biology. Lung de- and recellularization strategies, although not directly targeting COPD, have revealed fundamental knowledge about the role of the COPD lung extracellular matrix (ECM) and cell-matrix interactions in COPD pathophysiology. These and related areas are reviewed below.

Cell-based Therapies for COPD

The idea of administering a stem cell, either systemically or directly into the lung, to repair damaged or dysfunctional lung had significant genesis from work published 2001 by Diane Krause and colleagues at Yale University (13). This paradigm-shifting publication suggested that systemically hematopoietic stem cells could engraft in various tissues, including lung, and acquire tissue-specific phenotype and possibly even function. This initiated a broad-based effort attempting to demonstrate that various types of adult stem and progenitor cells including hematopoietic stem cells, MSCs, endothelial progenitor cells, as well as embryonic stem cells could engraft in diseased lung and participate in both structural and functional repair (reviewed in Reference 14). These early studies were subsequently found to predominantly represent artifacts and suboptimal use of photomicroscopy techniques, and the concept of stem cell engraftment in lung was not believed to be a biologic phenomenon of any significance (15, 16). However, data suggest that endogenous lung airway progenitor cells, for example, cytokeratin 5/14⁺ basal airway epithelial cells, or induced pluripotent stem cell-derived lung progenitor cells, may have more potent engraftment properties and potentially

contribute to functional as well as structural repair (3, 4). Although not the focus of this review, this is an exciting and rapidly progressing area that may provide significant reconsideration of potential structural repair of the lung by being further able to manipulate these cells *in situ* or by their exogenous administration.

Emphasis in the study of MSCs for use in COPD and other lung diseases accordingly has shifted to both understanding and exploiting their paracrine and other properties, independent of their differentiation abilities (17). This is particularly important for COPD as it is a chronic inflammatory as well as destructive disease with ongoing pulmonary and systemic inflammation even after smoking cessation. Although the full spectrum of MSC paracrine activities is still being determined, a partial list of actions includes the ability to sense local inflammatory environments through expression and activation of damage- and pathogen-associated molecular pattern receptors such as the Toll-like receptors (18). The MSCs will then release a spectrum of antiinflammatory and other ostensibly reparative mediators including antiinflammatory cytokines, antibacterial peptides, proangiogenic factors, and extracellular vesicle particles that can affect both lung epithelial cells as well as resident inflammatory and immune cells including alveolar macrophages (17, 18). Mitochondrial transfer from MSCs to damaged lung epithelial cells has also been observed (19).

These mechanisms have been observed in *in vitro* studies in which exposure to MSCs or their secreted products affects lymphocyte proliferation and function as well as macrophage phenotype and activation (16, 17). Accordingly, there have been a growing number of publications in which systemic or intratracheal MSC administration results in amelioration of injury in a range of preclinical lung disease models in rodents, larger animals (sheep and pigs), and explanted human lungs (reviewed in References 3 and 20). These include a number of studies in rodent COPD models including those induced by exposure to elastase, papain, or cigarette smoke (21–44). Although these models have known limitations in accurately reflecting clinical COPD structural and physiologic pathology, particularly those utilizing papain and

elastase, improvements in experimental outcomes were observed in many of these studies. For example, in mice exposed to cigarette smoke for a 24-week period, systemic administration of either mouse bone marrow- or human adipose-derived MSCs every other week for the last 2 months resulted in decreased pulmonary (lung inflammation and histologic injury) as well as systemic effects (decreased weight loss and increased subcutaneous fat) (29). Various paracrine pathways, including mitochondrial transfer, have been suggested as potential mechanisms by which the MSCs are having protective effects (36, 38, 39, 42, 44). These results suggest a potential role for MSC administration in clinical therapeutics for COPD. It is to be emphasized that, although there may be effects of MSCs on existing endogenous progenitor cells in the lung, current paradigms of MSC actions in both preclinical models of COPD and clinical trials focus on antiinflammatory paracrine actions rather than structural repair (3, 4, 14, 38). Conversely, several studies have also demonstrated the damaging effects of cigarette smoke on MSC functions, and this will need to be considered in clinical trials of MSCs (45–47).

However, it is critical to understand the biology of MSCs in the context of a chronic inflammatory and destructive lung disease such as COPD. MSCs reside in many tissues, most notably in bone marrow and adipose, but have also been identified in the lung. However, unlike bone marrow neutrophil populations, MSCs are not recruited out of the marrow or adipose in response to inflammatory signals. Rather, they are extracted from either bone marrow or adipose tissue (autologous) or from those tissues in an external source, for example, healthy normal volunteers, expanded in culture, and used either directly or after freezing and thawing. After systemic administration, MSCs lodge in the lung as the first major capillary bed encountered, predominantly through cell surface integrin actions still being defined (16, 17). Once in the lung the cells do not engraft but rather release a range of soluble mediators and may also have some direct cell-cell interactions with resident pulmonary epithelial or vascular endothelial cells. Available data demonstrate that the MSCs are then cleared from the lungs over a 1- to 3-day period. Similar observations have been made after direct airway (intratracheal) MSC

administration. Clearance mechanisms are still being defined but include phagocytosis/efferocytosis by alveolar macrophages, induction of apoptosis, and other mechanisms (17). Whether effects rendered on resident inflammatory cells or immune cells recruited to the lungs, effects presumably conducive to repair of inflammation and injury, persist is still being elucidated.

Accordingly, despite encouraging results in rodent models, the mechanisms by which systemically or intratracheally administered MSCs can have meaningful clinical effects in a chronic disease such as COPD are not clear. There have been several clinical trials of MSC-based therapies in COPD to date (reviewed in References 4 and 48). These have been predominantly phase 1 safety trials and (in those trials that have published results) uniformly demonstrate no obvious safety issues including no evidence of infusional toxicities (cell emboli) and no significant adverse effects for follow-up periods as long as 2 years (49–52). However, none of these have suggested efficacy in either pulmonary function or quality of life indicators. A decrease in circulating C-reactive protein was observed in subjects who received MSCs versus vehicle control in patients with elevated baseline C-reactive protein at study entry (49). Comparably, decreases in several circulating inflammatory mediators and changes in circulating T-cell populations were observed in patients with stable COPD after systemic MSC administration (52). These provide important hypothesis-generating data even in the absence of clinical efficacy.

Possible explanations for the lack of beneficial effects on clinical outcomes are multiple and include MSC source (bone marrow vs. other), administration route, dosage, and frequency and timing of administration(s) as well as host factors. Less well understood is the fate of the MSCs or their secreted products, including extracellular vesicle particles, after systemic or intratracheal administration to patients with COPD (52). These will likely be critical factors in determining whether a relatively short-lived cell infusion (days) will ever have a chance for therapeutic efficacy.

One unfortunate and troubling outcome of the growing interest in cell-based therapies for COPD has been the expansion of unproven cell therapies and stem cell medical tourism both in the United

States and globally (53). These unproven and often unsafe approaches are aggressively marketed and highly visible to desperate patients through the Internet and other forms of social media. Organizations such as the International Society for Cell and Gene Therapy (ISCT) and the International Society for Stem Cell Research (ISSCR) have taken strong public stances against this stem cell medical tourism, and the U.S. Food and Drug Administration (FDA) is beginning to take stronger actions (53, 54). The American Thoracic Society (ATS) Assembly on Respiratory Cell and Molecular Biology Stem Cell Working Group has been active in this area and has advocated with other respiratory groups worldwide for intensification of communication and collaboration between patients and respiratory health professionals, including patient and caregiver information on the ATS website (55, 56).

One particular area of concern is the listing of clinical trials on the NIH ClinicalTrials.gov website. As of May 7, 2018, 73 trials of MSC-based therapies for lung disease, including COPD, were listed on the www.clinicaltrials.gov website. Some of these are well-designed, peer-reviewed, FDA-sanctioned trials compliant with contemporary ethical, scientific, and regulatory standards. However, a significant number of listings are studies of dubious scientific rationale that do not comply with ethical and legal norms governing human subject research. Further, many of these charge participants \$7,500 to \$20,000 USD. This creates a situation in which patients can be misled into participating in unproven therapies at significant cost to themselves. The ATS Stem Cell Working Group, along with a number of other national and international organizations, is working with the FDA to rectify this situation (54, 57).

Lung Bioengineering

In parallel with approaches for clinical administration of MSCs and studies of other cell types as potential therapeutic strategies for COPD, there is widespread interest in approaches for growing functional lung tissue *ex vivo* for potential use in transplantation and for the study of lung biology and pathophysiology. As such, engineering a functional gas exchange system, whether suitable for implantation and use in lung transplantation or for use as

an external lung assist device, is an exciting yet challenging area of active growth. Various approaches are briefly considered below, with particular reference to application in COPD.

Lung Organoids

An explosion of interest in organoid technologies has paralleled advances in understanding of endogenous lung progenitor cell biology and the increasing sophistication in deriving lung progenitor as well as airway and alveolar epithelial cells from embryonic and induced pluripotent cells. Organoid technology is a powerful tool for studying lung developmental biology, cell–cell interactions, and cell–matrix interactions, and further serves as a pharmaceutical platform for screening and evaluating small molecules and other potential new therapeutic agents (8, 58–61). Organoid technology has not yet been extensively utilized to study defective epithelial cell biology in COPD but will be a powerful tool with which to do so.

Three-Dimensional Bioprinting and Lung-on-a-Chip

Increasing technical progress has been made in the sophistication of 3D bioprinters along with advances in biomaterial science including ECM-based bioinks (62). This includes increasing ability to include live cells in the printing processes and creation of complex multilayered printed tissues. A growing number of studies are using these approaches for clinical management of diseases of the larger airways (trachea/mainstem bronchi), usually in the setting of congenital defects, cancer, or trauma (63, 64). Although promising, the field has been undermined by scandal involving cardiothoracic surgeon Paolo Macchiarini and as such highlights the need for solid supporting preclinical data and appropriate rigor in clinical applications (65). Three-dimensional printing has had less progress in lung parenchymal applications as yet, in large part due to the current limitations on the printing resolution of current 3D bioprinters. Several groups have printed surface areas to use for study of the air–liquid interface and lung epithelial cell–cell and cell–matrix interactions (7). Increasing advances in technique such as stereolithography add power to this approach but at present, 3D bioprinting of alveoli remains an elusive goal. Lung-on-a-chip technologies are comparably powerful

tools with which to investigate cell–cell, cell–matrix, and cell–environment interactions. Incorporation of cyclic mechanical stretch and different oxygen tensions offers a means to comprehensively assess multiple variables in a tightly controlled manner (8, 66, 67). Lung-on-a-chip technologies also provide a strong platform for pharmaceutical testing. However, neither 3D bioprinting nor lung-on-a-chip has yet been extensively utilized to study lung epithelial or progenitor cells and/or matrix obtained from patients with COPD. These are anticipated to be highly fruitful areas of future studies.

Ex Vivo Lung Generation: De- and Recellularization

A promising and growing area of investigation is that of *ex vivo* bioengineering of functional lung tissue that could then be implanted into patients with end-stage COPD or other lung diseases. Either biologic scaffolds or fabricated 3D matrices are seeded with stem, progenitor, or other lung cells and cultured in bioreactor chambers to recapitulate gas exchange function. Ideally, the cells utilized would be obtained from the individual transplant recipient. If successful, this would then help to alleviate the ongoing shortage of donor

lungs available for transplantation and to minimize subsequent immune rejection. These approaches have been increasingly successfully utilized in regeneration of other tissues including skin, vasculature, cartilage, bone, and trachea and more recently more complex organs including trachea, heart, and liver. The majority of work in this area has focused on biologic scaffolds utilizing lungs treated to remove resident cells and cell debris, leaving behind the intact ECM macro- and microarchitecture scaffolding (decellularization) (68). This includes preservation of native airway and vascular structure and provides a native acellular matrix for cell seeding and structural and functional recellularization. This approach also provides a novel culture system with which to study cell–matrix interactions and the effect of environmental factors such as mechanical stretch on lung cell growth and development (69–72). A range of studies have demonstrated the feasibility of this approach in small (rodent), large (pig), and human lungs (9, 68).

However, despite much progress, functional lung tissue that exhibits robust gas exchange has yet to be produced. This reflects a number of considerations summarized in Figure 1, including the

decellularization method utilized and the subsequent quality of the remaining ECM in terms of remaining ECM and matrisome proteins, the recellularization strategies utilized, consideration of relevant environmental factors such as cyclic mechanical stretch, and the bioreactor technologies utilized (9, 68). Further technologic developments are required to overcome these hurdles, and a range of strategies including use of xenogeneic scaffolds of decellularized pig lungs seeded with human lung cells are being investigated (73, 74). Nonetheless, it will likely be a number of years before this approach yields functional gas exchange tissue that can be clinically utilized. Notably, decellularized lungs from both rodents with experimentally induced emphysema and patients with COPD are significantly altered compared with lungs obtained from normal mice or patients (75, 76). This provides a powerful tool with which to assess the role of deranged ECM in the pathogenesis of COPD.

Cell Sources for *ex Vivo* Lung Bioengineering

An important consideration for the above-mentioned approaches is the origin of cells

Figure 1. Schematic demonstrating considerations for utilizing decellularized lung scaffolds. ECM = extracellular matrix; ESC = embryonic stem cell; iPS = induced pluripotent stem (cell). Reprinted by permission from Reference 68.

to be utilized, particularly for a clinically translatable product. Ideally, cells derived from the eventual transplant recipient would mitigate immune responses and potential rejection. How to best achieve this is unclear as the number of cells, be they, for example, differentiated lung epithelial cells or airway epithelial progenitor cells, that might be obtained from lung biopsy or airway brushings is limited, as is the ability to expand these in culture. Further, the range of cell types to be utilized, for example, in recellularization strategies, is quite large and there is the additional challenge of seeding them into the proper locations in the decellularized scaffolds (68). Use of induced pluripotent stem cells (iPSCs), including the use of gene-editing techniques applied to correct any genetic or acquired chromosomal defects, offers a significant potential advantage given the progress in deriving functional airway and alveolar epithelial cells as well as other types of lung cells and the ability to expand these cells more effectively in culture (77). Work demonstrates that iPSC-derived inflammatory

cells rather than epithelial or other lung structural cells, for example, alveolar macrophages, may also be useful in clinical applications for disease such as pulmonary alveolar proteinosis (78). This raises the possibility of seeding decellularized scaffolds with autologous, gene-edited immune cells that will further contribute to other non-gas exchange functions of the lung.

Lung Assist Devices

ECMO devices have a significant role in short-term acute neonatal respiratory diseases and a more limited role in acute adult respiratory diseases. However, ECMO requires hospitalization in critical care units and specialized health care providers and has a number of significant complications including inflammation and clotting (79, 80). As such, it is not a practical or cost-effective option for long-term bridging to lung transplant or for use in patients with end-stage COPD or other lung diseases and who will not qualify for transplant. Despite some limited technologic advances (81, 82), new innovative, cost-effective,

easily implementable technologies are desperately needed. Developments in wearable portable artificial lungs (83) or consideration of decellularized avian lungs recellularized with mammalian lung cells (avian lung assist device) (84) demonstrate the wide ranging potential of and further room for novel and innovative engineered devices.

Summary

A number of regenerative medicine approaches for lung diseases are in active development and offer hope for patients with COPD and other lung diseases. However, none has yet reached fruition and despite promise, care has to be taken to deliver an accurate and consistent message to patients and their families to minimize the growing influence of stem cell medical tourism for COPD and other lung diseases. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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