Title
Steps toward Cell Therapy for Cystic Fibrosis

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Cystic fibrosis (CF) is an autosomal recessive genetic disorder affecting ~70,000 persons worldwide (1). Mutations in the *CFTR* (CF transmembrane conductance regulator) gene that affect production, processing, and function of the CFTR protein dramatically alter the biophysical properties of airway mucus leading to airway obstruction, chronic infection, and inflammation, eventually culminating in organ damage and failure. Recent advances in pharmacotherapy (2) led to U.S. Food and Drug Administration approval of triple-combination CFTR modulating therapy for individuals with at least one copy of the most common *CFTR* mutation, p.Phe508 del (commonly referred to as ΔF508). Although this therapy is generally well tolerated, it involves indefinite adherence to a twice-daily dosing regimen, effects on long-term outcomes are still under investigation, and ~10% of individuals with CF are not candidates for this or other currently available pharmacotherapeutic regimens because of the nature of their *CFTR* mutations.

Using gene therapy to introduce a functional *CFTR* gene or repair mutant *CFTR* has obvious appeal and has been eagerly pursued over the three decades since *CFTR* was identified (3). Clinical trials involving liposome- or viral vector–mediated delivery of *CFTR* transgenes to the airway failed to achieve desired clinical outcomes because of inefficiencies in transgene delivery and other obstacles. New gene-delivery systems and the emergence of powerful gene-editing methods such as CRISPR promise to improve our ability to introduce and repair airway epithelial-cell genes. However, using these tools *in vivo* presents significant challenges, including those imposed by the need to deliver transgenes or editing systems across barriers formed by mucus and epithelial tight junctions. Cell therapy is an alternative approach. Here, autologous cells undergo *ex vivo* correction of *CFTR* gene mutations and are then engrafted into the airways, avoiding the need for immunosuppression. Although advances in our understanding of stem-cell biology may open avenues for reprogramming of cells from other sites, one appealing approach involves the use of airway epithelial cells for CF cell therapy. Based on mouse models, it has been estimated that 60 million cells will be required for each cell-therapy treatment (4). Because the number of airway epithelial cells that can be safely harvested using bronchoscopy or other approaches is considerably smaller, it will be important to use culture methods that allow for expansion of epithelial cells while still maintaining "stemness" characteristics and capacity for differentiation into critical *CFTR*-expressing cell types.

In this issue of the *Journal*, Lee and colleagues (pp. 374–385) address these challenges by investigating how human bronchial epithelial-cell culture conditions affect cell proliferation and cell function (5). Airway epithelial cells have conventionally been propagated on placental or hide collagen–coated plates in bronchial epithelial growth medium, a method that results in limited cell expansion. The discovery that the Rho kinase inhibitor Y-27632, in combination with fibroblast feeder cells, induces indefinite proliferation of primary epithelial cells (6) and the subsequent application of this conditionally reprogrammed cell (CRC) method to human airway epithelial-cell culture (7, 8) provided a means to overcome this limitation. As expected from earlier studies (8–10), Lee and colleagues (5) found that the CRC method, compared with the conventional bronchial epithelial growth medium method, generated cells that proliferated much more rapidly, yielding ~20 population doublings (~10^6-fold expansion) over just 3 weeks in culture. The CRC method also maintained higher expression of the low-affinity NGFR (nerve growth factor receptor), a marker for a highly proliferative subset of airway basal cells (11). The CRC method generated a large population of NGFR-high cells even when starting with NGFR-negative cells from first-passage conventional cultures, implying that less proliferative basal cells are reprogrammed to be highly proliferative basal cells by the CRC method. A competitive repopulation assay was used to model the effects of introducing cultured non-CF cells into a population of CF epithelial cells. First-passage conventional and CRCs performed similarly, but by passage 3, CRCs substantially outperformed conventional cells as measured by cell abundance and CFTR function. Restoration of a substantial amount of CFTR ion transport could be accomplished with a modest proportion of non-CF cells: there was measurable improvement in transport with starting ratios as low as 1 non-CF cell to 99 CF cells and 50% restoration of function when non-CF cells represented only ~10% of all cells in mature cultures. This suggests that cell therapy could be successful, even with limited cell engraftment. A novel feature of this study is that Lee and colleagues (5) examined the generation of ionocytes, a recently described rare cell type with high *CFTR* expression, and found that CRCs produced four times more ionocytes than conventional conditions after a single passage. As the authors note, the observation that this increase in ionocytes was not accompanied by a substantial difference in ion transport function in the cultures raises questions about whether ionocytes are major contributors to net ion transport in the airway epithelium.

The report from Lee and colleagues (5) adds to a growing body of evidence that indicates that it is possible to massively expand basal or basal-like epithelial cells while retaining their capacity to differentiate into multiple cell types that serve distinct functions in the airway epithelium. The CRC method used by Lee and

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colleagues requires feeder cells, which could raise safety concerns for cell therapy applications. Other methods for cell reprogramming that have recently been described may provide similar or greater efficiency without a need for feeder cells (12, 13). Progress in culture systems must be accompanied by progress in other steps required for cell therapy of CF. Although earlier efforts concentrated on introducing a *CFTR* transgene, recent work has increasingly turned to using gene editing, including a recent report using Cas9 and adeno-associated virus 6 to correct the endogenous *CFTR* gene in cultured airway basal stem cells from CF patients with the ΔF508 mutation (14). We (15) and others (16) recently developed methods for efficient gene targeting in cultured human airway epithelial cells via direct delivery of guide RNA–recombinant Cas9 complexes by electroporation, without a requirement for plasmids, viruses, or antibiotic selection. Although this approach was designed for gene inactivation, other plasmid- and virus-free CRISPR approaches that correct *CFTR* mutations by homology-directed repair or base editing could be used for cell therapy. After *CFTR* correction and cell expansion, cells will need to be engrafted in individuals with CF. Despite considerable progress in understanding the complex biology relevant to lung repair and regeneration (17), much more needs to be done to understand how to prepare the airways, deliver cells, and monitor engraftment efficiency *in vivo*. To provide long-lasting benefits, engrafted cells will need to serve as self-renewing precursors for critical *CFTR*-expressing cells types. Clinically meaningful benefits of cell therapy may require widespread engraftment of cells in both airways and glands because both are involved in CF, although results from Lee and colleagues (5) suggest that replacement of even a modest fraction of epithelial cells may have physiologically meaningful effects. Although many challenges lay ahead, advances reported by Lee and colleagues and other groups are cause for optimism about the potential of cell therapy for CF. ■

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