Transplantation of Airway Epithelial Stem/Progenitor Cells: A Future for Cell-Based Therapy

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Abstract

Cell therapy has the potential to cure disease through replacement of malfunctioning cells. Although the tissue stem cell (TSC) is thought to be the optimal therapeutic cell, transplantation of TSC/progenitor cell mixtures has saved lives. We previously purified the mouse tracheobronchial epithelial TSCs and reported that *in vitro* amplification generated numerous TSCs. However, these cultures also contained TSC-derived progenitor cells and TSC repurification by flow cytometry compromised TSC self-renewal. These limitations prompted us to determine if a TSC/progenitor cell mixture would repopulate the injured airway epithelium. We developed a cell transplantation protocol and demonstrate that transplanted mouse and human tracheobronchial epithelial TSC/progenitor cell mixtures are 20–25% of airway epithelial cells, actively contribute to epithelial repair, and persist for at least 43 days. At 2 weeks after transplantation, TSCs/progenitor cells differentiated into the three major epithelial cell types: basal, secretory, and ciliated. We conclude that cell therapy that uses adult tracheobronchial TSCs/progenitor cells is an effective therapeutic option.

Keywords: cell therapy; tissue stem cell; progenitor cell; airway epithelium

Clinical Relevance

The data presented in this study support the conclusion that cell therapy that uses adult tracheobronchial tissue stem/progenitor cells is an effective therapeutic option.

Cell therapy for chronic disease will likely require transplantation of a cell that has extensive mitotic and differentiation potential. A tissue stem cell (TSC) is a progenitor subtype that self-renews (replaces itself) and generates each of the cell types found in the home tissue of the TSC (acts as a multipotential progenitor) (1). Thus, cell therapy initiatives often focus on TSCs.

Functional analyses from our group and others demonstrated that the adult mouse tracheobronchial epithelium is maintained and repaired by a TSC (2–9). We reported that the mouse tracheobronchial epithelial TSC is a CD49f^{Bright}/Sca1^{+}/Aldefluor^{Bright} basal cell subtype (3). These TSCs are extremely rare: there are approximately 100 TSCs per airway, and TSCs are only 0.05% of all epithelial cells.

We also reported that the mouse tracheobronchial TSCs generated a unique clone type, the rim clone, when cultured *in vitro* (2, 3). TSC culture as rim clones permitted TSC amplification and maintained TSC self-renewal and multipotential

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differentiation potential, the functional properties required of a therapeutic cell. However, TSC-derived rim clones also contained non-TSC basal progenitor cells, and repurification of amplified TSCs from rim clones by flow sorting compromised TSC self-renewal, as assayed by clone formation. These limitations caused us to question whether cell therapy using a TSC/progenitor inoculum was feasible.

The purpose of this study was to test the hypothesis that a mixture of adult TSCs/progenitor cells would repopulate the airway epithelium after injury. We report successful development of a transplantation assay that uses a well characterized epithelial injury/repair model. Using this model, we demonstrate that mouse TSCs/progenitor cells reconstitute the conducting airway epithelium of mice after transplantation and exhibit multilineage differentiation. We extend these results to humans by demonstrating that unfractionated human basal progenitor cells can also be used as an effective form of cell therapy.

Materials and Methods

Complete methods are provided in the online supplement.

Animals

All procedures involving animal use were approved by the National Jewish Health (Denver, CO) Institutional Animal Care and Use Committee. Mice were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care-approved facility and screened for pathogens on a quarterly basis. The strains varied by experiment, and are detailed subsequently here.

Naphthalene Treatment

Naphthalene (NA) was prepared and administered as previously reported (10). NA dose was selected to cause greater than 95% depletion of the Clara cell secretory protein (CCSP)$^+$ cell population by Recovery Day 3.

TSC Quantification

Female FVB/n mice, 6–8 weeks old, were treated with NA. On Recovery Days 6, 40, and 80, tracheal epithelial cells were recovered by dispase/collagenase/trypsin digestion and clone-forming cell frequency was determined by limiting dilution (3).

Mouse TSC Purification and Expansion

CD49f^{Bright}/Sca1$^+$/Aldefluor^{Bright} tracheal TSCs were purified from Rosa-LacZ mice by flow sorting (3). TSC were expanded on irradiated NIH3T3 feeder layers (3). Stem/progenitor cells were recovered by double trypsinization and resuspended in 1× PBS.

Tracheal Xenografts

Test cells, 1×10^6 irradiated fibroblast feeder cells, or mouse stem/progenitor cells were introduced into the lumen of freeze–thawed Swiss-Webster rat tracheas. This xenograft was positioned in a subcutaneous pocket in nonobese diabetic (NOD)/severe combined immunodeficiency (SCID) mice. The xenograft and host trachea were recovered at various time points.

Human Cells

Human tracheobronchial tissue was procured from National Disease Research Interchange under National Jewish Institutional Review Board–approved protocols. Epithelial cells were purified and cultured in bronchial epithelial growth medium (11, 12). Passage 3 cells were transduced with MISSION pLKO.1-puro TurboGFP lentiviral particles following the manufacturer's instructions (Sigma, St. Louis, MO). At 72 hours after transduction, cells were collected and resuspended in sterile 1× PBS. Approximately 80% of cells were green fluorescent protein (GFP)$^+$.

Transplantation Protocol

The protocol was developed using passage 1 stem/progenitor cells (test cells) that were recovered from ROSA26-LacZ transgenic mice. Pilot studies used NOD/SCID and C57Bl/6 mice that were treated with corn oil or NA. On Recovery Day 2 or 4, mice were anesthetized and the vocal cords visualized. Aliquots of 5×10^4, 2.5×10^5, or 10^6 cells in 30 μl 1× PBS were instilled into the trachea (13). For intravenous cell delivery studies, cells were injected into the tail vein with a 26-gauge needle. Subsequent studies used the optimal transplantation protocol, NOD/SCID host mice, and an inoculum of 2.5×10^5 test cells.

Histology

Xenografts, tracheal tissue, and lung tissue were fixed, paraffin embedded, and immunostained using published methods (10, 14–18). Mouse test cells were detected by 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside staining (14, 15). Human test cells were identified via immunodetection of the GFP tag. Differentiated cell type frequency and contribution to the epithelial cell pool were quantified as previously described (15, 16).

Statistical Analyses

All statistical analyses were performed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). For normally distributed data sets, differences were evaluated using Student's *t* test. For nonnormally distributed data sets, differences were evaluated by the Mann–Whitney test. *P* values less than 0.05 were considered significant. All analyses included trachea or lung tissue samples recovered from three to four mice.

Results

Mouse TSCs/Progenitor Cells Undergo Limited Differentiation *Ex Vivo*

The lung has a remarkable host defense system that imposes multiple barriers to successful cell transplantation. Thus, we first evaluated mouse TSC/progenitor cell differentiation using the rat tracheal xenograft system (19, 20).

Histological analysis of the host mouse trachea demonstrated that most mouse basal cells were keratin (K) 5$^+$/K14$^-$/tumor protein 63 (Trp63)$^+$ (Figure 1, *left column*). Luminal cells expressed K8 and K19, and subsets of cells were positive for the ciliated cell marker, acetylated tubulin, and the secretory cell marker, CCSP. Trachea scaffolds that were seeded with irradiated fibroblast feeder cells did not develop an epithelial lining (Figure 1, *middle column*). In contrast, scaffolds that were seeded with TSCs/progenitor cells developed an epithelial lining that was populated

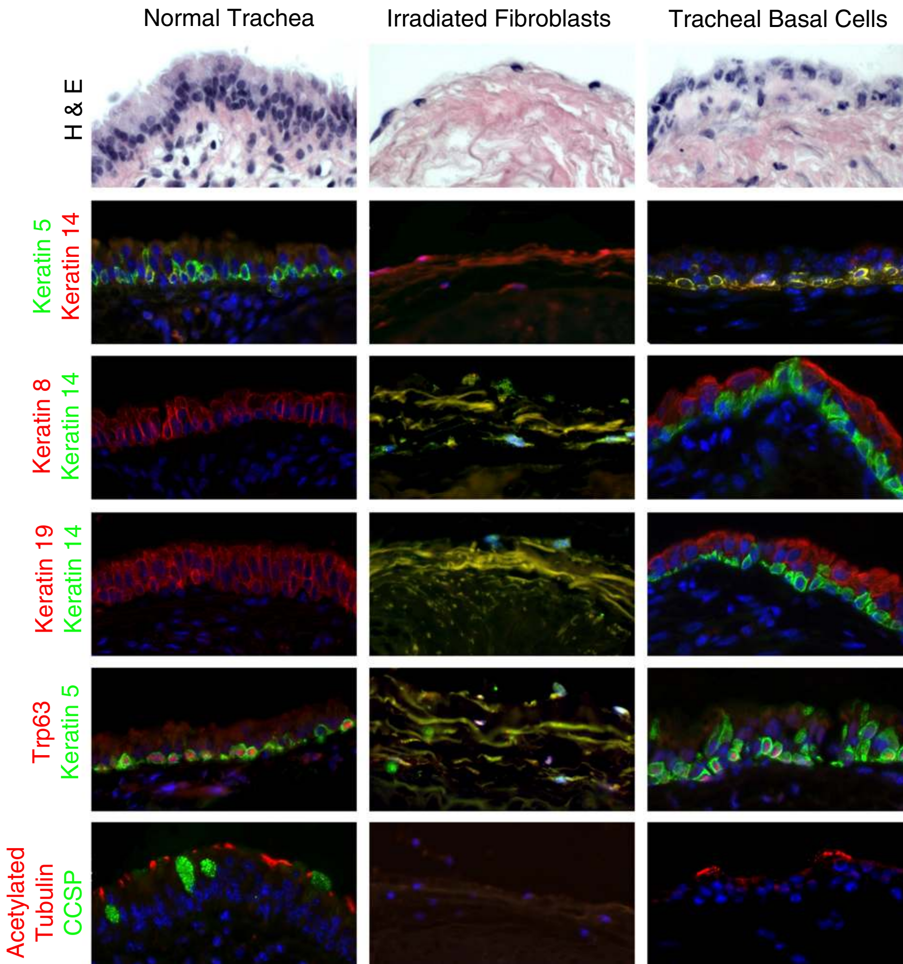

Figure 1. Mouse tissue stem cell (TSC)/progenitor cell differentiation in rat tracheal xenografts. Rat tracheal scaffolds were seeded with 1×10^6 irradiated NIH3T3 fibroblasts or viable mouse TSCs/progenitor cells and implanted in a dorsal–lateral subcutaneous pocket of nonobese diabetic (NOD)/severe combined immunodeficiency (SCID)/TSC-γ knockout mice. Xenografts and the host trachea were recovered on Day 14. Histological sections were stained as indicated. All images are ×200 magnification. Data are representative of four experiments. CCSP, Clara cell secretory protein; H&E, hematoxylin and eosin; Trp63, tumor protein 63.

by $K5^+$/$K14^+$/$TRP63^+$ basal cells, as well as $K8^+$ and $K19^+$ luminal cells (Figure 1, *right column*). Although rare ciliated cells were observed, no $CCSP^+$ cells were noted. These data are in agreement with our report that TSC/progenitor differentiation required paracrine signals from an as-yet undefined tracheal cell type (3), and suggested that a lung transplantation assay was needed to test the therapeutic potential of mouse TSCs/progenitor cells.

The TSC Pool Is Depleted by NA Injury

Host TSC depletion is thought to promote tissue repopulation by transplanted TSCs (reviewed in Reference 21), a concept that was recently reinforced in the context of embryonic stem cell (ESC) transplantation to the airways (22). Because NA injury is frequently used to activate mouse airway epithelial TSCs *in vivo* (2, 14, 15), and this model was also used in combination with total body irradiation for ESC transplantation, we determined if NA treatment depleted the TSC population.

Groups of adult female FVB/n mice were treated with vehicle or 300 mg/kg NA and recovered for 6, 40, or 80 days. Tracheal cells were recovered by dispase/collagenase/trypsin digestion, and TSC frequency was determined by the limiting dilution method (3). This study demonstrated that NA treatment significantly decreased TSC frequency on Recovery Days 6 and 40 (Figure 2). In contrast, TSC frequency was normal on Recovery Day 80. These data suggested that NA injury could be used to condition the host before transplantation of TSCs/progenitor cells.

Transplantation Protocol Development

Our first goal was to determine if repopulation of the conducting airway epithelium was facilitated by NA injury. Pilot studies (summarized in Figure 3) demonstrated that LacZ-labeled TSCs/progenitor (test) cells failed to repopulate the airways of vehicle-treated mice (uninjured). In contrast, the trachea and intrapulmonary airways of NA-injured mice were repopulated by test cells. Test cells did not repopulate the parenchyma, a lung region that is not injured by NA exposure.

Second, we determined the optimal route, dose, and timing for test cell transplantation. We show that transplantation on Recovery Day 2 was successful. In contrast, neither the airways nor the parenchyma were repopulated by test cells that

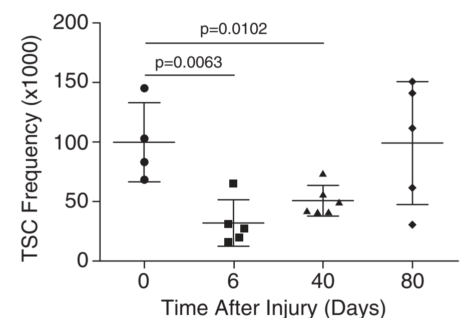

Figure 2. Naphthalene (NA) treatment depletes TSCs. Female FVB/n mice were treated with corn oil or 300 mg/kg NA and recovered for 6, 40, or 80 days. Trachea were recovered, single-cell suspensions were generated, and cells were plated on irradiated NIH3T3 fibroblast feeder layers. Progenitor cell frequency was determined using the limiting dilution method. Cultures were fixed, stained, and quantified on Day 12. Data are presented as the mean (±SD) ($n = 4$). *Symbols* indicate the value for individual mice.

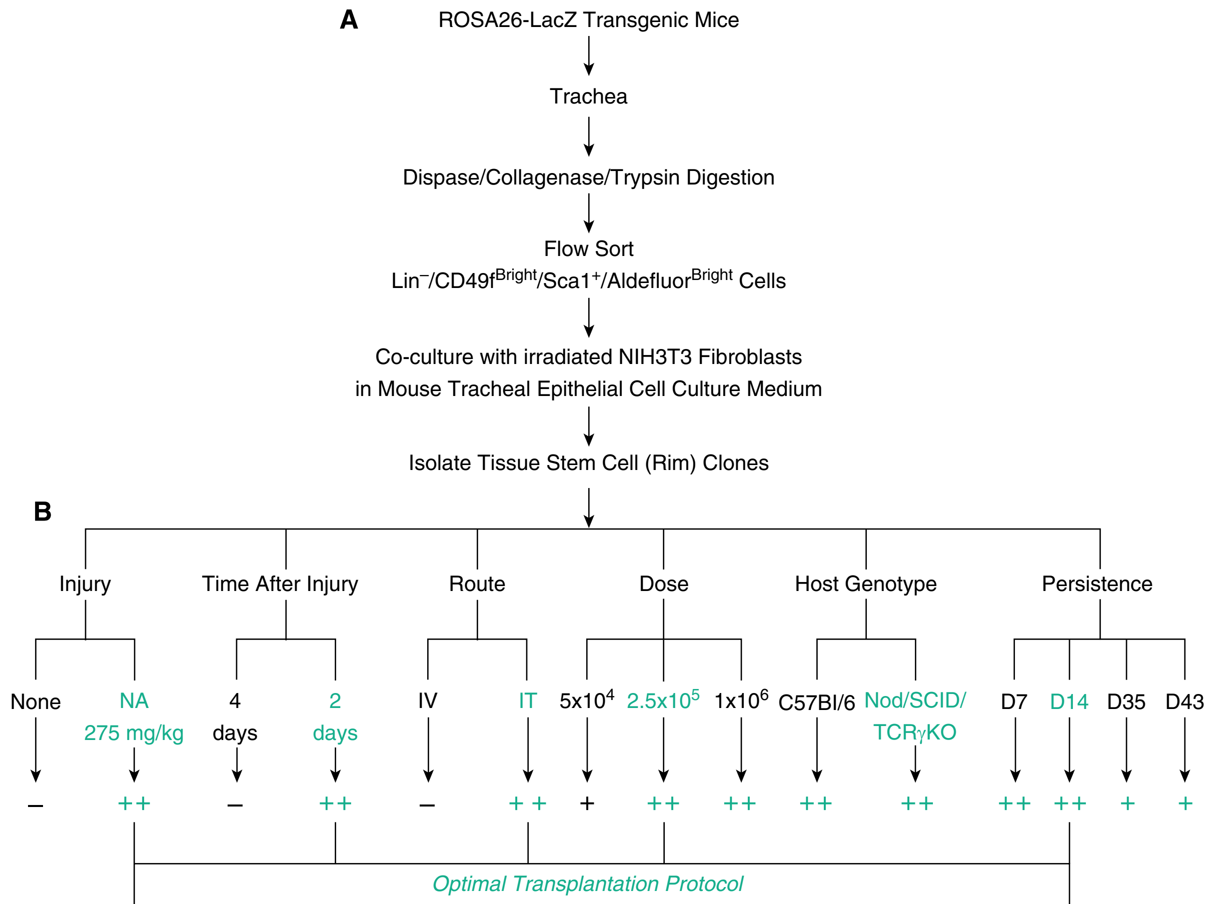

Figure 3. Optimal transplantation protocol. (*A*) Test cell preparation. (*B*) The steps used to determine the optimal transplantation parameters. IT, intratracheal; IV, intravenous; KO, knockout; Lin^-, cells are negative for CD45/CD31/Ter119; NA, not applicable; TCRγ, T-cell receptor-γ. *Green text* indicates the optimal condition in each subcategory.

were transplanted on Recovery Day 4. Intratracheal instillation permitted airway-specific repopulation, whereas intravenous infusion was unsuccessful. A dose–response study used intratracheal instillation on Recovery Day 2, and showed that as few as 50,000 TSCs/progenitor cells repopulated the airway epithelium, and as many as one million cells were well tolerated.

Third, we determined if test cells repopulated the airway epithelium of syngeneic C57Bl/6 mice or immune-compromised NOD/SCID/T-cell receptor (TCR)-γ knockout (NOD/SCID) hosts. These studies demonstrated successful and specific repopulation of the tracheal and intrapulmonary airway epithelium by test cells in both host types. Finally, we used the optimal transplantation parameters (Figure 3), NOD/SCID host mice, and an inoculum of 2.5×10^5 test cells were used to evaluate test cell persistence. Test-derived cells were detected 7, 14, 35, and 43 days after transplantation (Figure 3).

Transplanted Mouse TSCs/Progenitor Cells Repopulate the Conducting Airway Epithelium

We used the optimal transplantation protocol (Figure 3), NOD/SCID host mice, and an inoculum of 2.5×10^5 test cells to evaluate test cell location, abundance, and differentiation. Whole-mount analysis of the tracheobronchial airways identified clusters of test-derived cells in the trachea and main stem bronchi (Figures 4A–4C). These clusters were common throughout the trachea and its subregions (Figure 4E). However, cluster size varied within a single host animal and across hosts (Figure 4F). The bronchial airways were also repopulated with clusters of test-derived cells (Figures 4D and 4E). As for the trachea, the frequency of test-derived cells in the bronchial epithelium varied within and among hosts (Figure 4G). Few test-derived cells were identified in the terminal bronchioles, and none were located in the parenchyma.

Transplanted TSCs/Progenitor Cells Exhibit Multilineage Differentiation

Test-derived cell differentiation was evaluated by codetection of the LacZ tag and differentiation markers in the trachea (Figures 5A–5C) and intrapulmonary airways (Figures 5E–5H) 2 weeks after transplantation. Quantification demonstrated that test-derived cells generated basal and secretory cells at a frequency that was equivalent to that of host-derived ($LacZ^-$) tracheal cells (Figure 5D). In contrast, differentiation of test-derived cells to the ciliated cell phenotype was significantly less than

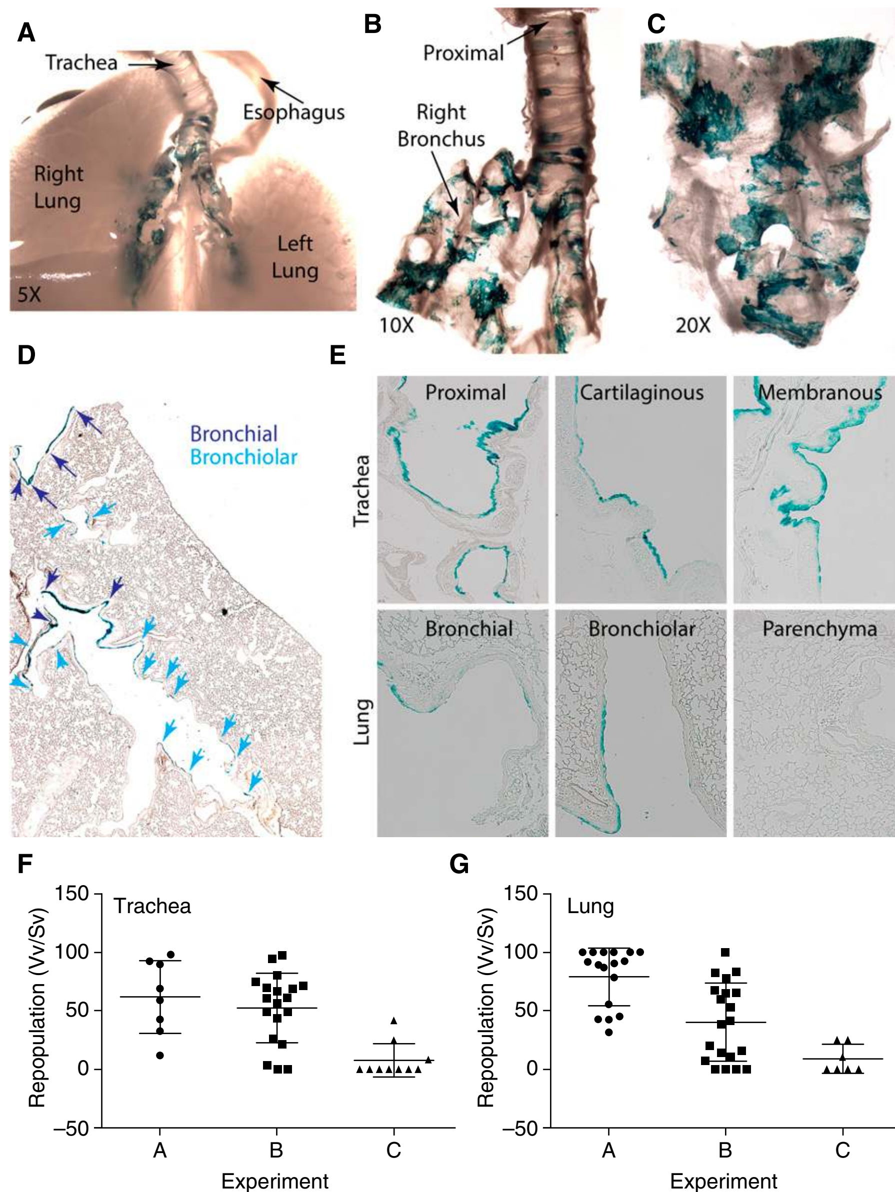

Figure 4. Transplanted mouse TSCs/progenitor cells repopulate the tracheobronchial and intrapulmonary airways. Mouse TSCs were recovered from ROSA26-LacZ transgenic mice, expanded as rim clones, and these "test" cells were instilled into the trachea of Nod/SCID/TCR-γ knockout mice on NA Recovery Day 2. (*A*) Whole-mount analysis of the trachea and lungs. Test-derived cells are positive for the LacZ protein product β-Gal$^+$ (*blue*). (*B*) Whole-mount analysis of the trachea and luminal surface of the right bronchus. (*C*) Whole-mount analysis of the luminal surface of the left bronchus. (*D*) Tissue section analysis of the lower right lung lobe. *Arrows*: *dark blue*, bronchial region, *light blue*, bronchiolar region. (*E*) Section analysis of tracheal and lung subregions. Original magnification, ×200. (*F* and *G*) Volume fraction of the tracheal (*F*) and lung (*G*) epithelium that is repopulated by transplanted cells. Mean (±SD). *Points* indicate individual high-power fields. (*A*–*C*) Distinct host mice transplanted with mouse rim clone cells.

that observed for host-derived cells. In the intrapulmonary airways, test-derived cell differentiation to basal and ciliated cells was similar to the host (Figure 5I). However, test-derived cells generated significantly fewer secretory cells than did host-derived cells.

Transplanted Human TSCs/Progenitor Cells Repopulate the Tracheobronchial and Intrapulmonary Airways

One-million GFP-labeled human TSCs/progenitor (test) cells were transplanted using the optimal transplantation protocol. Histological studies identified GFP$^+$ test-derived cells in the trachea (Figures 6A–6C) and intrapulmonary airways (Figures 6G–6I) 2 weeks after transplantation. Test-derived cells were not detected in the parenchymal epithelium.

In the trachea, test-derived cells were typically less abundant than host-derived cells (Figure 6D). In mice that were well repopulated, host- and test-derived cells were equally represented throughout the proximal-to-distal axis of the trachea (Figure 6E). The majority of test-derived cells did not express the basal cell markers, K5 (Figure 6F) or K14 (data not shown). Differentiation of test-derived cells to a mucus cell phenotype was significantly greater than the host cells (Figure 6F), whereas differentiation of test-derived cells to the ciliated cell phenotype was significantly less than the host cells (Figure 6F). In agreement with these data, the contribution of test-derived cells to the basal and ciliated cell pools was less than host-derived cells, whereas their contribution to the mucus cell pool was greater than host-derived cells (data not shown).

Human test-derived cells were also identified in the intrapulmonary airways (Figures 6A–6C). Test-derived cells were most frequent along the main axial pathway, where they comprised approximately one-half of the epithelium (Figure 6J). However, test-derived cells were less frequent than host-derived cells in the bronchial and terminal bronchiolar epithelium (Figure 6K). In the main axial airway, a minority of test-derived cells express K5 (Figure 6L), whereas the majority expressed secretory (Mucin 5B) and ciliated cell markers (Figure 6L). Test-derived cells were a rare contributor to the basal cell pool in bronchial airways, but comprised approximately one-half of the secretory and ciliated cell pools (Figures 6F, 6H, and 6J).

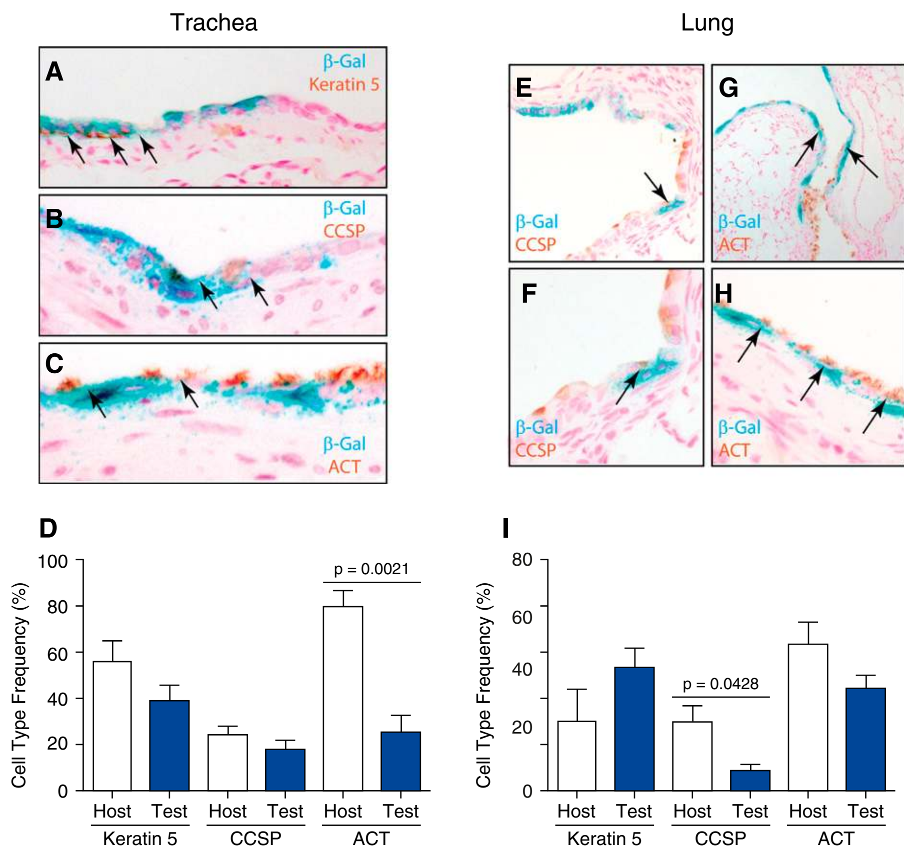

Figure 5. Transplanted mouse TSCs/progenitor cells exhibit multilineage differentiation after transplantation. Mice were transplanted as indicated in Figure 4. (*A–C*) Section analysis of the tracheal epithelium: test-derived cells are positive for the LacZ protein product, β-galactosidase (β-Gal)$^+$ (*blue*). (*A*) Keratin 5 (*brown*); (*B*) CCSP (*brown*); (*C*) acetylated tubulin (ACT; *brown*). (*D*) Quantification of host-derived (*white*) and test-derived (*blue*) basal cells (keratin 5), secretory cells (CCSP), and ciliated cells (ACT). Mean (±SD); $n = 3$; β-gal (*blue*). (*E–H*) Section analysis of the intrapulmonary airway epithelium: test-derived cells are β-gal$^+$ (*blue*). (*E* and *F*) CCSP (*brown*); (*G* and *H*) ACT (*brown*). (*E* and *G*) 20×; (*F* and *H*) 40×. (*I*) Quantification of host- (*white*) and test-derived (*blue*) basal cells (Keratin 5), secretory cells (CCSP), and ciliated cells (ACT). Mean (±SD); $n = 15–50$ high-power fields. *Arrows* signify cell type–specific antigen-positive test cells.

Discussion

Cell therapy has the potential to cure lung disease through replacement of malfunctioning cells. Although the TSC is thought to be the optimal therapeutic cell, evidence from nonpulmonary tissues demonstrated that transplantation of TSC/progenitor cell mixtures was an effective treatment approach (e.g., *see* Reference 23). We now show that transplanted mouse and human tracheobronchial TSC/progenitor cell mixtures reconstitute the injured airway epithelium within 2 weeks. We conclude that adult tracheobronchial TSCs/progenitors can be used as cell therapy.

Because chronic lung disease frequently involves the conducting airway epithelium, cell therapy has frequently focused on this tissue (Tables 1 and 2). Previous studies used a variety of methods (host conditioning, delivery route, and treatment time [22, 24–26]). The present study narrows these options and provides a transplantation model that can be used to test additional stem and progenitor cell preparations.

We and others have reported that NA-induced injury is dependent on dose and genetic background (27–30). For example, high-dose NA treatment results in 95% depletion of the CCSP$^+$ cell population on Recovery Day 3. This injury level can be achieved by varying the NA dose according to genetic background (e.g., FVB/n mice, 300 mg/kg [10]; C57Bl/6 mice, 250 mg/kg [30]). In the present study, NOD/SCID host mice were treated with 275 mg/kg NA. This dose caused extensive depletion of the airway epithelial progenitor cell pool, and was associated with effective repopulation by transplanted TSCs/progenitor cells. In contrast with our study, Rosen and colleagues (22) reported that NA injury did not permit effective cell therapy by ESC. Based on our knowledge of NA injury, we speculate that ineffective transplantation in C57Bl/6 mice conditioned with 200 mg/kg NA was due to suboptimal airway epithelial injury.

Previous studies also demonstrated that NA toxicity is limited to the conducting airway epithelium, and that it did not injure the parenchyma. In the present study, transplanted TSCs/progenitor cells repopulated the airway epithelium, but not parenchymal tissues. These data suggest that the lack of parenchymal injury in NA-treated mice prevents aberrant seeding of the alveolar epithelium. Furthermore, they also suggest that cell therapy for chronic airway disease should use a host-conditioning regimen that causes extensive and specific injury to the conducting airway epithelium.

Previous studies and preliminary analysis (data not shown) indicate that NA injury has no detectable effect on vasculature integrity, whereas many agents that are used to study epithelial injury and repair (bleomycin, influenza, and radiation) target multiple lung tissues, including the vasculature. In the present study, we report that intravenous delivery of TSCs/progenitor cells to NA-treated mice did not result in effective repopulation by transplanted TSCs/progenitor cells. These data are in contrast to the report that intravenous delivery was an effective route in mice that were conditioned with a combination of low-dose NA and total body irradiation (22). Knowing that radiation compromises vascular integrity, we suggest that the Rosen group's successful use of infused cells was due radiation-induced loss of vascular integrity. Collectively, these data suggest

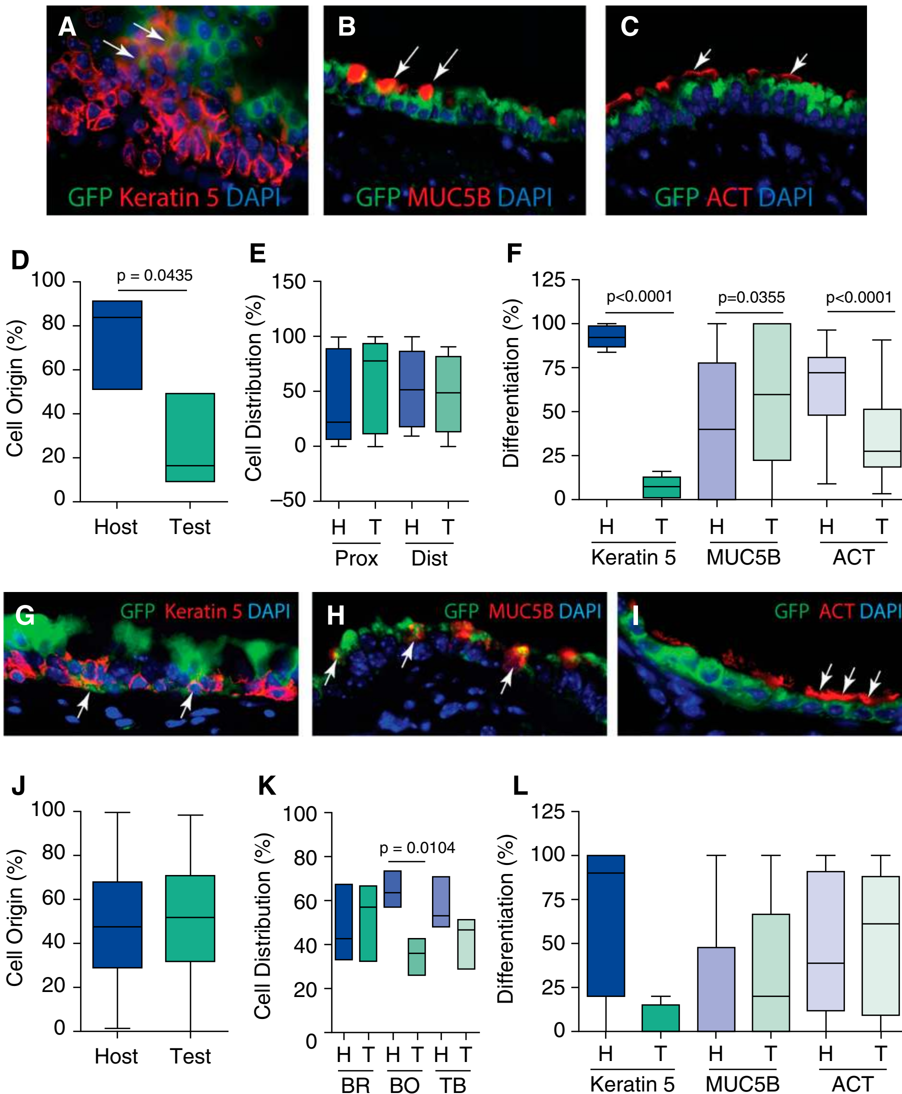

Figure 6. Transplanted human TSCs/progenitor cells repopulate the tracheal and intrapulmonary epithelium. Green fluorescent protein–positive human bronchial basal cells were transplanted as indicated in Figure 4. (*A*–*C*) Immunofluorescence detection of differentiation antigens in the trachea (80×). (*D*) Quantification of tracheal epithelial cell origin. Minimum/maximum with *line* at median; $n = 3$. (*E*) Quantification of host-derived (H; *blue bars*) and test-derived (T; *green bars*) cell distribution in the proximal (Prox) and distal (Dist) trachea. (*F*) Differentiation of host- and test-derived cells to basal cells (keratin 5), secretory cells (mucin 5B [MUC5B]), or ciliated cells (ACT). *Box* indicates the range; *line* indicates the mean; $n = 45$ high-power fields. (*G*–*I*) Immunofluorescence detection of differentiation antigens in the intrapulmonary airways (80×). (*J*) Quantification of intrapulmonary epithelial cell origin. (*K*) Quantification of epithelial cell origin in airway subregions. BO, bronchiolar; BR, bronchial; H, host; T, test; TB, terminal bronchiolar. Minimum/maximum with *line* at median; $n = 3$. (*L*) Quantification of cell differentiation. *Bars* indicate the range; *box* indicates the mean; *line* indicates the mean; $n = 15$–26 high-power fields. *Arrows* signify cell type–specific antigen-positive test cells. DAPI, 4′,6-diamidino-2-phenylindole; GFP, green fluorescent protein.

that the host-conditioning method determines the effective cell delivery route.

High-dose NA–induced airway epithelial injury is repaired in two stages. First, the uninjured epithelial cells spread and re-establish the epithelial barrier (29, 31). Second, tracheobronchial and bronchiolar TSCs proliferate and generate differentiated progeny that repopulate the epithelium (14–16). Ciliated cells are among the first differentiated cells that are replaced by the tracheobronchial TSCs, and are first detected on Recovery Day 3 (10).

We report that transplantation on Recovery Day 2 resulted in effective repopulation, whereas transplantation on Recovery Day 4 was unsuccessful. Knowing that ciliated cells sweep the extracellular lining fluid and associated particles from the airway lumen, we speculate that the effective timeframe for cell transplantation is limited by ciliated cell–mediated clearance of transplanted cells. These data suggest that effective host conditioning in diseases that exhibit ciliated cell hypoplasia will require little host conditioning, whereas a host-conditioning regimen that compromises ciliated cell function will be needed in patients with normal ciliated cell frequency and function.

Successful TSC transplantation is thought to be dependent on host-conditioning regimes that decrease TSC frequency in the niche. In the present study, we show that high-dose NA exposure decreases the TSC population by approximately 75% on Recovery Day 6 (Figure 2). This depletion indicates that NA treatment meets one criterion for effective host conditioning.

TSC depletion is also thought to increase TSC homing to the niche after transplantation. Previously identified respiratory epithelial TSC niches include the submucosal gland duct junction, the bronchiolar neuroepithelial body, and the bronchoalveolar duct junction (32–36). In the present study, we report that test-derived mouse and human cells are found throughout the conducting airway epithelium. These data suggest that transplanted TSCs/progenitor cells did not preferentially repopulate the known niches. In light of our previous report that TSCs were located throughout the cartilaginous tracheal epithelium (2), we suggest two explanations for the relatively uniform distribution of test-derived cells in the tracheobronchial region. First, the tracheobronchial epithelium may contain multiple niches that are differentially sensitive to epithelial injury. The submucosal gland duct niche was defined by injuries that eliminated TSCs and progenitors in the

Table 1. Mouse Transplantation Studies

	First Author (Reference)			
	Rosen (22)	Vaughan (24)	Zuo (25)	Ghosh (Present Study)
Test	Unfractionated embryonic mouse lung cells	Integrin β4$^+$ adult lung cells	Keratin 5$^+$/Trp63$^+$ tracheobronchiolar or distal basal cells	Rim clone cells
Host	C57Bl/6 mice	Adult C57Bl/6 mice	Adult C57Bl/6 mice	Nod/SCID/TCRγ-KO
Conditioning	Naphthalene and total body irradiation	Influenza infection	Influenza infection	Naphthalene
Timing	2 d postinjury	9 d postinfection	5 d postinfection	2 d postinjury
Dose	1×10^6	1×10^5	1×10^6	5×10^4 to 1×10^6
Route	Intravenous	Intranasal	Intratracheal	Intratracheal
Duration	4–8 wk	12 d	35 d	9 d
Results	Epithelial (airway and alveolar), endothelial, and mesenchymal chimerism	Alveolar epithelial chimerism	Bronchiolar and/or alveolar epithelial chimerism	Proximal conducting airway epithelial chimerism

Definition of abbreviations: KO, knockout; Nod, nonobese diabetic; SCID, severe combined immunodeficiency; TCRγ, T-cell receptor-γ; Trp63, tumor protein 63.

surface airway epithelium (e.g., 350 mg/kg NA in FVB/n mice [33], acid or detergent [32]). In contrast, the injury achieved in our transplantation studies may have spared the submucosal gland duct niche, and TSC retention may have prevented repopulation by test-derived cells. Second, the TSC may generate its own niche (3, 9). We previously reported that TSCs generated rim clones, and that the rim domain of the rim clone sequestered label-retaining cells (3). Subsequent purification of these label-retaining cells demonstrated that they exhibited the TSC-specific phenotype, and that they generated rim clones with label-retaining TSCs *in vitro* (2). These data suggest that the observed distribution of test-derived cells may stem from the TSC's intrinsic ability to create the niche *de novo*.

As previously shown for nonpulmonary tissues, the present study substantiates the notion that TSC/progenitor cell mixtures can be used as cell therapy for lung disease. Knowing that airway epithelial cells are long lived, additional studies are needed to determine if transplanted cells exhibit the same longevity. Similarly, the benefits of cell therapy require maintenance of the TSCs after transplantation. Additional single-cell lineage tracing and self-renewal studies are needed to address this aspect of cell therapy that uses a TSC/progenitor cell mixture.

Although the current transplantation model is appropriate to studies that evaluate the fundamental aspects of cell therapy, at least two limitations to the use of NA are recognized. First, NA-induced injury was used to condition the host. Although this treatment effectively prepared the mouse lung for cell therapy, the CyP-450 enzyme profile of the human airway epithelium (37) makes it unlikely that NA exposure will effectively condition the human lung. However, extensive knowledge of NA-induced injury in mice allows us to establish the characteristics of an effective host-conditioning strategy for airway-targeted cell therapy. We posit that extensive

Table 2. Human Cell Transplantation Studies

	First Author (Reference)		
	Rosen (22)	Kajstura (26)	Ghosh (Present Study)
Test	Embryonic human lung cells	cKit$^+$ adult human lung cell	Bronchial basal cells
Host	Nod/SCID	C57Bl/6	Nod/SCID/TCRγ-KO
Conditioning	Naphthalene and total body irradiation	Cyclosporine A immunosuppression and puncture with an ice-cold steel probe	Naphthalene
Timing	2 d postinjury	Shortly after puncture	2 d postinjury
Dose	1×10^6	2×10^4	1×10^6
Route	Intravenous	Wound injection	Intratracheal
Duration	4–8 wk	10–14 d	9 d
Results	Epithelial (airway and alveolar), endothelial, and mesenchymal chimerism	Epithelial (airway and alveolar), endothelial, and chimerism	Conducting airway epithelial chimerism

Definition of abbreviations: KO, knockout; Nod, nonobese diabetic; SCID, severe combined immunodeficiency; TCRγ, T-cell receptor-γ.

airway-specific epithelial injury will be needed for effective conditioning. Second, the present study used NA injury to mimic the epithelial injury observed in some chronic lung diseases. Although NA is a well established acute injury model, it does not recapitulate the chronic epithelial injury and remodeling that is characteristic of human lung disease or the inflammatory process present in many of these diseases. Consequently, additional studies are needed to test TSC/progenitor cell therapy under conditions that mimic these aspects of human chronic lung disease. ■

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