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Circulating Progenitor Epithelial Cells Traffic via CXCR4/CXCL12 in Response to Airway Injury

Brigitte N. Gomperts,* John A. Belperio,† P. Nagesh Rao,‡ Scott H. Randell,§ Michael C. Fishbein,∥ Marie D. Burdick,‡ and Robert M. Strieter‡

Recipient airway epithelial cells are found in human sex-mismatched lung transplants, implying that circulating progenitor epithelial cells contribute to the repair of the airway epithelium. Markers of circulating progenitor epithelial cells and mechanisms for their trafficking remain to be elucidated. We demonstrate that a population of progenitor epithelial cells exists in the bone marrow and the circulation of mice that is positive for the early epithelial marker cytokeratin 5 (CK5) and the chemokine receptor CXCR4. We used a mouse model of sex-mismatched tracheal transplantation and found that CK5⁺ circulating progenitor epithelial cells contribute to re-epithelialization of the airway and re-establishment of the pseudostratified epithelium. The presence of CXCL12 in tracheal transplants provided a mechanism for CXCR4⁺ circulating progenitor epithelial cell recruitment to the airway. Depletion of CXCL12 resulted in the epithelium defaulting to squamous metaplasia, which was derived solely from the resident tissue progenitor epithelial cells. Our findings demonstrate that CK5⁺CXCR4⁺ cells are markers of circulating progenitor epithelial cells in the bone marrow and circulation and that CXCR4/CXCL12-mediated recruitment of circulating progenitor epithelial cells is necessary for a normal pseudostratified epithelium after airway injury. These findings support a novel paradigm for the development of squamous metaplasia of the airway epithelium and for developing therapeutic strategies for circulating progenitor epithelial cells in airway diseases. *The Journal of Immunology, 2006, 176: 1916–1927.

The airway epithelium is in direct contact with the environment and, as such, is at constant jeopardy of injury from environmental toxins and pathogens. Therefore, an efficient mechanism for regeneration and repair of the airway epithelium is essential to maintain this barrier and protect the host. Our understanding of this repair process is limited and, until recently, was thought to occur only from resident tissue progenitor cells. Basal resident progenitor epithelial cells are located in the submucosal glands and ducts and have been shown to be capable of differentiating into all airway epithelial cell types (1–3). They possess the capacity for self-renewal, divide slowly, retain a BrdU label, and express markers of early epithelial differentiation (i.e., cytokeratin 5[CK5] and CK14) (1–6). CK5 and CK14 are type I and type II intermediate filament proteins that are expressed together in basal regenerating cells of complicated epithelia. Injury to the airway results in an initial increase in the number of CK5⁺CK14⁺ basal cells, with a return to normal CK5⁺CK14⁺ resident progenitor cell numbers once the airway is repaired, suggesting a negative feedback mechanism (2).

Under homeostatic conditions, it is likely that there are sufficient resident progenitor epithelial cells to replace the continuous loss of airway epithelial cells. However, at times of airway injury a contemporary concept suggests that the resident progenitor cells may be overwhelmed and that circulating progenitor epithelial cells contribute to repair. Evidence of this recruitment comes from human sex-mismatched lung transplants where tissue biopsies have shown pulmonary epithelial cells from the recipient contributing to the repair of donor lungs, implying that circulating progenitor epithelial cells do contribute to regeneration of the airway epithelium (7, 8). Furthermore, the circulating progenitor epithelial cells likely originate from the bone marrow, because serial transplantation of a single GFP⁺ bone marrow cell into lethally irradiated mice results in GFP expression in the bronchi as well as other tissues (9). However, these cells represent a very small population of cells and are not yet well understood. Therefore, identifying and characterizing these circulating progenitor epithelial cells and their mechanisms of trafficking is essential for our understanding of the repair process and for harnessing their potential therapeutic applications.

We hypothesized that circulating progenitor epithelial cells traffic to the injured airway in response to the CXCL12/CXCR4 biological axis and contribute to repair. To test this postulate, we created a mouse model of airway injury and used specific biomarkers to track the recruitment of circulating progenitor epithelial cells. Our studies demonstrated that circulating progenitor epithelial cells contributed to repair of the injured airway in a model of tracheal transplantation. Furthermore, the CXCR4/CXCL12 biological axis played a critical role in the trafficking of these cells to the site of airway injury. In addition, neutralizing Abs to CXCL12 resulted in a phenotype of squamous metaplasia in the regenerated epithelium, which was devoid of circulating progenitor epithelial cells. These findings may ultimately result in the use of circulating progenitor epithelial cells as a novel therapy for airway injury and...
further our understanding of the pathogenesis of airway squamous metaplasia.

**Materials and Methods**

### Mouse tracheal transplant model

We used a well established, reproducible murine model of tracheal epithelial regeneration using syngeneic s.c. tracheal transplants from C57BL/6 females into C57BL/6 males for our sex-mismatched model (10, 11). Male tracheas transplanted into male mice and female tracheas transplanted into female mice served as our sex-matched controls. For the experiments with GFP mice, wild-type C57BL/6 female tracheas were transplanted into C57BL/6 male GFP mice (The Jackson Laboratory). For the experiments with CK5-GFP mice, wild type C57BL/6C3H tracheas were transplanted into C57BL/6C3H CK5-GFP mice.

### CXCL12 mobilization and enrichment of bone marrow

The mobilization and enrichment of bone marrow was performed by s.c. injection of CXCL12 (100 μg), as compared with control vehicle, in mice. Six groups of mice (n = 5) were treated in the following manner. Groups 1 and 2 were treated with CXCL12 or control vehicle, respectively, at time 0 and were sacrificed in 1 h for analysis of the number of CK5 cells in both the buffy coat and bone marrow. Groups 3 and 4 were treated with CXCL12 or control vehicle, respectively, at time 0 and received a second dose of CXCL12 or control vehicle, respectively, at 24 h followed by sacrifice in 1 h for analysis of the number of CK5 cells in both the buffy coat and bone marrow. Groups 5 and 6 were treated with CXCL12 or control vehicle, respectively, at time 0, received a second dose of CXCL12 or control vehicle, respectively, at 24 h, and a third dose of CXCL12 or control vehicle, respectively, at 48 h followed by sacrifice in 1 h after the last dose for analysis of the number of CK5 cells in both the buffy coat and bone marrow (12).

### Depletion of CXCL12

F(ab')₂ of either neutralizing goat anti-mouse CXCL12 Ab or control F(ab')₂ were injected i.p. 1 h before tracheal transplant and then every other day until the animals were euthanized (13). Five milligrams of Ab were injected per dose as described previously (13). All animal experiments for these studies were approved by the Department of Laboratory Animal Medicine at the David Geffen School of Medicine at the University of California, Los Angeles, CA.

### Quantitative real-time PCR

Quantitative real-time PCR was performed using the 7700 sequence detection system (Applied Biosystems). Quantitative real-time PCR was performed for CXCL12 and CCR4 from tracheal transplant cDNA as described previously (10, 11).

### Chromogenic in situ hybridization (CISH)

Tracheal transplant tissue was fixed in 4% paraformaldehyde overnight and paraffin-embedded for sectioning. Deparaffinization was performed before boiling in CISH tissue heat pretreatment solution and digesting in pepsin per the manufacturer’s protocol (Zymed Laboratories). The biotinylated Y chromosome probe (Zymed Laboratories) and the tissue were denatured at 94°C for 5 min, and hybridization was performed overnight at 37°C. Immunodetection was performed per the manufacturer’s protocol.

### Fluorescent in situ hybridization (FISH)

Four-micrometer sections of paraformaldehyde-fixed, paraffin-embedded tissue were deparaffinized, incubated in sodium thiocyanate at 80°C for 30 min, and digested with 0.1% pepsin in 0.1 N HCl for 20 min. Tissues and probes were denatured at 60°C for 10 min and then hybridized at 37°C with FITC-labeled mouse Y chromosome probe (Zymed Laboratories) and the tissue were denatured at 94°C for 5 min, and hybridization was performed overnight at 37°C. Immunodetection was performed per the manufacturer’s protocol.

### Immunofluorescence

Tissue sections were deparaffinized, and Ab retrieval and blocking were performed as described above for immunohistochemistry. The primary Abs used were anti-mouse CK5 (dilution 1/200 (Covance Research Products), anti-GFP (dilution 1/200 (Santa Cruz Biotechnology), and anti-mouse p63 (dilution 1/200) (Santa Cruz Biotechnology). Biotinylated secondary Ab (Jackson ImmunoResearch Laboratories) followed by streptavidin-Cy3 (Jackson ImmunoResearch Laboratories) was used to amplify the signal. For dual immunostaining, avidin and biotin (Vector Laboratories) blocking steps were performed before repeating the above protocol with the second primary Ab. Microscopy was performed with a Zeiss microscope as described above.

### FACS analysis of buffy coat, bone marrow cells, and cells from tracheal transplants

FACS staining was performed in 96-well disposable plates. Bone marrow was isolated from mouse femurs by flushing with PBS through a 26-gauge needle into RPMI 1640 medium. Collagenase A (Roche) and DNase (Sigma-Aldrich) were added to the bone marrow, thoroughly mixed, and incubated at room temperature for 20 min to create a single cell suspension. The RBCs were lysed, and 1 × 10⁶ cells were plated in each well of the 96-well plate for immediate FACS analysis. Peripheral blood was obtained from mice by retro-orbital bleeding. Five hundred microliters of mouse whole blood was collected from a single mouse in an Eppendorf tube with 50 μl of heparin to prevent clotting. Single mouse samples were not combined. The whole blood was centrifuged at 3,000 rpm for 10 min to separate out the fractions. The layer of nucleated cells between the plasma layer and the RBC layer forms the buffy coat. The buffy coat was aspirated, and RBC was lysed using ammonium chloride potassium lysing buffer. Cells (1 × 10⁶) were placed into each well of a 96-well plate for immediate FACS analysis. The tracheal transplants were diced with a clean blade and then incubated in collagenase A (Roche) and DNase (Sigma-Aldrich) at 37°C for 30 min to create a single cell suspension. Cells were plated immediately into FACS plates for analysis. The Abs used were FITC-conjugated anti-mouse-CK5 (Covance Research Products), PerCP mouse anti-mouse CD45 (BD Biosciences), PE-anti-mouse CCR4 (BD Biosciences), PE-anti-mouse-CXCR4 (BD Biosciences), PE anti-Ly-6 (BD Biosciences), PE anti-NK1.1 (BD Biosciences), PE anti-CD3 (BD Biosciences), PE anti-CD4 (BD Biosciences), PE anti-CD8 (BD Biosciences), PE anti-CD117 (c-Kit) (BD Biosciences). Cells were permeabilized with Cytotix/Cytoperm (BD Biosciences) after surface staining but before CK5 staining because CK5 is an intracellular protein. Isotype Ab controls were performed for all of the Abs used and were added either pre- or post-cell permeabilization, depending on whether the Ag was intracellular or extracellular. We also used the purified rat anti-mouse C16/CD32 (FcγRII/II receptor) mAb (mouse BD Fc block; 0.5 μg/well) in all of our conditions to reduce nonspecific Ab binding to the FcγRII/II receptor. Fluorescent cell staining was analyzed with a bead multiplex system (CXCL12; Upstate Biotechnology) as described previously (14). Briefly, whole tissue extracts were made from tracheal transplants at 21 days posttransplant and incubated in complete protease inhibitor (Roche). Samples were incubated for 2 h at room temperature with anti-CXCL12 Abs in a 96-well plate. Biotinylated anti-CXCL12 Abs were added to each well, for 1.5 h, and the plate was incubated for 20 min at room temperature. Streptavidin-PE was added to the wells, and the plate was incubated for 30 min at room temperature. Next, 0.2% paraformaldehyde was added to the wells, and the plate was read on a Luminex100 IS instrument. The concentration of cytokine was

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determined from a standard curve assayed at the same time with known amounts of recombinant CXCL12.

Statistical analysis
Data were analyzed on a PC using the StatView 4.5 statistical package (Abacus Concepts). Comparisons were evaluated by the unpaired t test. Continuous data were expressed as mean ± SEM. Data were considered statistically significant if p values were ≤0.05.

Results
FACS analysis of CK5⁺ cells from the bone marrow and circulation
Our aim was to determine whether a population of CK5⁺ progenitor epithelial cells were detectable by FACS analysis in the bone marrow and circulation of mice. We first gated on the CK5⁺ cells and then determined the percentage of these cells that were CD45⁺ and CXCR4⁺. Under homeostatic conditions we found that 5 ± 0.5% of mononuclear cells isolated from the bone marrow expressed CK5, of which almost 100% were CD45⁺ (n = 5). Of these cells, 90 ± 3.5% were CXCR4⁺ (Fig. 1, i–iii). In the circulation, 8 ± 1.5% of mononuclear cells expressed CK5. Of the CK5⁺ cells, 76 ± 6% were also CD45⁺ (R2); of this group of CK5⁺ CD45⁺ cells (R2), 56 ± 3.9% were CXCR4⁺ (Fig. 1, iv–viii; n = 5). Isotype Ab controls were performed for CK5, CXCR4, and CD45 on bone marrow and buffy coat cells, and Fig. 1, iv and viii, are representative of all of the control Abs stained.

FACS analysis of bone marrow and buffy coat cells from CK5-GFP-transgenic mice that express GFP under control of the CK5 promoter confirmed our findings for CK5⁺ cells of the regenerating epithelium (Fig. 2). By day 21, male cells were present in the submucosal gland ducts along with the basal cells of the regenerating epithelium (Fig. 2A, iii and iv). By day 21

expressed Sca-1, and 31.7 ± 1.8% of CK5⁺ cells expressed c-kit. In the buffy coat, 28.2 ± 2.5% of CK5⁺ cells expressed CD34, 22 ± 1.7% of CK5⁺ cells expressed Sca-1, and 21.3 ± 2.9% of CK5⁺ cells expressed c-kit.

Male CK5⁺ circulating progenitor epithelial cells contribute to regeneration of female tracheal airway epithelium
To test the hypothesis that CK5⁺CXCR4⁺ circulating progenitor epithelial cells contribute to repair of the airway epithelium, female tracheas were transplanted into the flanks of syngeneic male mice, and male cells were identified by Y chromosome expression in the female trachea. This heterotopic tracheal transplant mouse model has been well characterized, especially as an allograft model system to study transplant rejection where syngeneic controls have demonstrated normal regeneration of the pseudostratified airway epithelium by day 21 posttransplantation (15, 16). The model is associated first with tracheal ischemia, followed by reperfusion from neovascularization posttransplantation. The airway injury is associated with complete sloughing of the epithelium from the basement membrane with gradual re-epithelialization starting by day 3 posttransplantation. The sloughed epithelium and recipient inflammatory cells form a luminal plug that organizes over time. Normal re-epithelialization following this injury is seen as early as day 7, and full regeneration of the pseudostratified columnar epithelium occurs by day 21 posttransplantation (10, 11, 15, 16). The syngeneic tracheal transplant is associated with minimal inflammatory changes after day 7 posttransplantation (10, 11, 15, 16).

Our aim was to determine the contribution of circulating cells to the repairing airway epithelium, we therefore performed both CISH and FISH in situ hybridization for the Y chromosome in sex-mismatched tracheal transplants. These two methods used biotin- and FITC-labeled DNA probes, respectively, for the murine Y chromosome. Male cells were detected in the luminal epithelial cells as well as the submucosal glands and ducts at day 3 posttransplant (Fig. 2A, i and ii). By day 7 posttransplant, male cells were present in the submucosal gland ducts along with the basal cells of the regenerating epithelium (Fig. 2A, iii and iv). By day 21

FIGURE 1. CK5⁺ cells are present in the bone marrow and buffy coat of mice. i, FACS analysis of bone marrow mononuclear cells revealed a population of cells (~85%) that were CD45⁺. ii, Dual immunostaining demonstrated a population of CK5⁺CD45⁺ cells (R1) (5 ± 0.5%; n = 5). iii, Gating on these cells (R1) demonstrated that 90 ± 3.5% of these cells expressed CXCR4 on their surface (n = 5). iv, Isotype Ab CK5 bone marrow control is representative of all the control Abs stained. v, FACS analysis of mouse circulating buffy coat cells showed a population of CD45⁺ cells. vi, 8 ± 1.5% of mononuclear cells from the buffy coat were CK5⁺, and 76 ± 6% of the CK5⁺ cells were also CD45⁺ (R2) (n = 5). vii, Gating on this population (R2) demonstrated 56 ± 3.9% of these cells to be CXCR4⁺ (n = 5). viii, Isotype Ab CK5 buffy coat control is representative of all the control Abs stained.
posttransplant, male cells were present in the submucosal gland ducts and the basal and apical epithelial cells (Fig. 2A, v). Morphometric analysis and quantitation of CISH-positive cells demonstrated 0% male cells in tracheal epithelia from female tracheal transplants in female mice on days 3, 7, and 21 posttransplantation. In comparison, morphometric analysis and quantitation of CISH-positive cells demonstrated 8.2 ± 2.6, 12.9 ± 3.8, and 18.4 ± 1.4% male cells in sex-mismatched tracheal transplants at days 3, 7, and 21 posttransplantation, respectively (p values of 0.001, 0.015, and 0.004, respectively; Table I).

**FIGURE 2.** A, CISH and FISH for the Y chromosome in female tracheas transplanted into male recipients. i, CISH, day 3 posttransplant with Y chromosome-positive cells seen on the luminal surface (brown dots; arrows). ii, FISH, day 3 posttransplant with Y chromosome-positive cells in the luminal surface, submucosal glands, and ducts (fluorescent green dots; arrows). iii, CISH, day 7 posttransplant with Y chromosome-positive cells contributing to the regenerating epithelium, submucosal glands, and ducts (arrows). iv, FISH, day 7 posttransplant with Y chromosome-positive cells contributing to the basal (white arrows), parabasal (yellow arrow), and apical cells (red arrow) of the regenerating epithelium, as well as submucosal cells (dashed white arrows). v, CISH, at day 21 posttransplant with chimerism for Y chromosome-positive and -negative cells in the submucosal glands, ducts, and pseudostratified epithelium (arrows). B, i, Male control trachea with Y chromosome-positive cells (arrows). ii, Female control nontransplanted trachea with no Y chromosome-positive cells. Scale bars, 10 μm. C, i–iv, Immunostaining and immunofluorescence of GFP in the tracheal epithelium. i, Wild-type female trachea transplanted into a wild-type female mouse demonstrated no immunostaining for GFP. ii, Homozygous GFP+ male trachea transplanted into a male GFP− mouse showed GFP+ immunostaining in all cells at day 21 posttransplant. iii and iv, DAB staining (arrows) and FITC staining (white arrows) at day 7 posttransplantation of a wild-type female trachea into a male GFP mouse and iv, v and vi, Immunostaining with DAB and FITC, respectively, at day 21 posttransplantation of a wild-type female trachea into a male GFP mouse and iv, demonstrated GFP+ cells (white arrows) contributing to regeneration of the epithelium. Scale bars, 10 μm. For quantitation, see Table I. D, i–vii, Immunohistochemistry for GFP in airway epithelium from wild-type and CK5-GFP mice at day 21 post-tracheal transplantation. i, GFP expression by DAB (arrows) in wild-type tracheas transplanted into CK5-GFP mice. ii, CK5-GFP tracheas transplanted into CK5-GFP mice demonstrate immunostaining in all and iv, the basal cells of the regenerating airway epithelium (arrows). iii, Wild-type tracheas transplanted into wild-type mice demonstrated no immunostaining and iv, for GFP. Scale bars, 10 μm.
The findings for CISH-positive male cells in sex-mismatched tracheal transplants were confirmed by FISH. Morphometric analysis and quantitation of FISH-positive cells demonstrated 0% male cells in female tracheal epithelia from sex-matched tracheal transplants. In comparison, morphometric analysis and quantitation of FISH-positive cells demonstrated 6.3 ± 1.8 and 12.3 ± 2.1% male cells in sex-mismatched tracheal transplants at day 3 and 7 posttransplantation, respectively (p values of 0.013 and 0.005, respectively; Table I).

To further confirm the validity of our morphometric analysis, we examined Y chromosome expression in male epithelium from sex-matched tracheal transplants. The Y chromosome was only detected in 42.5 ± 2.5% of control male tracheal cells by CISH and 53 ± 2.3% by FISH at day 21 posttransplantation (Fig. 2B, i, Table I, and data not shown). Other authors have also shown that not all male cells are positive for the Y chromosome by FISH, which may reflect the plane of sectioning of individual nuclei (17, 18). Control female tracheal sections from female mice did not demonstrate any hybridization with the Y-labeled probe (Fig. 2B, ii).

To further demonstrate that male circulating progenitor epithelial cells contribute to tracheal epithelial repair, we transplanted wild-type sex-mismatched tracheas into the flanks of homozygous GFP transgenic mice, thereby using GFP as a marker of circulating recipient cells. For controls, wild-type sex-mismatched tracheal transplants were transplanted into wild-type mice or male GFP transplanted into wild-type mice demonstrated GFP cells in the regenerating basal cells of the airway (Fig. 2D, i). C5K-GFP tracheas transplanted into C5K-GFP mice demonstrated GFP expression in all basal cells (Fig. 2D, ii), and wild-type tracheas transplanted into wild-type mice did not demonstrate any GFP expression (Fig. 2D, iii).

To further confirm that the GFP-labeled cells in the regenerating female tracheal epithelium were truly epithelial cells, dual immunofluorescence for mouse CK5 and GFP was performed on tracheal sections from female wild-type tracheas transplanted into male GFP mice (Fig. 3). In female wild-type tracheas transplanted into sex-matched wild-type mice, we determined there was no evidence for GFP cells (Fig. 3, iii). The isotype Ab control for CK5 demonstrated no staining for CK5 (Fig. 3, i). Dual CK5 and GFP expression was seen in the basal cells of the GFP male mouse tracheas transplanted into sex-matched GFP mice by yellow fluorescence, as demonstrated by colocalization of GFP-FITC and C5K-Cy3 (Fig. 3, ii). As another control, CK5 expression was seen in the basal cells of female wild-type tracheal epithelium transplanted into sex-matched wild-type mice with Cy3 staining of CK5 and no GFP-FITC staining (Fig. 3, iii). In wild-type female tracheas transplanted into male GFP mice, CK5 basal cells colocalized with circulation-derived GFP cells at both day 7 (9.2 ± 1.2%; p = 0.003; Fig. 3, iv, and Table I) and day 21 (18.6 ± 5.1%; p = 0.012; Fig. 3, v, and Table I) posttransplant. Cells that were only positive for GFP represented other circulating cell populations that had trafficked to the transplant (Fig. 3, v, green arrow) as well as apical cells of the regenerating airway epithelium (Fig. 3, v, green arrows).

CXCCL2 induces early mobilization and later enrichment of the bone marrow with CK5 progenitor cells

We hypothesized that CXCCL2 may mobilize the CK5 progenitor cells and enrich the bone marrow with CK5 progenitor cells. We therefore performed s.c. injection of mice with CXCCL2 (100 μg), as compared with control vehicle. Six groups of mice (n = 5) were treated in the following manner. Groups 1 and 2 were treated with CXCCL2 or control vehicle, respectively, at time 0 and sacrificed in 1 h for analysis of the number of CK5 cells in both the buffy coat and bone marrow. Groups 3 and 4 were treated with CXCCL2 or control vehicle, respectively, at time 0 and received a second dose of CXCCL2 or control vehicle, respectively, at 24 h followed by sacrifice in 1 h for analysis of the number of CK5 cells in both the buffy coat and bone marrow. Groups 5 and 6 were treated with CXCCL2 or control vehicle, respectively, at time 0, received a second dose of CXCCL2 or control vehicle, respectively, at 24 h, and received a third dose of CXCCL2 or control vehicle, respectively, at 48 h followed by sacrifice in 1 h after the last dose for analysis of the number of CK5 cells in both the buffy coat and bone marrow using a method described previously (12). For group 1, we found 3.2 × 104 ± 4.4 × 104 CK5 cells in the buffy coat and 9 × 105 ± 1.8 × 105 CK5 cells in the bone marrow, as compared with 5.39 × 104 ± 4.37 × 105 CK5 cells in the buffy coat and 4.7 × 105 ± 9.4 × 105 CK5 cells in the bone marrow from group 2. This represented a 6-fold increase in the mobilization of CK5 cells in the circulation and a 2-fold increase in the enrichment of the bone marrow CK5 cells in animals treated with CXCCL2. For group 3, we found 2.7 × 104 ± 5.4 × 104 CK5 cells in the buffy coat and 2.2 × 106 ± 1.8 × 106 CK5 cells in the bone marrow as compared with 6.0 × 104 ± 1.2 × 105 CK5 cells in the buffy coat and 5.6 × 105 ± 1.1 × 105 CK5 cells in the bone marrow from group 4. This represented a 4.5-fold increase in mobilization of CK5 cells in the circulation.

Table I. Quantitation of circulating progenitor cell chimerism

<table>
<thead>
<tr>
<th>Positive Cells/Total Cells Counted</th>
<th>Positive Cells (%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CISH Y+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>8/97</td>
<td>8.2 ± 2.6</td>
</tr>
<tr>
<td>Day 7</td>
<td>12/93</td>
<td>12.9 ± 3.8</td>
</tr>
<tr>
<td>Male</td>
<td>35/189</td>
<td>18.4 ± 1.4</td>
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<tr>
<td>Male</td>
<td>65/200</td>
<td>42.5 ± 2.5</td>
</tr>
<tr>
<td>Female</td>
<td>0/200</td>
<td>0</td>
</tr>
<tr>
<td>FISH Y+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>11/163</td>
<td>6.3 ± 1.8</td>
</tr>
<tr>
<td>Day 7</td>
<td>21/170</td>
<td>12.3 ± 2.1</td>
</tr>
<tr>
<td>Male</td>
<td>88/165</td>
<td>53 ± 2.3</td>
</tr>
<tr>
<td>Female</td>
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<tr>
<td>GFP +</td>
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<td>Day 7</td>
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<td>20 ± 3.8</td>
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<tr>
<td>Wild-type CK5/GFP +</td>
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</tr>
<tr>
<td>Day 7</td>
<td>12/131</td>
<td>9.2 ± 1.2</td>
</tr>
<tr>
<td>Day 21</td>
<td>55/296</td>
<td>18.6 ± 5.1</td>
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and a 3.9-fold increase in enrichment of the bone marrow CK5+ cells in animals treated with CXCL12. For group 5, we found $2 \times 10^5 \pm 4.1 \times 10^3$ CK5+ cells in the buffy coat and $2.8 \times 10^8 \pm 5.5 \times 10^5$ CK5+ cells in the bone marrow as compared with $4.4 \times 10^4 \pm 8.7 \times 10^3$ CK5+ cells in the buffy coat and $5.3 \times 10^5 \pm 1.1 \times 10^5$ CK5+ cells in the bone marrow from group 6. This represented a 4.6-fold increase in mobilization of CK5+ cells in the circulation and a 5.2-fold increase in enrichment of the bone marrow CK5+ cells in animals treated with CXCL12 (Fig. 4).

**Temporal and spatial expression of CXCL12 during regeneration of airway epithelium**

Because the CXCR4/CXCL12 biological axis has been found to be essential for adult stem cell homing and specific subpopulations of leukocyte recruitment (12, 19–21), we postulated that the CXCR4/CXCL12 axis could play a role in the recruitment of circulating progenitor epithelial cells. The justification for our hypothesis was that CXCR4 expression has been detected on other epithelial

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**FIGURE 3.** Colocalization of circulating male GFP+ cells with CK5 by dual immunofluorescence with primary Ab to CK5 labeled with Cy3 and GFP labeled with FITC in a tracheal epithelium. i, Isotype Ab control demonstrated background Cy3 staining of the basement membrane but no staining of cells. ii, Control nontransplanted homozygous male GFP+ trachea with colocalization of CK5-Cy3 and GFP-FITC resulting in yellow staining of the basal cells (arrows). iii, Control nontransplanted wild-type female trachea with red CK5-Cy3 staining of basal cells (arrows). iv, Wild-type female trachea day 7 posttransplant into a male GFP+ mouse with colocalization of CK5-Cy3 and GFP-FITC (yellow staining) in basal cells (white arrows) and CK5-Cy3 staining only in other cells (red staining, dashed white arrows). v, Day 21 posttransplant with chimerism of basal cells with staining for CK5-Cy3 and GFP-FITC (yellow staining, white arrows) and other cells expressing only CK5 (red staining, dashed white arrows). GFP-FITC staining is seen in apical epithelial cells (FITC staining, green arrows), illustrating chimerism in the regenerating epithelium. Scale bars, 10 μm. See Table I for quantitation.

**FIGURE 4.** CXCL12 mobilization and enrichment of bone marrow. A, Time course of mobilization in response to multiple s.c. injections of 100 μg of CXCL12 (groups 1, 3, and 5) or control vehicle (groups 2, 4, and 6) in C57BL/6 mice given on day 1, days 1 and 2, or days 1, 2, and 3 given 24 h apart and analyzed 1 h after the last injection (five mice per group). Significant mobilization of CK5+ cells is seen at all time points measured in response to CXCL12, although the most mobilization was seen at 1 h post the initial dose of CXCL12 (group 1). *, p < 0.01; **, p < 0.001. B, Time course of expansion of CK5+ cells in the bone marrow in response to multiple s.c. injections of 100 μg of CXCL12 (groups 1, 3, and 5) or control vehicle (groups 2, 4, and 6) in C57BL/6 mice given on day 1, days 1 and 2, or days 1, 2, and 3 given 24 h apart and analyzed 1 h after the last injection. (five mice per group). Significant expansion of CK5+ cells in the bone marrow is seen at all time points measured in response to CXCL12, although the greatest expansion was seen in the group of mice that received three doses of CXCL12 (group 5). *, p < 0.01; **, p < 0.001.
progenitor cell types, such as the crypts of intestinal epithelium (19), and that CXCL12 expression occurs after hypoxic injury, which appears to create a chemotactic gradient in ischemic tissue for the migration of CXCR4+ cells (19, 21). Thus, CXCR4+ circulating progenitor epithelial cells would be expected to home to the regenerating epithelium after tracheal transplantation.

Our aim was to demonstrate that the CXCR4/CXCL12 biological axis is important in the trafficking of these circulating progenitor epithelial cells. Therefore, we first compared CXCL12 mRNA expression at 3 and 7 days posttransplant by quantitative real-time PCR and found CXCL12 to be markedly increased over uninjured tracheal levels (20-fold at day 3 (p = 0.01) and 50-fold at day 7 (p < 0.001) posttransplant, respectively) (Fig. 5A). Next, we assessed the kinetic expression of CXCL12 protein in sex-mismatched tracheal transplants as compared with naive tracheas. CXCL12 protein was measured from tracheal tissue homogenates using the Luminex assay. We found that CXCL12 protein levels were the highest at day 3 and were persistently elevated from day 3 out to day 21 posttransplantation (Fig. 5B). We next performed immunohistochemistry of CXCL12 in tracheal transplant tissue to examine its temporal and spatial expression. CXCL12 was expressed in the lumen of the submucosal glands and ducts at day 3 posttransplant (Fig. 5C, i and ii). The CXCL12 staining was present in the mucus glands within the mucus vacuoles, consistent with the ability of CXCL12 to bind to glycosaminoglycans present in mucus. When CXCL12 is bound to glycosaminoglycans, its biological half-life is long-lived and is cleared by binding to the CXCR4 receptor (19). By day 7 posttransplant the expression of CXCL12 changed spatially from the submucosal glands and duct epithelium to the basal cells (Fig. 5C, iii). By day 21 posttransplant, CXCL12 expression spatially moved to the apical surface of the pseudostratified columnar epithelium (Fig. 5C, iv). The spatial expression of CXCL12 in the pseudostratified epithelium at day 21 posttransplant was similar to its location seen in uninjured tracheal epithelium. Morphometric analysis of epithelium from proximal to distal tracheal sections demonstrated similar patterns of CXCL12 expression for all of the tracheal transplantations at each time point. The temporal and spatial change in the pattern of expression of CXCL12 directly correlated with that of the spatial orientation of progenitor epithelial cells as they formed a pseudostratified epithelium.

**CXCR4/CXCL12 biological axis mediates recruitment of circulating progenitor epithelial cells and regeneration of pseudostratified airway epithelium**

Because the temporal and spatial expression of CXCL12 was coincident with the regeneration of sex-mismatched chimeric pseudostratified epithelium in female tracheas transplanted into male mice, we hypothesized that the CXCR4/CXCL12 biological axis is important for the recruitment of circulating progenitor epithelial cells. Therefore, our aim was to determine whether the CXCR4/CXCL12 biological axis is important in recruiting the circulating progenitor epithelial cells. We thus performed passive immunization experiments with either neutralizing anti-CXCL12 or control F(Ab’)_2 Abs in male GFP+ mice that had received a female wild-type trachea by using a modification as described previously (13). Based on the fact that complete regeneration of pseudostratified epithelium was noted by day 21 post-tracheal transplantation, we used this time point to assess the effect of neutralizing Abs on circulating progenitor epithelial cells during the regeneration of the tracheal epithelium. Regeneration of normal pseudostratified columnar epithelium was seen consistently throughout the tracheal transplants from mice treated with control Ab (Fig. 6, i and iii). Whereas re-epithelialization of the tracheal epithelium was seen in

**FIGURE 5.** CXCL12 is temporally and spatially expressed in the airway during re-epithelialization of the pseudostratified epithelium. A. Quantitative real-time PCR of CXCL12 mRNA expression. CXCL12 expression was increased in the tracheal transplants >20-fold above that of uninjured control trachea at day 3 posttransplant (p = 0.01) (n = 3). CXCL12 mRNA increased further in the tracheal transplants, >50-fold above uninjured trachea at day 7 posttransplant (p < 0.001) (n = 3). B. Kinetic analysis of CXCL12 protein levels of sex-mismatched tracheal transplants as compared with naive control tracheas. CXCL12 protein was measured from tracheal tissue homogenates using the Luminex assay. C. Immunohistochemistry of CXCL12 in tracheal transplants. i and ii, CXCL12 is expressed in the mucus vacuoles in the lumen of the submucosal glands and ducts of the trachea at day 3 posttransplant (arrows). iii, CXCL12 expression changed spatially to the submucosal gland cells, ducts, and basal cells at day 7 posttransplant. iv, In tracheas at day 21 posttransplant, CXCL12 expression was always present on the apical surface of the pseudostratified columnar epithelium (arrow) and was similar to that in naive tracheas (not shown). Scale bars, 10 μm.
the anti-CXCL12-treated group at day 21, the phenotype of the epithelium was squamous metaplasia (Fig. 6, ii and iv). Morphometric analysis of tracheal transplant sections of the entire transplant showed squamous metaplasia throughout the epithelium in the anti-CXCL12-treated group. The mean percentage of squamous metaplasia of the tracheal circumference was 71.2 ± 4.1% (p < 0.0001). To assess the epithelium for GFP male cells, we assessed both immunohistochemistry and immunofluorescence of GFP cells. The areas of squamous metaplasia of sex-mismatched wild-type tracheas transplanted into GFP male mice had re-establishment of the epithelium derived solely from female resident progenitor epithelial cells, with no contribution of male GFP circulating progenitor epithelial cells by immunohistochemistry and immunofluorescence for GFP cells, respectively (Fig. 7, i and ii). In contrast, the regeneration of normal pseudostratified epithelium in sex-mismatched tracheas transplanted into GFP male mice treated with control Ab demonstrated the presence of both resident (female) and circulating GFP male progenitor epithelial cells (Fig. 7, iii). FACS analysis showed a decrease in the CK5+CXCR4 population of cells in the anti-CXCL12-treated mouse tracheal transplants, with the absolute CK5+CXCR4 cell numbers decreasing from $11 \times 10^5 \pm 2.3 \times 10^4$ to $4 \times 10^5 \pm 0.9 \times 10^5$, respectively (p = 0.03; Fig. 8). FACS analysis of leukocyte subpopulations showed no significant difference in the numbers of polymorphonuclear cells, lymphocyte populations, and NK cells in the tracheas from either the control or anti-CXCL12 Ab-treated groups at day 21 posttransplantation. However, there was a statistical decrease in the number of macrophages in the anti-CXCL12-treated tracheal transplants (p = 0.014; Fig. 9).

p63 is a member of the p53 family of proteins and is a recognized marker of the reserve cell hyperplasia reflective of the basal regenerative cells of many epithelial tissues as well as squamous

FIGURE 6. Depletion of CXCL12 inhibits the recruitment of CXCR4+ circulating progenitor epithelial cells and leads to squamous metaplasia of the regenerating airway epithelium in response to injury, as seen by H&E staining. i and iii, Representative example of regeneration of an airway pseudostratified columnar epithelium day 21 posttransplant in mice treated with control Ab (magnifications, ×200 and ×400 for i and iii, respectively). ii and iv, Representative example of squamous metaplasia of the regenerated airway epithelium at day 21 posttransplant in animals treated with neutralizing Abs to CXCL12 (magnifications, ×200 and ×400 for ii and iv, respectively). Scale bars, 10 μm.

FIGURE 7. Squamous metaplasia of transplant airway epithelium is derived solely from female resident tissue progenitor epithelial cells at day 21 in mice treated with neutralizing anti-CXCL12 Abs. i, Lack of peroxidase staining for GFP cells in areas of squamous metaplasia, with a recipient cell staining for GFP in the submucosal area (arrow) in female tracheal transplants from GFP male mice treated with anti-CXCL12 Abs. ii, Lack of GFP cell immunofluorescence in areas of squamous metaplasia, with a few recipient cells staining for GFP in the submucosal area (arrow) in female tracheal transplants from GFP male mice treated with anti-CXCL12 Abs. iii, Immunofluorescence for GFP cells in female tracheal transplants from GFP male mice treated with control Abs. The trachea demonstrates chimerism for GFP+ and GFP− cells in the regenerated pseudostratified epithelium. Scale bars, 10 μm.
metaplasia (22). Therefore, our aim was to determine whether the areas of squamous metaplasia expressed p63. We thus examined p63 expression by immunofluorescence in the tracheal transplants. In mice treated with neutralizing anti-CXCL12, p63 expression was not only found in the basal nuclei but in nuclei throughout the areas of squamous metaplasia of the regenerated epithelium at day 21 posttransplant, compatible with reserve cell hyperplasia of female resident basal cells (Fig. 10, i). In contrast, mice treated with control Abs only demonstrated p63 expression in the nuclei of the basal cells at day 21 posttransplant (Fig. 10, ii). An isotype control Ab showed no immunofluorescence (Fig. 10, iii).

Discussion

Our findings demonstrate that there is a population of progenitor epithelial cells in the bone marrow that is CK5<sup>+</sup>CD45<sup>−</sup>CXCR4<sup>+</sup>. Other groups have shown the presence of mature cytokeratins such as pancytokeratin and CK19-expressing populations in the bone marrow (23, 24). However, to our knowledge this is the first report of CK5<sup>+</sup> progenitor epithelial cells present in the bone marrow. This population of cells was also identified in the circulation under homeostatic conditions. Moreover, these cells can be found recruited to the airway epithelium in response to injury, implying that the cells may be mobilized into the circulation in response to tissue injury and repair. This finding is supportive of the hypothesis that repair and regeneration of the airway epithelium may be enhanced by circulating progenitor epithelial cells derived from the bone marrow. Our FACS analysis studies identified a higher percentage of CK5<sup>+</sup> cells in the bone marrow and circulation of mice than we would have predicted under homeostatic conditions. This may be a phenomenon unique to mice, because we have detected a much smaller percentage of CK5<sup>+</sup> cells in the buffy coat of humans (0.5–1%) under homeostatic conditions. We propose that the circulating progenitor epithelial cells may represent a precursor population of cells for a number of epithelial tissues that would include the airway, lung parenchyma, skin, and prostate, with CK5 basal cells representing an important component of the regenerating epithelium of these tissues. The possibility exists in the future that culturing these cells in vitro will allow us to further characterize these CK5<sup>+</sup> progenitor cells. Furthermore, we cannot exclude the possibility that the higher number of CK5<sup>+</sup> cells in circulation of mice, as compared with humans, may represent turnover of fur.

Our explanation for the detection of more progenitor epithelial cells in the circulation than in the bone marrow may be related to the fact that these cells are most likely in a state of transition as they mobilize and traffic with gain of CK5 expression. In addition, a previous study by Phillips et al. (13) demonstrated with another circulating progenitor cell population, namely fibrocytes, that they gain the more differentiated marker of α-smooth muscle actin expression as they traffic to a bleomycin-injured lung.

We have demonstrated that in the bone marrow the CD45<sup>−</sup>CK5<sup>+</sup> cells are uniformly positive for CXCR4, but in the circulation approximately half of the cells lose CXCR4. CXCR4 is down-regulated after transition from bone marrow to the peripheral blood because of the change in oxygen tension and protein degradation. In addition, a previous study by Phillips et al. (13) demonstrated that differentiation of a fibrocyte to an α-smooth muscle actin-positive cell was associated with reduced expression of CXCR4 under normoxic conditions. Therefore, we hypothesize that maturation of a progenitor cell may contribute to the loss of CXCR4 expression. In the tracheal transplants the number of CK5<sup>+</sup>CXCR4<sup>+</sup> cells may be underestimated, because CXCR4 positivity would be expected to decrease as the cells bind to their CXCL12 ligand and the chemokine receptor is internalized and degraded.

The CK5<sup>+</sup> cells in the bone marrow and circulation express CD45 and CXCR4. Whereas CXCR4 is expressed by neutrophils, CXCR4 is the salient chemokine receptor found on several leukocytes and progenitor cells, and, therefore, these progenitor cells may use CXCR4 to traffic into tissue. CXCR4 is included in membrane lipid rafts to more efficiently interact with other surface receptors such as downstream proteins of signal transduction pathways and, therefore, to induce chemotaxis. The receptor-like protein tyrosine phosphatase CD45, the common leukocyte Ag, can also be found in lipid rafts (25) and, therefore, may be important in facilitating the interaction of CXCL12 with the CXCR4 receptor, allowing membrane-proximal signaling events to proceed. Therefore, the expression of CD45 on progenitor cells that traffic via the CXCL12/CXCR4 pathway is not unexpected.

Bone marrow mesenchymal cells have been shown to form pulmonary epithelial cells of both proximal and distal airways (9, 26, 27). These studies used markers of bone marrow-derived cells such...
During lung transplantation the airway is exposed to significant epithelial injury and sloughing of the airway epithelium. The ischemia-reperfusion injury results in complete sloughing of the epithelial basement membrane, which may explain why we saw a relatively high percentage of circulation-derived progenitor epithelial cells in the epithelial repair process. This type of injury may appear severe; however, the injury is clinically relevant to lung transplantation. During lung transplantation the airway is exposed to significant ischemia and reperfusion injury, because the bronchial circulation is usually not reanastomosed at the time of reimplantation. In addition, other forms of airway injury such as severe viral infections related to respiratory syncytial virus or influenza may result in significant epithelial injury and sloughing of the airway epithelium and, therefore, may be relevant to our mouse model.

Using markers for both the Y chromosome and GFP+ recipient cells, we saw similar numbers of recipient circulating progenitor epithelial cells that contributed to regeneration of the normal airway pseudostratified epithelium. These cells were also found to contribute to the resident progenitor epithelial cells seen in the submucosal gland ducts. The recipient circulating progenitor epithelial cells were present and increased in number at day 21 post-transplantation. There is controversy as to whether cell fusion of circulating progenitor epithelial cells occurs with local resident progenitor cells during regeneration of the airway epithelium (29, 30). However, we failed to identify any binucleate cells in histologic sections of the chimeric tracheas and found only one Y chromosome per cell by either FISH or CISH techniques. These findings support the notion that stable cell fusion did not occur, but we acknowledge that our experiments were not designed to detect reductive division of fused cells.

Wang et al. (31) recently demonstrated the ability of bone marrow cells to develop a pulmonary epithelial cell phenotype when cocultured with pulmonary epithelial cells. These cells were amenable to retroviral transduction and gene therapy (31). Grover et al. (32) found similar results with bone marrow-derived mesenchymal stromal cells in vivo. The findings from these two studies have implications for therapy using bone marrow-derived epithelial cells in lung diseases such as cystic fibrosis. Our data suggests that isolation of CK5+ progenitor epithelial cells from the bone marrow and/or circulation could be useful for identifying cells ideal for ex vivo manipulation before autologous transplantation.

Our study demonstrated a mechanism for recipient circulating progenitor epithelial cell trafficking to the injured airway through the CXCL12/CXCR4 biological axis. This biological axis has been found to be critical for the regulation of trafficking of other adult stem cells in response to injury and repair of tissues such as the liver bile duct epithelium (33). We found significant and early mobilization of CK5+ cells in the circulation of mice treated with CXCL12, whereas enrichment of CK5+ cells in the bone marrow appeared to occur later. However, we cannot fully exclude the possibility that these circulating progenitor epithelial cells may be derived from a source other than the bone marrow and could just be “hiding out” in the bone marrow as other authors have suggested (23, 34). Other tissue sources are possible, including the possibility of peripheral differentiation of leukocyte subpopulations. However, given the protected environment of the bone marrow and its high levels of CXCL12, it is feasible that the bone marrow could be the home of both hemopoietic and nonhemopoietic progenitor cells.

The presence of CXCR4 on undifferentiated epithelial cells in tissues such as pulmonary and intestinal epithelia suggests a wider role for CXCL12/CXCR4 in the regulation of cell homing (13, 19, 20). In addition, the CXCL12/CXCR4 biological axis is critical in the normal development of several organs, suggesting that this biological axis may play a role in the migration of most adult progenitor cells (19). We demonstrated a temporal and spatial increase in the expression of CXCL12 in the epithelium after airway injury related to tracheal transplantation, which provided a gradient for infiltration of CXCR4+ recipient circulating progenitor epithelial cells (21). The presence of CXCR4 on CK5+ bone marrow- and circulation-derived progenitor epithelial cells further supports the role of this receptor in the recruitment of circulating progenitor epithelial cells. In addition, the temporal/spatial change of CXCL12 expression during the regeneration of the airway epithelium mimicked the temporal and spatial recruitment and orientation of progenitor epithelial cells during re-epithelialization of the basement membrane.
The depletion of CXCL12 with specific neutralizing Abs was associated with the loss of the normal pseudostratified epithelium and a change of the phenotype of the epithelium to squamous metaplasia at day 21 posttransplant. Moreover, the areas of squamous metaplasia were completely devoid of sex-mismatched cells, suggesting that recruitment of recipient CK5+/CXCR4+ circulating progenitor epithelial cells is both necessary and sufficient to re-establish the normal airway pseudostratified columnar epithelium. The areas of the tracheal transplant epithelium that had undergone squamous metaplasia under conditions of CXCL12 depletion represented 71.2 ± 4.1% of the total epithelium by morphometric analysis, which suggests that we were not able to completely block all circulating CK5+/CXCR4+ cells from contributing to repair of the epithelium. This is compatible with our FACS data showing a significant decrease in the numbers of CK5+/CXCR4+ cells in the tracheal transplants after CXCL12 depletion but not complete abrogation.

Although we cannot fully exclude the possibility of an indirect effect related to depletion of CXCL12 leading to squamous metaplasia, we would however argue in favor of a direct effect as follows: 1) CK5+ cells in circulation express CXCR4; 2) CK5+ cells from the recipient (Y chromosome-positive, GFP+, and CK5-GFP+) are present in the regenerating epithelium of the tracheal transplant; 3) CXCL12 and CXCR4 are both temporally and spatially present during the regeneration of the airway epithelium in the tracheal transplant; 4) depletion of CXCL12 leads to squamous metaplasia with loss of recruited recipient CK5+ cells and, at the same time, metaplasia of resident epithelial progenitor cells undergoing reserve cell hyperplasia with p63; and 5) depletion of CXCL12 is directly related to a marked reduction in dual CK5+/CXCR4+ cells in the regenerating airway epithelium of the tracheal transplant.

Squamous metaplasia results from reserve cell hyperplasia, and continuous exposure to environmental stimuli subjects these cells to epigenetic events and may predispose them to oncogenic transformation. The changes of squamous metaplasia in our model are analogous to those seen in the airways of patients with chronic bronchiitis and may invoke the notion that repetitive airway injury in these patients may be associated with inadequate recruitment of circulating progenitor epithelial cells that could contribute to re-establishment of the normal pseudostratified epithelium. In support of this concept, patients with congenital bone marrow failure syndromes have been found to be at increased risk for the development of squamous carcinomas (35). Although the etiology for the epithelial cancers seen in these patients is presumed to be related to a defect in DNA repair, it is plausible that these same patients may lack bone marrow-derived progenitor epithelial cells. As a consequence, these patients may have abnormal epithelial repair in areas with high cellular turnover that may predispose the resident cells to epigenetic events (35).

Houghton et al. (36) recently described a model of chronic gastric epithelial injury leading to gastric cancer. In this study, bone marrow-derived cells repopulated the injured gastric mucosa and developed metaplasia, dysplasia, and cancer over an extended period of time. In contrast to that study, we found the opposite findings, where inhibiting the recruitment of recipient circulating progenitor epithelial cells by blocking CXCL12 resulted in squamous metaplasia, which was derived exclusively from resident female donor progenitor epithelial cells. It is possible that various tissues respond differently to injury, and in gastric mucosa, where very few bone marrow-derived cells contribute to the regenerating epithelium, the signals for normal differentiation may be entirely different than those in the trachea. It is not known whether persistent inhibition of the trafficking of circulating progenitor epithelial cells in the airway in the setting of chronic injury results in dysplasia and subsequent carcinoma in situ. This possibility is currently being investigated in our laboratory.

The ΔNp63α splice variant of p63 is the predominant form of p63 in basal epithelial cells. p63 functions as a dominant-negative inhibitor of the tumor suppressor gene p53. p63 counteracts the apoptotic and cell cycle inhibitory role of p53 and contributes to cell renewal of the pseudostratified epithelium. p63 has been shown to be highly expressed in areas of abnormal cellular proliferation characterized by squamous metaplasia as well as squamous cell carcinomas and, therefore, may play an oncogenic role in epithelial transformation. Whether p63 plays a causative role or is merely temporally and spatially expressed during tumor development remains to be fully elucidated (22). Our finding of p63 expression in all cells associated with squamous metaplasia in mice treated with an anti-CXCL12 Ab suggests that the abnormal proliferation of donor resident progenitor epithelial cells in the absence of recipient circulating progenitor epithelial cells may be due to p63 inhibition of p53. Future studies will determine whether the persistence of p63 expression will lead to oncogenic transformation of the squamous metaplastic epithelium in the absence of the trafficking of circulating epithelial progenitor cells.

Our studies demonstrate the following sequence of events: 1) a population of oriented progenitor cells expressing the epithelial marker CK5 is harvested in the bone marrow; 2) these cells passing into the circulation provide a cellular pool able to repair damaged tracheal epithelium; 3) the CXCL12/CXCR4 axis is involved in epithelial precursor mobilization and recruitment at sites of injury; 4) CK5+/CXCR4+ cells have a crucial role in the re-epithelialization of tracheal transplants; and 5) CXCL12 blocking prevents precursor recruitment and appropriate epithelial repair and favors squamous metaplasia. This sequence has major implications for the therapeutic use of circulating progenitor epithelial cells in a number of respiratory diseases where normal airway repair and regeneration is required, such as infections like respiratory syncytial virus, severe acute respiratory syndrome, influenza, and inflammatory conditions such as cystic fibrosis, asthma, and chronic obstructive pulmonary disease. Furthermore, our studies demonstrate that, if circulating progenitor epithelial cell recruitment is impaired, the airway epithelium develops a squamous metaplastic phenotype, creating a novel paradigm for the development of squamous metaplasia consisting of only resident epithelial progenitor cells.

Disclosures
The authors have no financial conflict of interest.

References


