Assessment of cystic fibrosis transmembrane conductance regulator (CFTR) activity in CFTR-null mice after bone marrow transplantation

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Several studies have demonstrated that bone marrow (BM)-derived cells give rise to rare epithelial cells in the gastrointestinal (GI) and respiratory tracts after BM transplantation into myeloablated recipients. We investigate whether, after transplantation of cystic fibrosis transmembrane conductance regulator (CFTR)-positive BM-derived cells, BM-derived GI and airway epithelial cells can provide CFTR activity in the GI tract and nasal epithelium of recipient cystic fibrosis mice. CFTR/−/− mice were transplanted with wild-type BM after receiving different doses of irradiation, and CFTR activity was assessed in vivo in individual mice over time by using rectal and nasal potential difference analyses and in vitro by Ussing chamber analysis. The data suggest that rare BM-derived epithelial cells in the GI and nasal epithelium detected in CFTR/−/− transplanted mice provide a modest level of CFTR-dependent chloride secretion. Detection of CFTR mRNA and protein in tissues of transplanted CFTR/−/− mice supports these data.

bone marrow stem cells | chloride channel | epithelial cells

Cystic fibrosis (CF) is a recessive genetic disease characterized by loss of CF transmembrane conductance regulator (CFTR)-dependent Cl− transport on the apical membrane of epithelial cells (1). Due to the high incidence and severity of CF, there is great motivation to develop strategies, such as gene therapy and pharmacological approaches, for restoring the defective gene product. Significant obstacles have limited the effectiveness of these approaches. The use of bone marrow (BM)-derived cells (BMDCs) as a potential strategy to replace nonfunctional epithelial cells is an exciting direction of research. CF would be a good candidate for this approach because (i) CF is a multiorgan disease characterized by epithelial dysfunction, (ii) replacement of just 10% of epithelial cells could be sufficient to correct the phenotype (2), and (iii) cell therapy may be combined with gene therapy, as suggested by data demonstrating that BMDCs can be stably transduced in vitro and retain their ability to differentiate into lung epithelium while maintaining long-term transgene expression (3). Furthermore, BMDCs can differentiate into epithelial cells in vitro and express functional CFTR (4).

BMDCs give rise to various nonhematopoietic cells and, in particular, to rare epithelial cells of the gastrointestinal (GI) and respiratory tracts. Although several papers strongly support the notion that after BM transplantation epithelial cells can develop from BMDC in mice as well as in humans, there is much controversy regarding the percentage of marrow-derived epithelial cells, the subpopulation of BM cells that is responsible, the mechanism(s) underlying BMDC plasticity, and whether or not BM-derived epithelial cells perform the normal functions of epithelial cells (5).

The goal of the work presented here was to assess whether BM-derived epithelial cells, even if rare, are functional. Specifically, we test whether these cells can partially restore CFTR-dependent Cl− secretion after transplantation into CFTR/−/− mice. Although the lungs are the most affected organs in CF patients, CFTR/−/− mice have limited lung pathology, perhaps because of redundant Cl− channels in the lung (6) but have severe GI disease. Therefore, our studies focused primarily on functional CFTR in the gut. CFTR/−/− mice were myeloablated by irradiation and transplanted with CFTR/+; GFP + BM cells, and CFTR activity was assessed in individual mice over time by using rectal and nasal potential difference. CFTR activity in the GI tract was also measured in vitro by Ussing chamber analysis, the gold standard for assessing CFTR activity.

Results

Before testing whether BM transplantation could restore CFTR-mediated Cl− secretion in CF-affected epithelia, we first determined the in vivo and in vitro ion transport properties of the GI and respiratory epithelia of CFTR+/- and CFTR−/− mice. Similar to humans with CF, CFTR−/− mice have ion transport abnormalities characterized by hyperabsorption of Na+ and lack of cAMP-dependent Cl− secretion by both GI and respiratory epithelia (7, 8). Average values of rectal potential difference (RPD), nasal potential difference (NPD), and Ussing chamber analysis in CFTR+/- and CFTR−/− mice are summarized in Table 1. For a detailed description and evaluation of the CFTR activity as assessed by forskolin (FSK)-induced change in potential difference (ΔRPD and ΔNPD) and short circuit current (Isc)/cm2 (Ussing chamber), see Fig. 4 and Supporting Text, which are published as supporting information on the PNAS web site.

Six-week-old CFTR−/− mice were myeloablated with 700 (n = 3), 750 (n = 4), or 800 (n = 6) rads, transplanted with WT-GFP + BM cells, and analyzed by RPD at 6, 12, and 24 weeks post-BM transplantation. As controls to evaluate whether irradiation itself might induce electrophysiological changes, we transplanted five CFTR−/− mice with BM isolated from CFTR−/− mice (CFintoCF) irradiated with 750 rads (Table 1). All mice survived the procedure, suggesting that irradiation did not increase the risk of death in these mice. No change in RPD was detected between CFTR−/− and CFintoCF mice at any time posttransplantation (Fig. 1A). Although we did not observe a significant difference in RPD between the 700 rads WIntoCF group and either of the CFTR−/− or CFintoCF control groups at any time post-BM transplantation (6 and 12 weeks), the groups of CFTR−/− mice that received 750 and 800 rads showed a FSK-induced hyperpolarization of the tissue indicative of CFTR activity (Fig. 1A and Table 1). Six weeks post-BM transplantation, the ΔRPD of the 750-rads WIntoCF group was not significantly different from

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Abbreviations: BM, bone marrow; BMDC, BM-derived cells; CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; CK, cytokeratin; FSK, forskolin; GI, gastrointestinal; Isc, short circuit current; Iso, isoproterenol; PD, potential differences; NPD, nasal PD; RPD, rectal PD; Y, Y chromosome; G/C, Cl−-free bicarbonate Kreb’s solution.

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untransplanted CFTR−/− and CFiintoCF mice (P = 0.42). However, at later times, the ΔRPD was statistically significant (12 weeks, ΔRPD = 1.9 ± 1.0, P = 0.04; 24 weeks, ΔRPD = 1.5 ± 1.5, P = 0.03) (Fig. 24 and Table 1). In contrast, at all times the ΔRPD for the 800-rads WTiintoCF group was robust and significantly different (P < 0.05) from that of untransplanted CFTR−/− and CFiintoCF mice (Fig. 1A and Table 1). There was a modest decay in the response over time, which was not statistically significant (6 vs. 12 weeks, P = 0.19; 12 vs. 24 weeks, P = 0.46; 6 vs. 24 weeks, P = 0.21). Thus, CFTR−/− mice transplanted with higher doses of irradiation acquired CFTR activity in the GI tract. We also tested by RPD WTiintoCF+/− (n = 3) mice, which were irradiated with 800 rads. As expected, there was no difference in FSK response between this group and untransplanted CFTR+/− mice (data not shown). Fig. 1B shows in vivo RPD traces for representative CFTR+/− (Top), CFTR−/− (Middle) and WTiintoCF (Bottom) mice. The black-headed arrow points to the FSK-induced hyperpolarization indicative of CFTR activity in the WTiintoCF mouse. The distribution of ΔRPD for each experimental group at 6 weeks post-BM transplantation is shown in Fig. 1C. Transplanted mice that received 700 or 750 rads have a ΔΔ in a range similar to that of CFTR−/− and CFiintoCF mice (above the dashed line). In contrast, all mice in the 800-rads group and one mouse in the 700-rads group had a more negative ΔΔ.

To determine the kinetics of marrow-derived CFTR activity, we monitored CFTR activity in individual mice before and over time after BM transplantation. In the representative mouse shown in Fig. 1D, 1 week before transplantation the baseline RPD was ~0 mV, and there was a FSK-induced depolarization of approximately +0.5 mV. In contrast, 5, 8, and 24 weeks post-BM transplantation, the baseline PD was between +0.5 and ~1 mV, and there was a FSK-induced hyperpolarization of approximately ~3 mV, consistent with CFTR activity. These data suggest that the CFTR activity from BMDC is stable over time.

We also measured the in vivo NPD in WTiintoCF (750 and 800 rads) and CFiintoCF mice 24 weeks post-BM transplantation. The ΔNPD in response to Cl−-free bicarbonate Kreb’s solution (0Cl−) amiloride was not different between untransplanted CFTR−/− and CFiintoCF mice (1.4 ± 1.0 mV vs. 1.9 ± 0.9 mV, P = 0.25), and none of the transplanted mice had a detectable difference in the 0Cl− response (data not shown). However, in response to isoproterenol (Iso), there was a significant difference in ΔNPD between CFTR−/− and CFiintoCF mice. In CFTR−/−, Iso induced almost no change in NPD (0.0 ± 0.2 mV), and in CFiintoCF mice, Iso induced a modest depolarization of 1.7 ± 0.6 mV (P < 0.05), suggesting that radiation itself may induce a perturbation of ion transport in respiratory epithelia that is sensitive to Iso. In the 750- and 800-rads WTiintoCF groups, the ΔNPD in response to Iso was 0.08 ± 0.4 mV and 1.27 ± 0.7 mV, respectively (Fig. 1E and Table 1), which was not statistically significant compared with untransplanted CFTR−/− mice (750 rads, P = 0.3; 800 rads, P = 0.05). However, when compared with CFiintoCF, which represent a more appropriate control, the Iso-induced ΔNPD was significantly different in both groups of mice (750 rads, P = 0.03; 800 rads, P = 0.01). The NPD for WTiintoCF mice was 1.6 mV (750 rads) and 2.9 mV (800 rads) more negative than the NPD in CFiintoCF mice (1.7 ± 0.6 mV). Consistent with the in vivo RPD data, these data suggest that WT BMDC can engraft as nasal epithelial cells that express functional CFTR in CFTR−/− mice.

All mice were killed 24 weeks post-BM transplantation, except the group that was transplanted with 700 rads, which were killed 6 (n = 2) and 12 (n = 1) weeks post-BM transplantation. Engraftment in the BM assessed by FACS analysis for GFP+ cells, was between 34% and 100% at the time of death (Table 1). Because of the high dose of BM cells administered, no difference in hematopoietic engraftment occurred after different doses of irradiation. We examined the change in ΔΔc/ΔΔ by Ussing chamber analysis on the distal colon of all mice first in response to apical 0Cl− and then to FSK. The ΔΔc/ΔΔ in response to 0Cl− for CFiintoCF (37 ± 9.1 μA) and WTiintoCF (700 rads, 33.6 ± 4.3 μA; 750 rads, 47.2 ± 10.6 μA; and 800 rads, 41 ± 5.6 μA) was not significantly different from CFTR+/− and untransplanted CFTR−/− mice (39.5 ± 5.6 μA and 39.5 ± 5.6 μA, respectively) (data not shown). These data might suggest that, in contrast to respiratory epithelium, certain 0Cl−-dependent currents in CFTR−/− mice do not depend on CFTR in the GI tract.

After FSK stimulation, the CFiintoCF group had a ΔΔc of −16 ± 2.7 μA/cm², which was not different from untransplanted CFTR−/− mice (P = 0.32) (Fig. 2A). The FSK-generated ΔΔc in the distal colon of WTiintoCF mice from the 700-, 750-,
800-rads groups was $-3.7 \pm 5.0, -6.6 \pm 1.8$, and $-2.1 \pm 3.4 \mu A/cm^2$, respectively (Fig. 2A and Table 1), showing an increase in $I_{sc}/cm^2$ compared with untransplanted CFTR$^-/-$ mice. Compared with the CFintoCF mice, this change represents an increase of 12.3, 9.4, and 13.9 $\mu A/cm^2$, respectively. The difference in the FSK-dependent $\Delta I_{sc}/cm^2$ was statistically significant for the WTintoCF mice transplanted with 800 ($P = 0.006$), 750 ($P = 0.03$), and 700 rads ($P = 0.03$) compared with CFintoCF mice.

To further investigate whether the FSK-dependent $\Delta I_{sc}$ in transplanted mice was truly because of CFTR, we used a thiazolidinedione type CFTR inhibitor (CFTRinh-172), which interacts with the NBD1 domain of CFTR and specifically blocks CFTR-dependent anion transport (9, 10). CFTRinh-172 efficiently blocks CFTR in airway epithelial cells but is less efficient in colonic cells unless the tissue has been permeabilized (11). In our experiments, 20 $\mu M$ CFTRinh-172 bilaterally produced an $I_{sc}$ decay of $-18 \pm 2 \mu A$ in CFTR$^+/-$ tissue. As confirmation of the specificity of CFTRinh-172, no effect was observed in CFTR$^-/-$ or CFintoCF mice (Fig. 2B and C and Table 1). In contrast, in WTintoCF colonic tissues, the blocker induced a decrease in $I_{sc}$ of $-1.3 \pm 1.2 \mu A$ (750 rads) and $-1.8 \pm 0.5 \mu A$ (800 rads), confirming that part of the current generated by FSK stimulation in these tissues was CFTR-dependent (Fig. 2B and C). The 800-rads WTintoCF group had a statistically significant $\Delta I_{sc}$ in response to CFTRinh-172 ($P = 0.01$) compared with CFintoCF mice. No difference in $I_{sc}$ decay occurred between CFTR$^-/-$ and CFintoCF mice ($P = 0.17$).

The in vivo and in vitro functional data obtained prove that CFTR is expressed in epithelial cells of at least some of the WTintoCF mice. We measured the level of CFTR expression with quantitative RT-PCR for CFTR. Quantitative PCR of multiple tissues from WT mice ($n = 3$) revealed no significant difference in CFTR expression between different parts of the GI tract (duodenum, jejunum, ileum, colon, and rectum) or between nasal epithelium and lung ($P > 0.05$). CFTR expression in the trachea was 5-fold lower than in the other CFTR-expressing tissues ($P = 9 \times 10^{-6}$). CFTR mRNA expression was higher in the GI tract than in the airways ($P < 0.05$). Although no CFTR expression was detected in BM, blood, or purified macrophages with standard PCR (EtBr staining), by quantitative PCR (with its increased sensitivity) low levels of CFTR mRNA were detectable in BM but not in blood or macrophages. Comparative analysis of the threshold values revealed that the level of CFTR in WT BM is $\approx 0.017\%$ of that in WT GI tissue.

The percentage of CFTR mRNA in different tissues of transplanted WTintoCF mice in comparison with WT tissues is summarized in Table 2, which is published as supporting information on the PNAS web site. In the GI tract of WTintoCF mice, CFTR was expressed at levels ranging from 0% to 0.015% of that measured in the same tissues of WT mice. CFTR mRNA was not detected in the duodenum or jejunum of mice transplanted with 700 rads ($n = 3$). In contrast, CFTR mRNA was detected in the duodenum and jejunum of 2 of 5 WTintoCF mice transplanted with 800 rads (Table 2) supporting the finding that, at higher radiation doses, there is...
more epithelial engraftment. In individual mice, CFTR mRNA was not detected in all portions of GI, suggesting that the distribution of BM-derived epithelial cells is not uniform in accordance with previous findings (12). Although there was no significant correlation between mRNA levels and CFTR functional activity, there were striking correlations at the single mouse level. For example, in the colon and rectum of WT mice, CFTR mRNA was 0.279% of that in WT tissues, with the highest ratios of relative CFTR mRNA in 0 Cl−/amiloride solution (first 4 min), FSK was added bilaterally (f). After 7–10 min, in which the FSK effect usually reached its peak, 20 μM CFTRinh-172 was added bilaterally, and the I0 recorded (b). Arrows indicate solution changes and the star (Lower) indicates the decrease in I0 observed after with CFTRinh-172. (C) ΔI0 is the difference between the I0 before (one reading) and after (three readings) blocker addition. *, P < 0.05, statistically significant difference between CFIntOCF and WTIntOCF groups.

donor-derived epithelial cells were found, suggesting that they do not engraft as GI stem cells. We found that ∼1 in 10,000 to 1 in 100,000 epithelial cells are donor-derived. More important, we identified marrow-derived CFTR+ GI epithelial cells costaining for GFP and CFTR in WTIntOCF mice. (Fig. 3 D–F). Ameen et al. (13) showed that CFTR high-expressing epithelial cells (CHE) have a distinct pattern of immunostaining with some signal in the cytoplasm and a stronger signal at the luminal surface. In WT mice, CHE represent a subpopulation of epithelial cells in the duodenum and jejunum and are not present in the colon and rectum. In CFTR−/− mice, no CFTR+ cells were detected (data not shown).

In WTIntOCF mice, CFTR protein was detected in a subpopulation of epithelial cells in the duodenum of a transplanted CFTR−/− mouse. Functional CFTR activity and CFTR mRNA were because of engraftment in the GI tract of mouse recipients. In the colon and rectum of WT mice, CFTR+ epithelial cells are located in the crypts, where the immunofluorescence signal is more diffuse than that of CHE cells. Immunofluorescent staining, therefore, was not sensitive enough to detect rare CFTR+ cells in the large GI of transplanted CFTR−/− mice. Tissues from WT GFP+ mice were used as positive controls for staining, and tissues from CFIntOCF BMT recipients were used as negative controls for CFTR and GFP staining in each experiment (data not shown).
Discussion

Based on data from our laboratory and data of others that BMDC can become epithelial cells in vivo (14), we tested whether CFTR+/−; GFP+ BM cells can engraft as functional epithelia in the GI and respiratory tracts in CFTR−/−/ mice. Consistent with published data, we found rare BM-derived epithelial cells in WTintoCF mice by detection of GFP+ and/or Y−/CK−/CD45 negative donor cells in the GI tract, and we detected CFTR mRNA in the GI tract and airways (14). The frequency of BM-derived epithelial cells was between 1 in 104 and 1 in 105. Quantitative RT-PCR for CFTR mRNA showed a similar degree of epithelial engraftment. The relative percentage of CFTR expression in WTintoCF tissues compared with CFTR+/−/ tissues was between 0.001% and 0.27%, with the highest relative expression levels in the colon and trachea. Because CFTR is expressed at very low levels in fresh BM cells (15), the detection of CFTR mRNA expression in the different tissues could be because of infiltrating nonepithelial BMDC rather than the presence of epithelial cells. However, CFTR expression in the epithelial tissues of the transplanted mice was higher than BM, and CFTR+ epithelial cells were detected by immunofluorescence for apical CFTR and GFP, to identify donor-derived cells.

We show the functionality of BM derived CFTR+ cells by in vivo and in vitro electrophysiological studies. Consistent with the immunostaining and CFTR mRNA analyses, there was detectable CFTR activity by in vivo PD analysis in the GI tract and nasal epithelium of some of the transplanted mice. This CFTR-dependent Cl− transport was confirmed by in vitro Ussing chamber analysis of colonic tissues. By using as controls CFTR−/−/ mice that had been myeloablated and transplanted with CFTR−/−/ BM, we excluded that detection of CFTR activity was because of the transplantation process. The electrophysiological data suggest that the number of BM-derived epithelial cells was higher in mice transplanted with higher doses of irradiation, consistent with data suggesting that BM-derived epithelial engraftment occurs in response to tissue damage (16, 17). We postulate that radiation damage to the GI tract early post-BM transplantation may induce migration of BMDC to the gut, where the cells find themselves in a microenvironment that is conducive to epithelial engraftment, and that this engraftment is stable over time. Histological analysis of GI tissues of WTintoCF mice 24 weeks post-BM transplantation did not reveal any abnormalities compared with untransplanted CFTR−/−/ mice.

Exciting data presented at the North American Cystic Fibrosis Society in 2004 suggest that the extensive pulmonary inflammatory response to *Pseudomonas aeruginosa* observed in CF patients and CFTR−/−/ mice, may be because of immune dysfunction of CFTR−/−/ BM-derived hematopoietic cells, rather than lack of CFTR in the respiratory epithelium.3 CFTR−/−/ mice were transplanted with CFTR+/−/ BM and CFTR+/+/ mice with CFTR−/−/ BM. The CFintotWT mice fared worse than WT mice, suggesting that hematopoietic BMDC may play a role in the excessive immune response of CF mice to *P. aeruginosa* infection. In this study, <1% of the epithelial cells in the lung were BM-derived, which may be too low to justify the survival advantage in the WTintoCF mice (18).

Our data suggest that BM-derived epithelial cells can, although at low levels, reestablish some Cl− transport across the epithelia, which suggests that this restoration could have an ameliorative effect on the CF phenotype if engraftment levels could be increased. This hypothesis is supported by recent studies of the electrophysiology of epithelia generated from mixtures of CFTR+/+/ and CFTR−/−/ human airway cells (19) and from extensive electrophysiological analyses of CF mouse models with varied levels of CFTR mRNA expression. In these studies, CFTR expression in a small fraction of cells was sufficient to correct the CF epithelial Cl− transport defect (20, 21).

A controversy regarding BM to epithelial differentiation is whether the BM-derived cells derive from cell–cell fusion or, alternatively, from direct reprogramming of the gene expression profile by the microenvironment (for review, see ref. 14). The present study was not designed to establish whether cell fusion occurs. However, no binucleate marrow-derived epithelial cells were observed, consistent with Okamoto et al. (12), suggesting that stable fusion does not occur. In separate studies, we have shown, by using CFTR+ mice, that the derivation of epithelial cells from BM does not require cell fusion (22). Because unfractonated BM was used for transplantation, the identity of the cells that differentiate into mature GI epithelial cells cannot be determined. Recent in vitro studies suggest that marrow stromal stem cells can contribute to the airway epithelium (4). Also, a recent in vivo study suggests that freshly isolated BM cells can contribute to the respiratory tract (23).

The level of epithelial engraftment detected here (<1%) is well below that which could be therapeutic (~10%), as confirmed by the fact that the transplanted WTintoCF mice needed to be maintained on a liquid diet to survive. However, these findings open avenues of investigation for possible treatments for CF. Future efforts must focus on understanding the mechanism(s) that drive BMDC to engraft in the tissues. In conclusion, our studies provide compelling evidence that BMDC can engraft as functional epithelial cells in the GI and upper respiratory tracts. Whether this approach will ever be of clinical use is not known yet.

**Materials and Methods**

**Mouse Colonies.** Transgenic CFTR−/−/knockout (B6.129P2- Cfttm1Unc)(24), C57BL/6J-Tg(Actb-EGFP)10sb/J, and WT C57BL/6J mice from The Jackson Laboratory were bred in the Yale University Animal Facility and genotyped with standard protocols. To allow CFTR−/−/ mice to reach adulthood, they were fed with 9F food (Teklad, Madison, WI) and the drinking water was supplemented with 17.5 g/250 ml of Colyte (Schwarz Pharma, Milwaukee, WI). Mice that underwent BM transplantation were fed a liquid diet of Peptam (Nestle clinical nutritional product, Breinigsville, PA) (25). All procedures were performed in compliance with relevant laws and institutional guidelines and were approved by the Yale University Institutional Animal Care and Use Committee.

**BMDC Isolation, Transplantation, and Engraftment Evaluation.** BM transplantation was performed as described in ref. 26. Recipients were irradiated with 700, 750, or 800 rads by using a cesium irradiator and transplanted IV 4 h postirradiation with 1.0–1.5 × 107 total GFP + BM cells per mouse. Engraftment in the blood and BM was assessed by flow cytometry for GFP. For transplantation of female CFTR−/−/ mice with male CFTR−/−/ BM (CFintotCF), engraftment was evaluated by Y chromosome FISH (27).

**RPD and NPD Measurement.** RPD was assessed as described in ref. 8. Mice were anesthetized with 110 mg/kg ketamine and 10 mg/kg xylazine. A 3 M KCl-agar bridge was inserted 0.5 cm into the rectum of the mice and connected through an Ag–AgCl electrode to a digital voltmeter. The PD was measured with two consecutive solutions: (i) Cl−/free/5 mM barium/0.1 mM amiloride for 15 min and (ii) Cl−/free/5 mM barium/0.1 mM amiloride supplemented with 10−2 M FSK for 15 min. The NPD was measured as described (28) with perfusion of four solutions: (i) Ringer’s, (ii) Ringer’s/10−5 M amiloride, (iii) 0Cl−/10−3 M amiloride, and (iv) 0Cl−/10−5 M amiloride/10−5 M isoproterenol (Isuprel; Abbott Laboratory, North Chicago, IL). The NPD was sensed with a 3 M KCl-agar bridge inserted ∼0.3 cm into one nostril and connected through an Ag–AgCl electrode to a digital voltmeter.

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Short-Circuit Current and Potential Difference Measurement by Using Ussing Chamber Analysis. Short-circuit current in vitro analysis was performed as described by Grubb et al. (29) with minor modifications. The distal colonic tissue was removed, washed, and mounted in Ussing chambers with an aperture size of 0.3 cm² (VCC MC2 multichannel voltage-current clamp; Physiology Instruments, San Diego, CA). Both surfaces (serosal/apical) were equilibrated in Krebs Bicarbonate Ringer’s solution (KBS) for 20 min, and baseline PD values were recorded. Then the apical membrane was exposed to 10⁻⁵ M FSK was added bilaterally, and the PD was recorded for 15 min. For the CFTRmax−172 inhibitor studies, after the tissue reached maximum response to FSK (8–10 min), 20 μM CFTRmax−172 was added bilaterally. All solutions were 37°C and continuously gassed with 95% O₂/5% CO₂. Tissue viability was assessed at the end of each experiment by confirming that the baseline PD in KBS solution had not changed from the initial value (7). Studies were performed under open-circuit conditions, and tissues were pulsed with 1-μA current for 1 s every 60 s. From the change in the transepithelial voltage, the resistance and equivalent short-circuit current were calculated by Ohm’s law.

**BM-Derived Macrophage Isolation.** BM-derived macrophages were grown for 4 days in vitro in the presence of M-CSF as described (30).

**RNA Isolation and CFTR cDNA Analysis.** Total RNA was isolated from 20 mg of tissue or ~1 × 10⁷ cells by using Qiagen RNA mini kits or Qiagen RNA blood mini kits (Valencia, CA). After RNase-free DNaseI treatment (Roche Molecular Biochemicals), 2 μg of total RNA was reverse transcribed by using Superscript II RNaseH− Reverse Transcriptase (Invitrogen) with either 100 ng of random hexamers or 2 pmol of gene-specific primers (CF12; GAPDH-3).

Real-time PCR analysis was performed with a BioRad Cycler by using the iQSYBR green supermix (Bio-Rad). Two microliters of cDNA were PCR amplified with primers mCF7 and mCF15. The relative percentage of CFTR expression in transplanted mice was determined by the comparative threshold cycle method by using as calibrator tissue-specific CFTR expression in CFTR+/+ mice. Copy number was normalized to GAPDH levels (primers: GAPDH-1 and GAPDH-2). See Table 4, which is published as supporting information on the PNAS web site, for primer sequences.

**Y FISH and Immunofluorescence.** Isootype, serum, or no primary antibody controls were included for each sample in the immunostaining protocols. Negative and positive control tissues were processed in each staining run. For Y FISH/CD45/CK, 3-μm sections were deparaffinized, hydrated, incubated in BD Biosciences Retrieval A solution, stained for BD by using the M.O.M kit (Vector Laboratories), and detected with streptavidin–Texas red (Molecular Probes), and stained for GFP as described above, except the GFP signal was developed with streptavidin–FITC (Molecular Probes). The slides were then stained as stated above for anti-CD45, F4/80, and pan-keratin. However, the antibody anti-CD45 (Clone F11; BD Biosciences) was used.

For CFTR staining, deparaffinized slides were biotin blocked, incubated in 3% goat serum, incubated in 1:200 anti-CFTR antibody (R3195; a kind gift from C. Marino, University of Tennessee, Memphis) overnight at 4°C, incubated in 1:100 biotinylated anti-rabbit antibody (Chemicon), detected with 1:500 streptavidin–Texas red (Molecular Probes), and stained for GFP as described above, except the GFP signal was developed with streptavidin–FITC (Molecular Probes).

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Figure 4

A: RPD

B: 

ΔRDP (mV)

C: NPD

D: 

ΔNPD (mV)

E: Ussing chamber

F: 

ΔIsc/cm²(μAmp)