Gene complementation of airway epithelium in the cystic fibrosis mouse is necessary and sufficient to correct the pathogen clearance and inflammatory abnormalities

Delvac Oceandy¹, Brendan J. McMorran¹, Stephen N. Smith², Rainer Schreiber³, Karl Kunzelmann³, Eric W.F.W. Alton², David A. Hume¹ and Brandon J. Wainwright¹,*

¹Institute for Molecular Biosciences and ²Department of Gene Therapy, Imperial College of Medicine at the National Heart and Lung Institute, Manresa Road, London SW3 6LR, UK and ³Department of Physiology and Pharmacology, The University of Queensland, St Lucia, QLD 4072, Australia

Received January 3, 2002; Revised and Accepted March 11, 2002

Increasingly, cystic fibrosis (CF) is regarded as an inflammatory disorder where the response of the lung to Pseudomonas aeruginosa is exaggerated as a consequence of processes mediated by the product of the CF gene, CFTR. Of importance to any gene-replacement strategy for treatment of CF is the identification of the cell type(s) within the lung milieu that need to be corrected and an indication whether this is sufficient to restore a normal inflammatory response and bacterial clearance. We generated G551D CF mice transgenically expressing the human CFTR gene in two tissue compartments previously demonstrated to mediate a CFTR-dependent inflammatory response: lung epithelium and alveolar macrophages. Following chronic pulmonary infection with P. aeruginosa, CF mice with epithelial-expressed but not macrophage-specific CFTR showed an improvement in pathogen clearance and inflammatory markers compared with control CF animals. Additionally, these data indicate the general role for epithelial cell-mediated events in the response of the lung to bacterial pathogens and the importance of CFTR in mediating these processes.

INTRODUCTION

The major cause of death in patients with cystic fibrosis (CF) is lung disease (1). After the cloning and characterization of the cystic fibrosis transmembrane conductance regulator (CFTR) gene (2,3), which is mutated in CF, a number of investigators have reported experiments to correct the defect by genetic reconstitution. Both in vitro and in vivo analyses have demonstrated that delivering the CFTR gene can correct the ion transport defect in CF cell lines (4,5) and mouse models (6,7). Restoration of ion conductance in the CF airway tract can be achieved by delivering CFTR cDNA liposome complexes into the airway by nebulization (6) or direct tracheal instillation (7). Transgenic complementation of CF mice with a human CFTR YAC transgene, which included the entire 250 kb human genomic CFTR gene, resulted in correction of chloride channel activity (8), indicating that the transgenic CFTR is active in mouse tissues. Expression of CFTR in intestinal epithelial cells using the fatty acid binding protein gene promoter restored normal function to CF mouse intestine (9). In contrast, replacement of endogenous mouse Cfr with human CFTR cDNA in a ‘knock-in’ experiment only resulted in partial correction of electrophysiology and pathology in lung and intestine (10). Despite these encouraging studies, the important link between gene replacement and correction of more clinically relevant markers such as bacterial colonization and lung inflammation has not been made.

CF lung disease is characterized by persistent bacterial infection, predominantly by Pseudomonas aeruginosa. There are several theories as to how a defect in CFTR leads to airway disease, none of which are mutually exclusive. One possibility is that altered fluid flux causes depletion of airway surface fluid volume and increasing viscosity leading to airway obstruction and altered bacterial clearance (11). Secondly, CFTR-dependent ion transport abnormalities may cause elevation of salt concentration in airway surface liquid, which could inactivate natural antibiotics in the lung such as defensins (12,13). Other than mucociliary clearance or defensin-mediated killing, it is thought that P. aeruginosa are cleared from the lung after internalization by epithelial cells and subsequent shedding of the cells containing the pathogen. CFTR is proposed to be a receptor for internalization of P. aeruginosa.
(14–16); therefore defective CFTR function may alter bacterial elimination in the lung. Other studies have revealed that binding of bacteria to CF airway cells is increased because of elevated production of bacterial receptor asialoGM1 (17).

An excessive lung inflammatory response to bacterial infection is also a major feature of the CF lung. CF respiratory epithelial cells exhibit an exaggerated inflammatory response to a number of stimuli compared with non-CF cells (18–22). CF mice with the null S489X mutation (Cftr\textsuperscript{m1UNC}) when infected with P. aeruginosa embedded in agar beads produce more proinflammatory cytokines such as tumor necrosis factor \( \alpha \) (TNF-\( \alpha \)), macrophage inflammatory protein 2 (mip-2) and KC compared with wild-type animals (23). Another CF mouse model, the G551D mouse (Cftr\textsuperscript{G551D/G551D}), exhibits abnormal lung inflammation in response to bacterial lipopolysaccharide (LPS), and the bone-marrow-derived macrophages (BMM) from these animals demonstrate hypersensitivity to LPS-induced cytokine production (24). Similar data from human subjects (25,26) suggest that defective CFTR within inflammatory cells could contribute independently to the exaggerated lung host response.

In order to dissect the role of airway epithelial cells and alveolar macrophages in CF lung disease, we conducted genetic complementation studies. We generated G551D CF mice expressing human CFTR cDNA in airway epithelia, or in macrophages using lineage-specific promoters. The G551D homozygous mice have a reduced capacity to clear bacteria compared with normal mice after pulmonary infection with P. aeruginosa embedded in beads (27). We show that expression of human CFTR in the airway epithelium improved the bacterial clearance capacity and reduced cytokine production in the lung. In contrast, expression of CFTR in macrophages had no effect on bacterial clearance or cytokine production.

RESULTS

Establishment of the G551D-K18 and G551D-fms bitransgenic mice

We used the human cytokeratin 18 (K18) promoter to direct CFTR cDNA expression selectively to respiratory epithelium. Expression of the K18 promoter is restricted to epithelial cells, including lung and intestine, and previous studies in transgenic mice have shown that K18 transgene expression matched that of the human CFTR in these tissues (28,29). Four transgenic founders were generated and two of these successfully transmitted the transgene to offspring.

We also generated transgenic mice selectively expressing CFTR in alveolar macrophages using the mouse c-fms gene promoter. The c-fms gene encodes the receptor for the lineage-specific growth factor, macrophage colony-stimulating factor (CSF-1, M-CSF) and is exclusively expressed in macrophages (30,31). The 6.7 kb promoter construct contains 3.5 kb flanking the first coding exon plus the first downstream intron (32). The start codon was specifically mutated to provide a convenient cassette, and we called this promoter cassette \( \Delta \text{6.7fms} \). In a separate study, we have found that the \( \Delta \text{6.7fms} \) promoter directs specific expression of green fluorescent protein (GFP).

Figure 1. Immunofluorescence analyses of lung sections. Immunofluorescence staining with anti-human CFTR monoclonal antibody in lung sections of (A) K18-CFTR, (B) non-transgenic and (C) \( \Delta \text{6.7fms} \)-CFTR mice. Arrows indicate lung epithelium. Results are representative of three or four independent mice.
to macrophages, including alveolar macrophages (T. Sasmono, D.A. Hume et al., manuscript submitted). We fused the Δ6.7fms promoter with a CFTR cDNA containing the SV40 poly(A) signal. From three transgenic founders bearing the Δ6.7fms–CFTR transgene, two transmitted the transgene to their offspring.

We then mated K18–CFTR mice and Δ6.7fms–CFTR mice with G551D CF mice (Cftr<sup>G551D/G551D</sup>). Then, by intercross breeding of F<sub>1</sub> mice, we generated bitransgenic Cftr<sup>G551D/G551D</sup>Δ18–CFTR<sup>+/−</sup> (G551D–K18), which are CF mice expressing human CFTR in the respiratory epithelial cells, and Cftr<sup>G551D/G551D</sup>Δ6.7fms–CFTR<sup>+/−</sup> (G551D–fms) which are CF mice expressing human CFTR in macrophages. Subsequent studies were focused on those mice together with G551D CF mice (Cftr<sup>G551D/G551D</sup>) and normal mice (Cftr<sup>+/+</sup> and Cftr<sup>+/+</sup>) that are the littermates of both G551D–K18 and G551D–fms as controls.

The K18-CFTR transgene is expressed in pulmonary epithelial cells

We examined the expression pattern of K18–CFTR in the lung by immunofluorescence analysis. We used anti-human CFTR antibody (clone M3A7), which can specifically detect the human CFTR (33,34), and found strong staining in airway epithelial cells of mice carrying the K18–CFTR transgene, whereas no positive staining was evident in animals without the transgene (Fig. 1A, B). To further analyze whether the K18-CFTR transgene is selectively expressed in epithelial cells and not in macrophages, we isolated the alveolar, peritoneal and bone marrow macrophages from K18–CFTR mice and stained them with anti-human CFTR antibody. We found that there is no expression of the transgene in the macrophages of K18–CFTR mice (Fig. 2G–I).

The Δ6.7fms–CFTR transgene is expressed in macrophages

To characterize the expression of human CFTR under the control of the Δ6.7fms promoter, we isolated macrophages from bronchoalveolar and peritoneal lavage fluid and grew BMM from mice carrying the Δ6.7fms–CFTR transgene. Immunofluorescence analysis showed that transgene expression was strong in alveolar and peritoneal macrophages that express high levels of the CSF-1 receptor, whereas BMM demonstrated weaker but significant CFTR expression (Fig. 2A–C). We also performed immunofluorescence analysis on lung sections of

Figure 2. Immunofluorescence analyses of isolated macrophages. Immunofluorescent detection of human CFTR in alveolar (A,D,G), peritoneal (B,E,H) and bone marrow (C,F,I) macrophages of Δ6.7fms–CFTR transgenic (A,B,C), non-transgenic (D,E,F), and K18–CFTR (G,H,I) mice. Cells were stained with monoclonal anti-human CFTR antibody. Nuclear counterstain was performed using DAPI. Results are representative from three or four independent animals.
Transgenic CFTR protein is functionally active

To test the functional activity of the human CFTR protein expressed in the transgenic mice, we analyzed the bioelectric properties of tracheal and intestinal epithelium. The electrophysiological responses to IBMX and forskolin were examined in excised tracheal and caecal tissues from G551D, G551D–K18, G551D–fms and normal mice. In trachea, the change in short-circuit current (ΔI_{SC}) after IBMX/forskolin challenge was significantly reduced in G551D mice compared with normal animals (P < 0.05) (Fig. 3A). In the G551D–K18 mice, the ΔI_{SC} was significantly higher (P = 0.01) and the average was increased by approximately 2.5 fold compared with G551D animals, indicating that the human transgene had restored function to the murine CF trachea. By contrast, the IBMX/forskolin response in the trachea of G551D–fms animals was not different from that in G551D mice, suggesting that expression of the transgene in the macrophages did not restore the chloride channel activity in the trachea.

We also analyzed the bioelectric properties in the cecum of the mice. The forskolin response in the cecum was markedly reduced in G551D compared with normal mice (P < 0.001), and in G551D–K18 mice the ΔI_{SC} was increased by approximately 40% of the value of normal mice and significantly higher (P < 0.05) than G551D animals (Fig. 3B). As in the trachea, the forskolin response in the cecum of G551D–fms mice was comparable to that in G551D mice. These findings demonstrated that the expression of exogenous human CFTR protein in epithelial cells but not in macrophages can restore the chloride channel activity in both airway and digestive tract.

Expression of human CFTR in airway epithelial cells corrects P. aeruginosa clearance in the lung

To test whether complementation of human CFTR in airway epithelial cells or in alveolar macrophages corrected the pathogen clearance defect in CF mice, we studied the effects of chronic lung infection in G551D–K18 and G551D–fms mice. Chronic endobronchial infection was modeled by intratracheal instillation of agar beads containing mucoid P. aeruginosa and the bacterial clearance capacity was determined by measuring the number of colony-forming units remaining in the lung after 3 days of infection.

After 3 days of infection, G551D mice had a significantly higher (P < 0.05) number of P. aeruginosa in the lung than normal mice (Fig. 4A and Table 1), indicating an impaired clearance capacity. These data have been reproduced extensively in a separate detailed study (27). The bacterial burden in the lung of G551D–K18 mice was comparable to that in normal mice and significantly (P = 0.01) lower than that in G551D mice. However, there was no significant difference between G551D and G551D–fms animals. These results indicate that expression of exogenous human CFTR in lung epithelial cells corrected the bacterial clearance defect in CF mice, whereas expression of the same transgene in alveolar macrophages did not improve the bacterial clearance defect.

The mortality rate of the animals indicated the severity of the infection. One out of 14 (7.1%) G551D mice and 2 out of 11 (18.2%) G551D–fms mice died by 3 days after infection, whereas none of the G551D–K18 and normal mice died (Table 1). Although it did not reach statistical significance, this result supports the quantitative bacterial clearance assay that G551D and G551D–fms mice are more susceptible to P. aeruginosa lung infection.

An indirect indicator of the failure of mice to control a gram-negative infection is weight loss due to the induction of cachexia by LPS-induced inflammatory cytokines. Two days after infection, weight loss stabilized in normal and G551D–K18 mice, whereas it continued in G551D and G551D–fms mice (Fig. 4B). Thus, by day 3 after infection, G551D and G551D–fms mice lost more weight than G551D–K18 and normal mice, although this did not reach statistical significance (P < 0.2).

The levels of proinflammatory cytokine and chemokines are correlated with bacterial clearance capacity

To investigate the inflammatory response in the lungs of P. aeruginosa-infected mice, we measured the levels of proinflammatory cytokine/chemokines in bronchoalveolar lavage (BAL) fluid. Levels of TNF-α, mip-2 and KC were significantly...
<p>P < 0.05) elevated in the G551D mice compared with normal animals after 3 days of infection with P. aeruginosa agar beads (Fig. 5). In the G551D-K18 mice, the concentrations of these molecules were comparable to those in normal mice, and significantly lower (P < 0.01) than there in G551D mice. The major source of TNF-α, mip-2, and KC is believed to be</p>
activated macrophages (35–37). If upregulation of the inflammatory response in CF mice is caused by altered CFTR function in macrophages, we anticipated correction in the G551D–fms mice. However, levels of these cytokines were not significantly different in these mice compared to G551D animals. Further, we examined the correlation between bacterial burden and cytokine/chemokine production. Levels of TNF-α, mip-2 and KC were directly correlated with the bacterial load in the lung in all groups of mice (Fig. 6). Thus, the higher concentration of cytokine/chemokines in the BAL fluid of G551D and G551D–fms mice was most likely a consequence of ineffective bacterial clearance by the pulmonary epithelium, resulting in the accumulation of bacterial products and stimulation of macrophages.

**DISCUSSION**

The major features of CF lung disease are the failure to eliminate bacterial infection and an excessive inflammatory response. Although most CF mice do not exhibit spontaneous lung pathology, abnormalities can be elicited in response to infection with common CF lung pathogens. Previous studies showed that CF mice with an insertional mutation in exon 10 (Cftr<sup>ln1HGU</sup> mice) had an impaired capacity to clear aerosolized Staphylococcus aureus and Burkholderia cepacia (38). Further, congenic C57/B6 CF (Cftr<sup>ln1UNC</sup>-B6) mice exhibit impaired bacterial clearance after intratracheal instillation with agar beads containing P. aeruginosa (39). In keeping with these findings, G551D CF mice that carry a type III mutation in the Cftr gene also show reduced bacterial clearance after infection with P. aeruginosa-laden agar beads (27). A higher mortality rate accompanied with excessive inflammatory response was also found in CF mice (Cftr<sup>ln1UNC</sup>) infected with P. aeruginosa agar beads compared with non-CF littermates (23).

Here we demonstrate that expression of human CFTR in airway epithelial cells but not macrophages corrected the pathogen clearance and inflammatory defects in CF mice. On average, the G551D mice retained 12-fold more bacteria in the lung than normal mice after 3 days of P. aeruginosa infection (Table 1). In contrast, the bacterial clearance capacity of G551D–K18 mice was comparable to that of normal animals and significantly better than that of G551D mice. However, the bacterial clearance defect was not corrected in the G551D–fms mice - suggesting that expression of exogenous CFTR in macrophages had no effect in eliminating bacterial infection. These results indicate that the presence of CFTR in airway epithelial cells is vital in limiting bacterial infection.

Thomas and co-workers (24) reported that G551D CF mice have an abnormal inflammatory response in the lung as well as in isolated macrophages after bacterial LPS induction. Macrophages isolated from CF patients also produce higher amounts of proinflammatory cytokines than do non-CF macrophages (25,26). In CF infants, alveolar macrophages have been found to produce higher amounts of interleukin-8 (IL-8) (40), (25,26). In CF infants, alveolar macrophages have been found to produce higher amounts of interleukin-8 (IL-8) (40), suggesting the possibility of macrophage involvement in developing an exaggerated inflammatory response in CF. Thus, if these observations are biologically significant, expression of CFTR in macrophages of CF mice should improve bacterial clearance and/or cytokine levels. However, G551D–fms mice exhibited an excessive inflammatory response after P. aeruginosa infection, as indicated by higher concentrations of TNF-α, mip-2 and KC in BAL fluid. In contrast, G551D–K18 mice showed reduced cytokine/chemokine production, comparable to normal levels. Pearson product moment correlation analysis revealed that the levels of TNF-α, mip-2 and KC were directly correlated with the number of bacteria retained in the lung. These data suggest that cytokine levels depend on bacterial clearance capacity. On the basis of in vitro studies, Scheid and co-workers (19) suggest that higher cytokine expression in CF...
epithelial cells treated with P. aeruginosa is due to greater adherence of the bacteria to CF cells. If bacterial concentrations were equilibrated so that the numbers of adherent bacteria in CF and non-CF cells were similar, the level of cytokine expression was comparable (19). Our in vivo data are in agreement with these studies, and thus correction of bacterial clearance by the pulmonary epithelium is necessary to reduce the excessive immune response to P. aeruginosa infection.

Davies and colleagues (41) reported that the CF respiratory epithelium binds more P. aeruginosa than normal cells, and this abnormality can be corrected in vitro by CFTR gene transfer. It has also been reported that bacterial killing activity was reduced in CF airway cells and can be restored by CFTR gene transfer (42). Our data provide the first in vivo evidence that restoration of bacterial clearance can be achieved by expressing the CFTR gene in airway epithelial cells, and suggest that replacement of CFTR in airway epithelial cells is both necessary and sufficient to achieve therapeutic benefit.

MATERIALS AND METHODS

Generation of bitransgenic mice

The K18-CFTR construct, provided by Dr J. Hu (University of Toronto, Toronto, ON), consists of the K18 promoter followed by human CFTR cDNA and SV40 polyadenylation site as previously described (28). TheΔ6.7fms-CFTR was constructed by fusing a 6.7 kb regulatory sequence of the murine c-fms gene (32) with the human CFTR cDNA. The transgenes were excised from the vector and microinjected into the pronuclei of single-cell embryos (C57Bl/6 x CBA F1) using standard techniques (43). Transgenic founders were identified by Southern blotting and mated with CD1 mice to generate individual lines. Female transgenic offspring were mated with Cfr−/− males (129Sv/CD1), which are homozygous for the G551D mutation in the murine Cftr gene (44). Bitransgenic mice were obtained by intercross breeding of F1 offspring. Mice carrying human CFTR were identified by polymerase chain reaction (PCR) analysis of tail DNA using the following primers: 5’-GAGGACAATCCTCAAGTTGGCA-GAG-3’ and 5’-TGTTGGATGTGTTGTCTTTCCG-3’ (producing a 791 bp PCR product). PCR followed by restriction enzyme digestion was used to identify the G551D mutation as a 791 bp PCR product. PCR followed by restriction enzyme digestion was used to identify the G551D mutation as described (44). All mice were maintained in specific pathogen-free conditions before experimentation.

Mice were used for experimentation between the ages of 8 and 14 weeks. All animal experimentation was carried out in accordance with National Health and Medical Research Council of Australia guidelines and was approved by the University of Queensland Animal Ethics Committee.

Immunofluorescence studies

Lungs from K18-CFTR+/−, Δ6.7fms-CFTR+/− and non-transgenic mice were fixed in 4% paraformaldehyde in PBS and frozen in OCT (Tissue-Tek: Sakura, Torrance, CA). Immunofluorescence was conducted on 10μm cryosections using mouse anti-human CFTR monoclonal antibody clone M 3A7 (Chemicon International Inc., Temecula, CA) and goat anti-mouse IgG–Alexa Fluor conjugate (Molecular Probes, Eugene, OR). Alveolar and peritoneal macrophages were isolated by bronchoalveolar and peritoneal lavage, and plated overnight on glass coverslips in RPMI 1640 medium (BioWhittaker, Walkersville, MD) containing 10% FCS. BMM were grown as described previously (24). Adherent cells were fixed in 4% paraformaldehyde and subjected to standard immunofluorescence using mouse anti-human CFTR (R-domain-specific) (Genzyme, Cambridge, MA) and rabbit anti-mouse Ig–rhodamine conjugate (Boehringer Mannheim, GmbH, Germany) or using anti-human CFTR clone M 3A7 and goat anti-mouse IgG–Alexa Fluor conjugate.

Electrophysiological measurements

Electrophysiological studies were performed in excised trachea and cecum from G551D, bitransgenic G551D–K18, G551D–fms and normal mice. Transepithelial voltages of mouse tracheal epithelia were assessed in a perfused micro Ussing chamber under open-circuit conditions, and equivalent short-circuit currents were calculated. The changes in Isc after addition of 10μM forskolin (Sigma) and 100μM IBMX (Sigma) were analyzed. Analysis of electrophysiological properties in cecum were performed as described previously (44,45). Briefly, cecal segments were mounted in Ussing chambers under short-circuit conditions. Tissues were circulated with Krebs-Henseleit solution of composition (mM): Na+ 145.0, Cl− 126.0, K+ 5.9, Ca2+ 2.5, Mg2+ 1.2, HCO3− 26.0, PO43− 1.2, SO42− 1.2, glucose 5.6, circulated by 95% O2-5% CO2. Isc was continuously recorded and once stable values were attained, tissues were treated with 10μM forskolin and the change in Isc was recorded.

P. aeruginosa infection studies

To model chronic P. aeruginosa endobronchial infection, the agar bead method was used with modification as described by McMorran et al. (27). In brief, mucoid P. aeruginosa strain M57–15 was grown to log phase and mixed with warm (52°C) Trypticase Soy Agar (TSA) (BBL). The TSA–bacteria mixture was added to mineral oil (Sigma) that was equilibrated at 52°C and rapidly stirred for 6 minutes at room temperature, followed by cooling of the mixture on ice for 10 minutes. The bacteria-containing beads were recovered by centrifugation at 9000 g for 20 minutes at 4°C, followed by three washes with PBS. The beads were filtered through sterile nylon mesh (200μm hole) (Australian Filter Specialist Pty Ltd, Australia) and then verified for size (70–150μm) and uniformity by microscopic examination. Quantitative bacteriology was performed on an aliquot of homogenized beads to determine the number of bacterial colony-forming units (CFU).

Mice were anesthetised with a mixture of ketamine hydrochloride (25 mg/kg body weight) (Bayer, Australia) and tiletone/zolasepam (60 mg/kg body weight) (Virbac, Austral-ia) injected intraperitoneally. A 25μl inoculum containing 105 CFU of viable P. aeruginosa entrapped in agar beads was introduced into the lungs via the trachea using non-surgical methods. Briefly, mice were hung in a supine position by their top incisors from a wire frame (7 cm high) and a cold light source was placed against the throat. The animal’s tongue was
extended with lined forceps and the beads delivered via a 21-
gauge blunt-end needle to the back of the tongue above the
trachea opening. Successful delivery of the beads to the lungs
was evidenced by choking of the mouse immediately after
instillation followed by rapid breathing. Animals were weighed
prior to inoculation with P. aeruginosa beads and then daily for
up to 3 days following infection.

Bronchoalveolar lavage
At day 3 post infection, mice were anesthetized and
exsanguinated. Bronchoalveolar lavage (BAL) was performed
by canulating the trachea with a blunted 23G needle, instilling
3 × 1 ml of sterile PBS, and collecting the fluid by gentle
aspiration. The total lavage fluid recovered was approximately
2.5 ml per animal. The fluid was centrifuged at 10 000 g for 10
minutes and the supernatants were stored at −20°C after
addition of PM SF and EDTA at final concentrations of 100 μM
and 5 mM respectively.

Quantitative bacteriology
Following BAL, the lungs and trachea were aseptically
sectected and homogenized in 3 ml of sterile PBS. Samples of lung homo-
genates were serially diluted and cultured on Pseudomonas
isolation agar (Difco) supplemented with 5% fetal calf serum
(Biowhittaker). Mucoid colonies of P. aeruginosa were
visualized after incubation for 24 hours at 37°C.

ELISA
Recovered BAL fluid was assayed for TNF-α, mip-2 and KC
using sandwich enzyme immunoassays according to the
manufacturer’s recommended protocol (R&D Systems, Min-
neapolis, MN). The concentrations were corrected for the
respiratory epithelial lining fluid (ELF) volume using the urea
method as described previously (46).

Statistical analysis
Data are expressed as the mean ± standard error of the mean
(SEM). Student's t-test was used to compare differences
between experimental groups when the data were distributed
normally, otherwise the Mann-Whitney rank sum test was
used. The relationships between bacterial burden and cytokine
concentrations were analyzed using the Pearson correlation
coefficient. The chi-square test was used to analyze the
mortality rate. The criterion for statistical significance was
P < 0.05.

ACKNOWLEDGEMENTS
We are grateful to Jim Hu for the gift of the K18-CFTR
construct and thank Anna van Heekeren for providing the
P. aeruginosa M57-15 cells. D.O. is the recipient of an
International Postgraduate Research Scholarship and a
University of Queensland Postgraduate Research Scholarship.
This study was funded by the National Health and Medical
Research Council of Australia, the Cystic Fibrosis Trust UK
and a Wellcome Trust Senior Clinical Fellowship (E.A.).

REFERENCES
The Metabolic and Molecular Bases of Inherited Disease. McGraw-Hill,
2. Riordan, J.R., Rommens, J.M., Kerem, B., Alon, N., Rozmahel, R.,
Identification of the cystic fibrosis gene: cloning and characterization of
3. Rommens, J.M., Iannuzzi, M.C., Kerem, B., Drum, M.L., M elmer,
(1989) Identification of the cystic fibrosis gene: chromosome walking and
of the cystic fibrosis defect in vitro by retrovirus-mediated gene transfer.
Cell, 62, 1227–1233.
Expression of cystic fibrosis transmembrane conductance regulator corrects
defective chloride channel regulation in cystic fibrosis airway epithelial
et al. (1993) Non-invasive liposome-mediated gene delivery can correct
the ion transport defect in cystic fibrosis mutant mice. Nature Genet., 5,
135–142.
Correction of the ion transport defect in cystic fibrosis transgenic mice
8. Manson, A.L., Treslize, A.E., M acVinish, L.J., Kasschau, K.D., Birchall,
N., Episkopou, V., Vassaux, G., Evans, M.J., Colledge, W.H., Cuthbert,
A.W. et al. (1997) Complementation of null CF mice with a human CFTR
YAC transgene. EMBO J., 16, 4238–4249.
J.A. (1994) Correction of lethal intestinal defect in a mouse model of
cystic fibrosis by human CFTR. Science, 266, 1705–1708.
10. Rozmahel, R., Gzymorey, K., Pyle, S., Nguyen, V., Wilchanski, M.,
fibrosis transmembrane conductance regulator defective mice by the human
CFTR cDNA. Hum. Mol. Genet., 6, 1153–1162.
and Boucher, R.C. (1998) Evidence for pericyclic liquid layer depletion,
not abnormal ion composition, in the pathogenesis of cystic fibrosis airways
in lung that is inactivated in cystic fibrosis. Cell, 88, 553–560.
fibrosis airways epithelium fail to kill bacteria because of abnormal airway
surface fluid. Cell, 85, 229–236.
14. Pier, G.B., Grout, M., Zaidi, T.S., Olsen, J.C., Johnson, L.G., Yankaskas,
conductance regulator is an epithelial cell receptor for clearance of
Pseudomonas aeruginosa from the lung. Proc. Natl Acad. Sci. USA, 94,
12088–12093.
regulator in innate immunity to Pseudomonas aeruginosa infections.
asialoGM1 which is increased on the surface of cystic fibrosis epithelial
epithelial cell cytokine production in cystic fibrosis. J. Allergy Clin.
Immunol., 104, 72–78.
Inflammation in cystic fibrosis airways: relationship to increased bacterial


