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Characterization of immortal cystic fibrosis tracheobronchial gland epithelial cells

(simian virus 40/cAMP/calcium ionophore/cystic fibrosis transmembrane conductance regulator)

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ABSTRACT **Tracheobronchial glands were isolated and cultured from a patient with cystic fibrosis (CF). Cultured epithelial cells were transformed with pSVori⁻. All transformed cell lines express cytokeratin filaments and at early passages express the junctional complex molecule cellCAM 120/80, indicating their epithelial origin. Several gland cell lines express antigens that localize to secretory cells *in vivo*. Cl^- transport measured by ^{36}Cl efflux shows that CF gland epithelial cells, like CF surface airway and nasal polyp epithelial cells, are unable to respond to increases in intracellular cAMP. However, they do produce an increase in intracellular cAMP after treatment with isoproterenol or forskolin. One CF gland cell line shows increased intracellular calcium in response to a number of agents and increased Cl^- efflux comparable to that observed in a non-CF airway surface epithelial cell line after addition of calcium ionophore. All cell lines express CF transmembrane conductance regulator mRNA, as measured by PCR amplification of first-strand cDNA. The CF tracheobronchial gland cell lines described here are compound heterozygotes, having a single copy of the ΔF508 mutation.**

Cystic fibrosis (CF) is a recessive genetic disorder, affecting ≈1/2500 Caucasian live births (1). Epithelia from individuals with CF, particularly of sweat glands, intestines, pancreas, and airways, have altered Cl^- transport and secretion (2). In airways, this results in production of thick, viscous mucus secretion that appears to be a determinant in CF-associated lung disease. The gene defective in CF has been cloned and sequenced and encodes a protein of 1480 amino acids, the CF transmembrane conductance regulator (CFTR) (3). About 70% of CF chromosomes contain a 3-base-pair (bp) deletion that results in loss of a phenylalanine at amino acid 508 (ΔF508) (4). Protein structure comparisons suggest that the CFTR gene encodes a membrane protein similar to the multidrug-resistance protein (P-glycoprotein) (3, 5). While transfection with constructs containing CFTR cDNA confers the ability to generate cAMP-activated Cl^- currents on both CF epithelial cells (6, 7) and nonepithelial cells (8, 9), these studies do not preclude other roles for the CFTR gene product.

It is not clear whether abnormal mucus present in airways of CF patients is due to defective regulation of airway secretory cells or is a secondary effect of altered epithelial cell ion transport (1). One recent study suggests that it is a consequence of defective acidification of intracellular organelles in CF (10). To fully understand the relationship between the CF defect and mucus secretion, it will be necessary to study cells that are responsible for airway secretions. The tracheobronchial submucosal glands are lined with secretory mucous and serous epithelial cells and may be responsible for the majority of airway secretions (11). Gland cells have been isolated and cultured *in vitro*, producing cell monolayers with a mixed serous/mucous cell phenotype (12). These cultures retain tight junctions and directional, stimulated ion transport (13), while cultures of CF gland cells show altered ion transport (14). We describe here properties of CF airway gland cells transformed with pSVori⁻.

METHODS

Cells and Cell Transformation. Tracheobronchial gland cells were isolated and cultured as described (12). Nontransformed gland, surface airway, and nasal polyp cells from different individuals with CF were grown in modified serum-free LHC-9 medium (MLHC-8e) on tissue culture plastic that had been precoated with fibronectin/collagen/bovine serum albumin (15, 16). Pure cultures of gland epithelial cells were transfected as described (16). Cells grown in 100-mm precoated tissue culture dishes to 70–80% confluence were transfected with linearized pSVori⁻, a plasmid containing a replication-deficient simian virus 40 (SV40) genome (17) via the calcium phosphate precipitation method. Transfected cultures were grown in MLHC-8e medium at 37°C under 5% CO_2/95% air until cells with altered growth characteristics appeared. Colonies of transformants were isolated and expanded for further characterization.

To enhance the ability of cells to survive crisis (generally, ≈15 passages posttransfection), cells were transferred before crisis to Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics. Subsequently, all colonies of pSVori⁻ transformants (CF and non-CF) were grown in supplemented MEM as described above.

Immunocytochemical Staining. Cells were grown on well slides (Lab-Tek) coated with fibronectin/collagen/bovine serum albumin to various stages of confluence. After washing, fixing, and drying, the slides were rehydrated and stained for immunofluorescence as described (18). Primary antibodies used in these studies were the L19 monoclonal antibody against the SV40 large tumor antigen (T antigen) (19), the AE1/AE3 anti-cytokeratin antibody (Boehringer Mannheim), and the E9 monoclonal antibody against the junctional complex adhesion protein, cellCAM 120/80 (20). SV40 T antigen is expressed only in successfully transformed cells. Cytokeratin and cellCAM 120/80 are expressed only in epithelial cells.

For light microscopic immunocytochemistry, slides were fixed as described (12). Staining was by a modification of a

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Abbreviations: CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; FBS, fetal bovine serum; SV40, simian virus 40; T antigen, large tumor antigen.

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biotin/avidin procedure (21). Primary antibodies used in these studies included monoclonal antibodies that recognize antigens expressed by goblet, serous, and mucous airway secretory cells (22) and polyclonal antibodies against lactoferrin (Sigma) and lysozyme (DAKO, Santa Barbara, CA).

ΔF508 Mutation Analysis. Analysis of cells for the presence of the ΔF508 mutation was carried out as described by Kerem *et al.* (4). Genomic DNA was prepared from transformed epithelial cell lines and amplified by PCR with a GeneAmp kit (Perkin–Elmer/Cetus). Oligonucleotide primers C16B (5′-GTTTTCCTGGATTATGCCTGGGCAC-3′) and C16D (5′-GTTGGCATGCTTTGATGACGCTTC-3′) (4) were used for amplification of the CFTR region around the ΔF508 deletion site. DNA was amplified for 28 cycles with the following cycle times: denaturation for 60 s at 94°C, annealing for 45 s at 62°C, and extension at 72°C, starting with 120 s and increasing to 7 min on the final cycle. The PCR products were separated on 1.4% agarose gels and transferred to GeneScreen*Plus* membranes (DuPont/NEN). Oligonucleotide probes (10 ng each) for normal CFTR DNA (5′-CACCAAAGATGATATTTTC-3′) and for the ΔF508 deletion (5′-AACACCAATGATATTTTCTT-3′) were labeled with [γ-^{32}P]ATP. Filters were hybridized overnight at 37°C with labeled oligonucleotide in a solution containing 6× SSC (1× SSC is 150 mM NaCl/15 mM trisodium citrate), 1% SDS, 1 g of Ficoll per liter, 1 g of bovine serum albumin fraction V per liter, 1 g of polyvinylpyrrolidone 6000 per liter, and 100 μg of sonicated salmon sperm DNA per ml. Membranes were washed twice with 2× SSC at room temperature for 5 min, twice at 45°C in 2× SSC/0.1% SDS for 30 min, and once at room temperature in 0.1× SSC for 30 min. Positively hybridizing bands were visualized autoradiographically.

CFTR mRNA Expression by PCR Amplification of First-Strand cDNA. Cytoplasmic RNA was prepared from 10^6 to 10^8 cells (23). RNA (1 μg) was denatured by heating to 95°C for 2 min and then reverse transcribed with the PCR RNA GeneAmp kit, according to the manufacturer's instructions (Perkin–Elmer/Cetus). First-strand cDNA was amplified with oligonucleotide designed to span intron–exon boundaries, thereby eliminating amplification of contaminating genomic DNA. These were 5′-ACTTTAAAGCTGTCAAGCCGTG-3′ and 5′-CTGTATTTTGTTTATTGCTCCAA-3′, giving a predicted 627-bp product (nucleotide positions 622–1248 of the CFTR gene). Thirty-five cycles of PCR amplification were used, with cycle times as follows: denaturation 30 s at 94°C, annealing 30 s at 55°C, and extension 1 min at 72°C. The extension time was increased by 5 s per cycle. The amplification products were separated by electrophoresis on 1.5% agarose gels.

cAMP Analysis. Intracellular cAMP levels were measured as described (18). Cells were grown to confluence in MLHC-8e medium or in FBS supplemented MEM. Production of cAMP was stimulated by addition of 10 μM isoproterenol or 10 μM forskolin. After a 1-min incubation at 37°C, the medium was replaced with ice-cold 10% trichloroacetic acid. Cells were harvested, sonicated, and centrifuged for 25 min at 5000 × *g* at 4°C. The pellet was dried to measure total protein. An aliquot of supernatant was extracted with water-saturated ether and then evaporated to dryness at 75°C. The cAMP content of each sample was measured with a DuPont/NEN cAMP [^{125}I] RIA kit. Results were adjusted to account for a 95% efficiency of recovery of cAMP from the sample.

Intracellular Calcium Measurements. 2CFSMEo$^-$ or 9HTEo$^-$ cells were incubated with acetoxymethyl ester, fluo-3 (1–3 μM), and pluronic acid at room temperature for 40 min. Cultures were washed and the fluorescence of individual cells was visualized with a Zeiss standard microscope equipped with transmitted illumination (Zeiss 6 V, 15 W illuminator and epi-illumination; Zeiss 50 W dc high-pressure mercury lamp). Incident light traverses an iris, neutral density filters, an interference filter (480–490 nm), and a fluorescein filter [Zeiss interference filter (450–490 nm) and dichroic filter (510 nm)]. Fluorescence was passed through an 520-nm emission filter and an iris adjusted to the cell diameter. The emission signal was measured with a photomultiplier [Hamamatsu (Middlesex, NJ); model R464S]. The cytosolic concentration of calcium was calculated from the K_d value of the fluo-3 and the difference between fluorescence observed in a calcium-free medium versus a millimolar calcium medium in the presence of the calcium ionophore A23187 (20 μM). All studies were carried out at 37°C.

Cl$^-$ Efflux. Cl$^-$ transport was measured by radioactive ^{36}Cl$^-$ efflux (24–26). Cells were grown to confluence in MEM with 10% FBS. Cultures were rinsed twice with 2 ml of efflux buffer (140 mM NaCl/3.3 mM KH_2PO_4/0.83 mM K_2HPO_4/1 mM $CaSO_4$/1 mM $MgSO_4$/10 mM Hepes, pH 7.4/10 mM glucose). Fresh efflux buffer (1 ml) with 2 μCi of ^{36}Cl$^-$ per ml (1 Ci = 37 GBq) was added to each dish and incubated for 2 h at 37°C. Dishes were washed by dipping each into 200 ml of efflux buffer for a wash time of 8–10 s. After washing, 1 ml of efflux buffer was added, and cells were again incubated at 37°C. Samples were removed at 1-min intervals and replaced with fresh buffer. After 3 min, 1 ml of efflux buffer containing 10 μM isoproterenol, forskolin, or A23187 or 0.5 μM ionomycin was added. Additional samples were removed and replaced at 1-min intervals. The ^{36}Cl$^-$ remaining in the cells was extracted with 0.1 M HCl (1 ml) overnight at 4°C. Samples were counted in scintillation cocktail. The percentage efflux per 1-min time point was calculated as follows (24): % efflux/min = [(cpm for sample)/(average cpm in the cells for that minute)] × 100. The average cpm/1-min time interval = $(cpm_t + cpm_{t-1})/2$, where cpm_t is the cpm for the sample at min t and cpm_{t-1} is the cpm for the sample the preceding minute.

RESULTS

Four transformed cell lines were isolated from CF tracheobronchial gland cells. The origin and nomenclature of all transformed cell lines used in this study are summarized in Table 1. Immunocytochemical staining showed the SV40 T antigen in the nucleus (Fig. 1*A*). All cell lines showed cytoplasmic staining with an anti-keratin antibody (Fig. 1*B*), although the pattern is more diffuse in most postcrisis clones. Precrisis cells showed characteristic pericellular staining with the E9 monoclonal antibody directed against the cell-CAM 120/80 epithelial cell adhesion molecule (20), suggesting the presence of junctional complexes (Fig. 1*C*). The

Table 1. Colony characteristics

Cell line	Tissue origin	ΔF508	cAMP/Cl$^-$ transport	Ca^{2+}/Cl$^-$ transport
2CFSMEo$^-$	Airway	±	−	+
6CFSMEo$^-$*	submucosal	±	−	−
21CFSMEo$^-$*	gland	±	NT	NT
3ACFSMEo$^-$*		±	NT	NT
12CFBE22o$^-$*	Bronchus	−	−	−
CFNPE9o$^-$	Nasal polyp	−	−	+
CFNPE14o$^-$*	Nasal polyp	±	NT	NT
3HTEo$^-$	Trachea	−	−	+
9HTEo$^-$	Trachea	−	+	+
56FHTE8o$^-$	Fetal trachea	−	+	+
T84	Colon carcinoma	−	+	+

With the exception of T84, numbers preceding the lettering indicate colony number and number following the letters denotes tissue sample number. The o$^-$ indicates that the cells were transformed with an origin of replication-deficient SV40 genome. ±, One ΔF508 allele is present; cAMP/Cl$^-$, cAMP-dependent Cl$^-$ transport; Ca^{2+}/Cl$^-$, calcium-dependent Cl$^-$ transport; NT, not tested.
*Cell lines not previously described.

presence of antigens that localize to mucous or serous cells *in vivo* was detected in individual cell lines indicating secretory cell-specific properties (Table 2). In some cases, expression of specific secretory antigens was lost postcrisis.

Presence of the ΔF508 deletion was determined from Southern blot hybridization of PCR amplified genomic DNA probed with normal or ΔF508 specific radiolabeled oligonucleotides (Fig. 2). DNA from individuals known to be homozygous or heterozygous for the ΔF508 mutation was included as a control. Cell lines 2CFSMEo$^-$, 21CFSMEo$^-$, 3ACFSMEo$^-$, and 6CFSMEo$^-$ and nontransformed nasal polyp cells from another individual with CF (CFNPE14) are heterozygous for the ΔF508 mutation. Nasal polyp (CFNPE9o$^-$) and surface airway (CFBE22) epithelial cells do not have the ΔF508 mutation.

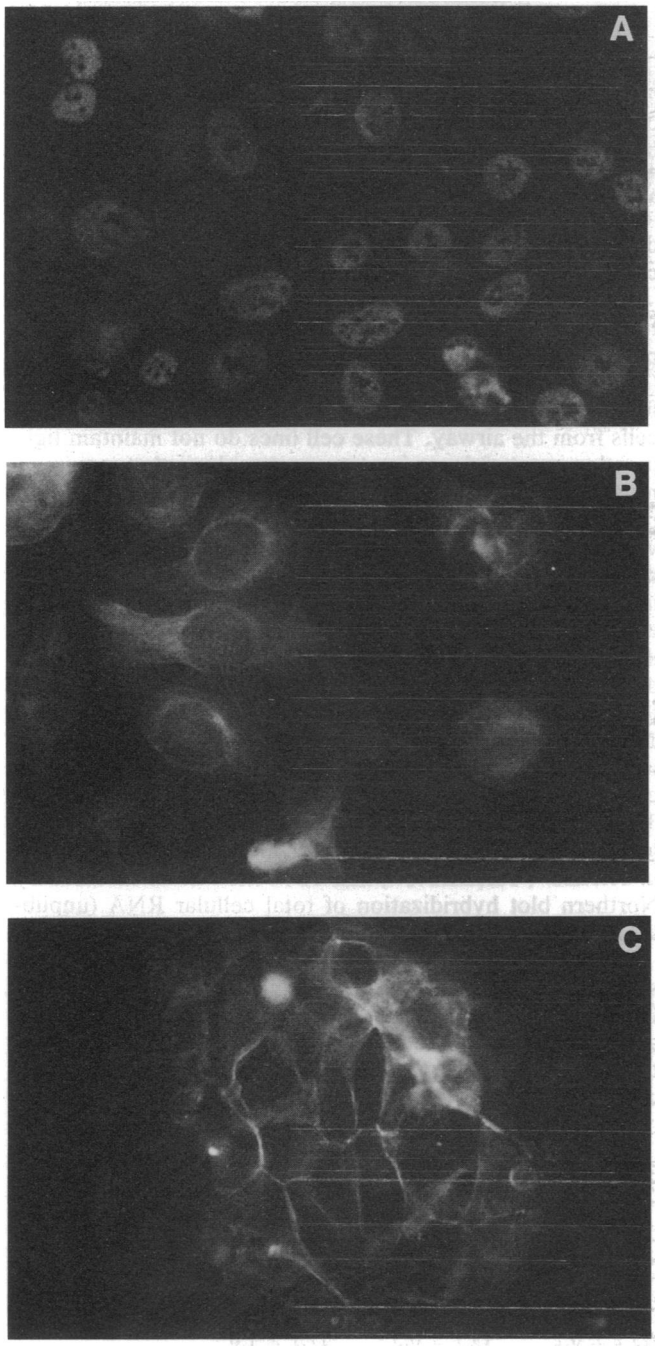

FIG. 1. Immunocytochemical staining of immortalized CF cells. Cells were stained with primary antibodies raised against SV40 T antigen (*A*), cytokeratin (*B*), or adhesion molecule cellCAM 120/80 (*C*). Cell lines are 2CFSMEo$^-$ (*A*), 6CFSMEo$^-$ (*B*), and 12CFBE22o$^-$ (*C*). Note that in the 12CFBE22o$^-$ cell line only patches of cells retain expression of the cellCAM 120/80 antigen, even at early passages.

Table 2. Antibody staining

Antibody	2CFSMEo$^-$	3ACFSMEo$^-$	6CFSMEo$^-$	*In vivo* localization
A1D3	<25/0	<25	<25/0	G, M, S
A1E11	<25/0	0	0/NT	G, M
B6E8	0/0	<25	0/NT	G, M
B6G6	NT/0	0	<25/0	G, M
B8C3	NT/0	100	0/0	G, S
B1F8	100/0	100	<25/<25	M
A8E4	<25/<25	NT	NT/0	M
A2E7	100/0	100	NT/100	S
A3B7	100/100	0	<25/NT	S
Lactoferrin	<25/<25	0	100/NT	S
Lysozyme	NT/<25	0	100/NT	S

Immunoperoxidase staining of individual clones of pSVori$^-$ transformed tracheobronchial gland epithelial cells with antibodies that recognize cell secretory products. Data are expressed as percentage of cells staining in precrisis/postcrisis cells. Data for 3ACFSMEo$^-$ are for precrisis cells only. G, goblet cell; M, mucous cell; S, serous cell; NT, not tested.

Expression of CFTR mRNA in 2CFSMEo$^-$ and 6CFSMEo$^-$ gland cell lines has been investigated. Both cell lines express CFTR message as confirmed by the presence of the predicted 627-bp fragment spanning exons 5–7 (Fig. 3). This is compared with amplified first-strand cDNA from both non-CF and surface airway CF cell lines. Thus, the CFTR mutations in the CF gland cell lines do not eliminate production of CFTR mRNA.

CF nasal polyp and gland cells were assayed for production of intracellular cAMP in response to the β-adrenergic agonist, isoproterenol, and forskolin, an activator of adenylyl cyclase. When cells were grown in MEM with 10% FBS, this analysis showed that tracheobronchial gland epithelial cells elevated cAMP in response to both isoproterenol and forskolin (Fig. 4), similar to that in nasal polyp (CFNPE9o$^-$) and non-CF transformed airway epithelial cells (18). However, response to isoproterenol and forskolin was greatly attenuated in cells grown in MLHC-8e, which contains 2.7 μM epinephrine (15, 16) (Fig. 4).

The $^{36}Cl^-$ efflux assay was used to measure Cl^- transport (Table 3). Neither CF submucosal gland cells (2CFSMEo$^-$ and 6CFSMEo$^-$) nor surface airway epithelial cells (12CFBE22o$^-$) nor nasal polyp (CFNPE9o$^-$) responded to isoproterenol or forskolin, as would be expected for CF cells. However, transformed non-CF cells responded very poorly to these agents. Surface epithelial cells (3HTEo$^-$) were unable to increase Cl^- efflux above background after addition of isoproterenol or forskolin, while fetal airway epithelial cells (56FHTE8o$^-$) showed increased Cl^- efflux after expo-

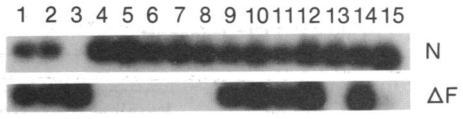

FIG. 2. Allele-specific oligonucleotide hybridization for detection of the ΔF508 mutation. Amplified DNA around the site of the ΔF508 mutation was hybridized to radiolabeled oligonucleotides specific to either the ΔF508 (ΔF) or non-ΔF508 (N) sequence at this region of the CFTR gene. DNA samples were prepared from known ΔF508/non-ΔF508 heterozygous individuals (lanes 1 and 2), a known ΔF508 homozygous CF patient (lane 3), non-CF cell lines, for comparison (lanes 4–8), and CF cell lines 2CFSMEo$^-$ (lane 9), 6CFSMEo$^-$ (lane 10), 3ACFSMEo$^-$ (lane 11), 21CFSMEo$^-$ (lane 12), CFNPE9o$^-$ (lane 13), CFNPE14 (lane 14), and CFBE22 (lane 15).

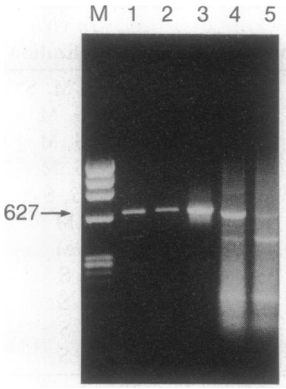

FIG. 3. CFTR mRNA expression in immortalized CF cell lines. First-strand cDNA was prepared and amplified by PCR with primers from exons 5 and 7 of the CFTR gene. Amplification products were separated by electrophoresis on a 1.5% agarose gel and stained with ethidium bromide. Arrow, predicted product (for a nonmutant CFTR gene) of 627 bp, spanning nucleotides 622–1248 of the CFTR gene. Lanes: 1, non-CF cell line 56FHTE8o$^-$ shown for comparison; 2, 2CFSMEo$^-$; 3, 6CFSMEo$^-$; 4, 12CFBE22o$^-$; 5, CFNPE9o$^-$.

sure to isoproterenol. When assayed by whole-cell patch clamp, both of these non-CF cell lines showed increased Cl^- current in response to an increase in intracellular cAMP (J.A.W., unpublished results).

Elevation of intracellular calcium was measured in response to a number of biological mediators. In 2CFSMEo$^-$ cells, histamine produced a sustained increase in internal calcium, while carbachol showed a wave-like pattern in internal calcium (Fig. 5*A*). Bradykinin produced a single spike of internal calcium (Fig. 5*A*). Three of six cells responded to 20–50 μM carbachol, five of five cells responded to 20–50 μM histamine, and four of four cells responded to 0.5–1 μM bradykinin. Similar results were obtained in 9HTEo$^-$ cells, although the repetitive "spike" of calcium release was more pronounced in these cells (Fig. 5*B*) (carbachol, 16/24; histamine, 16/17; bradykinin, 8/10) and in normal primary tracheal cells (carbachol, 2/3; histamine, 4/4; bradykinin, 1/1) (data not shown).

CF gland cells, CF nasal polyp, and non-CF surface airway cells increased $^{36}Cl^-$ efflux in response to calcium ionophore A23187 or ionomycin, agents that increase intracellular calcium concentration. However, the 12CFBE22o$^-$ cell line did not respond in this fashion (Table 3).

DISCUSSION

Analysis of the biochemical defect in CF has been hampered by the availability of suitable tissue. Isolated airway and sweat gland epithelial cells have a reduced permeability to Cl^- (1, 2). In the airways, this is believed to cause increased water uptake from the lumen, leading to production of characteristic thick, underhydrated mucus (1, 2). Cl^- secretion can be stimulated in normal, but not in CF, cells by increasing intracellular cAMP (27–29), even though accumulation of intracellular cAMP after stimulation is normal in CF cells (27, 28, 30). Regulation of calcium-dependent Cl^- currents is apparently unaffected in CF (24, 27–31). One type of calcium-dependent Cl^- current distinct from cAMP-dependent current is activated via multifunctional calcium/calmodulin-dependent protein kinase (31).

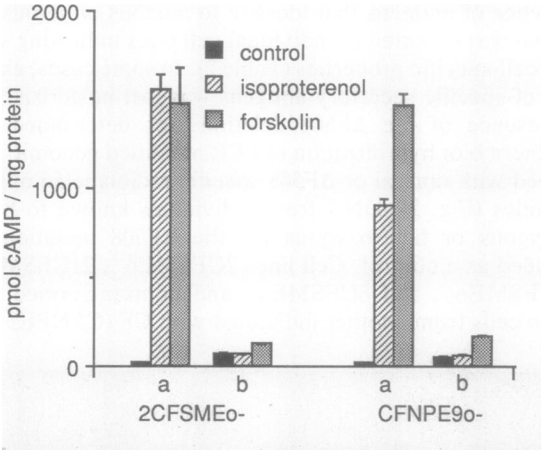

FIG. 4. Accumulation of intracellular cAMP in immortalized CF cells, grown either in serum-containing medium (MEM/10% FBS) (*A*) or in serum-free medium (MLHC-8e) (*B*) after a 1-min stimulation with either forskolin or isoproterenol. cAMP was measured by RIA and normalized to cell protein content, assuming a recovery of 95%.

Recently, several groups have described the immortalization of CF epithelial cell lines from nasal polyps (32–35), a pancreatic adenocarcinoma (36), sweat glands (35), bronchus (37), and genital duct (38). Here we describe *in vitro* transformation and immortalization of CF and non-CF epithelial cells from the airway. These cell lines do not maintain tight junctions postcrisis, so ion transport analysis that requires a monolayer with tight junctions to generate a transepithelial resistance was not possible. Instead, the $^{36}Cl^-$ efflux assay was used and indicated that tracheobronchial gland cell lines retain the CF phenotype, since they do not respond to intracellular increases in cAMP. The non-CF surface airway epithelial cells (3HTEo$^-$) also did not respond to isoproterenol or forskolin in this assay, while the fetal epithelial cells derived from tracheal explants (56FHTE8o$^-$) responded only to isoproterenol. Two other cell lines, 1HAEo$^-$ (39) and 16HBE14o$^-$ (unpublished observations), respond well to isoproterenol and forskolin in this assay, as does the colon carcinoma cell line T84. The poor responsiveness in the 3HTEo$^-$ and the 56FHTE8o$^-$ cell lines correlates with low levels of CFTR mRNA evident only by PCR. CFTR mRNA in 1HAEo$^-$, 16HBE14o$^-$, and T84 cells can be detected by Northern blot hybridization of total cellular RNA (unpublished observations).

The whole-cell patch clamp assay measures ion currents in single cells and has been used to demonstrate the presence of Cl^- currents in human airway epithelial cells (40). In this assay, both 2CFSMEo$^-$ and CFNPE9o$^-$ cells fail to respond to the lipid-soluble cAMP analogue 8-(4-chlorophenylthio)-cAMP (CPT-cAMP) (31). In contrast, 1HAEo$^-$ and 3HTEo$^-$

Table 3. Cl^- transport

Cell line	Cl^- efflux, % efflux per min				
	Baseline	Isoproterenol	Forskolin	A23187	Ionomycin
2CFSMEo$^-$	13.7 ± 1.5	13.6 ± 0.7	13.1 ± 0.3	12.8 ± 0.7	20.7 ± 0.5*
6CFSMEo$^-$	19.8 ± 1.4	19.6 ± 0.3	18.7 ± 0.5	NT	25.2 ± 0.04
12CFBE22o$^-$	8.2 ± 1.0	8.9 ± 0.3	7.8 ± 0.9	NT	9.3 ± 0.7
CFNPE9o$^-$	14.2 ± 0.7	13.4 ± 0.8	13.2 ± 0.2	19.7 ± 0.3	17.1 ± 1.0*
3HTEo$^-$	12.4 ± 0.8	13.4 ± 2.0	11.2 ± 0.8	22.4 ± 1.4	NT
56FHTE8o$^-$	6.9 ± 0.6	13.5 ± 2.4	7.6 ± 0.3	NT	17.8 ± 2.0

NT, not tested. Data were collected in the first minute after addition of the drug. Numbers are means ± SD (n = 3).

*Separate experiment, where baselines for 2CFSMEo$^-$ and CFNPE9o$^-$ ionomycin data are 9.4 ± 0.2 and 6.6 ± 0.8, respectively.

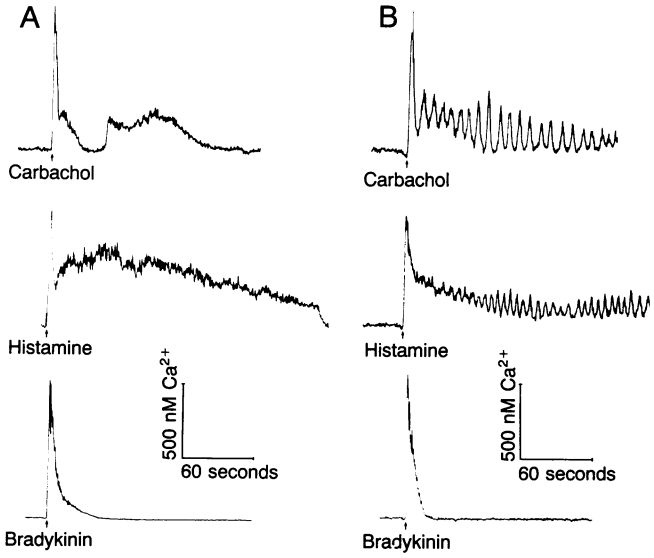

FIG. 5. Increase in intracellular calcium as measured in response to carbachol (20–50 μM), histamine (20–50 μM), and bradykinin (0.5–1 μM). (*A*) 2CFSMEo$^-$ cells. (*B*) 9HTEo$^-$ cells.

cells produce Cl^- currents in response to CPT-cAMP or forskolin (J.A.W. and P. Gardner, unpublished observations). Transformed fetal cells (56FHTE8o$^-$) also produce Cl^- currents in response to CPT-cAMP (31). This assay therefore readily differentiates normal and CF cells.

Our results indicate no gross abnormalities in calcium signaling between transformed normal airway epithelial cells and transformed CF tracheobronchial gland cells. Since calcium-dependent regulation of Cl^- current is normal in CF (31), our results suggest that activation of calcium signaling and calcium-dependent Cl^- current by cell-surface receptors might be used to circumvent defective cAMP regulation of Cl^- current. Future investigation may reveal whether different patterns of calcium signaling, such as those induced by bradykinin and histamine, produce different effects on calcium-dependent Cl^- currents.

These cells provide a valuable resource for the study of the CF defect *in vitro* and of the role of the CF gene product CFTR. Although the cell lines have lost the ability to produce tight junctions, Cl^- channels, whose activities can be measured, are still present. As is evident from the studies described above, it has become increasingly clear that growth conditions (e.g., growth medium and stage of confluence) at which experiments are conducted are critical and must be standardized to analyze Cl^- transport, mucus secretion, and/or CFTR expression.

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1. Boat, T. F. & Cheng, P. W. (1989) *Acta Paediatr. Scand. Suppl.* **363**, 25–30.
2. Quinton, P. M. (1990) *FASEB J.* **4**, 2709–2717.
3. Riordan, J. R., Rommens, J. M., Kerem, B.-S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.-L., Drumm, M. L., Iannuzzi, M. C., Collins, F. S. & Tsui, L.-C. (1989) *Science* **245**, 1066–1073.
4. Kerem, B. S., Buchanan, J. A., Durie, P., Corey, M. L., Levison, H., Rommens, J. M., Buchwald, M. & Tsui, L. C. (1989) *Am. J. Hum. Genet.* **44**, 827–834.
5. Hyde, S. C., Emsley, P., Hartshorn, M. J., Mimmack, M. M., Gileadi, U., Pearce, S. R., Gallagher, M. P., Gill, D. R., Hubbard, R. E. & Higgins, C. F. (1990) *Nature (London)* **346**, 362–365.
6. Rich, D. P., Anderson, M. P., Gregory, R. J., Cheng, S. H., Paul, S., Jefferson, D. M., McCann, J. D., Klinger, K. W., Smith, A. E. & Welsh, M. J. (1990) *Nature (London)* **347**, 358–363.
7. Drumm, M. L., Pope, H. A., Cliff, W. H., Rommens, J. M., Marvin, S. A., Tsui, L. C., Collins, F. S., Frizzell, R. A. & Wilson, J. M. (1990) *Cell* **62**, 1227–1233.
8. Kartner, N., Hanrahan, J. W., Jensen, T. J., Naismith, A. L., Sun, S. Z., Ackerley, C. A., Reyes, E. F., Tsui, L. C., Rommens, J. M., Bear, C. E. & Riordan, J. R. (1991) *Cell* **64**, 681–691.
9. Anderson, M. P., Gregory, R. J., Thompson, S., Souza, D. W., Paul, S., Mulligan, R. C., Smith, A. E. & Welsh, M. J. (1991) *Science* **253**, 202–205.
10. Barasch, J., Kiss, B., Prince, A., Saiman, L., Gruenert, D. & Al-Awqati, Q. (1991) *Nature (London)* **352**, 70–73.
11. Davis, B. (1988) in *Textbook of Respiratory Medicine*, eds. Murray, J. F. & Nadel, J. A. (Saunders, Philadelphia), pp. 374–388.
12. Sommerhoff, C. P. & Finkbeiner, W. E. (1990) *Am. J. Respir. Cell Mol. Biol.* **2**, 41–50.
13. Yamaya, M., Finkbeiner, W. E. & Widdicombe, J. H. (1991) *Am. J. Physiol.* **261**, L485–L490.
14. Yamaya, M., Finkbeiner, W. E. & Widdicombe, J. H. (1991) *Am. J. Physiol.* **261**, L491–L494.
15. Lechner, J. F. & LaVeck, M. A. (1985) *J. Tissue Cult. Methods* **9**, 43–48.
16. Gruenert, D. C., Basbaum, C. B. & Widdicombe, J. H. (1990) *In Vitro Cell. Dev. Biol.* **26**, 411–418.
17. Small, M. B., Gluzman, Y. & Ozer, H. L. (1982) *Nature (London)* **296**, 671–675.
18. Gruenert, D. C., Basbaum, C. B., Welsh, M. J., Li, M., Finkbeiner, W. E. & Nadel, J. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5951–5955.
19. Harlow, E., Crawford, L. V., Pim, D. C. & Williamson, N. M. (1981) *J. Virol.* **39**, 861–869.
20. Wheelock, M. J., Buck, C. A., Bechtol, K. B. & Damsky, C. H. (1987) *J. Cell. Biochem.* **34**, 187–202.
21. Beckstead, J. H. (1985) *J. Histochem. Cytochem.* **33**, 954–958.
22. Finkbeiner, W. E. & Basbaum, C. B. (1988) *Am. J. Pathol.* **131**, 290–297.
23. Laski, F. A., Alzner-Deweerd, B., Raj Bhandary, U. L. & Sharp, P. A. (1982) *Nucleic Acids Res.* **10**, 4609–4626.
24. Clancy, J. P., McCann, J. D., Li, M. & Welsh, M. J. (1990) *Am. J. Physiol.* **258**, L25–L32.
25. Rugolo, M., Romeo, G. & Lenaz, G. (1986) *Biochem. Biophys. Res. Commun.* **134**, 233–239.
26. Venglarik, C. J., Bridges, R. J. & Frizzell, R. A. (1990) *Am. J. Physiol.* **259**, C358–C364.
27. Widdicombe, J. H. (1986) *Am. J. Physiol.* **251**, R818–R822.
28. Welsh, M. J. & Liedtke, C. M. (1986) *Nature (London)* **322**, 467–470.
29. Frizzell, R. A., Rechkemmer, G. & Shoemaker, R. L. (1986) *Science* **223**, 558–560.
30. Boucher, R. C., Cheng, E. H. C., Paradiso, A. M., Stutts, M. J., Knowles, M. R. & Earp, H. S. (1989) *J. Clin. Invest.* **84**, 1424–1431.
31. Wagner, J. A., Cozens, A. L., Schulman, H., Gruenert, D. C., Stryer, L. & Gardner, P. (1991) *Nature (London)* **349**, 793–796.
32. Scholte, B. J., Kansen, M., Hoogeveen, A. T., Willemse, R., Rhim, J.-S., Vander Kamp, A. W. M. & Bijman, J. (1989) *Exp. Cell. Res.* **182**, 559–571.
33. Jefferson, D. M., Valentich, J. D., Marini, F. C., Grubman, S. A., Iannuzzi, M. C., Dorkin, H. L., Li, M., Klinger, K. W. & Welsh, M. J. (1990) *Am. J. Physiol.* L496–L505.
34. Jetten, A. M., Yankaskas, J. R., Stutts, M. J., Willumsen, N. J. & Boucher, R. C. (1989) *Science* **244**, 1472–1475.
35. Buchanan, J. A., Yeger, H., Tabcharani, J. A., Jensen, T. J., Auerbach, W., Hanrahan, J. W., Riodan, J. R. & Buchwald, M. (1990) *J. Cell Sci.* **95**, 109–123.
36. Schoumacher, R. A., Ram, J., Iannuzzi, M. C., Bradbury, N. A., Wallace, R. W., Hon, C. T., Kelley, D. R., Schnid, S. M., Gelder, F. B., Rado, T. A. & Frizzell, R. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4012–4016.
37. Zeitlin, P. L., Lu, L., Rhim, J., Cutting, G., Stetten, G., Kieffer, K. A., Craig, R. & Guggino, W. B. (1991) *Am. J. Respir. Cell Mol. Biol.* **4**, 313–319.
38. Coleman, L. & Harris, A. (1991) *J. Cell Sci.* 85–89.
39. Cozens, A. L., Yezzi, M. J., Chin, L., Simon, E. M., Friend, D. S. & Gruenert, D. C. (1991) *Adv. Exp. Med. Biol.* **290**, 187–196.
40. McCann, J. D., Li, M. & Welsh, M. J. (1989) *J. Gen. Physiol.* **94**, 1015–1036.