Generation and phenotype of cell lines derived from CF and non-CF mice that carry the H-2Kb-tsA58 transgene

MARCIA TAKACS-JARRETT, WILLIAM E. SWEENEY, ELLIS D. AVNER, AND CALVIN U. COTTON

Departments of Pediatrics and Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio 44106-4948

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Takacs-Jarrett, Marcia, William E. Sweeney, Ellis D. Avner, and Calvin U. Cotton. Generation and phenotype of cell lines derived from CF and non-CF mice that carry the H-2Kb-tsA58 transgene. Am J Physiol Cell Physiol 280: C228–C236, 2001.—Tracheal, renal, salivary, and pancreatic epithelial cells from cystic fibrosis (CF; cystic fibrosis transmembrane conductance regulator (CFTR) −/−) and non-CF mice that carry a temperature-sensitive SV40 large T antigen (ImmortoMouse) were isolated and maintained in culture under permissive conditions (33°C with interferon-γ). The resultant cell lines have been in culture for >1 year and 50 passages. Each of the eight cell lines form polarized epithelial barriers and exhibit regulated, electrogenic ion transport. The four non-CF cell lines (mTEC1, mCT1, mSEC1, and mPEC1) express cAMP-regulated Cl− permeability and cAMP-stimulated Cl− secretion. In contrast, the four CFTR −/− cell lines (mTEC1-CF, mCT1-CF, mSEC1-CF, and mPEC1-CF) each lack cAMP-stimulated Cl− secretory responses. Ca2+-activated Cl− secretion is retained in both CF and non-CF cell lines. Thus we have generated genetically well-matched epithelial cell lines from several tissues relevant to cystic fibrosis that either completely lack CFTR or express endogenous levels of CFTR. These cell lines should prove useful for studies of regulation of epithelial cell function and the role of CFTR in cell physiology.

Cystic fibrosis; murine epithelial cell lines; ts-sv40 large T antigen; cystic fibrosis transmembrane conductance regulator; calcium-activated chloride secretion

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) are responsible for cystic fibrosis (CF), and the primary defect in CF is the loss of cAMP-regulated anion conductance in the apical plasma membrane of epithelial cells in affected tissues (23, 33). Identification, cloning (34, 35), and heterologous expression of CFTR and mutant forms of CFTR provide direct evidence that the protein functions as a cAMP-regulated anion channel (2, 3). It is not certain how loss of apical membrane Cl− conductance alone leads to the wide spectrum of phenotypic abnormalities associated with defective CFTR (37), including aberrant regulation of Na+ channels (9, 10, 41) and non-CFTR Cl− channels (38), altered regulation of exocytosis and endocytosis (13), abnormal composition of macromolecules, and increased adherence of certain bacteria to airway epithelial cells (12). A number of mechanisms have been proposed to explain CF pathophysiology (4, 38, 39); however, unifying hypotheses are few, and the relevance of certain observations to native epithelial cell function is unclear. Furthermore, most studies of CFTR function have been performed using airway and intestinal (T84) epithelial cells (7, 8, 43) or heterologous expression systems (5). Epithelial cells from other tissues affected by CF have received relatively little attention, primarily due to lack of access to appropriate human biological material (6). The development of the CF mouse has expanded the number of epithelial cell types available for study (16, 21, 40).

Primary culture of human epithelial cells (9) and immortalized epithelial cell lines (24, 25, 27, 28, 45, 46) have been widely used in biomedical research and have proven particularly useful for studies of CF and CFTR. Many of the immortalized CF and non-CF epithelial cell lines were generated by in vitro transfection of human airway epithelial cells with either SV40 large T antigen (24, 27, 28, 46) or human papilloma virus (45). The degree of differentiation appears to vary widely, as many of the cell lines do not form functional epithelial tight junctions (24, 46). However, the CF phenotype, namely loss of cAMP-stimulated Cl− permeability, is retained (24, 27, 28, 45, 46). Immortalized cell lines have also been developed from genetically modified mice that carry an SV40 large T antigen transgene (25). Recently, Jat and coworkers (26) produced a transgenic mouse line (H-2Kb-tsA58; ImmortoMouse) that carries a thermolabile mutant of SV40 large T antigen under the control of a ubiquitous interferon-γ (IFN-γ)-inducible promoter. This mouse line has been used to generate several conditionally immortalized cell lines to date (14, 26, 32, 42, 44). The major theoretical advantages of cell lines generated in this way are 1) the avoidance of unpredictable characteristics of in vitro transfection with oncogenes (e.g., variable copy number and multiple sites of integration), 2) the ability...
to immortalize a heterogeneous population of cells from an epithelium (e.g., ciliated, goblet, and basal cells from the airway) from which clones with specific properties may be selected at a later time, and 3) the opportunity to control large T antigen levels and thereby promote differentiation by switching the cells from permissive to nonpermissive culture conditions (36). The goal of this work was to cross the Immortomouse (26) with the University of North Carolina (UNC) CF knockout mouse (CFTR S489X) (40) and develop genetically well-matched, conditionally immortalized CF and non-CF epithelial cell lines.

**METHODS**

**Animals**

Male mice, homozygous for a temperature-sensitive SV40 large T antigen transgene (Immortomouse; CBA/ca X C57Bl/10 strain; Charles River Laboratories) (26), were bred with female mice that were heterozygous for the S489X CFTR mutation (UNC; CFTR +/−; C57BL/6J X F129 strain) (40). The offspring were genotyped by polymerase chain reaction (PCR) analysis of DNA extracts from tale sections. Sections of ~1 cm in length were digested overnight at 55°C with 700 μl proteinase K [Fisher; 10 mg/ml NTES buffer: 100 mM NaCl, 50 mM Tris (pH 8.0), 50 mM EDTA, and 1% SDS]. DNA was extracted from the digests by the phenol method, and pooled DNA was rinsed with 70% ethanol and dried. DNA was extracted from the digests by the phenol method, and separated DNA was rinsed with 70% ethanol and dried. DNA was resuspended in 5 mM glucose in phosphate-buffered saline (PBS). The cell suspension was plated onto tissue culture dishes (Falcon 1058; Falcon-Becton Dickinson, Lincoln Park, NJ) coated with agglutinin (DBA; 4°C, 10 minutes) and spooled DNA was rinsed with 70% ethanol and dried. DNA was extracted from the digests by the phenol method, and separated DNA was rinsed with 70% ethanol and dried. DNA was resuspended in 5 mM glucose in phosphate-buffered saline (PBS). The cell suspension was plated onto tissue culture dishes (Falcon 1058; Falcon-Becton Dickinson, Lincoln Park, NJ) coated with agglutinin (DBA; 4°C, 10 minutes) and spooled DNA was rinsed with 70% ethanol and dried.

**Pathway analysis**

- The offspring were genotyped by polymerase chain reaction (PCR) of DNA extracts from tail sections. Section of ~1 cm in length was digested overnight at 55°C with 700 μl proteinase K (Fisher; 10 mg/ml NTES buffer: 100 mM NaCl, 50 mM Tris (pH 8.0), 50 mM EDTA, and 1% SDS). DNA was extracted from the digests by the phenol method, and pooled DNA was rinsed with 70% ethanol and dried. DNA was resuspended in 5 mM glucose in phosphate-buffered saline (PBS). The cell suspension was plated onto tissue culture dishes (Falcon 1058; Falcon-Becton Dickinson, Lincoln Park, NJ) coated with agglutinin (DBA; 4°C, 10 minutes) and spooled DNA was rinsed with 70% ethanol and dried.

**Isolation**

- The tissues were placed in a HEPES-buffered Ringer solution (HR) that contained 0.25 mg/ml collagenase type IV, and 0.1 mg/ml soy bean trypsin inhibitor. The tissues were minced with scissors and digested for 45 min at 37°C. At 15-min intervals, the tissue fragments were disrupted by repeated passage through a plastic pipette. The resulting tissue digest was passed through a nylon filter (149 × 149 μm), and the material trapped by the filter was retained for culture. The tissue fragments were resuspended in exocrine media and plated on tissue culture dishes. A combination of serum-free media and differential trypsinization was used to eliminate fibroblast contamination. The resulting cell line is identified as mPEC1. An identical procedure was used to isolate pancreatic epithelial cells from four mice that carried at least one copy of the H-2Kb-tsA58 transgene with both CFTR alleles disrupted. The resulting cell line is identified as mCT1-CF.

**Salivary gland cell lines.** Three mice that carried at least one copy of the H-2Kb-tsA58 transgene and were heterozygous for the CFTR allele were killed, and the pancreati were removed. The resulting tissue digest was passed through a nylon filter (149 × 149 μm), and the material trapped by the filter was retained for culture. The tissue fragments were resuspended in exocrine media and plated on tissue culture dishes. A combination of serum-free media and differential trypsinization was used to eliminate fibroblast contamination. The resulting cell line is identified as mPEC1-CF.

**Salivary gland cell lines.** Three mice that carried at least one copy of the H-2Kb-tsA58 transgene and were heterozygous for the CFTR allele were killed, and the submandibular salivary glands were removed. The tissues were disrupted by repeated passage through a plastic pipette. At the end of this period, the cell suspension was layered over a 4% bovine serum albumin (BSA) in HR solution and was allowed to settle for 10 min on ice. The supernatant was removed, and the resulting pellet was resuspended in exocrine media and plated on tissue culture dishes. After several passages, the contaminating fibroblasts were removed by differential trypsinization. The resulting cell line is identified as mSEC1. An identical procedure was used to isolate salivary gland epithelial cells from three mice that carried at least one copy of the H-2Kb-tsA58 transgene with both CFTR alleles disrupted. The resulting cell line is identified as mSEC1-CF.

**Tracheal epithelial cell lines.** Three mice that carried at least one copy of the H-2Kb-tsA58 transgene and were heterozygous for the CFTR allele were killed, and tracheas were removed. Isolated tracheas were cleaned of connective tissue, opened along the posterior surface, and pinned to expose the epithelial surface. The tissues were exposed to 0.1% protease (type XIV, Sigma) and 0.1% collagenase (type IV, Sigma) in Ca2+- and Mg2+-free HBSS that contained 5 mM EDTA at 37°C for 20 min. The epithelium was freed from tracheal rings and lamina propria by pipetting a steady, forceful
stream of fluid over the partially digested tracheal tissue. Tracheal epithelial cells were rinsed, centrifuged, resuspended in small airways growth media (SAGM; Clonetics), and plated on vitrogen gel-coated six-well culture plates. Cells were grown on vitrogen gels for three passages. Tracheal cell cultures were subsequently expanded and maintained on tissue culture dishes. The resulting cell line is identified as mTEC1. An identical procedure was used to isolate tracheal epithelial cells from two mice that carried at least one copy of the H-2Kb-tsA58 gene with both CFTR alleles disrupted. The resulting cell line is identified as mTEC1-CF.

**Cell culture.** Renal cell lines were maintained in culture media (CT media) that contained a 1:1 mix of Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 medium supplemented with 1.3 μg/ml sodium selenite, 1.3 μg/ml 3,5,3'-triodo-l-thyronine, 5 mg/ml insulin, 5 mg/ml transferrin, 25 μg/ml prostaglandin E1, 2.5 mM glutamine, 5 mM dexamethasone, 50,000 U/ml nystatin, 50 mg/ml streptomycin, 30 mg/ml penicillin G, and 10,000 U/ml recombinant mouse IFN-γ. mCT1 and mCT1-CF cells were maintained on plastic tissue culture dishes in CT media in a humidified 33°C incubator with 5% CO2. Media was changed every other day, and cells were passaged weekly. Cells used for experiments reported here were between passages 15 and 25. Pancreatic and salivary gland epithelial cell lines were maintained in culture media (exocrine media) that contained 1:1 mix of DMEM and Ham's F-12 medium supplemented with 0.5 mM isobutyl methyl xanthine, 2 mM glutamine, 10 μg/ml epidermal growth factor, 100 μg/ml streptomycin sulfate, 60 mg/ml penicillin G, 50,000 U/ml nystatin, 2.5% FBS, and 10,000 U/ml IFN-γ. Salivary and pancreatic epithelial cells were maintained on plastic tissue culture dishes in exocrine media in a humidified 33°C incubator with 5% CO2. Media was changed every other day, and cells were passaged weekly. Cells used for experiments reported here were between passages 10 and 25. Tracheal epithelial cells were maintained in SAGM (Clonetics) supplemented with 500 mg/ml BSA, 0.5 μg/ml human epidermal growth factor, 5 μg/ml insulin, 6.5 μg/ml 3,5,3'-triodo-l-thyronine, 10 μg/ml transferrin, 30 mg/ml bovine pituitary extract, 0.5 mg/l epinephrine, 0.5 mg/l hydrocortisone, 0.1 mg/l retinoic acid, 50 mg/l gentamycin sulfate, 50 μg/l amphotericin B, and 10,000 U/ml IFN-γ. Tracheal epithelial cells were maintained on plastic tissue culture dishes in SAGM in a humidified 33°C incubator with 5% CO2. Media was changed every other day, and cells were passaged weekly. Cells used for experiments reported here were between passages 15 and 30.

**Immunolocalization of ZO-1.** Cells were seeded onto Costar Transwell clear polyester filters and maintained under permissive conditions until the cultures became confluent. The epithelial monolayers were rinsed with PBS and fixed with 4% formaldehyde for 10 min at room temperature. The monolayers were then washed three times with PBS, permeabilized by exposure to 0.1% Triton X-100 in PBS for 5 min, and then washed three times with PBS. The monolayers were blocked with 10% FBS in PBS and then incubated with primary antibody (diluted 1:100; ZO-1; R26.4C; obtained from the Developmental Studies Hybridoma Bank at the Univ. of Iowa) for 60 min at room temperature. The cells were washed three times with PBS and then exposed to secondary antibody (diluted 1:100; FITC-conjugated AffiniPure goat anti-rat IgG; Jackson Immunochemicals) for 60 min at room temperature. The monolayers were washed three times with PBS, and a section of the filter was cut, mounted on a glass slide with a drop of Slow Fade (Molecular Probes), covered with a coverslip, and sealed with clear nail polish. The samples were examined using a confocal microscope.

**Transepithelial electrical measurements.** Cells were seeded (1-3 × 10^5 cells/filter) on collagen-coated, permeable supports (Millicell-CM 12 filters, Millipore) cut to a height of 4 mm, with the “feet” removed (17). The filter surface was coated with 125 μl/cm² calf skin collagen (Sigma) dissolved in acetic acid (7.5 mg/ml 0.2% glacial acetic acid) and allowed to dry. The collagen coating was cross-linked by exposure to ammonium hydroxide vapors (3.5% solution) for 10 min followed by immersion in glutaraldehyde (2.5%) for 10 min. This procedure was followed by a thorough rinsing in distilled water, 70% ethanol, distilled water, and finally, culture media. Filter-grown cells were cultured with IFN-γ at 33°C for 7-14 days. Media was changed every 48 h. Cell monolayers grown on modified supports were clamped between Lucite flux chambers and bathed on both sides by equal volumes (usually 6-10 ml) of Krebs-Ringer bicarbonate (KRB) solution. The solutions were circulated through the water-jacketed glass reservoir by gas lifts (95% O2, 5% CO2) to maintain solution temperature at 37°C and pH at 7.4. Transepithelial voltage difference (V_T) was measured between two Ringer-agar bridges, each positioned within 3 mm of the monolayer surface. Calomel half-cells connected the bridges to a high-impedance voltmeter. Current from an external direct current source was passed by silver-silver chloride electrodes and Ringer-agar bridges to clamp the spontaneous V_T to 0. The current required (short-circuit current, I_sc) was corrected for solution and filter series resistance. Monolayers were maintained under short-circuit conditions except for brief 3- to 5-s intervals when the current necessary to clamp the voltage to a nonzero value (usually +2 mV) was measured to calculate transepithelial resistance (R_T).

36Cl efflux. Efflux assays were performed as described previously (1, 31). Briefly, cells were grown to confluence in 35-mm tissue culture dishes. The monolayers were then washed three times with HR to remove media, and monolayers were incubated with 36Cl- (NaCl, 5 μCi/ml; Amersham, Arlington Heights, IL) in 1 ml HR for 1 h. After the cells were loaded with 36Cl-, they were rapidly washed (3 times with warmed isotope-free HR) to remove extracellular 36Cl-. Efflux of 36Cl– was measured at 30-s intervals for 8 min. The effect of elevation of cAMP on 36Cl– efflux was determined by adding forskolin (10 μM) and isobutyl methyl xanthine (100 μM) during time intervals of 2 to 8 min. After the last sample was removed, the cells were lysed by the addition of 0.5 ml of 1 N HCl for 20 min. The sample was neutralized with NaOH. All samples were mixed with liquid scintillation fluid (Ecolite, ICN) and assayed for 36Cl– activity (LS 5801; Beckman Instruments, Fullerton, CA). The apparent rate constant (r, in min–1) was calculated for each efflux interval from the following equation: r = ln(C1) - ln(C2)/(t2 - t1), where ln(C1) and ln(C2) are the natural logs of the percentage of counts remaining in the cell layer, at times t1 and t2, respectively.

**Solutions and chemicals.** HR was composed of (in mM) 10 HEPES, 138 NaCl, 5 KCl, 2.5 NaHPO4, 1.8 CaCl2, 1 MgSO4, and 10 glucose. KRB was composed of (in mM) 115 NaCl, 25 NaHCO3, 5 KCl, 2.5 NaHPO4, 1.8 CaCl2, 1 MgSO4, and 10 glucose.

**RESULTS**

Epithelial cell lines were successfully derived from trachea, pancreas, salivary gland, and renal CT from CF and non-CF mice that carried the temperature-
sensitive SV40 transgene. Each of the eight cell lines had been maintained in culture under permissive conditions (33°C plus INF-γ) for >1 yr with >50 passages. Multiple attempts to generate cell lines from the small intestine and colon were not successful. The cell lines grow as epithelial monolayers, and each cell line expresses the epithelial tight junction protein ZO-1 (Fig. 1), consistent with the formation of functional tight junctions (as revealed by measurements of transepithelial electrical resistance).

**Cell Line Genotypes**

The genotypes of the cell lines were determined by PCR analysis of genomic DNA, and the results are shown in Fig. 2. The CF cell lines (mTEC1-CF, mCT1-CF, mSEC1-CF, and mPEC1-CF) were negative for the wild-type CFTR allele (faint 200-bp bands are non-CFTR PCR products) and positive for the S489X neo-disrupted allele of CFTR. In contrast, three of the non-CF cell lines (mTEC1, mCT1, and mPEC1) carried

![Fig. 1. Immunolocalization of ZO-1 in confluent monolayers of cystic fibrosis (CF) and non-CF epithelial cells. Cells were grown to confluence on uncoated Transwell filters under permissive conditions. The monolayers were fixed, permeabilized, stained, and examined for ZO-1 expression. In each of the 8 cell lines, the ZO-1 immunoreactivity is restricted to the lateral surface of the epithelial cells near the apical membrane. A: mTEC1 cells; B: mTEC1-CF cells; C: mCT1 cells; D: mCT1-CF cells; E: mSEC1 cells; F: mSEC1-CF cells; G: mPEC1 cells; H: mPEC1-CF cells. Magnification, ×270.](http://apcell.physiology.org/Downloaded/from/10.220.33.3)
both the wild-type CFTR allele and the S489X neodisrupted allele, whereas one of the non-CF cell lines (mSEC1) was positive for the wild-type CFTR allele and negative for the S489X neodisrupted allele of CFTR. All eight cell lines were positive for the Immorto gene. The 4 CF cell lines were negative for the wild-type CFTR allele (CFTR) and positive for the disrupted CFTR allele (NEO). Three of the non-CF cell lines (mTEC1, mCT1, and mSEC1) were heterozygous for the CFTR allele (CFTR, +/−), and 1 cell line (mSEC1) carried 2 copies of wild-type CFTR (CFTR). pos con and neg con, Positive and negative control samples, respectively.

cAMP-Regulated Cl− Permeability

The CF phenotype of the resulting cell lines was determined by measuring cAMP-stimulated 36Cl− efflux from the cells. As illustrated in Fig. 3, plasma membrane Cl− permeability of each of the four non-CF epithelial cell lines was increased by exposure to forskolin/isobutyl methyl xanthine. The time course for the response was the same in each of the four cell lines (i.e., 30- to 60-s delay) and was similar to what we have observed previously in bovine pancreatic duct cells (1, 31). Cl− efflux from the CF pancreatic and CF salivary epithelial cells (mPSEC1-CF and mSEC1-CF) was increased slightly after an ~180-s delay. The increase in 36Cl− efflux observed in these two cell lines was prevented by addition of bumetanide (10 μM) to the efflux media, suggesting that the small increase in Cl− permeability was due to stimulation of Na−-K+−2Cl− co-transport-mediated efflux (data not shown). In contrast, there was no response to elevation of cAMP in the CF renal CT and CF tracheal cells (mCT1-CF and mTEC1-CF).

Electrogenic Ion Transport

Transepithelial electrical measurements were made on epithelial monolayers to determine whether the cell lines formed polarized epithelial barriers and expressed the appropriate Cl− transport phenotype. Cells were cultured on permeable supports and mounted in Ussing chambers for measurements of electrogenic ion transport (Isc). As summarized in Table 1, each of the eight cell lines formed polarized epithelial barriers and exhibited electrogenic ion transport. Amiloride-sensitive Isc was observed in renal, tracheal, and pancreatic cell lines but not in salivary cell lines. As illustrated in Fig. 4 and summarized in Table 1, each of the four non-CF cell lines exhibited cAMP-stimulated ion transport. In contrast, all four of the CF cell lines failed to respond to elevation of cAMP. However, subsequent exposure to extracellular ATP (100 μM added to the luminal bath to elevate intracellular Ca2+) elicited a large, transient increase in Isc in both non-CF and CF cell lines. Thus cAMP-stimulated Cl− secretion (CFTR-mediaged) is expressed in each of the non-CF cell lines and is missing in the CF cell lines, whereas Ca2+-stimulated Cl− secretion is common to all eight cell lines.

Effects of Nonpermissive Culture Conditions on Ion Transport Properties of mCT1 Cells

The cell lines described in this report were derived from animals that carry a temperature-sensitive form of large T antigen. We have previously shown that the amount of large T antigen and the rate of cell proliferation are reduced by placing the cultures at 39°C and removing the INF-γ from the culture media (42). To determine the effects of the switch from permissive to nonpermissive conditions on electrogenic ion transport, confluent monolayers of mCT1 cells were maintained under permissive conditions for 12 days or under permissive conditions for 8 days followed by an additional 4 days under nonpermissive conditions. Amiloride-sensitive Na+ absorption and cAMP-stimulated Cl− secretion were measured in paired monolayers. As shown in Fig. 5, cultures maintained under nonpermissive conditions for 4 days (a time when large T antigen levels fell by >95%; see Ref. 42) exhibited an increase in amiloride-sensitive Na+ absorption and a decrease cAMP-stimulated Cl− secretion.

DISCUSSION

The purpose of this work was to generate genetically well-matched, immortalized epithelial cell lines from CF and non-CF mice. The results of the 36Cl− efflux studies demonstrate that the non-CF cell lines each respond to elevation of cAMP with a characteristic increase in Cl− permeability. The small increase in 36Cl− efflux seen in mSEC1-CF and mPEC1-CF cells (after a 3-min delay) suggests that in these cell types, elevation of cAMP stimulates bumetanide-sensitive Na−-K+−2Cl− cotransport. Without concurrent stimulation of apical Cl− conductance, activation of basolat-
eral Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{−} cotransport would not be expected to increase $I_{sc}$ (see Fig. 4). The transepithelial electrical measurements provide direct evidence for cAMP-activated Cl\textsuperscript{−} secretion in non-CF cell lines but not in the corresponding CF cell lines. Therefore, as expected, the cAMP-dependent Cl\textsuperscript{−} secretory phenotype of the cell lines accurately reflects the genotypes of the animals from which the cells were derived (9, 11, 28).

**Table 1. Summary of bioelectric properties of epithelial monolayers**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>$R_T$, Ω·cm\textsuperscript{2}</th>
<th>$I_{sc}$, μA/cm\textsuperscript{2}</th>
<th>$\Delta I_{sc}$, μA/cm\textsuperscript{2}</th>
<th>$\Delta I_{sc}$, μA/cm\textsuperscript{2}</th>
<th>$\Delta I_{sc}$, μA/cm\textsuperscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>mTEC1</td>
<td>108 ± 7</td>
<td>3.2 ± 0.3</td>
<td>−1.0 ± 0.2*</td>
<td>1.4 ± 0.2*</td>
<td>27.4 ± 3.3*</td>
</tr>
<tr>
<td>mTEC1-CF</td>
<td>116 ± 50</td>
<td>2.3 ± 1.2</td>
<td>−0.6 ± 0.2*</td>
<td>0.2 ± 0.2†</td>
<td>16.7 ± 4.6*</td>
</tr>
<tr>
<td>mCT1</td>
<td>183 ± 54</td>
<td>19.3 ± 6.0</td>
<td>−16.2 ± 6.1*</td>
<td>4.3 ± 0.8*</td>
<td>24.2 ± 3.3*</td>
</tr>
<tr>
<td>mCT1-CF</td>
<td>563 ± 80†</td>
<td>2.2 ± 0.5†</td>
<td>−1.8 ± 0.4†</td>
<td>−0.1 ± 0.2†</td>
<td>27.7 ± 3.2*</td>
</tr>
<tr>
<td>mSEC1</td>
<td>382 ± 70</td>
<td>2.9 ± 0.2</td>
<td>−0.1 ± 0.1</td>
<td>9.9 ± 0.7*</td>
<td>56.9 ± 15.1*</td>
</tr>
<tr>
<td>mSEC1-CF</td>
<td>288 ± 34†</td>
<td>3.7 ± 0.5†</td>
<td>−0.1 ± 0.1†</td>
<td>−0.1 ± 0.1†</td>
<td>67.6 ± 6.7*</td>
</tr>
<tr>
<td>mPEC1</td>
<td>179 ± 18</td>
<td>4.2 ± 0.9</td>
<td>−1.0 ± 0.3*</td>
<td>9.7 ± 1.6*</td>
<td>63.2 ± 12.2*</td>
</tr>
<tr>
<td>mPEC1-CF</td>
<td>80 ± 17†</td>
<td>2.9 ± 0.9</td>
<td>−1.4 ± 0.2*</td>
<td>0.0 ± 0.2†</td>
<td>171.9 ± 6.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE; 4–5 monolayers of each cell type were assayed. Cells were seeded onto collagen-coated filters and maintained under permissive conditions for 8–12 days. The filters were mounted in Ussing chambers and bathed on both sides by Krebs-Ringer bicarbonate solution at 37°C. The short-circuit current ($I_{sc}$) and the transepithelial resistance ($R_T$) were measured (Basal). Amiloride (10 μM) was added to the luminal bathing solution to inhibit amiloride-sensitive Na\textsuperscript{+} absorption. Five minutes later, forskolin (10 μM) and IBMX (100 μM) were added to the basolateral bathing solution to elevate intracellular cAMP. Ten minutes later, ATP (100 μM) was added to the luminal bathing solution to increase intracellular Ca\textsuperscript{2+} activity. The steady-state changes in $I_{sc}$ following addition of amiloride and forskolin/IBMX and the peak increase in $I_{sc}$ in response to ATP are reported. *Drug-induced change in $I_{sc}$ is significantly different from 0 ($P < 0.05$, paired $t$-test). †Basal value or drug-induced change in $I_{sc}$ is significantly different between cystic fibrosis (CF) and non-CF cell lines ($P < 0.05$, unpaired $t$-test).
A variety of secondary defects have been identified in CF cells, including hyperabsorption of Na\(^+\) (9, 20, 23) and altered cAMP-dependent regulation of epithelial Na\(^+\) channels (10, 37, 41) and outward-rectifying Cl\(^-\) channels (38). Six of the eight cell lines that we generated exhibited small, amiloride-sensitive currents; however, Na\(^+\) hyperabsorption was not observed in the CF cell lines. This is not unexpected, since freshly isolated CF mouse trachea does not exhibit Na\(^+\) hyperabsorption compared with non-CF trachea (22). The reason for tissue- and species-specific Na\(^+\) hyperabsorption (9, 20, 23) in CF is not known but may depend on the relative and absolute levels of expression of CFTR and epithelial Na\(^+\) channel. CFTR is also known to regulate cAMP-activated, DIDS-sensitive Cl\(^-\) secretion, perhaps via release of ATP, although this hypothesis remains controversial (37, 38). CFTR-dependent activation of DIDS-sensitive Cl\(^-\) secretion is observed in mPEC1 monolayers but not in mPEC1-CF cells.
(data not shown). Thus murine pancreatic cell lines appear to retain abnormal regulation of DIDS-sensitive Cl⁻ channels and may be useful for studies of the interaction of CFTR and non-CFTR Cl⁻ channels.

It is difficult to make comparisons between the cell lines that we generated and native murine epithelia due to the paucity of ion transport data from freshly isolated tissues. Transepithelial ion transport data are not available from native murine pancreatic ducts; however, Githens et al. (19) reported that primary cultures of mouse pancreatic ducts expressed amiloride-sensitive Na⁺ absorption and cAMP-stimulated Cl⁻ secretory responses. The responses of the mPEC1 cell line resembled those reported by Githens et al. (19), but quantitative comparisons cannot be made since they showed only single traces with no summary data. We are unaware of transepithelial ion transport data from murine salivary ductal epithelial cells. A number of renal epithelial cell lines have been generated from various nephron segments, including CT (36, 42). Nearly all of the CT cell lines express amiloride-sensitive Na⁺ absorption and cAMP-stimulated Cl⁻ secretion, similar to the results obtained with the mCT1 cell line. The relatively small amiloride-sensitive Iₛｃ of mCT1-CF cells was unexpected but is probably unrelated to the lack of CFTR expression, since amiloride-sensitive Na⁺ absorption appears to be poorly retained in epithelial cell lines. We have previously shown that mCT1 cells express aquaporin-2 and vasopressin receptors (42), properties characteristic of CT principal cells. The ion transport properties of freshly isolated tissues and primary cultures of murine tracheal epithelium have been established. Grubb and coworkers (22) reported cAMP-activated Cl⁻ secretion of ~10 μA/cm² in both non-CF and CF mouse trachea, whereas primary cultures of non-CF and CF tracheal epithelium responded to elevation of cAMP with ~5 and 0 μA/cm², respectively (15). The anomalous secretory response of CF trachea to cAMP is thought to be mediated by cAMP-dependent release of Ca²⁺ and activation of Ca²⁺-stimulated Cl⁻ secretion (22). The response is absent in primary cultures of CF tracheal cells (15) and in the immortalized mTEC1-CF cell line (this report). The CAMP-activated secretory response of mTEC1 cells might be enhanced by modifications in culture conditions such as addition of cholera toxin to the media and/or growth at an air-liquid interface. The Cl⁻ secretory response elicited by elevation of intracellular Ca²⁺ in CF and non-CF trachea (~25–30 μA/cm²) (15) and primary cultures of non-CF and CF tracheal cells (~20–40 μA/cm²) (20) is similar to that observed in our immortalized tracheal cell lines (~17–27 μA/cm²) (this report).

The strategy used to generate the cell lines (CF knockout mice crossed with the ImmortoMouse) avoided the problems (variable integration site and transgene copy number) associated with in vitro immortalization of primary cell cultures. Furthermore, the use of a temperature-sensitive SV40 large T antigen provides an opportunity to control large T antigen levels and cell proliferation. The results presented above were obtained from cells maintained under permissive growth conditions (33°C with IFN-γ); however, several cell lines derived from the ImmortoMouse show tissue-specific differentiation when the cells are placed under nonpermissive growth conditions (14, 26, 42, 44). The mCT1 cells are derived from principal cells of the CT and are expected to exhibit amiloride-sensitive Na⁺ absorption. Thus the increase in amiloride-sensitive Na⁺ absorption coupled with the decrease in cAMP-stimulated Cl⁻ secretion (Fig. 5) when the cells are maintained under nonpermissive conditions is consistent with acquisition of a more differentiated transport phenotype. Additional studies will be required to examine the effect of nonpermissive growth conditions on each of the cell lines.

The cell lines that we have generated are unique in several regards: 1) they represent the first cell lines obtained from CF knockout mice, 2) the lines are derived from several tissues relevant to CF, 3) the lines are genetically well matched, and 4) the use of a temperature-sensitive SV40 large T antigen oncogene provides an opportunity to control large T antigen activity, since amiloride-sensitive Na⁺ absorption and cAMP-stimulated Cl⁻ secretion (22). The response is absent in primary cultures of murine tracheal epithelium (this report). The cAMP-activated secretory response of mCT1 cells might be enhanced by elevation of intracellular Ca²⁺ in CF and non-CF trachea (~25–30 μA/cm²) (15) and primary cultures of non-CF and CF tracheal epithelium (~20–40 μA/cm²) (20) is similar to that observed in our immortalized tracheal cell lines (~15–27 μA/cm²) (this report).

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