Gene transfer of CFTR to airway epithelia: low levels of expression are sufficient to correct Cl⁻ transport and overexpression can generate basolateral CFTR

Sara L. Farmen, Philip H. Karp, Philip Ng, Donna J. Palmer, David R. Koehler, Jim Hu, Arthur L. Beaudet, Joseph Zabner, and Michael J. Welsh. Gene transfer of CFTR to airway epithelia: low levels of expression are sufficient to correct Cl⁻ transport and overexpression can generate basolateral CFTR. Am J Physiol Lung Cell Mol Physiol 289:L1123–L1130, 2005. First published August 5, 2005; doi:10.1152/ajplung.00049.2005.—Gene transfer of CFTR cDNA to airway epithelia is a promising approach to treat cystic fibrosis (CF). Most gene transfer vectors use strong viral promoters even though the endogenous CFTR promoter is very weak. To learn whether expressing CFTR at a low level in a fraction of cells would correct Cl⁻ transport, we mixed freshly isolated wild-type and CF airway epithelial cells in varying proportions and generated differentiated epithelia. Epithelia with ~20% wild-type cells generated ~70% transepithelial Cl⁻ current of epithelia containing 100% wild-type cells. These data were nearly identical to those previously obtained with CFTR expressed under control of a strong promoter in a CF epithelial cell line. We also tested high level CFTR expression using the very strong cytomegalovirus (CMV) promoter as well as the cytokeratin-18 (K18) promoter. In differentiated airway epithelia, the CMV promoter generated 50-fold more transgene expression than the K18 promoter, but the K18 promoter generated more transepithelial Cl⁻ current at high vector doses. Using functional studies, we found that with marked overexpression, some CFTR channels were present at the basolateral membrane where they shunted Cl⁻ flow, thereby reducing net transepithelial Cl⁻ transport. These results suggest that very little CFTR is required in a fraction of CF epithelial cells to complement Cl⁻ transport because transepithelial Cl⁻ flow is limited at the basolateral membrane. Thus they suggest a broad leeway in promoter strength for correcting the CF gene transfer, although at very high expression levels CFTR may be mislocalized to the basolateral membrane.

cystic fibrosis; gene therapy; promoter; cytomegalovirus; cytokeratin-18

Cystic fibrosis (CF) is a common, lethal, autosomal recessive disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channel (35). Lung disease is the main source of CF morbidity and mortality, and in the lung, airway epithelia are a location of CFTR expression and a major site of disease (46). Gene transfer to airway epithelia could represent an important new treatment for CF lung disease (7, 13, 21, 45). Although earlier studies demonstrated that gene transfer vectors could express CFTR in the airway epithelia of people who have CF, clinical benefit has not yet been achieved in the 15 years since the gene was identified. Many questions remain in the attempt to develop CF gene transfer. For example, uncertainty persists about the percentage of corrected cells that will be required and the level of CFTR expression that will be needed.

Earlier studies showed that the endogenous CFTR promoter is quite weak, producing approximately one to two transcripts per cell (41). Even less CFTR expression may be sufficient for normal function because studies of humans with splice site variations suggest that as little as 8% of normal transcripts preserve normal lung function (6). Likewise, mouse intestine expressing 5% of normal CFTR levels showed no intestinal disease and retained ~50% of normal Cl⁻ transport (9). However, in these cases the endogenous CFTR promoter drove expression in most if not all of the epithelial cells. In contrast, a gene transfer vector will express CFTR in a smaller fraction of cells. These considerations raise the question of what promoter strength might be required in a gene transfer vector that targets <100% of airway cells and whether CFTR overexpression might have unexpected consequences.

A previous report focused on the percentage of cells expressing CFTR that were required to correct the CF Cl⁻ transport defect (16). That study used a retroviral vector with the Maloney murine leukemia virus long terminal repeat (LTR) promoter to stably express CFTR in a CF epithelial cell line. Previous studies suggested that this promoter generates expression at a level comparable to that of the cytomegalovirus (CMV) promoter (14, 23, 25), which drives CFTR expression at very high levels in airway epithelia (15, 49, 51). Cells expressing CFTR were mixed with uncorrected cells. When 20% of cells expressed CFTR, the epithelia generated ~70% of the cAMP-stimulated transepithelial Cl⁻ current that was obtained in epithelia composed entirely of corrected cells. Whether similar results would be obtained in differentiated human airway epithelia is uncertain. Moreover, the percentage of cells required to repair Cl⁻ transport with a weaker promoter driving CFTR expression remains unknown.

Many gene transfer vectors have used viral promoters because they generate high-level expression in numerous different cell types. However, there are some suggestions that overexpressing CFTR might have a deleterious effect. Studies of cultured cell lines report that overexpression reduced the rate of cell proliferation in NIH-3T3 fibroblasts with CFTR...
under transcriptional control of the LTR promoter (38), in a monkey kidney cell line (BTS) with CFTR driven by the simian virus-40 promoter (36), and in a CF bronchial epithelial cell line with a tetracycline-inducible cytokeratin-18 (K18) promoter controlling CFTR (48). In contrast, well-differentiated primary cultures of CF airway epithelia overexpressing CFTR from an LTR promoter remained stable for >6 mo (43); if CFTR had a toxic effect, we would expect those cells expressing CFTR to have died or failed to proliferate. Moreover, in transgenic mice, intestinal overexpression from the fatty acid binding promoter (53) and lung overexpression from the surfactant protein C promoter (47) had no apparent adverse effect. Thus any effect on proliferation may depend on the absolute level of expression, the cell type, and whether the cells are polarized and differentiated in an epithelium.

In this study, we further explored how the level of CFTR expression influences Cl⁻ transport in airway epithelia. For these experiments we used well-differentiated primary cultures of human airway epithelia (18) and adenovirus vectors, including helper-dependent adenovirus vectors (HD-Ad) (33, 52). We also expressed CFTR at different levels using the CMV promoter/enhancer, which generates very high levels of expression (15, 49, 51), and the K18 promoter, which is normally active in airway epithelia (4, 5).

**METHODS**

**Airway epithelia.** Airway epithelial cells were obtained from trachea and bronchi of lungs removed for donation and prepared by the University of Iowa In Vitro Models and Cell Culture Core by methods previously described (18). Cells were isolated by enzyme digestion, and freshly isolated cells were seeded onto collagen-coated Mill cell polycarbonate filters (Millipore, Bedford, MA). The cells were maintained at 37°C in a humidified atmosphere of 5% CO₂ and air. Twenty-four hours after plating, the apical medium was removed and the cells were maintained at the air-liquid interface. These cultures develop a ciliated surface within 14 days of seeding. The culture medium consisted of a 1:1 mix of Dulbecco’s modified Eagle’s medium (Gibco, Gaithersburg, MD) and freshly isolated cells were seeded onto collagen-coated Mill cell filters (Millipore, Bedford, MA). The cells were maintained at 37°C in a humidified atmosphere of 5% CO₂ and air.

**Methods.** Transepithelial ion transport. The short-circuit current (Iₛₑₜ) was measured in modified Ussing chambers (Jim’s Instruments, Iowa City, IA) as previously described (29). Epithelia were treated with forskolin (10⁻⁷ M) and 3-isobutyl-1-methylxanthine (IBMX, 10⁻⁴ M) for 18–24 h before study in Ussing chambers to minimize basal CFTR current. For conditions with symmetrical Cl⁻ concentrations, solutions on both surfaces of the epithelium contained (in mM): 135 NaCl, 5 HEPES, 1.2 MgCl₂, 1.2 CaCl₂, 2.4 K₂HPO₄, 0.6 KH₂PO₄, and 5 dextrose. To create a Cl⁻ concentration gradient, NaCl was replaced with Na-glucuronate on the apical side. For studies with an Cl⁻ concentration gradient, solutions on both surfaces of the epithelium contained (in mM): 5 HEPES, 1.2 MgSO₄, 1.2 CaSO₄, 2.4 K₂HPO₄, and 0.6 KH₂PO₄; the basolateral solution contained 139.8 mM NaCl, and the apical solution contained 135 mM Na-Glucuronate and 4.8 mM NaCl. Transepithelial voltage was clamped to zero for all studies. To measure transepithelial electrical conductance (Gₛₑₜ), the transepithelial voltage was clamped to 0.5 mV for 1 s at intervals of 50 s, and the change in current was measured. After measuring baseline current, we added the following agents sequentially: 1) apical amiloride (10⁻⁴ M), which inhibits apical Na⁺ channels and hyperpolarizes the apical membrane, thereby generating a driving force for a Cl⁻ secretory current; 2) apical 4,4'-disothiocyanostilbene-2,2'-disulfonic acid (DIDS, 10⁻⁴ M), which inhibits non-CFTR Cl⁻ channels; 3) forskolin (10⁻⁶ M), which increases cellular levels of cAMP leading to phosphorylation of CFTR (we refer to this intervention as CAMP agonists); and 4) basolateral bumetanide (10⁻⁴ M), which inhibits basolateral Cl⁻ cotransport. Under these conditions, bumetanide-sensitive Iₛₑₜ provides a measure of CFTR-dependent transepithelial Cl⁻ transport (18).

**Measurement of β-galactosidase activity.** We measured total β-galactosidase activity using a commercially available method (Galacto-Light; Tropix, Bedford, MA). After being rinsed with PBS, cells were removed from filters by incubation with 120 μl of lysis buffer (25 mM Tris-phosphate (pH 7.8), 2 mM dithiothreitol, 2 mM 1,2-diaminocyclohexane-N,N',N',N'-tetraacetic acid, 10% glycerol, and 1% Triton X-100) for 15 min 200 μl of 1:100 Galacto-Light reaction buffer was incubated for 1 h with 4 μl of each sample. Just before measuring luminescence, we added 300 μl of Galacto-Light Accelerator. Light emission was quantified in a luminometer (Analytical Luminescence Laboratory, San Diego, CA).

**Immunoprecipitation, Western blot, and phosphorylation.** Lysates made by incubation in lysis buffer (50 mM Tris·HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 2 mM Na₃VO₄, 10 mM sodium pyrophosphate, 100 mM NaF, phenylmethylsulfonyl fluoride, leupeptin, pepstatin, and aprotinin) with 1% Triton X-100 was homogenized and spun, and the soluble fraction was immunoprecipitated with anti-β-galactosidase antibody (Novus, 1:500) and complexed with protein G-Sepharose (Pierce). Protein was eluted by incubation in sample buffer (4% SDS, 100 mM dithiothreitol, 20% glycerol, 0.005% bromphenol blue, and 0.065 M Tris, pH 6.8), boiled, separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinylidene fluoride membrane (Immobilon-P, Millipore). Membranes were
we measured transepithelial Cl\(^-\) current under short-circuit conditions (Fig. 1). Interestingly, Cl\(^-\) current did not increase, and instead, it fell. Failure of Cl\(^-\) current to increase under these conditions suggests that with endogenous levels of wild-type CFTR the apical membrane does not limit transepithelial Cl\(^-\) flow. Below we further address the reduction of Cl\(^-\) current.

**Low-level CFTR expression in a fraction of cells**

We also asked whether expressing CFTR at a very low level in a fraction of epithelial cells would correct Cl\(^-\) transport. To do this we tested cells expressing CFTR from the endogenous *CFTR* promoter. We mixed freshly isolated wild-type and CF (homozygous ΔF508) airway epithelial cells in varying proportions and then used them to generate well-differentiated cultures of airway epithelia. After Ussing chamber studies, we used PCR to quantitatively determine the percentage of each cell type. This approach has the advantage that we can accurately determine the percentage of wild-type cells in the epithelium. In contrast, with gene transfer the expression level may vary from cell to cell and preclude an accurate assessment of the percentage of transduced cells.

Figure 2 shows that as the percentage of non-CF cells increased, bumetanide-sensitive Cl\(^-\) transport increased. Epithelia with ~20% wild-type cells generated ~70% of the transepithelial Cl\(^-\) current of epithelia containing 100% wild-type cells. The relationship between the percentage non-CF cells and transepithelial Cl\(^-\) current was nearly identical to that obtained with CFTR expressed under control of a retroviral LTR promoter in a CF epithelial cell line (16). These results indicate that a relatively small fraction of CFTR-expressing cells is sufficient to correct the CF epithelial Cl\(^-\) transport defect. Finding a similar relationship in studies done with the retroviral LTR promoter and the very weak endogenous *CFTR* promoter also suggests that once epithelia express a small amount of CFTR, the limitation to transepithelial Cl\(^-\) transport resides at the basolateral membrane. This conclusion is also consistent with our finding that expressing CFTR in non-CF epithelia did not increase Cl\(^-\) current.

**The CMV promoter produces more protein**

Because experiments were obtained from multiple cultures, data are presented as percentage of *I\(_{sc}\)* measured in cultures containing 100% non-CF cells. Each point is mean \(\pm\) SE of current from 6 epithelia.
(4, 40). For additional controls, we used the K18 and CMV promoters to express the β-galactosidase reporter.

Figure 3A shows that as the dose of HD-Ad-CMV-lacZ increased, β-galactosidase activity increased. This likely results from a dose-dependent increase in the number of cells transduced and an increase in expression of β-galactosidase in individual cells (single cells may be infected with more than a single adenovirus vector). HD-Ad-CMV-lacZ generated much more β-galactosidase activity than did HD-Ad-K18-lacZ. We also found similar results using the CMV promoter to produce β-galactosidase in an FG-Ad vector and an HD-Ad vector, indicating that the difference was due to the promoters rather than the vector. As an independent assay of transgene expression, we used immunoprecipitation and Western blot to assess β-galactosidase protein production (Fig. 3B). The data confirmed the β-galactosidase activity measurements, indicating that in differentiated human airway epithelia, the CMV promoter produced more protein than did the K18 promoter.

However, we were surprised to find the opposite when these promoters drove CFTR expression (Fig. 3C). At the highest dose, the K18 promoter produced more transepithelial Cl− current in CF epithelia measured as the bumetanide-sensitive $I_{sc}$ than the CMV promoter. As we observed with the β-galactosidase reporter, the CMV promoter produced much more CFTR protein than the K18 promoter (Fig. 3D). Thus, even though the CMV promoter produced more CFTR, it generated less Cl− current than the K18 promoter.

We considered several explanations for this seemingly paradoxical result. Toxicity from CFTR overexpression seemed unlikely because these epithelia maintained their transepithelial transport and electrical resistance (not shown). For the studies shown in Fig. 3, we applied vectors to the basolateral surface to allow access to basolateral adenovirus receptors (34, 42). We considered the possibility that basolateral infection preferentially infected basal cells that are not involved in transepithelial Cl− transport. To exclude this possibility, we applied vector to the apical surface in two ways. Complexing adenovirus in a CaPi coprecipitate (11, 24) and incorporating it in a thixotropic solution (37) enhance the efficiency of infection from the apical solution. As with basolateral application, when we used these infection procedures the CMV promoter generated much more transgene expression (Fig. 4). Finally, we hypothesized that CFTR overexpression might result in mislocalization to the basolateral membrane. Because in our hands CFTR immunocytochemistry has not been sufficiently sensitive to detect endogenous or small levels of CFTR, we turned to very sensitive functional assays and performed several studies to test this hypothesis.

The CMV promoter, but not the K18 promoter, generates basolateral CFTR. Under short-circuit conditions (transepithelial voltage clamped to 0 mV and the same solutions on both apical and basolateral surfaces) and in the presence of amiloride (which blocks apical Na+ channels and hyperpolarizes the apical membrane), airway epithelia transport Cl− from the submucosal to the mucosal surface (46). Most Cl− enters the cell through basolateral, electrically neutral, bumetanide-sensitive Na+−K+−Cl− cotransporters. Cl− then exits via apical

Fig. 3. β-Galactosidase activity and bumetanide-sensitive $I_{sc}$ in differentiated airway epithelia following infection with vectors containing the cytomegalovirus (CMV) and cytokeratin-18 (K18) promoters. A: β-galactosidase activity. Increasing doses of FG-Ad-CMV-lacZ, HD-Ad-K18-lacZ, or HD-Ad-CMV-lacZ were applied to the basolateral surface. Three days later, β-galactosidase activity was measured; $n = 4$. Similar results were obtained in 2 other experiments. *$P < 0.05$ compared with HD-Ad-K18-lacZ. Expression from FG-Ad-CMV-lacZ and HD-Ad-CMV-lacZ were not significantly different at any vector dose. RLU, relative light unit. B: β-galactosidase protein following expression with different vectors. Western blot analysis of differentiated airway epithelia infected with 5,000 particles/cell of HD-Ad-K18-lacZ, HD-Ad-CMV-lacZ, or FG-Ad-CMV-lacZ applied basolaterally. Three days after infection, immunoprecipitation and Western blot for β-galactosidase were performed. Arrow indicates β-galactosidase. C: bumetanide-sensitive $I_{sc}$ in CF epithelia. Epithelia were studied 3 days after basolateral infection with CMV-CFTR or K18-CFTR. *$P < 0.05$ compared with FG-Ad-CMV-CFTR. In this and other figures, symbols sometimes obscure error bars. Uninfected CF epithelia do not produce bumetanide-sensitive current (30, 44). D: CFTR expression in differentiated airway epithelia following infection with adenovirus vectors expressing CFTR from the CMV and K18 promoters. Panel shows autoradiogram following immunoprecipitation and phosphorylation of CFTR. Fully glycosylated (band C) and immature (band B) CFTR are indicated. Lane with HD-Ad-CMV-lacZ is a negative control; a nonspecific band migrates above band C in all 3 lanes.
CFTR Cl\(^-\) channels. We reasoned that if the CMV promoter drove very high levels of CFTR production, then some of the protein might inappropriately localize to the basolateral membrane. In this case, basolateral Cl\(^-\) channels could shunt some of the Cl\(^-\) that entered through Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporters back to the basolateral solution and thereby reduce net active transepithelial Cl\(^-\) transport from the submucosal to the mucosal solution. Thus, under short-circuit conditions, the CMV promoter might produce more protein but less transepithelial Cl\(^-\) transport than the K18 promoter. To test this hypothesis, we did several experiments.

First, opening apical CFTR Cl\(^-\) channels by cAMP stimulation will increase Gt. If CFTR is also present basolaterally, then cAMP agonists should also increase the conductance of that membrane and thereby further increase Gt. Although the K18 promoter generated greater transepithelial Cl\(^-\) current than the CMV promoter at high vector doses (Fig. 3C), the opposite pattern emerged from Gt measurements (Fig. 5). cAMP agonists increased Gt more when the CMV promoter drove CFTR.

Second, we reasoned that if we established a basolateral-to-apical Cl\(^-\) concentration gradient, then basolateral CFTR Cl\(^-\) channels would provide an additional pathway for Cl\(^-\) entry across the basolateral membrane and their presence would increase net Cl\(^-\) current in CF epithelia. In contrast to data obtained with symmetrical Cl\(^-\) concentrations, with a Cl\(^-\) concentration gradient more transepithelial current was produced by vector with the CMV promoter driving CFTR (Fig. 6A).

Third, if CFTR resides in the basolateral membrane, then in the presence of a transepithelial Cl\(^-\) concentration gradient it would provide a bumetanide-insensitive pathway for Cl\(^-\) entry and thus transepithelial Cl\(^-\) current. In CF epithelia expressing CFTR under control of the K18 promoter, basolateral bumetanide prevented a cAMP-induced increase in current (Fig. 6B). However, even in the presence of bumetanide, cAMP stimulated a large transepithelial current when CFTR was expressed from the CMV promoter.

Fourth, CFTR shows a significant iodine (I\(^-\)) permeability (39), whereas the basolateral Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter is relatively I\(^-\) impermeable (20). Therefore, we predicted that in Cl\(^-\)-free solutions with a transepithelial I\(^-\)–concentration gradient, cAMP agonists would stimulate current only if CFTR was present in both apical and basolateral membranes. Figure 6C shows a much greater current in CF epithelia expressing CFTR from the CMV compared with the K18 promoter.

Fifth, we hypothesized that coinfection with both the HD-Ad-K18-CFTR and the FG-Ad-CMV-CFTR vectors would decrease Cl\(^-\) current compared with infection with the K18 vector alone because of the presence of basolateral CFTR. We infected CF epithelia with the two vectors alone and together and measured transepithelial Cl\(^-\) transport under short-circuit
conditions (i.e., symmetrical Cl− concentrations). As before, we found that the K18 promoter generated more Cl− transport than the CMV promoter (Fig. 6D). However, epithelia infected with both vectors produced less current than those infected with HD-Ad-K18-CFTR alone.

All these data indicate that overexpression from the CMV promoter leads to some CFTR function in the basolateral membrane. In contrast, with the K18 promoter, functional CFTR appeared to be exclusively apical. Thus these results explain in part the apparent discrepancy between the amount of protein produced by the two promoters and the amount of Cl− current generated in epithelia.

Overexpression of CFTR from the CMV promoter in non-CF epithelia. Finding that overexpressing CFTR caused some CFTR mislocalization to the basolateral membrane could explain our finding that overexpressing CFTR from the CMV promoter reduced Cl− current in non-CF airway epithelia (Fig. 1). To further test this, we treated non-CF epithelia with FG-Ad-CMV-CFTR and found that, in contrast to the reduced Cl− current measured with symmetrical Cl− concentrations, transepithelial Cl− flow increased when there was a Cl− concentration gradient (Fig. 7A). Moreover, in the presence of a Cl− concentration gradient, bumetanide prevented cAMP-stimulated Cl− current in non-CF epithelia, but with CFTR overexpression a significant current persisted after bumetanide (Fig. 7B). Finally, cAMP stimulated a much larger I− current when epithelia were infected with Ad-CMV-CFTR (Fig. 7C).

These results support our conclusion that when CFTR is overexpressed from the CMV promoter some of the channels end up in the basolateral membrane.

DISCUSSION

In developing gene transfer to CF airway epithelia, much effort has appropriately focused on the vectors and delivery methods (12, 19). However, implementing gene transfer may also require knowledge of the proportion of epithelial cells that should be targeted and the level of CFTR expression per cell that is required to correct the CF Cl− transport defect. The need for this information also becomes apparent when one considers the assays used to evaluate gene transfer. For example, the number of vector-derived transcripts and the total activity of reporter transgenes are sometimes quantitated to evaluate various interventions (28, 40). While certainly of value, interpretation of such studies would be influenced by knowledge of whether the total level of CFTR expression or the percentage of targeted cells is more important for complementing the Cl− transport defect.

By generating epithelia from mixtures of CF and non-CF cells, we found that epithelia with ~20% wild-type cells generated ~70% of wild-type Cl− current. These results have several important implications. They indicate that expressing CFTR in a relatively small fraction of cells is sufficient to correct the CF epithelial Cl− transport defect. This result is consistent with an earlier study that used an airway cell line and a viral LTR to drive CFTR expression (16). These data also suggest that even with a relatively small fraction of cells, very little CFTR expression is sufficient to correct the CF epithelial Cl− transport defect; that is, the weak endogenous CFTR promoter was as effective as a viral LTR promoter driving recombinant CFTR expression. Thus these findings emphasize the importance of the percentage of cells expressing CFTR rather than the level of CFTR expression in individual cells.

How is it that a very low level of CFTR expressed in only a fraction of cells is sufficient to complement the CF Cl− transport defect? The nonlinear relationship between the percentage wild-type cells in the epithelium and Cl− transport suggests an explanation. First, there must be cytosolic coupling between epithelial cells. Otherwise, as the number of wild-type cells progressively increased, Cl− transport would gradually increase in a linear fashion. This conclusion is consistent with evidence for gap junctions between transporting epithelial cells (2, 3). Second, the relationship suggests that Cl− movement across the basolateral membrane must limit transepithelial Cl− flow, even with very low endogenous levels of CFTR. This conclusion is supported by our finding that expressing CFTR in non-CF epithelia did not increase Cl− current. It is also consistent with our finding that an adenovirus vector express-
ing CFTR from the CMV promoter generated no more Cl− current in CF epithelia than a vector using the K18 promoter. Although these data suggest wide latitude in the promoter strength that could be useful for CF gene transfer, they also show an unexpected effect of very high-level CFTR expression. Even though the K18 promoter produced ~2% as much transgene expression as the CMV promoter, it generated more Cl− current. We found that when markedly overexpressed, some CFTR resided in the basolateral membrane. Earlier immunohistological studies from our lab and others showed that when the CMV promoter was used for expression, CFTR was located at the apical but not the basolateral membrane (10, 29). However, it has been difficult to detect endogenous CFTR at the apical membrane because of the limited sensitivity of immunocytochemistry, and when we again evaluated immunostaining for the present studies we could not reliably detect basolateral CFTR (not shown). Likewise, we were not able to reliably biotinylate and precipitate basolateral CFTR (not shown). Thus our data emphasize the sensitivity of these functional analyses vs. immunostaining and biochemical approaches and clearly reveal the presence of basolateral CFTR Cl− channels.

These studies raise questions about the consequences of basolateral CFTR expression in human airways. First, it would not likely alter the major conclusions of previous in vivo studies of gene transfer to people with CF. In such studies, CFTR Cl− channel function was assessed by measuring the voltage across the nasal epithelium in the presence of a Cl− concentration gradient. If a vector with a strong promoter generated some basolateral CFTR, the signal might have been quantitatively greater, but not less than that produced by an equivalent vector with a weak promoter. Second, by increasing basolateral Cl− conductance, CFTR Cl− channels might depolarize basolateral membrane voltage and thereby secondarily reduce the driving force for transepithelial Na+ and Cl− transport. Whether such a change would be beneficial or deleterious for CF airway surface liquid ion concentrations and volume is unknown. Third, evidence that mislocalization of CFTR might have a physiological consequence comes from the study of the S1455X mutation, which was located in the basolateral membrane in airway epithelial cells (27). Although this mutation did not appear to cause CF (pulmonary and pancreatic function were normal), the mutation was associated with an elevated sweat Cl− concentration (26). Fourth, because the basolateral membrane is not the normal location of CFTR, it may be prudent to avoid very high-level overexpression in developing gene therapy.

Our data suggest the potential utility of the K18 promoter for CF gene transfer vectors. The K18 promoter also targeted expression to cell types in which CFTR is normally expressed (4, 5), a potential advantage if CFTR expression in cells that do not normally express it has adverse consequences. One potential disadvantage of the K18 promoter is its size of ~2.5 kb (4). This size precludes its use, at least in its present form, in adeno-associated virus vectors that show promise for gene therapy approaches and clearly reveal the presence of basolateral CFTR Cl− channels.

ACKNOWLEDGMENTS

We thank Daniel Vermeer, Pary Weber, Tammy Nesselhauf, Janice Launspach, and Theresa Mayhew for excellent assistance. We appreciate valuable assistance from the University of Iowa In Vitro Models and Cell Culture Core [supported by the National Heart, Lung, and Blood Institute (NHLBI) (HL-51670), the Cystic Fibrosis Foundation (R458-CR02 and ENGLH9850), and the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDKD) (DK-54759)], the University of Iowa Gene Transfer Vector Core [supported by the Roy J. Carver Charitable Trust, the NHLBI, the Cystic Fibrosis Foundation, and NIDDKD (DK-54759)]. M. J. Welsh is an Investigator of the Howard Hughes Medical Institute.

GRANTS

This work was supported by NHLBI Grants HL-51670 and HL-59314.

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