Olfactory epithelia exhibit progressive functional and morphological defects in CF mice

Barbara R. Grubb,1 Troy D. Rogers,1 Heather M. Kulaga,2 Kimberlie A. Burns,1 Robert L. Wonseller,1 Randall R. Reed,2 and Lawrence E. Ostrowski1

1Cystic Fibrosis/Pulmonary Research and Treatment Center, The University of North Carolina, Chapel Hill, North Carolina; and 2Center for Sensory Biology and Department of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, Maryland

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The sustentacular or “supporting” cells are interdigitated among the olfactory dendrites emanating from the ORNs (30), and although their apical borders are also in contact with the external environment, they are thought to play no direct role in odor detection (50). Various functions have been attributed to the sustentacular cells, including structural support of the ORNs (50), biotransformation of noxious chemicals (34, 50), phagocytosis (45, 46, 50), vectorial K+ transport (49, 50), and the maintenance of salt (and presumably water) balance in the neuroepithelial mucosal layer surrounding the olfactory cilia (29, 35). If the sustentacular cells are involved in maintenance of the environment in which the olfactory cilia are bathed, then a defect in ion transport in the sustentacular cell might be expected to alter this environment, possibly leading to a compromise in the integrity of the ORN structure/function.

Cystic fibrosis (CF) results from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, a cAMP-activated Cl− channel. The absence of CFTR function results in a defect in ion transport (absence of Cl− secretion and an increase in Na+ absorption) in human airways, leading to dehydration of airway surface mucus and severe lung disease (for review, see Ref. 5). In this report, we present data suggesting that ion transport defects in the OE, most likely in the sustentacular cells, of the CF mouse lead to severe progressive morphological and functional defects in the OE of this mouse model. The results may provide insight into new mechanisms regulating the loss and regeneration of OE.

EXPERIMENTAL PROCEDURES

Neonatal and adult CF mice (cfttmut/mut mice: mixed strain, BALB/c, C57BL/6, DBA/2, and 129/SVEV) as well as wild type (WT: mice heterozygous for CFTR; littermates when possible) were studied. Adult mice were euthanized by CO2 inhalation, and neonates were euthanized by an overdose of ketamine-xylazine. All studies were approved by the Institutional Animal Care and Use Committee at The University of North Carolina or Johns Hopkins University.

Genotyping. To confirm the genotype of experimental animals, genomic DNA was isolated from tail snips, using the DNeasy tissue kit (Qiagen, Valencia, CA). DNA (100 ng) was amplified with AmpliTaq Gold (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. Primers specific for the WT and the null allele (Jackson ImmunoResearch Laboratories, West Grove, PA) were used in separate reactions, and products were analyzed by agarose gel electrophoresis. The endogenous CFTR gene produces a 526-bp product, whereas the null allele produces a 357-bp product.

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Histology. Tissues for light microscopic analysis were fixed in neutral buffered formalin and embedded in paraffin, and thin sections were cut and processed for staining or immunohistochemistry using routine procedures. For electron microscopy, nasal epithelia were carefully removed by dissection (typically from the dorsal meatus), fixed in 2% glutaraldehyde-2% formaldehyde in 0.1 M Sorensen’s buffer, and post-fixed in 1% osmium tetroxide in Sorensen’s buffer. Samples for transmission electron microscopy were embedded in epon resin (Poly/Bed812; Polysciences, Warrington, PA), and 90-nm sections were cut, stained with 7% uranyl acetate-0.3% lead citrate, and viewed using a Zeiss EM900 electron microscope. Samples for scanning electron microscopy were critical point-dried using the Bal-Tec 1 CPD 030, mounted on stubs with 12-mm carbon-conductive tabs (Ted Pella, Redding, CA), coated with gold-palladium using the Hummer 10.2 Sputtering System, and viewed on the ETEC Autoscan scanning electron microscope.

Immunohistochemistry. After deparaffinization and rehydration, antigen retrieval was performed by incubating in Vector antigen unmasking solution (Vector Laboratories, Burlingame, CA) for 40 min at 99°C. Endogenous peroxidase was inactivated by incubation in 0.6% hydrogen peroxide in methanol. Nonspecific binding sites were blocked with 2% fish gelatin and 2% BSA in PBS with 0.05% Tween 20 for 2 h at room temperature. The sections were then incubated overnight at 4°C with primary antibody or control serum in blocking solution. Sections were washed and incubated with the appropriate biotin-labeled secondary antibody, followed by incubation with peroxidase-conjugated streptavidin and detection with diaminobenzidine (Sigma-Aldrich, St. Louis, MO). Antisera against olfactory marker protein (OMP; Wako Chemicals USA, Richmond, VA) was used at 1:40,000, and antisera against the sustentacular cell marker CYP2A5 [generous gift from Dr. X. Ding (15)] was used at 1:5,000. All other reagents were obtained from Jackson ImmunoResearch Laboratories.

Electroolfactograms. Electroolfactograms (EOGs) were performed on olfactory tissue from mice ranging in age from 27 to 547 days, as previously described (23). The investigators were blinded as to the mouse’s genotype. Structurally divergent odontanacs were selected for stimuli to ensure activation of a broad repertoire of receptors. All EOGs were performed at Johns Hopkins University.

RT-PCR analysis of CFTR expression. Total RNA was isolated from freshly isolated mouse tissues, using the Qiagen RNeasy kit, and reverse-transcribed into cDNA using SuperScript (Invitrogen, Carlsbad, CA). PCR was performed using standard procedures and AmpliTaq Gold (Applied Biosystems). Controls included amplifications performed on samples prepared identically with no reverse transcriptase (−RT) and amplifications performed with no added substrate (water control). Primers used to amplify mouse CFTR were 5’-ACGTTCACACCCAACTCAGGCTCC-3’ and 5’-GAAGCAGC-CAACCTCAACCCAGAAA-3’.

Nasal bioelectricity. Olfactory epithelia, easily identified by a thicker, more dense appearance than the respiratory (ciliated) epithelia, were removed from the dorsal meatus, as far posterior in the nasal cavity as possible, from age-matched WT (mean age 165.5 ± 10.5 days, n = 8 mice) and CF mice (mean age 170 ± 4.7 days, n = 7 mice). (Epithelia from both sides of the nose were studied for most mice.) This olfactory tissue was mounted on the Ussing chamber for bioelectric study, as previously described (14). The electrical measurements were made under open-circuit conditions, and the equivalent short-circuit current (Isc) was calculated from Ohm’s law, using the spontaneous electrical potential difference (PD) and PD response to constant current pulses (14). In the present study, the aperture size of the chambers was reduced to 0.015 cm² to minimize the chance of contamination of the olfactory tissue with respiratory epithelia. All tissues were studied in bilateral Krebs-Ringer bicarbonate, as described previously (14). After the tissue had equilibrated for 30 min, amiloride (10⁻⁴ M) was added to the apical side. After this response had stabilized (5 min), forskolin (10⁻⁵ M) was added to the apical side of the tissue. Drugs were purchased from Sigma. At the end of the experiment, each piece of tissue was removed from the Ussing chamber and fixed in neutral buffered formalin for histological study.

Statistics. Data are means ± SE. The data were analyzed using the Student’s t-test when only two groups were being compared. If more than two groups were compared, a one-way analysis of variance (ANOVA) or a covariance analysis (ANCOVA) was used as appropriate. The Newman-Keuls test was used to isolate differences between groups when a difference was detected using an ANOVA. The Pearson product-moment correlation coefficient was used to determine whether a significant relationship between age and EOG response was present.

RESULTS

Morphology of the CF mouse OE. Examination by light microscopy revealed a striking loss of ORNs in the OE from adult CF mice, compared with the typical multilayered appearance of the WT animals (Fig. 1, A and B). In the WT preparations, the olfactory cilia, emanating from each dendirct knob of the ORN (see Fig. 5A), lay parallel to the epithelial surface and were embedded in the neuroepithelial mucus, thus giving the surface layer (~3.3 μm, see I-bracket in Fig. 1A) a dense appearance. In the CF preparations, this surface layer was much less dense and appeared disorganized due to the absence of olfactory cilia (Fig. 1B; also see Fig. 5). Staining of thin plastic sections with Richardson’s stain revealed areas of extensive apical blebbing in the CF animals (Fig. 1D). Similar apical protrusions were infrequently observed in sections of OE from WT mice (Fig. 1C). To confirm the loss of ORNs in the CF mice, sections of CF and WT mice were immunostained for olfactory marker protein (OMP) (3). The number of OMP-positive cells was clearly reduced in the CF compared with WT animals (Fig. 2).

In the CF olfactory preparations, the sustentacular microvilli on the apical surface were more clearly visible, whereas they were obscured by the olfactory cilia in the WT OE (Fig. 1, A and B). The sustentacular cell layer appeared to be largely intact in the CF preparations, with the sustentacular cells and their apically localized nuclei clearly visible in both the WT and CF preparations (arrow, Fig. 1, A and B). However, in the CF preparations, the cytoplasm below these nuclei was greatly expanded and filled with a uniformly staining material. In contrast, in the normal preparations, a thin extension of the sustentacular cytoplasm extended to the basal lamina and was frequently obscured by the ORN cell bodies. Immunostaining with an antisera against CYP2A5, a cytochrome P-450 that is highly expressed in sustentacular cells (15), produced intense staining of the sustentacular cells of WT mice (Fig. 2E). Sustentacular cells of the CF mouse showed a generally similar pattern of staining; however, the intensity of staining was reduced compared with WT (Fig. 2F).

Age-related changes in CF OE. The degeneration in the OE of the CF mice was progressive, since there was no aberrant sustentacular cell morphology or detectable loss of ORN in the OE of the neonatal CF mouse (36 h old) compared with that of WT littermates (Fig. 3, A and B). Neither the number of ORNs nor the number of sustentacular cells differed between genotypes (Table 1). However, we could identify the CF mice, even as early as 27–30 days of age (done in a blinded study), by the aberrant appearance of the OE (Fig. 4, A and B). In these mice, the OE in the dorsal meatus region exhibited a variable but significant loss of ORNs (Fig. 4B) compared with the WT
Fig. 1. Olfactory epithelium (OE) of the adult mouse (~6 mo old) from wild-type (WT; A) and cystic fibrosis animals (CF; B). The I- brackets on the apical surface mark the zone containing the olfactory cilia and the sustentacular microvilli. The solid arrow indicates the row of sustentacular cell nuclei. The nuclei of the olfactory receptor neurons (ORNs) are seen as numerous columns of cells throughout the tissue in the WT preparation. In the CF preparation, there are only a few scattered nuclei of the ORNs present. Tissue was taken from the middle meatus at about level 14–16. Both preparations were stained with hematoxylin and eosin (H&E). OE from a WT (C) and CF mouse (D) were stained with Richardson’s stain, demonstrating the “blebbing” of the ORNs in the CF preparation.

tissue (Fig. 4A and Table 1). This degeneration extended into the region of the ethmoturbinate in the posterior cavity. The number of sustentacular cells did not differ between the CF and WT mice in the 30-day-old group (Table 1). The degree of OE degeneration in the older mice (5 mo and older, Fig. 1B) was extensive, with the OE in the entire nasal cavity (from the nasal turbinate through the ethmoturbinate; see Ref. 31 for review of nasal anatomy) exhibiting some degree of degeneration. However, the region of the dorsal meatus exhibited the most marked degree of degeneration. There was a highly significant reduction in the number of ORNs in CF compared with WT mice, but the number of sustentacular cells was not significantly decreased compared with those in the aged WT mice (Table 1).

Electron microscopy. To examine the degeneration of the OE at higher resolution, tissue from CF and WT animals (both 4 mo old) was processed for electron microscopy. With transmission electron microscopy, olfactory cilia and dendritic knobs emanating from the ORNs were regularly observed in sections of the WT animals (Fig. 5A). These were rarely observed in the CF animals. In addition, it was clear that the blebs observed with the Richardson’s stain (Fig. 1D) were extending from the underlying cells, most likely the ORNs (Fig. 5B). Scanning electron microscopy of OE from adult animals clearly revealed the morphological degeneration of the CF tissue (Fig. 5, D and F). The border between the OE and the ciliated respiratory epithelia was clearly visible in both the WT (Fig. 5C) and CF samples (Fig. 5D). In samples from WT mice, the olfactory cilia formed a tangled web, covering the surface of the epithelia (when viewed using scanning electron microscopy; Fig. 5, C and E). In contrast, the OE from CF mice showed a markedly different morphology due to the presence of the cellular protrusions (blebs), the absence of the sensory cilia, and the microvilli of the sustentacular cells now being clearly visible (Fig. 5, D and F).

Electroolfactograms. To determine whether the morphological degeneration of the OE resulted in a loss of function (ability to smell), we recorded EOGs from WT and CF mice ranging in age from 27 to 547 days. For these studies, the electrical responses to three concentrations (10^{-3}, 10^{-4}, and 10^{-5} M) of three odors [amyl acetate (AA), acetophenone (AC), and 7-al heptaldehyde (7AL)] previously shown to produce robust responses in WT mice (23) were measured.

Because there is a progressive degeneration of the OE in CF mice with age, we first examined the effect of age on the EOG response. For the WT mice, there was no significant effect of age on the response to any of the odorants at any of the concentrations tested (Supplemental Table 1S). (Supplemental data for this article is available online at the American Journal of Physiology-Cell Physiology website.) However, for the CF mice, the responses to each odorant at each concentration were highly negatively dependent on age, with the response being much more attenuated as the animal aged (with the exception of the CF response to AA at 10^{-5} M, which was not significantly correlated with age; Supplemental Table 1S).

To determine whether there was a significant difference in the magnitude of the EOG response exhibited by WT mice compared with that of the CF mice, for each odorant (at each concentration) an ANCOVA was run on the data [WT (age vs. EOG response) vs. CF (age vs. EOG response); see Supplemental Table 2S]. In each case, there was a significant difference in the response between the genotypes. For visual presentation, the EOG response calculated from the regression equations (Supplemental Table 1S) for 30-day-old WT and CF mice for each of the odorants at 10^{-3} M is plotted in Fig. 6A. For each odorant, the response of the CF animals was de-
creased 42–50% compared with the WT response. We also calculated the expected EOG response for 365-day-old WT and CF mice (again using the regression equations; Supplemental Table 1S) and plotted these data in Fig. 6B. For these mice, again, the CF EOG response was markedly decreased (65–70%) for each odorant compared with the response of the WT mice. Thus CF mice exhibit a deficit in EOG responses by 30 days of age, and the deficit becomes more severe as the animals age.

Expression of CFTR in OE. To confirm that CFTR is expressed in the OE, RNA isolated from the OE of the dorsal meatus was analyzed by quantitative RT-PCR. The results demonstrated that CFTR mRNA was clearly present in the OE, at a level greater than that found in tracheal tissue (~700 copies/ng of RNA compared with 200 copies for tracheal tissue, data from 2 mice). All three subunits of epithelial Na⁺ channel (ENaC) were also present in the RNA isolated from the OE. These data are in agreement with the in situ studies by Rochelle et al. (35). Together these results suggest that the absence of CFTR may be directly responsible for the degeneration of the OE in the CF mouse.

Bioelectrics of OE. OE were removed from the dorsal meatus of age-matched WT and CF mice and studied open circuit on the Ussing chamber. Histological examination of the tissue in the aperture of the Ussing chamber, following the Ussing chamber studies, confirmed that only OE were included in the bioelectric data (not shown). The baseline $I_{eq}$ of these tissues was $17.8 \pm 7.3$ ($n = 15$) and $38.3 \pm 10.4 \mu A/cm^2$ ($n = 14$) for WT and CF mice, respectively. The bioelectric data demonstrated that the magnitude of the amiloride-sensitive $I_{eq}$.
a measure of electrogenic $\text{Na}^+$ absorption (14), was ~12-fold greater in the CF preparations compared with the WT preparations (Fig. 7). The response to forskolin (which induces an increase in cAMP that stimulates $\text{Cl}^-$ secretion through CFTR) was significantly greater in the WT preparations than that exhibited in the CF tissue. The small response to forskolin in the CF tissue did not differ significantly from zero. These results demonstrate that CFTR may play a similar role in regulating ion transport in the OE of the mouse as in human airway epithelia (5).

Table 1. Number of olfactory cells in WT or CF olfactory epithelium

<table>
<thead>
<tr>
<th>Age</th>
<th>Genotype</th>
<th>Sustentacular</th>
<th>ORN</th>
</tr>
</thead>
<tbody>
<tr>
<td>36 h</td>
<td>WT</td>
<td>33.5 ± 2.5</td>
<td>106.5 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>32.3 ± 4.3</td>
<td>89.8 ± 4.3</td>
</tr>
<tr>
<td>30 days</td>
<td>WT</td>
<td>27.5 ± 0.99</td>
<td>163 ± 8.5*</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>24.5 ± 1.5</td>
<td>104 ± 10.7</td>
</tr>
<tr>
<td>6 mo</td>
<td>WT</td>
<td>20 ± 0.6</td>
<td>108.9 ± 0.82*</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>17 ± 1.5</td>
<td>46.7 ± 7.9</td>
</tr>
</tbody>
</table>

Values represent the number of sustentacular cells or olfactory receptor neurons (ORN) per 100 $\mu$m of basement membrane in wild-type (WT) or cystic fibrosis (CF) mice ($n = 3$ mice/group for 36 h and 6 mo; $n = 5$ mice/group for 30 days). *$P < 0.001$. 

Fig. 3. OE from neonatal (36 h old) mice. A: tissue from WT mouse. B: tissue from CF mouse. No apparent loss of ORNs can be seen in CF neonatal olfactory tissue. Tissue was from the dorsal meatus. Preparations were stained with H&E.

Fig. 4. OE from 27-day-old mice. A: tissue from WT mouse. B: tissue from CF mouse with obvious loss of ORNs. Tissue was from the dorsal meatus region at approximately the same level as the adult and neonatal preparations.

DISCUSSION

The role of CFTR in the airway epithelium, including that in the nasal cavity, has been studied extensively in CF, because in human CF patients, airway disease is the major cause of morbidity and mortality. Bioelectric defects in nasal epithelia of CF mice have been shown to be nearly identical to those exhibited by human CF airway (13), and in this respect, the CF mouse is a good model for human CF airway disease. However, a major difference between the two species is that the OE comprises ~50% of the epithelia in the murine nasal cavity (10) and only ~3% in the human nasal cavity (16). CFTR is clearly expressed in OE [this study, Rochelle et al. (35)];
however, there is little information available in the literature concerning the contribution of specific cell types to the bioelectric defects in the nasal cavity of the CF mouse (33).

In this study, the OE of CF mice were found to exhibit both progressive morphological and functional degeneration. At birth, the OE of CF mice appeared normal, but as early as ~1 mo of age, degeneration of the OE was evident. By 4–6 mo of age, many CF mice exhibited an almost complete absence of ORNs and olfactory sensory cilia, especially in the dorsal meatus region. Although not all of the OE was as severely affected as the tissue shown in Fig. 1B, very little of the OE in the entire nasal cavity of the older CF mouse appeared normal. Interestingly, although there was a marked reduction in the number of ORNs in the CF preparations, the thickness of the OE in most of the CF preparations appeared similar to that in the normal preparations (Fig. 1, A and B). In contrast, in WT mice that have undergone olfactory bulbectomy, which causes a massive loss of ORNs (but not sustentacular cells), there is a marked decrease in thickness of the olfactory layer (37). In the CF mice, it appears that the sustentacular cells may increase in

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Fig. 5. Transmission electron micrographs are shown of OE from 4-mo-old WT (A) and CF mice (B). The dendritic knobs extending from the ORNs are clearly visible (solid arrows) in the WT OE. Olfactory cilia (broken arrows) can be seen emanating from the dendritic knobs (A). In contrast, large “blebs” rather than dendritic knobs appear to emanate from the ORNs in the CF preparation (B). There are no visible cilia in the CF OE, but numerous sustentacular microvilli are clearly visible in both A and B. Scanning electron micrographs are shown of OE from WT (C and E) and CF mice (D and F). The border between the respiratory ciliated epithelia and the OE is clearly visible in the nasal tissue from both genotypes (C and D); arrows demarcate the border between the respiratory epithelia (top right) and OE (bottom left). There is more contrast between the border of the OE and the respiratory epithelia in the CF preparation (D), because the overlying OE cilia are absent and the sustentacular microvilli (as well as blebs) are clearly visible (F). In contrast, in the WT preparation, the apical OE surface is dominated by the olfactory cilia (C and E). Images are representative of 8–10 animals of each genotype.
mice have shown that ablation of genes important in the olfactory receptor signaling cascade [Golf (4), type III adenyl cyclase (53), or cyclic nucleotide-gated channel (6)] ablate the EOG response and disrupt olfactory-dependent behavior (53). In all three of these anosmic mouse models, most of the pups died at birth because of an inability to locate the mother’s nipple. However, the OE in these animals appeared histologically normal (4, 6, 53). In contrast, CF mice are able to nurse normally and only develop an olfactory deficit over time. For each of the three odorants tested in our study, the CF mice exhibited a significant decrease in the EOG response (at all 3 concentrations) compared with the response of WT animals. The progressive age-related loss of EOG response in CF mice appears to be correlated with the progressive degeneration of the ORNs.

Ussing chamber studies of CF OE revealed that the OE exhibited hyperabsorption of Na⁺ (amiloride-sensitive response), with the rate of Na⁺ absorption almost 12-fold greater in the CF than in the WT olfactory tissue. This is much greater than the difference in the rate of Na⁺ absorption between WT and CF nasal epithelia that we (14) and others (26) have previously reported (CF ~4–6-fold greater than WT). This difference most likely reflects the fact that no attempt was made to study OE exclusively in these earlier studies. Clearly, in the WT mice, both sustentacular cells and ORNs are present, and thus the bioelectric signals we recorded from our Ussing chamber preparations may have originated from either or both cell types. However, in our CF mice, the preparations we studied had a markedly reduced number of ORNs; thus the bioelectric signal was likely largely a response of the sustentacular cells. In further support of this hypothesis, we have found that the amiloride-sensitive $I_{eq}$ was not negatively correlated with age, as one would expect if the amiloride-sensitive $I_{eq}$ were originating from the ORN. In contrast, the amiloride-sensitive $I_{eq}$ measured in our 5- to 6-mo-old CF mice (31.3 ± 7.2 μA/cm²) was actually more than double that measured in 30-day-old CF mice (13.4 ± 4.1 μA/cm², n = 8). There have been few studies on the bioelectric properties of sustentacular cells. However, one report, in which sustentacular cells were studied using patch-clamp techniques identified a “leak conductance” that was partly inhibited by replacing external Na⁺.

To assess olfactory neuronal function in our mice, we recorded EOGs, which measure changes in voltage across the OE (summed generatory potential) originating from the ORNs in response to a variety of odorants (38). Previous studies in size to fill the spaces left by the degenerating ORNs. Others have reported that as rats age, the number of ORNs decreases and the volume of the sustentacular cells increases (51), which may be similar to what we observe in our CF olfactory preparations.

The ORNs, unlike other adult mammalian neurons, not only have the capacity to restore their neuronal population after injury, but functional restoration occurs as well (20). This is important for the OE, because this is the only neuronal tissue directly exposed to the external environment. The neuroepithelial mucus layer provides a carefully controlled environment for the OE and helps to protect it from environmental insults. The basal cell layer in the OE is composed of globose and horizontal basal cells (37), but the former are thought to be the primary multipotent progenitors of both the neuronal and nonneuronal cells following certain types of injury (20). In our CF OE, the basal cell layer appeared intact. However, we cannot distinguish horizontal from globose basal cells. Despite the presence of a basal cell layer, the ORNs in the CF OE do not appear to regenerate, or alternatively, degenerate at a much greater rate than they are replaced.

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![Fig. 6. A: EOG response for 30-day-old mice calculated from the regression equations (see Supplemental Table 1S, age vs. EOG response) for each genotype for the odorants at 10⁻³ M. B: EOG data calculated for 365-day-old mice using the regression equations (Supplemental Table 1S, age vs. EOG response) for each genotype for the odorants at 10⁻³ M. [Data at the other two concentrations (not shown) looked similar.] Open bars are data from WT animals, and filled bars are data from CF animals. Odorants were amyl acetate (AA), acetophenone (AC), and 7-al heptaldehyde (7AL).](image)

![Fig. 7. Ussing chamber data from intact murine OE removed from WT mice (open bars) or CF mice (filled bars). Data shown are the changes in equivalent short-circuit current ($I_{sc}$) in response to apical amiloride (10⁻⁴ M) or apical forskolin (10⁻⁵ M) addition. The response to each drug differed between genotypes (⁎P ≤ 0.01). Data are means ± SE in tissue from WT (n = 15) and CF mice (n = 14).](image)
with N-methyl-d-glucamine; thus this conductance was in part due to inward Na\(^+\) conductance (50). Although the authors did not test the effect of amiloride on this inward Na\(^+\) conductance, it may have been what we have measured as the amiloride-sensitive \(I_{eq}\) in our preparations. In addition, because the sustentacular cell microvilli, rather than the receptor cilia of the ORNs, have been reported to contain most of the amiloride-sensitive Na\(^+\) channels (29), it is likely that the sustentacular cells are the site of Na\(^+\) hyperabsorption in our Ussing chamber OE preparations. Because CFTR has been shown to down-regulate ENaC function (43), hyperabsorption of Na\(^+\) in the OE of the CF mouse implies that CFTR is also present in sustentacular cells.

Indeed, we also found a defect in cAMP-mediated ion conductance, with the response to forskolin being reduced almost sevenfold in the CF tissue compared with that in the WT OE tissue. Because it is well known that an increase in cAMP induces CFTR-mediated Cl\(^-\) secretion, the lack of response to forskolin in the CF OE could be interpreted as a defect in this Cl\(^-\) conductance. However, cAMP also acts as the second messenger for odor transduction (6, 53) via the cyclic nucleotide-gated channel, and activation of this channel would be expected to generate a current of the same polarity as that which would be seen with CFTR activation. Thus an attenuated response to forskolin in the CF preparations may in part reflect a diminished number of ORN, and thus an attenuated response in the olfactory signaling cascade, unrelated to CFTR.

The enhanced rate of Na\(^+\) absorption, possibly coupled with a defect in cAMP-mediated Cl\(^-\) secretion, would be expected to reduce the depth of the surface liquid layer (27, 28). In CF mice (cftr\(^{mT/lnc}\)), we have found that the depth of the airway surface liquid layer (ASL) was reduced almost 35% (from ~7 \(\mu\)m to 4 \(\mu\)m) on the nasal septal epithelia compared with that measured on the nasal epithelia of the WT mouse (47). It is likely that the volume of the neuroepithelial mucus layer (NML) would be similarly reduced, especially because of the very high rate of Na\(^+\) absorption that we have measured in the isolated OE. We hypothesize that the loss of sensory cilia in CF OE is a result of epithelial damage secondary to a reduced volume of NML. If the volume of NML is reduced, the olfactory cilia may be inadequately covered and the exposed olfactory cilia would be especially vulnerable to damage due to desiccation.

It is also possible that other components of the NML layer may be perturbed in CF murine nasal epithelia, which may lead to an unfavorable milieu for the sensory cilia and their associated cells. There are several studies in the literature in which the composition of the tracheal ASL in the CF mouse has been determined. One of these studies reports no significant difference in the ASL composition between WT and CF genotypes (8), whereas another study reports significantly higher concentrations of Na\(^+\) and Cl\(^-\) in the tracheal ASL of CF mice compared with that in the WT mouse (22). Another study reported that the nasal ASL layer of the CF mouse was found to have an elevated K\(^+\) concentration compared with the WT mouse, whereas the concentrations of Na\(^+\) and Cl\(^-\) did not differ between the two genotypes (12). Therefore, it may be that the compositional milieu of the NML layer is perturbed in the OE of CF mice, which may also contribute to the ciliary and ORN demise. Indeed, in zebrafish, a mutation in the oval locus affecting a component of the ciliary transport mechanism (intraflagellar transport) results in early loss of sensory cilia (48). Following loss of cilia, neuronal sensory cells, which rely on these cilia for olfactory reception, degenerate. Our studies did not resolve whether cilia loss is sufficient to induce neuronal loss. However, it is plausible that in our murine CF model, the olfactory sensory cilia degenerate as a result of desiccation, which then results in subsequent degeneration of the ORNs. It is also possible that absence of CFTR in the sustentacular cells in the CF mice may perturb the structure or function of these cells in other ways and indirectly cause loss of ORNs, or that ORNs themselves require CFTR expression. Future studies are needed to distinguish these possibilities.

Interestingly, there are studies reporting a decrease in olfactory function in human CF patients (1, 18, 19, 32, 39). One study reported 55% of the CF patients studied (mean age 28 yr) had a decreased sensitivity of smell (1). This study found (as was the case with our CF mice) that the decreased sensitivity of smell in the CF patients (age 14–53 yr, mean age 28 ± 8 yr) was negatively correlated with age of the patients \((P < 0.01)\). This loss of function appears well before an age-related decline in olfaction in a population of normal individuals (40). In human CF patients, it has not previously been suggested that the decrease in olfactory function may be related to a defect in the OE. Rather, it has been suggested that sinus infection or obstruction may be a likely cause of the CF-related partial anosmia (1, 32). [There is one early study in which CF children were reported to have an enhanced sense of smell compared with normal controls (17). However, virtually every subsequent study has refuted this finding (1, 18, 19, 39)].

In conclusion, we have identified a progressive morphological and functional defect in the OE of CF mice. We hypothesize that in the CF murine OE, similarly to human CF airway, hyperabsorption of Na\(^+\) secondary to a loss of CFTR function in the sustentacular cells leads to an enhanced rate of fluid absorption from the OE, which in turn leads to a reduction in the volume of surface liquid. Because the olfactory sensory cilia reside in this NML layer, a reduction in fluid volume would expose them to desiccation. This in turn may lead to destruction of the cilia, loss of ORN, and a loss of olfactory acuity. Although the ORN are capable of regeneration, this does not appear to take place in the CF mouse at a rate sufficient to overcome loss of ORNs. Further studies are needed to test this hypothesis.

Neuronal differentiation, survival, and regeneration are critical to the development and maintenance of normal sensory function. The importance of these pathways has led to renewed interest in understanding the molecular mechanisms responsible for these activities in normal individuals and how these processes are compromised in disease (23, 36). The CF mouse will provide a valuable tool to study various aspects of factors controlling neuronal cell degeneration/regeneration in a model of a debilitating human disease. In addition, since the nasal epithelium, in its entirety, of the CF mouse has been studied extensively and used as a model for the lower airways of the human CF patient (2, 11, 13, 14, 21, 26, 41, 42, 52, 54), our study points out the need to characterize the ion transport properties of the olfactory and respiratory epithelia independently to better model human CF airway disease and to test potential therapies.
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