CFTR and Differentiation Markers Expression in Non-CF and ΔF 508 Homozygous CF Nasal Epithelium

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Abstract

Human nasal polyps from non-CF and ΔF 508 homozygous CF patients were used to compare the expression of CFTR and markers of epithelial differentiation, such as cytokeratins (CK) and desmoplakins (DP), at the transcriptional and translational levels. mRNA expression was assessed by semiquantitative RT/PCR kinetic assays while the expression and distribution of proteins were evaluated by immunofluorescence analysis. In parallel, for each nasal tissue specimen, the importance of surface epithelium remodeling and inflammation was estimated after histological observations. Our results show that the steady-state levels of CFTR, CK13, CK18, CK14, or DP 1 mRNA transcripts in ΔF 508 CF nasal polyps were not significantly different from those of non-CF tissues. A variability in the CFTR mRNA transcript level and in the pattern of CFTR immunolabeling has been observed between the different tissue samples. However, no relationship was found between the level of CFTR mRNA transcripts and the CFTR protein expression and distribution, either in the non-CF or in the CF group. The histological observations of non-CF and CF nasal polyp tissue indicated that the huge variations in the expression and distribution of the CFTR protein were associated with the variations in the degree of surface epithelium remodeling and inflammation in the lamina propria. A surface epithelium, showing a slight basal cell hyperplasia phenotype associated with diffuse inflammation, was mainly characterized by a CFTR protein distribution at the apex of ciliated cells in both non-CF and CF specimens. In contrast, in a remodeled surface epithelium associated with severe inflammation, CFTR protein presented either a diffuse distribution in the cytoplasm of ciliated cells, or was absent. These results suggest that abnormal expression and distribution of the CFTR protein in CF airways is not only caused by CFTR mutations. Airway surface epithelium remodeling and inflammation could play a critical role in the posttranscriptional and/or the posttranslational regulation of the CFTR protein expression in non-CF and CF airways. (*J. Clin. Invest.* 1995. 95:1601–1611.) Key words: cystic fibrosis • nasal surface epithelium remodeling • inflammation • cytokeratins • desmoplakins

Introduction

Cystic fibrosis (CF),[1] the most common and severe autosomal recessive disease amongst the Northern American and European population, is characterized by a defect in cyclic AMP-dependent chloride channel activity in a number of tissues, in particular the respiratory tract tissue (1). It is caused by mutations in the gene coding for the cystic fibrosis transmembrane conductance regulator protein (CFTR) (2, 3, 4). In human airways, the gene coding for CFTR is expressed very early in the fetus, where the CFTR protein is restricted to the cytoplasm in nondifferentiated airway epithelial cells, and to the apical domain of the ciliated cells in differentiated airway epithelium (5), as previously described in adults (6, 7). It has been shown in other epithelial tissues, such as the human intestine, that the levels of CFTR mRNA increase with cellular differentiation (8). Nevertheless, the increased levels of CFTR mRNA in differentiated cells may be accompanied by a decreased CFTR protein level that indicates a translational control of CFTR (9). As reported by Sood et al. (9), the functions of the CFTR protein are modulated by complex interactions of regulatory elements after translation. The regulation of CFTR expression is probably rather more complex to apprehend in pathological situations such as cystic fibrosis which associates a mutation in the CFTR gene with changes in the epithelial cell phenotype, due to injury of the airway epithelium by infection and inflammation. In CF, there is evidence that suggests that the failure of the predominant mutation, deletion of the amino acid residue Phe 508 (ΔF 508) was due both to the absence of the protein on the apical membrane and to the reduced time of opening of the mutated CFTR channel (10, 11). The diffuse localization of the major mutant ΔF 508 CFTR in the cytoplasm of airway surface epithelial cells (7) is considered as being directly related to an intracellular abnormal maturation and trafficking of the protein (10). Recently, we demonstrated that abnormalities of CFTR protein expression and distribution, similar to that present in ΔF 508 homozygous CF respiratory tissue, can be observed in remodeled airway surface epithelium of non-CF patients (12). We have also observed that protein markers of epithelial differentiation, such as cytokeratins and desmoplakins, could be very useful to define the dedifferentiation and remodeling of the airway surface epithelial cells observed in nasal polyps.

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1. *Abbreviations used in this paper:* CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; CK, cytokeratin; DP, desmoplakin; PA, *Pseudomonas aeruginosa;* RT, reverse transcription, SE, surface epithelium.

Both several genetic and acquired factors may be implicated in the regulation of CFTR expression at the airway level. Thus, since surface epithelium injury is particularly frequent in CF airways, the altered ΔF 508 CFTR distribution in CF respiratory tract may not only be associated with the CFTR gene mutation but also with the surface epithelium dedifferentiation and remodeling. To better define the pathway by which the expression and distribution of the wild-type and the ΔF 508 proteins may vary in epithelial respiratory cells, we have analyzed, in association with the CFTR mRNA transcript levels, the pattern of CFTR immunolabeling in non-CF and ΔF 508 homozygous CF nasal tissue. To take into account the degree of airway surface epithelium remodeling, we have also analyzed the mRNA transcript levels and the protein distribution of epithelial cell differentiation markers, such as cytokeratins (CK) and desmoplakins (DP). The degree of inflammation was also evaluated after histological observations.

Our present study indicates no evidence of significant correlation between the CFTR mRNA level and the pattern of CFTR protein distribution in non-CF and ΔF 508 CF nasal polyp tissue. However, this data shows that inflammation and surface epithelium remodeling may influence the expression and distribution of the wild-type and the ΔF 508 mutant CFTR protein in nasal tissue. Thus, we have surprisingly detected a normal expression and distribution of CFTR in the respiratory epithelium of one ΔF 508 homozygous CF patient presenting low levels of inflammation and remodeling. Our present study suggests that environmental factors, such as the inflammation of the lamina propria and the remodeling of the nasal surface epithelium, could represent acquired factors responsible for an abnormal CFTR distribution that may increase the severity of the CF airway disease and directly affect the pulmonary phenotype.

Methods

Human nasal tissue. Nasal polyps, obtained from non-CF or ΔF 508 homozygous CF patients were immediately frozen after resection and stored in liquid nitrogen until used.

Non-CF patients. Nasal polyps were removed from eight non-CF subjects (aged 22–81 yr, median: 60 yr) for reasons of discomfort and obstruction. None of the patients suffered from allergies or any other immunological disorder.

CF Patients. Nasal polyps were collected in five ΔF 508 homozygous CF patients (aged 7.5–27.6 yr, median: 16.2 yr). The diagnosis of CF was suspected due to the typical symptoms of gastrointestinal and pulmonary disease and was subsequently confirmed by pathological chloride concentrations (> 90 mmol Cl^-/L in all patients) in Gibson-Cooke pilocarpine ionophoresis sweat tests. The median age at diagnosis was 3 mo (range 0–25 mo). Patients were identified to be homozygous for the ΔF508 deletion by 12% PAGE of CFTR exon 10 PCR products (13). All patients were pancreas insufficient. Table I compiles the clinical status at the date of nasal polyps resection. The youngest patient (CF3) was born with a meconium ileus. It is important to note that one polyp specimen came from a transplanted CF patient (CF 1). Patient CF1 suffered a dramatic downhill course with his lung disease during early adulthood. Vital capacity dropped from 110% predicted by the age of 19 yr to 50% predicted one year later. After becoming immobilized and orthodyspneic, he received a heart-lung transplant at the age of 22 yr. Thereafter, weight, height, and physical activity returned to normal.

RNA extraction. Before extraction of RNA from frozen nasal polyp tissues, glassware was sterilized overnight at 180°C, and all solutions and plasticware were treated for at least 12 h with 0.1% (by vol) aqueous diethylpyrocarbonate solution to inactivate RNases. RNA was prepared twice from each polyps. Frozen nasal tissue was disrupted with an ultraturrax homogeniser by two pulses of 2 s, lysed with a seven- to ninefold excess volume of 6 M guanidinum isothiocyanate, 5 mM sodium citrate, pH 7.0, 0.1 M 2-mercaptoethanol and 0.5% lauroylsarcosine. The total RNA was then pelleted by ultracentrifugation (15 h, 20°C; 35,000 rpm, SW50.1 rotor; Beckman Instrs., Inc., Fullerton, CA) through a CsCl cushion (14).

Quantitation of mRNA levels by RT/PCR kinetic. The amounts of mRNA transcripts of CFTR (exons 3-6A), CK 13 (marker of squamous metaplasia), CK 18 (marker of ciliated and secretory epithelial cells), CK 14 (marker of basal cells), and DP (desmosomal proteins) were quantified by reverse transcription (RT) and PCR kinetic assays. Aldolase mRNA was chosen as the internal standard of a constitutively expressed housekeeping gene, in order to assess any degradation of the RNA and to allow a quantitative sample-to-sample comparison (15). Oligodeoxynucleotides were synthesized using the phosphoamidite method. The following primers were made for the detection of mRNA transcripts:
aldolase sense primer oald 141N 5′-GGCAAGGGCATCCTGGCTGCAGA, aldolase antisense primer oald583T 5′-TAACGGGCCAGAACATTGGCATT, CFTR sense primer (exon 3) 5′-AGAATGGGATAGAGAGCTGGCTTC, CFTR antisense primer (exon 5/exon 6A boundary) for reverse transcription 5′-GTGCCAATGCAAGTCCTTCATCAA, CFTR antisense primer (exon 5) for PCR 5′-TTCATCAAATTTGTTCAGGTTGTTG, cytokeratin 13 sense primer 5′-CTGTTGCAGAGACGGAGTGC, cytokeratin 13 antisense primer 5′-GCCACCAGAGGCATTAGAGG, cytokeratin 18 sense primer 5′-AGTCTGTGGAGAACGACATCC, cytokeratin 18 antisense primer 5′-TGGTGCTCTCCTCAATCTGC, cytokeratin 14 sense primer 5′-GAGGAGACCAAAGGTCGCTAC, cytokeratin 14 antisense primer 5′-GTGAAGTGCTTGGGCAGGAGAG, desmoplakin 1 and 2 sense primer 5′-CCGACTGACTTATGAGATTGAAG, desmoplakin 1 and 2 antisense primer 5′-GATTTTCACCAGAAGGCTCTCTC.

For cDNA synthesis, 10 U avian myoblastoma virus reverse transcriptase (Stratagene Inc., San Diego, CA) were added to the 10-μl reaction volume of 1 μg denatured RNA, 1 μM oligonucleotide primer, 0.2 mM of each dNTP, 75 mM KCl, 10 mM dithiotreitol, 3 mM $MgCl_2$, and 50 mM Tris-HCl, pH 8.3, and then incubated for 30 min at 37°C. The PCR assay (50-μl reaction volume covered with 50 μl mineral oil) contained 0.5 μM of each oligonucleotide primer, 0.2 mM of each dNTP, 3 μl of the RT reaction mix, 0.002% gelatine, 1.5 mM $MgCl_2$, 50 mM KCl and 10 mM Tris-HCl, pH 8.0. After DNA denaturation at 92°C for 5 min, PCR was run for 18–38 cycles in a thermal cycler, 7-μl aliquots were withdrawn in intervals of four cycles and subjected to 2% agarose gel electrophoresis. We titrated for the first reaction cycle, when the cDNA became visible by ethidium bromide fluorescence during the late exponential phase of PCR (16) and could be quantified by a benchtop scanner. This procedure yields an estimate of mRNA concentration within half an order of magnitude, if the cDNA is smaller than 800 bp in size and the initial template concentration is within the range of 10^5–10^8 transcript molecules/μg RNA (15). The thermal cycler amplified cDNA product (1.78±0.02-fold) during one reaction cycle of the exponential phase of PCR, i.e., the amount of PCR product increased by one order of magnitude within four reaction cycles.

Histological characteristics of the surface epithelium. For the histological observations of non-CF or CF nasal polyps, frozen tissue samples were embedded in OCT (Miles Tissue Tek, Elkhart, IN), immersed in liquid nitrogen, and stored at −80°C. 5 μm thick cryosections deposited onto gelatine-coated glass slides were then air-dried and stained with hematoxylin/phloxin/safranin (HPS). Sections were then analyzed throughout the length of the surface epithelium varying from 5 to 10 mm corresponding to ~ 500–1,000 cells. Five surface epithelium types were distinguished according to their morphological aspect that corresponded either to (*a*) slight basal cell hyperplasia (2–4 basal cell layers; nearly a normal surface epithelium), (*b*) moderate basal cell hyperplasia, (*c*) advanced basal cell hyperplasia, (*d*) squamous metaplasia, or (*e*) exfoliated surface epithelium.

Table I. Clinical and Respiratory Function Characteristics of ΔF 508 Homozygous CF Patients at the Date of Nasal Polyp Resection

Patient	Age	Age at diagnosis	Sex	Pancreatic status	Onset of *Pseudomonas aeruginosa* colonization	Respiratory function		Shwachman score
						FVC	FEV1	
	yr	*mo*			*yr*	%	%	
CF1	27.6	25	Male	PI	19	70	50	60
CF 2	21.5	3	Male	PI	9	70	65	75
CF 3	7.5	0	Male	PI	—	65	57	90
CF 4	12.2	2	Female	PI	—	79	58	90
CF 5	12.1	4	Female	PI	10	72	69	90

PI, pancreatic insufficiency

Immunohistochemistry of CFTR protein and of epithelial markers of differentiation. For immunohistochemical analysis, the procedures of sample embedding, cryofixation, and cryosection were similar to those used for histological analysis. The sections were stored at −20°C after air-drying. The samples were then rehydrated in 0.1 M PBS, pH 7.2, for 10 min and rinsed in PBS-1% bovine serum albumin. Indirect immunofluorescence assays were carried out as previously described (12) using the following mouse monoclonal primary antibodies: anti CFTR (MATG 1061; Transgène, Strasbourg, France, raised against a synthetic peptide corresponding to the amino acid sequence 503–515 without the residue 508, thus equivalent to the ΔF 508 epitope in the NBF1 domain of the human CFTR protein, dilution 1:200; MATG 1104, Transgène, raised against a synthetic peptide corresponding to the amino acid sequence 722–734 of the R domain of the human CFTR protein, dilution 1:200; mAb 24-1 (Genzyme Corporation, Cambridge, MA) raised against the amino acid sequence 1377–1480 of the COOH-terminal domain of CFTR, dilution 1:200), anti CK 13 (clone KS-1A3, ref. C-0791, 1:800 dilution; Sigma Chemical Co., St Louis, MO), anti CK 18 (clone CY-90, dilution 1:2,000; Sigma Chemical Co.), anti CK 14 (LL002, dilution 1:10; Dr. E.B. Lane, CRC Cell Structure Research Group, Dundee DD1 4HN, UK), anti desmoplakin 1 and 2 (clone DP 1 and 2-2.15, dilution 1:10; Boehringer Mannheim France S.A., Meylan, France). Negative controls were performed using nonimmune mouse IgG fraction (ref. M-7769; Sigma Chemical Co.). Secondary antibodies goat biotinylated anti-mouse IgG fractions (Boehringer Mannheim) and streptavidin-fluorescein isothiocyanate (Amersham International, Amersham, UK) were used at a 1:50 dilution. The sections were counterstained with Harris hematoxylin solution for 10 s, mounted in citifluor antifading solution (Agar Scientific, Stansted, UK), and observed with a Zeiss Axiophot microscope (Zeiss, Le Pecq, France) using epifluorescence and Nomarski differential interference illumination.

Inflammation, surface epithelium remodeling, and CFTR scores. To characterize the different nasal polyp specimens, scores of inflammation, of surface epithelium remodeling and of CFTR protein distribution were applied to each tissue sections. The ranking was done blinded.

The score of inflammation was qualitatively evaluated on HPS-stained tissue sections by analyzing the presence of inflammatory cells, in either the surface epithelium or the lamina propria. Three scores were identified according to the inflammatory cell density (1, rare and diffuse inflammation; 2, moderate inflammation; 3, severe inflammation).

The score of surface epithelium remodeling, varying from 1 to 5, was determined by both morphological observations and immunocytochemical analysis of markers of epithelial differentiation (Table II).

Since we have previously shown that CFTR protein is normally localized at the apical membrane of ciliated cells in non-CF pseudostratified surface epithelium (7), we applied to each tissue section a coefficient, the CFTR score, which corresponded to the percentage of ciliated cells multiplied by the ratio of the apical CFTR (0 to 1). This semiquantitative value took into account both the proportion of ciliated cells and the presence of apical distribution of the CFTR protein. Three groups were separated for the ciliated cells ratio: ≥ 50% corresponding to the normal value, 50–10%; 10–0%. The ratio of apical CFTR, assessed to apprehend the distribution of CFTR in apical plasma membrane, was semiquantitatively evaluated after immunohistolocalization of CFTR by analysis of 10 different microscopic fields of nasal tissue sections (×40). The ratio of apical CFTR was scored 1 when apical CFTR was observed in 100% of the ciliated cells, 0.5 when only 50% of ciliated cells presented an apical CFTR labeling, or 0 when the apical CFTR was not observed in any of the epithelial cells.

For example, a nearly normal surface epithelium, presenting an apical CFTR distribution in ciliated cells, was characterized by a score of $50 \times 1 = 50$, and surface epithelium associated with no apical CFTR protein expression was scored 0.

Statistical analysis. The results of the quantitation of CFTR mRNA transcripts in the non-CF and the CF population were expressed as geometric mean values. Statistical differences among these geometric means were analyzed by the Mann and Whitney nonparametric test. The correlations between different scores were analyzed by the Spearman nonparametric regression test.

Results

mRNA transcripts of CFTR and markers of epithelial differentiation. To examine the contents of the mRNA transcripts of CFTR, CK 13, CK 18, CK 14, and DP 1 in the total mucosa of five non-CF and five ΔF 508 homozygous CF nasal polyps, we used RT/PCR kinetic assays which exploit the sensitivity and specificity of PCR and allow the quantitation of the mRNA transcript levels over five orders of magnitude (Fig. 1). Confidence intervals for the relative amounts of mRNA transcripts ranged within a factor of two to five, if at least 10^4 molecules of specific transcript were present in the tissue sample. By incorporating radioactive nucleotide into PCR product, the level of aldolase mRNA in nasal polyps was determined to be 40–70 amol/μg RNA (~ 0.1% of total mRNA) (16). Table III displays the relative amounts of CFTR, CKs and DP 1 mRNA transcripts normalized to aldolase mRNA transcript in the non-CF and ΔF 508 homozygous CF nasal polyps. The mRNA transcript levels in the non-CF and the CF group were evaluated by the geometric means of each individual sample value. For each sample, the value for mRNA transcript level corresponded to the geometric mean of at least three independent RT/PCR kinetics. The assay-to-assay variance varied between a factor of 2.6 to 3.3 for the five different analyzed transcripts (CFTR, CK 13, CK 18, CK 14, DP 1), i.e., about two cycles of PCR during the exponential phase. The sample-by-sample variation was either within (DP 1) or just 1.5–3-fold larger (CFTR, CK 18, CK 14) than the assay-to-assay variance of single specimens. The maximal sample-to-sample variations were 25-fold

Table II. Classification of Surface Epithelium Remodeling (SE) Scores According to the Morphological Aspect and the Immunocharacterization of Nasal Polyp Tissue

SE remodeling score	Morphological aspect	CK 13	CK 18	CK 14	DP 1 and 2
1	Slight basal cell hyperplasia (2 to 4 basal cell layers)	+ Basal cells	+ Suprabasal cells	+ Basal cells	+
2	Moderate basal cell hyperplasia (≥ 4 basal cell layers, presence of ciliated cells)	+ Basal cells	+ Suprabasal cells	+ Basal cells	+
3	Advanced basal cell hyperplasia (≥ 4 basal cell layers, depletion or absence of ciliated cells)	+ Basal cells	+ Suprabasal cells	+ Basal cells	+
4	Squamous metaplasia	+ Superficial cells	−	−	++
5	Exfoliated SE				

CKs are detected in basal cells, suprabasal cells (located above the basal cell layers) or squamous superficial cells. Desmoplakins 1 and 2 (DP 1 and 2) labeling presents a characteristic punctuate pattern observed between cells. ++, intense positive labeling; +, positive labeling; −, negative for negligible labeling.

for CFTR, 5-fold for CK 18, 11-fold for CK 14, and 4-fold for DP 1 mRNAs. In contrast, CK 13 mRNA content was identified in highly variable amounts. Relative levels ranged from 0.3 to 180% of the aldolase mRNA standard. The mRNA contents of CFTR, CK 14, CK 18, and DP 1 were maintained within a small range. Wild-type and ΔF 508 CFTR mRNAs were found to be low abundant transcripts in nasal polyps. The 0.1–2 amol CFTR mRNA/μg RNA, i.e., ~ $0.5–10 \times 10^5$ copies/μg RNA are 3–10-fold less than the amount of CFTR mRNA found in epithelial cells that had been derived from nasal polyps by 5–10-d primary culture (16). The mRNA transcripts encoding CK 14, CK 18, and DP 1 were found in similar amounts of ~ 10% of aldolase mRNA. The steady-state levels of CFTR, CK 13, CK 18, CK 14, and DP 1 mRNAs of ΔF 508 CF samples were compared to those of non-CF to define whether mRNA content of CFTR and markers of epithelial differentiation could be different. The statistical analysis indicates that mRNA transcripts levels were not significantly different between the two distinct populations. The steady-state levels of CFTR mRNA transcripts were compared to the steady-state levels of CK 13, CK 18, CK 14, or DP 1 to determine whether a relationship exists at the transcription level between CFTR and markers of differentiation. No correlation was demonstrated between CFTR with either CK 13, CK 18, CK 14, or DP 1.

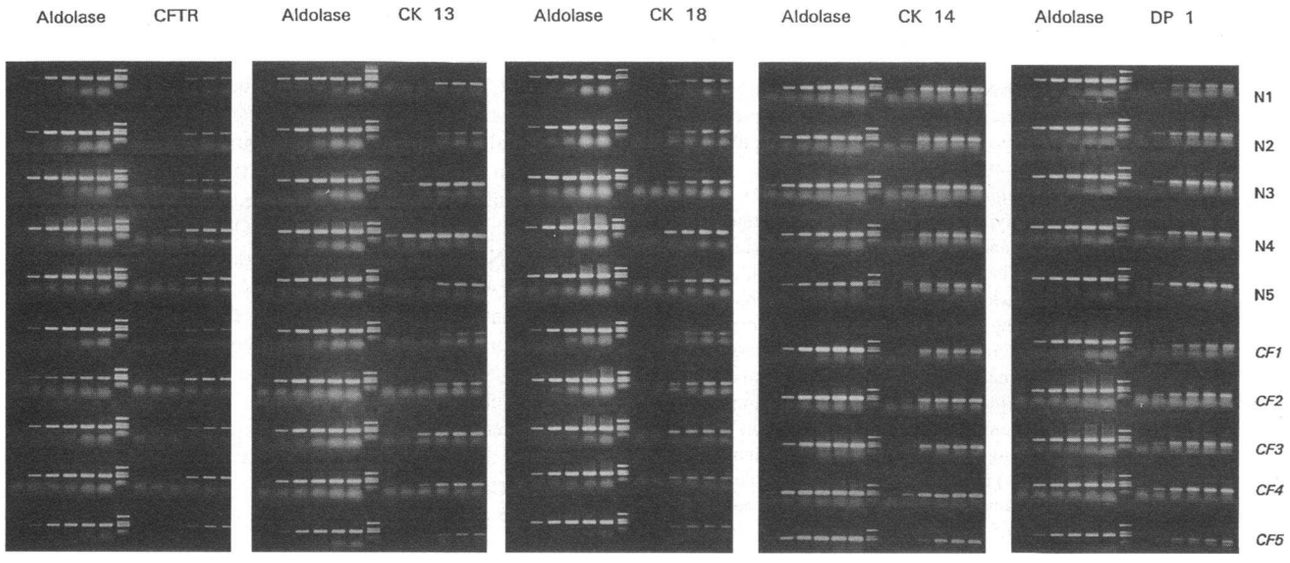

Figure 1. Semiquantitative evaluation of CFTR, cytokeratins 13, 18, 14, and desmoplakin 1 mRNA transcripts by RT/PCR kinetics in nasal polyps of non-CF (*N*) and ΔF 508 homozygous CF patients (*CF*) in correlation to the amount of aldolase mRNA as an internal standard (*left*). The RT assay was performed with 1 μg RNA as described in Methods. A 30% aliquot of the RT reaction mixture was subjected to PCR, and 7-μl aliquots were withdrawn at 18, 22, 26, 30, 34, and 38 reaction cycles (*from left to right, all panels*). The gel-separated PCR products were visualized by ethidium bromide stain. A DdeI digestion of pUC 8 DNA was applied in the middle lanes as size markers (889, 540, [426, 409] [290, 166] bp).

Table III. Relative Amounts q of CFTR, CKs and DP 1 mRNA Transcripts Normalized to Aldolase mRNA Transcript in Non-CF and ΔF 508 Homozygous CF Nasal Polyps

	Patients			
	NON-CF (n = 5)		CF (n = 5)	
	Geometric mean	Range	Geometric mean	Range
CFTR	6	1–10	15	4–25
CK 13	420	4–1800	50	3–150
CK 18	180	70–380	140	70–180
CK 14	120	20–220	110	80–180
DP 1	100	50–180	130	80–180

$q = 10^3\ c_{X\ mRNA}/c_{aldolase\ mRNA}$

$c_{X\ mRNA}/c_{aldolase\ mRNA} = 1.78^{-m-n}$

m and n, cycle when the PCR product for X and aldolase, respectively, first appears

Table IV. Inflammation, Remodeling and CFTR scores of Surface Epithelium from Non-CF and ΔF 508 Homozygous CF Nasal Polyp Tissue Samples

		Inflammation score			
	Sample code	Surface epithelium	Lamina propria	Remodeling score	CFTR score
Non-CF (n = 8)	N 1	1	1	1	50
	N 2	2	3	3	0
	N 3	1	1	1	50
	N 4	1	2	4	0
	N 5	1	2	3	0
	N 6	1	2	2	10
	N 7	1	2	2	15.5
	N 8	2	2	2	0
CF (n = 5)	CF 1	2	1	1	50
	CF 2	n.d.	3	5	0
	CF 3	3	3	3	1
	CF 4	1	2	3	2.5
	CF 5	2	2	2	6.5

Inflammation is evaluated as rare and diffuse (1), moderate (2), or severe (3). Surface epithelium remodeling corresponds to: slight basal cell hyperplasia phenotype (*1*), moderate basal cell hyperplasia phenotype (*2*), advanced basal cell hyperplasia (*3*), squamous metaplasia (*4*), or epithelium exfoliation (*5*).
n.d., not determined.

Morphological characteristics of the surface epithelium: inflammation and remodeling. The alteration of nasal polyps was estimated in terms of inflammation and surface epithelium (SE) remodeling. For each tissue sample, the degree of alteration was evaluated by observation of a unique tissue section (~ 5 mm), since the homogeneity of inflammation and surface epithelium remodeling within a specimen has been previously observed (data not shown). The importance of inflammation and remodeling in non-CF and CF tissue samples was evaluated by scores reported in Table IV. The degrees of inflammation correspond to either diffuse, moderate, or severe inflammation. The degrees of SE remodeling, correspond to slight basal cell hyperplasia (nearly a normal SE), typical basal cell hyperplasia (moderate or severe), squamous metaplasia, and exfoliated SE. For 10 out of the 13 samples, the inflammation or surface epithelium remodeling was strictly homogenous all along the tissue sections while the other samples presented a local heterogeneity: besides the scores evaluated in the majority of sections of the respective specimen (Table IV), 20% of sample N4 scored 3 for the lamina propria inflammation, 40% of sample N7 and 20% of sample CF4 scored 3 and 1, respectively, for the surface epithelium remodeling. In the non-CF population, 88% of the tissue samples exhibited a diffuse or moderate inflammation in the lamina propria, and 12% exhibited a severe inflammation. The morphological structure of normal pseudostratified SE (with a single basal cell layer) was never observed and slight basal cell hyperplasia (two to four basal cell layers) was present in only 25% of the tissue samples. The remaining 75% of the non-CF nasal tissue specimens corresponded to moderate or advanced basal cell hyperplasia (68%) and squamous metaplasia (7%). In the CF population, diffuse or moderate inflammation in the lamina propria was present in 60% of the samples and the remaining 40% exhibited severe inflammation. Slight basal cell hyperplasia was identified in only 20% of samples, and 80% of the tissue samples were characterized by moderate or advanced basal cell hyperplasia (60%) or SE exfoliation (20%). The comparison of CF with the non-CF population did not show any significant difference in the degree of inflammation or SE remodeling. Nevertheless, the tissue samples characterized by the highest inflammation and remodeling scores were of CF origin.

Relationship between the degree of inflammation, the degree of surface epithelium remodeling, and CFTR score. To analyze the expression and distribution of the CFTR protein in the surface epithelium of all the tissue samples, immunofluorescence analysis was performed using three different anti-CFTR monoclonal antibodies raised against three different domains of CFTR. In earlier studies, specificity of those antibodies has been demonstrated (7, 12). For different sections of a same tissue sample, CFTR labeling patterns appeared homogenous all along the surface epithelium and were similar, whatever the antibody used (Fig. 2). In contrast to this low sample-by-sample variability, we observed a large variability in the intensity and cellular distribution of anti-CFTR antibody-reactive protein, present in either the non-CF or the CF population. A CFTR score, depending on the proportion of ciliated cells with an apical distribution of CFTR, was attributed to each tissue sample. These semiquantitative values varied from 0 to 50 (Table IV). As it is clearly shown in Figs. 3 and 4, this variability was associated with a huge variability in the morphology of the surface epithelium, present in either the non-CF or the CF population. This observation was confirmed by statistical analysis: significant correlations were observed in the non-CF and CF nasal tissue between the SE remodeling and the CFTR score ($r_s = -0.875$, $P < 0.005$ and $r_s = -0.975$, $P < 0.006$, respectively). Figs. 3 and 4 also indicate that the pattern of CFTR labeling in the non-CF population may have many similarities with the patterns observed in the CF population. This observation was statistically confirmed by the nonsignificant differences between CFTR scores in the non-CF and ΔF 508 CF nasal polyps. Moreover, significant correlations were observed, in non-CF and CF nasal tissue, between the inflammation of the lamina propria and the CFTR score ($r_s = -0.79$, $P < 0.02$ and

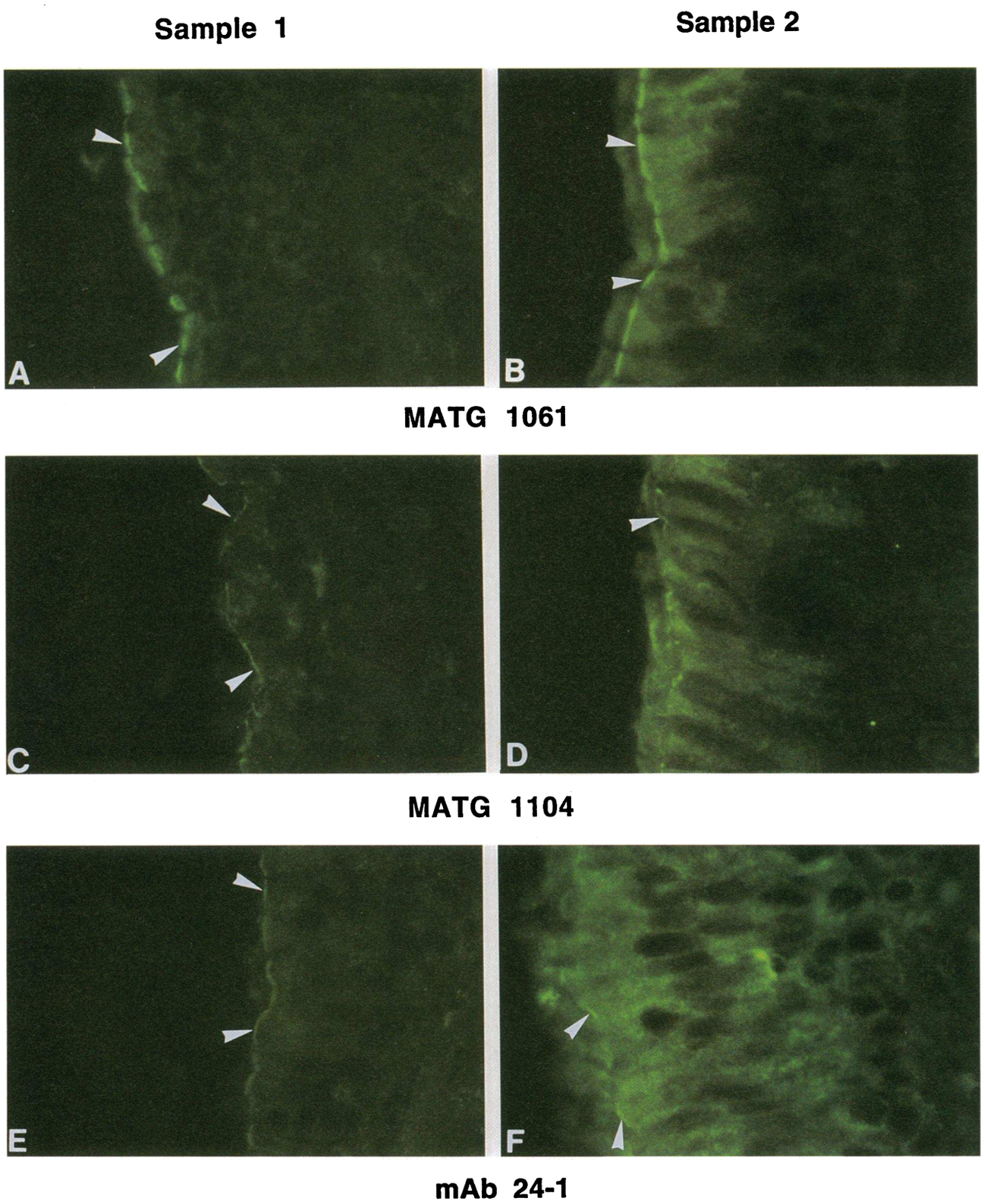

Figure 2. Comparison of CFTR immunodetections on two different nasal polyps of the same ΔF 508 homozygous CF patient (CF1) using indirect immunofluorescence with different antibodies. For different sections of the same tissue sample, the CFTR distribution is identical using three different anti-CFTR antibodies (MATG 1061 against NBF1 domain, MATG 1104 against R domain, mAb 24-1 against the COOH-terminal domain of the CFTR). (*A, C,* and *E*) The CFTR labeling is strictly detected at the apical plasma membrane of ciliated cells in the nasal polyp sample 1 (*arrowheads*). (*B, D,* and *F*) The CFTR labeling is detected at the apical plasma membrane (*arrowheads*) but also in the cytoplasm of ciliated cells in the nasal polyp sample 2.

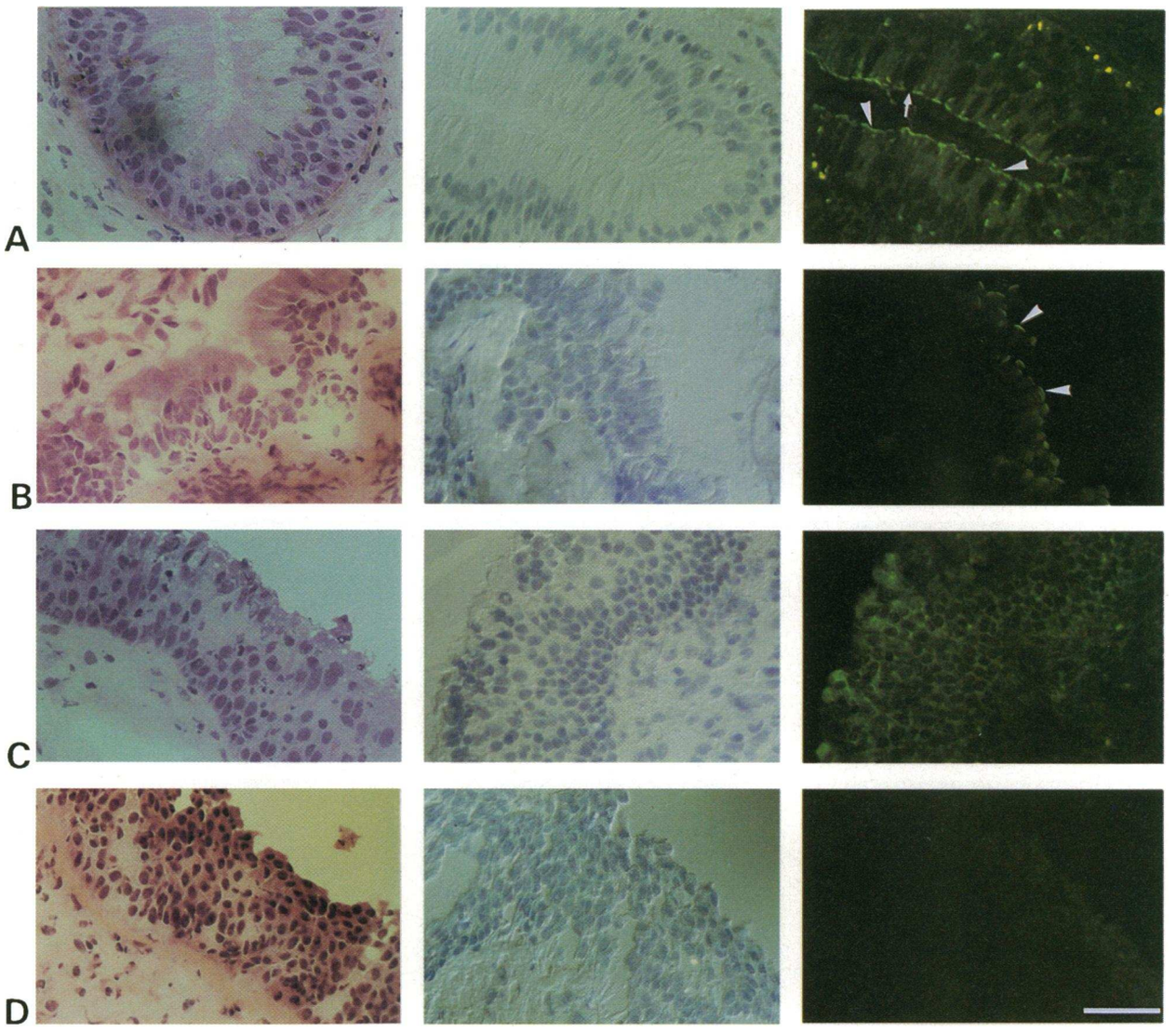

Figure 3. Diversity in the morphology and the CFTR expression and distribution observed in non-CF nasal surface epithelium. (*Left*) Views of HPS-stained sections. (*Center*) Nomarski pictures corresponding to CFTR immunodetections. (*Right*) immunodetections of CFTR by indirect immunofluorescence (MATG 1061 antibody). (*A*) CFTR is found at the apical plasma membrane of ciliated cells present in surface epithelium with slight basal cell hyperplasia phenotype (*arrowheads*) (N3 sample). Goblet cells are negative for CFTR immunodetection. (*B*) Areas of ciliated cells show typical apical CFTR labeling (*arrowheads*) and some ciliated cells have a diffuse cytoplasmic CFTR labeling (N6 sample). (*C*) CFTR labeling is diffusely present in the cytoplasm of numerous cell layers (N8 sample). (*D*) No CFTR labeling is observed in highly remodeled surface epithelium close to squamous metaplasia (N5 sample). Bar = 50 μm. (A similar magnification [×220] has been used for all the pictures.)

$r_s = -0.95$, $P < 0.02$, respectively). Furthermore, a significant correlation was also observed between the surface epithelium inflammation and remodeling scores in non-CF tissue ($r_s = 0.77$, $P < 0.03$).

A CFTR score of 50 was found in 2 out of 8 non-CF (N1 and N3) and in 1 out of 5 (CF1) CF nasal polyp tissues which were characterized by a surface epithelium with the morphological aspect of a slight basal cell hyperplasia. The almost normal surface epithelium contained two to four basal cell layers (Figs. 3 *A* and 4 *A*). Compared to a normal pseudostratified surface epithelium, the proportion of ciliated cells was similar. By indirect immunofluorescence, intense CFTR labeling was localized along the apical plasma membrane of all ciliated cells (Figs. 3 *A* and 4 *A*). A similar CFTR score of 50 was attributed for two non-CF and one CF specimen for tissue sections exhibiting either a strictly apical CFTR labeling in the apex of ciliated cells (Fig. 2 *A, C,* and *E*), either a very faint CFTR labeling just beneath the apical plasma membrane of ciliated cells (Fig. 3 *A*) or a strong cytoplasmic CFTR labeling in addition to the apical labeling (Fig. 2 *B, D, E,* and Fig. 4 *A*). An intermediate CFTR score (between 0 and 50) was also found to correspond to the slight basal cell hyperplasia phenotype. In comparison with the previous case, the ciliated cell ratio remained unchanged, but CFTR protein was detected in patches of ciliated cells either with apical labeling or with diffuse cytoplasmic labeling. In the typical morphological phenotype of basal cell hyperplasia, CFTR immunolabeling demonstrated an accumulation of the protein in the cytoplasm of cells constituting numerous cell layers, and ciliated cells were rare along the superficial cell layer (Figs. 3 *C* and 4 *C*). Although anti-CFTR labeling

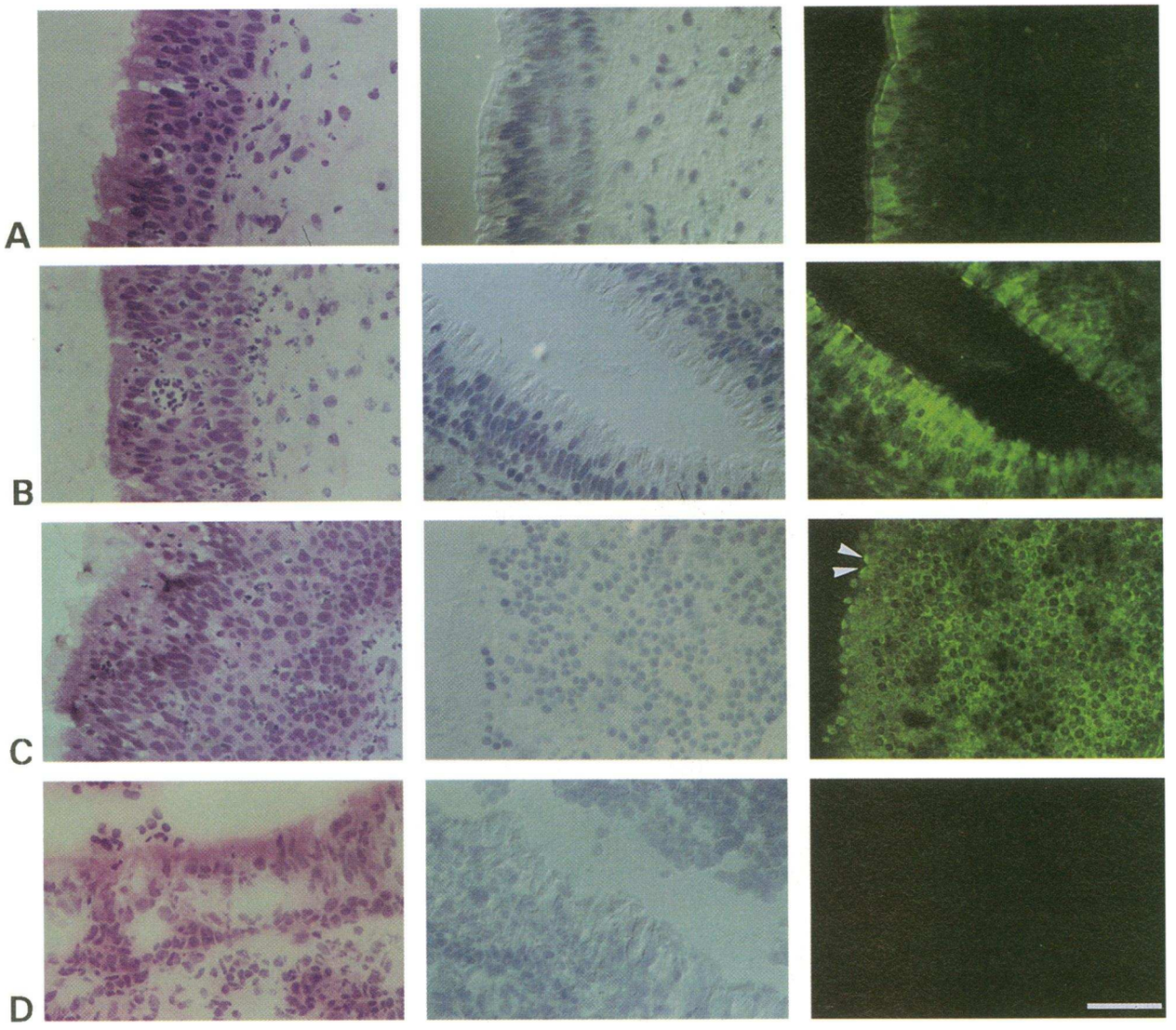

Figure 4. Diversity in morphology and CFTR distribution observed in Δ F508 homozygous CF nasal surface epithelium. (*A*) CFTR labeling (MATG 1061 antibody) is marked along the apical plasma membrane and in the cytoplasm of ciliated cells (CF1 sample). (*B*) CFTR labeling is observed in the cytoplasm of all the ciliated cells. Some ciliated cells present also an apical fluorescent staining (*arrowheads*) (CF5 sample) (*C*) An intense and diffuse cytoplasmic CFTR labeling is observed in numerous successive cell layers. Some ciliated cells, persisting in the more superficial cell layer, present CFTR both at the apical plasma membrane and in the cytoplasm (CF5 sample). (*D*) CFTR is undetectable in a highly remodeled surface epithelium, showing evidence of alteration and severe inflammation (CF3 sample). Bar = 50 μm. (A similar magnification [×220] has been used for all the pictures.)

was identified in most of the cells with a high intensity, CFTR was abnormally distributed in comparison to the normal pseudostratified epithelium, and the CFTR score was zero. A similar CFTR score was attributed to highly remodeled tissue samples close to the squamous metaplasia phenotype (Figs. 3 *D* and 4 *D*). In this latter epithelium phenotype, ciliated cells were absent and no CFTR labeling was observed, in contrast to basal cell hyperplasia.

Relationship between the CFTR mRNA transcript level and the CFTR protein score. To determine whether a relationship could exist between CFTR mRNA and protein expression, the quantitation of CFTR mRNA transcript has been associated with the immunodetection of the CFTR protein. The CFTR mRNA transcripts were analyzed in the total mucosa while the CFTR protein was evaluated at the level of the surface epithelial cells in a portion of tissue where RT/PCR quantitation could not be carried out at the same time. However, comparison between the two parameters could be performed since the CFTR labeling pattern appeared homogenous all along the surface epithelium for different sections of a same tissue sample, and were similar, whatever the anti-CFTR antibody used. As reported in Table III, the relative amounts of CFTR mRNA transcripts were not significantly different between the non-CF and the CF group and varied from 1 to 25. A variability in the intensity and cellular distribution of CFTR labeling was also observed by immunodetection. When considering all the samples together, either non-CF or CF, and analyzing the number of ciliated cells with an apical CFTR distribution, no significant correlation could be identified between the CFTR protein expression and CFTR mRNA transcripts level. These results are

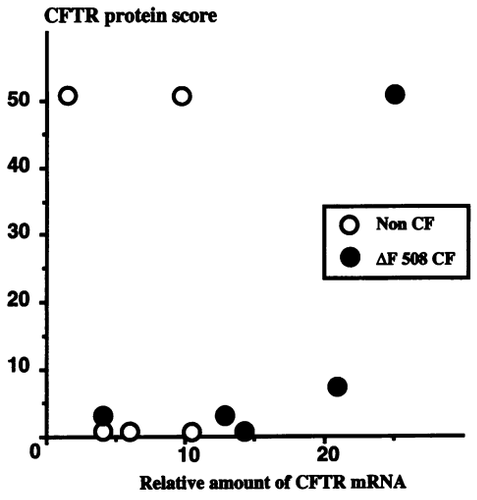

Figure 5. Relationship between the relative amount of CFTR mRNA transcripts and the CFTR score in five non-CF and five ΔF 508 homozygous patients. There is no significant correlation between the two parameters (r_s = 0.130; P = 0.72).

illustrated in Fig. 5 showing that the level of CFTR mRNA transcripts were not significantly correlated to the CFTR protein semi-quantitative score (r_s = 0.130, P = 0.72).

Relationship between clinical parameters and CFTR protein score in CF nasal polyps. In the group of ΔF 508 homozygous CF patients, the insufficient pancreatic status was very similar in all the patients, whereas the respiratory function and the Shwachman score were heterogeneous. Although we could not demonstrate any correlation between the clinical and respiratory function parameters and the expression of CFTR, it is striking to note that the CF patient who showed the highest CFTR score exhibited a good clinical state, up to the age of 19 yr, before being colonized by *Pseudomonas aeruginosa* (PA).

Discussion

In this study, we have demonstrated, using the semiquantitative method of RT/PCR, that the low steady-state mRNA transcript levels of CFTR in nasal polyps varied in a small range and did not appear to be significantly different in non-CF and ΔF 508 homozygous CF specimens. These results are in concordance with previous data showing that the wild-type and ΔF 508 CFTR mRNA transcripts are expressed in similar low amounts in human respiratory surface epithelial cells (17). Moreover, we have shown that CFTR mRNA transcript levels were not correlated with mRNA transcript levels of epithelial differentiation markers such as cytokeratins and desmoplakins. In contrast, a close and significant association was found between the CFTR protein expression and the protein expression of epithelial differentiation markers in non-CF and CF nasal polyp tissue. A marked remodeling of the nasal surface epithelium was generally associated with a low or even absent apical CFTR expression in the non-CF or the CF group. This data confirms our previous results (12) showing that a "CF phenotype", characterized by decreased expression and/or mislocalization of the CFTR protein, may be identified in non-CF nasal polyps. From our present results, the degree of inflammation and surface epithelium remodeling appear to be important factors closely correlated to the CFTR protein expression and distribution. Surprisingly, we have detected, in one out of five ΔF 508 CF nasal tissue samples (CF1, presenting low inflammation and surface epithelium remodeling score), a quasi-normal CFTR protein localization. We are confident of these results because CFTR apical distribution was reproducibly observed in two different samples of the CF1 patient using the three different specific anti-CFTR antibodies. The two different samples corresponded to different nasal polyps from the same resection, analyzed in a blind fashion. It is interesting to note that the histological and inflammation characteristics of those two samples from the same patient were also very similar and reproducible. In contrast to this sample-by-sample homogeneity, the sample-to-sample variability could be important and the tissue specimens could be dramatically different from one CF patient to another even though their genotype (ΔF 508 homozygosity) is similar. For example, the patient CF3 is characterized by a very low and reproducible CFTR score associated with a marked remodeling and inflammation score.

We have shown that ΔF 508 CFTR can have an apical distribution in CF airway ciliated cells. This data stands in contrast to the present state of knowledge indicating that the ΔF 508 mutation causes a temperature-sensitive defect in post-translational maturation and trafficking of the CFTR protein, unable to accumulate in the plasma membrane at 37°C (10, 18). Mislocalization of ΔF 508 CFTR in the cytosol and absence of ΔF 508 CFTR in apical membrane have been observed in vitro in primary cultures of nasal epithelial cells (19) and in vivo in ΔF 508 homozygous CF patients' sweat glands (20), nasal polyps (7), or submucosal airway glands (21). Nevertheless, in vitro experiments now provide evidence that ΔF 508 CFTR protein may reach its target in the plasma membrane of cells grown at a temperature below 30°C or in transfected cells overexpressing the mutant protein (18, 22). These and other studies also indicate that ΔF508 CFTR protein could be functional, at least partially, as a cAMP sensitive Cl^- channel if it was able to reach the plasma membrane although its half-life is sixfold lower than that of wild-type CFTR protein. (11, 18, 22–27). Thus, cAMP-regulated Cl^--channel activity was found in transfected mammalian (11, 18), amphibian (23) and insect cells (27) or in lipid bilayer reconstituted with purified ΔF 508 CFTR (27). All this data supports the idea that the "export incompetent" phenotype of ΔF508 CFTR may be leaky, at least in vitro. Our current study demonstrates that the ΔF 508 CFTR may be localized in the plasma membrane of CF epithelial cells in vivo, as previously suggested by Veeze et al. (28). Interestingly, these authors detected some residual chloride secretion in 4 out of 28 rectal biopsies of ΔF 508 homozygous CF patients.

The nasal polyp is a paradigm to mimic airway inflammatory diseases, in as much as it consists essentially of an oedematous stroma with numerous inflammatory cells, including neutrophils and most commonly eosinophils, plasma cells, mast cells, lymphocytes, and macrophages (29, 30) covered by a respiratory type epithelium with large areas of basal and mucous cell hyperplasia or even squamous cells metaplasia (12). In fact, neither RT/PCR analysis of CFTR mRNA nor immunodetection of CFTR protein allowed us to detect any striking differences in the non-CF and CF nasal polyps to identify the ΔF 508 homozygosity. As shown in our present data, non-CF and CF tissue specimens could not be distinguished in terms of inflammation and remodeling, probably due to the small number

of samples. However, the degree of remodeling and inflammation seems to be more marked in the group of CF patients compared to non-CF patients. The only marked difference between the non-CF and the CF inflamed nasal polyp tissue is the large number of neutrophils which infiltrate the CF surface epithelium. This situation is very similar to that described in CF airways where an intense chronic neutrophil-dominated epithelium inflammation is responsible for a chronic derangement of the fragile respiratory tissue (31). The nasal polyp is therefore an ideal model for answering the question whether, in non-CF and in CF airway tissue, the inflammation and remodeling of the surface epithelium can induce changes in the CFTR expression at the mRNA and protein level.

Our present data does not focus on the control mechanisms of CFTR expression. Nevertheless, they suggest a potential posttranscriptional regulation of CFTR by environmental factors. The regulation of the expression of CFTR in epithelial cells has been evaluated mostly in in vitro models of intestinal epithelial cells such as T84 or HT 29 cell lines. Such studies have shown that CFTR mRNA levels may be regulated in correlation with cellular differentiation, and more specifically that HT 29 intestinal epithelial cells express low levels of CFTR transcripts while lying in an undifferentiated state (8, 9). In contrast, other studies have reported that both undifferentiated and differentiated HT 29 cells can express similar levels of CFTR mRNA (32). Taken together, these results suggest that the levels of the CFTR mRNA and those of the CFTR protein do not correlate closely (8), and that CFTR expression, measured both at the mRNA and protein level, is not strictly linked with the ability to secrete chloride ions in response to a cAMP agonist (32). Recently, Morris et al. (33) demonstrated that cAMP-dependent Cl^- secretion in HT 29 cells requires the apical membrane targeting of CFTR. In airway tissue, the expression of CFTR mRNA transcripts in undifferentiated fetal epithelium appears to be very low (34). CFTR protein expression is localized in the apical domain of the ciliated cells by the 7th week of gestation (5). Interestingly, it has been reported that in rat regenerating liver the quantitative levels of CFTR gene activity is the result of transcriptional and posttranscriptional phenomena (35). The influence of inflammation on CFTR mRNA transcripts may be highly variable. It is well known that the influx of neutrophils into the airways is an important characteristic of CF airway epithelium, particularly during infection by PA (36). The PA products stimulate IL-8 production through the bronchial epithelial cells and through monocytes which, in turn, may induce an up-regulation of CFTR mRNA transcripts. In contrast, other cytokines, such as TNF, have been reported to down-regulate CFTR mRNA transcript levels in a dose and time-dependent fashion (37). More recently, it was demonstrated that TNF modifies the stability of CFTR mRNA transcripts, resulting in a mRNA half-life reduction (38). In respiratory tract epithelial cells, it has been also reported that a down-regulation of CFTR mRNA transcript levels, with the adoption of a "CF phenotype", may be induced by the protein kinase C activator PMA (39). Furthermore, post-translational control of CFTR expression is invoked to explain the defective processing and intracellular translocation of the CFTR ΔF508 mutation (10, 40). In addition, the phorbol ester PMA expedites degradation of CFTR protein, mediated by prolonged activation of protein kinase C (41). It is likely from our results that in inflamed and remodeled nasal surface epithelium the regulated expression of CFTR involves posttranscriptional mechanisms.

This study indicates that the patient CF1 with the highest CFTR protein score was found to have the lowest Shwachman score. This CF patient maintained a good clinical state until the onset of PA colonization at the age of 19. He deteriorated rapidly within three years into end-stage lung disease, but after heart–lung transplantation his clinical state improved dramatically. This patient is at present a full-time worker, his only inconvenience being a rapid growth of nasal polyps. Patient CF3, who is younger (7.5 yr) than patient CF1 (27.6 yr), exhibited a higher Shwachman score. It is interesting to note that CFTR mRNA transcripts were present in the nasal polyp tissue of both patients, although the levels were lower in the patient CF3 who maintained a low or even absent CFTR score compared to that quantified in patient CF1, characterized by the high CFTR score. Our study also clearly demonstrates that the severity of the clinical phenotype is quite heterogeneous even within a group of CF patients with a similar ΔF 508 homozygous genotype. Despite the close association between the cystic fibrosis genotype and the pancreatic phenotype, this present study reinforces the idea that factors other than those of cystic fibrosis genotype affect the pulmonary phenotype.

The present data shows that environmental factors, and particularly the histological and inflammatory state of the surface epithelium, appear to be able to influence the variability of CFTR protein expression in non-CF and ΔF 508 homozygous CF nasal polyps. The pathogenesis of the airway surface epithelium remodeling associated with PA infection remains an open question of critical importance. The possible expression of ΔF 508 CFTR at a correct cellular location, identified in one CF patient, gives hope that at least a small amount of the protein may escape the cellular quality control mechanisms. The variability of CFTR expression and CFTR targeting in such CF patients encourages the stimulation of the residual CFTR function by pharmacological means targeting the CFTR protein to the apical membrane.

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