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Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)-Mediated Residual Chloride Secretion Does Not Protect against Early Chronic *Pseudomonas aeruginosa* Infection in F508del Homozygous Cystic Fibrosis Patients

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ABSTRACT

Cystic fibrosis (CF) disease severity is characterized by a broad variability that has been attributed, in addition to the CF transmembrane conductance regulator (*CFTR*) genotype, to modulating factors such as CFTR-mediated residual chloride (Cl^-) secretion. Moreover, CFTR has been suggested to function as a receptor for *Pseudomonas aeruginosa* (PA). In this study, we investigated whether or not the presence of residual Cl^- secretion protects against early chronic PA colonization of patients' airways. Excluding influences on the phenotype caused by different *CFTR* mutations, we evaluated a cohort of F508del homozygous individuals with respect to the correlation between residual Cl^- secretion and the age of onset of PA colonization as an important marker of clinical phenotype. A group with early chronic PA colonization before the age of 7 y ($n = 14$) was compared with a cohort that had no initial PA detection at least until the age of 13 y ($n = 10$). We determined the Cl^- transport properties by using the intestinal current measurement in rectal suction biopsies. Residual Cl^- secretion, most likely due to the CFTR Cl^- channel, was observed in 63% of subjects, more frequently in early chronically PA colonized than among late or not colonized patients. These results demonstrate the presence of some active F508del-CFTR in the apical cell membrane and imply that factors other than the CFTR-mediated residual Cl^- secretion determine the age of onset of PA colonization. **(*Pediatr Res* 55: 69–75, 2004)**

Abbreviations

Ca^{2+}, calcium
cAMP, adenosine 3',5'-cyclic monophosphate
CF, cystic fibrosis
CFTR, cystic fibrosis transmembrane conductance regulator
Cl^-, chloride
DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid
FEVPerc, percentiles for predicted forced expiratory volume in 1 s
$\Delta I_{sc\ net}$, net change in short-circuit current
IC, intestinal current
K^+, potassium
PA, *Pseudomonas aeruginosa*
PD, potential difference

In CF, mutations in the *CFTR* gene, encoding for the CFTR Cl^- channel in the apical membrane of epithelial cells, result in an absent or significantly reduced Cl^- secretion of CFTR expressing epithelia (1). *CFTR* mutations such as A455E have been reported to give rise to residual Cl^- secretion and a milder disease phenotype, *i.e.* pancreatic sufficiency and less frequent PA colonization (2, 3). However, mildly diseased A455E heterozygous patients have been described who do not exhibit residual Cl^- secretion (4). The disease severity varies widely even in patients homozygous for the same *CFTR* mutation (5–7). In addition, the extent to which residual Cl^- secretion is observed is not exclusively determined by the *CFTR* mutation genotype as the expression of the basic defect varies considerable even among F508del homozygotes (8, 9). With respect to the amount of residual Cl^- secretion, monozygous F508del homozygous twins are more concordant than dizygous pairs

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indicating the influence of inherited factors other than the *CFTR* gene on total Cl^- secretion (8, 9).

The onset of chronic PA colonization is known as a hallmark of the clinical course in CF disease (10). The airways are chronically infected with PA in a complex process, which starts with initial intermittent detection of PA and ends in chronic colonization with mucoid PA (10). The transition from nonmucoid to mucoid PA stages is the decisive step that impairs pulmonary function irreversibly and can be delayed only by therapy in early colonization stages (11, 12). It has been reported that patients homozygous for the mutation F508del show an increased adherence of PA to the airway cells (13) and are earlier chronically colonized with PA (3, 6) than patients carrying mild *CFTR* mutation genotypes. Moreover, pancreatic sufficiency is associated with later acquisition of PA (14, 15). The mechanism by which PA adheres to the airways is only partially known. It has been reported that CFTR, apart from its well known role as a Cl^- channel, is also a receptor for PA in the airways and, consequently, unimpaired CFTR function must be vital for the clearance of the bacteria from the lungs (16–18). Recently, it has been suggested that dysfunctional CFTR leads to hyperacidification of the trans-Golgi network in CF lung epithelial cells, being the molecular basis for defective glycosylation and increased PA adherence (19). In summary, the molecular mechanism that links CFTR Cl^- channel dysfunction to the adhesion of PA to epithelial cells and to the persistence of respiratory colonization with mucoid PA in CF patients has not been satisfactorily resolved so far.

In this study, we elucidated whether the presence of residual Cl^- secretion is associated with a later onset of chronic PA colonization, which would indicate a clinical advantage for these patients. The measurement of residual Cl^- secretion was studied and is possible in either gastrointestinal (2, 8, 20, 21) or respiratory tract (9, 22–24), as the basic defect manifests itself in both tissues (25). Both CFTR and Cl^- channels other than CFTR (26–28) contribute to residual Cl^- secretion (8). Whereas CFTR is expressed in both lung and intestine (25), the molecular entities of Ca^{2+}-activated Cl^- secretion have been identified merely in part (28–30), and might be different in these epithelia. CFTR can be best discriminated from other Cl^- channels by blocking of non-CFTR Cl^- channels with DIDS (8, 31), as CFTR is not inhibited by this agent (32). However, DIDS is toxic and hence cannot be applied in an *in vivo* setup such as nasal potential difference (nPD). Consequently, we chose to evaluate residual Cl^- secretion by transepithelial current measurements from rectal suction biopsies by means of IC measurement. This *ex vivo* method allows one to differentiate CFTR-mediated from alternative Cl^- secretion, and, moreover, the minimally invasive procedure is applicable at all ages. We recruited only F508del homozygous patients to compare the influence of residual Cl^- secretion on the age of onset of chronic PA colonization within a cohort homogeneous for the *CFTR* disease–causing lesion.

METHODS

Patients. After study approval by the local medical ethics committee, 24 CF patients (13 males, 11 females; mean age, 16.9 ± 1.6 y; range, 4.2–33.7 y) who are homozygous for the mutation F508del were recruited with informed consent. Patients were selected from our local CF center database and grouped according to both extremes of the age at PA colonization. As it is well known that there is an age-dependent risk for CF patients to acquire PA and that early chronic PA colonization is related to a poorer survival (11), this selection strategy leads to groups with maximum contrast and allows to investigate the most informative phenotypes. The first group ($n = 14$) had an early chronic PA colonization before the age of 7 y (PA^+). In this age group the incidence of PA in German CF patients is known to be <20% (33). We defined the onset of chronic PA colonization as more than 50% positive sputum or deep throat swab cultures in a 12-mo period with either nonmucoid or mucoid PA (11). These patients were compared with a second group ($n = 10$), who had no PA detection at least until the age of 13 y (no/late initial) in the routinely performed sputum or deep throat swab cultures (first positive culture >13 y) (PA^-). In this age group, <30% of German CF patients are PA free (33). The PA^+ group had a significantly lower mean age at investigation than the PA^- group ($p < 0.005$). Patients from the PA^+ group had a significantly lower mean age at chronic PA colonization than the initial PA detection in 5 out of 10 patients from the PA^- group ($p < 0.01$). The other five patients showed no PA colonization up to now. Both PA^+ and PA^- groups differed significantly among the PA serum antibodies ($p = 0.001$). All patients' clinical data are shown in Table 1.

IC measurements. We examined all patients for the presence of residual Cl^- secretion by measuring the transepithelial short-circuit current (I_{sc}) in rectal suction biopsies as described in detail elsewhere (2, 8, 34). Briefly, fresh rectal suction biopsies were preserved in PBS on ice, mounted within 5 min in a micro-Ussing chamber with an exposed area of 1.13 mm^2, and incubated at 37°C with Meyler buffer solution (composition in mmol/L: Na^+ 126.2; Cl^- 114.3; HCO_3^- 20.2; HPO_4^{2-} 0.3; $H_2PO_4^-$ 0.4; HEPES 10; pH 7.4 when gassed with 95% O_2, 5% CO_2). Basal transepithelial resistance was determined by the voltage response to pulse currents of 1 μA and application of Ohm's law; the fluid resistance was taken into account. The basal I_{sc} before voltage clamping was calculated from the transepithelial resistance and the open-circuit PD across the tissue. Subsequently, the tissue was short-circuited using voltage clamps and the I_{sc} required to maintain the PD at 0 mV was recorded, which is a direct measure for the net movement of ions transported across the epithelium. After equilibration, the following substances were added to the mucosal (M) and/or serosal (S) side in a standardized sequence: glucose (10^{-2} mol/L, M+S) for the maintenance of cell metabolism followed by amiloride (10^{-4} mol/L, M) to block the contribution of the amiloride-sensitive sodium channel to the I_{sc}. Next, indomethacin (10^{-5} mol/L, M+S) was applied to inhibit the endogenous prostaglandin synthesis. Thereby, excessive cAMP production and cAMP-mediated Cl^- secretion is prevented. Subsequently, the cholinergic Cl^- secretion was stimulated by applying carbachol (10^{-4} mol/L, S), which causes opening of basolateral K^+ channels so that the resulting electrochemical gradient mediates apical Cl^-

Table 1. *Clinical data of all patients*

	Early chronic PA (PA+) group	No/late initial PA (PA−) group
No.	14	10
Age (y)	12.5 ± 1.2*; range 4.2–17.6	23.2 ± 2.5*; range 13.4–33.7
Sex		
Male	9	4
Female	5	6
F508del/F508del (%)	100	100
Pseudomonas (PA) colonization		
No [current age (y)]	—	18.7 ± 2.8; range 13.4–29.4 (n = 5)
Initial [age at initial PA (y)]	—	24.7 ± 2.9†; range 15.9–32.0 (n = 5)
Chronic [age at chronic PA (y)]	3.6 ± 0.5†; range 0.5–6.9 (n = 14)	—
PA serum antibodies positive (n)	14/14‡	3/9‡
Pulmonary function		
No.	13	10
FEV_1 (% predicted)	67.2 ± 4.2	70.5 ± 8.7
FEVPerc	27.9 ± 5.3§	65.5 ± 11.3§
Pancreatic insufficiency (%)	100	100
Nutritional status:		
BMI SDS_{LMS} (<18 y)	−0.5 ± 0.2 (n = 14)	−0.3 ± 0.2 (n = 4)
BMI (kg/m^2) (>18 y)	—	19.9 ± 1.2 (n = 6)
Meconium ileus (%)	21.4	10
Age at diagnosis (y)	0.6 ± 0.2	1.6 ± 0.6

FEVPerc, age-corrected percentiles for FEV_1 % predicted; BMI, body mass index; BMI SDS_{LMS}, standard deviation scores for BMI; meconium ileus, patients had been operated for newborn meconium ileus.

Values are expressed as mean ± SEM unless otherwise indicated. Differences between both groups: * $p < 0.005$, † $p < 0.01$ (t test); ‡ $p = 0.001$ (Fisher's exact test); § $p = 0.011$ (Mann-Whitney U test).

secretion. Furthermore, the carbachol-mediated increase in intracellular Ca^{2+} stimulates the protein kinase C (PKC)-dependent signaling pathway and thereby directly activates CFTR (35). Next, 8-bromo-cAMP (10^{-3} mol/L, M+S) and forskolin (10^{-5} mol/L, S) were applied for stimulation of the cAMP/protein kinase A (PKA)-linked Cl^- secretion. To evaluate the contribution of Cl^- conductances other than CFTR to the residual Cl^- secretion, these Cl^- channels were then inhibited by incubation with DIDS (2×10^{-4} mol/L, M) before reactivating the Ca^{2+}- and PKA/PKC-mediated Cl^- secretion with histamine (5×10^{-4} mol/L, S) (8, 36). All chemicals were obtained from Sigma Chemical (St. Louis, MO, U.S.A.).

Interpretation of IC measurements. The changes in I_{sc} in response to carbachol and histamine are the net result of opposite currents (34, 37), whereby an increase in I_{sc} indicates Cl^- secretion, and a decrease in I_{sc} represents K^+ efflux unmasked by the reduction or absence of CFTR-mediated Cl^- secretion (34, 38), as used for the CF diagnostics (2, 39). As demonstrated in Figure 1, the typical changes in I_{sc} after addition of carbachol or histamine are known to show a large transient increase in controls and a small transient decrease in most CF patients. In some CF patients the response is biphasic, *i.e.* an initial decrease in I_{sc} is followed by an increase that indicates residual Cl^- secretion (2, 8).

The activation of cAMP-linked CFTR Cl^- secretion by the PKA-agonists cAMP and forskolin is known to result in a sustained increase in I_{sc} in controls and no or a small increase in CF (8). Patients with contribution of Cl^- channels other than CFTR to the residual secretion can be identified by a positive carbachol response (residual Cl^- secretion) and a following reduced or negative histamine response (reduced or absent residual Cl^- secretion) after blocking the alternative Cl^- conductances with DIDS (8).

All results were expressed as the net maximal change in I_{sc} ($\Delta I_{sc\ net}$) evoked by the used secretagogues, which was defined as the sum of downward and upward peak responses. Additionally, the responses to carbachol were classified according to the earlier described residual Cl^- secretion $\Delta I_{sc\ net}$ types I–III (2) (Fig. 1). For the evaluation of the interexperimental error, duplicate biopsies were taken in all patients.

Pulmonary function tests. Records of forced expiratory volume in 1 s (FEV_1) were measured and expressed in FEV_1% predicted using Knudson's equilibrations (40). The results of each patient's best pulmonary function test in the last 6 mo were used.

To correct for the known CF-specific age decline of FEV_1% predicted, percentiles for age for FEV_1% predicted, called FEVPerc, have been calculated for the CF population based on the data compiled by the European CF registry (ERCF) report (41) as described previously (7). As these percentiles for FEV_1 are age independent, we could compare the pulmonary function in patients of different ages in our cohort.

Other clinical data. Medical records were evaluated for PA serum antibodies (anti-oprF IgG titer in all PA^+ and seven PA^- patients, exotoxin A/elastase/alkaline protease in two PA^- patients, not investigated in one PA^- patient), exocrine pancreatic function, the age at diagnosis, a history of being operated for newborn meconium ileus, and the nutritional status [BMI: body mass index (patients >18 y); BMI SDS_{LMS}: SD scores for BMI (42, 43) (patients <18 y)].

Statistical analysis. Data are presented as means ± SEM unless otherwise indicated. Statistical analysis of our results was performed by the Mann-Whitney U test for non-normally

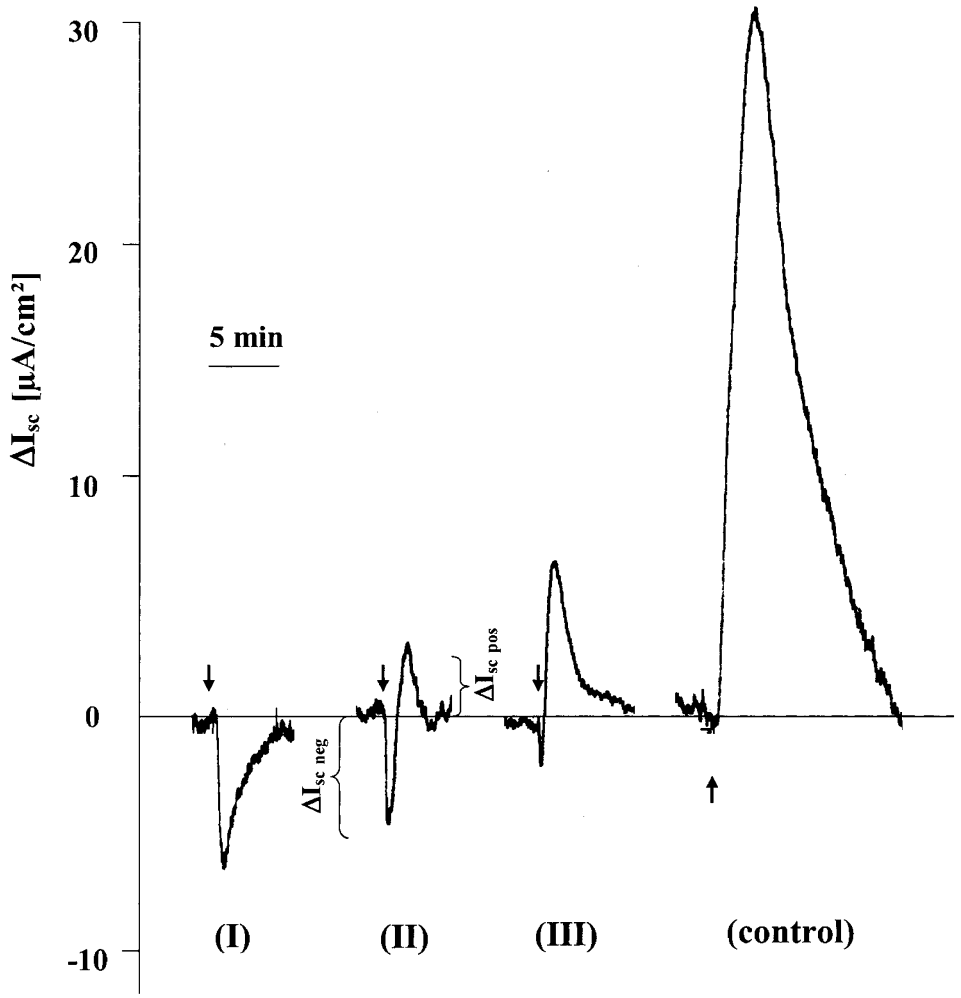

Figure 1. Typical original ΔI_{sc} responses in CF (I–III) and control after stimulation of Cl^- secretion by carbachol (10^{-4} mol/L, serosal, ↓). $\Delta I_{sc\ net} = \Delta I_{sc\ neg} + \Delta I_{sc\ pos}$. (I) $\Delta I_{sc\ net}$-type I: negative $\Delta I_{sc\ net}$ (downward response indicating K^+ secretion, no upward response), no Cl^- secretion. (II) $\Delta I_{sc\ net}$-type II: small negative $\Delta I_{sc\ net}$ (downward > upward response), low residual Cl^- secretion. (III) $\Delta I_{sc\ net}$-type III: positive $\Delta I_{sc\ net}$ (downward < upward response), high residual Cl^- secretion. (Control) large transient increase in ΔI_{sc} indicating normal Cl^- secretion that masks underlying K^+ secretion.

distributed data and the *t* test for normally distributed data. The χ^2 test and Fisher's exact test were used to compare frequencies. *P* values ≤ 0.05 were considered statistically significant. All significances are two-tailed. All statistical analysis was calculated with the SPSS statistics software (SPSS Inc., Chicago, IL, U.S.A.).

RESULTS

IC measurements. The basal tissue resistance R_t in CF amounted to 23.0 ± 1.3 $\Omega \cdot cm^2$; the basal short-circuit current $I_{sc\ basal}$ was 17.3 ± 2.6 $\mu A/cm^2$. There were no significant differences in the $I_{sc\ basal}$ between the three ΔI_{sc} net-type groups after stimulation with carbachol: I (18.9 ± 7.0 $\mu A/cm^2$; $n = 9$), II (13.4 ± 5.2 $\mu A/cm^2$; $n = 7$), III (17.8 ± 5.4 $\mu A/cm^2$; $n = 8$). The $I_{sc\ basal}$ in control was 42.2 ± 5.0 $\mu A/cm^2$ ($n = 42$ biopsies). All patients with interpretable duplicate biopsies ($n = 16$) had the same IC pattern type in both biopsies as defined by $\Delta I_{sc\ net}$ type I–III after addition of carbachol (Fig. 1). Most probably for technical reasons, such as size and thickness of the tissue, we could interpret only one of both tracings in 8 of 24 patients. In total, 40 responses to carbachol, 39 responses to 8-bromo-cAMP/forskolin, and 39 responses to histamine were determined. With respect to residual Cl^- secretion, each patient's biopsy with the highest amount of Cl^- secretion was included in the further analysis.

After stimulation of the rectal tissue with carbachol, 9 of 24 of the F508del homozygous CF patients showed no presence ($\Delta I_{sc\ net}$ type I: −10.1 ± 2.2 $\mu A/cm^2$), 7 of 24 low levels ($\Delta I_{sc\ net}$ type II: −2.1 ± 0.7 $\mu A/cm^2$), and 8 of 24 high levels of residual Cl^- secretion ($\Delta I_{sc\ net}$ type III: 4.5 ± 0.8 $\mu A/cm^2$) (Table 2). Individuals from the early chronic PA (PA^+) group revealed more often residual Cl^- secretion after carbachol than those from the no/late initial PA (PA^-) group ($p = 0.03$). In 14 of 15 individuals with some presence of residual Cl^- secretion after stimulation with carbachol, a small positive response to 8-bromo-cAMP/forskolin (1.71 ± 0.3 $\mu A/cm^2$) could be observed, which was also more frequent in the PA^+ group than in the PA^- group ($p = 0.04$). In 20 of 24 patients, the same individual $\Delta I_{sc\ net}$ type after addition of carbachol and histamine was

Table 2. *Differentiation of chloride channels by the IC measurement*

Residual Cl^- secretion	Signature typical for: CFTR	CaCC	ORCC	$\Delta I_{sc\ net}{}^-$ type	Early chronic PA (PA+) group ($n = 14$)	No/late initial PA (PA−) group ($n = 10$)	Difference p Value
After carbachol	+	+	+	I	2/14	7/10	0.03
				II	6/14	1/10	
				III	6/14	2/10	
After cAMP	+	–?	+		11/14	3/10	0.04

CaCC, calcium-dependent chloride channel; ORCC, outwardly rectifying chloride channel. Comparisons between the PA+ group and the PA− group were analyzed by the χ^2 test.

observed (Fig. 2), indicating that residual Cl^- secretion, if present, most likely was due to the CFTR Cl^- channel.

Pulmonary function tests. Pulmonary function tests could be performed in 23 of 24 patients; one patient from the PA^+ group was too young (4.2 y) for the procedure. Patients from both groups showed comparable levels of FEV_1 % predicted (Knudson) (Table 1). When calculating FEVPerc, which corrects for the CF-specific decline of FEV_1% predicted with age, the patients from the PA^- group had significant better pulmonary function as expressed by FEVPerc (65.5 ± 11.3) than the patients from the PA^+ group (27.9 ± 5.3) ($p = 0.011$) indicating a correlation between the PA colonization status and the lung function in these F508del homozygous individuals.

DISCUSSION

In this study, we addressed the issue of whether the manifestation of the CF basic defect, as assessed by the absence or presence of CFTR-mediated residual Cl^- secretion, is predictive for the onset of PA colonization, as CFTR has been shown to play a key role for the acquisition of PA (14, 17–19). Among patients who are homozygous for F508del, a considerable percentage show some residual Cl^- secretion (9), indicating that the *CFTR* mutation genotype does not exclusively predict the manifestation of the CF basic defect.

Our present study cohort was stratified to be highly informative by selecting only the extreme phenotypes concerning the PA colonization. This maximum contrast in characterization of the PA status between both groups with no overlap leads to apparently relatively small patient numbers.

By investigating the Cl^- transport properties in the both PA groups, residual Cl^- secretion was observed in 15 of 24 individuals (63%). It could be stimulated by the Ca^{2+}- (15/15) and cAMP-pathway (14/15) and was DIDS insensitive in most cases (11/15), indicating that the residual Cl^- secretion was most presumably mediated by CFTR. Previous reports on CFTR expression and function among F508del homozygotes (8, 9, 44) and the results of this study all demonstrate that the expression of F508del-CFTR *in vivo* is more variable than suggested on the basis of studies in heterologous expression systems that predicted the complete absence of F508del-CFTR from the apical membrane (45). The existence of F508del-CFTR in the apical cell membrane has been recently shown by immunohistochemical studies in airway, intestinal, and hepatobiliary patients' tissues (21, 44).

With respect to the onset of PA colonization, we could not confirm our reasonable hypothesis that CFTR-mediated residual Cl^- secretion protects against colonization with PA at an early age. In our cohort, patients with early onset of chronic PA colonization expressed residual Cl^- secretion more often than patients who were late initially colonized or without PA detection. Hence, other causes than the lack of residual Cl^- secretion, and therefore of CFTR expression, must be held responsible for the early onset of PA colonization in this patient subgroup. Our results are in accordance with the known wide variability of the CF disease phenotype among patients carrying the same *CFTR* mutation genotype (5–7). The smaller responses to carbachol, 8-bromo-cAMP/forskolin, and histamine observed among the PA^- group may be due to the significantly higher age of these patients at the day of investigation, as it has been noted earlier in controls that there might be some decline in the secretory responses to carbachol with increasing age (2). We did not observe such a decline in our control groups (data not shown). However, any age-dependent decline of the magnitude of responses to secretagogues is irrelevant for the outcome of this study, as the early chronically PA colonized patients were younger and exhibited residual Cl^- secretion. Hence, in F508del patients, the presence of residual CFTR-mediated Cl^- secretion does not protect against early chronic PA colonization, albeit we cannot exclude that the late colonized patients might have had an even higher residual response at a younger age. In our study cohort, stratified for the onset of PA colonization, the age at PA colonization correlated with the age-corrected lung function value FEVPerc and the PA serum antibody titers. Both PA^+ and PA^- groups differed in none of the other parameters known to correlate with variability in or to characterize the manifestation of CF disease severity such as *CFTR* mutation genotype, pancreatic, and nutritional status.

Other studies investigated the correlation of residual Cl^- secretion and clinical phenotype by nasal potential difference (nPD) and IC measurements, analyzing patients with different *CFTR* genotypes. Bronsveld *et al.* (9) reported in the European Twin and Sibling Study that, in F508del homozygous twins and siblings, the presence of a gluconate response in the respiratory tissue, pointing to residual Cl^- secretion and a DIDS-insensitive residual Cl^- secretion in the intestinal tissue, corresponded with a milder phenotype. We now wanted to analyze the hypothesis whether or not residual Cl^- secretion is associated with a protection against PA colonization additionally in the respiratory tract, as non-CFTR Cl^- channels might be expressed differently in respiratory and intestinal tissue. For this purpose, we have reviewed the data published previously by Bronsveld *et al.* (9) concerning the correlation of residual Cl^- secretion in the nPD and IC measurement with the age at

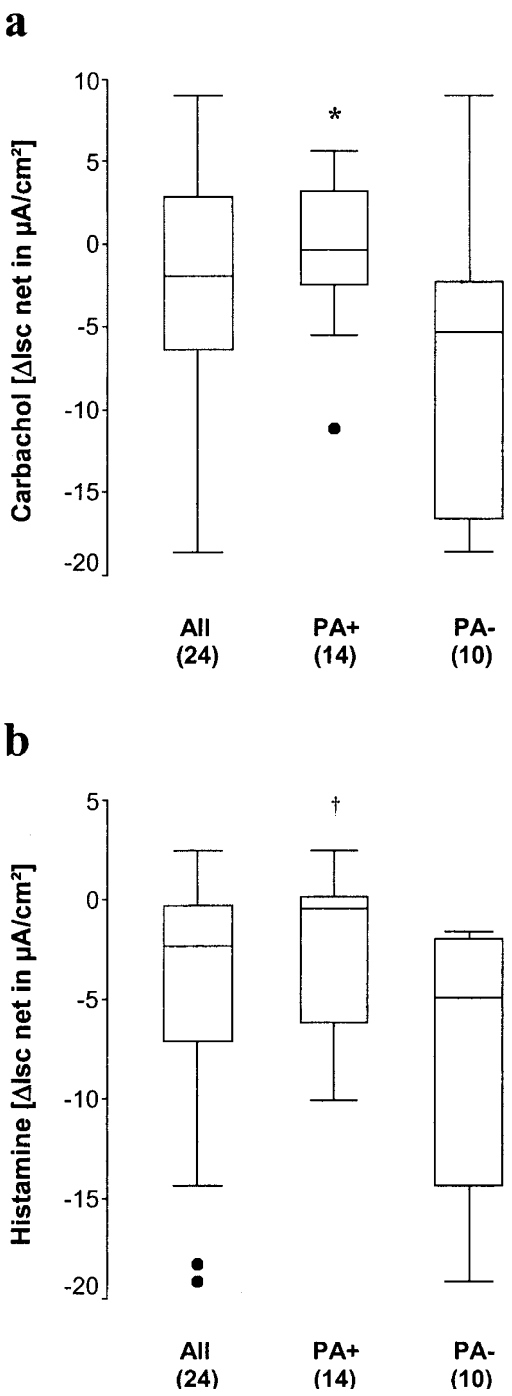

Figure 2. $\Delta I_{sc\ net}$ responses to carbachol (*a*) and histamine (*b*) in F508del homozygous CF. Results are given for the whole group (All), and separately again for the early chronic PA group (PA^+) and the no/late initial PA group (PA^-). For each group of data, the interquartile range (IQR) is depicted by a rectangle, with the upper horizontal line indicating the 75th percentile, followed by the median and the lower horizontal line presenting the 25th percentile. The spread for the rest of the data points in the group (range) is given by the upper and lower vertical lines initiating from the rectangle. Data points larger than 75th percentile +1.5 IQR, and data points smaller than 25th percentile −1.5 IQR are considered outliers and indicated as individual data points. Statistical comparisons were performed by the Mann-Whitney *U* test. $^*p < 0.05$ *vs* PA^-; †$p < 0.03$ *vs* PA^-.

PA colonization. Two subgroups were selected by extremes in the PA serum antibodies (anti-oprF IgG titers) and anamnestic data about onset of initial and chronic PA colonization according to the age criteria in our study. Thereby, in a subcohort of early colonized F508del homozygous twins and siblings, residual Cl^- secretion was observed by both nPD and IC (data not shown). The trend in this independently evaluated data set confirms the observation that residual Cl^- secretion does not protect against PA colonization of the airways as we could demonstrate in our present study in a cohort of unrelated F508del homozygotes that were characterized thoroughly to both extremes with respect to their PA colonization status.

CONCLUSIONS

In conclusion, in this work and in previously published studies (2, 8, 9), F508del homozygous CF patients show a wide variability of the electrophysiological phenotype even though the individuals of this cohort have the same disease-causing lesion in the CF causing *CFTR* gene. Residual Cl^- secretion by CFTR and alternative Cl^- conductances may modulate the basic defect in CF and subsequently alter the clinical course of CF disease, but our finding of residual Cl^- secretion among patients who were early chronically colonized with PA argues against a protective effect of CFTR residual function with respect to the onset of PA colonization. More likely, determinants of the specific and the innate immune response such as IgG subclass levels (46), alpha-1-antitrypsin (47), and mannose-binding lectin (48, 49) will decide on the susceptibility of CF patients to PA.

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