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**Title**

Electrophysiological evaluation of Cystic Fibrosis Conductance Transmembrane Regulator (CFTR) expression in human monocytes.

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**Abstract****Background**

Cystic Fibrosis is caused by mutations of CFTR gene, a protein kinase A-activated anion channel, and is associated to an persistent and excessive chronic lung inflammation, suggesting functional alterations of immune cells. Leukocytes express detectable levels of CFTR but the molecule has not been fully characterized in these cells.

**Methods**

Freshly isolated monocytes from healthy individuals and CF patients were assessed by protein expression, single cell electrophysiological and membrane depolarization assays.

**Results**

We recorded chloride currents by patch clamp in healthy monocytes, after administration of a CFTR stimulus. Currents were sensitive to a specific blocker of the CFTR channel, CFTR<sub>inh-172</sub> and were absent in CF monocytes. Next, we evaluated the effects of *ex vivo* exposure of monocytes from Cystic Fibrosis patients carrying the F508del mutation to a chemical corrector, Vertex-325. We found an increase in CFTR expression by confocal microscopy and a recovery of CFTR function by both patch clamp and single cell fluorescence analysis .

**Conclusions**

We confirm the expression of functional CFTR in human monocytes and demonstrate that blood monocytes can represent an adequate source of primary cells to assess new therapies and define diagnosis of CF.

**General Significance**

Tests to evaluate CFTR functional abnormalities in CF disease might greatly benefit from the availability of a convenient source of primary cells. This electrophysiological study promotes the use of monocytes as a minimally invasive tool to study and monitor CFTR function in individual patients.

**Abbreviations:**

CF, Cystic Fibrosis; CFTR, Cystic Fibrosis Conductance Transmembrane Regulator; CFTR<sub>inh-172</sub>, CFTR inhibitor; F508del, deletion of phenylalanine at position 508 of the *CFTR* gene; ICM, Intestinal Current Measurement; NPD, Nasal Potential Difference; S.E., Standard Error; Vertex-325, 4-cyclohexyloxy-2-(1-[4-(4-methoxy-benzenesulfonyl)-piperazin-1-yl]-ethyl)-quinazoline; WCR, Whole Cell Recording.

KEY WORDS: Cystic Fibrosis, Cystic Fibrosis Conductance Transmembrane Regulator, leukocytes, Patch Clamp.

## 1. Introduction

Cystic fibrosis (CF), one of the most severe genetic diseases with recessive inheritance among people of caucasian origin, is caused by mutations in the gene coding for Cystic Fibrosis Conductance Transmembrane Regulator (CFTR) [1, 2]. CFTR mutations impact cell types and the corresponding tissues differently, with various lung and gastrointestinal manifestations of CF and associated comorbidities among affected patients [4]. Although earlier pathogenic models proposed most of the pulmonary complications of CF to be a direct consequence of epithelial dysfunctions caused by the absence of functional CFTR, more recent clinical and experimental evidence has suggested that a defective CFTR alters the function of cells of the immune system slowing the resolution of inflammation and infection [5, 6]. Di and colleagues demonstrated that CFTR is expressed in alveolar macrophages, in which it has a critical role in the lysosomal acidification and production of cytokines [7, 8]. Other authors showed that CF-affected neutrophils have a decreased ability to kill microorganisms [8, 9]. Del Porto observed that macrophages derived from peripheral blood monocytes of healthy donors express CFTR and that its dysfunction alters the bactericidal activity against *Pseudomonas Aeruginosa* [10]. Altogether the decrease of CFTR expression in immune cells might therefore represent a leading mechanism involved in the pathogenesis of CF and in the development of chronic lung inflammation. As such a better understanding of the expression, regulation and function of CFTR in these cells is becoming a major topic in CF research.

To date a little less than 2000 mutations of the CFTR gene are known but only 23-28 have been related to CFTR dysfunction and consequently also to CF disease [11]. This underlines the importance of assays evaluating CFTR activity for diagnostic purposes. Currently CFTR function is tested using *ex vivo* (Intestinal Current Measurements, ICM) and *in vivo* (Nasal Potential Differences, NPD and Sweat test) assays [12]. The same assays can also be utilized to evaluate the efficacy of the recently developed therapies targeting the basic defect [3]. However the possibility to test the efficacy of these compounds *ex vivo* in a patient-specific context is becoming a major

goal of medical research in this field. In the last few years innovative approaches based on the evaluation of specific patient- derived cells have been developed [13, 14].

Along this line we have recently demonstrated CFTR expression in human peripheral monocytes and proposed a functional assay to evaluate its activity [15].

The aim of this work was to confirm and extend our previous findings. By applying whole-cell recordings in voltage clamp mode we first tested CFTR activity in human monocytes from CF and healthy individuals and we related it to results obtained by a fluorescence probe-based membrane depolarization assay. Subsequently, in order to explore the potential of both techniques to evaluate the expression and the functional recovery of CFTR in F508del mutation carrying monocytes we exposed these cells *ex vivo* to a chemical corrector, 4-cyclohexyloxy-2-(1-[4-(4-methoxy-benzenesulfonyl)-piperazin-1-yl]-ethyl)-quinazoline (Vertex-325) a well established compound capable to correct mutated CFTR processing and expression in epithelial cells [16].

## 2. Material and methods

### 2.1. Subjects and purification of monocytes from whole blood

Monocytes were obtained from 4–5 ml of fresh whole blood withdrawn from 4 non CF individuals (mean age 34 years, range 15–48 years; 3 males and 1 female) and 4 CF patients (mean age 34.5 years, range 24–51 years; 2 males and 2 females, Table 1). The purification protocol was described previously [15]. Written informed consent was obtained as approved by the local Ethical Committee (Azienda ospedaliera universitaria-Verona, protocol number 24737).

### 2.2. Vertex -325 treatment

Purified monocytes from 4 homozygous CF patients carrying F508del mutation (mean age 30 years, range 24–37 years; 4 males, Table 2) were incubated with 4-cyclohexyloxy-2-(1-[4-(4-methoxybenzenesulfonyl)-piperazin-1-yl]-ethyl)-quinazoline (Vertex-325, Chemical Compound Program – Cystic Fibrosis Foundation, U.S.A., 10  $\mu$  M) or vehicle (DMSO) at 37°C for 24 h and then analyzed by patch clamp and single cell fluorescence assay as described below.

### 2.3. Electrophysiology

Whole-cell recordings in voltage clamp mode were performed in monocytes 24–32 hours after purification [17]. All experiments were conducted at room temperature (22 – 24°C) using EPC-7 patch clamp amplifier (HEKA Elektronik GmbH, Lambrecht, Germany) and the pCLAMP 9.0 and CLAMPFIT 9.0 as acquisition and data analysis programs respectively (Axon Instruments, Burlingame, CA, USA). Patch-clamp pipettes had a resistance between 6–8 MOhms.

The pipette solution contained (in mM): 140 N-methyl D-glucamine; 40 HCl; 100 L-glutamic acid; 0.2 CaCl<sub>2</sub>; 2 MgCl<sub>2</sub>; 1 EGTA; 10 HEPES; and 2 ATP-Mg, pH 7.2. The bath solution contained (in mM): 140 N-methyl D-glucamine; 140 HCl; 2 CaCl<sub>2</sub>; 1 MgCl<sub>2</sub>; and 10 HEPES, pH 7.4. The Cl<sup>-</sup> equilibrium potential was -31 mV estimated by Nernst's equation. Cells were maximally stimulated by the addition of a cAMP-activating cocktail (400  $\mu$  M cAMP, 10  $\mu$  M forskolin, 1 mM IBMX) to the pipette solution with voltage steps ranging from -100 to +100 mV for 200 msec with increments of 10 mV from a holding potential of -40 mV. The CFTR inhibitor CFTR<sub>inh-172</sub> (Sigma C2992) was

added to the bath solution at a final concentration of 10  $\mu$  M to specifically inhibit CFTR activity [18, 19]. The reversal potential was calculated, in the different experimental conditions (basal, stimulus and stimulus+inhibitor), as the x-value in which the I/V relationship intercepts the X axis. While the cell membrane capacitance ( $C_m$ ) was determined by integration of the capacitive transients evoked in response to voltage steps ( $\pm 10$  mV) from the holding potential of -40 mV.

#### 2.4. Cell membrane depolarization assay

The potential-sensitive probe bis- (1,3-diethylthiobarbituric acid) trimethine oxonol (DiSBAC2(3), Life Technologies, Carlsbad, USA) was used to monitor CFTR-dependent membrane potential ( $V_m$ ) changes in monocytes from healthy donors and CF patients as described in detail previously [15]. Briefly, the cells were perfused at room temperature with Cl-free solution containing 100 nM DiSBAC2(3). A baseline was acquired for 5 minutes before addition of a CFTR stimulus, consisting of a cocktail containing 500  $\mu$ M 8-Br-cAMP (Sigma B5386), 10  $\mu$ M Forskolin and 100  $\mu$ M 3-Isobutyl-1-methylxanthine (Sigma I7018). Data are presented as percentage of signal variation ( $\Delta F$ ) as compared to the time of addition of the stimulus, according to the equation:  $\Delta F_t = [(F_t - F_0)/F_0] * 100$ , where  $F_t$  and  $F_0$  are the fluorescence values at time t and at the time of addition of the stimulus, respectively.

#### 2.5. Immunofluorescence

Monocytes were purified with RosetteSep (STEMCELL Technologies Inc, Vancouver, BC, Canada). Purified cells were fixed for 20 minutes at room temperature in Fixation buffer (BioLegend, San Diego, U.S.A.). After several washes with PBS, cells were incubated 20 minutes in permeabilization blocking solution (0.1% (v/v) Triton®X-100 and 10% (v/v) human serum (both Sigma-Aldrich, Missouri, U.S.A.) in PBS) and then 60 minutes with 1 mg/mL monoclonal anti-CFTR antibody clone 13-1 (R&D Systems, Minneapolis, U.S.A) diluted in permeabilization blocking solution. Excess antibodies were washed away by PBS1X-0,1% Tween and cells were then incubated 60 minutes with 2 mg/mL Goat anti-Mouse IgG/AF594 (Life Technologies, Carlsbad, U.S.A). Nuclei were stained with 3  $\mu$ M DAPI ((4',6-Diamidino-2-phenylindole



dihydrochloride, Sigma –Aldrich, Missouri, U.S.A.) and cover slip was added using anti-fading mounting medium to prevent loss of fluorescence. Cells were then analyzed with a Leica TCS-SP5 confocal microscope (Leica Microsystem, Wetzlar, Germany).

## 2.6. Statistics

Statistical analyses were performed by Prism5 software (GraphPad Software Inc., La Jolla, U.S.A.) A one-way ANOVA was used to compare means of variables between groups. All pair-wise comparisons were performed using the Tukey's post-hoc test. A significance threshold of  $P < 0.05$  was set for all statistical analyses.

## 3. Results

### 3.1. Non CF monocytes express functional CFTR channels

We examined the electrophysiological properties of CFTR in freshly isolated human blood monocytes from healthy and CF individuals, performing whole cell recording (WCR) in voltage clamp configuration. We added N-methyl D-glucamine in the extracellular and in the pipette solutions to inhibit potassium and sodium currents in order to record only  $\text{Cl}^-$  currents.

We recorded currents during the application of a train of voltage steps to monocytes from healthy and CF donors in: i) basal conditions, ii) after administration of an intracellular (pipette solution) stimulus cocktail, composed of  $400 \mu\text{M}$  cAMP,  $10 \mu\text{M}$  forskolin,  $1 \text{mM}$  IBMX and iii) after simultaneous administration of intracellular stimulus and CFTR inhibitor in bath solution (Fig. 1-2). After stimulus administration we recorded chloride currents in 15 out of 18 (83%) non CF monocytes (Fig. 1 panel a, Stimulus). The recorded currents were sensitive to CFTR<sub>inh-172</sub>, a specific blocker of the CFTR channel (Fig. 1 panel a, Stimulus+CFTR-inh).  $\text{Cl}^-$  currents were never recorded in 19 CF monocytes after cocktail stimulus exposure (Fig. 2, panel a) and CFTR<sub>inh-172</sub> did not modify recorded currents in CF monocytes. The graphs in panel c of figures 1 and 2 show average current/voltage relationships in healthy and CF monocytes respectively and indicate no consistent voltage dependence. Moreover, the reversal potential of  $-30 \text{mV}$  is close to the value

calculated from the Nernst's equation for a perfectly  $\text{Cl}^-$  selective channel (in our experimental condition is  $-31\text{mV}$ ).

To confirm our data we performed other two groups of recordings:

1. In the same healthy monocytes ( $n=7$ ) we administrated intracellular stimulus followed, after 3 minutes, by CFTR inhibitor in bath solution that significantly reduced the chloride current recorded (fig. S1).
2. Moreover to definitively show that the recorded current is chloride dependent we changed the  $\text{Cl}^-$  concentration in the bath solution to modify the reversal potential, shifting it to more positive values ( $-10\text{ mV}$ ) and repeated the treatment as before. We recorded a current sensitive to CFTR inhibitor with a reversal potential of about  $-10\text{ mV}$ , as expected for a chloride channel (fig. S1).

The log<sub>10</sub> current density values in basal condition were of  $-0.22\pm 0.07$  and  $-0.38\pm 0.07$  in healthy and CF individuals, respectively. Stimulus administration induced an increase only in healthy subjects ( $0.59\pm 0.08$  and  $-0.24\pm 0.04$  in healthy and CF individuals, respectively) while in the presence of CFTR<sub>inh-172</sub> current density dropped in cells from healthy subjects to values similar to those recorded in basal conditions ( $-0.30\pm 0.09$  and  $-0.22\pm 0.07$ , in stimulus and basal conditions, respectively: Fig.1 panel d and fig. S2)

Monocytes from the same subjects analyzed by WCR (healthy and CF donors) were studied in parallel by single-cell fluorescence assay (Fig. 1-2 panel b). We observed increased single cell fluorescence in non-CF monocytes, but not in CF monocytes, following administration of the stimulus, confirming that single cell depolarization assay can detect a CFTR dependent function. This conclusion is supported by the results obtained by the evaluation of the consequence of CFTR stimulation on whole-cell  $\text{Cl}^-$  currents.

### **3.2. Vertex-325 induces recovery of CFTR function in monocytes carrying F508del mutation.**

In order to evaluate whether monocytes could represent a suitable cell model to monitor the effect of drugs active on CFTR channel and to confirm the presence of a functional CFTR channel in

these cells, we tested the efficacy of a CFTR corrector on the most common CF mutation, the deletion of phenylalanine at position 508 of the *CFTR* gene (F508del) [20, 21]. This mutation causes protein misfolding and retention of the protein in the endoplasmic reticulum [22], a defect that can be at least partially rescued by small molecules, called "correctors" [23]. We tested a well-characterized corrector, Vertex-325, *ex vivo* in monocytes carrying the homozygous F508del mutation (Table 2). Monocytes from the same subject were incubated for 24h with Vertex-325 or with vehicle (DMSO). We then compared their response to the cocktail stimulus by WCR. In 15 of 17 Vertex-325 exposed monocytes (88%) we observed chloride currents, significantly higher than those detected in DMSO exposed-monocytes (7 monocytes studied, Fig. 3 panel a). Moreover we found that the chloride currents in Vertex-325 treated monocytes were sensitive to CFTR<sub>inh-172</sub> (7 monocytes studied, Fig. 3 panel c and d). We also evaluated in parallel the Vertex-325 exposed monocytes to CFTR stimulus by single cell fluorescence assay. Both assays confirmed that exposure to Vertex-325 induced a response associated to the recovery of CFTR function (Fig 3 panel b).

The relation Current/Voltage recorded by WCR (fig. 3 panel c) showed a reversal potential value (-30mV) of a selective Chloride channel and no voltage dependence identical to those observed in monocytes from healthy individuals.

The log<sub>10</sub> current density values after stimulus administration were significantly increased in monocytes exposed to Vertex-325 compared to control ( $0.42 \pm 0.03$  and  $-0.13 \pm 0.07$  in Vertex-325 and DMSO exposed monocytes respectively, fig. 3 panel d) while in presence of CFTR<sub>inh-172</sub> the current density values, in Vertex-treated monocytes, dropped to values similar to those observed in stimulated control monocytes, DMSO treated ( $-0.12 \pm 0.09$  and  $-0.13 \pm 0.07$ , respectively, fig. 3 panel d).

To further support the electrophysiological data we analyzed the CFTR expression, by confocal microscopy analysis, in Vertex-325 exposed monocytes. Consistently with the functional data, we observed a higher expression level of CFTR in Vertex-325 exposed monocytes as compared to

control (Fig. 3 panel e). CFTR expression was associated to increased protein expression and loss of perinuclear localization of the signal in many cells (figure 3 panel e).

#### 4. Discussion

CF disease typically feature an exaggerated lung inflammatory response whose mechanisms are not yet completely understood [5]. Therefore, the evaluation of CFTR expression and function in leukocytes, key players of innate immunity and inflammation, becomes crucial for the understanding of the role played in the pathogenesis of the disease.

In this study we extend our previous findings by describing the electrophysiological proprieties of CFTR expressed in human monocytes using a patch clamp technique (WCR), a method that directly measure transmembrane currents specifically associated to Cl<sup>-</sup> transport. Under conditions in which Na<sup>+</sup> and K<sup>+</sup> currents are abolished, Cl<sup>-</sup> conductance is: 1) increased by elevation of intracellular cAMP, 2) voltage independent as shown from the I-V relationship, 3) time independent during depolarization, 4) suppressed by a specific CFTR antagonist, CFTR<sub>inh-172</sub>, 5) totally absent in monocytes from CF patients and 6) restored in monocytes carrying the F508del mutation from homozygous subjects after treatment with the specific CFTR corrector, Vertex-325. Altogether these results not only formally demonstrate for the first time that human blood monocytes isolated from healthy subjects express functional CFTR on their plasma membrane, but that this expression is lost in CF patients and restored after an appropriate drug treatment.

The expression of ion channels in the cell of the immune system control their differentiation and function. A number of electrophysiological studies have identified the expression of voltage- and ligand-gated K<sup>+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup>, H<sup>+</sup> and cation channels in inflammatory cells [24, 25]. The functional role of these channels was investigated in monocytes and in monocyte-like cells [24, 26]. However there is not extensive information about the chloride channel identity and function in human blood monocytes. Kim et al. showed volume-sensitive Cl<sup>-</sup> channels in human blood monocytes that regulate migration of the cells [27], Cheng-yao Shi described an acidic extracellular pH-activated,

outward rectifying chloride channel in monocyte-derived macrophages and human peripheral monocytes [28] while another work found the involvement of Chloride channels in monocyte chemoattractant protein1-induced migration of monocytes [29].

Di et al., showed that in alveolar macrophages CFTR chloride channel participates in control of phagosomal pH, important to kill internalized microorganisms [7]. In our recent works we described that human peripheral monocytes express CFTR [15, 30]. Despite these findings the specific function of CFTR in monocytes is currently unknown even if the different membrane depolarization of CF monocytes in comparison with controls suggest alteration of membrane-associated functions. Along this line bone marrow transplants between CF and non-CF mice suggest that these cells are inherently different and that CFTR is involved directly in myeloid cell function. This imply that these cells contribute to the pathophysiological phenotype of the CF lung, a major target of disease whose dysfunction is associated to high morbidity and mortality in CF [31]. The biophysical and pharmacological properties of the CFTR  $\text{Cl}^-$  currents that we recorded in human monocytes were similar to those previously recorded in different other models (e.g. voltage independence in their I–V relationships, and time-independent activation during depolarization) [32]. Currents of monocytes from healthy individuals were sensitive to CFTR<sub>inh-172</sub>, a selective blocker of CFTR. Monocytes from CF patients showed negligible changes in conductance under similar experimental conditions, as previously shown also in alveolar macrophages from CFTR-null mice [7]. Interestingly, peak current density measured in human monocytes showed lower values than those recorded by Del Porto et al. in human *in vitro* differentiated macrophages [10]. However this result is not unexpected as it is likely that different amounts of CFTR are expressed in plasma membrane of different cell types. Indeed, Del Porto et al. found a lower quantity of CFTR mRNA in blood monocytes than monocyte derived macrophages [7]. Gallin et al. described that the expression of different ion channels in monocytes, macrophages and related cell lines is different and that it changes during the course of cell maturation, differentiation or activation [24]. Moreover, different cell culture conditions may affect CFTR expression level, which also may vary over time.

Brochiero et al. showed a 60% CFTR expression level reduction in cultures of pulmonary epithelium after 24h that was then significantly increased on day 4 [33].

Another study showed a cell cycle-dependent CFTR expression in fresh B-lymphocytes and in B- and T-lymphocyte cell lines. In particular they found low chloride permeability in G0 phase and a high permeability in G1-S and this was related to different CFTR expression levels [34]. Under our experimental conditions, 24-32 hours after purification, monocytes were in G0 phase as demonstrated by the lack of staining with the cell cycle-related antigen Ki67 (data not shown). We observed that 83% of recorded monocytes from healthy donors were sensitive to CFTR-stimulus administration. Moreover, in our former work, we showed CFTR membrane expression in monocytes 24 hours after preparation[15]. The readily detectable expression of CFTR in monocytes during G0 phase compared to lymphocytes might be due to different functions that CFTR is expected to have in these two different cell types.

Monocytes can represent a convenient source of primary cells derived from individual patients and we recently demonstrated their potential use as convenient source of primary cells useful to evaluate CFTR function to support the diagnosis of CF [35]. We now wondered whether these cells could be sensitive to a treatment aiming to the reconstitution of CFTR function. In the present study we exposed monocytes carrying F508del mutation ex vivo to Vertex-325, a well characterized chemical corrector able to rescue the biosynthetic defect of F508del-mutated CFTR protein and to increase its expression on the plasma membrane in different cell lines [36]. We focused our attention on the ability of patch clamp and fluorescence assay to detect the recovery of CFTR function in these cells. We indeed succeeded in measuring cAMP-induced chloride currents in CF monocytes exposed to Vertex-325 by WCR and by single cell fluorescent assay. We found that these currents were sensitive to CFTR antagonist, CFTR<sub>inh</sub>-172. Moreover, using confocal microscopy analysis, we observed an increase in CFTR expression after Vertex-325 treatment, consistent with electrophysiological analysis. Multiple correction mechanisms accounting for the rescue of F508del-CFTR have been described. Vertex-809, Vertex-325, and Corr-4a show variable

additive effects with the genetic revertants tested. Although other compounds are currently available and are being tested in clinic either alone or in combination [6, 37], Vertex-325 is taken as a point of reference to compare the effectiveness of new compounds and apparently acts on the NBD1:NBD2 dimerization interface displaying a mechanism of action distinct from that of Vertex-809 [16, 38, 39].

The limited success of Vertex-809 in clinical trials prompted the testing of combination therapies to achieve complementary rescue by distinct mechanisms of actions and full F508del-CFTR correction, so as to reach the functional CFTR threshold necessary to avoid CF. It is also known that some CFTR correctors act only on cells expressing recombinant, but not native, CFTR [40]. This highlights the importance to confirm activity of any experimental compound in primary cells before moving to animal and human studies.

Another critical point in Cystic Fibrosis is related to the diagnosis of disease when non conventional CFTR mutations variants are detected. Recent guidelines for CF diagnosis require genetic test and sweat test, but they are not always sufficient to diagnose CF or CFTR related disorders given the presence of a little less than 2000 different mutations, some of which of uncertain clinical significance [41, 42]. Intestinal current measurement (ICM) and nasal potential difference (NPD), evaluating currents and potential differences respectively, directly on epithelial cells of individuals are currently used in specialized clinics even if the issue of standardization is still matter of debate [43-45]. Monocytes represent easily accessible and relatively abundant cells while alternative sources of primary cells are scarce (nasal epithelium), more difficult to collect (rectal or colon epithelium) or virtually impossible to collect in a meaningful number of patients (lower airways). As such, monocytes may represent a valuable cell model for multiple tasks in both basic and applied science related to CF. Along this line we recently proposed a single cell fluorescence assay as a new test to evaluate CFTR function in blood monocytes and showed that their results were overlapping with those obtained by NPD measurements in the same CF subjects [15, 35].

In the present work we have strengthened and significantly expanded our previous findings as: i) we performed a more in-depth characterization of CFTR-dependent currents in monocytes by patch-clamp technique, because cell depolarization assay that we proposed in our previous work is less specific than WCR; ii) we further support the ability of a single cell fluorescence assay to detect CFTR –associated activity and moreover; iii) we show the ability of both techniques to detect the recovery of CFTR activity in monocytes from F508del mutation carrying patients, after *ex vivo* exposure to a prototype chemical corrector like Vertex-325. The aim of our work is to provide the proof-of-principle that WCR in human primary leukocytes can be utilized as a suitable approach to evaluate CFTR function and the effect of chemical correctors. The possibility exists to improve manual WCR using innovative medium and high-performance patch clamp recording system that can greatly improve the throughput capability of a direct measure of CFTR in these cells. Efforts are currently under way in our laboratories to explore this possibility.

Altogether these results highlight fluorescence assay and patch clamp as two valuable tools to assess CFTR activity and further support blood monocytes as a suitable and convenient source of primary cells for the study of the functional consequences of various CF mutations and for the evaluation of the efficacy of approaches aimed to correct the basic defect in CF.

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Conflict of Interest: The authors declare no conflict of interest.

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## FIGURE LEGENDS

**Figure 1. Functional activity of CFTR in Non CF monocytes.**

Whole-cell in voltage clamp configuration and single-cell fluorescent assay of human peripheral monocytes of healthy subject. Panel (a): representative currents in basal conditions (Basal, upper registration), after administration of a stimulus cocktail (Stimulus, middle registration) in pipette solution and after concomitant administration of stimulus and CFTR<sub>inh-172</sub> to the bath solution (Stimulus+ CFTR<sub>inh</sub>, lower registration). Currents were recorded applying 200 msec voltage steps from -100 to +100 mV with increments of 10 mV. Panel (b): single-cell fluorescent assay performed of monocytes from the same donor as those analyzed by patch clamp in panel (a). Percentage of fluorescence variation ( $\Delta F$ ) is shown over time in 8-Br-cAMP/forskolin/IBMX stimulus condition (circles), and in basal condition (diamonds). Panel (c): average current/voltage relationship in basal condition (diamonds, n=10), in presence of cocktail stimulus (circles, n= 15) and after administration of stimulus and CFTR<sub>inh-172</sub> (triangles, n=9). Panel (d): log<sub>10</sub> of current densities calculated at +100 mV in the experimental conditions described. Data in b and c are means  $\pm$  S.E., n indicates the number of recorded cells. \*\*p<0.001.

**Figure 2. Impaired CFTR function in monocytes from cystic fibrosis patients**

Whole-cell in voltage clamp configuration and single-cell fluorescent assay in human peripheral monocytes of CF subject. Panel (a): representative currents in basal conditions (Basal, upper registration), after administration of a stimulus cocktail (Stimulus, middle registration) in pipette solution, and after concomitant administration of stimulus and CFTR<sub>inh-172</sub> to the bath solution (Stimulus+ CFTR<sub>inh</sub>, lower registration). Currents were recorded applying 200 msec voltage steps from -100 to +100 mV with increments of 10 mV. Panel (b): single-cell fluorescent assay performed of monocytes from the same patient as those analyzed by patch clamp in panel (a). Percentage of fluorescence variation ( $\Delta F$ ) is shown over time in 8-Br-cAMP/forskolin/IBMX stimulus condition (circles), and in basal condition (diamonds). Panel (c): averaged current/voltage relationship in basal condition (diamonds, n=7), in presence of cocktail stimulus (circles, n=19) and

after administration of stimulus and CFTR<sub>inh-172</sub> (triangles, n=8). Panel (d): log<sub>10</sub> of current densities calculated at +100 mV in the experimental conditions described. Data in b and c are means ± S.E., n indicates the number of recorded cells.

**Figure 3. Functional recovery of CFTR in monocytes carrying F508del mutation after *ex vivo* exposure to Vertex-325**

Whole-cell recording in voltage clamp configuration and single-cell fluorescent assay in human peripheral monocytes from CF patients carrying F508del after *ex vivo* exposure to Vertex-325. Panel (a): representative currents after administration of a stimulus cocktail (Stimulus) in pipette solution, in monocytes exposed to Vertex-325 or to DMSO. Currents were recorded applying 200 msec voltage steps from -100 to +100 mV with increments of 10 mV. Panel (b): single-cell fluorescent assay performed of monocytes from same individual as those analyzed by patch clamp in panel (a). Percentage of fluorescence variation ( $\Delta F$ ) is shown over time in 8-Br-cAMP/forskolin/IBMX stimulus condition (circles), and in basal condition (diamonds). Panel (c): average current/voltage relationship in presence of cocktail stimulus in Vertex-325 (circles, n=17), in DMSO-treated monocytes (diamonds, n=7) and after stimulus and CFTR<sub>inh-172</sub> in Vertex-325 treated monocytes (triangles, n= 7). Panel (d): log<sub>10</sub> of current densities calculated at +100 mV under experimental conditions as described above. Panel (e) Confocal microscopy analysis performed using monoclonal anti-CFTR (clone 13-1) of Vertex-325 treated and control monocyte from a subject carrying the F508del mutation. Scale bar 10  $\mu$ m (figure represents one experiment out of 2 performed). Data in b and c are means ± S.E., n indicates the number of recorded cells. \*\*p<0.001.



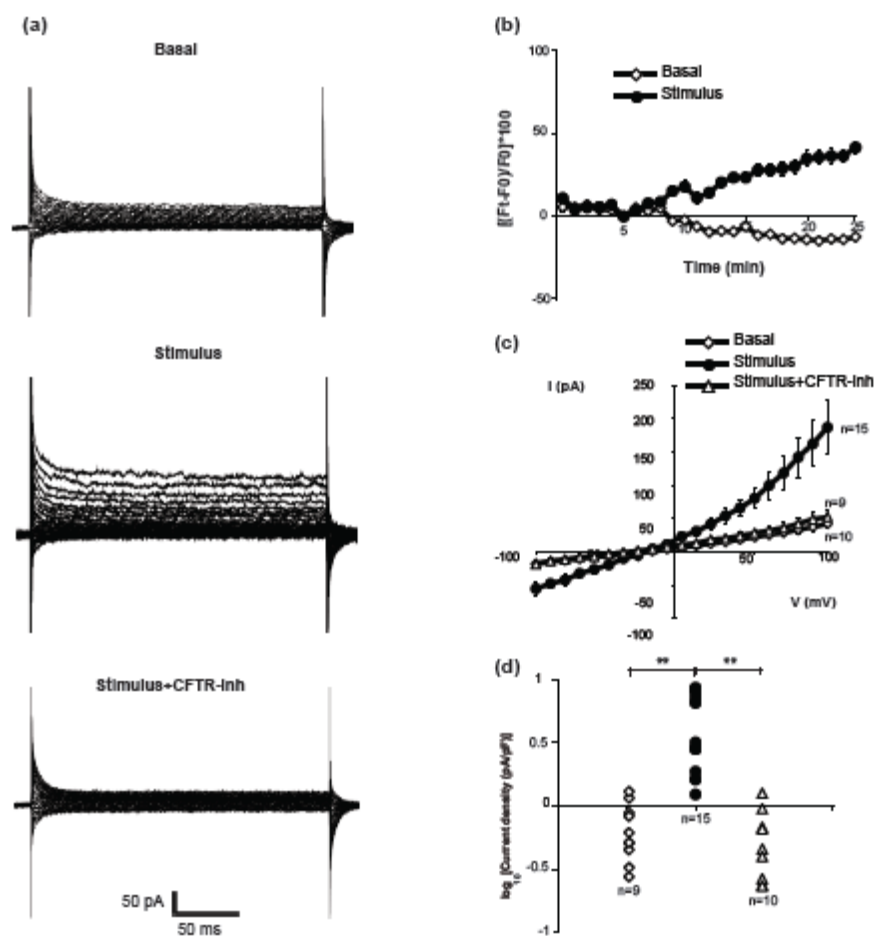
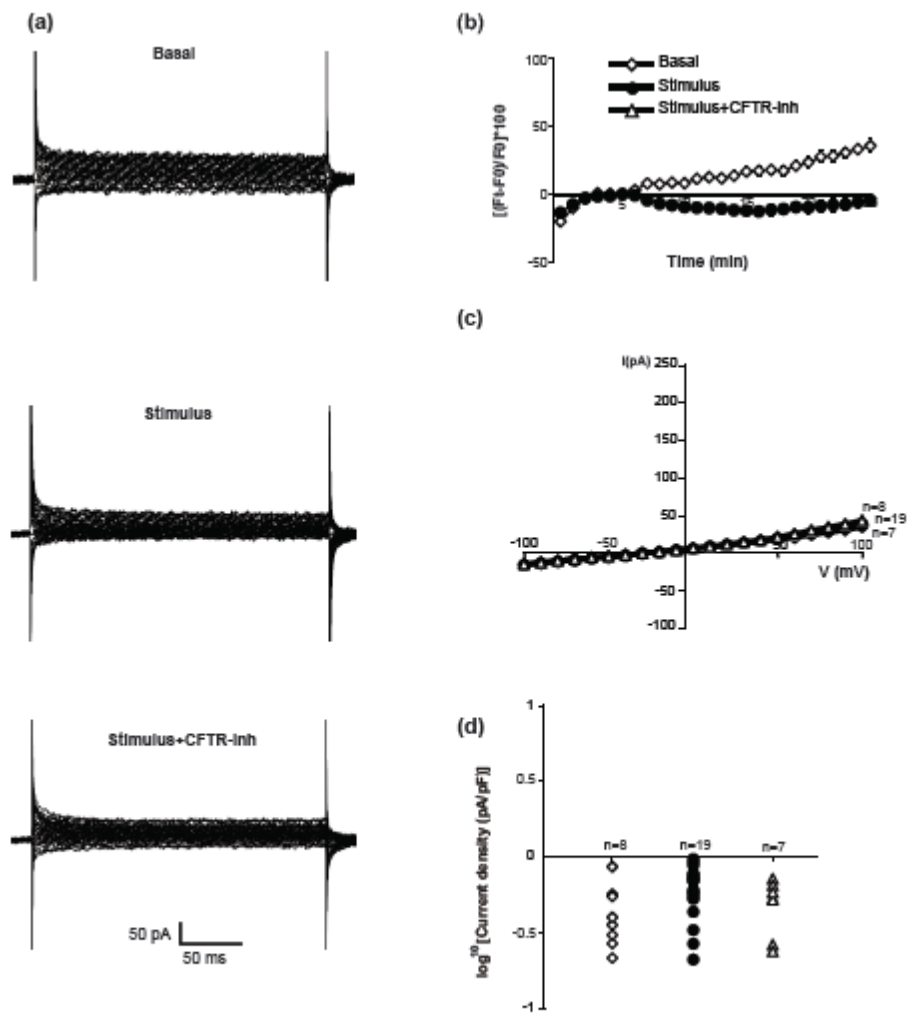


Figure 1



A

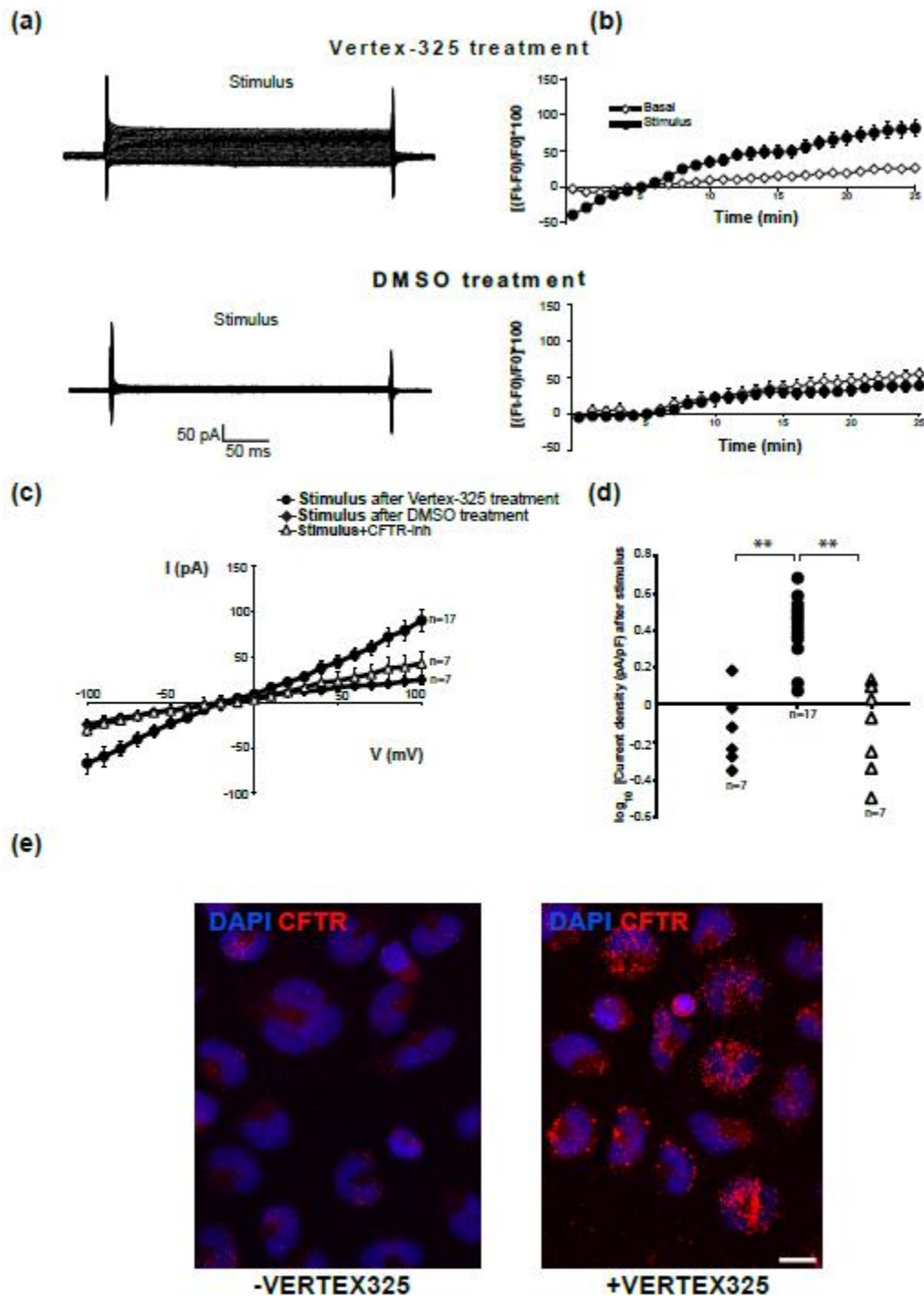


Figure 3

**Table 1. CF patients enrolled in the study.**

<b>Subject number</b>	<b>Gender</b>	<b>Genotype</b>	<b>Age (years)</b>
0948	F	F508del / 4382delA	51
0952	F	F508del / 1717-1G→A	24
0961	M	F508del / Q353X	32
0986	M	F508del / 1717-1G→A	31

**Table 2. CF patients whose monocytes were exposed ex vivo to Vertex-325.**

<b>Subject number</b>	<b>Gender</b>	<b>Genotype</b>	<b>Age (years)</b>
1139	M	F508del / F508del	37
1151	M	F508del / F508del	26
1152	M	F508del / F508del	35
1200	M	F508del / F508del	24

**Highlights**

- Human non CF monocytes express a functional CFTR.
- F508del CF monocytes recover CFTR function after *ex vivo* exposure to Vertex-325.
- Monocytes as a possible support for diagnosis and drug development for CF.