Antibiotic therapy affects functional behaviour in cystic fibrosis blood mononuclear cells

To the Editor:

Cystic fibrosis (CF) is the most common life-shortening genetic disorder in the Caucasian population and is due to mutations at the CF transmembrane conductance regulator (CFTR) gene leading to dysfunction of the protein, which normally acts as a chloride channel. This basic defect is associated with a progressive and lethal lung disease [1]. Opportunistic respiratory infections are common in CF patients’ lungs, making antibiotics an important part of the regular care.

In the present study, we investigated whether a course of intravenous antibiotic treatment (10 days) for a pulmonary exacerbation in CF patients resulted in improved pulmonary function that was associated with changes in the expression of proteins forming the macromolecular complexes required for correct CFTR function in lymphomonocytes (LMNs).

While polymorphonuclear neutrophils (PMNs) preferentially accumulate on the CF surface epithelium, mononuclear cells are the predominant cell population in areas of cartilaginous destruction. The role of mononuclear cells in CF lung disease is presently poorly understood, although recently, a predominance of lymphocytes has been shown to infiltrate the subepithelial bronchial tissue from children with CF [2]. Lymphocytes found in the bronchial submucosa of CF subjects produce high levels of interleukin-17, a proinflammatory cytokine that regulates granulopoiesis and neutrophil recruitment [3].

17 nonsmoking subjects with CF who were homozygous for the F508del mutation (mean age 27.3 years, range 11–43 years), all of whom were pancreatic insufficient, were enrolled at the time of admission for a clinically diagnosed pulmonary exacerbation at the CF Regional Centre (Bari, Italy). The study was approved by the ethics committee of the Azienda Ospedaliera Universitaria “Policlinico” (Bari) (n.1373/CE/2012) and performed in accordance with the 1964 Declaration of Helsinki, after written informed consent was obtained from the adult study subjects, or the next of kin, caretakers, or guardians on behalf of the enrolled children.

Initial antibiotic choices (one, two to three antibiotics per patient) included azithromycin (n=8), ciprofloxacin (n=6), tobramycin (n=5), cefazidime (n=4), minocycline (n=2), meropenem (n=1), levofloxacin (n=1), sulfamethoxazole/trimethoprim (n=1), amikacin (n=1), imipenem (n=1), teicoplanin (n=1) and linezolid (n=1). All treatments for acute exacerbation resulted in significant decreases in circulating PMNs (62.6% of total white blood cell (WBC) count pre- versus 57.5% of total WBC count post-antibiotics, p=0.01), which paralleled the changes in serum C-reactive protein (19.5 mg·dL$^{-1}$ pre- versus 11.3 mg·dL$^{-1}$ post-antibiotics, p=0.02). The antibiotic treatment also resulted in a significant amelioration of forced expiratory volume in 1 s (47.2% predicted pre- versus 52.4% predicted post-antibiotics, p=0.04) and forced vital capacity (59.8% predicted pre- versus 65.1% predicted post-antibiotics, p=0.04).

Furthermore, during acute exacerbations, CF patients’ LMNs had a severely impaired expression of the mature CFTR band with respect to LMNs from healthy subjects [4]. Here, in confirmation of that study, Figure 1a shows a typical Western Blot in which we loaded LMN lysates derived from a healthy donor and from a representative CF patient pre- and post-antibiotic treatment. In healthy LMNs, wild-type CFTR was expressed as the fully glycosylated mature form of the protein, band C (180 kDa), and the core glycosylated form, band B (160 kDa). As expected, in the CF LMNs pre-antibiotic treatment, F508delCFTR was almost completely expressed as the immature band B, although a small amount of the mature band C protein was also observed in three of them. Antibiotic treatment resulted in the appearance of band C, corresponding to a functional protein, as confirmed by spectrophuorimetric analysis using the chloride-sensitive dye, MQAE (N-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide). CFTR-dependent chloride efflux was not detectable in LMNs from CF patients with acute exacerbation whereas antibiotic therapy significantly increased efflux to levels similar to those found in non-CF LMNs (figure 1b). The observed restoration of CFTR functionality was not associated with a statistically significant increase in CFTR mRNA levels post-antibiotic treatment (data not shown), which may therefore indicate that antibiotic treatment indirectly regulates the trafficking of F508delCFTR in the cell membrane via modulation of chaperones and cochaperones (90-kDa heat-shock protein (Hsp90)/70-kDa heat-shock protein (Hsp70)) known to bind CFTR [5].
F508delCFTR activity rescue was instead correlated to expression changes of the components of the multiprotein complex CFTR–sodium/hydrogen exchanger regulatory factor (NHERF)1–ERM (ezrin–radixin–moesin)–actin known to be involved in the regulation of CFTR localisation and activity [6]. Indeed, antibiotic therapy significantly increased NHERF1 and moesin protein expression levels (64% and 45%, respectively) compared with levels observed in acute exacerbation, which were significantly lower than healthy LMNs (figure 1c and 1d), in line with our previous observations in human bronchiolar epithelial cells [7, 8].

As ERM proteins have been also implicated in other aspects of lymphocyte activation, such as migration [9], we investigated whether the observed differences in moesin protein expression could correlate with altered migration in CF LMNs. Results from transwell migration studies showed that circulating LMNs obtained from CF patients with acute exacerbation had increased migration rates whereas migration returned to non-CF values post-antibiotic treatment (figure 1e). It is worth noting that our assay did not use chemotactic factors, thus suggesting that CF LMNs inherently display this migration property, i.e. they are pre-activated in the peripheral circulation. This “primed” status may result from the shedding of pro-inflammatory cytokines from the lung, where they are produced at high levels in CF exacerbations [10].
It is known that ERM function is partly controlled by phosphorylation on a conserved threonine in the actin-binding domain [11]. Moreover, it has been reported that, unlike in epithelial cells, ERM proteins occur predominantly in their active phosphorylated conformation in lymphocytes [12]. In line with these findings, we observed more ERM protein phosphorylation in healthy LMNs compared with CF LMNs, as well as phosphorylation levels in CF LMNs post-antibiotic therapy that were almost comparable to healthy LMNs (figure 1f). These results suggest a critical physiological role of ERM protein activation in lymphocytes. ERM protein dephosphorylation is known to be affected by chemokine signalling and leads to microvillar collapse in LMNs, thereby promoting the transition from tethering and rolling to integrin-dependent cell–cell adhesion [13, 14]. Thus, it is tempting to speculate that a more relaxed cytoskeleton, in response to reduced levels of activated moesin, may promote the increased LMN migration observed in the acute exacerbation condition.

Among the different antibiotics used in CF patients, azithromycin significantly activates chloride efflux in CF human bronchial epithelial cells [15, 16]. Since this macrolide was used in many of the CF patients in this study, it could be, at least in part, responsible for the rescuing effect on CFTR and the macromolecular complex that allows correct positioning and function of CFTR on the plasma membrane.

In conclusion, we demonstrated, for the first time, that antibiotic treatment restores adequate levels of functional CFTR in LMNs, therefore contributing to improving the clinical status of CF patients. Our hypothesis is that antibiotic-mediated regulation of chaperones might allow increased folding and trafficking of mutant CFTR protein, thereby inducing partial CFTR function restoration. Despite the paucity of patients taken into consideration and the heterogeneity of antibiotic treatments, we found that their clinical status amelioration was consistently associated with increased functional expression of the defective CFTR chloride channel, increased expression of NHERF1 and moesin, and subsequent reduced LMN migration, probably suggesting that mononuclear cells may also take part in the inflammation-related damage of CF airways.

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Antibiotic therapy indirectly restores adequate levels of functional F508delCFTR in CF lymphomonocytes patients http://ow.ly/Ncydg

Lorenzo Guerra1,5, Maria Favia3, Stefano Castellani2, Giovanna Barbuti1, Pasqualina Montemurro3, Anna Diana4, Teresa Santostasi4, Angela M. Polizzi4, Maria A. Mariggiò1, Stephan J. Reshkin1, Antonio Manca4, Valeria Casavola1,6 and Massimo Conese2,6

1Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari, Italy. 2Department of Medical and Surgical Sciences, University of Foggia, Foggia, Italy. 3Department of Biomedical Sciences and Human Oncology, Section of General Pathology, University of Bari, Bari, Italy. 4Cystic Fibrosis Regional Center, Department of Biomedical and Human Oncology, Section Pediatrics, U.O. “B. Trambusti”, University of Bari, Bari, Italy. 5These authors contributed equally to this work. 6These authors share senior authorship.

Correspondence: Lorenzo Guerra, Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Via Orabona 4, Bari 70126, Italy. E-mail: lorenzo.guerra1@uniba.it

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References


