Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

Overview of CF lung pathophysiology

Giulio Cabrini[1,3], Alessandro Rimessi[2,3], Monica Borgatti[1,3], Paolo Pinton[2,3] and Roberto Gambari[1,3]

Abstract

Defects of the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) protein affect the homeostasis of chloride, bicarbonate, sodium, and water in the airway surface liquid, influencing the mucus composition and viscosity, which induces a severe condition of infection and inflammation along the whole life of CF patients. The introduction of CFTR modulators, novel drugs directly intervening to rescue the function of CFTR protein, opens a new era of experimental research. The review summarizes the most recent advancements to understand the characteristics of the infective and inflammatory pathology of CF lungs.

Addresses

[1] Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

[2] Department of Medical Sciences, University of Ferrara, Ferrara, Italy

[3] Center of Innovative Therapies for Cystic Fibrosis, University of Ferrara, Ferrara, Italy

Corresponding author: Cabrini, Giulio (giulio.cabrini@unife.it)

Current Opinion in Pharmacology 2022, **64**:102214

This review comes from a themed issue on **Respiratory**

Edited by **Carla M.P. Ribeiro** and **Martina Gentzsch**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.coph.2022.102214

Keywords

Cystic fibrosis, CFTR modulators, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Lung inflammation, Reactive oxygen species.

Abbreviations

CF, Cystic Fibrosis; CFTR, Cystic Fibrosis Transmembrane conductance Regulator; IL-8, Interleukin 8; *P aeruginosa*, *Pseudomonas aeruginosa*; ENaC, Epithelium Sodium Channel; ASL, Airway Surface Liquid; BALF, Bronchoalveolar Lavage Fluid; PMN, PolyMorphonuclear Neutrophil; PTEN, phosphatase and tensin homolog deleted in chromosome 10.

Introduction

CF is a genetic disease caused by mutations of the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene, localized on chromosome 7, which was correctly predicted as an ion transporter since the gene discovery [1–3]. More than 2000 CFTR gene variants have been identified so far, a few hundred being confirmed as disease causing mutations [4*].

In order to provide an operational synopsis of the effects of such a wide series of *CFTR* gene variants on CFTR biology, *CFTR* mutations have been categorized into six major classes depending on their main pathologic effects on CFTR protein, namely, Class I: absence of protein, Class II: protein trafficking defect, Class III: no function, Class IV: reduced function, Class V: reduced protein, Class VI: reduced protein stability [for review see the study by Bell et al. [5]].

As a result of CFTR protein mutations, CF disease affects the function of several organs, mainly exocrine pancreas, liver, intestinal tract, salivary glands, male reproductive organ, sweat glands, being the pathology in the respiratory tract the main cause of morbidity and reduction of life expectancy [6*]. Since the milestone of the *CFTR* gene discovery in 1989, tremendous progresses have been made in understanding the basic CF defect. This allowed to develop and apply novel drugs, termed "CFTR modulators," which act directly on the mutated protein by rescuing at least in part its defective function [7*]. In spite of these exciting therapeutic improvements, which make CF disease entering the era of the novel CFTR modulator Trikafta [8,9], different questions remain unanswered on CF lung pathophysiology.

Defective CFTR protein affects ion and fluid homeostasis in the respiratory mucosa

CFTR protein has been confirmed as an ATP-dependent, protein kinase A and C regulated, transporter of chloride and bicarbonate [10,11]. These ions are secreted from the apical membrane of different kinds of epithelial cells composing the airway mucosa and submucosa. The cell types that express CFTR gene/protein is a hot field of research. For example, recent single cell RNA-sequencing studies revealed CFTR-rich pulmonary ionocytes [12,13]. Furthermore, more recent studies on proximal and distal airways in control and CF airway epithelia suggest that secretory cells (but not ciliated cells, which had been previously considered as dominant CFTR-expressing cells [14]) are the dominant CFTR-expressing cell type [15**,16]. In addition to direct ion transport, CFTR inhibits the activation of the epithelial sodium channel (ENaC) [17] and

regulates other chloride channels [18]. Because of defective chloride and bicarbonate secretion and excessive sodium reabsorption, it is logically expected that the airway surface liquid (ASL) residing on top of the apical membranes of the epithelium of the CF airways should be altered. However, the equilibrium between different phases of fluid secretion and absorption made this issue extensively debated for years, with contradictory results due to the complexity of the *in vivo* measurements of the physic-chemical parameters of ASL in CF patients [19]. Although still under scrutiny, the prevalent consensus now considers that the ASL of CF lungs is dehydrated and more acidic in respect to non-CF ASL [20].

Impaired ASL homeostasis affects the biology of CF airway mucosa

The ASL consists of a hydro-gel mucus layer with gel-forming mucins constituting a mesh that traps dust and microorganisms. These are rapidly transported from distal airways to trachea by ciliary beating, the muco-ciliary clearance being considered the very basic innate defense mechanism in the lungs. The pH and the relative content of mucins and water in ASL is critical for the viscosity of the mucin hydrogel [20]. In CF, dehydration reduces the periciliary liquid and increases the viscosity of the mucin hydrogel mesh, which reduces the frequency of the muco-ciliary beating mechanism [21**]. Besides the reduced hydration, bronchoalveolar lavage fluid (BALF) obtained from young CF patients revealed increased concentration of mucins (MUC) 5B and 5AC [22**], although it is not clarified whether this depends on the primary CFTR defect or it results from a mechanism secondary to the advanced stages of the CF lung pathology. Interestingly, markers of inflammation were found elevated either in presence or in absence of bacterial infection [22**]. Thus, altered physicochemical properties of mucus in CF ASL are considered an upstream initiating, and later amplifying, pathogenic mechanism in between defective ion transport and the infective/inflammatory process [21**,22**].

The onset of CF lung disease has been demonstrated since the early months of the life of CF infants, even in the absence of overt respiratory symptoms, as the bronchioles of CF infants has been found filled with polymorphonuclear neutrophils (PMN) [23]. Lung disease starts from a clinically asymptomatic phase that is followed by recurrent, and lately, chronic bacterial infections, associated with inflammation, conditions leading to damages of the bronchial walls, with dilations (bronchiectasis) filled with mucopurulent sputum. A huge number of microorganisms and PMNs in lumen constitutes an infective/inflammatory condition leading to progressively severe obstructive respiratory insufficiency [23]. CF lung pathology could be summarized with the two key points of infection and inflammation: 1) recurrent infections with *Haemophilus influenzae* in childhood and with *Staphylococcus aureus* (*S. aureus*) in early life are progressively substituted in adulthood with recurrent infection with *Pseudomonas aeruginosa* (*P. aeruginosa*), which lately chronically infects CF airways; 2) CF cellular and humoral immune defenses, that are inefficient in clearing bacterial infections, mount an "exaggerated" inflammatory response characterized by a huge amount of PMNs filling the airway lumen. Both recurrent and chronic infection and exaggerated inflammation contribute to bronchial wall tissue damage and progressive airway flow obstruction, leading to progressively severe respiratory insufficiency.

CF airways mucosa as a pro-infective milieu

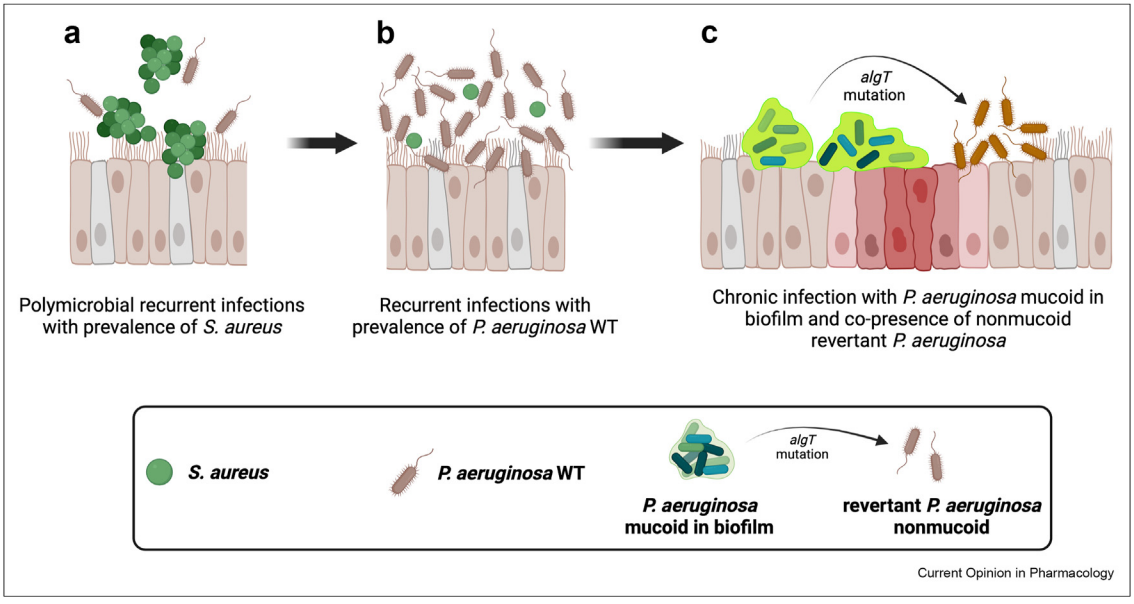
As mentioned, impaired muco-ciliary clearance and mucus abnormality is widely believed to constitute the favorable milieu for CF lung infections [21**,22**]. Although CF infections are polymicrobial along the whole life, including *H. influenzae, Stenotrophomonas maltophilia, Achromobacter xylosoxidans, Nontuberculous mycobacteria*, different fungi (e.g. *Aspergillus* and *Candida* species) and viruses (e.g. rhinovirus, influenza virus, respiratory syncytial virus) and other microorganisms, the main attention in clinics and research has been focused on *S. aureus* and *P. aeruginosa*, considered the major infectious effectors of CF lung tissue damage [24]. Predominance of *S. aureus* in lung expectorate early in life of CF patients has been related to a CF-specific defective immune response dependent on reduced pH in CF ASL, which favors survival of this bacterium [25]. In CF adults, *P. aeruginosa* becomes the predominant infection agent overtaking *S. aureus*, possibly by inducing the host secretion of the bactericidal enzyme type-IIA-secreted phospholipase A2, which kills *S. aureus* with limited lytic effects on *P. aeruginosa* [26], although coexistence of both microorganisms persists in CF adults [27].

The reason why *P. aeruginosa*, a ubiquitous environmental opportunistic microorganism causing disease almost only in immunocompromised individuals, becomes the predominant infectious agents in CF adults is still unknown, unless we consider the general hypothesis of CF as a mucosal immunodeficiency condition [28]. In this respect, it has been reported the association of the tumor suppressor phosphatase and tensin homolog deleted in chromosome 10 (PTEN) with defective CFTR, the latter acting as scaffold protein for PTEN. The CFTR-PTEN association induces mitochondrial metabolic dysfunction by increasing the release of itaconate and succinate, a condition promoting a selective advantage for *P. aeruginosa* infection in CF [29,30**]]. This mechanism could also be favored by the inhibition of the bacterial killing of CF macrophages exposed to the protease LasB, one of the virulence factors released by *P. aeruginosa* [31].

Evolution and adaptation of *P. aeruginosa* infection

Free flowing flagellated and piliated, so termed "wild type" or "environmental" or "planktonic", *P. aeruginosa* strains start infecting CF patients at a median age of one year [32]. During childhood, *P. aeruginosa* infections are recurrent and in principle antibiotics at high doses could eradicate them. Soon or later, *P. aeruginosa* infecting CF adults undergoes several genomic mutations leading to a flagellum- and pili-deprived nonmotile "mucoid" phenotype, an evolution intervening in CF patients at the median age of thirteen years [32]. Bacterium mutated with mucoid phenotype lives much better protected than wild-type planktonic one from mucosal anti-microbial peptides, innate and adaptive host immune system, and antibiotics while encapsulated in biofilms. The protecting effect on *P. aeruginosa* is thought to be dependent on at least three major exopolysaccharides produced by the bacterium itself, namely alginate, Pel and Psl, which sustain the antimicrobial tolerance [33]. Different a condition which practically impedes bacterial eradication and is considered a true irreversible chronic infection. The role of the huge amount of reactive oxygen species (ROS) and redox unbalance in CF airway mucosa in producing a series of mutations in the genome of *P. aeruginosa*, leading to mucoid phenotype, has been extensively studied [for summary see the study by Malhotra et al. [34**]]. Relevantly to pathophysiology and pharmacology, entering the irreversible stage of the chronic *P. aeruginosa* infection does not mean that all CF bronchial tree is irreversibly and exclusively infected by the mucoid strains growing in biofilms. Actually, mucoid *P. aeruginosa* is known since long time to be instable in CF lung *in vivo* and to revert frequently back to planktonic form, because of accumulation of new genomic mutations. The switch from mucoid to nonmucoid, and again back to mucoid phenotypes, is assumed as a frequent process *in vivo* CF lung [35–37]. Interestingly, as recently reported by analyses of BALF obtained selectively from 6 pulmonary lobes of CF patients, different lung areas are infected at the same time by mucoid or by planktonic non-mucoid, again by mucoid/non-mucoid mixed strains [38**], suggesting to us a "leopard skin spots" heterogeneous model of *P. aeruginosa* infection in adult C6F lungs (Figure 1). Importantly, the heterogeneous type of *P. aeruginosa* phenotypes, co-existing in the same lung of chronically infected CF patients, leads to different

Figure 1



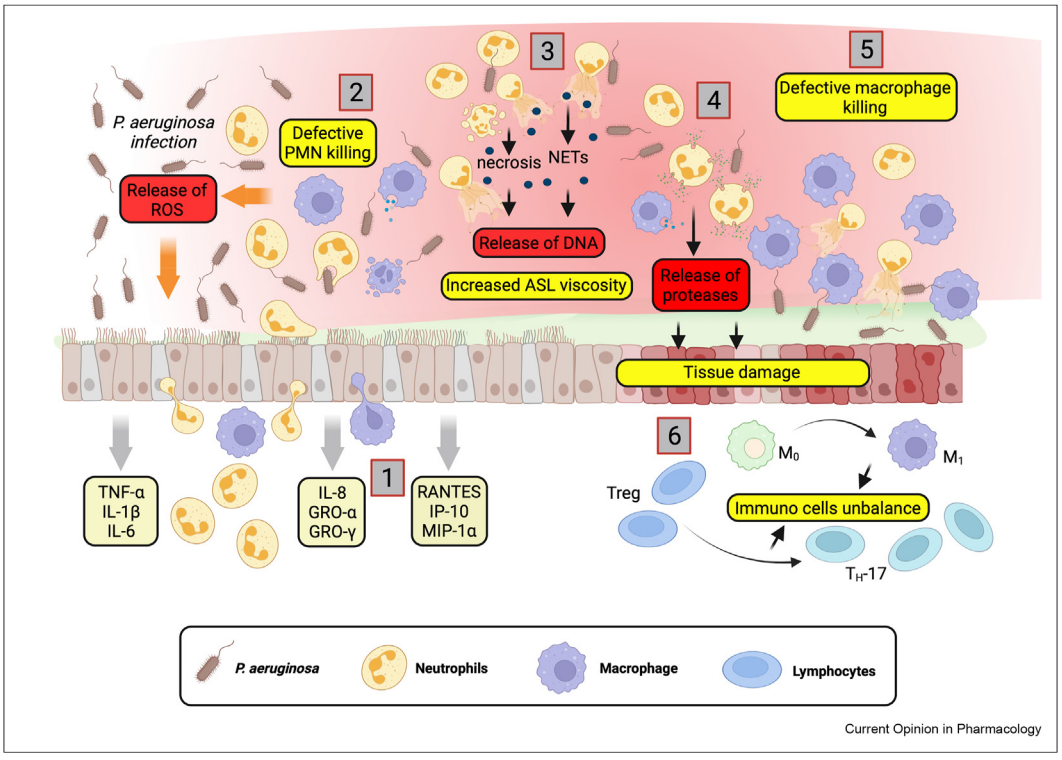
Evolution of bacterial infection in CF lung. The basic defect of *CFTR* leading to altered ASL with more viscous and acidic mucus favors bacterial infections since the early years of life of CF patients [17**,18**]. (**a**) During childhood, recurrent bacterial infections are mainly polymicrobial with *S. aureus* becoming progressively prevalent, possibly due to the acidic ASL environment [25]; (**b**) During adolescence, *P. aeruginosa,* which is present in CF airways since the early years of life, eradicates the *S. aureus* infection, possibly by action of a specific phospholipase providing selective advantage [26]; (**c**) During adulthood, the genome of wild type *P. aeruginosa* undergoes a series of mutations, possibly dependent on the CF pro-oxidative milieu of the ASL, loosing flagellum and pili and secreting alginate to build a biofilm, inside which it results more protected from the host immune responses and antibiotics [32]. The mutant *P. aeruginosa* recurrent infection thus evolves as a chronic one, sustained by the presence of mucoid strains. These mucoid strains further evolve towards revertant nonmucoid strains expressing flagellum and pili [34**,35–38]. The lungs of chronically infected CF patients therefore harbor at the same time *P. aeruginosa* mucoid strains in biofilms, nonmucoid revertant with flagellum and pili and mixed phenotypes, resembling a "leopard-like skin heterogeneous model" of infection and inflammation [34**]. Graph created with BioRender.com.

degrees of inflammatory/immune response among the different lobes [38**]. These findings contradict the scenario of a topographically homogeneous infective phenotype, corresponding to a homogeneous immune response in whole CF lung. In addition, the heterogeneity of chronic *P. aeruginosa* infection seems to best fit the previously advanced hypothesis that exacerbations, periodically affecting chronically infected CF patients and severely worsening their lung function, reflect most likely the spread of the disease from more infected to less/not-infected areas of the same lung, rather than being dependent on an intensification of the bacterial load [39]. This hypothesis on the main mechanism of CF infective exacerbations is, in our opinion, more easily explained by the co-presence of motile, planktonic bacteria, not only mucoid bacteria strictly confined in biofilms firmly adherent to bronchial walls.

The CF inflammatory response is ineffective and harmful

Whether the innate immune inflammatory response in CF lungs precedes or follows the bacterial infection has been debated for years. Although contradictory reports are presented, which leave this issue still unsettled, bacterial infection intervenes very early in CF life, either inducing or amplifying a series of host inflammatory responses modulated by different mucosal cell components [40,41] (Figure 2). CF mucosal epithelium is not a simple physical barrier to protect the airway mucosa from penetrating microorganisms but is known to orchestrate the strong chemotactic process driving PMNs in the CF lumen [42]. The huge amount of PMNs filling CF mucosa are inefficient in avoiding *P. aeruginosa* chronic infection but their presence, continuously activated by bacterial products, results in a

Figure 2



Key features of the host immune response in CF airway mucosa. Graphical summary of the hallmarks of the mucosal host response in advanced CF airways disease. **1)** CF bronchial epithelium exposed to pathogen activated molecular patterns (PAMPs) activates the expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and chemokines recruiting PMNs (IL-8, GRO-α/γ) and mononuclear immune cells (RANTES, IP-10, MIP-1α) mainly, but not exclusively, through MyD88-dependent toll-like receptors 2, 4, and 5 signaling [42]; **2)** CF PMNs present a defective bacterial killing in respect to *P. aeruginosa* and their continuous stimulation by bacterial PAMPs induce massive release of reactive oxygen species (ROS), which contribute to unbalance the redox equilibrium of CF ASL towards a pro-oxidative status [41], which affects CFTR protein stability [65]; **3)** huge amount of PMN DNA derived from Neutrophil Extracellular Traps (NETs) and PMN hypoxic necrosis further worsen the viscosity of CF ASL [44*,45]; **4)** besides ROS and DNA, CF PMNs exposed to continuous bacterial stimulation release a series of proteases, first of all neutrophil elastase, which contribute to bronchial wall damage and dilation resulting in bronchiectasis, anatomical niches favoring bacterial stasis, thus recurrent bacterial exacerbations; **5)** CF macrophages present defective *P. aeruginosa* killing and defective removal of dead neutrophils, the latter being a critical process that is necessary to reestablish tissue homeostasis [47]. Moreover, they contribute to release of ROS in CF ASL and are implicated in selecting an environment favoring *P. aeruginosa* chronic infection [29–31,48–50]; **6)** as a result of M_1 preferential polarization of CF macrophages, a T cell unbalanced pattern has been found, where T_H-17 pro-inflammatory cells are prevalent of Treg cells [54–56]. Graph created with BioRender.com.

series harmful effects, such as: 1) the release of ROS, contributing to a pronounced pro-oxidative redox unbalance [43]; 2) the spread of DNA on mucosal surface, both from PMN necrosis and release of neutrophil extracellular traps, which further increase ASL viscosity and are associated with lung disease severity [44*]; 3) the exocytosis of proteases, which contribute to damage the fibers of the extracellular matrix of the bronchial walls and to amplify the inflammatory response, with very limited effect on bacterial killing [45]. Overall, the presence of a huge number of activated neutrophils is thought to have a critical role in CF lung pathology, thus the neutralization of neutrophil elastase or the fine regulation of recruitment of these cells in the airway lumen by the chemokine IL-8/CXCL8 are considered relevant therapeutic targets [46]. Besides neutrophils, CFTR mutations are implicated in immune functions of macrophages, which show reduced efficiency in removing dead neutrophils [47], a critical process to reestablish tissue homeostasis. Macrophages are gaining a critical role in further explaining the defective CF host response to *P. aeruginosa* and to sustain a proinflammatory status by preferential polarization to M1 phenotype [47–52]. Although M1 polarization of macrophages is mainly applicable to *in vitro* investigation with limited translation into *in vivo* setting [53], a possible consequence of CF macrophage dysfunction and M1 polarization has been also suggested as regards an imbalance of anti-inflammatory regulation of the adaptive immune branch, where a reduced Tregs cells presence has been observed in CF patients and accompanied by preferential increments of Th-17 cells (the latter actively involved in PMNs recruitment in CF bronchi [54,55]), an imbalance that does not seem corrected by CFTR modulators [56].

Entering the era of the novel CFTR modulator Trikafta, CF lung pathophysiology should consider not only its effects on chronic infection and inflammation on bronchial tissue damage, but also on the efficiency of rescue of mutant CFTR in CF patients treated with CFTR modulators. Several reports consistently indicate that the rescue by CFTR modulators of the most common F508del-mutated CFTR protein in bronchial epithelial cells is strongly reduced by planktonic *P. aeruginosa* [57–64], the proposed mechanisms including quorum sensing [62], LasB protease [63], the interference with expression of the CFTR scaffolding protein NHERF1 [59]. In parallel, the ROS abundantly released in the CF ASL inflammatory milieu, as a result of activated PMNs by bacterial infections, have been shown to strongly reduce wild type and F508del-CFTR expression through a mixed lineage kinase-3 dependent activation inducing CFTR proteolysis [65]. In contrast, it has been reported that the supernatant obtained from mucopurulent material (SMM) of CF lungs augments *in vitro* the rescue of F508del-CFTR by CFTR modulators [66**], a recent finding that will open a series of further investigations.

Conclusions

A defect in muco-ciliary clearance of the airways due to viscous respiratory mucus has been suggested since the early clinical identification of cystic fibrosis (CF), originally termed "mucoviscidosis." *CFTR* gene identification and the intense clinical and experimental research have confirmed the role of altered airway mucus in the onset of inflammation and infection, although the final answer revealing the mechanism of the selectivity of *P. aeruginosa* is still debated. In the years, a condition of relative deficiency of CF mucosal defenses is gaining consensus, as either CFTR defects seem to involve directly or indirectly the bacterial killing efficiency of CF PMNs and macrophages. The chronic infective/inflammatory condition has implications not only on bronchial wall damage but also on the efficiency of novel CFTR modulators. Whether the cascade of events starting from CFTR ion transport defects down to chronic infection and inflammation is becoming consistently clear, that the novel CFTR modulators will be *per se* sufficient to completely revert lung infection and inflammation of adult CF patients to a reasonably stable respiratory function is presently under scrutiny.

Author contributions

Conceptualization: GC, AR, MB. Data curation and formal analysis: GC, AR, MB, PP, RG. Methodology: GC, AR, MB. Writing - original draft: GC. Writing - review and editing: GC, AR, MB, PP, RG. All authors read and approved the final manuscript.

Availability of data and material

Not applicable.

Funding

The work was funded by grants from local founds from University of Ferrara, FIR-2021, the Italian Ministry of Health (GR-2016-02364602) and the Italian Ministry of Education, University and Research (PRIN Grant 2017XA5J5N) to A.R., by grant FFC #7/2019 of the Italian Cystic Fibrosis Research Foundation to M.B. and G.C., by grant FFC #7/2018 to R.G.

Ethics approval and consent to participate

Not applicable

Conflict of interest statement

GC, AR, MB, PP, RG report no conflict of interest.

Acknowledgments

GC is grateful to Alice Prince (Columbia University, New York, NY, U.S.A.) and to Lhousseine Touqui (Pasteur Institute, Paris, F) for helpful discussions.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, *et al.*: **Identification of the cystic fibrosis gene: genetic analysis**. *Science* 1989 Sep 8, **245**:1073–1080, https://doi.org/10.1126/science.2570460. PMID: 2570460.

2. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, *et al.*: **Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA**. *Science* 1989 Sep 8, **245**:1066–1073, https://doi.org/10.1126/science.2475911. Erratum in: Science 1989 Sep 29;245(4925):1437. PMID: 2475911.

3. Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, *et al.*: **Identification of the cystic fibrosis gene: chromosome walking and jumping**. *Science* 1989 Sep 8, **245**: 1059–1065, https://doi.org/10.1126/science.2772657. PMID: 2772657.

4. * https://cftr2.org.
A website database of *CFTR* gene variants which have been reported to affect the clinical state of CF patients, a very useful source for genetic counseling, clinical care and research.

5. Bell SC, De Boeck K, Amaral MD: **New pharmacological approaches for cystic fibrosis: promises, progress, pitfalls**. *Pharmacol Ther* 2015 Jan, **145**:19–34, https://doi.org/10.1016/j.pharmthera.2014.06.005. Epub 2014 Jun 14. PMID: 24932877.

6. * Shteinberg M, Haq IJ, Polineni D, Davies JC: **Cystic fibrosis**. *Lancet* 2021, **397**:2195–2211, https://doi.org/10.1016/S0140-6736(20)32542-3.
This review gives an update of the CF disease by recalling the organ manifestations and summarizing the state of the art of the main pharmacological approaches and their limitations.

7. * Bell SC, Mall MA, Gutierrez H, Macek M, Madge S, Davies JC, Burgel PR, Tullis E, Castaños C, Castellani C, Byrnes CA, Cathcart F, Chotirmall SH, Cosgriff R, Eichler I, Fajac I, Goss CH, Drevinek P, Farrell PM, Gravelle AM, Havermans T, Mayer-Hamblett N, Kashirskaya N, Kerem E, Mathew JL, McKone EF, Naehrlich L, Nasr SZ, Oates GR, O'Neill C, Pypops U, Raraigh KS, Rowe SM, Southern KW, Sivam S, Stephenson AL, Zampoli M, Ratjen F: **The future of cystic fibrosis care: a global perspective**. *Lancet Respir Med* 2020 Jan, **8**:65–124, https://doi.org/10.1016/S2213-2600(19)30337-6. Epub 2019 Sep 27. Erratum in: Lancet Respir Med. 2019 Dec;7(12):e40. PMID: 31570318.
A state of the art of the perspectives in the clinical care of CF disease, summarizing the main results of the clinical trials with the novel CFTR modulators, advantages and limitations.

8. Middleton PG, Mall MA, Dřevínek P, Lands LC, McKone EF, Polineni D, RamseyBW, Taylor-Cousar JL, Tullis E, Vermeulen F, Marigowda G, McKee CM, Moskowitz SM, Nair N, Savage J, Simard C, Tian S, Waltz D, Xuan F, Rowe SM, Jain R: **VX17-445-102 Study Group. Elexacaftor-Tezacaftor-Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele**. *N Engl J Med* 2019 Nov 7, **381**:1809–1819, https://doi.org/10.1056/NEJMoa1908639. Epub 2019 Oct 31. PMID: 31697873; PMCID: PMC7282384.

9. Heijerman HGM, McKone EF, Downey DG, Van Braeckel E, Rowe SM, Tullis E, Mall MA, Welter JJ, Ramsey BW, McKee CM, Marigowda G, Moskowitz SM, Waltz D, Sosnay PR, Simard C, Ahluwalia N, Xuan F, Zhang Y, Taylor-Cousar JL, McCoy KS: **VX17-445-103 Trial Group. Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial**. *Lancet* 2019 Nov 23, **394**:1940–1948, https://doi.org/10.1016/S0140-6736(19)32597-8. Epub 2019 Oct 31. Erratum in: Lancet. 2020 May 30;395(10238):1694. PMID: 31679946; PMCID: PMC7571408.

10. Tabcharani JA, Chang XB, Riordan JR, Hanrahan JW: **Phosphorylation-regulated Cl- channel in CHO cells stably expressing the cystic fibrosis gene**. *Nature* 1991 Aug 15, **352**: 628–631, https://doi.org/10.1038/352628a0. PMID: 1714039.

11. Poulsen JH, Fischer H, Illek B, Machen TE: **Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmembrane conductance regulator**. *Proc Natl Acad Sci USA* 1994 Jun 7, **91**:5340–5344, https://doi.org/10.1073/pnas.91.12.5340. PMID:7515498;PMCID: PMC43990.

12. Montoro DT, Haber AL, Biton M, Vinarsky V, Lin B, Birket SE, Yuan F, Chen S, Leung HM, Villoria J, Rogel N, Burgin G, Tsankov AM, Waghray A, Slyper M, Waldman J, Nguyen L, Dionne D, Rozenblatt-Rosen O, Tata PR, Mou H, Shivaraju M, Bihler H, Mense M, Tearney GJ, Rowe SM, Engelhardt JF, Regev A, Rajagopal J: **A revised airway epithelial hierarchy includes CFTR-expressing ionocytes**. *Nature* 2018 Aug, **560**: 319–324, https://doi.org/10.1038/s41586-018-0393-7. Epub 2018 Aug 1.; PMCID: PMC6295155.

13. Plasschaert LW, Žilionis R, Choo-Wing R, Savova V, Knehr J, Roma G, Klein AM, Jaffe AB: **A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte**. *Nature* 2018 Aug, **560**:377–381, https://doi.org/10.1038/s41586-018-0394-6. Epub 2018 Aug 1.; PMCID: PMC6108322.

14. Kreda SM, Mall M, Mengos A, Rochelle L, Yankaskas J, Riordan JR, Boucher RC: **Characterization of wild-type and deltaF508 cystic fibrosis transmembrane regulator in human respiratory epithelia**. *Mol Biol Cell* 2005 May, **16**:2154–2167, https://doi.org/10.1091/mbc.e04-11-1010. Epub 2005 Feb 16. 1; PMCID: PMC1087225.

15. ** Carraro G, Langerman J, Sabri S, Lorenzana Z, Purkayastha A, Zhang G, Konda B, Aros CJ, Calvert BA, Szymaniak A, Wilson E, Mulligan M, Bhatt P, Lu J, Vijayaraj P, Yao C, Shia DW, Lund AJ, Israely E, Rickabaugh TM, Ernst J, Mense M, Randell SH, Vladar EK, Ryan AL, Plath K, Mahoney JE, Stripp BR, Gomperts BN: **Transcriptional analysis of cystic fibrosis airways at single-cell resolution reveals altered epithelial cell states and composition**. *Nat Med* 2021 May, **27**:806–814, https://doi.org/10.1038/s41591-021-01332-7. Epub 2021 May 6. PMID: 33958799.
This article widens the horizon on the different bronchial mucosal cells expressing CFTR by single-cell expression analyses, providing new hints on the cellular targets for future gene editing therapies for CF lung disease.

16. Okuda K, Dang H, Kobayashi Y, Carraro G, Nakano S, Chen G, Kato T, Asakura T, Gilmore RC, Morton LC, Lee RE, Mascenik T, Yin WN, Barbosa Cardenas SM, O'Neal YK, Minnick CE, Chua M, Quinney NL, Gentzsch M, Anderson CW, Ghio A, Matsui H, Nagase T, Ostrowski LE, Grubb BR, Olsen JC, Randell SH, Stripp BR, Tata PR, O'Neal WK, Boucher RC: **Secretory cells dominate airway CFTR expression and function in human airway superficial Epithelia**. *Am J Respir Crit Care Med* 2021 May 15, **203**:1275–1289, https://doi.org/10.1164/rccm.202008-3198OC. PMCID: PMC8456462.

17. Gentzsch M, Dang H, Dang Y, Garcia-Caballero A, Suchindran H, Boucher RC, Stutts MJ: **The cystic fibrosis transmembrane conductance regulator impedes proteolytic stimulation of the epithelial Na+ channel**. *J Biol Chem* 2010 Oct 15, **285**: 32227–32232, https://doi.org/10.1074/jbc.M110.155259. Epub 2010 Aug 13. PMID:20709758; PMCID: PMC2952223.

18. Gabriel SE, Clarke LL, Boucher RC, Stutts MJ: **CFTR and outward rectifying chloride channels are distinct proteins with a regulatory relationship**. *Nature* 1993 May 20, **363**:263–268, https://doi.org/10.1038/363263a0. PMID: 7683773.

19. Verkman AS: **Lung disease in cystic fibrosis: is airway surface liquid composition abnormal?** *Am J Physiol Lung Cell Mol Physiol* 2001 Aug, **281**:L306–L308, https://doi.org/10.1152/ajplung.2001.281.2.L306. PMID: 11435202.

20. Haq IJ, Gray MA, Garnett JP, Ward C, Brodlie M: **Airway surface liquid homeostasis in cystic fibrosis: pathophysiology and therapeutic targets**. *Thorax* 2016 Mar, **71**:284–287, https://

doi.org/10.1136/thoraxjnl-2015-207588. Epub 2015 Dec 30. PMID: 26719229.

21. Boucher RC: **Muco-obstructive lung diseases**. *N Engl J Med* 2019 May 16, **380**:1941–1953, https://doi.org/10.1056/NEJMra1813799. PMID: 31091375.
** A state of the art of the mucus alterations in the airway surface liquid of patients with CF, analyzing different biochemical and physiological aspects, with implications towards clinical therapeutics.

22. Esther Jr CR, Muhlebach MS, Ehre C, Hill DB, Wolfgang MC, Kesimer M, Ramsey KA, Markovetz MR, Garbarine IC, Forest MG, Seim I, Zorn B, Morrison CB, Delion MF, Thelin WR, Villalon D, Sabater JR, Turkovic L, Ranganathan S, Stick SM, Boucher RC: **Mucus accumulation in the lungs precedes structural changes and infection in children with cystic fibrosis**. *Sci Transl Med* 2019 Apr 3, **11**, eaav3488, https://doi.org/10.1126/scitranslmed.aav3488. PMID: 30944166; PMCID: PMC6566903.
** Here the report of the analyses of the ASL composition, inflammatory and infectivity markers in the bronchoalveolar lavage fluids (BALFs) obtained from 46 preschool children with CF and 16 non-CF diseased controls enrolled in the Australian Respiratory Early Surveillance Team (AREST) for CF study. Due to its invasiveness, the study is one of the few available reporting results from *ex vivo* sampling of young CF patients. The results focus on the role of CF airway mucus in relation to both infection and inflammation and provides further hints on the origin of the inflammatory process and its dependence on bacterial infection.

23. Stoltz DA, Meyerholz DK, Welsh MJ: **Origins of cystic fibrosis lung disease**. *N Engl J Med* 2015 Jan 22, **372**:351–362, https://doi.org/10.1056/NEJMra1300109. PMID: 25607428; PMCID: PMC4916857.

24. Zemanick ET, Hoffman LR: **Cystic fibrosis: microbiology and host response**. *Pediatr Clin* 2016 Aug, **63**:617–636, https://doi.org/10.1016/j.pcl.2016.04.003. PMID: 27469179; PMCID: PMC4967239.

25. Simonin J, Bille E, Crambert G, Noel S, Dreano E, Edwards A, Hatton A, Pranke I, Villeret B, Cottart CH, Vrel JP, Urbach V, Baatallah N, Hinzpeter A, Golec A, Touqui L, Nassif X, Galietta LJV, Planelles G, Sallenave JM, Edelman A, Sermet-Gaudelus I: **Airway surface liquid acidification initiates host defense abnormalities in Cystic Fibrosis**. *Sci Rep* 2019 Apr 24, **9**:6516, https://doi.org/10.1038/s41598-019-42751-4. Erratum in: Sci Rep. 2019 Nov 21;9(1):17535. PMID: 31019198; PMCID: PMC6482305.

26. Pernet E, Guillemot L, Burgel PR, Martin C, Lambeau G, Sermet-Gaudelus I, Sands D, Leduc D, Morand PC, Jeammet L, Chignard M, Wu Y, Touqui L: **Pseudomonas aeruginosa eradicates Staphylococcus aureus by manipulating the host immunity**. *Nat Commun* 2014 Oct 7, **5**:5105, https://doi.org/10.1038/ncomms6105. PMID: 25290234.

27. Fischer AJ, Singh SB, LaMarche MM, Maakestad LJ, Kienenberger ZE, Peña TA, Stoltz DA, Limoli DH: **Sustained coinfections with *Staphylococcus aureus* and *Pseudomonas aeruginosa* in cystic fibrosis**. *Am J Respir Crit Care Med* 2021 Feb 1, **203**:328–338, https://doi.org/10.1164/rccm.202004-1322OC. PMID: 32750253; PMCID: PMC7874317.

28. Cohen TS, Prince A: **Cystic fibrosis: a mucosal immunodeficiency syndrome**. *Nat Med* 2012 Apr 5, **18**:509–519, https://doi.org/10.1038/nm.2715. PMID: 22481418; PMCID: PMC3577071.

29. Riquelme SA, Hopkins BD, Wolfe AL, DiMango E, Kitur K, Parsons R, Prince A: **Cystic fibrosis transmembrane conductance regulator attaches tumor suppressor PTEN to the membrane and promotes anti *Pseudomonas aeruginosa* immunity**. *Immunity* 2017 Dec 19, **47**:1169–1181.e7, https://doi.org/10.1016/j.immuni.2017.11.010. Epub 2017 Dec 12. PMID: 29246444; PMCID: PMC5738266.

30. Riquelme SA, Lozano C, Moustafa AM, Liimatta K, Tomlinson KL, Britto C, Khanal S, Gill SK, Narechania A, Azcona-Gutiérrez JM, DiMango E, Saénz Y, Planet P, Prince A: **CFTR-PTEN-dependent mitochondrial metabolic dysfunction promotes *Pseudomonas aeruginosa* airway infection**. *Sci Transl Med* 2019 Jul 3, **11**, eaav4634, https://doi.org/10.1126/scitranslmed.aav4634. PMID: 31270271; PMCID: PMC6784538.
* This paper completes a series of previous observations which identify CFTR protein as a scaffold protein for PTEN. Defective CFTR therefore affects PTEN function inducing mitochondrial stress and altered production of itaconate and succinate, which affect *P. aeruginosa* infection. This and previous papers by this group also highlight the cross-talk between CF macrophages and the bacterium.

31. Bastaert F, Kheir S, Saint-Criq V, Villeret B, Dang PM, El-Benna J, Sirard JC, Voulhoux R, Sallenave JM: ***Pseudomonas aeruginosa* LasB subverts alveolar macrophage activity by interfering with bacterial killing through downregulation of innate immune defense, reactive oxygen species generation, and complement activation**. *Front Immunol* 2018 Jul 23, **9**:1675, https://doi.org/10.3389/fimmu.2018.01675. PMID: 30083156; PMCID: PMC6064941.

32. Li Z, Kosorok MR, Farrell PM, Laxova A, West SE, Green CG, Collins J, Rock MJ, Splaingard ML: **Longitudinal development of mucoid *Pseudomonas aeruginosa* infection and lung disease progression in children with cystic fibrosis**. *JAMA* 2005 Feb 2, **293**:581–588, https://doi.org/10.1001/jama.293.5.581. PMID: 15687313.

33. Jennings LK, Dreifus JE, Reichhardt C, Storek KM, Secor PR, Wozniak DJ, Hisert KB, Parsek MR: **Pseudomonas aeruginosa aggregates in cystic fibrosis sputum produce exopolysaccharides that likely impede curren therapies**. *Cell Rep* 2021 Feb 23, **34**:108782, https://doi.org/10.1016/j.celrep.2021.108782. PMID: 33626358; PMCID: PMC7958924.

34. Malhotra S, Hayes Jr D, Wozniak DJ: **Cystic fibrosis and *Pseudomonas aeruginosa*: the host-microbe interface**. e00138-18 *Clin Microbiol Rev* 2019 May 29, **32**, https://doi.org/10.1128/CMR.00138-18. PMID: 31142499; PMCID: PMC6589863.
** This review provides an excellent presentation of different key aspects of *P. aeruginosa* CF lung infection mainly, but non-exclusively, from the microorganism side. It includes the evasion of innate immunity, the adaptation during chronic infection evolution, the mechanisms of mutagenesis and alginate biofilm synthesis, the mucoid conversion with instability and its nonmucoid reversion, the heterogeneity of *P. aeruginosa* phenotypes in the CF chronically infected lung.

35. Schurr MJ, Martin DW, Mudd MH, Deretic V: **Gene cluster controlling conversion to alginate-overproducing phenotype in Pseudomonas aeruginosa: functional analysis in a heterologous host and role in the instability of mucoidy**. *J Bacteriol* 1994 Jun, **176**:3375–3382, https://doi.org/10.1128/jb.176.11.3375-3382.1994. PMID: 8195094; PMCID: PMC205510.

36. DeVries CA, Ohman DE: **Mucoid-to-nonmucoid conversion in alginate-producing Pseudomonas aeruginosa often results from spontaneous mutations in algT, encoding a putative alternate sigma factor, and shows evidence for autoregulation**. *J Bacteriol* 1994 Nov, **176**:6677–6687, https://doi.org/10.1128/jb.176.21.6677-6687.1994. PMID: 7961421; PMCID: PMC197025.

37. Ciofu O, Lee B, Johannesson M, Hermansen NO, Meyer P, Høiby N: **Investigation of the algT operon sequence in mucoid and non-mucoid Pseudomonas aeruginosa isolates from 115 Scandinavian patients with cystic fibrosis and in 88 in vitro non-mucoid revertants**. *Microbiology (Read)* 2008 Jan, **154**(Pt 1):103–113, https://doi.org/10.1099/mic.0.2007/010421-0. PMID: 18174130.

38. Malhotra S, Hayes Jr D, Wozniak DJ: **Mucoid *Pseudomonas aeruginosa* and regional inflammation in the cystic fibrosis lung**. *J Cyst Fibros* 2019 Nov, **18**:796–803, https://doi.org/10.1016/j.jcf.2019.04.009. Epub 2019 Apr 26. PMID: 31036488; PMCID: PMC6815243.
** Malhotra and Coll. study a cohort of 14 adults CF patients chronically colonized with *P. aeruginosa* performing in each patient a bronchoalveolar lavage in 6 different lobes to isolate and characterize bacterial phenotypes and the degree of inflammatory response in different areas of their lungs. Results demonstrate the heterogeneity of *P. aeruginosa* phenotype during chronic colonization by finding in different regional areas either mucoid or nonmucoid strains together with mixed colonies in the same patient. Interestingly, the different phenotypes were found associated with different degrees of inflammatory response, as studied by measuring the concentration of the classical cyto-chemokines found in CF lungs (e.g. TNF-alpha and IL-8). The article provides strong *ex vivo* proof of the heterogeneity of infective/inflammatory status in each adult CF patient chronically infected with *P.aeruginosa*.

39. Boucher RC: **On the pathogenesis of acute exacerbations of mucoobstructive lung diseases**. Suppl 2 *Ann Am Thorac Soc* 2015 Nov, **12**(Suppl 2):S160–S163, https://doi.org/10.1513/AnnalsATS.201507-460AW. PMID: 26595733; PMCID: PMC4722836.

40. Roesch EA, Nichols DP, Chmiel JF: **Inflammation in cystic fibrosis: an update**. *Pediatr Pulmonol* 2018 Nov, **53**:S30–S50, https://doi.org/10.1002/ppul.24129. Epub 2018 Jul 12. PMID: 29999593.

41. Ribeiro CMP, McElvaney NG, Cabrini G: **Editorial: novel anti-inflammatory approaches for cystic fibrosis lung disease: identification of molecular targets and design of innovative therapies**. *Front Pharmacol* 2021, **12**:794854, https://doi.org/10.3389/fphar.2021.794854.

42. Cabrini G, Rimessi A, Borgatti M, Lampronti I, Finotti A, Pinton P, Gambari R: **Role of cystic fibrosis bronchial epithelium in neutrophil chemotaxis**. *Front Immunol* 2020 Aug 4, **11**:1438, https://doi.org/10.3389/fimmu.2020.01438. PMID: 32849500; PMCID: PMC7427443.

43. Galli F, Battistoni A, Gambari R, Pompella A, Bragonzi A, Pilolli F, Iuliano L, Piroddi M, Dechecchi MC, Cabrini G: **Working Group on Inflammation in Cystic Fibrosis. Oxidative stress and antioxidant therapy in cystic fibrosis**. *Biochim Biophys Acta* 2012 May, **1822**:690–713, https://doi.org/10.1016/j.bbadis.2011.12.012. Epub 2011 Dec 28. Erratum in: Biochim Biophys Acta. 2014 Dec;1842(12):2531. PMID: 22226887.

44. * Keir HR, Shoemark A, Dicker AJ, Perea L, Pollock J, Giam YH, Suarez-Cuartin G, Crichton ML, Lonergan M, Oriano M, Cant E, Einarsson GG, Furrie E, Elborn JS, Fong CJ, Finch S, Rogers GB, Blasi F, Sibila O, Aliberti S, Simpson JL, Huang JTJ, Chalmers JD: **Neutrophil extracellular traps, disease severity, and antibiotic response in bronchiectasis: an international, observational, multicohort study**. *Lancet Respir Med* 2021 Aug, **9**:873–884, https://doi.org/10.1016/S2213-2600(20)30504-X. Epub 2021 Feb 17. PMID: 33609487.
This clinical study examines the association of NETs with lung exacerbations and progression of the disease, suggesting NETs as useful biomarkers of exacerbations and underlying the importance of PMNs as key cellular targets for CF lung disease.

45. Clancy DM, Sullivan GP, Moran HBT, Henry CM, Reeves EP, McElvaney NG, Lavelle EC, Martin SJ: **Extracellular neutrophil proteases are efficient regulators of IL-1, IL-33, and IL- 36 cytokine activity but poor effectors of microbial killing**. *Cell Rep* 2018 Mar 13, **22**:2937–2950, https://doi.org/10.1016/j.celrep.2018.02.062. PMID: 29539422.

46. McElvaney OJ, McElvaney NG: **Targeting IL-8 in cystic fibrosis: enough but not too much**. *Am J Respir Cell Mol Biol* 2018 Oct, **59**:401–402, https://doi.org/10.1165/rcmb.2018-0145ED.

47. Vandivier RW, Fadok VA, Hoffmann PR, Bratton DL, Penvari C, Brown KK, Brain JD, Accurso FJ, Henson PM: **Elastase-mediated phosphatidylserine receptor cleavage impairs apoptotic cell clearance in cystic fibrosis and bronchiectasis**. *J Clin Invest* 2002 Mar, **109**:661–670, https://doi.org/10.1172/JCI13572. PMID: 11877474; PMCID: PMC150889.

48. Simonin-Le Jeune K, Le Jeune A, Jouneau S, Belleguic C, Roux PF, Jaguin M, Dimanche- Boitre MT, Lecureur V, Leclercq C, Desrues B, Brinchault G, Gangneux JP, Martin-Chouly C: **Impaired functions of macrophage from cystic fibrosis patients: CD11b, TLR-5 decrease and sCD14, inflammatory cytokines increase**. *PLoS One* 2013 Sep 30, **8**, e75667, https://doi.org/10.1371/journal.pone.0075667. PMID: 24098711; PMCID: PMC3787056.

49. Bruscia EM, Bonfield TL: **Cystic fibrosis lung immunity: the role of the macrophage**. *J Innate Immun* 2016, **8**:550–563, https://doi.org/10.1159/000446825. Epub 2016 Jun 24. PMID: 27336915; PMCID: PMC5089923.

50. Zhang S, Shrestha CL, Kopp BT: **Cystic fibrosis transmembrane conductance regulator (CFTR) modulators have differential effects on cystic fibrosis macrophage function**. *Sci Rep* 2018 Nov 20, **8**:17066, https://doi.org/10.1038/s41598-018-35151-7. PMID: 30459435; PMCID: PMC6244248.

51. Hazlett HF, Hampton TH, Aridgides DS, Armstrong DA, Dessaint JA, Mellinger DL, Nymon AB, Ashare A: **Altered iron metabolism in cystic fibrosis macrophages: the impact of CFTR modulators and implications for Pseudomonas aeruginosa survival**. *Sci Rep* 2020 Jul 2, **10**:10935, https://doi.org/10.1038/s41598-020-67729-5. PMID: 32616918; PMCID: PMC7331733.

52. Gillan JL, Davidson DJ, Gray RD: **Targeting cystic fibrosis inflammation in the age of CFTR modulators: focus on macrophages**. *Eur Respir J* 2021 Jun 4, **57**:2003502, https://doi.org/10.1183/13993003.03502-2020. PMID: 33303535.

53. Sica A, Mantovani A: **Macrophage plasticity and polarization: in vivo veritas**. *J Clin Invest* 2012 Mar, **122**:787–795, https://doi.org/10.1172/JCI59643. Epub 2012 Mar 1. PMID: 22378047; PMCID: PMC3287223.

54. Iannitti RG, Carvalho A, Cunha C, De Luca A, Giovannini G, Casagrande A, Zelante T, Vacca C, Fallarino F, Puccetti P, Massi-Benedetti C, Defilippi G, Russo M, Porcaro L, Colombo C, Ratclif L, De Benedictis FM, Romani L: **Th17/Treg imbalance in murine cystic fibrosis is linked to indoleamine 2,3-dioxygenase deficiency but corrected by kynurenines**. *Am J Respir Crit Care Med* 2013 Mar 15, **187**:609–620, https://doi.org/10.1164/rccm.201207-1346OC. Epub 2013 Jan 10. PMID: 23306541.

55. Hector A, Schäfer H, Pöschel S, Fischer A, Fritzsching B, Ralhan A, Carevic M, Öz H, Zundel S, Hogardt M, Bakele M, Rieber N, Riethmueller J, Graepler-Mainka U, Stahl M, Bender A, Frick JS, Mall M, Hartl D: **Regulatory T-cell impairment in cystic fibrosis patients with chronic *pseudomonas* infection**. *Am J Respir Crit Care Med* 2015 Apr 15, **191**:914–923, https://doi.org/10.1164/rccm.201407-1381OC. PMID: 25632992.

56. Westhölter D, Beckert H, Straßburg S, Welsner M, Sutharsan S, Taube C, Reuter S: ***Pseudomonas aeruginosa* infection, but not mono or dual-combination CFTR modulator therapy affects circulating regulatory T cells in an adult population with cystic fibrosis**. *J Cyst Fibros* 2021 May 21, https://doi.org/10.1016/j.jcf.2021.05.001. S1569-1993(21)00128-4.

57. Swiatecka-Urban A, Moreau-Marquis S, Maceachran DP, Connolly JP, Stanton CR, Su JR, Barnaby R, O'toole GA, Stanton BA: ***Pseudomonas aeruginosa* inhibits endocytic recycling of CFTR in polarized human airway epithelial cells**. *Am J Physiol Cell Physiol* 2006 Mar, **290**:C862–C872, https://doi.org/10.1152/ajpcell.00108.2005. Epub 2005 Oct 19. PMID: 16236828.

58. MacEachran DP, Ye S, Bomberger JM, Hogan DA, Swiatecka-Urban A, Stanton BA, O'Toole GA: **The *Pseudomonas aeruginosa* secreted protein PA2934 decreases apical membrane expression of the cystic fibrosis transmembrane conductance regulator**. *Infect Immun* 2007 Aug, **75**:3902–3912, https://doi.org/10.1128/IAI.00338-07. Epub 2007 May 14. PMID: 17502391; PMCID: PMC1951978.

59. Rubino R, Bezzerri V, Favia M, Facchini M, Tebon M, Singh AK, Riederer B, Seidler U, Iannucci A, Bragonzi A, Cabrini G, Reshkin SJ, Tamanini A: ***Pseudomonas aeruginosa* reduces the expression of CFTR via post-translational modification of NHERF1**. *Pflügers Archiv* 2014 Dec, **466**:2269–2278, https://doi.org/10.1007/s00424-014-1474-6. Epub 2014 Mar 5. PMID: 24595473.

60. Stanton BA, Coutermarsh B, Barnaby R, Hogan D: ***Pseudomonas aeruginosa* reduces VX- 809 stimulated F508del-CFTR chloride secretion by airway epithelial cells**. *PLoS One* 2015 May 27, **10**, e0127742, https://doi.org/10.1371/journal.pone.0127742. PMID: 26018799; PMCID: PMC4446214.

61. Trinh NT, Bilodeau C, Maillé É, Ruffin M, Quintal MC, Desrosiers MY, Rousseau S, Brochiero E: **Deleterious impact of *Pseudomonas aeruginosa* on cystic fibrosis transmembrane conductance regulator function and rescue in airway epithelial cells**. *Eur Respir J* 2015 Jun, **45**:1590–1602, https://doi.org/10.1183/09031936.00076214. Epub 2015 Mar 18. PMID: 25792634.

62. Maillé É, Ruffin M, Adam D, Messaoud H, Lafayette SL, McKay G, Nguyen D, Brochiero E: **Quorum sensing down-regulation counteracts the negative impact of *Pseudomonas aeruginosa* on CFTR channel expression, function and rescue in human airway epithelial cells**. *Front Cell Infect Microbiol* 2017 Nov 10, **7**:470, https://doi.org/10.3389/fcimb.2017.00470. PMID: 29177135; PMCID: PMC5686086.

63. Saint-Criq V, Villeret B, Bastaert F, Kheir S, Hatton A, Cazes A, Xing Z, Sermet-Gaudelus I, Garcia-Verdugo I, Edelman A, Sallenave JM: ***Pseudomonas aeruginosa* LasB protease impairs innate immunity in mice and humans by targeting a lung epithelial cystic fibrosis transmembrane regulator- IL-6-antimicrobial-repair pathway**. *Thorax* 2018 Jan, **73**:49–61, https://doi.org/10.1136/thoraxjnl-2017-210298. Epub 2017 Aug 8. PMID: 28790180; PMCID: PMC5738602.

64. Laselva O, Stone TA, Bear CE, Deber CM: **Anti-infectives restore ORKAMBI® rescue of F508del-CFTR function in human bronchial epithelial cells infected with clinical strains of *P. aeruginosa***. *Biomolecules* 2020 Feb 19, **10**:334, https://doi.org/10.3390/biom10020334. PMID: 32092967; PMCID: PMC7072183.

65. Hegde RN, Parashuraman S, Iorio F, Ciciriello F, Capuani F, Carissimo A, Carrella D, Belcastro V, Subramanian A, Bounti L, Persico M, Carlile G, Galietta L, Thomas DY, Di Bernardo D, Luini A: **Unravelling druggable signalling networks that control F508del-CFTR proteostasis**. *Elife* 2015 Dec 23, **4**, e10365, https://doi.org/10.7554/eLife.10365. PMID: 26701908; PMCID: PMC4749566.

66. ** Gentzsch M, Cholon DM, Quinney NL, Martino MEB, Minges JT, Boyles SE, Guhr Lee TN, Esther Jr CR, Ribeiro CMP: **Airway epithelial inflammation *in vitro* augments the rescue of mutant CFTR by current CFTR modulator therapies**. *Front Pharmacol* 2021 Mar 30, **12**:628722, https://doi.org/10.3389/fphar.2021.628722. PMID: 33859562; PMCID: PMC8042279.
This investigation tests the effect of the supernatant of mucopurulent material (SMM), a pool of sputum extracted from different adult CF lungs, filtered from living bacteria, which contains a large series of molecules, on F508del-CFTR rescue with CFTR modulators on CF airway epithelia grown polarized at the air-liquid interface *in vitro*. The results 1) exclude that novel generation CFTR modulators has anti-inflammatory effect by reducing the expression and release of the pro-inflammatory mediator IL-8 (CXCL8) from CF epithelium and 2) show that F508del-CFTR rescue is augmented by the SMM. The article opens a new perspective and suggests investigation on the underlying mechanisms activated by SMM and how to reconcile them with previous reports showing the opposite effects observed with living *P. aeruginosa* on rescue of mutated F508del-CFTR operated by CFTR modulators.