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Review

The impact of impaired macrophage functions in cystic fibrosis disease progression

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Abstract

The underlying cause of morbidity in cystic fibrosis (CF) is the decline in lung function, which results in part from chronic inflammation. Inflammation and infection occur early in infancy in CF and the role of innate immune defense in CF has been highlighted in the last years. Once thought simply to be consumers of bacteria, macrophages have emerged as highly sensitive immune cells that are located at the balance point between inflammation and resolution of this inflammation in CF pathophysiology. In order to assess the potential role of macrophage in CF, we review the evidence that: (1) CF macrophage has a dysregulated inflammatory phenotype; (2) CF macrophage presents altered phagocytosis capacity and bacterial killing; and (3) lipid disorders in CF macrophage affect its function. These alterations of macrophage weaken innate defense of CF patients and may be involved in CF disease progression and lung damage.

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Keywords: Cystic fibrosis; Macrophage; Inflammation; Lipid homeostasis; Phagocytosis

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Abbreviations: AA, arachidonic acid; AMs, alveolar macrophages; BAL, bronchoalveolar lavage; BM, bone marrow; BMDMs, bone marrow-derived macrophages; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; DHA, docosahexaenoic acid; G-CSF, granulocyte-colony stimulating factor; GM-MDMs, granulocyte macrophage-colony stimulating factor monocyte derived macrophages; HETEs, hydroxyeicosatetraenoic acid; IFN, interferon; IL, interleukin; KETEs, ketoeicosatetraenoic acids; LOX, lipoxygenase; LPS, lipopolysaccharide; LXR, liver X receptors; MCP, monocyte chemoattractant protein; M-MDMs, macrophage-colony stimulating factor monocyte derived macrophages; PIs, peritoneal macrophages; PPAR, peroxisomal proliferator activated receptors; PRR, pattern recognition receptors; ROS, reactive oxygen species; TLRs, toll-like receptors; TNF, tumor necrosis factor; WT, wild-type.

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1. Introduction

Patients with CF are susceptible to bacterial infections, which induce an intense inflammatory response even in those with only modest pulmonary disease. Impairment of immune cellular defense in CF patients is now commonly accepted, affecting mostly the lungs but also the pancreas, liver and intestine [1]. The ongoing response of immune cells to this chronic infection results in progressive lung destruction and increased disease exacerbations in CF patients, responsible partly for increasing morbidity and mortality. Even in the absence of clinically apparent viral or bacterial infections, inflammation is often present in CF airways, as evidenced by neutrophil/macrophage accumulation and excessive concentrations of interleukin (IL)-8 and proteases in bronchoalveolar lavage (BAL) and sputum [2,3]. Thus, it seems that besides epithelial cells, other CF immune cells, have an inherent altered phenotype that exists without signs of infection. Recruited phagocytes have a role in regulating the innate host response against infection, and resolving inflammation. While the role of macrophages in response to pathogens infection is not completely understood, numerous recent studies have shown an alteration of macrophage function that could contribute to inflammation and to its pathological evolution. In this Review, we summarize the current data on the hyper-inflammatory behavior, reduced scavenger ability, altered phagocytosis capacity and lipid disorders in CF macrophages, in relation to the different tissue origin (alveolar, peritoneal, monocyte-derived, bone marrow-derived) and the species (mice, human).

2. Macrophage is an innate surveillance cellular system impaired in CF

Macrophages are remarkable for the diverse activities in which they engage. Many of these activities appear to be opposing in nature: pro-inflammatory vs anti-inflammatory, immunogenic vs tolerogenic, and tissue destructive vs tissue restorative. The macrophage stands as the guardian of the tissue–blood interface, serving as the front line of cellular defense against pathogens in all organs affected in CF, especially in the lungs. Alveolar macrophages (AMs) are the primary phagocytic cells of the innate immune system present in airways, allowing the clearance of air spaces from infectious, toxic, or allergic particles that were not eliminated by the mechanical defenses of the respiratory tract. Indeed, the roles of macrophages are respectively to phagocytize and to kill toxic organisms within endocytic vacuoles rich in oxygen metabolites, lysozymes, antimicrobial peptides, and proteolytic enzymes, allowing the destruction of the pathogen [4]. Thus, AMs work as regulators of innate alveolar defense against respiratory infections. When facing large amounts of infectious toxins or highly virulent microbes, these macrophages can synthesize and secrete a wide range of cytokines and chemokines such as IL-1, IL-6, tumor necrosis factor (TNF)-α, IL-8, and some arachidonic acid (AA) metabolites such as leukotrienes and prostaglandins. By using these mediators, AMs can initiate inflammatory response and recruit therefore activated neutrophils into the alveolar spaces [5].

In CF, lungs are highly susceptible to environmental toxins and the front-line cellular defense represented by the macrophages has been shown to be defective, allowing the airway invasion by these pathogens. The number of AMs in non-infected CF children has been reported to be elevated compared to non-CF individuals [2,3,6], suggesting an early and constitutive mononuclear inflammation in CF. Besides, the high number of AMs in CF patients BAL was correlated with an increased concentration of the monocyte chemoattractant chemokine (MCP)-1 CCL2 [2,7]. A similar phenotype has been observed in the BAL of several CF mouse models [8,9] suggesting the important role of macrophage in spontaneous lung inflammation.

The adequate activation of an inflammatory response for a subsequent resolution of infection requires the balanced and coordinated activation of macrophage subpopulations with M1 or M2 phenotypes. Classical M1 macrophages are activated by exposure to IFN-γ and GM-CSF, or to bacterial products such as LPS. M1 macrophages produce high levels of the proinflammatory cytokines TNF-α, IL-6, IL-1β, IL-12, IL-23 and CCL2, low levels of IL-10 and increased levels of reactive oxygen species that aid in the clearance of invading pathogens [10]. M2 macrophages are polarized by stimulation with Th2 cytokines such as IL-4 and IL-13, as well as M-CSF, and have upregulated expression of scavenger and mannose receptors, the IL-1 receptor antagonist (IL-1RA) and arginase-1. M2 macrophages are associated with anti-inflammatory and homeostatic functions linked to wound healing and tissue repair. Macrophages are highly plastic and during the progression of inflammatory response the phenotype switching from M1 to M2 enables the dual role of macrophages in orchestrating the onset of inflammation and subsequently promoting healing and repair [11]. Attempts to study macrophage polarization in CF have been
made; however, results have been inconclusive so far. Indeed, analysis of the activation status of macrophages from CF sputa or BAL fluids revealed that macrophages from *Pseudomonas aeruginosa* infected patients exhibited a M2 phenotype characterized by the upregulation of the mannose receptor and high arginase activity [12]. On the contrary, another study revealed that macrophages isolated from CF nasal polyp explants showed a M1 profile [13]. However, it is important to note that, in the last study, these markers were analyzed in total extracts, not in purified macrophages exposed to lipopolysaccharide (LPS) ex vivo. Furthermore nasal polyps may not faithfully reflect the inflammatory response in the lower airways and it must be considered that nasal polyposis do not constitute a primary manifestation of CF but a frequent complication [6,14]. Also, a recent study in macrophages from human sputum shows that M1- or M2-polarization status is distinct among patient grouped by molecular phenotype where these expression signatures did not significantly correlate with clinical characteristics [15]. Elsewhere, studies on murine CF AMs suggested that in the absence of any inflammatory triggers, the macrophages express a M1 polarization profile, and remain therefore in a pro-inflammatory state [16].

Interestingly it has been proposed that the encounter of murine PMs with apoptotic cells represents an additional polarizing trigger that induces Th2-like responses, as well as the expression of arginase-1 and 12/15-lipoxygenase (LOX). Thus in CF, chronic exposure to apoptotic neutrophils could drive macrophages toward the M2 phenotype [17].

Macrophages are plastic cells and their physiological functions are in part dependent on their origin, which overall influence their phenotype. Further studies are needed in order to evaluate the polarization and phenotype of macrophages in CF patients and to understand how they contribute to the pathogenesis of the disease.

### 3. CFTR expression in macrophages

Based on these studies, it remains to be considered whether macrophages express functional CFTR (cystic fibrosis transmembrane conductance regulator), and whether the observed macrophage phenotypes in CF are cell-intrinsic. The expression of CFTR in non-epithelial tissues has already been described [18]. Besides, even though the impact of non-functional CFTR in non-epithelial cells is not very clear, it is however becoming better understood with the development of mouse models in which the expression of CFTR can be abolished in a tissue-specific manner [19].

At low levels, the CFTR protein is detectable in both murine [20] and human macrophages [21,22]. Furthermore, CFTR-like C1− conductance has been recorded in human monocytes and human and murine macrophages [20,21,23–25], suggesting that in these cells, CFTR operates as a cAMP-dependent chloride channel.

In addition, inhibition of functional CFTR in wild-type (WT) macrophages was shown to lead to a phenotype similar to CF macrophages [21–24]. Therefore, macrophages express functional CFTR, suggesting that CF macrophages dysfunctions could be partially a consequence of CFTR defect.

### 4. Inflammatory phenotype of CF macrophages

CF associated airway inflammation is characterized by increased levels of pro-inflammatory cytokines and chemokines, as well as a massive influx in neutrophils which, through the release of oxidants and proteases, can cause damages to the respiratory epithelium causing progressive injury of the airway structure [26].

At present, a growing body of evidences indicates that macrophages play a key role in the effusive production and secretion of cytokines/chemokines characteristic of CF disease (Table 1).

#### 4.1. Murine CF macrophage inflammatory phenotype

The contribution of macrophages in the abnormal immune response was first investigated in murine models of CF. Peritoneal macrophages (PMs) and AMs from *Cftrtm1Eur* mice displayed a pro-inflammatory phenotype after *in vitro*...
stimulation, characterized by enhanced secretion of IL-1β, but reduced secretion of IL-10 [9,16,28]. Similarly, it has been demonstrated that, in response to LPS, bone marrow-derived macrophages (BMDMs), AMs and PMs from CF mice over-produce TNF-α, IL-1α, IL-6, G-CSF and MCP-1/CCL-2, suggesting that CFTR could also regulate the signaling of receptors involved in the recognition of microbial stimuli [8,29,30]. Toll-like receptors (TLRs), through the recognition of a variety of microbial or cellular stimuli (including neutrophil elastase, bacterial LPS, and other microbial products), mediate inflammation in part by activating NF-κB, which governs a molecular pathway that induces the production of inflammatory mediators [31,32]. Indeed, in murine cftr−/− macrophages, excessive inflammatory response was associated with abnormal TLR-4 trafficking and degradation, leading to increased LPS-induced activation of the NF-κB, MAPK and IRF-3 pathways [23]. Furthermore, reduced CFTR expression in AMs from mice resulted in increased activation of NF-κB and IL-8 secretion [33]. In contrast, Zhang et al. reported that CFTR deficiency did not affect the release of CCL-2 and IL-8 by murine AMs stimulated with P. aeruginosa [34]. The most compelling evidence that macrophages contribute to excessive CF lung inflammation derives from in vivo studies showing that, in response to LPS from P. aeruginosa, CF mice transplanted with WT bone marrow (BM) displayed lower proinflammatory cytokine secretion and neutrophilic recruitment compared to mice that received CF BM [8]. In addition, pulmonary instillation of P. aeruginosa in mice, in which CFTR was selectively/conditionally inactivated in myeloid cells, resulted in increased mortality, elevated lung inflammation and bacterial load compared to controls [19].

4.2. Human CF macrophage inflammatory phenotype

In humans, increased levels of inflammatory mediators have been consistently documented in sputa and BALs from CF patients [3,35]. Lung secretions from patients with CF contain large concentrations of TNF-α, IL-1β, IL-6, and IL-8 [36–38]. However few studies investigated the inflammatory phenotype of human macrophages in CF. Furthermore these studies were performed in various types of macrophages including AMs, monocytes-derived macrophages spontaneously differentiated with sera or by the addition of M-CSF (M-MDMs) or GM-CSF (GM-MDMs).

The prototypic pro-inflammatory cytokine TNF-α is mainly released from monocytes and macrophages and has pleiotropic effects central to innate immunity, including inflammatory cell recruitment and induction of cytokines secretion. In CF, GM-MDMs secrete at baseline significantly higher levels of TNF-α [22]. Also, the basal levels of TNF-α mRNA and protein secretion in human CF AMs were higher than in non-CF AMs and the absolute increase after LPS stimulation was greater in CF than in non CF AMs and MDMs [39,40]. Recently, the over-production of TNF-α in LPS-stimulated human CF AM was associated with an increased activation of IRE1/XBP1 branch of the ER-stress signaling pathway. Indeed CF AMs expressed significantly higher levels of XBP-1 mRNA and treatment with the IRE1α inhibitor 4μ8C significantly decreased LPS-induced XBP-1 mRNA expression and TNF-α secretion in such cells. In addition while inhibition of CFTR in non-CF macrophages had no effect on cytokine secretion, the exposure to the infectious/inflammatory CF airway milieu increased TNF-α and IL-6 secretion and XBP-1 expression, suggesting that the greater inflammatory response of CF AMs is not a consequence of the absence of CFTR function but rather reflects a response to persistent inflammatory stimulation [39].

Similar to TNF-α, IL-1β is mainly released from macrophages/monocytes and is actively involved in inflammation. Studies investigating the production of IL-1β by human CF macrophages are very limited. Kopp et al. reported that IL-1β production was significantly increased in CF MDMs infected with Burkholderia cenocepacia [27]. In addition, Simonin et al. documented that under basal conditions, GM-MDMs express higher level of IL-1β protein and mRNA than non-CF cells [22].

Chemoattractant chemokines are regulated by cytokines, such as IL-1β, TNF-α, and IL-6, and they play an important role in both the recruitment of macrophages and neutrophils to the lung and the resolution of inflammation. IL-8 represents the principal neutrophil chemoattractant and its elevated concentration characterizes CF lung inflammation. High levels of IL-8 have been positively correlated with the infection status of CF patients [41]. Furthermore, whole blood IL-8 levels were related to the severity of CF lung disease [41–48]. Alterations in the IL-8 production by human CF macrophages have been consistently documented even though airway epithelial cells are the source of most IL-8 in the lung. In particular, IL-8 production between non CF and CF human M-MDMs showed no difference in response to LPS stimulation, however significantly higher constitutive IL-8 production in CF MDMs compared to non CF cells was observed [23,49]. Accordingly, higher levels of IL-8 mRNA were detected in human AMs isolated from CF children free of any bacterial colonization [50]. In addition, although IL-8 basal level and mRNA expression were not significantly increased in human macrophages from adult CF patients, higher IL-8 levels were observed in CF patients with FEV1 ≤ 55% [22]. Thus, CF macrophages hyper-responsiveness may contribute to the exuberant migration of other immune cells to the lungs, such as neutrophils, and this excessive inflammation may provide an environment suitable to bacterial growth.

As discussed above, CF macrophages display over-expression of multiple proinflammatory cytokines, however, whether this phenomenon is associated with a decrease in the production of suppressive cytokines has not been clearly established yet. IL-10 is a critical anti-inflammatory protein that exerts its immunomodulatory effect by inhibiting the synthesis of proinflammatory cytokines. Early studies revealed that AMs from CF patients express significantly higher concentration of IL-10 compared to non CF AMs [37]. Furthermore, MDMs from CF individuals secreted significant higher level of IL-10 together with IL-1β, MCP-1 and Interferon (IFN)-γ in response to B. cenocepacia J2315 infection [27]. Finally, a significant increase in the
constitutive production of IL-10 was demonstrated in CF MDMs [22]. These findings suggest that, independently from the origin and the procedure of cell differentiation, macrophages carrying dysfunctional CFTR preserve the ability to produce IL-10. Therefore, it is likely that downregulation of IL-10 secretion by CF epithelial cells mainly contributes to the observed decrease in this cytokine in the BAL fluid of CF individuals [36,37].

Human CF macrophages were found to display a constitutive proinflammatory status that was not observed in murine CF macrophages. However, human and murine CF macrophages show a hyper-responsiveness to microbial stimuli (Table 1). These features have been attributed to several signaling abnormalities in CF macrophages, especially in the NF-κB, MAPK and PI3K/AKT pathways [23,30,51,52], as well as to the alteration of LPS-induced metabolic pathways (e.g. PPAR/LXR) [29]. Interestingly, secretion of proinflammatory cytokines was positively correlated with the amount of functional CFTR [51].

5. Alteration of pattern recognition receptors in CF macrophages

Given the long-term and intimate contact of pathogens with the lining CF airway, the mechanisms by which these pathogens are recognized by the host are of key relevance for the understanding of innate immunity in CF disease. In general, pattern recognition receptors (PRR), including TLRs representing the prototypic PRR, are traditionally considered as ‘signaling receptors’ that, upon recognition of a variety of microbial components, induce the synthesis of pro-inflammatory cytokines [53]. In addition, recognition of pathogens may occur through dependent or independent receptors on serum opsonins including scavenger receptors, C-type lectins complement receptors and Fcγ receptors [54].

Expressions of TLRs and bona fide phagocytic receptors in CF macrophages have been investigated by several groups. Studies of TLR expression in CF monocytes/macrophages vs control cells demonstrated no significant differences in the expression of TLR-2 but highlighted various results for TLR-4. Otherwise, mCD14 (LPS receptor) and HLA-DR (MHC Class II) expressions were significantly reduced on AMs from CF children and M-MDMs from CF adults [57,58] whereas no change in their expression was observed in GM-MDMs from CF adults [22]. Consequently, it is likely that the observed discrepancies may be attributed to differences in the age of patients, in the origin of macrophage and in the experimental procedure of cell differentiation (M-CSF vs GM-CSF), some important factors that can lead to different macrophage phenotypes.

Even though a deficiency in the expression of the macrophage receptor with collagenous structure (MARCO) and CD206 (mannose receptors) in sputum macrophages from CF patients was documented [3], its effects on the phagocytosis of CF pathogens have not been evaluated in this model.

Besides, expression of Fcγ receptors CD16 and CD64, which recognize the immunoglobulin Fe domain that constitutes an opsonin, were unchanged in human GM-MDMs [22].

However, an important decrease in CD11b expression was demonstrated in human CF GM-MDMs [22]. CD11b or CR3, an heterodimer integrin composed of α- and β-subunits, is the major receptor for opsonic phagocytosis of many bacteria [59,60]. A similar strong reduction in CD11b expression was reported in CF human monocytes and was associated with a reduced capacity of monocytes to phagocytose opsonized P. aeruginosa [61].

Previous studies have also shown the nonopsonic ingestion of microbial pathogens, such as Mycobacterium tuberculosis [62,63] Leishmania [64], and P. aeruginosa [65] through CR3-mediated phagocytosis. Also defects in the chemoattractants mediated activation of integrins have been documented in CF human and murine monocytes [66]. In these cells chemoattractant triggered activation of RhoA and CDC42 Rho small GTPases was strongly deficient leading to an almost complete absence of adhesion and chemotaxis finally altering the traffic of monocytes in the lung [66].

In addition to the ineffective uptake of pathogens, it has been reported that human CF macrophages show an impairment in the removal of apoptotic cells (efferocytosis). This was ascribed to reduced expression of the phosphatidylserine receptor as a consequence of the proteolytic cleavage by neutrophil elastase. Thus the elastase rich CF airway milieu delaying apoptotic cell clearance perpetuates inflammation and causes tissue damage [67].

All together, these data demonstrate that multiple innate phagocytic and/or signaling receptors are dysregulated in CF macrophages, implying their important contribution to the enhanced lung susceptibility to infections and tissue damage in CF patients.

6. CF macrophages present altered phagocytosis capacity and bacterial killing

By the production of oxygen metabolites, lysozyme, antimicrobial peptides and proteases, and through the processes of phagocytosis and intracellular killing, macrophages can eliminate pathogens. They patrol the body and engulf pathogens in nascent organelles called phagosomes, subsequently matured into phagolysosomes that display antimicrobial activities [68].

Previous studies on the host defense function of human CF AMs [58] demonstrated that phagocytosis was significantly reduced in pulmonary phagocytes from pediatric CF lung. However since these changes were not observed in peripheral blood phagocytes, it was suggested that they depended on the lung microenvironment rather than on genetic factors. More
recently an increasing body of data indicates that the absence or mutations in CFTR gene might directly affect the bactericidal activity of macrophages. 

In particular, an impairment in intracellular *P. aeruginosa* killing was described for the first time in AMs from CFTR −/− mice [20]. This finding was further confirmed in AMs isolated from mutant mice expressing the two most common disease-causing CFTR mutations, F508del and G551D [24]. Also, defective elimination of the pathogen *B. cenocepacia* was allocated to BMDMs expressing the F508del mutation [69]. These data have been confirmed in humans. A significant increase in *P. aeruginosa* and *B. cepacia* survival was indeed observed in CF MDMs and AMs vs non CF cells [21,22,27,70,71]. Although these data provided clear evidences that dysfunctional CFTR affects bacterial killing in macrophages, the bactericidal mechanisms impaired by CFTR mutations have been partially identified.

### 6.1. Defective phagolysosome acidification in CF macrophages

Different mechanisms have been proposed to draw a link between dysfunctional CFTR and defective bactericidal activity in macrophages. In 2006, Di et al. reported that phagolysosomes in AMs from CFTR null mice were ∼2 pH units more alkaline than those from WT mice [20]. Since an effective antimicrobial activity within the macrophage phagolysosome requires a low pH of ∼4.5, it was proposed that CFTR contributes to maintain lysosomes at low pH that consequently limits the growth of ingested bacteria. This observation was further substantiated by the demonstration that pharmacological inhibition of CFTR with the CFTRinh-172 led to a significant increase in phagolysosome pH of AMs. However, these findings were challenged by other investigators who, by using a radiometric approach to measure phagosomal pH, demonstrated that phagosomal acidification was not dependent on CFTR channel activity in macrophages and was not different in F508del murine AMs compared to WT cells [72]. Further, Zhang et al. reported that the absence of CFTR altered the acidification of a particular subset of murine AMs intracellular vesicles, likely to be secretory lysosomes [34]. This resulted in an imbalance in the activity of pH-sensitive enzymes (sphinomyelinase acid) involved in ceramide metabolism, thus impairing the formation of ceramide enriched membrane platforms, which cluster and stimulate the activity of NADPH oxidase in response to bacterial infection. This complex triggers the production of reactive oxygen species (ROS) such as superoxide (O$_2^-$) which mediate the killing of *P. aeruginosa*. In accordance, murine CFTR deficient AMs infected by *P. aeruginosa* failed to release ROS and to eliminate *P. aeruginosa* [34]. However, the role of CFTR in controlling vesicle pH remains controversially discussed [73,74] and the observed discrepancies may be likely due to differences in experimental settings. An additional observation is that the phagosome CI− concentration can directly modulate the behavior of intracellular bacteria by altering bacterial protein activity or host factors. For instance, the CFTR-mediated CI− flux in macrophages contributes to the *L. monocytogenes* phagosomal escape toward the host cytosol by promoting its hemolytic activity [75]. Moreover, the evaluation of the generation of the oxidative burst and the effect of NADPH oxidase inhibition on the intracellular survival of *P. aeruginosa* in non CF and CF M-MDMs revealed no differences in the ROS mediated *P. aeruginosa* killing between the two groups, suggesting that defects in other microbicidal pathways are influenced by CFTR mutations in humans [71].

### 6.2. Defective autophagy in CF macrophages

Defects in autophagy, which is a physiologic process that enhances innate responses against intracellular pathogens, have been also related to the impairment of bacterial killing by CF macrophages. In 2011, Abdulrahman et al. demonstrated an increase in the survival of *B. cenocepacia* within BMDMs from F508del mice compared to WT cells. This was explained by differences in the nature of bacteria containing vacuoles which in WT cells acquired the specific autophagy marker LC3 and subsequently fused with the lysosomes for bacterial destruction. In contrast, in murine F508del BMDMs, *B. cepacia* -containing vacuoles did not acquire autophagosomes markers and did not merge with the lysosomes. Later it was demonstrated that inefficient autophagosome formation was a consequence of the sequestration of the essential autophagy molecules BECN1 by p62 aggregates in murine F508del BMDMs. Accordingly, depletion of p62 in these cells promoted *B. cepacia* uptake by autophagosomes and decreased the bacterial burden [76]. The causative link between defective autophagosome formation and increased *B. cenocepacia* survival was confirmed in human CF MDMs in which stimulation of autophagy through IFN-γ treatment led to bacterial clearance [77].

### 7. Lipid disorders in CF macrophages

CFTR dysfunction in dendritic cells and macrophages can affect membrane structure and lipid metabolism, which may contribute to the abnormal immune response and impaired phagocytosis observed in CF [78–80].

#### 7.1. Caveolin disorder

Caveolae, small flask-shaped invaginations of the plasma membranes, mediate non clathrin-dependent endocytosis, and regulate internalization of particles including viruses and bacteria. Caveolin-1 (Cav-1) is the major structural component of caveolae, and has been involved in the regulation of several signaling membrane receptors [81]. Indeed, in murine BMDMs, Cav-1 colocalizes with TLR-4 and heme oxygenase-1 (HO-1) [82], acting therefore as a potent negative regulator of TLR-4 activation. The attenuation of TLR-4 signaling by Cav-1 was related to the production of carbon monoxide by HO-1 activity. Indeed upon LPS stimulation, Cav-1 allows the trafficking of HO-1 to the caveolae in a p38 MAPK-dependent manner, leading to a down-regulation of the TLR-4 pro-inflammatory signaling. These phenomena reveal an anti-inflammatory network involving Cav-1 and HO-1 [81]. In murine CF BMDMs, stimulation with LPS fails to upregulate Cav-1 expression as well as the
subsequent compartmentalization of HO-1 to the plasma membrane, leading to impaired TLR-4 regulation and exacerbation of the inflammatory response to the bacterial stimulus. This defect was confirmed in MDMs from patients with CF [82] and the reduced expression of Cav-1 in human CF M-MDMs and murine CF BMDMs was attributed to blunted PI3K/AKT signaling, leading therefore to high miR-199a-5p levels [52]. Interestingly, Cav-1 also plays important roles in receptor trafficking and degradation [83] which may contribute to the abnormal TLR-4 trafficking and its reduced degradation in CF murine BMDMs and CF human M-MDMs [23]. At variance gene expression analysis in dendritic cell (DC) generated from naïve CF and WT mice revealed decreased expression of Cav-1 in the CF DC compared to WT DC [84]. Consistently, protein and activity levels of sterol regulatory element binding protein (SREBP) were increased in CF DC [84]. Following exposure to P. aeruginosa, expressions of 3β-hydroxysterol-Delta-7 reductase (DhcR7) and stearoyl-CoA desaturase 2 (SCD2), two enzymes involved in lipid metabolism and also upregulated by SREBP, showed a smaller decrease in the CF DC compared to WT DC [84]. Indeed, CFTR influences cellular lipid metabolism in bone marrow DC, which may affect lipid raft composition, pathogen up-take and clearance, intracellular signaling events, and therefore cause inadequate inflammatory response.

7.2. Sphingolipid disorder

Localization of CFTR into lipid rafts, cellular lipid membrane domains enriched in cholesterol and ceramide, is described following infection with P. aeruginosa, and is crucially involved in signal transduction events. Several recent studies underline the important role of sphingolipids, in particular ceramide, in the pathogenesis of CF [32,34,78,79,85–90]. Since the enzymes that control ceramide metabolism in acidic vesicles are pH-dependent, an alkalization of these vesicles, as described above, has a dramatic effect on ceramide concentrations [91]. Indeed, alkalization of lysosomes in macrophage results in an imbalance of the activities of the acid sphingomyelinase and the ceramidase, leading to ceramide accumulation in CF AMs from mice [34]. However, CFTR deficiency prevents the acute ceramides release in murine CF AMs exposed to P. aeruginosa. This defect results in failure to stimulate the activation of NADPH oxidase and, thus, in an inability to release ROS and to kill P. aeruginosa [34].

7.3. Fatty acid disorder

One possible cause of the tendency to inflammation in CF is the alteration in phospholipid-bound fatty acids within CFTR regulated cells. The eicosanoid pathway plays a crucial role in inflammation by producing bioactive products that modulate both the onset and resolution of inflammation. The importance of relative levels of AA and docosahexaenoic acid (DHA) and their ratio in macrophages is a good indicator of inflammatory state. Imbalance suggests that the defects observed in phospholipid-bound fatty acid levels cannot be explained by aberrant oxidation. They have however an impact on phosphorylation of ERK1/2 which has a direct effect on the increased expression of inflammatory mediators. Nevertheless, no reduction in DHA levels were observed in CF murine PMs compared to WT [29]. Neither are how CFTR dysfunction leads to the fatty acid abnormalities nor the mechanism of action by which DHA ameliorates the inflammatory response known.

However, since fatty acids are ligands to peroxisomal proliferator activated receptors (PPAR), alterations in the fatty acid profile of CF macrophages could provide an explanation for the decreased PPAR activity. Therefore, low DHA levels are not the explanation as to why PPARs are dysfunctional in murine CF PMs [29]. PPAR and liver X receptors (LXR) could provide the link between these processes [92]. These nuclear receptors are well known regulators of fatty acid and cholesterol metabolism. PPAR and LXR activation inhibits pro-inflammatory cytokine expression both in vitro and in vivo, and are important for the resolution of inflammation [93,94]. Indeed, defective regulation of proinflammatory pathways, due to impaired PPAR and LXR expression/function in macrophages, may be a contributing factor to the excessive inflammation in CF. DHA treatment increased PPAR activity, decreased NF-κB activity, and decreased TNF-α secretion from CF PMs [29]. DHA acts, at least in part, through PPAR-α and -γ in macrophages in order to reduce CF associated inflammation which may provide a potential therapeutic pathway for CF treatment.

Interestingly, the end product of the n-6 fatty acid pathway, 22:5, was increased in CF PMs which could suggest an increased metabolism in the n-6 fatty acid pathway [29]. Concurrently to cyclooxygenases, LOXs are non-heme iron lipid-peroxidizing enzymes that catalyze the oxygenation of polyunsaturated fatty acids to their corresponding hydroperoxy derivatives. Although LOXs are best known to generate free acid eicosanoids, it was shown that they also produce phospholipid-esterified eicosanoids such as hydroxyeicosatetraenoic acid (HETE) attached to phosphatidylethanolamine that regulate cytokines produced in macrophages and monocytes [95,96]. Those can be further oxidized to form electrophilic ketoeicosatetraenoic acids (KETEs). Phospholipid-esterified lipids from this pathway are distinct from free eicosanoids and are signaling molecules on their own. Hammond et al. [97] have demonstrated the 15-KETE-PE synthesis in BAL fluids from patients with lung diseases including CF, as well as in human monocytes from healthy subjects cultured with IL-4. Furthermore, 15-KETE-PE induces CD36 in human monocytes and activates PPAR-γ in murine PMs notably by forming adducts by combining with amino acids [97]. It has been also shown that several oxidized phospholipids, including 15-HETE-PE, can attenuate inflammatory signaling through inhibition of TLR-4, a well-known inhibitor of PPAR-γ expression/activity [97,98]. In the case of an esterified KETE, this could generate a lipid capable of membrane anchoring. Thus, the identification and characterization of esterified KETE as a family of lipid signaling mediators on their own is relevant for CF macrophage functions.

In summary, alterations in lipid membrane composition in CF macrophage may play a role in chronic infections in CF patients. Future studies are expected to investigate free fatty acids, cholesterol and sphingolipid metabolism and its significance in CF macrophage dysfunction.
8. Conclusion

Following the increasing number of reports that describe the expression of CFTR in hematopoietic cells involving dysregulation of macrophage functions to control innate immune defense, CF can be described as a disease having all of the features of a primary immunodeficiency disease. Knowledge of the molecular mechanisms behind this defect may help understanding and targeting immune defense systems to the treatment of CF disease. These findings underpin the complexity of the initiation, maintenance, resolution and perpetuation of inflammation in CF involving macrophages and neutrophils within the environment of epithelial cells. We can therefore consider that CF macrophages are unable to properly recognize pathogens, and that their decreased phagocytic capacity leads to persistent infection. Chronic infection induces a continuous stimulation of macrophages, contributing to anarchical production of pro-inflammatory cytokines and chemokines. Persistence of inflammation/infection maintained by CF macrophage further exacerbates the inflammatory state in CF patients that strongly disturbs macrophage lipid homeostasis, membrane fluidity and phagocytosis. Moreover, data sharply suggests that changes in macrophage functions involved in the resolution of inflammation could be responsible for chronic infection (Fig. 1).

Adding to the complexity of host-pathogen interactions, macrophages are not a homogeneous cell population. Macrophage location and activation state can markedly influence its interactions with microbes, and various phenotypes exist between macrophages, depending on their tissue origins (alveolar, peritoneal, monocyte-derived, bone marrow-derived) and on the species (mice or humans). Therefore, the macrophage phenotype may influence interpretation and comparisons of data obtained from different laboratories. In conclusion CF murine AMs represent a good model for studying CF macrophage physiopathology in an infectious context whereas human AMs and MDMs models are closer to constitutive innate immune defect in CF.

Significant inflammation in the airway of CF patients with clinically mild lung disease were observed, even in patients with a mean FEV1 > 95% of predicted [1,6,99]. Consequently, intervention aimed at reducing ongoing infection and destructive inflammatory response may be beneficial, even when patients do not have signs or symptoms of acute exacerbations.

Fig. 1. Impaired CF macrophage functions against *P. aeruginosa*. The figure illustrates the altered molecules and pathways causing an exaggerated inflammatory response and reduced *P. aeruginosa* elimination in CF macrophages. In healthy macrophage (left panel), upon encountering the bacteria, the engagement of the pathogen recognition receptors TLR4 and TLR5 activates a signaling cascade leading to the synthesis of pro-inflammatory mediators. TLR5-induced IL-1β production concurs to *P. aeruginosa* phagocytosis and killing in the endosomal compartment. Removal of extracellular bacteria is mediated by non-opsonic and opsonized receptors and CR-3 (CD11b/CD18) is mainly involved in the uptake of opsonized *P. aeruginosa*. Ingested bacteria are finally destroyed in phagolysosomes. In addition the release of ceramide during bacterial infection in healthy macrophages promotes the formation of ceramide-enriched membrane platforms (raft), which mediate signal transduction, contributing to bacterial killing. In CF macrophage (right panel), TLR4 signaling is increased leading to the over-production of pro-inflammatory mediators while TLR5 is not expressed. In addition CF macrophage has a fatty acids imbalance characterized by increased arachidonic acid (AA) and decreased docosahexaenoic acid (DHA), a condition promoting inflammation. Reduced expression of CR-3 in CF macrophages impairs opsonized bacteria uptake promoting bacterial persistence. CFTR-deficiency results in the alkalinization of intracellular vesicles leading to increase in cellular ceramide impairing the formation of lipid rafts impacting signal transduction and bacterial killing. In addition alkalinization of phagolysosomes impairs bacterial killing further promoting intracellular bacteria survival. IL, interleukin; CCL2/MCP1, monocyte chemoattractant protein 1; LTs, leukotriens; PGs, prostaglandins; TLR, Toll like receptor; TNF-α, tumor necrosis factor-α. Illustration made from Servier Medical Art.
in order to preserve their lung function. Based on the present review, therapeutically approaches in order to change the functional phenotype of the macrophages would be beneficial.

Conflict of interest

The authors declare that there is no conflict of interests between them and regarding the publication of this paper.

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