Macrophage dysfunction in cystic fibrosis: Nature or nurture?

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Abstract
Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) affect the homeostasis of chloride flux by epithelial cells. This has deleterious consequences, especially in respiratory epithelia, where the defect results in mucus accumulation distinctive of cystic fibrosis. CFTR is, however, also expressed in phagocytic cells, like macrophages. Immune cells are highly sensitive to conditioning by their environment; thus, CFTR dysfunction in epithelia influences macrophages by affecting the lung milieu, but the mutations also appear to be directly consequential for intrinsic macrophage functions. Particular mutations can alter CFTR’s folding, traffic of the protein to the membrane and function. As such, understanding the intrinsic effects of CFTR mutation requires distinguishing the secondary effects of misfolded CFTR on cell stress pathways from the primary defect of CFTR dysfunction/absence. Investigations into CFTR’s role in macrophages have exploited various models, each with their own advantages and limitations. This review summarizes these methodologic approaches, discussing their physiological correspondence and highlighting key findings. The controversy surrounding CFTR-dependent acidification is used as a case study to highlight difficulties in commensurability across model systems. Recent work in macrophage biology, including polarization and host–pathogen interaction studies, brought into the context of CFTR research, offers potential explanations for observed discrepancies between studies. Moreover, the rapid advancement of novel gene editing technologies and new macrophage model systems makes this assessment of the field’s models and methodologies timely.

KEYWORDS
CFTR, cystic fibrosis, macrophage, phagosome

1 | INTRODUCTION

The cystic fibrosis transmembrane conductance regulator (CFTR) is an ATP-gated ABC transporter, which primarily acts as a channel for chloride ions, and is abundantly expressed on the apical membrane of epithelial cells, especially the pH-regulating subset referred to as ionocytes. Mutations in the Cftr gene have devastating consequences for people with cystic fibrosis (PWCF), and an estimated 1 in 3000 babies of Caucasian descent is born with this autosomal recessive disease. In cystic fibrosis (CF), disequilibrium in chloride ion regulation leads to defects in secretory and respiratory epithelia. The effects in the airways are especially detrimental to PWCF, with osmotically induced dehydration of the air–surface liquid interface of airway epithelial cells making mucus more viscous and refractory to mucociliary clearance. The disease is characterized by long-term chronic dysregulation of the pulmonary environment, inflammation, and consequently enhanced susceptibility to bacterial and fungal infections, which can be life threatening (reviewed in ref. 4). In addition to being expressed by epithelial cells, CFTR is expressed in neutrophils and macrophages.

“Cystic fibrosis macrophage” has multivalent meaning, given that immune cells are conditioned by their milieu, and since there are...
several CFTR mutations that can lead to CF phenotypes, which may have differential effects on macrophage function. This review seeks to answer the following questions: why is studying CFTR’s role in macrophages relevant? What are the challenges in understanding the effects of CFTR mutations in macrophages? Why is modeling CF macrophage function difficult?  

Studying the role of CFTR in macrophages is complicated by the specificities of Cftr gene mutations and the commensurability of model systems used. The consequences of CFTR mutations can be extrinsic or intrinsic. Extrinsic effects refer to the consequences of epithelial dysfunction on the lung milieu because this milieu dictates the differentiation and modality of the immune cells including macrophages. Intrinsic effects include the loss of CFTR function (primary effect), as well as secondary consequences, like increased cell stress due to CFTR misfolding and intracellular protein aggregation. Parsing these 3 effect types, extrinsic, primary, and secondary, is further complicated by the inherent limitations of how CF can be modeled. The multiplicity of models and methodologies used to study CF macrophages exposes the inherent difficulty of working with this heterogenous cell type.  

This review expands on a 2016 review by Bruscia and Bonfield, where the authors delineate intrinsic and acquired factors accounting for macrophage alteration in CF. We argue that “intrinsic” should be parsed in terms of the effects of CFTR’s absence or dysfunction (primary), but also in terms of the influence of CFTR protein misfolding on cellular stress pathways (secondary). With this framework in mind, we critically assess methodologies used for evaluating CFTR’s role in macrophages, with a focus on the models themselves.

2 | SPOTLIGHTING THE CF MACROPHAGE

2.1 | The CF lung milieu conditions macrophages

The perturbed equilibrium of the pulmonary environment in PWCF predisposes the lung toward proinflammatory cytokine production and phagocyte recruitment. The lung-resident alveolar and interstitial macrophages are yolk sac derived, and seeded during embryonic hematopoiesis. Alveolar macrophages act as sentinels of the lung, maintaining homeostasis and, upon activation, secreting proinflammatory cytokines to recruit neutrophils, whereas interstitial macrophages play a regulatory role in lung tissue and contribute to the adaptive immune response. Pathogens encounter alveolar macrophages as part of the first-line of defense in the airway lumen but in CF, mucus accumulation compromises host–pathogen interaction. In CF, infiltrating neutrophils release reactive oxygen species (ROS) and elastase, and continued response without resolution of inflammation leads to airway epithelium damage and promotes fibrosis (reviewed in ref. 10). This tissue damage, with compromised mucociliary clearance, exacerbates the risk of infection. Elastases also cleave receptors necessary for host–pathogen recognition, making phagocytosis less efficient (reviewed in ref. 9).  

*Pseudomonas aeruginosa* and *Burkholderia cepacia* complex are among several opportunistic bacterial pathogens often encountered in pulmonary infections in CF. These bacteria can survive intracellularly in macrophage phagosomes, and their presence elicits an augmented immune response in CF versus wild-type (WT) phagocytes. These bacteria also adapt to the lung environment, so isolates obtained later in the course of infection may have altered expression profiles in response to the selective pressure within the lung (reviewed in ref. 16). In parallel, the plasticity of macrophages enables them to adapt to environmental cues. These iterative interactions are difficult to model but have implications for disease progression, especially because CF models have shown that CFTR mutations compromise bacterial responses. Delineating the effect of CFTR mutations on macrophages, including their adaptability, will enable better understanding of evolving host–pathogen interactions in the CF lung.

The phagocyte dysfunction observed in PWCF cannot be fully attributed to conditioning by the proinflammatory lung milieu. Bone marrow transplants from mice with the Cftr knockout (KO) mutation into WT mice, and vice versa, suggested that CFTR-deficient myeloid cells contribute to exaggerated inflammatory response in mice. Moreover, myeloid-specific depletion of CFTR in a mouse model led to an immune response phenotype intermediate between WT and CF mice, with compromised resolution of inflammation and infection. Studies using human CFTR-deficient monocyte-derived macrophages (MDMs) indicate that CFTR mutations are associated with intrinsic effects on macrophage function. Collectively, these studies suggested that CFTR dysfunction has functional implications for immune cells beyond conditioning by the CF lung milieu.

2.2 | CFTR is expressed in macrophages

The influence of CFTR mutations in macrophages requires elucidation because CF is characterized by opportunistic bacterial infections, and it is likely that immune cell dysfunction contributes to the chronicity of infection. CFTR is expressed in human macrophages and neutrophils as demonstrated by the isolation of Cftr mRNA as early as 1991; hence, mutated CFTR in these cells may contribute to immunodysregulated phenotypes. CFTR is a large, glycosylated transmembrane

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**Key Questions**

- What are the extrinsic and intrinsic (direct and indirect) contributions of CFTR mutations on macrophage function?
- How do mutations differ?
- Do CFTR mutations have different consequences in macrophages of differing ontogeny and tissue residency?
- What are the advantages and caveats in macrophage models, and what are the implications for commensurability?
- (How) Do macrophage polarization and bacterial effectors influence phagosome acidification dynamics? Does CFTR mutation have any effect?
- How can we leverage CF macrophage research to treat bacterial infection in people with CF?
protein, which can complicate efficient detection by immunoblotting. However, due to the multitude of available antibodies (including mutation- and phospho-sensitive ones), CFTR has been detected in macrophages both at the surface and intracellularly by immunoblotting, flow cytometry, and immunofluorescence microscopy. Human peripheral blood MDMs and monocytes express CFTR, albeit with variation in abundance between blood donors. CFTR expression is reduced on the surface of monocytes from PWCF. Investigating CFTR’s contributions to macrophage function has become more practicable with advances in gene editing tools and macrophage models. CFTR’s chloride efflux functionality has been investigated in CF and non-CF macrophages from humans and other species using whole-cell patch-clamp assays and fluorescence-based chloride ion flux measurement.

Confirming CFTR expression in tissue-resident alveolar macrophages has been challenging. Di et al. used immunoblotting and RT-PCR to establish that CFTR was expressed in alveolar macrophages from humans and mice, and in the J774A.1 murine macrophage-like cell line. They concluded CFTR was present at the plasma membrane based on voltage-clamp analyses for murine alveolar macrophages and electric current sensitivity to inhibitor CFTRinh-172. However, their immunofluorescent staining shows that CFTR has a perinuclear distribution in these cells, relocalizing to phagosomes. Others found negligible basal expression of Cfr mRNA in cultured primary human alveolar macrophages, as well as in mouse alveolar macrophages, whereas Zhang et al. immunolocalized CFTR protein in the lysosomes of alveolar macrophages from the same mouse strain. Conceivably, techniques for procuring and culturing alveolar macrophages influence CFTR expression, as the isolation technique can alter the transcriptome of other tissue-resident immune cells. A phagosomal/lysosomal localization in alveolar macrophages contrasts with the surface CFTR distribution observed in MDMs. Numerous studies show CFTR expression (albeit at varying levels) in macrophages (reviewed in ref. 9), and evidence shows that disrupting CFTR affects macrophage function (see below). It has been difficult to establish consensus on certain attributes of CF macrophage dysfunction (reviewed in ref. 13), and whether these factors are extrinsic, primary, or secondary consequences of CFTR mutations, which suggests the need for targeted elucidation work.

3 | THE MULTIFACETED CONSEQUENCES OF CFTR MUTATIONS

3.1 | The CFTR mutations in lung epithelia influence phagocytes

CFTR mutations in epithelial cells can extrinsically influence macrophage function by contributing to lung dysfunction because macrophages’ inflammatory profile depends on cues from the lung environment (for a detailed review, see ref. 9). The CF lung milieu differs starkly from the healthy lung, with altered inflammatory cytokines, neutrophil effectors, and hydrostatic pressure. CF modifier genes also influence the lung environment.

Mucopurulent material harvested from CF airways is sufficient to recapitulate the CF inflammatory phenotype in non-CF alveolar macrophages. However, both pulmonary macrophages and MDMs with the CFTR mutation have an exaggerated cytokine response to bacterial lipopolysaccharide, and gene expression profiles are altered in monocytes and MDMs from PWCF. Since MDMs are not exposed to the airways, pulmonary environmental conditioning is not the only factor in inflammation. Systemic cytokine levels are altered in PWCF, which could influence MDM phenotype. The cytokine profiles of the CF lung are conducive to both proinflammatory and reparatory types of polarization. Both proinflammatory “M1”- and reparatory “M2”-polarized alveolar macrophages increased in ΔF508-CFTR mice, complicating the immune response phenotype. The evolving understanding of macrophage activation from “M1”/”M2” polarization to a spectrum model of intermediate phenotypes could inform interpretations of previous work, spotlighting the influence of CF environmental cues in macrophage polarization.

3.2 | Pleiotropic dysfunction of CFTR mutations in macrophages

Mutations in CFTR have consequences for the folding, trafficking, and degradation of the protein. Over 2000 mutations in the Cfr gene have been recorded, and these are classified according to their effects: absence or reduction of the protein, defective anion transport, or membrane localization (reviewed in ref. 40). Accordingly, parsing the contributions of misfolding or CFTR dysfunction to pathology is complicated. The most common Cfr gene mutation (class II) observed in PWCF results in the loss of a phenylalanine residue at position 508 (ΔF508), and occurs in over two-thirds of PWCF worldwide. Misfolded CFTR is targeted for endoplasmic reticulum (ER)-associated degradation. Although most of ΔF508-CFTR is degraded in this way, misfolded CFTR trafficked to the plasma membrane can be removed by a peripheral protein quality control system. This means that the ΔF508 mutation has primary consequences, affecting CFTR’s chloride channel activity, but also secondary consequences for endocytic recycling, ER stress, and the unfolded protein response in cells, as misfolded CFTR is targeted for ubiquitination and proteasome degradation. A recent study using peripheral blood monocytes from PWCF suggested that mutated CFTR did not inherently contribute to inflammasome activation in these cells, but rather that inflammasome activation was a secondary consequence of cell stress induced by the burden of processing misfolded protein.

CFTR mutation in phagocytes is associated with alterations in cytokine profiles, bactericidal activity, lysosome maturation, adhesion, and dysfunction of other ion channels. Treatment of “M1”- and “M2”-polarized non-CF human MDMs decreased ability to phagocytose Escherichia coli bioparticles, recapitulating the phenotypes observed in CF MDMs. MDMs isolated from PWCF are less effective at killing intracellular P. aeruginosa than non-CF macrophages, but CFTR deficiency does not compromise
ROS-mediated killing.\textsuperscript{48} These findings suggest that whatever leads to compromised bacterial killing in CF macrophages is independent from the oxidative burst. However, PWCF have defective Ca\textsuperscript{2+}-dependent PKC activation of NADPH oxidase in response to Burkholderia cenocepacia and compromised bacterial killing.\textsuperscript{49} Differences in the way bacterial species interface with macrophages may account for these discrepancies.

Even at potentially low levels, CFTR (dys)function seems to be physiologically relevant for macrophage defense activities, albeit by undefined mechanisms. Defective CFTR appears to enhance bacterial survival in the macrophage phagolysosome, as demonstrated in CFTR-defective alveolar macrophages infected with \textit{P. aeruginosa}.\textsuperscript{5} \textit{Burkholderia} delay phagosome maturation in RAW 264.7 cells (a murine macrophage line): this effect was exaggerated with the CFTR inhibitor, suggesting that CF macrophages may have compounded difficulties clearing the intracellular infection.\textsuperscript{46} This may be related to compromised autophagy because defective autophagy has been observed in \textit{AF508-CFTR} epithelia,\textsuperscript{50,51} and also in MDMs from PWCF.\textsuperscript{15} Autophagy is cytoprotective, and CFTR dysfunction led to protein aggregation in these epithelial cells. Functional autophagy is necessary for the clearance of \textit{B. cenocepacia} in human CF MDMs.\textsuperscript{52} as inducing autophagy by rapamycin enhanced bacterial clearance in CF macrophage models.\textsuperscript{17} Although CFTR-containing protein aggregates have not been directly shown in macrophages, Abdulrahman et al.\textsuperscript{53} have observed that Beclin 1, a critical component of the early stages of autophagosome formation sequestered in mutant CFTR aggresomes, forms aggregates in \textit{AF508-CFTR} murine macrophages. Together, these findings cumulatively suggest that a secondary effect of CFTR mutation could be dysfunctional autophagy and the creation of a bacterial niche in CF macrophages.\textsuperscript{54} Additionally, the phenomena observed in immune cells with dysfunctional CFTR include up-regulated proinflammatory cytokine production, altered TLR4 activity, and elevated MMP12 activity, suggesting CFTR’s interactions with other proteins could augment inflammation.\textsuperscript{40}

Chloride ion is an important signaling effector in cells (reviewed in ref. 55). Thus, altered chloride ion flux due to CFTR dysfunction has implications for signaling pathways in addition to contributing to airway surface liquid dehydration. Intracellular chloride levels influence Ca\textsuperscript{2+} signaling\textsuperscript{56} and can promote inflammation in airway epithelium.\textsuperscript{57} CFTR inhibition enhances Ca\textsuperscript{2+} efflux in macrophages.\textsuperscript{30} Treatment with CFTR\textsubscript{mut-172} blocked forskolin-induced Cl\textsuperscript{-} efflux in healthy murine macrophages, but neither forskolin nor CFTR\textsubscript{wt-172} treatment influenced CF macrophages’ Cl\textsuperscript{-} efflux,\textsuperscript{58} suggesting CFTR is a functional ion channel in non-CF macrophages.

Whether macrophage-specific features of CF are indirect or direct consequences of CFTR mutation requires further elucidation. The endosomal recycling of WT and G551D-CFTR are similar in a heterologous expression model\textsuperscript{59} and protein maturation efficiency has been assessed only in epithelial cell lines and other heterologous expression systems,\textsuperscript{60} not yet in phagocytic models. Even if mutant CFTR proteins fold efficiently and reach maturation, it remains unknown whether endosomal recycling, interaction with peripheral protein quality control is the same as in WT cells, especially if the complement of chaperones and ER-associated degradation proteins differs from epithelial cells.\textsuperscript{41}

Delineating extrinsic, primary and secondary phenotypic consequences may help to tailor treatment according to mutation type, if particular consequences are characteristic of certain mutations. Mechanistic knowledge about CFTR in macrophages could inform investigation of how CFTR mutations disrupt signaling pathways in phagocytes and influence their ability to fight bacteria. The contribution of CFTR misfolding to pathology may turn out to be negligible, but at present the models available to delineate how mutated CFTR influences macrophage biology have limitations. These models and methods are the theme of the next section.

4 | THE MULTIPLECTICITY OF MACROPHAGE MODELS

4.1 | Ex vivo models

Pulmonary macrophages can be studied in CF lung tissue obtained through autopsy or explant material, yielding visual data about bacterial infection of these cells.\textsuperscript{61,62} Isolation of peripheral or alveolar blood cells is laborious and often results in phenotypically heterogeneous batches of cells. This means that these models are not conducive to reproducible studies and are thus not ideal for mechanistic work. However, they valuably inform our understanding of the alveolar macrophage microenvironment during CF disease progression. Ex vivo experiments can make use of modulators (like ivacaftor and lumacaftor) and certain other tools but these cells are short lived so their tractability is limited. Finding a model that recapitulates in vivo leukocytes remains challenging: leukocytes isolated from peripheral blood are not representative of alveolar and tissue leukocytes\textsuperscript{63} because the immune plasticity of these cells means they are greatly influenced by their ontogeny, tissue residency, and milieu. Parsing the individual and interacting contributions of epithelial and phagocytic cells in CF is further complicated by the pleiotropic dysfunctionality of CFTR mutations.

4.1.1 | Mouse macrophages

Rosen et al.\textsuperscript{64} have recently reviewed animal models used for CF disease phenotype studies, so here we focus on how animal models have been used for studies of macrophages specifically. Mouse models with CFTR dysfunction do not adequately recapitulate the propensity to develop bacterial lung infection observed in humans with CF.\textsuperscript{64} Even so, several mouse models have been used in studies of immune cell function in CF (reviewed in ref. 65).

The first mouse models were generated from the C57BL/6 strain in 1992, and initial work was epithelium focused. Dorin et al.\textsuperscript{66,67} knocked out exon 10 of the Cfr gene, creating mice with low, but not absent, expression of CFTR, due to alternative splicing. Alveolar macrophages from these \textit{Cfr}\textsuperscript{MHH} mice have higher levels of ceramide.\textsuperscript{24} Snouwaert et al.\textsuperscript{68} generated a mouse model with a disrupted \textit{Cfr} gene by adding a premature stop codon in exon 10 after
Ser489 (S489X). Alveolar macrophages from S489X-CFTR mice have been isolated from bronchoalveolar lavage (BAL) and macrophages have been derived from bone marrow (differentiated using M-CSF). TLR4 internalization is abnormal in murine BM-derived S489X-CFTR macrophages and the rate of protein degradation in lysosomes is also slower.

The ΔF508-CFTR mouse (129/Ola X FVB/N background) reproduces the phenylalanine deletion observed in human CF. Levels of alveolar macrophages and CCL2 were elevated in these mice, with isolated cells showing enhanced LPS-induced proinflammatory mediator production. Moreover, monocytes from these mice exhibited compromised adhesion to ICAM-1, with reduced trafficking in vivo. Shenoy et al. used ΔF508-CFTR and S489X-CFTR mice to investigate CI− efflux in WT and CF bone-marrow-derived macrophages, concluding that functionality was compromised in the latter, albeit without discussing if there were differences between their 2 CF models. Macrophages from mouse lines are tractable for experimental work because genetic heterogeneity can be mitigated. However, models with different types of CFTR mutation are not necessarily commensurable.

A conditional Cftr KO line has been used to create a myeloid-specific Δexon10-Cftr mouse, which had survival and inflammatory profiles intermediate between a full Cftr KO and healthy control mouse following bacterial challenge. Creating a macrophage-specific KO would be challenging, given the complicated ontogeny of these cells. Interestingly, genetic complementation of Cftr in the airway epithelium was sufficient to correct inflammatory abnormalities in mice with the G551D-Cftr mutation. This finding suggests that the effects of epithelial dysfunction induced by this mutation were more consequential for immune cells than intrinsic Cftr mutation, at least in the mouse model.

### 4.1.2 Human macrophages

Human alveolar macrophages can be obtained through BAL, or peripheral blood monocytes can be induced to differentiate into macrophages through treatment with M-CSF or GM-CSF. The genetic heterogeneity of CF and non-CF controls, as well as differences in ontogeny, may account for discrepancies in cytokine profiles reported for CF and non-CF macrophages. Although ex vivo cells are not tractable for reproducible studies, their phenotypic features are informative. For example, a “small macrophage” phenotype was observed by flow cytometry of BAL cells from PWCF and macrophages from PWCF have an altered DNA methylation profile. Ex vivo CF monocytes and macrophages have been profiled for cytokine production and assessed for inflammatory responses and bacterial phagocytosis/killing. Isolated cells are amenable to analyses by immunofluorescence, flow cytometry, and immunoblotting.

### 4.1.3 CFTR modulators

Immune cells from PWCF, treated with pharmaceutical CFTR modulators, can be isolated for study, or isolated cells can be treated with these potentiators/correctors (reviewed in ref. 43). They were developed primarily to treat CFTR misfolding in epithelial cells, but are known to have effects on other cell types (reviewed in ref. 77). Modulators can be categorized as potentiators, like ivacaftor, which enhance CFTR channel function and correctors, like lumacaftor, which chaperone CFTR during folding. Ivacaftor and lumacaftor are combined in Orkambi treatment, but it is worth dwelling on how their distinct modes of action have been shown to influence CF macrophages. Conceptually, ivacaftor fixes the primary defect of chloride channel dysfunction, whereas lumacaftor ameliorates the secondary effects of CFTR misfolding as well as restoring CFTR expression, and hence primary function. Orkambi restores chloride efflux and CFTR expression and glycosylation in immune cells. Treatment of CF monocytes with correctors restored integrin function, and ivacaftor/lumacaftor decreased levels of NLRP3-inflammasome signature markers, suggesting CFTR dysfunction is imbricated with other cellular pathways. However, the synergistic activity of potentiators and correctors for macrophage CFTR requires further articulation. Ivacaftor/lumacaftor and ivacaftor alone both enhanced CFTR expression and maturation in ΔF508-CFTR monocytes and MDMs, but ivacaftor-only treatment restored MDMs’ ability to phagocytose P. aeruginosa, whereas ivacaftor/lumacaftor did not. Ivacaftor decreased P. aeruginosa bacterial load, and may have bactericidal properties, as evidenced by its antimicrobial activity against several clinical respiratory bacterial isolate species.

### 4.2 Cell line-based models as tools for recapitulating CF phenotype

CF cell lines were initially developed mostly from epithelial cells but also in fibroblasts. Mechanistic work regarding folding, trafficking, and stability of CFTR and its mutants has made use of heterologous expression in Hela and Cos7 models, in addition to human bronchial epithelial cell lines. Myeloid cell lines with CFTR mutations are rarer, and arguably less likely to be physiologically representative than their epithelial counterparts (for example, ref. 82 includes a discussion of the physiologic representativeness of THP-1, a non-CF human monocyte-like cell line). CRISPR/Cas9 has been used to modify HL-60 (a promyelocytic cell line that can be differentiated into neutrophils), creating a neutrophilic cell line with ΔF508-CFTR. A Cftr+/- murine monocyte cell line (with Cftr+/+ cell line from littermate) has been derived from the bone marrow of C57BL/6 mice (described in ref. 46). C. neoformans induces delay of acidification in this line. A finding corroborated in RAW264.7 (murine macrophage cell line) treated with CFTRinh-172.

Heterogeneity and the effects of the lung milieu confound direct comparison of CF and non-CF cells. Hence, inhibitors CFTRinh-172 (a thiazolidinone) and GlyH-101 (a glycine hydrazide) have been used to pharmacologically mimic the effects of CFTR loss. However, there are concerns about these inhibitors’ CFTR-independent effects on Cl− conductance, off-target mitochondrial effects, and antibiotic properties. Concerns about CFTRinh-172’s off-target effects notwithstanding, many of these studies support the theory that intrinsic CFTR defects have consequences for leukocyte function. Moreover, simply inhibiting CFTR does not holistically recapitulate
the ΔF508-CFTR phenotype, as exemplified in a macrophage Listeria monocytogenes infection model. In epithelial cells, CFTR also acts as a signaling hub through its interaction with numerous kinases and adapter proteins (reviewed in ref. 90). This regulatory network includes the actin cytoskeleton, which is known to be dysregulated in cells with mutated CFTR (reviewed in ref. 91). CFTR may similarly contribute to signaling coordination in immune cells. For this reason, the mutated CFTR phenotype cannot be simply recapitulated by inhibiting CFTR’s channel function.

THP-1 treated with CFTRinh-172 has been used to represent the human CF phenotype. THP-1 cells can be differentiated into macrophages, with PMA treatment more closely recapitulating the phenotype of macrophages (based on surface markers) than vitamin D3 or M-CSF treatment (reviewed in ref. 82). There are difficulties in representing macrophage phenotypes in vitro given the significance of environmental cues in conditioning cells. Additionally, macrophages are sensitive to nutrient deprivation and thus the use of serum (and batch variation in serum supplements like FBS) further complicates commensurability and replicability of studies. These difficulties notwithstanding, cell lines can offer stability, reproducibility, and tractability. Moreover, they are amenable to genetic modification.

4.3 The contested role of CFTR in phagosome acidification: a case study to highlight interdisciplinarity contribution

CFTR’s contribution to lysosomal acidification through chloride channel activity has been controversial. There have been several contradictory studies in both macrophage and epithelial models. Experiments have used human and murine cells; epithelia and leukocytes; primary cells differentiated in vitro and cell lines (along with stark differences in measurement protocols and fluorescent dyes), making meaningful commensurability challenging, as many of the authors acknowledge. The methodology and findings of these studies are reviewed elsewhere with the authors concluding that lysosomal acidification appears to be CFTR independent. Quantification of acidification was based on ratiometric imaging using pH-sensitive and pH-insensitive dyes. Although the chloride ion flux data have been interpreted to support a role for CIC7 (a Cl−/H+ antiporter) in reacidification, by and large the consensus from these studies is that cation counterion flux is key to lysosome acidification, and CFTR does not play a direct role. This is also supported by a more recent study using surface-enhanced Raman spectroscopy-based nanosensors showing that phagosomal acidification was the same for CF and non-CF MDMs, although the authors argue that dynamics of bacterial phagocytosis may differ from that of nanosensors.

Could acidification of bacteria-containing phagosomes be influenced by CFTR dysfunction? Certain bacterial species induce delayed lysosome maturation, which alters acidification. Most of the aforementioned studies used dyes conjugated to zymosan or dextran for pH measurements and thus cannot answer this question. The question is worth raising given that the impetus for investigating CF macrophage acidification came from observed differences in bacterial survival in CF cells’ phagosomes, which could have been neatly explained by dysfunctional acidification. Nelson’s group highlighted several dysfunctional phagosomal attributes in Cfr−/− macrophages following P. aeruginosa ingestion, including deficient microbicidal activity. However, their zymosan ingestion-based phagosomal pH measurements, which suggested that acidification is CFTR dependent, could not be replicated by others. Studies using ratiometric fluorescent probes or P. aeruginosa strain PAO1 expressing FITC did not show differences in the acidification of phagosomes from CF and non-CF macrophages. In another study, ceramide accumulation in the secretory lysosomes differed in CF but not non-CF macrophages, compromising killing of P. aeruginosa. The hypothetic mechanism was differential activity of ceramide sphingomyelinase at different pH values, but whether CFTR directly contributed was not established.

The work on P. aeruginosa usefully illustrates the complexity of pathogen-specific dependencies for CFTR. Del Porto et al. demonstrated that bactericidal activity against P. aeruginosa was compromised in MDMs from PWCF. In epithelial cells, lipid rafts rich in CFTR and caveolin-1 are needed to internalize P. aeruginosa, and with the CFTR/lipopolysaccharide interaction being critical. P. aeruginosa CFTR inhibiting factor interferes with CFTR-mediated signaling in epithelial cells (reviewed in ref. 102), and there are known orthologues in several other bacterial genera, like Burkholderia. These findings could be leveraged to inform host–pathogen studies in CFTR-deficient macrophages. It remains to be determined why CF macrophages have differential phagolysosomal acidification in response to other bacterial species, and whether CFTR mutation is relevant. Lysosome maturation is delayed postphagocytosis of live but not heat-killed B. cenocepacia. In Cfr−/− macrophages, phagosomes containing live B. cenocepacia ultimately acidified and fused with lysosomes, but Cfr−/− cells (and macrophages treated with CFTRinh-172) remained alkaline even 8 h postinfection. These findings suggest that phagosome acidification based on zymosan conjugates might not recapitulate phagosome acidification with certain live bacterial species. Bacterial interference in phagosome maturation is varied (reviewed in ref. 104). L. monocytogenes exploits CFTR to escape from the phagosome into cytosol, and CFTR mutation appears to suppress this escape, possibly because chloride ion disequilibrium alters host or pathogen factors. Since CF macrophage lysosome maturation is delayed postinfections of B. cenocepacia, but not for Salmonella enterica, species-specific bacterial effectors may affect CF macrophage phagosomes. Serum starvation can profoundly influence macrophage response to infection, and thus the culturing conditions for cells (or cell lines) are methodologically relevant.

Setting aside the question of bacterial effectors, differential phagolysosomal acidification occurs in other contexts. Cantor et al. have shown that there are differences in acidification kinetics in differentially polarized human monocyte-derived macrophages, using a protocol based on ratiometric imaging of FITC fluorescence (Since FITC’s pKₐ of 6.3 limits its dynamic range, these data are not readily commensurable with the Oregon Green-based phagolysosomal pH data in CFTR-deficient cells.). These data suggest that “M1”- and “M2”-polarized macrophages have different phagosome maturation
(and acidification), with "M1" phagosomes being near neutral, whereas "M2"'s rapidly dropped to below pH 5. The authors hypothesize that proton consumption through ROS generation contributes to more alkaline conditions in "M1", along with "M1"'s delayed fusion with V-ATPase-containing lysosomes. Although they did not investigate whether bacterial ingestion influenced acidification dynamics, their work demonstrates that phagosomal acidification should not be considered monolithically. After all, phagocytosis of pathogens and efferocytosis are mechanistically similar, but these phagosomes perform different roles in the cell (reviewed in ref. 106).

Perhaps functional CFTR influences intracellular survival of certain bacteria, or its presence alters the release of bacterial effectors that influence phagolysosome maturation. Alternatively, CFTR may influence whether macrophages form "M1"- or "M2"-type phagosomes upon bacterial ingestion. Bacterial infection influences polarization (reviewed in ref. 107). Several polarized macrophage phenotypes are present in the CF lung. Tarique et al. have shown that dysfunctional CFTR affects cytokine responsiveness in "M2" but not "M1" polarization in human MDMs, but how polarization is coordinated in the infected CF lung requires further elucidation. CFTR activity is compromised in acidic environments, so if CFTR influences acidification in any way, its role is likely to be indirect.

5 | CONCLUDING REMARKS

CFTR mutations can extrinsically, indirectly or directly, influence alveolar macrophage function but there is no ideal model system for parsing these consequences. The consequences are further complicated by the type of macrophage (tissue or monocyte-derived, mouse or human) and its activation state. Furthermore, live engulfed bacteria can affect phagocytosis, which has implications for studies assessing the influence of CFTR mutation on phagosome dynamics. This complexity could partially account for observed discrepancies in findings. We have also highlighted how differences in models and methods could account for differences between studies. Contextualizing these studies within more recent work on the macrophage polarization spectrum and phagocyte–pathogen biology demonstrates the importance of revisiting previous work in the light of new research.

One means of delineating the primary and secondary effects of CFTR mutation in CF macrophages would be to create a set of stable monocyte cell lines expressing different forms of the protein: WT, ΔF508-CFTR, and other mutations. CRISPR/Cas9 mutation of CFTR has already been done in a promyelocytic cell line (HL-60), and the protocol could be adapted for THP-1 cells. Alternatively, modified induced pluripotent stem cells could be differentiated to evaluate the consequences of these mutations (reviewed in ref. 108), as this model has been leveraged to study macrophage infection. The multifaceted consequences of CFTR mutations for CF macrophages, and the influence of milieu and bacterial effectors, complicate interpretation of data. New models have their own limitations but, informed by recent work in macrophage ontology and polarization, could powerfully inform our understanding of immune cell infection in relation to CF.

AUTHORSHIP

K.B.T. wrote the manuscript. R.J.I. and M.A.V. edited the manuscript and contributed to the development of its structure and content.

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DISCLOSURES

The authors declare no conflicts of interest.

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