Chapter 6

Role of Neutrophils in Cystic Fibrosis Lung Disease

Massimo Conese, Stefano Castellani, Susanna D'Oria, Sante Di Gioia and Pasqualina Montemurro

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67798

Abstract

Cystic fibrosis (CF) is a genetic syndrome caused by mutations in the *CF Transmembrane Conductance Regulator (CFTR)* gene. In CF patients, chief morbidity and mortality are due to pulmonary manifestations. CFTR lack/dysfunction brings an altered ion flux through the airway epithelium and ablation of mucociliary clearance, which in turn ensues in colonization and infection by opportunistic bacterial pathogens and subsequent neutrophil-dominated inflammation. This response eventually leads to the damage of the lung tissue. A host of inflammatory mediators attract, activate, and reprogramme neutrophils to survive (avoiding apoptosis) and produce a wealth of proteases and radical oxygen species. The protease/antiprotease imbalance and oxidative stress have multiple downstream effects, including impaired mucus clearance, increased and self-perpetuating inflammation, and impaired immune responses, thus facilitating and fostering bacterial infections. On the other hand, CFTR lack or dysfunction is likely responsible for alterations in neutrophils concerning chemotaxis, phagocytosis, oxidative burst, degranulation, and neutrophil extracellular trap (NET) formation. A good opportunity to reveal new and non-invasive biomarkers of CF lung disease is the evaluation of circulating neutrophils. Indeed, neutrophil responses are now investigated as outcomes of the aetiological therapies in CF, such as hypertonic saline, antiproteases, CFTR correctors and potentiators.

Keywords: neutrophils, cystic fibrosis, proteases, NETs, oxidative burst, degranulation, chemotaxis

1. Introduction

Cystic fibrosis (CF) is a rare autosomal recessive disease whose average birth incidence rate is now 2.9/10,000 (i.e. 1/3500) in Europe [1] and prevalence is 100,000 globally [2]. Although CF is a chronic disease affecting many organs, the lung manifestations are still today the major cause of morbidity and mortality of these individuals and are the consequences of an ongoing inflammatory process, which stems either in the absence or in the presence of opportunistic bacterial infections. Lung inflammation and respiratory infections affect the prognosis of CF patients [3, 4]; indeed, they are associated with the progressive destructive changes that are responsible for most of the morbidity and mortality in CF [5]. Over 1000 microbial species (viruses, bacteria, mould, and fungi) have been found in the airways of CF patients [6]. *Staphylococcus aureus* and *Haemophilus influenzae* are the most common pathogens isolated from the sputum in the first decade of life, while *Pseudomonas aeruginosa* is found to dominate numerically in the second and third decades of life [7]. However, according to the Cystic Fibrosis Foundation Registry, *P. aeruginosa* is no longer the most common pathogen cultured in individuals with CF in the USA, and there has been an increase in the prevalence of *S. aureus* and *Stenotrophomonas maltophilia* [8].

Mutations in the 250-kb *CF transmembrane conductance regulator (CFTR)* gene are responsible for CF, but other environmental and genetic modifiers are thought to play a role in the phenotype of lung disease [9]. The CFTR gene encodes for a chloride channel that is expressed on the apical membrane of epithelial cells residing in organs with absorptive/secretory properties (**Figure 1(a)**). More than 2000 mutations have been identified at the moment (www.genet.sickkids.on.ca/cftr/), which can be classified in six classes (**Table 1**).

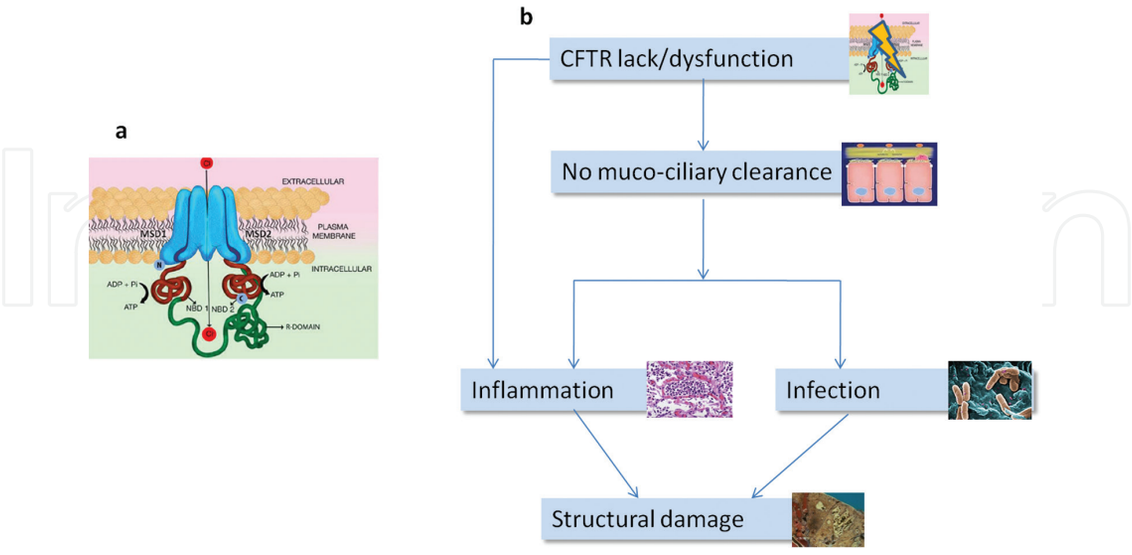

Figure 1. CFTR structure and CF lung disease. (a) A supposed CFTR structure when inserted in the plasma membrane. CFTR is composed of a two-membrane spanning domain (MSDs), each linked to nucleotide-binding domains (NBD1 and NBD2). Unique to CFTR, NBD1 is connected to the NBD2 by a regulatory domain (R). (b) The pathophysiological cascade of CF lung disease.

http://dx.doi.org/10.5772/67798

CFTR mutation class	Example	Effect on CFTR protein
Class I (stop mutation)	G542X	No expression
Class II (trafficking mutants)	F508del	Very low expression
Class III (low ATP binding)	G551D	Very low function
Class IV (low conductance)	R117H	Low function
Class V (low synthesis)	A455E	Low expression
Class VI (high turnover)	120del23	Low expression

Table 1. The six classes of CFTR mutations and their effects at the protein level.

The hallmark of the CF lung disease is a neutrophil-dominated inflammatory response; however, the link between CFTR mutations and the complex inflammatory milieu of the CF lungs is largely still poorly understood. The pathophysiological cascade which leads from the lack/dysfunction of CFTR chloride channel activity to the airway inflammation and infection, and eventually to tissue damage and destruction, is represented in **Figure 1(c)**. In the airways, the low excretion of chloride ions and bicarbonate, along with the hyperabsorption of sodium by the epithelial sodium channel (ENaC) and subsequently of water, contributes to the volume depletion from the periciliary liquid and its acidification. Thus, the loss of CFTR reduces the effectiveness of at least two defences—mucociliary transport and antimicrobial activity [10–12]. This eventually brings the colonization and infection by opportunistic bacterial pathogens and opposing inflammation, which, far from being resolutive, seems to be dysregulated, becoming chronic. In this context, polymorphonuclear leukocytes (PMNs) are thought to play a fundamental role on the onset and progression of lung tissue damage. Observational clinical studies made in the past have ascertained that infants with CF do show an airway inflammation prior to overt infection [13], indicating that the inflammatory response is dysregulated a priori before any bacterial infection and also suggesting that CFTR mutations are implicated in this abnormal response (**Figure 1(b)**). This is supported by the findings showing that free and bound airway neutrophil elastase is detected very early in CF infants and predicts the development of bronchiectasis later in life [14]. Furthermore, it has been found that CFTR is involved in some functions of innate immune cells that are diverted by CFTR mutations. We will discuss these evidences in Section 4.

2. Recruitment and activation of neutrophils in CF lungs

Neutrophils are the main cell types involved in the first-line defence of many organs, including the respiratory tract. However, they remain in the blood circulation unless they are recruited in the tissue. In the airways, they are marginated along the endothelium of capillaries and are ready to migrate first through the endothelium and then across the respiratory epithelium [15]. Marginated neutrophils are recruited rapidly to sites of inflammation, where their primary role is to kill invading bacteria and certain fungal species through phagocytosis and production of a range of oxygen species within the phagolysosomes and

also by preformed granular enzymes and proteins. Tissue inflammation results in the release of multiple inflammatory mediators and subsequent neutrophil priming. Priming results in a marked change in neutrophil shape and rheology that leads to their increased stiffness and retention within the capillary microvascular bed of the lung [16]. These mediators include an early wave comprised of cytokines, such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, and pathogen-associated molecular patterns (PAMPs) such as endotoxin, the ligand of Toll-like receptor (TLR)-4, followed by a late wave of chemoattractants and growth factors including IL-8, leukotriene B_4 (LTB_4), and granulocyte-macrophage colony-stimulating factor (GM-CSF).

In the airways, macrophages and epithelial cells are the main cell types which sense the pathogens and secrete a wealth of factors both inducing priming and full activation of neutrophils, as well as their extravasation. Upon exposure to bacteria, respiratory epithelial cells release reactive oxygen species (ROS) as an innate anti-infective mechanism, together with several anti-microbial peptides such as human beta-defensins (hBD-1/2/4) and cathelicidins (LL-37). The major pro-inflammatory cytokines (e.g. IL-1β, TNF-α, and IL-6) are initially expressed and released by surface epithelial cells of the conductive airways, which also release chemokines directed to recruit neutrophils (e.g. IL-8, GRO-α/γ) [17–22]. Besides the phagocytosis of inhaled pathogens and apoptotic cells, alveolar macrophages (AMs) play an important role in orchestrating innate immune defences by releasing inflammatory mediators. One of the important regulatory functions of AMs may be to dampen immune responses [23] so that dysfunction of AMs in CF could be related to increased inflammation. Both airway epithelial cells and AMs have been shown to be dysfunctional in CF, contributing to the onset and progression of chronic lung disease [24, 25]. This is reflected by the high burden of cytokines, chemokines, and other mediators found in the airway secretion of CF patients [26]. The CF airways contain massive amounts of cytokines and chemoattractants for neutrophils such as TNF-α, IL-1β, IL-6, IL-8, IL-17, IL-33, LTB_4, C5a, high-mobility group box 1 (HMGB1), proline-glycine-proline (PGP), and N-acetyl PGP [27–32]. For example, TNF-α enhances the neutrophil oxidative capacity, the granule release, and, with IL-1β, induces the priming of neutrophils [33]. The concentration of IL-8 in bronchoalveolar lavage (BAL) fluid is generally elevated and often correlates with the number of neutrophils in the airways [34]. It is thought that both extrinsic (e.g. microbes) and intrinsic (e.g. *CFTR* mutations) contribute to the alterations of the respiratory epithelium and AMs, ensuing in a hyper-inflammatory state and defect in immune defence.

Besides chemokines, such as CXCL8 (IL-8) [35], and lipid products, such as LTB_4 [36], other mediators have also been recently implicated in the recruitment of neutrophils into the CF airways. UDP-glucose levels are abnormally elevated in lung secretions from CF patients and from a mouse model of CF/chronic bronchitis, the βENaC-Tg transgenic mouse [37]. Moreover, instillation of UDP-glucose into mouse lung resulted in robust accumulation of neutrophils in BAL. Levels of damage-associated molecular patterns (DAMP), HMGB1, were found elevated in CF sputum and in BAL from βENaC-Tg transgenic mouse and shown to be chemotactic for neutrophils [38]. Upon activation, neutrophils secrete matrix metalloproteinases (MMP)-8 and -9, which perform an initial digestion of collagen from the macromolecule's size. Subsequently, neutrophils release prolyl endopeptidase (PE), a serine protease

http://dx.doi.org/10.5772/67798

previously only known to be a processor of neuropeptides. PE performs the final digestion of collagen to the tri-peptide PGP, which, upon binding to the same receptors as IL-8, CXCR1 and CXCR2, acts as a neutrophil chemoattractant and activator [39]. Thus, release of this peptidic collagen fragment provides a positive feedback mechanism that contributes to persistent neutrophilic inflammation in the CF lung [40]. During the adaptive immune response phase, neutrophils are recruited to the lung via the IL-23/IL-17A axis. Dendritic cells, activated by bacterial antigens, produce IL-23, which, in turn, binds to IL-23 receptor on T cells and stimulates them to produce IL-17A. This cytokine induces granulopoiesis via the induction of G-CSF and neutrophil recruitment via induction of chemotactic mediators such as IL-8. Both IL-23 and IL-17A have been found at high levels in the sputum from CF patients in acute exacerbation [41] and in stable condition [42], amplifying the extravasation and activation of neutrophils already induced by the innate immune response.

Once extravasated, neutrophils locate all along the CF bronchial tree and particularly in segmental bronchi, where they preferentially locate at the level of the lamina propria and in the lumen [43]. In this position, they are already activated and try to phagocytose microbes (e.g. *P. aeruginosa*) which have adapted to the hypoxic environment by producing an exopolysaccharide called alginate [44]. This frustrated phagocytosis leads to neutrophil hyperactivation which is more harmful than protective.

In the following subsections, we shall revise the main features of neutrophil physiology and how these are modified in the CF airway microenvironment (**Figure 2**).

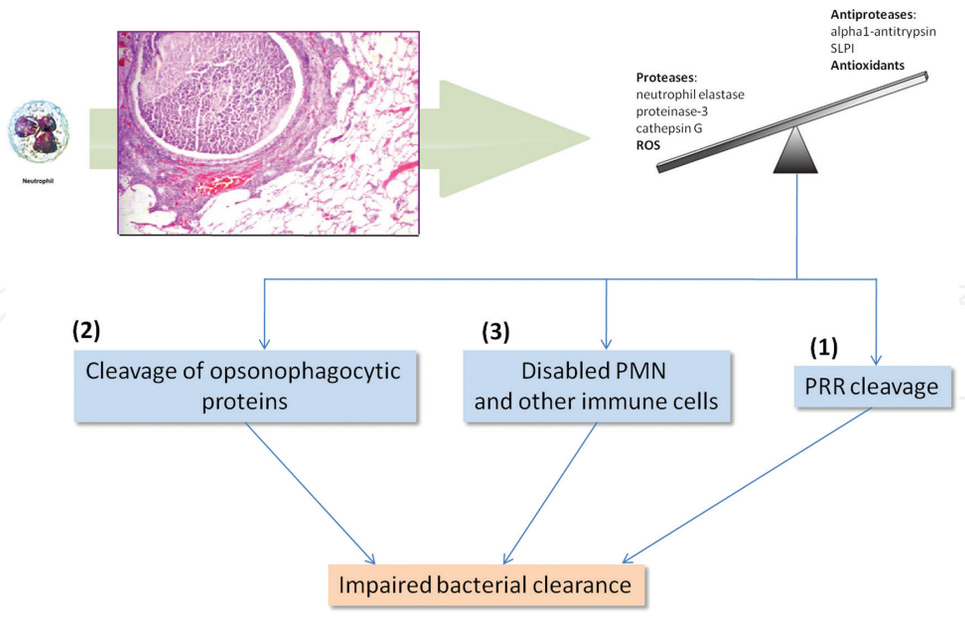

Figure 2. The role of neutrophils in maintaining inflammation and respiratory infections. The increased burden of neutrophils in the CF airways is the hallmark of the mucus plugs contained in the bronchioles lumen. From this location, PMNs secrete proteases and reactive oxygen species that overwhelm antiproteases and antioxidants, respectively, ensuing in various effects: (1) cleavage of pattern recognition receptors (PRR), (2) cleavage of opsonophagocytic receptors, and (3) disabling PMNs themselves and other immune cells. All these alterations facilitate bacterial infections.

2.1. Activation

Neutrophils recruited from the blood into the CF airway environment undergo marked functional changes. They express high levels of markers conventionally found on long-lived antigen-presenting cells (APCs), including class II molecules of the Major Histocompatibility Complex (MHC), the costimulatory molecule CD80, and the chemoattractant receptor of Th2 cells (CD294), all of which suggest profound reprogramming [45]. CF airway neutrophils present marked increases in glucose, amino acid, and phosphate transporters as compared with blood neutrophils [46], indicating that metabolic adaptation of neutrophils occurs as they are recruited to CF airways. However, these changes are not equal for all neutrophil subsets found in CF airways.

2.2. Apoptosis and resolution of inflammation

Apoptosis is a physiological process necessary for the clearance of inflammatory cells. Neutrophils are short-living cells which undergo apoptosis at the end of the inflammatory response, attracting macrophages which eventually ingest apoptotic cells in a process called *efferocytosis*. The removal of apoptotic cells is relevant to avoid secondary necrosis and the release of pro-inflammatory mediators that disrupt tissue homeostasis [47]. In CF, the lung disease is characterized by an alterated balance of pro- and anti-inflammatory mediators. Studies have shown that CF airways are deficient in several anti-inflammatory molecules, including IL-10 and lipoxin-A_4 (LXA_4) [48]. IL-10 inhibits the pro-inflammatory activities of cytokines, chemokines, and transcription factors and induces neutrophil apoptosis [49]. Not surprisingly, IL-10 knockout mice inoculated with *P. aeruginosa* that was embedded in agarose beads, in order to mimic a chronic Pseudomonas infection, had more drastic weight loss, greater neutrophil infiltration, larger inflammatory exudate of the lungs, and higher concentrations of pro-inflammatory cytokines in BAL compared to wild-type mice [50, 51]. Lipoxins are arachidonic metabolites generated by a lipoxigenase transcellular pathway involving neutrophils with epithelia, endothelia, monocytes, and platelets. In particular, LXA_4 acts to down-modulate acute inflammation by inhibiting neutrophil transmigration induced by LTB_4 and IL-8 and stimulating macrophage phagocytosis of apoptotic PMNs [52, 53]. LXA_4 levels have been found to be reduced in BAL fluid from CF patients, along with a significant suppression of LXA_4/neutrophil ratios [54, 55].

It seems for a number of reasons that neutrophils are resistant to apoptosis when they have extravasated into the CF airways; for example, it has been suggested that the oversecretion of cytokines might be responsible of apoptosis inhibition of airway neutrophils. The release of G-CSF or GM-CSF by epithelial cells, stimulated by *S. aureus* or *P. aeruginosa,* inhibits apoptosis of CF neutrophils [56], suggesting that increased expression of cytokines by CF airway cells not only induces neutrophil response but also enhances their survival, perpetuating an inflammatory process. Also, it has been described that PMNs from CF patients showed delayed constitutive and TNF-α or GM-CSF-induced phosphatidylinositol 3-kinase (PI3K)-dependent apoptosis [57]. CF airway neutrophils also undergo strong activation of CREB and mTOR's pro-survival pathways [58]. Moreover, it has been postulated that delayed phosphatidylserine externalization and mitochondria depolarization might be responsible for delayed

apoptosis of CF neutrophils [59]. In another study [60], neutrophils isolated from CF patients showed enhanced survival and upregulation of p21/Waf1, a cyclin-dependent kinase inhibitor and partner of proliferating cell nuclear antigen (PCNA). As also suggested by in vivo studies in p21(−/−) mice with *P. aeruginosa* lipopolysaccharide (LPS) challenge, p21/Waf1 is involved in the apoptotic response occurring during the resolution of inflammation [60]. In order to dissect the early phases of interaction between CF neutrophils and airway epithelial cells, it was found in co-culture experiments that a high number of non-apoptotic airway PMNs adhered to the CF airway epithelium in the presence of elevated levels of IL-6 and IL-8 [61], indicating another mechanism involved in enhanced inflammatory responses in airways of CF patients. Finally, independent of the sensitivity to apoptosis of CF cells, it has been shown that clearance of apoptotic cells by efferocytosis is defective in CF due to elastase-mediated degradation of macrophage phosphatidylserine receptors and that accumulation of such cells may contribute to ongoing inflammation [62].

2.3. Phagocytosis, oxidative burst, and degranulation

In cystic fibrosis, there is a tendency for bacterial colonization that may be due to dysfunction of phagocytosis. Airway neutrophils of CF patients showed a blunted phagocytic capacity and a reduced expression of cell surface recognition receptors, namely TLRs, leading to impaired bacterial killing [63]. Recent studies have demonstrated that CF neutrophils display an absence or dysfunction of CFTR at the level of phagolysosomes [64]. Likely due to this defect, CF neutrophils are impaired in chlorination of engulfed pathogens due to defective hypochlorous acid (HOCl) production [65].

One of the major mechanisms through which neutrophil phagocytosis kills pathogens entrapped inside the phagolysosomal vacuole is the release of high quantities of ROS [66]. The activation of the nicotinamide adenine dinucleotide phosphate oxidase (NOX2) in the neutrophils induces the production of superoxide anion and consequently the other ROS. Excessive activation of the neutrophil NOX2 results in exaggerated ROS release in the external surroundings, which increases the oxidative damage to tissues [67]. Furthermore, the inflammatory response can be enhanced by imbalance created by excessive release of pro-oxidative and impaired release of anti-oxidative molecules. While some authors have reported that ROS production by CF blood PMNs can be higher than or identical to that of healthy controls [68, 69], others have demonstrated that ROS generation varied according to the infecting pathogen [70] or to the method employed to detect respiratory burst activity [71]. For example, it has been shown that an extracellular polysaccharide of non-mucoid *P. aeruginosa* strain (Psl) inhibits opsonization and reduces ROS production by neutrophils [72]. Montemurro et al. [73] have established that CF blood neutrophils at the baseline are characterized by a higher ROS release as compared with controls PMNs and that the antibiotic therapy does not change this pattern. Nevertheless, ROS production is reduced in airway neutrophils compared to blood neutrophils that have different ROS oxidant activity profiles [74].

Neutrophils are identified by the presence of cytoplasmic primary (azurophilic), secondary (specific), and tertiary (gelatinase) granules as well as the secretory vesicles [75]. Focusing on granules, neutrophils abundantly express a cell-type specific set of neutrophil serine pro-

teases, namely cathepsin G, proteinase 3, and neutrophil elastase (NE), which are stored in the azurophilic granules. Also, myeloperoxidase (MPO) is stored in primary granules. Secondary granules are characterized by the presence of lactoferrin and cathelicidins, such as hCAP-18, while tertiary granules are enriched with gelatinase, an old name for MMPs, in particular MMP-9.

A dysregulated neutrophil degranulation capacity in CF has been shown. Neutrophils obtained from CF patients have an increased capacity to release primary granule contents such as MPO and NE [76]. In the airways, CF neutrophils undergo active exocytosis of primary granules, leading to a massive release of enzymes (e.g. NE, MPO) that damage the airway tissue and perpetuate inflammation [45]. On the other hand, Pohl et al. [77] have demonstrated that blood neutrophils obtained from CF patients can release less secondary (lactoferrin and hCAP-18) and tertiary (MMP-9) granule components compared with cells obtained from healthy individuals. The dysfunction of CFTR channel in neutrophils results in the deactivation of the GTP-binding protein Rab27a and in an impaired granule exocytosis. Interestingly, hypoxia, which is a hallmark of the CF bronchiolar environment, augmented neutrophil degranulation and possibly enhanced damage to respiratory airway cells in a hypoxia-inducible factor (HIF)-independent but PI3Kγ-dependent mechanism [78].

2.4. NETosis

The neutrophils are the first immune cells to achieve the site of injury or infection and are key players in microbial killing, because they are equipped with three main anti-bacterial weapons: phagocytosis, release of ROS, and granule release. Aside from these traditional mechanisms, neutrophils are also able, upon activation, to release DNA fibres decorated with anti-microbial proteins or neutrophil extracellular traps (NETs) to immobilize and to kill bacteria. NETs are composed of chromatin fibres coated with anti-microbial proteins, such as histones, NE, MPO, and α-defensins [79–82]. Moreover, NETs and their associated molecules are able to directly induce epithelial death, and massive NET formation has been reported in several pulmonary diseases including CF [83]. NETs are present in excess in CF sputum and the normal host defence functions become pathological [84]. CF patients with poor pulmonary functions presented higher levels of NETs compared to patients with mild lung disease, and the G protein-coupled receptor (GPCR) CXCR2 mediates NOX2-independent NET formation [85]. Histones and protease-coated DNA structures are released by neutrophils in response to respiratory bacteria (whole cells or virulence factors such as LPS, pilus, pyocyanin) or to inflammatory mediators (IL-8, interferon type I [IFN I], C5a) [86]. The exotoxin pyocyanin, a virulence factor of *P. aeruginosa*, enhances NET formation and requires NOX2 for its action [87]. Another pro-inflammatory cytokine, macrophage migration inhibitory factor (MIF), is able to stimulate NET release by promoting mitogen-activated protein kinase and thus exacerbating the inflammation [88]. Finally, *P. aeruginosa* triggers the release by lung epithelial cells of the eicosanoid hepoxilin A3, a neutrophil chemoattractant that induces NETosis [89]. Besides, MPO and NE expressed on NET fibres may induce the degradation of proteins of the connective tissue and of endothelial heparan sulphate proteoglycan at the site of inflammation [90, 91], contributing to lung pathology of CF patients. Furthermore,

there is growing evidence of NET escape by pathogens. NET release might be inhibited by down-regulation of inflammatory responses, or NET degradation might be induced by bacteria, including *H. influenzae*, by deoxyribonuclease [92]. Also *P. aeruginosa,* a very mutable bacterium, is able to acquire resistance to NET-mediated killing [93].

2.5. Cytokine production and immune regulation

As already pointed out above, there are many synergistic mediators which prime, activate, and attract neutrophils in the CF airways. Neutrophils also contribute to the CF airway environment by producing mediators that are pro-inflammatory and modify the function of other immune cells. CF airway neutrophils were found to increase TLR-4 expression on their surface and produce excessive IL-8 at the baseline, while failing to increase secretion in response to LPS or repress it in response to IL-10 [94]. Neutrophils in the sputum and blood of F508del CF subjects at the time of pulmonary exacerbation were found to express IL-17 RNA and protein as well as IL-23 receptor [95]. These investigators also showed a positive correlation between percent-IL-17-producing neutrophils and the total sputum activity of NE and MMP-9 and that IL-17 was absent following antibiotic treatment. IL-17 production by neutrophils may therefore contribute to tissue damage in the lungs of patients with CF.

Neutrophilic myeloid-derived suppressor cells (MDSC) are innate immune cells that are functionally characterized by their potential to suppress T- and natural killer (NK)-cell responses. Circulating neutrophilic MDSC have been found to be increased in patients with CF infected with *P. aeruginosa* as compared with age-matched healthy control subjects, their percentages correlating with lung function in those patients [96]. Further studies have revealed in an in vivo animal model of respiratory infection that *P. aeruginosa* triggers the recruitment of neutrophilic MDSC into the pulmonary compartment and enhances their suppressive capacity towards T cells [97]. Interestingly, they also showed that MDSC obtained from $Cftr^{-/-}$ mice were generated and recruited as in wild-type mice but were impaired in suppressing T-cell proliferation compared to their $Cftr^{+/+}$ counterpart cells. Thus, neutrophils contribute to the escape of *P. aeruginosa* from the adaptive immune response, and *CFTR* mutations may contribute to the bacterial infection.

3. Neutrophils and the effect of *CFTR* mutations

While bacteria and their products, cytokines and chemokines, are important triggers of neutrophil activation in CF airways, it is an emerging picture that a primary CFTR defect in cells of the innate immune system, including neutrophils, monocytes, and lymphocytes, contributes significantly to CF lung pathology [24]. Pharmacologic inhibition of CFTR and genetic mutation (F508del) in murine neutrophils activated the nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) and increased macrophage inflammatory protein-2 (MIP-2) and TNF-α production, as compared to non-inhibited and control neutrophils. Interestingly, under LPS challenge, neutrophil-depleted wild-type mice reconstituted with F508del neutrophils displayed a more severe lung inflammation in comparison with neutrophil-depleted

wild-type mice reconstituted with wild-type neutrophils [98]. Altogether, these data strongly indicate that the lack of functional CFTR could result in excessive NF-κB activation in neutrophils and therefore propagate a hyper-inflammatory response.

CF neutrophils have a reduced phagocytic activity [19, 99] and defects in the respiratory burst, attributed to disrupted chloride transport to the phagolysosome [65, 100–102]. While wild-type CFTR is transported to neutrophil phagosomes, the F508del protein is not targeted efficiently to these organelles [64], explaining why a correct chlorination of phagosomes in CF does not occur and hence the bactericidal defect. A still debated question is, however, the CFTR expression in neutrophils. Morris and colleagues, although found a defect in iC3b-mediated phagocytosis, did not detect CFTR in circulating and airway neutrophils by either immuno-labelling or a Western blot [99]. Others found that CFTR expression was limited or undetectable in neutrophils by flow cytometry and also that no role for CFTR in neutrophil-mediated phagocytosis was observed [103]. On the other hand, Zhou and colleagues found CFTR at the phagosome level, although a lentiviral-expressing system was used to achieve high protein levels. It might be that CFTR, expressed in hematopoietic stem/progenitor cells [104, 105], is down-regulated to low levels during neutrophil maturation, which is nevertheless sufficient for neutrophil phagocytic and killing activities. The lack/dysfunction of CFTR in the bone marrow may lead to an irreversible functional defect. In this context, it is worth mentioning that knocking out CFTR in the myeloid compartment of mice resulted in poor survival, increased inflammation with recruitment of neutrophils, elevated cytokine production, and inability to resolve infection upon challenge with *P. aeruginosa*-loaded agarose beads to mimic a chronic pulmonary infection [106].

4. Disabling neutrophils and other immune cells in CF airways

Excess neutrophil recruitment to the lungs results in the discharge of their destructive weapons not only directed to kill pathogens (see Section 2) but also to damage the lung and airway tissue. A large number of mediators produced by neutrophils, mainly oxidants and proteases, escape from neutrophils during cell death and phagocytosis. NE, a serine protease capable of digesting several substrates including structural proteins, is a direct mediator degrading elastin, which drives towards bronchiectasis and bronchomalacia [18]. Importantly, NE is associated with lung function decline [107]. In the lung, the main protease inhibitors, the prototypical α1-antitrypsin (α1-AT) secreted by hepatocytes and secretory leukoprotease inhibitor (SLPI) produced by the respiratory epithelium in bronchi and bronchioles, are designed to oppose free proteases and prevent their deleterious effects. These protease inhibitors are eventually overwhelmed by the protease burden in the lung and degraded by bacterial and human NE. It has been documented that despite normal antigenic concentrations of α1-AT and SLPI in children with CF, the majority of α1-AT and SLPI were complexed and/or degraded [108]. In addition, CF airways are exposed to ROS (O_2, H_2O_2, HOCl), derived mainly from the host's immune response. This oxidative stress exacerbates pulmonary deterioration and advances bronchiectasis in patients with CF [109]. Similar to the protease/antiprotease balance, antioxidants produced by airway epithelial cells (reduced glutathione [GSH] and thiocyanate [SCN^-]) are overwhelmed by the burden of oxidants in the CF airways. Activated

neutrophils are also capable of oxidizing glutathione by HOCl [110], contributing to GSH deficiency in CF airways. Hypochlorous acid is also able to oxidize calprotectin thereby inhibiting its ability to sequester manganese and zinc ions and consequently to limiting the growth of *S. aureus* and *P. aeruginosa* [111]. Moreover, it has been documented that ROS suppresses CFTR function [112] and that NE degrades CFTR [113], further worsening the CF pathophysiologic vicious cycle.

An important role in the degradation of structural proteins in CF airways is played synergistically by serine proteases, such as NE, proteinase 3, and cathepsin G [114]. In cystic fibrosis, neutrophil activation and degranulation result in the excessive release of proteinase 3, cathepsin G, and NE into the extracellular medium as active enzymes. Part of these serine proteases are exposed at the cell surface of immune cells and are important as modulators of the inflammatory response. Proteinase 3 has been shown to convert IL-8 to more potent, amino-terminally truncated forms [115], indicating that neutrophil proteases released in the inflamed lung convert IL-8 to enhance its chemotactic activity. Besides serine proteases, neutrophil-derived metalloproteinases, including MMP-8 and MMP-9, have also been involved in CF lung disease and chronic neutrophilic inflammation [116]. NE contributes to MMP-9 activation early in CF disease as the ratio of active/pro-enzyme MMP-9 was found to be higher in the presence of free neutrophil elastase activity, but not infection, and active MMP-9 was associated with progression of bronchiectasis [117]. In the context of CF, it is important to recall that neutrophil proteases increase mucin secretion in the airways and reduce ciliary beat frequency, contributing to the impairment in mucociliary clearance [118, 119], induce airway epithelial cells to produce neutrophil chemoattractants [120], and activate the apical epithelial sodium channel ENaC [121].

Unopposed serine proteases and metalloproteinases are responsible for degradation of soluble pattern recognition receptors (PRRs). NE proteolytic activity present in the CF sputum has been shown to degrade the prototypic long pentraxin PTX3, explaining the low levels of this PRR in CF airway secretions [122]. Released cathepsin G upon neutrophil activation degrades both components of the extracellular matrix and the surfactant protein A, a peptide that facilitates bacterial clearance by alveolar macrophages [123]. MMP-9 cleaves the pulmonary collectin surfactant protein D (SP-D) more efficiently than NE; this cleavage causes SP-D to no longer be able to agglutinate bacteria and affects SP-D's innate immune functions, as bacteria are no longer efficiently phagocytosed by alveolar macrophages in vitro [124].

High levels of neutrophil proteases further worsen the immune response by disabling immune cell functions. NE has several potential roles in disabling neutrophils including cleavage of opsonophagocytosis proteins, such as iC3b, complement receptor 1 (CR1) and C5a receptor [125–127], the chemokine receptor CXR1 [128], and TIM3 receptor leading to decreased galectin-9/TIM3 interactions [129]. Overall, the loss of these proteins is responsible for suboptimal local neutrophil priming and bacterial clearance. PMN-derived cathepsin G also thwarts efficient phagocytosis by macrophages, resulting in the cleavage of receptors and causing inefficient opsonization and impaired bacterial killing [18]. Cathepsin G cleavage of serum amyloid P component (SAP) renders it anti-opsonic, as evidenced by the increased binding of SAP to *P. aeruginosa* LPS and inhibition of phagocytosis in vitro [130], thus sequestering bacteria within the lung and potentially contributing to persistent infections in CF. Cathepsin G also interferes with removal of neutrophilic apoptotic bodies, since it mediates the degradation of

the macrophage phosphatidylserine receptors with failure to resolve inflammation because of the lack of efferocytosis [62, 131]. Also, NK cells and lymphocytes are disabled by neutrophil serine proteases. Cathepsin G determines a proteolytic cleavage of NKp46, a crucial activating receptor expressed on NK cells, an effect also determined by the CF sputum [132]. NE cleaves T-cell receptors CD2, CD4, CD8, and CD14, impairing monocyte activation and also blocking dendritic cell maturation and antigen presentation [133, 134].

5. Neutrophils as biomarkers of CF lung disease

The mainstays of CF lung disease management are commenced early in infancy and presently include chest physiotherapy to remove mucus plugs from the airways and antibiotic therapy to control infections [12]. Other therapeutic approaches such as hypertonic saline, finalized to increase mucociliary clearance, should be corroborated by efficacy data [135]. Recombinant human DNAse (Dornase alpha) is a strong mucolytic which improves lung function [136] but is given to CF infants only on indication due to its cost [137]. The recent breakthrough in CF, represented by the use of CFTR-correcting therapies, is a milestone in the clinical management of these patients. Ivacaftor (Kalydeco®, Vertex Pharmaceuticals, USA) is a CFTR potentiator given successfully to patients with class III gating mutations. This drug not only improves lung function and normalizes sweat chloride in children above 6 years of age [138], but its efficacy has also been proven in preschoolers [139].

At whatever age, the control of therapeutic efficacy of medications is granted by functional respiratory tests. However, more specific and sensitive assays are urgently needed to monitor the halt in the progression of lung disease, especially now that we entered the era of personalized medicine in CF [140]. Neutrophils, the main cell type involved in the onset and progression of CF lung disease, are clearly an interesting target in this context and are being evaluated for such a purpose. The best indication that neutrophils and their products are sensitive biomarkers of CF lung disease comes from the clinical data about NE. Sputum NE levels have been validated as the most predictive biomarker of lung decline and reduced survival [107, 141], being, however, of no utility in non-expectorating young children. Being easy to isolate from the peripheral blood, circulating neutrophils are more at hand to being studied. Conese et al. [142] analysed blood neutrophils by microarray gene expression in 10 CF patients, homozygous for the F508del mutation, given a course of parenteral antibiotics for an acute exacerbation, before and after therapy. mRNAs of three genes were found downregulated in CF patients before therapy and returned to 'healthy' levels after therapy: phorbol-12-myristate-13-acetate-induced protein 1 (*PMAIP1*), hydrogen voltage-gated channel 1 (*HVCN1*), and β-arrestin 1 (*ARRB1*). Recently, we validated neutrophil *HVCN1* mRNA as a biomarker following the treatment of seven CF patients, homozygous or heterozygous for class III mutations, with ivacaftor, confirming that its expression levels are lower as compared with healthy controls before therapy, while they are increased after CF patients were treated for 6 months (Guerra et al., submitted). Overall, these data strongly indicate that *HVCN1* mRNA level is a neutrophil biomarker sensitive to therapy. In another study [77], ivacaftor treatment resulted in normalized ion homeostasis and corrected Rab27a activation as well as degranulation in blood neutrophils

http://dx.doi.org/10.5772/67798

obtained from six CF patients with the genotype *F508del*/*G551D*. In line with these findings, extracellular Pseudomonas killing by CF neutrophils obtained from CF patients during treatment was significantly increased. Activated CD11b was investigated as a marker of neutrophil activation and whether it was downregulated by ivacaftor treatment in five patients with *F508del*/*G551D* and *G551D*/*N1303K* genotypes [143]. A cytofluorimetric assay showed that activated CD11b on PMNs was significantly higher at baseline in the CF patients compared to controls. However, after treatment, this marker was not significantly different from healthy controls, suggesting that ivacaftor treatment results in a decrease, towards normalization, of the activation status of blood neutrophils in vivo.

6. Conclusion

CF neutrophils display a number of abnormalities including increased survival, hyperactivation with increased protease and ROS production, defects in phagocytosis, and increased NET formation. Altogether, these neutrophil anomalies are derived from an intrinsic CFTR defect and are compounded by bacterial products. The unbalanced protease/antiprotease ratio in favour of proteases is responsible, together with excess oxidative stress, for the structural damage of CF airways and for secondary defects in an innate immune response as well as a skewed adaptive immune response. The neutrophil protease production is thus one of the main targets for therapy today to be explored. CF neutrophils can be also envisaged as a biomarker of therapies. The sensitivity to therapy of neutrophil genes is worthy of further investigation in the clinical setting. A higher number of patients are needed for studies aimed to consider neutrophils and their products as predictors of acute exacerbation and follow up.

Acknowledgements

This work was supported by the Italian Ministry of Health (Law 548/93). Stefano Castellani is a researcher funded by Intervento Cofinanziato dal Fondo di Sviluppo e Coesione 2007–2013—APQ Ricerca Regione Puglia 'Programma regionale a sostegno della specializzazione intelligente e delle sostenibilità sociale ed ambientali—Future In Research'.

Author details

Massimo Conese[1]*, Stefano Castellani[1], Susanna D'Oria[2], Sante Di Gioia[1] and Pasqualina Montemurro[2]

*Address all correspondence to: massimo.conese@unifg.it

1 Department of Medical and Surgical Sciences, University of Foggia, Foggia, Italy

2 Section of General Pathology, Department of Biomedical Sciences and Human Oncology, University of Bari, Bari, Italy

References

[1] Scotet V, Dugueperoux I, Saliou P, Rault G, Roussey M, et al. Evidence for decline in the incidence of cystic fibrosis: a 35-year observational study in Brittany, France. Orphanet J Rare Dis. 2012; **7**: 14.

[2] Davies JC, Ebdon AM, Orchard C. Recent advances in the management of cystic fibrosis. Arch Dis Child. 2014; **99**: 1033–6.

[3] Dakin CJ, Numa AH, Wang H, Morton JR, Vertzyas CC, et al. Inflammation, infection, and pulmonary function in infants and young children with cystic fibrosis. Am J Respir Crit Care Med. 2002; **165**: 904–10.

[4] Pereira LC, Moreira EA, Bennemann GD, Moreno YM, Buss Zda S, et al. Influence of inflammatory response, infection, and pulmonary function in cystic fibrosis. Life Sci. 2014; **109**: 30–6.

[5] Pillarisetti N, Williamson E, Linnane B, Skoric B, Robertson CF, et al. Infection, inflammation, and lung function decline in infants with cystic fibrosis. Am J Respir Crit Care Med. 2011; **184**: 75–81.

[6] Bhagirath AY, Li Y, Somayajula D, Dadashi M, Badr S, et al. Cystic fibrosis lung environment and *Pseudomonas aeruginosa* infection. BMC Pulm Med. 2016; **16**: 174.

[7] Coutinho HD, Falcao-Silva VS, Goncalves GF. Pulmonary bacterial pathogens in cystic fibrosis patients and antibiotic therapy: a tool for the health workers. Int Arch Med. 2008; **1**: 24.

[8] Cystic Fibrosis Foundation. Patient registry 2015 annual report. Bethesda, MD: Cystic Fibrosis Foundation; 2015.

[9] Cutting GR. Cystic fibrosis genetics: from molecular understanding to clinical application. Nat Rev Genet. 2015; **16**: 45–56.

[10] Boucher RC. Airway surface dehydration in cystic fibrosis: pathogenesis and therapy. Annu Rev Med. 2007; **58**: 157–70.

[11] Stoltz DA, Meyerholz DK, Welsh MJ. Origins of cystic fibrosis lung disease. N Engl J Med. 2015; **372**: 351–62.

[12] Proesmans M. Best practices in the treatment of early cystic fibrosis lung disease. Ther Adv Respir Dis. 2017; **11**: 97–104.

[13] Armstrong DS, Grimwood K, Carlin JB, Carzino R, Gutièrrez JP, et al. Lower airway inflammation in infants and young children with cystic fibrosis. Am J Respir Crit Care Med. 1997; **156**: 1197–204.

[14] Sly PD, Gangell CL, Chen L, Ware RS, Ranganathan S, et al. Risk factors for bronchiectasis in children with cystic fibrosis. N Engl J Med. 2013; **368**: 1963–70.

[15] Downey DG, Bell SC, Elborn JS. Neutrophils in cystic fibrosis. Thorax. 2009; **64**: 81–8.

[16] Summers C, Rankin SM, Condliffe AM, Singh N, Peters AM, et al. Neutrophil kinetics in health and disease. Trends Immunol. 2010; **31**: 318–24.

[17] Conese M, Copreni E, Di Gioia S, De Rinaldis P, Fumarulo R. Neutrophil recruitment and airway epithelial cell involvement in chronic cystic fibrosis lung disease. J Cyst Fibros. 2003; **2**: 129–35.

[18] Cohen TS, Prince A. Cystic fibrosis: a mucosal immunodeficiency syndrome. Nat Med. 2012; **18**: 509–19.

[19] Cohen-Cymberknoh M, Kerem E, Ferkol T, Elizur A. Airway inflammation in cystic fibrosis: molecular mechanisms and clinical implications. Thorax. 2013; **68**: 1157–62.

[20] Bezzerri V. Proinflammatory signal transduction in epithelial cells: the model of cystic fibrosis lung disease. Ph.D. thesis, University of Verona, Verona, 2012.

[21] Jacquot J, Tabary O, Le Rouzic P, Clement A. Airway epithelial cell inflammatory signalling in cystic fibrosis. Int J Biochem Cell Biol. 2008; **40**: 1703–15.

[22] Holtzman MJ, Byers DE, Alexander-Brett J, Wang X. The role of airway epithelial cells and innate immune cells in chronic respiratory disease. Nat Rev Immunol. 2014; **14**: 686–98.

[23] Takabayshi K, Corr M, Hayashi T, Redecke V, Beck L, et al. Induction of a homeostatic circuit in lung tissue by microbial compounds. Immunity. 2006; **24**: 475–87.

[24] Ratner D, Mueller C. Immune responses in cystic fibrosis: are they intrinsically defective? Am J Respir Cell Mol Biol. 2012; **46**: 715–22.

[25] Xu Y, Worgall S. Immune dysfunction in cystic fibrosis. In: Sriramulu D, editor. Cystic fibrosis—renewed hopes through research. Rijeka: InTech; 2012, pp. 273–88.

[26] Sagel SD, Chmiel JF, Konstan MW. Sputum biomarkers of inflammation in cystic fibrosis lung disease. Proc Am Thorac Soc. 2007; **4**: 406–17.

[27] Elizur A, Cannon CL, Ferkol TW. Airway inflammation in cystic fibrosis. Chest. 2008; **133**: 489–95.

[28] Dhooghe B, Noel S, Huaux F, Leal T. Lung inflammation in cystic fibrosis: pathogenesis and novel therapies. Clin Biochem. 2014; **47**: 539–46.

[29] Di Gioia S, Sardo C, Castellani S, Porsio B, Belgiovine G, et al. From genesis to revelation: the role of inflammatory mediators in chronic respiratory diseases and their control by nucleic acid-based drugs. Curr Drug Deliv. 2017; 14: 234–52.

[30] Roussel L, Farias R, Rousseau S. IL-33 is expressed in epithelia from patients with cystic fibrosis and potentiates neutrophil recruitment. J Allergy Clin Immunol. 2013; **131**: 913–6.

[31] Sass LA, Hair PS, Perkins AM, Shah TA, Krishna NK, et al. Complement effectors of inflammation in cystic fibrosis lung fluid correlate with clinical measures of disease. PLoS One. 2015; **10**: e0144723.

[32] Gaggar A, Jackson PL, Noerager BD, O'Reilly PJ, McQuaid DB, et al. A novel proteolytic cascade generates an extracellular matrix-derived chemoattractant in chronic neutrophilic inflammation. J Immunol. 2008; **180**: 5662–9.

[33] Hostoffer RW, Krukovets I, Berger M. Enhancement by tumor necrosis factor-alpha of Fc alpha receptor expression and IgA-mediated superoxide generation and killing of Pseudomonas *aeruginosa* by polymorphonuclear leukocytes. J Infect Dis. 1994; **170**: 82–7.

[34] Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, et al. Early pulmonary inflammation in infants with cystic fibrosis. Am J Respir Crit Care Med. 1995; **151**: 1075–82.

[35] Bodas M, Vij N. The NF-kappaB signaling in cystic fibrosis lung disease: pathophysiology and therapeutic potential. Discov Med. 2010 **9**: 346–56.

[36] Ringholz FC, Buchanan PJ, Clarke DT, Millar RG, McDermott M, et al. Reduced 15-lipoxygenase 2 and lipoxin A4/leukotriene B4 ratio in children with cystic fibrosis. Eur Respir J. 2014; **44**: 394–404.

[37] Sesma JI, Weitzer CD, Livraghi-Butrico A, Dang H, Donaldson S, et al. UDP-glucose promotes neutrophil recruitment in the lung. Purinergic Signal. 2016; **12**: 627–35.

[38] Rowe SM, Jackson PL, Liu G, Hardison M, Livraghi A, et al. Potential role of high-mobility group box 1 in cystic fibrosis airway disease. Am J Respir Crit Care Med. 2008; **178**: 822–31.

[39] Guess TA, Gaggar A, Hardison MT. New frontiers in the diagnosis and treatment of chronic neutrophilic lung diseases. In: Kayembe J-M, editor. Oncogenesis, inflammatory and parasitic tropical diseases of the lung. Rijeka: InTech; 2013, pp. 1–24.

[40] Gaggar A, Rowe SM, Matthew H, Blalock JE. Proline-glycine-proline (PGP) and high mobility group box protein-1 (HMGB1): potential mediators of cystic fibrosis airway inflammation. Open Respir Med J. 2010; **4**: 32–8.

[41] McAllister F, Henry A, Kreindler JL, Dubin PJ, Ulrich L, et al. Role of IL-17A, IL-17F, and the IL-17 receptor in regulating growth-related oncogene-alpha and granulocyte colony-stimulating factor in bronchial epithelium: implications for airway inflammation in cystic fibrosis. J Immunol. 2005; **175**: 404–12.

[42] Decraene A, Willems-Widyastuti A, Kasran A, De Boeck K, Bullens DM, et al. Elevated expression of both mRNA and protein levels of IL-17A in sputum of stable cystic fibrosis patients. Respir Res. 2010; **11**: 177.

[43] Hubeau C, Lorenzato M, Couetil JP, Hubert D, Dusser D, et al. Quantitative analysis of inflammatory cells infiltrating the cystic fibrosis airway mucosa. Clin Exp Immunol. 2001; **124**: 69–76.

[44] Cantin AM, Hartl D, Konstan MW, Chmiel JF. Inflammation in cystic fibrosis lung disease: pathogenesis and therapy. J Cyst Fibros. 2015; **14**: 419–30.

[45] Tirouvanziam R, Gernez Y, Conrad CK, Moss RB, Schrijver I, et al. Profound functional and signaling changes in viable inflammatory neutrophils homing to cystic fibrosis airways. Proc Natl Acad Sci USA. 2008; **105**: 4335–9.

[46] Laval J, Touhami J, Herzenberg LA, Conrad C, Taylor N, et al. Metabolic adaptation of neutrophils in cystic fibrosis airways involves distinct shifts in nutrient transporter expression. J Immunol. 2013; **190**: 6043–50.

[47] McCubbrey AL, Curtis JL. Efferocytosis and lung disease. Chest. 2013; **143**: 1750–7.

[48] Bonfield TL, Konstan MW, Berger M. Altered respiratory epithelial cell cytokine production in cystic fibrosis. J Allergy Clin Immunol. 1999; **104**: 72–8.

[49] Cox G. IL-10 enhances resolution of pulmonary inflammation in vivo by promoting apoptosis of neutrophils. Am J Physiol. 1996; **271**: L566–71.

[50] Chmiel JF, Konstan MW, Knesebeck JE, Hilliard JB, Bonfield TL, et al. IL-10 attenuates excessive inflammation in chronic Pseudomonas infection in mice. Am J Respir Crit Care Med. 1999; **160**: 2040–7.

[51] Chmiel JF, Konstan MW, Saadane A, Krenicky JE, Lester Kirchner H, et al. Prolonged inflammatory response to acute Pseudomonas challenge in interleukin-10 knockout mice. Am J Respir Crit Care Med. 2002; **165**: 1176–81.

[52] Karp CL, Flick LM, Yang R, Uddin J, Petasis NA. Cystic fibrosis and lipoxins. Prostaglandins Leukot Essent Fatty Acids. 2005; **73**: 263–70.

[53] Higgins G, Ringholz F, Buchanan P, McNally P, Urbach V. Physiological impact of abnormal lipoxin A(4) production on cystic fibrosis airway epithelium and therapeutic potential. Biomed Res Int. 2015; **2015**: 781087.

[54] Karp CL, Flick LM, Park KW, Softic S, Greer TM, et al. Defective lipoxin-mediated anti-inflammatory activity in the cystic fibrosis airway. Nat Immunol. 2004; **5**: 388–92.

[55] Starosta V, Ratjen F, Rietschel E, Paul K, Griese M. Anti-inflammatory cytokines in cystic fibrosis lung disease. Eur Respir J. 2006; **28**: 581–7.

[56] Saba S, Soong G, Greenberg S, Prince A. Bacterial stimulation of epithelial G-CSF and GM-CSF expression promotes PMN survival in CF airways. Am J Respir Cell Mol Biol. 2002; **27**: 561–7.

[57] McKeon DJ, Condliffe AM, Cowburn AS, Cadwallader KC, Farahi N, et al. Prolonged survival of neutrophils from patients with Delta F508 CFTR mutations. Thorax. 2008; **63**: 660–1.

[58] Makam M, Diaz D, Laval J, Gernez Y, Conrad CK, et al. Activation of critical, host-induced, metabolic and stress pathways marks neutrophil entry into cystic fibrosis lungs. Proc Natl Acad Sci USA. 2009; **106**: 5779–83.

[59] Moriceau S, Kantari C, Mocek J, Davezac N, Gabillet J, et al. Coronin-1 is associated with neutrophil survival and is cleaved during apoptosis: potential implication in neutrophils from cystic fibrosis patients. J Immunol. 2009; **182**: 7254–63.

[60] Martin C, Ohayon D, Alkan M, Mocek J, Pederzoli-Ribeil M, et al. Neutrophil-expressed p21/waf1 favors inflammation resolution in *Pseudomonas aeruginosa* infection. Am J Respir Cell Mol Biol. 2016; **54**: 740–50.

[61] Tabary O, Corvol H, Boncoeur E, Chadelat K, Fitting C, et al. Adherence of airway neutrophils and inflammatory response are increased in CF airway epithelial cell-neutrophil interactions. Am J Physiol Lung Cell Mol Physiol. 2006; **290**: L588–96.

[62] Vandivier RW, Fadok VA, Hoffmann PR, Bratton DL, Penvari C, et al. Elastase-mediated phosphatidylserine receptor cleavage impairs apoptotic cell clearance in cystic fibrosis and bronchiectasis. J Clin Invest. 2002; **109**: 661–70.

[63] Donnelly LE, Barnes PJ. Defective phagocytosis in airways disease. Chest. 2012; **141**: 1055–62.

[64] Zhou Y, Song K, Painter RG, Aiken M, Reiser J, et al. Cystic fibrosis transmembrane conductance regulator recruitment to phagosomes in neutrophils. J Innate Immun. 2013; **5**: 219–30.

[65] Painter RG, Valentine VG, Lanson NA, Jr., Leidal K, Zhang Q, et al. CFTR Expression in human neutrophils and the phagolysosomal chlorination defect in cystic fibrosis. Biochemistry. 2006; **45**: 10260–9.

[66] Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: from mechanisms to disease. Annu Rev Immunol. 2012; **30**: 459–89.

[67] El-Benna J, Hurtado-Nedelec M, Marzaioli V, Marie JC, Gougerot-Pocidalo MA, et al. Priming of the neutrophil respiratory burst: role in host defense and inflammation. Immunol Rev. 2016; **273**: 180–93.

[68] Vaisman N, Kerasin E, Hahn T, Trifon S, Voet H, et al. Increased neutrophil chemiluminescence production in patients with cystic fibrosis. Metabolism. 1994; **43**: 719–22.

[69] McKeon DJ, Cadwallader KA, Idris S, Cowburn AS, Pasteur MC, et al. Cystic fibrosis neutrophils have normal intrinsic reactive oxygen species generation. Eur Respir J. 2010; **35**: 1264–72.

[70] Fruhwirth M, Ruedl C, Ellemunter H, Bock G, Wolf H. Flow-cytometric evaluation of oxidative burst in phagocytic cells of children with cystic fibrosis. Int Arch Allergy Immunol. 1998; **117**: 270–5.

[71] Witko-Sarsat V, Allen RC, Paulais M, Nguyen AT, Bessou G, et al. Disturbed myeloperoxidase-dependent activity of neutrophils in cystic fibrosis homozygotes and heterozygotes, and its correction by amiloride. J Immunol. 1996; **157**: 2728–35.

[72] Mishra M, Byrd MS, Sergeant S, Azad AK, Parsek MR, et al. *Pseudomonas aeruginosa* Psl polysaccharide reduces neutrophil phagocytosis and the oxidative response by limiting complement-mediated opsonization. Cell Microbiol. 2012; **14**: 95–106.

[73] Montemurro P, Mariggio MA, Barbuti G, Cassano A, Vincenti A, et al. Increase in interleukin-8 production from circulating neutrophils upon antibiotic therapy in cystic fibrosis patients. J Cyst Fibros. 2012; **11**: 518–24.

[74] Houston N, Stewart N, Smith DS, Bell SC, Champion AC, et al. Sputum neutrophils in cystic fibrosis patients display a reduced respiratory burst. J Cyst Fibros. 2013; **12**: 352–62.

[75] Pham CT. Neutrophil serine proteases: specific regulators of inflammation. Nat Rev Immunol. 2006; **6**: 541–50.

[76] Koller DY, Urbanek R, Gotz M. Increased degranulation of eosinophil and neutrophil granulocytes in cystic fibrosis. Am J Respir Crit Care Med. 1995; **152**: 629–33.

[77] Pohl K, Hayes E, Keenan J, Henry M, Meleady P, et al. A neutrophil intrinsic impairment affecting Rab27a and degranulation in cystic fibrosis is corrected by CFTR potentiator therapy. Blood. 2014; **124**: 999–1009.

[78] Hoenderdos K, Lodge KM, Hirst RA, Chen C, Palazzo SG, et al. Hypoxia upregulates neutrophil degranulation and potential for tissue injury. Thorax. 2016; **71**: 1030–8.

[79] Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, et al. Neutrophil extracellular traps kill bacteria. Science. 2004; **303**: 1532–5.

[80] Urban CF, Reichard U, Brinkmann V, Zychlinsky A. Neutrophil extracellular traps capture and kill *Candida albicans* yeast and hyphal forms. Cell Microbiol. 2006; **8**: 668–76.

[81] McCormick A, Heesemann L, Wagener J, Marcos V, Hartl D, et al. NETs formed by human neutrophils inhibit growth of the pathogenic mold *Aspergillus fumigatus*. Microbes Infect. 2010; **12**: 928–36.

[82] Jenne CN, Wong CH, Zemp FJ, McDonald B, Rahman MM, et al. Neutrophils recruited to sites of infection protect from virus challenge by releasing neutrophil extracellular traps. Cell Host Microbe. 2013; **13**: 169–80.

[83] Saffarzadeh M, Juenemann C, Queisser MA, Lochnit G, Barreto G, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. PLoS One. 2012; **7**: e32366.

[84] Yoo DG, Floyd M, Winn M, Moskowitz SM, Rada B. NET formation induced by *Pseudomonas aeruginosa* cystic fibrosis isolates measured as release of myeloperoxidase-DNA and neutrophil elastase-DNA complexes. Immunol Lett. 2014; **160**: 186–94.

[85] Marcos V, Zhou Z, Yildirim AO, Bohla A, Hector A, et al. CXCR2 mediates NADPH oxidase-independent neutrophil extracellular trap formation in cystic fibrosis airway inflammation. Nat Med. 2010; **16**: 1018–23.

[86] Wartha F, Beiter K, Normark S, Henriques-Normark B. Neutrophil extracellular traps: casting the NET over pathogenesis. Curr Opin Microbiol. 2007; **10**: 52–6.

[87] Rada B, Jendrysik MA, Pang L, Hayes CP, Yoo DG, et al. Pyocyanin-enhanced neutrophil extracellular trap formation requires the NADPH oxidase. PLoS One. 2013; **8**: e54205.

[88] Dwyer M, Shan Q, D'Ortona S, Maurer R, Mitchell R, et al. Cystic fibrosis sputum DNA has NETosis characteristics and neutrophil extracellular trap release is regulated by macrophage migration-inhibitory factor. J Innate Immun. 2014; **6**: 765–79.

[89] Douda DN, Grasemann H, Pace-Asciak C, Palaniyar N. A lipid mediator hepoxilin A3 is a natural inducer of neutrophil extracellular traps in human neutrophils. Mediators Inflamm. 2015; **2015**: 520871.

[90] Logters T, Margraf S, Altrichter J, Cinatl J, Mitzner S, et al. The clinical value of neutrophil extracellular traps. Med Microbiol Immunol. 2009; **198**: 211–9.

[91] Fujie K, Shinguh Y, Inamura N, Yasumitsu R, Okamoto M, et al. Release of neutrophil elastase and its role in tissue injury in acute inflammation: effect of the elastase inhibitor, FR134043. Eur J Pharmacol. 1999; **374**: 117–25.

[92] Storisteanu DM, Pocock JM, Cowburn AS, Juss JK, Nadesalingam A, et al. Evasion of neutrophil extracellular traps by respiratory pathogens. Am J Respir Cell Mol Biol. 2016 Nov 17. [Epub ahead of print].

[93] Young RL, Malcolm KC, Kret JE, Caceres SM, Poch KR, et al. Neutrophil extracellular trap (NET)-mediated killing of *Pseudomonas aeruginosa*: evidence of acquired resistance within the CF airway, independent of CFTR. PLoS One. 2011; **6**: e23637.

[94] Petit-Bertron AF, Tabary O, Corvol H, Jacquot J, Clement A, et al. Circulating and airway neutrophils in cystic fibrosis display different TLR expression and responsiveness to interleukin-10. Cytokine. 2008; **41**: 54–60.

[95] Taylor PR, Bonfield TL, Chmiel JF, Pearlman E. Neutrophils from F508del cystic fibrosis patients produce IL-17A and express IL-23-dependent IL-17RC. Clin Immunol. 2016; **170**: 53–60.

[96] Rieber N, Brand A, Hector A, Graepler-Mainka U, Ost M, et al. Flagellin induces myeloid-derived suppressor cells: implications for *Pseudomonas aeruginosa* infection in cystic fibrosis lung disease. J Immunol. 2013; **190**: 1276–84.

[97] Oz HH, Zhou B, Voss P, Carevic M, Schroth C, et al. *Pseudomonas aeruginosa* airway infection recruits and modulates neutrophilic myeloid-derived suppressor cells. Front Cell Infect Microbiol. 2016; **6**: 167.

[98] Su X, Looney MR, Su HE, Lee JW, Song Y, et al. Role of CFTR expressed by neutrophils in modulating acute lung inflammation and injury in mice. Inflamm Res. 2011; **60**: 619–32.

[99] Morris MR, Doull IJ, Dewitt S, Hallett MB. Reduced iC3b-mediated phagocytotic capacity of pulmonary neutrophils in cystic fibrosis. Clin Exp Immunol. 2005; **142**: 68–75.

[100] Painter RG, Bonvillain RW, Valentine VG, Lombard GA, LaPlace SG, et al. The role of chloride anion and CFTR in killing of *Pseudomonas aeruginosa* by normal and CF neutrophils. J Leukoc Biol. 2008; **83**: 1345–53.

[101] Painter RG, Marrero L, Lombard GA, Valentine VG, Nauseef WM, et al. CFTR-mediated halide transport in phagosomes of human neutrophils. J Leukoc Biol. 2010; **87**: 933–42.

[102] Bonvillain RW, Painter RG, Adams DE, Viswanathan A, Lanson NA, Jr., et al. RNA interference against CFTR affects HL60-derived neutrophil microbicidal function. Free Radic Biol Med. 2010; **49**: 1872–80.

[103] Van de Weert-van Leeuwen PB, Van Meegen MA, Speirs JJ, Pals DJ, Rooijakkers SH, et al. Optimal complement-mediated phagocytosis of *Pseudomonas aeruginosa* by monocytes is cystic fibrosis transmembrane conductance regulator-dependent. Am J Respir Cell Mol Biol. 2013; **49**: 463–70.

[104] Piro D, Rejman J, Conese M. Stem cell therapy for cystic fibrosis: current status and future prospects. Expert Rev Respir Med. 2008; **2**: 365–80.

[105] Trotta T, Guerra L, Piro D, d'Apolito M, Piccoli C, et al. Stimulation of beta2-adrenergic receptor increases CFTR function and decreases ATP levels in murine hematopoietic stem/progenitor cells. J Cyst Fibros. 2015; **14**: 26–33.

[106] Bonfield TL, Hodges CA, Cotton CU, Drumm ML. Absence of the cystic fibrosis transmembrane regulator (Cftr) from myeloid-derived cells slows resolution of inflammation and infection. J Leukoc Biol. 2012; **92**: 1111–22.

[107] Sagel SD, Wagner BD, Anthony MM, Emmett P, Zemanick ET. Sputum biomarkers of inflammation and lung function decline in children with cystic fibrosis. Am J Respir Crit Care Med. 2012; **186**: 857–65.

[108] Birrer P, McElvaney NG, Rudeberg A, Sommer CW, Liechti-Gallati S, et al. Protease-antiprotease imbalance in the lungs of children with cystic fibrosis. Am J Respir Crit Care Med. 1994; **150**: 207–13.

[109] Galli F, Battistoni A, Gambari R, Pompella A, Bragonzi A, et al. Oxidative stress and antioxidant therapy in cystic fibrosis. Biochim Biophys Acta. 2012; **1822**: 690–713.

[110] Kettle AJ, Turner R, Gangell CL, Harwood DT, Khalilova IS, et al. Oxidation contributes to low glutathione in the airways of children with cystic fibrosis. Eur Respir J. 2014; **44**: 122–9.

[111] Magon NJ, Turner R, Gearry RB, Hampton MB, Sly PD, et al. Oxidation of calprotectin by hypochlorous acid prevents chelation of essential metal ions and allows bacterial growth: relevance to infections in cystic fibrosis. Free Radic Biol Med. 2015; **86**: 133–44.

[112] Cantin AM, Bilodeau G, Ouellet C, Liao J, Hanrahan JW. Oxidant stress suppresses CFTR expression. Am J Physiol Cell Physiol. 2006; **290**: C262–70.

[113] Le Gars M, Descamps D, Roussel D, Saussereau E, Guillot L, et al. Neutrophil elastase degrades cystic fibrosis transmembrane conductance regulator via calpains and disables channel function in vitro and in vivo. Am J Respir Crit Care Med. 2013; **187**: 170–9.

[114] Twigg MS, Brockbank S, Lowry P, FitzGerald SP, Taggart C, et al. The role of serine proteases and antiproteases in the cystic fibrosis lung. Mediators Inflamm. 2015; **2015**: 293053.

[115] Padrines M, Wolf M, Walz A, Baggiolini M. Interleukin-8 processing by neutrophil elastase, cathepsin G and proteinase-3. FEBS Lett. 1994; **352**: 231–5.

[116] Gaggar A, Hector A, Bratcher PE, Mall MA, Griese M, et al. The role of matrix metalloproteases in cystic fibrosis lung disease. Eur Respir J. 2011; **38**: 721–7.

[117] Garratt LW, Sutanto EN, Ling KM, Looi K, Iosifidis T, et al. Matrix metalloproteinase activation by free neutrophil elastase contributes to bronchiectasis progression in early cystic fibrosis. Eur Respir J. 2015; **46**: 384–94.

[118] Amitani R, Wilson R, Rutman A, Read R, Ward C, et al. Effects of human neutrophil elastase and *Pseudomonas aeruginosa* proteinases on human respiratory epithelium. Am J Respir Cell Mol Biol. 1991; **4**: 26–32.

[119] Sommerhoff CP, Nadel JA, Basbaum CB, Caughey GH. Neutrophil elastase and cathepsin G stimulate secretion from cultured bovine airway gland serous cells. J Clin Invest. 1990; **85**: 682–9.

[120] Nakamura H, Yoshimura K, McElvaney NG, Crystal RG. Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. J Clin Invest. 1992; **89**: 1478–84.

[121] Caldwell RA, Boucher RC, Stutts MJ. Neutrophil elastase activates near-silent epithelial Na+ channels and increases airway epithelial Na+ transport. Am J Physiol Lung Cell Mol Physiol. 2005; **288**: L813–9.

[122] Hamon Y, Jaillon S, Person C, Ginies JL, Garo E, et al. Proteolytic cleavage of the long pentraxin PTX3 in the airways of cystic fibrosis patients. Innate Immun. 2013; **19**: 611–22.

[123] Rubio F, Cooley J, Accurso FJ, Remold-O'Donnell E. Linkage of neutrophil serine proteases and decreased surfactant protein-A (SP-A) levels in inflammatory lung disease. Thorax. 2004; **59**: 318–23.

[124] Bratcher PE, Weathington NM, Nick HJ, Jackson PL, Snelgrove RJ, et al. MMP-9 cleaves SP-D and abrogates its innate immune functions in vitro. PLoS One. 2012; **7**: e41881.

[125] Berger M, Sorensen RU, Tosi MF, Dearborn DG, Doring G. Complement receptor expression on neutrophils at an inflammatory site, the Pseudomonas-infected lung in cystic fibrosis. J Clin Invest. 1989; **84**: 1302–13.

[126] Tosi MF, Zakem H, Berger M. Neutrophil elastase cleaves C3bi on opsonized Pseudomonas as well as CR1 on neutrophils to create a functionally important opsonin receptor mismatch. J Clin Invest. 1990; **86**: 300–8.

[127] van den Berg CW, Tambourgi DV, Clark HW, Hoong SJ, Spiller OB, et al. Mechanism of neutrophil dysfunction: neutrophil serine proteases cleave and inactivate the C5a receptor. J Immunol. 2014; **192**: 1787–95.

[128] Hartl D, Latzin P, Hordijk P, Marcos V, Rudolph C, et al. Cleavage of CXCR1 on neutrophils disables bacterial killing in cystic fibrosis lung disease. Nat Med. 2007; **13**: 1423–30.

[129] Vega-Carrascal I, Bergin DA, McElvaney OJ, McCarthy C, Banville N, et al. Galectin-9 signaling through TIM-3 is involved in neutrophil-mediated gram-negative bacterial killing: an effect abrogated within the cystic fibrosis lung. J Immunol. 2014; **192**: 2418–31.

[130] Farberman MM, Akers KT, Malone JP, Erdman-Gilmore P, Townsend RR, et al. Airway proteins involved in bacterial clearance susceptible to cathepsin G proteolysis. Eur Respir J. 2010; **35**: 410–7.

[131] Vandivier RW, Fadok VA, Ogden CA, Hoffmann PR, Brain JD, et al. Impaired clearance of apoptotic cells from cystic fibrosis airways. Chest. 2002; **121**: 89S.

[132] Valayer A, Brea D, Lajoie L, Avezard L, Combes-Soia L, et al. Neutrophils can disarm NK cell response through cleavage of NKp46. J Leukoc Biol. 2017; **101**: 253–9.

[133] Doring G, Frank F, boudier C, Herbert S, Fleischer B, et al. Cleavage of lymphocyte surface antigens CD2, CD4 and CD8 by polymorphonuclear leukocyte elastase and cathepsin G in patients with cystic fibrosis. J Immunol. 1995; **154**: 4842–50.

[134] Le-Barillec K, Si-Tahar M, Balloy V, Chignard M. Proteolysis of monocyte CD14 by human leukocyte elastase inhibits lipopolysaccharide-mediated cell activation. J Clin Invest. 1999; **103**: 1039–46.

[135] Reeves EP, McCarthy C, McElvaney OJ, Vijayan MS, White MM, et al. Inhaled hypertonic saline for cystic fibrosis: reviewing the potential evidence for modulation of neutrophil signalling and function. World J Crit Care Med. 2015; **4**: 179–91.

[136] Yang C, Chilvers M, Montgomery M, Nolan SJ. Dornase alfa for cystic fibrosis. Cochrane Database Syst Rev. 2016; **4**: CD001127.

[137] Borowitz D, Parad RB, Sharp JK, Sabadosa KA, Robinson KA, et al. Cystic Fibrosis Foundation practice guidelines for the management of infants with cystic fibrosis transmembrane conductance regulator-related metabolic syndrome during the first two years of life and beyond. J Pediatr. 2009; **155**: S106–16.

[138] Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med. 2011; **365**: 1663–72.

[139] Davies JC, Cunningham S, Harris WT, Lapey A, Regelmann WE, et al. Safety, pharmacokinetics, and pharmacodynamics of ivacaftor in patients aged 2–5 years with cystic fibrosis and a CFTR gating mutation (KIWI): an open-label, single-arm study. Lancet Respir Med. 2016; **4**: 107–15.

[140] Amaral MD. Novel personalized therapies for cystic fibrosis: treating the basic defect in all patients. J Intern Med. 2015; **277**: 155–66.

[141] Mayer-Hamblett N, Aitken ML, Accurso FJ, Kronmal RA, Konstan MW, et al. Association between pulmonary function and sputum biomarkers in cystic fibrosis. Am J Respir Crit Care Med. 2007; **175**: 822–8.

[142] Conese M, Castellani S, Lepore S, Palumbo O, Manca A, et al. Evaluation of genome-wide expression profiles of blood and sputum neutrophils in cystic fibrosis patients before and after antibiotic therapy. PLoS One. 2014; **9**: e104080.

[143] Bratcher PE, Rowe SM, Reeves G, Roberts T, Szul T, et al. Alterations in blood leukocytes of G551D-bearing cystic fibrosis patients undergoing treatment with ivacaftor. J Cyst Fibros. 2016; **15**: 67–73.

IntechOpen