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Review article

Cytokines and inflammatory mediators in cystic fibrosis

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Abstract

Airway disease in cystic fibrosis (CF) is characterised by a continuous cycle of chronic infection and inflammation dominated by a neutrophilic infiltrate. This inflammation is characterised by an increased production of pro-inflammatory cytokines in the lung. The relationship between the abnormal CFTR gene product and the development of inflammation and progression of lung disease in CF is not fully understood. This review article studied the mechanisms of pulmonary inflammation in CF, the profiles of cytokines and inflammatory mediators in the lung in CF, the mechanisms that predispose to chronic *Pseudomonas aeruginosa* infection, cytokine involvement in diseases other than CF and reviewed current therapeutic strategies for CF. Imbalances of cytokine secretion are now better understood due to recent advances in understanding CF at a molecular level and it is increasingly thought that the normal inflammatory process is deranged in CF early in the course of the disease and may occur in the absence of detectable infection. However, the relationship between this unbalanced cytokine production, the mutations in CFTR and its actual consequence for pathogenesis need further investigation.

Keywords: Inflammation; Cytokines; *Pseudomonas aeruginosa*; Remodelling

Contents

1. Introduction

Airway disease in cystic fibrosis (CF) is characterised by chronic infection and an inflammatory response dominated by a neutrophilic infiltrate (Fig. 1). There is incomplete understanding of the relationship between the abnormal

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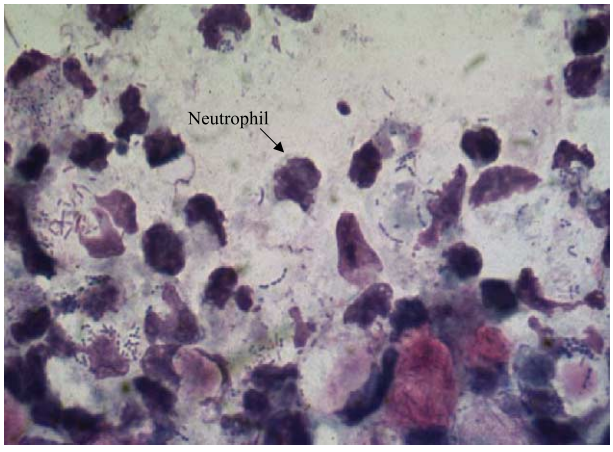

Fig. 1. Neutrophil in CF sputum.

CFTR gene product and the development of inflammation and progression of lung disease in CF [1].

Evidence suggests that airway inflammation in CF is associated with increased production of pro-inflammatory cytokines in the lung. Airway epithelial cells, macrophages, and neutrophils are all capable of producing cytokines. Several studies have found elevated concentrations of pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-8, and tumour necrosis factor-alpha (TNFα) in the sputum and bronchoalveolar lavage fluid (BALF) of patients with CF [1]. Their synthesis is promoted by the transcription factor nuclear factor-κB (NF-κB), which plays an important role in intracellular signalling for the production of pro-inflammatory cytokines [2]. IL-10, IL-1 receptor antagonist protein (IRAP), and soluble TNFα receptor (TNFsR) are anti-inflammatory cytokines that are relatively down-regulated in CF airway cells [3]. The principle action of IL-10 is to increase the synthesis of I-κB, the inhibitor of NF-κB. Downregulation of IL-10 leads to increased pro-inflammatory cytokines due to less inhibition of NF-κB actions [2]. Table 1 lists the actions of both the pro-inflammatory and anti-inflammatory cytokines. The aim of this review is to give an overview of the role of cytokines and their dysregulation in the pathogenesis of lung disease in CF, and to discuss current and future anti-inflammatory treatments in CF.

2. Injury and remodelling

The most characteristic feature of inflammation in the CF lung is neutrophil infiltration into the airways. The excessive release of oxidants and proteases, including neutrophil elastase by infiltrating neutrophils, plays an important role in tissue damage [4]. This results in disruption of the elastin fibres and other matrix proteins, and increased degradation in infected patients [4,5]. Anti-protease defenses of the CF lung are overwhelmed by a combination of endogenous and bacterial proteases rather than a primary abnormality in the production of protease inhibitors [4], so there is uninhibited proteolytic enzyme activity. Elastase directly damages the airway wall by digesting elastin and other structural proteins; it increases mucus secretion, cleaves vital opsonins and receptors necessary for phagocytosis, and promotes the generation of chemoattractants, thus fueling the vicious cycle of infection and inflammation that leads to lung destruction [6]. Other serine proteases such as the cathepsins may also be important in the process of lung injury.

Matrix metalloproteinases (MMPs), a group of calcium- and zinc-dependent endopeptidases involved in tissue breakdown and repair, cell migration, and turnover of extracellular matrix (ECM) components [7,8], are also thought to be important in the normal remodelling process [9] and in the increased ECM destruction seen in many diseases [10,11]. More than 20 MMPs have now been described, which are inactivated by specific tissue inhibitors (TIMPs) [12]. MMP-8 and MMP-9 are the most important in the CF airways as they are mainly derived from neutrophils in the lower respiratory tract [13]. A recent study by Ratjen et al. [13] demonstrated that MMP-8 and MMP-9 are increased in the BALF of CF patients with mild lung disease, with a rise in TIMP not in proportion to the elevated MMPs. Although the role of MMPs in CF lung disease is not clearly understood, it is thought that excess MMPs play a role in the continuing cycle of inflammation and injury.

Lung injury also results in the synthesis of transforming growth factor-alpha (TGFα) [14]. TGFα is thought to play a role in the regulation of remodelling following injury in the lungs of individuals with CF as its expression is increased in CF lung tissues compared to those without CF [14]. Remodelling of the airways in vitro has been found to be associated with the continual presence and release of

Table 1
Actions of cytokines

Cytokines	Actions
IL-1	Primes neutrophils
	Increases adhesion of neutrophils to endothelium
IL-8	Increases chemotaxis of neutrophils to site of inflammation
	Activates neutrophils
	Increases expression of adhesion molecules
TNFα	Increases chemotaxis of neutrophils to site of inflammation
	Increases adhesion of neutrophils to endothelium
	Induces synthesis of chemoattractant neutrophils
	Increases intermediary metabolism
IL-6	Mediates acute-phase reaction
	Matures B-lymphocytes
	Activates T-lymphocytes
IL-10	Inhibits secretion of TNFα and other cytokines
	Inhibits antigen presentation
IRAP	Inhibits IL-1 receptor binding
	Antagonises activities of IL-1

IL-1, interleukin-1; IL-8, interleukin-8; TNFα, tumour necrosis factor-alpha; IL-6, interleukin-6; IL-10, interleukin-10; IRAP, interleukin-1 receptor antagonist protein.

inflammatory mediators and Th2 cytokines (especially IL-13) during cycles of epithelial injury and repair [15].

3. Inflammation before infection?

Excessive inflammation in the airways may be due to the persistence of stimuli for cytokine production, such as bacteria, or a constitutive abnormality in the regulation of cytokine production by cells, or both. Airway inflammation in CF patients is often viewed as a response to infection, but studies have shown that inflammation and infection are early events in CF lung disease in infants without clinically apparent lung disease [16]. There is uncertainty regarding what actually initiates the cycle of persistent inflammation and infection leading to lung injury and destruction.

Several studies of BALF from infants with CF have documented the presence of increased inflammatory markers often in the absence of CF-related pathogens [17–19]. Two studies have shown that some infants with CF had airway inflammation in the apparent absence of infection, although in the study by Khan et al. [17] and Armstrong et al. [20], the patients had respiratory symptoms. However, in the infants, the degree of inflammation was less when compared to the BALF of CF infants in whom microorganisms were isolated. In one of these studies, aspiration lung disease was felt to be responsible for inflammation in the absence of infection in two infants. One possible conclusion is that inflammation precedes infection by some direct contribution of the defective CFTR [6]. An alternative explanation is that bacteria that are below detectable levels are generating an inflammatory response. It is also possible that the inflammatory response and/or treatment effectively cleared the infection, but the inflammation then persisted in the absence of bacterial or viral stimuli [6]. A further hypothesis is that endogenous signals may be generated, leading to an intense inflammatory response with the production of factors that could damage the airway surface, and so favour infection and bacterial colonisation [21].

Airway epithelial cells with abnormal CFTR have a lower threshold for bacterial adhesion and an augmented cytokine production response to adherent bacteria [22–24]. In vitro data have shown that lung epithelial cells that express defective CFTR have elevated production of pro-inflammatory cytokines and increased activation of NF-κB [25–27]. CF mononuclear cells have also demonstrated selective cytokine dysregulation after maximal activation. Moss et al. showed a reduced interferon-gamma (IFN-γ) secretion and increased IL-10 mRNA without increased production or secretion, suggesting that the cytokine imbalance already described in epithelial cells also occurs in immunoregulatory cells, further suggesting a link between CFTR mutations and cytokine dysregulation [28].

4. Cytokine profile

The inflammatory response in the CF lung is the result of a complex balance between pro-inflammatory and anti-inflammatory mediators. CF cell lines produce more pro-inflammatory cytokines than normal cell lines in response to *Pseudomonas aeruginosa* infection [29] and it has been shown that they are elevated in the epithelial lining fluid (ELF) of CF patients compared to healthy controls [30]. Not only is there increased neutrophil infiltration into CF airways—these neutrophils probably differ from normal neutrophils in that they have an increased propensity to release their granule proteins when stimulated, which may be as a direct result of CFTR mutation [31,32].

The ELF of CF patients compared to healthy controls has reduced levels of anti-inflammatory cytokine IL-10 [3], which inhibits the production of TNFα, IL-1β, IL-6, and IL-8 by macrophages [33,34]. Two other anti-inflammatory cytokines are IRAP and TNFsR. IRAP is produced by macrophages in response to an inflammatory stimulus [35] and is a specific antagonist to IL-1α and IL-1β. Two types of receptors exist for TNFα: those attached to the cell membrane and those soluble in extracellular fluid, both of which act as natural antagonists competing for TNFα binding. Bonfield et al. [30] showed that IRAP and TNFsR were increased in the ELF of CF patients compared to controls. This rise was proportionately less than that of the pro-inflammatory cytokines, resulting in a relative deficiency of these natural antagonists [30]. The abnormal regulation of cytokine release from CF epithelial cells may be due to CFTR dysfunction, but as yet the link is unclear [6]. Thus, if there is a reduction in the levels of anti-inflammatory cytokines (actual or relative) as well as increased production of pro-inflammatory cytokines, excessive and persistent inflammation will be the result.

Most of the tissue damage in CF is due to activated neutrophils despite the chronic ongoing nature of the inflammation [36], indicating a predominant humoral response and weak cellular response to infection [37,38]. Since the demonstration that the mouse T helper (Th) cell clones into either Th1 (IFN-γ-producing) or Th2 (IL-4-, IL-5-, or IL-10-producing) cells, the outcome of chronic infections has been thought to depend on the differences in the specific Th cell response [39,40]. Animal studies have demonstrated that in chronic *P. aeruginosa* lung infection in mice with a pulmonary Th1 response, there was lower mortality, faster clearance of bacteria, and milder lung inflammation in comparison to mice reacting with a Th2 response [41,42]. In CF patients with chronic infection, those with the highest IFN-γ production had the best lung function [43]. Peripheral blood mononuclear cells from these patients stimulated with *P. aeruginosa* antigen demonstrated a Th2-dominating response in CF patients with stable chronic *P. aeruginosa* lung infection as compared to CF patients without chronic *P. aeruginosa* lung infection [43]. A high RNA expression of IFN-γ and

transforming growth factor-beta (TGF-β) in bronchial biopsies was associated with milder disease in CF patients [44]. The predominance of a Th1 or Th2 response is thought to depend on the type of dendritic cell that is responsible for the priming of the T cells to new antigens [45,46]. This process is in turn thought to be determined by the granulocyte–macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) balance in CF [45]. The skewed T-helper response in CF in favour of Th2 may be an important factor in the outcome of infection with microorganisms.

Some studies have found that blood levels of pro-inflammatory cytokines are undetectable, in contrast to the greatly elevated concentrations in the sputum and BALF of CF patients indicating a florid inflammatory response that is compartmentalised to the local environment of the lungs [47]. Salva et al. [48] made reference to several studies that documented elevated concentrations of inflammatory mediators such as IL-1, IL-6, IL-8, and TNFα when not only sputum or BALF but also serum of patients with CF were compared with normal controls. In CF patients with chronic *P. aeruginosa* infection studied for 6 months prior to death, there were increased circulating levels of C-reactive protein (CRP), TNFα, and elastase complex (an inflammatory marker that reflects neutrophil activity) [49]. This suggests that the increased inflammatory activity present in the CF lung is reflected in the systemic circulation.

The inflammatory response also has systemic effects contributing to other features of CF such as cachexia, hyperglobulinaemia, and osteopenia [35,50–53]. TNFα in the systemic circulation alters intermediary metabolism, increasing resting energy expenditure (REE), stimulating lipolysis and catabolism, and causing anorexia and weight loss [54,55]. Clinically stable CF patients who are chronically infected with *P. aeruginosa* have elevated circulating levels of TNFα compared to healthy controls [56]. The levels of TNFα are increased further during an acute pulmonary exacerbation. Patients with CF have an increased REE and tend to be underweight [57]. An association between raised circulating levels of TNFα and measures of body wasting and intermediary metabolism (REE) has been demonstrated [58]. Bell et al. [59] showed that there was a relationship between raised REE, inflammatory markers, and catabolic status in patients with chronic *P. aeruginosa* infection at the start of an acute pulmonary exacerbation. These factors showed a subsequent parallel reduction following intravenous antibiotics, which is further evidence suggesting a link between cytokines and intermediary metabolism in patients with CF.

Aris et al. [60] showed an important link between pulmonary infection and inflammation in CF and unfavourable alterations in bone metabolism. These data supported the possibility that lung-derived inflammatory cytokines promote bone resorption and diminished bone formation, but did not demonstrate an actual loss in bone mass. Many CF patients suffer from low bone mineral density from osteoporosis or osteomalacia, fractures, and kyphosis [61,62]. The pathogenesis of bone disease in CF is not well understood [63], but it is undoubtedly multifactorial. Studies have shown that pro-inflammatory cytokines, especially TNFα and IL-1β, are important promoters of bone resorption and inhibit bone formation [60,64,65]. Further studies will be needed to determine which factors are most important in the bone disease that occurs in CF and the role played by cytokines.

Airway epithelial cells may be involved directly in the excess inflammation by several mechanisms. Pro-inflammatory cytokines arise from airway epithelial cells, as well as from macrophages and infiltrating neutrophils (Table 2). Airway epithelial cells also express large numbers of the important pro-inflammatory adhesion molecule, ICAM-1. This adhesion molecule is a ligand for neutrophils, and adhesion is thought to result in increased IL-8 production, leading to persistence of neutrophils in the airway [6]. *P. aeruginosa* pili bind to airway epithelial cells using the asialo-GM 1 receptors. It has been suggested that a CF mutation-related sialyation defect may be the cause of increased numbers of these receptors on CF cells [66,67]. The fact that epithelial cells themselves are involved in cytokine release and are thought to play a major role in the local inflammation leads to further speculation that defective CFTR function (expressed most importantly in the epithelial cells) may be directly related to excessive inflammation.

Airway surface epithelial cells may also contribute to the pro-inflammatory status of the airway by altering the composition of airway surface fluid (ASF). Salt-sensitive antibacterial peptides may be rendered inactive by the NaCl concentration in ASF, changing from normally hypotonic (85 mM) to hypertonic (>115 mM), which has been suggested as a result of defective CFTR function [68,69]. Tabary et al. [70] showed that CF human bronchial gland cells (HBGs) had increased IL-8 production even in hypotonic NaCl concentration when compared to non-CF HBG cells, and that this IL-8 production was further increased with isotonic and hypertonic NaCl.

Table 2
Cellular source of cytokines produced in human airways

	Neutrophils	Epithelial cells	Macrophages	Lymphocytes
IL-1β	+	+	+++	
IL-6	+	+	+++	+
IL-8	+++	++	++	+
IL-10		++	+	++
TNFα	++	+	+++	+
IRAP	+		+++	
TNFsR	++	+	+	

IL-1β, interleukin-1beta; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; TNFα, tumour necrosis factor-alpha; IRAP, interleukin-1 receptor antagonist protein; TNFsR, tumour necrosis factor soluble receptor.

5. Response to *P. aeruginosa*

Despite its multiorgan involvement, the progression of pulmonary disease decides the clinical outcome in the majority of patients. *P. aeruginosa* is the most important pathogen in CF. In spite of a high antibody response to *P. aeruginosa* both in the lungs and in the circulation, the bacteria are not eradicated after the infection is established, leading to chronic infection with destruction of the airways and lung function decline. Several hypotheses exist to explain the high prevalence and chronic nature of *P. aeruginosa* infection in CF. Some studies have suggested that the abnormal CFTR results in increased *P. aeruginosa* adhesion to airway epithelial cells [21–24], which in turn results in an augmented cytokine response, specifically IL-8 production [66]. The absence of CFTR at the apical surface of the epithelial cells is thought to result in impaired internalisation and killing of *P. aeruginosa* [71].

Another hypothesis suggests a reduced activity of beta-defensin-1 peptides due to a high concentration of NaCl in CF ASF [72–74]. But in vitro and in vivo data have demonstrated that ASL is isotonic, thus making this mechanism less likely [75–77].

In vitro studies by Worlitzsch et al. [78] demonstrated that the periciliary liquid layer is volume-depleted, resulting in reduced mucus clearance, which, along with raised oxygen consumption by CF epithelia, leads to thick hypoxic mucus. *P. aeruginosa* then penetrates and grows in the hypoxic zones and proliferates to form biofilm-like macrocolonies, making the infection more resistant to defense mechanisms.

Whatever the actual features of the host–bacterial relationship that lead to the establishment of the chronic *P. aeruginosa* infection in CF, it seems clear that this is a critical event in beginning the inflammatory process that is eventually responsible for most of the morbidity and mortality in CF.

6. Cytokines and clinical disease

Although it has been shown that concentrations of inflammatory mediators are markedly elevated in the sputum or BALF of CF patients when compared with control subjects, few studies find a correlation between cytokine concentrations and clinical status.

Induced sputum is increasingly being used as a research tool as it is a safe, reproducible method of studying the airways of patients with respiratory disease [79]. The inflammatory cell population and microbiology in induced sputum are representative of CF airways [80]. Bronchoalveolar lavage (BAL) is a reproducible, well-validated method used for the study of markers of inflammation and inflammatory cells in numerous lung diseases including CF [81,82]; however, it is more invasive than sputum induction.

Wolter et al. [47] and Salva et al. [48] were unable to find a correlation between levels of inflammatory mediators in sputum and clinical parameters during periods of well-being and during acute respiratory exacerbations, indicating that these markers are unreliable as predictors of disease status. A possible explanation that may be used to account for this lack of correlation is that the presence of acute-on-chronic inflammation may not allow for further augmentation in IL-8 or other cytokine production [48]. Alternatively, the ability of older patients to produce IL-8 may be reduced [49]. Some cross-sectional studies, however, have found a correlation between cytokine levels and clinical status [20,83]. IL-6 has been shown to be a potent inducer of the acute phase response and CRP is a surrogate marker for IL-6. Inflammatory markers such as CRP, TNFα, and elastase complex have been found to be elevated during symptomatic deterioration and subsequently have decreased during appropriate antibiotic therapy [49]. This may have clinical relevance, but the benefits of monitoring cytokines in the clinical setting have yet to be demonstrated.

7. Other diseases

Cytokines are known to be involved in regulating normal physiological processes, but excess or decreased cytokine production is associated with disease [84,85]. Altered cytokine profiles are thought to play a major role in various diseases such as sepsis, ARDS, rheumatoid arthritis (RA), and inflammatory bowel disease. For example, in RA, mononuclear cells, synovial fibroblasts, chondrocytes, and osteoclasts are stimulated to release cytokines especially TNFα, IL-1, and IL-6, which in turn result in synovial inflammation and the clinical features associated with RA [86].

Therapy directed at cytokine response has obvious potential to modify disease, yet significant impact on clinical status has yet to be proven. Anti-TNF therapies have been approved for RA and inflammatory bowel disease, and although they do provide symptomatic relief, the long-term effects of these treatments are as yet unknown [84].

8. Anti-inflammatory therapy

Inflammation is a vital process needed to counteract infection, but it is increasingly thought that the normal inflammatory process is deranged in CF early in the course of the disease [6,87]. The aim of anti-inflammatory therapy therefore is to gain the balance between ‘useful’ and ‘inappropriate’ inflammation [66]. Several approaches to anti-inflammatory treatment in CF have been studied in the last few years, including oral and inhaled corticosteroids (ICS), nonsteroidal anti-inflammatory drugs (NSAIDs), and, more recently, macrolide antibiotics and a range of antioxidants and antiproteases. Long-term oral corticosteroids have been shown to lower immunoglobulin G (IgG) levels and cytokine concentrations in CF patients [88], but the signifi-

cant side effect profile limits their prolonged use. A multicentre trial by Eigen and Rosenstein [89] demonstrated an improvement in lung function in patients with mild to moderate lung disease treated with alternate day prednisolone for a period of up to 24 months. An increase in adverse events was noted if treatment extended beyond 24 months. Questions still remain, however, as to the timing of steroid use in relation to the age of the patient and the severity of lung function [89]. At the present time, there is no place for the routine long-term use of oral corticosteroids. ICS seem a reasonable treatment due to their anti-inflammatory properties but with limited systemic absorption. However, there is limited conclusive data in support of the efficacy of ICS in CF. There is a need for larger multicentre clinical trials to examine efficacy and safety [90,91].

The good safety profile and potent anti-inflammatory action of NSAIDs have resulted in clinical trials to assess their potential role in CF. High-dose ibuprofen has been shown to inhibit neutrophil recruitment to mucosal sites in vivo [92]. It has been demonstrated that patients treated with high-dose ibuprofen had a slower rate of FEV_1 decline, better maintained weight, and chest X-ray scores with no significant adverse events in comparison with placebo [93]. Despite this, the use of high-dose ibuprofen as an anti-inflammatory agent in CF remains quite low due to its complex pharmacokinetics, fears of adverse events, and lack of multicentre trials [90].

There is increasing interest in macrolide antibiotics following their possible anti-inflammatory effects seen in Japanese panbronchiolitis [94]. Their mechanism of action is still unclear but may be due to an anti-inflammatory action of decreasing production of pro-inflammatory mediators or due to antimicrobial activity especially against *P. aeruginosa* [95]. In a 3-month study, adult CF patients treated with azithromycin demonstrated maintenance of lung function, fewer courses of intravenous antibiotics, improved quality of life, and a decrease in CRP compared to placebo [96]. Equi et al. [97] studied the effects of long-term azithromycin in children with CF and found a significant beneficial group response in lung function. Neither study revealed any significant adverse event associated with treatment. A large multicentre placebo-controlled, double-blinded trial of azithromycin in adult patients with CF who were chronically infected with *P. aeruginosa* showed a sustained beneficial treatment effect in lung function, detectable after 28 days of treatment. Azithromycin was well tolerated in this study in which there was also a reduction in the number of exacerbations and an improvement in body mass index [98].

The relative lack of antiproteases in the CF airway has led to trials investigating the anti-inflammatory properties of exogenous α_1 antitrypsin (the major antiprotease in the lung). Initial clinical studies with serum-derived, aerosolized α_1 antitrypsin have shown a reduction in neutrophil elastase activity [99,100]; however, a large multicentre trial did not demonstrate any beneficial effects [101].

Ramdin et al. [102] suggested that heparin had immunomodulatory effects in vivo by demonstrating the binding and inhibition of IL-8. However, due to the complex genetics of heparin clearance, therapeutically, it is difficult to assess the dose of heparin required to modify cytokine activity. Further clinical trials are required to assess the potential for heparin as an anti-inflammatory agent in CF [102].

The complexity of the inflammatory process in the CF airways has led to interest in other possible anti-inflammatory agents including antioxidants, leukotriene receptor antagonists, and anti-TNFα therapies that need further evaluation as potential therapeutic agents in CF [90].

9. Conclusion

The balance of pro-inflammatory and anti-inflammatory mediators and the recruitment of neutrophils by chemoattractant cytokines are very important in the pathophysiology of CF. Imbalances of cytokine secretion are now better understood due to recent advances in understanding CF at a molecular level. However, the relationship between this unbalanced cytokine production, the mutations in CFTR, and its actual consequence for pathogenesis needs further investigation. The bacteria–host immune response interaction comprises a vicious cycle of chronic infection and inflammation. Antibiotic therapy alone addresses only the microbiological component of the interaction and, at present, there is increasing interest in the adjunctive use of anti-inflammatory agents. Oral corticosteroids have significant anti-inflammatory actions, but adverse effects with long-term treatment limit their use. More recently, work has focused on less toxic oral agents and inhaled anti-inflammatory therapies such as ICS; however, studies demonstrating their value so far have not been conclusive. Increased knowledge and understanding of the mechanisms involved in lung injury should result in advances in immunomodulatory therapies that might lead to major contributions to the treatment of the disease in the future [47,96].

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