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The role of airway epithelium and blood neutrophils in the inflammatory response in cystic fibrosis

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Abstract

Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, which accounts for the cAMP-modulated chloride conductance of airway epithelial cells. CFTR also regulates other membrane proteins like the negative regulation of the amiloride-sensitive epithelial sodium channel (ENaC). Mutations in the CFTR gene lead to hyperabsorption of sodium chloride and a reduction in the periciliary salt and water content which leads to impaired mucociliary clearance. It seems that a lack of functional CFTR leads to abnormal function of the NF-κB pathway in submucosal gland cells, causing an increased production of pro-inflammatory cytokines and the chemokine IL-8, and a pro-inflammatory environment. CFTR is also expressed in neutrophils and several neutrophil functions like cytokine production, migration, phagocytosis and apoptosis seem altered in CF. In this review we describe the role of airway epithelium and blood neutrophils in the viscious circle of inflammation and infection seen in CF.

Keywords: CFTR; Blood neutrophils; Airway epithelium; Inflammation; Bacterial binding

1. Introduction

The hallmarks of the lung pathology in CF are bacterial colonisation and infection of the airways (particularly with *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and an apparently exaggerated, sustained and extended inflammatory response, characterised by influx of neutrophils and high concentrations of interleukin-8 in bronchoalveolar lavage fluid (BALF) [1–8]. Several pathophysiological mechanisms have been postulated to explain the pulmonary infections and inflammation. In this review we will focus on the role of mutant CFTR on various aspects of the airway epithelium including mucociliary clearance, submucosal gland cell products and bacterial binding. We will furthermore focus on the role of neutrophils in airway inflammation in CF, since these cells play a key role in the ongoing inflammatory response.

2. Defence mechanisms by airway epithelium

2.1. Mucociliary clearance

Airway epithelium is covered on its apical surface by a thin liquid layer called the airway surface liquid (ASL). The ASL is the first line of defence against inhaled pathogens and is mandatory for effective mucociliary clearance [9]. ASL is composed of a mucus gel and a periciliary sol layer (PCL) [10] that are propelled upwards by coordinated ciliary beating. The PCL of the ASL is an aqueous solution with a height, equalling the height of extended cilia (~7 μm), and a relatively low viscosity enabling effective ciliary beating and cell surface lubrication. The mucus layer is a gel-like aqueous layer in which soluble compounds are mixed with mucus polymers and

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aggregates of mucins and other high molecular-weight glycoproteins, proteoglycans, defence molecules (like lactoferrin, lysozyme and defensins), DNA, and actin. The two major mucins present in human respiratory mucosa are MUC5AC and MUC5B [11,12]. The diversity of the carbohydrate side chains within the mucin gel allows entrapment of a wide variety of particles, like bacteria, for ultimate clearance from the airway [13].

Effective mucus transport requires a well-defined PCL liquid layer and the capacity of the airway epithelium and mucus layer to maintain the PCL layer at the appropriate height [14]. The mucus layer has the propensity to swell and shrink by accepting liquid from or donating liquid to the PCL layer as needed [15]. The airway epithelium seems to have the capacity to both absorb liquid from the PCL and to secrete liquid into the PCL as necessary, a process that is believed to be mediated by isotonic (100–150 mM NaCl) volume transport [16–18]. Still relatively little is known about this tightly regulated process such as: what are the sensors of ASL volume and how is information transmitted to the various effectors (ion channels) in the apical cell membrane [15]. Under physiological conditions, the airway epithelium defence system, by virtue of endogenously produced antimicrobial peptides like lactoferrin and lysozyme can suppress bacterial growth for 3 to 6 h [19], after which bacteria are cleared from the airways by mucociliary clearance within 6 h [13].

In CF, loss of CFTR function results in altered salt transport of the airway epithelium. This has led to the proposal of basically two hypotheses, addressing the mechanism by which altered salt transport, associated with the loss of CFTR, leads to a breakdown of lung defence mechanisms in CF and to persistent endobronchial infections [20,21]. The first hypothesis is the *isotonic* "low volume" hypothesis with resultant abnormalities in mucociliary clearance. This hypothesis proposes that a lack of CFTR inhibition of epithelium sodium channels (EnaC) causes increased Na^+ absorption. On top of this, mutant CFTR also fails to initiate cAMP-dependent Cl^- secretion. This causes an increased water absorption, leading to a decreased volume of the PCL: components of the ASL (see Fig. 1) [22,23]. The decrease in ASL volume also decreases mucociliary clearance and causes mucus stasis. The concentrated mucus adheres to the cell surface leading to a reduced efficacy of mucociliary clearance by coughing. The ciliated cells require an increased metabolic activity to sustain the excessive salt and water absorption and this, together with the accumulated mucus leads to hypoxia in adherent mucus plaques near the epithelial cell surface [24,25]. The combination of mucus plaques and mucus hypoxia probably promotes the accumulation of bacteria. The innate antimicrobial peptides present in the mucucs are no longer able to control bacterial growth, and the formation of bacterial biofilms can result in the acquisition of chronic bacterial infection [26]. An important argument in the low volume hypothesis is EnaC dysregulation. Several studies have shown that CFTR functions as a regulator of ENaC [27–30] but the exact molecular interaction between CFTR and ENaC remains to be elucidated.

The second and opposite hypothesis is the "compositional" hypothesis with hypertonic ASL salt concentrations in CF, inactivating salt-sensitive antimicrobial peptides [31]. This hypothesis proposes that a lack of CFTR leads to defective cellular chloride absorption, leading to an increased Cl^- concentration of ASL in patients with CF. This increased Cl^- concentration of ASL inactivates the salt-sensitive antimicrobial peptides like defensins [31–33]. More recently studies in mouse-models on the ASL ionic composition as well as studies in CF-patients however failed to detect differences in ASL ionic composition between normal and CF, i.e. both normal and CF ASL are isotonic [34–36]. These results therefore would favour the isotonic "low volume" hypothesis but the "compositional" hypothesis cannot be entirely refuted yet. It should be kept in mind that there are significant technical limitations of collecting and assaying ASL from the upper and lower airways and it is not feasible yet to perform the necessary experiments in vivo, in actual human airways.

2.2. Submucosal gland cell products

Serous cells secrete a variety of nonmucin products like lysozyme, lactoferrin, secretory IgA, peroxidase and protease inhibitors [38]. They also secrete several defensins, salt-sensitive antimicrobial substances important in airway defence [31,39,40]. CFTR is highly expressed in the

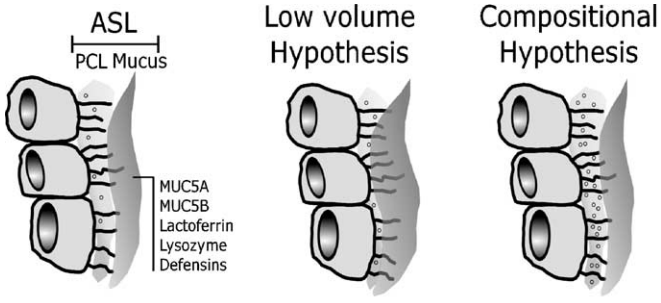

Fig. 1. Hypothetical models on ASL dysregulation in CF (see text for further explanation).

serous epithelial cells of submucosal glands [41] and these serous cells are considered to be the primary defence cells of the airway mucosa [38]. The presence of CFTR in the secretory granules of serous cells [38] suggests that CFTR contributes mechanistically to secretion of glycoproteins, either by providing a regulated Cl^- conductance in secretory granule membranes or by contributing to cAMP-mediated secretory vesicle exocytosis. Loss of CFTR function may lead to an alteration in the macromolecular composition of the serous cell secretions and thereby also change mucus viscosity and may adversely affect mucociliary clearance [13,37,42,43].

In CF the bronchial and tracheal gland cells show an altered cytokine production profile with a significant decrease of IL-10 content and a reduced ability of CF epithelial cells (and $CD4^+$ T-lymphocytes) to produce IL-10 in response to inflammatory stimuli [44–46]. Furthermore there is a constitutive upregulation of the pro-inflammatory chemokine IL-8 by CF airway epithelial cells and an increased IL-8 mRNA expression in bronchial epithelial cells in young CF-patients [44,47–52]. The synthesis of all these pro-inflammatory cytokines and chemokines elevated in CF is regulated by the transcription factor nuclear factor-κB (NF-κB) [53]. In epithelial cell lines derived from CF-patients, a higher and constitutive activation of NF-κB has been described [49,50,54]. It is suggested that a lack of functional CFTR leads to abnormal function of the NF-κB pathway [49,55]. It is however also suggested that a decrease or absence in IL-10 locally, which is described in patients with CF [44,56], causes increased expression of IκB α/β kinases. IκB α/β kinases are required for NF-κB activation [49]. The combination of reduced IL-10 and increased IκB α/β kinases may lead to uncontrolled NF-κB activation (see also Fig. 2) [57].

2.3. Bacterial binding and colonozation

CFTR appears to play an indirect, but also a direct role in the ability of CF pathogens like *S. aureus*, *Haemophilus influenzae* and *P. aeruginosa* to bind to epithelial membrane in cell culture systems [58–60]. The finding that significant numbers of bacteria do not bind to differentiated epithelia of native airway secretions [61,62] but are bound to injured epithelia [63–66] suggest that direct epithelial–bacterial cell interactions require antecedent epithelial injury. Bacterial–epithelial adhesion may be important once infection and inflammation have caused epithelial injury but appears less likely to play a critical role in the initiation of infection.

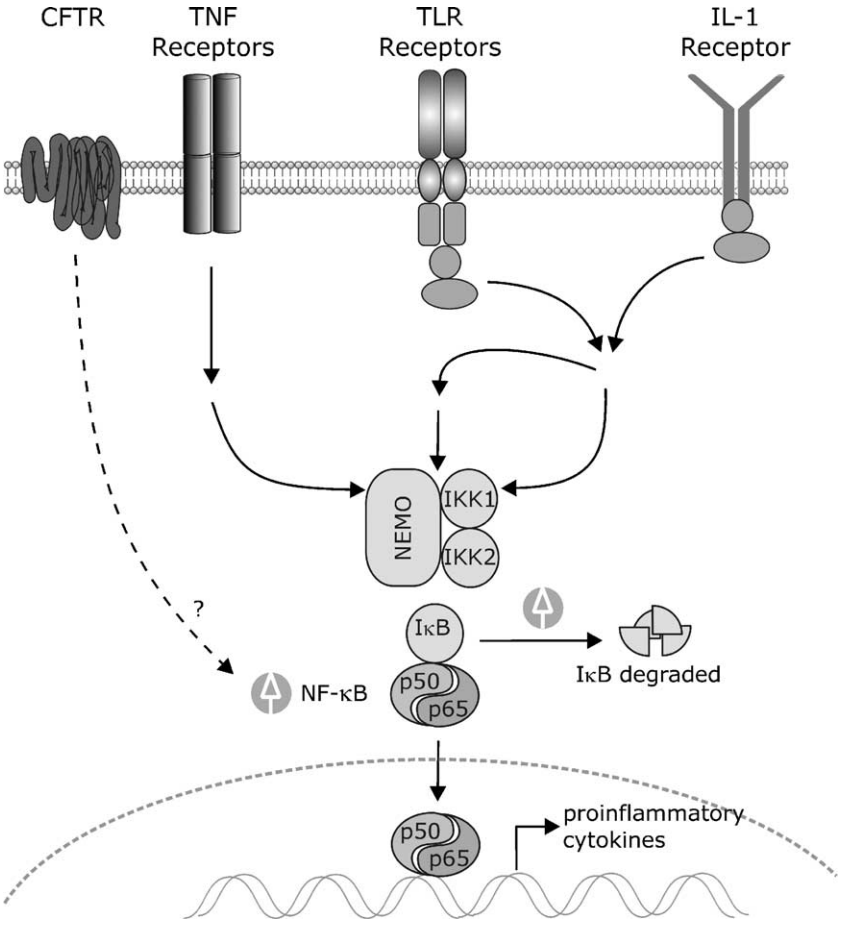

Fig. 2. Central role of NF-κB in signalling routes of inflammation receptors. Pathways/activities found to be upregulated in CF indicated by circled arrowheads.

A direct role for CFTR is suggested by the finding that CFTR serves as a pattern recognition molecule for *P. aeruginosa* lipopolysaccharide-core oligosaccharide. Expression of mutant CFTR leads to a defective internalisation of *P. aeruginosa* and defective epithelial cell phagocytosis of these bacteria [67–69]. The relative importance of epithelial phagocytosis in innate immune defense against *P. aeruginosa* is uncertain.

2.4. Neutrophils

Neutrophils are the major cellular players of the innate immune system, both by phagocytosis of infectious agents but also by their capacity to limit the growth of some microbes. Both mechanisms serve to keep an infection under control until adaptive (specific) immunological responses can develop.

2.5. Priming

Neutrophils exist in a dormant (resting) state unless they become activated. Over the last decade it has become clear that this "all or nothing" scenario is too simplistic and that various intermediate stages exist, such as the primed state. Priming is a mechanism whereby dormant neutrophils acquire a state of preactivation that generates a more powerful response to microbial activity [70]. In patients with CF, neutrophils show an increased primed phenotype measured by expression of phagocyte opsonin receptors, like complement receptor (CR)1 (CD35) and CR3 (CD11b), response capacity, even in uninfected children with CF [71]. These data, which are compatible with data from our own group [72], may point towards CF as a disease with autoinflammatory characteristics.

2.6. Cytokine and chemokine production

During an inflammatory response, chemotactic factors of different origin and pro-inflammatory cytokines signal the recruitment of neutrophils to sites of infection and/or injury. Not only CF epithelial cells, but also CF neutrophils display an abnormal release pattern of inflammatory mediators. Blood neutrophils from patients with CF constitutively secrete higher amounts of IL-8 and lower amounts of the anti-inflammatory cytokine IL-1 receptor antagonist (IL-1 RA) [73]. Spontaneous release of IL-8 and IL-1 RA by CF airway neutrophils is even higher, indicating a modification of the response by the local environment [73]. The finding that spontaneous release of IL-8 was significantly lower in airway neutrophils from children with dyskinetic cilia syndrome however provides support for a genetic component.

2.7. Migration

Neutrophil migration to the site of infection/inflammation is a multistep process that consists of tethering (capture), rolling, slow rolling, firm adhesion and transmigration (see also Fig. 3) [74,75]. The initial event is caused by the appearance of new adhesion molecules (E-selectin and P-selectin (pre-stored in the Weibel-Palade bodies) on the endothelium adjacent to the inflamed site. Expression of these adhesion molecules is induced by inflammation

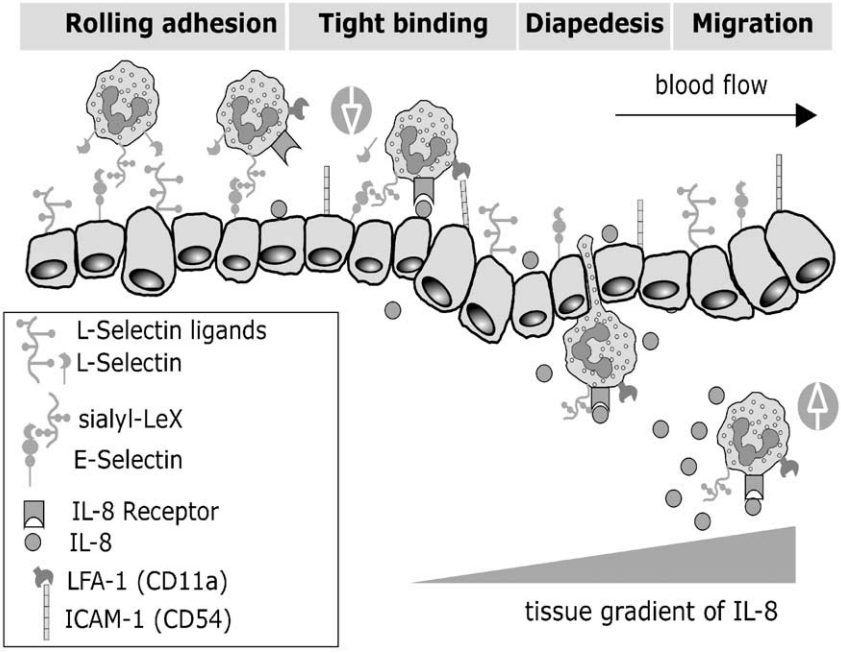

Fig. 3. Cellular and molecular interactions during neutrophil migration. Pathways/activities found to be upregulated or downregulated in CF indicated by circled arrowheads. See text for further details.

mediators released by damaged tissues, like TNF-α and bacterial lipopolysaccharide (LPS). In postcapillary venules, the slow flow rate allows for a short transient interaction (or tethering) between the neutrophil and the endothelium. Neutrophils roll along microvascular walls via low affinity interaction of selectins (like L-selectin (CD62L), present on neutrophils and E-selectin on endothelial cells) with specific endothelial carbohydrate ligands. This leads to the activation of neutrophil-integrins (for example $\alpha_M\beta_2$ (CD11b/CD18, also known as CR3)) and subsequent firm adhesion to intercellular adhesion molecules on the surface of activated endothelial cells in postcapillary venules. Under the influence of a locally generated chemotactic gradient and by diffusion of chemoattractants from the infection site, neutrophils penetrate the endothelial layer and migrate through connective tissue to sites of inflammation/infection (transmigration), where they finally congregate and adhere to extracellular matrix components such as laminin and fibronectin. In the pulmonary capillaries however selectin-mediated rolling of neutrophils does not occur, presumably due to spatial constraints [76] and selectins are often even not required during the acute response of neutrophils [77,78].

Two different pathways have been described through which neutrophils can emigrate in the alveoli and distal bronchioles that are fed by the pulmonary circulation. Neutrophils can emigrate through a pathway that requires CD11/CD18 and one that does not require CD11/CD18 [79]. Neutrophil emigration in response to acute *P. aeruginosa* exposure occurs through adhesion pathways that require CD11/CD18. Via this pathway shedding of L-selectin only occurs after the neutrophil has emigrated from the circulation into the alveolar interstitium [80]. In contrast, chronic *Pseudomonas aeruginosa* exposure shifts the migration pathway to the CD11/CD18-independent route, and is accompanied by a decrease in the number of neutrophils migrating to the lung [81,82]. In this pathway L-selectin shedding occurs in the vessels [80]. Neutrophils from both CF and non-CF subjects showed similar upregulation of CD11b, but CF neutrophils showed significantly less L-selectin shedding than control subjects upon stimulation with IL-8 or fMLP [83]. It has therefore been suggested that the reduced L-selectin shedding observed in CF-patients may reflect the maintenance of a heightened 'acute-type' (CD18-dependent) response to *P. aeruginosa*.

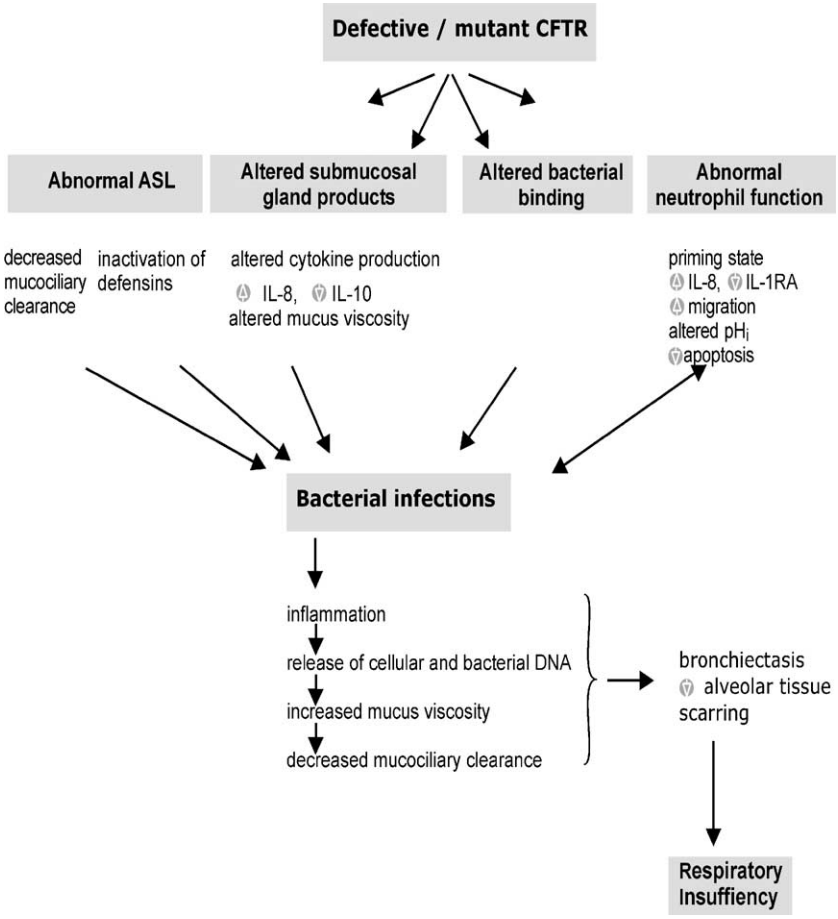

Fig. 4. Summary of proposed pathophysiological links between defective/mutant CFTR, airway defence mechanisms and neutrophils in the development of CF lung disease. Defects in CFTR lead to abnormal airway surface liquid, altered submucosal gland cell products and to abnormal neutrophil functions. Via mechanisms outlined, bacterial infection results and supports the vicious circle of ongoing inflammation and infection in CF lung disease. This further impairs mucociliary clearance and promotes chronic infection.

Migratory response towards IL-8 in neutrophils from clinically stable CF-patients is increased [84]. Neutrophils from acutely infected patients with CF however show decreased chemotaxis, possibly due to bacterial exoproducts, and show lower numbers of IL-8 receptors [85,86]. Reduced responsiveness of CF neutrophils to IL-8 seems to be associated with receptor desensitisation as a result of exposure to high systemic levels of IL-8 and this might contribute to the persistence of chronic endobronchial infections [85,86].

2.8. Phagocytosis

Once neutrophils have transmigrated to the site of infection phagocytosis can begin. This is a complex process consisting of several morphological and biochemical steps. After recognition and particle binding to the phagocyte surface, ingestion (engulfment), phagosome origination, phagolysosome formation (fusion of phagosome with lysosomes), killing and degradation of ingested cells or other material proceed. Antimicrobial efficiency of neutrophils is dependent on the generation of reactive oxygen species (ROS) by assembly and activation of NADPH-oxidase. Activation of the oxidative metabolism, known as the respiratory burst first involves NADPH-oxidase, an enzymatic complex that generates superoxide anion (O_2^-) which can dismutate in H_2O_2. Generation of O_2^- leads to production of various reactive oxidants, including halogenated oxidants generated through the myeloperoxidase (MPO) pathway. MPO, an enzyme contained in azurophilic granules of neutrophils, catalyzes the H_2O_2-dependent oxidation of chloride (Cl^-) to hypochlorous acid (HOCl).

In neutrophils many functional responses, including triggering of secretion in azurophilic granules (containing neutrophil elastase (NE) and MPO), oxidant production and microbe killing, are pH dependent.

Intracellular pH in CF neutrophils after phorbol ester activation is more acidic, indicating an intrinsic defect in CF neutrophil pH regulation possibly linked to mutant CFTR [87]. This "hyperacidification" has also been described in *trans*-Golgi network in CF lung epithelial cells [88]. Neutrophils of both CF homozygotes and heterozygotes display normal NADPH activity but increased myeloperoxidase-dependent oxidant activity [89].

CF neutrophils also release increased amounts of neutrophil elastase in response to TNF-α and IL-8 [90]. NE plays a major role in the pathophysiology of chronic inflammation in CF [91,92]. It directly contributes to tissue damage by degrading structural proteins, such as elastin, collagen, and proteoglycans, and has many other detrimental biological activities in the CF airways. NE enhances macromolecular secretion from serous gland cells and promotes hypertrophy and hyperplasia of the mucus-secreting apparatus and inhibits ciliary beating in vitro. NE also facilitates the persistence of infection by cleaving immunoglobulins, complement components and opsonin receptors, such as CR1, on the surface of phagocytes, and thus has an important impact on opsonophagocytosis [93]. These effects may further impair mucociliary and bacterial clearance and exacerbate airway obstruction in CF-patients.

2.9. Apoptosis

After the successful elimination of an invading pathogen, the inflammatory response should come to an end in order to avoid further tissue damage. Therefore, neutrophils ultimately are removed by apoptosis. CF pathogens like *P. aeruginosa* and *S. aureus* stimulate epithelial expression of G-CSF and GM-CSF, thereby counteracting induction of apoptosis and thus prolonging the inflammatory response [94]. The hyperacidification of CF cells as mentioned above also has an anti-appoptotic effect [95].

In summary there is a complex pathophysiological cascade leading to lung damage in CF, as summarized in Fig. 4. The resultant excessive inflammatory response not only fails to clear infection, but contributes to its persistence and is mostly responsible for lung damage and the progression of CF lung disease. Next to keeping control over the infections, it is therefore probably equally important to keep control over the (pulmonary) inflammation in CF.

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