The International Journal of Biochemistry & Cell Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

# Current concepts of immune dysregulation in cystic fibrosis☆

N. Rieber, A. Hector, M. Carevic, D. Hartl*

*CF Research Group, Department of Pediatrics I, University of Tübingen, Tübingen, Germany*

ARTICLE INFO

*Article history:*
Available online xxx

*Keywords:*
Cystic fibrosis
Immunity
Innate
Toll-like receptors
Neutrophils

ABSTRACT

Cystic fibrosis (CF) lung disease is caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene and is characterized by a perpetuated feedback loop of bacterial infection and inflammation. Both intrinsic (CFTR-dependent) and extrinsic (CFTR-independent) mechanisms contribute to the inflammatory phenotype of CF lung disease. Innate immune cells, initially recruited to combat bacterial pathogens, are acting in a dysregulated and non-resolving fashion in CF airways and cause harm to the host by releasing proteases and oxidants. Targeting harmful immune pathways, while preserving protective ones, remains the challenge for the future. This review highlights current concepts of innate immune dysregulation in CF lung disease.

This article is part of a Directed Issue entitled: Cystic Fibrosis: From o-mics to cell biology, physiology, and therapeutic advances.



## 1. Immunity in cystic fibrosis: more questions than answers

Cystic fibrosis (CF) lung disease is characterized by a chronic and non-resolving activation of the innate immune system with release of chemokines and an infiltration of neutrophils into the airways. Neutrophil-derived oxidants and proteases cause harm to multiple cellular and humoral factors and have recently been shown to impair Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) functionality itself (Le Gars et al., 2013). Beyond neutrophils, the immune response in CF lungs is dysregulated at several levels, including impaired (i) ceramide homeostasis, (ii) apoptosis, (iii) autophagy, (iv) macrophage polarization and functionality, (v) T-cell responses (Th2/Th17 predominance) and other deviations as reviewed recently (Hartl et al., 2012). Research in innate immunity in CF is aimed at (i) understanding the key mechanisms driving this pro-inflammatory vicious circle, (ii) identifying biomarkers that predict the course of disease and (iii) targeting specific components of the innate immune system as future therapeutics. However, our precise understanding of innate immunity in CF is handicapped by several uncertainties in the field:

1. *The chicken-and-egg issue*: what comes first? Inflammation or bacterial infection in the early course of CF lung disease? Is there intrinsic CFTR-dependent inflammation also without any prior infection or were our methods to detect infection in previous studies just not sensitive enough to assess microbial impact? Recent insights from culture-independent microbial detection techniques have to be taken into account when addressing this question (Rabin and Surette, 2012). Countries with CF newborn screening and bronchoalveolar lavage (BAL) studies have shed light on this relationship (Sly et al., 2013) and new animal models, primarily the CF pig (Rogers et al., 2008), will help to answer this question.
2. *Inter-species differences:* Basic immunology relies on murine models to dissect pathways that contribute to disease. However, both (i) the lung anatomy and (ii) the myeloid cell composition differ substantially between mouse and man. New animal models, namely the CF ferret and the CF pig, present major advantages to overcome these hurdles, but studies are required to comprehensively characterize their immune responses and to assess their potential as therapeutically useful model systems to target inflammation in CF lung disease.
3. *Who's bad?* Are neutrophils, the most abundant inflammatory cell in CF airways, good or bad? If we deplete them, our first cellular shield against bacteria and fungi is lacking, but there is compelling evidence that neutrophil-derived products, mainly proteases, cause harm to the extracellular pulmonary matrix. Therefore, one might target neutrophil products, for instance proteases. However, these approaches showed limited success so far in clinical studies. As there are several functionally distinct phenotypes of neutrophils (Borregaard, 2010; Kolaczkowska and Kubes, 2013), one could target harmful subtypes while preserving protective ones. But which markers discriminate these subtypes? What is the role of myeloid-derived

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* Corresponding author. Tel.: +49 70712981460.
*E-mail address:* dominik.hartl@med.uni-tuebingen.de (D. Hartl).

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http://dx.doi.org/10.1016/j.biocel.2014.01.017

suppressor cells (MDSCs) that dampen overshooting T cell activation (Gabrilovich and Nagaraj, 2009)? What about macrophages? They are required as potent phagocytic cell type, clearing both pathogens and – maybe more important for advanced CF lung disease – apoptotic cells through efferocytosis (Vandivier et al., 2009). Macrophages can be subdivided into classical M1 and M2 macrophages, the latter being induced in a Th2-environment and driving fibrosis and extracellular matrix remodeling. Data on M1/M2 macrophage phenotypes in CF are controversial, so it's too early to target one of them, at least from our point of view. Data on other innate immune cell types, such as dendritic cells, natural killer (NK) cells, NKT cells and innate lymphoid cells (ILC) are scarce in the field of CF and additional studies are required to define their potential roles and CF disease relevance.

4. *Diagnostic consequences:* Which biomarkers could indicate patients who are prone to a rapid loss of lung function and should be monitored with greater caution? A recent study shows that free elastase in BAL fluid may represent such a predictive biomarker (Sly et al., 2013). However, BAL is hardly implementable in many clinical CF routines. Elastase in sputum could serve as an alternative, but is not – or hardly – feasible in infants below five years of age and requires standardized pre-analytical processing for reproducible biomarker analyses.
5. *Therapeutic consequences:* The cycle of inflammatory cell recruitment and unopposed immune cell activation causes tissue damage and leads to irreversible loss of lung function. Proteases and reactive oxygen species (ROS), released by infiltrating myeloid cells, are two key components in this cycle. Consequently, using anti-proteases and/or anti-oxidants are two mechanistic anti-inflammatory approaches in CF lung disease. However, several attempts to target these pathways were only partially successful, presumably due to (i) too low drug concentrations deposited at the pulmonary target site and (ii) inclusion of older CF patients with advanced lung disease characterized by irreversibly-remodeled and – damaged lung structure (Griese et al., 2008, Gaggar et al., 2011). What do we learn from this? How early do we have to start targeting proteases and oxidants to achieve clinical success? What about the compartmentalization of candidate drugs? It is critical that compounds reach their target in the midst of the acidic, oxidative, hyperviscous and hyperproteolytic micro-environment of the CF airway lumen. Are small-molecule compounds superior to natural protease inhibitors or do they show severe side effects as they also inhibit intracellular proteases that are involved in bacterial killing? Accordingly, it would make sense to engineer cell-impermeable compounds. All these issues have to be considered for new drug developments in CF lung disease.

In the section below, we summarize and discuss selected recent findings in the field of innate immunity in CF lung disease.

## 2. Immunity in cystic fibrosis: recent findings

### 2.1. CFTR in myeloid cells

Previous studies provided convincing evidence that human neutrophils express the CFTR protein, localized in the membrane of secretory vesicles (Painter et al., 2006). Optimal microbicidal activity of neutrophils relies on the generation of toxic agents such as hypochlorous acid (HOCl) within phagosomes (Hampton et al., 1998). HOCl formation, in turn, requires chloride ion transported from the cytoplasm into phagosomes mediated by chloride channels (Nauseef, 2007). Studies demonstrated that neutrophils pretreated with a CFTR inhibitor or siRNA-mediated CFTR knock-down in neutrophil-like HL-60 cells reduced $Cl^-$ transport into phagosomes and impaired killing of phagocytosed bacteria (Painter et al., 2006, 2010; Bonvillain et al., 2010). Therefore, CFTR seems to play a role in regulating antimicrobial neutrophil activities. Zhou et al. (2013) recently confirmed and extended these studies by showing that nearly all mature phagosomes of human peripheral blood neutrophils are CFTR positive. The authors further stably expressed enhanced green fluorescent protein (EGFP) together with wt-CFTR or dF508-CFTR, respectively, in a promyelocytic cell line, where EGFP-wt-CFTR associated with phagosomes. In contrast, significantly less dF508-CFTR was found in phagosomes, indicating a defective targeting of the molecule to the organelle. Notably, the CFTR corrector compound VRT-325 facilitated the recruitment of dF508-CFTR to phagosomes. When viewed in combination, these data demonstrated the possibility of pharmacologic correction of impaired recruitment of mutant CFTR to the phagosome. This approach might enhance the chloride supply into the phagosomes of neutrophils in CF patients and increase their antimicrobial function (Zhou et al., 2013). However, promyelocytic cell lines, such as HL-60 cells or PLB-985 cells, used in the latter study, are only partially adequate models to study phagocytosis and other neutrophil functionalities, since their vesicle and granule compositions differ from primary human neutrophils and are therefore restricted as cellular model systems when recapitulating the physiological sequence of vesicle and granule membrane fusion events involved in phagocytosis by mature neutrophils.

In contrast to patients with primary (inherited) immunodeficiencies in neutrophil function, such as chronic granulomatous disease, CF patients are not affected by recurrent invasive infections in general. Hence, the nature of neutrophil dysfunction in CF patients, has to be more subtle and is probably only of major disease relevance for (i) the extravascular pulmonary site of infection and for (ii) defined stages of host–pathogen interactions, an issue that, however, remains poorly understood. Recent bone marrow transplant studies corroborated the functional impact of myeloid *Cftr in vivo* (Su et al., 2011). Using conditional inactivation of *Cftr* in myeloid cells by means of a myeloid-targeted Cre-recombinase, Bonfield et al. demonstrated that the conditional "*myeloid CF mice*" displayed survival and inflammatory characteristics between wild-type and full genotypic *Cftr*−/− mice in *P. aeruginosa* infection models, depending on the stage of infection (Bonfield et al., 2012). In particular, the contribution of myeloid CFTR was most critical at later post-infection time points compared to the whole CFTR-deficient mice, supporting the notion that epithelial CFTR is involved in acute anti-bacterial host defense, while myeloid CFTR comes one step later when myeloid cells have entered the airways. Effects on other CF-associated phenotypes, such as epithelial ion transport, growth reduction or intestinal obstruction, as observed in the total absence of functional *Cftr*, were not detected in the myeloid-targeted conditional CF mouse model. In contrast to these studies, Oceandy et al. reported earlier in another CF mouse model that *Cftr* gene complementation in airway epithelium was sufficient to normalize pathogen clearance and inflammatory anomalies (Oceandy et al., 2002), suggesting that epithelial rather than myeloid CFTR is essential. The differing results may be due to the mouse models and the *Cftr* complementation/targeting methods used. Further comparative studies in mice and men are required to precisely define the contribution of myeloid CFTR to CF lung disease.

### 2.2. Myeloid-derived suppressor cells

Myeloid-derived suppressor cells are innate immune cells generated in cancer and pro-inflammatory microenvironments (Gabrilovich and Nagaraj, 2009). These specialized innate immune

Please cite this article in press as: Rieber N, et al. Current concepts of immune dysregulation in cystic fibrosis. Int J Biochem Cell Biol (2014), http://dx.doi.org/10.1016/j.biocel.2014.01.017