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Original Article

Protease–antiprotease imbalances differ between Cystic Fibrosis patients' upper and lower airway secretions

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Abstract

Background: Balanced levels of proteases and anti-proteases are essential in host defense systems. In CF patients' lungs, elevated protease/anti-protease-ratios contribute to damage of airway tissue and premature death with the inherited disease. Little is known about upper airway protease equilibrium in CF.
Methods: Neutrophil elastase (NE), Secretory leukocyte protease inhibitor (SLPI), matrix metalloproteinase (MMP)9, tissue inhibitors of metalloproteinase (TIMP)1, cathepsin S (CTSS) and the corresponding cellular distribution were assessed in the nasal lavage (NL) and sputum of 40 CF patients.
Results: Concentrations of all proteases and anti-proteases were markedly higher in sputum than in NL (NE: 10-fold, SLPI: 5000-fold). Interestingly, the NE/SLPI ratio was 726-fold higher in NL compared to sputum, while the MMP9/TIMP1 ratio was 4.5-fold higher in sputum compared to NL.
Discussion: This first study to compare protease/anti-protease networks of CF upper and lower airways by NL and sputum reveals substantial differences between both compartments' immunological responses. This finding may have implications for sinonasal and pulmonary treatment, possibly leading to new therapeutic approaches.

Keywords: Protease; Neutrophil elastase; SLPI; Cathepsin; Nasal lavage; Sinonasal; Lung

Abbreviations: BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; BOS, bronchiolitis obliterans syndrome; CF, Cystic Fibrosis; CRS, chronic rhinosinusitis; CTSS, cathepsin S; ECM, extracellular matrix; IFN, interferon; IL, interleukin; LAW, lower airways; LTx, lung transplantation; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; MN, mononuclear; NE, neutrophil elastase; NLF, nasal lavage fluid; NL, nasal lavage; PMN, polymorphonuclear; SLPI, secretory leukocyte protease inhibitor; TCC, total cell count; TIMP, tissue inhibitors of metalloproteinase; TNF, tumor necrosis factor; UAW, upper airways; DTT, Dithiothreitol; $PA^{-}SA^{-}$, not colonized with *P. aeruginosa* or *S. aureus*; $PA^{-}SA^{+}$, not colonized with *P. aeruginosa* but with *S. aureus*; $PA^{+}SA^{-}$, colonized with *P. aeruginosa* but not with *S. aureus*; $PA^{+}SA^{+}$, colonized with *P. aeruginosa* and *S. aureus*.

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Table 1
Adapted from Fischer et al. [25].

Clinical characteristics (total n = 40)	
Sex female	21 (52.2%)
Mean age, yrs	27 (5–72)
Mean FEV1, % pred.	68 (19–120)
Mean BMI *	21 (16.2–26.0)
Pancreatic insufficiency	31 (77.5%)
Diabetes	15 (37.5%)
Allergy in the patient's history	26 (65%)
Allergic rhinitis in the patient's history	18 (45%)
ABPA	3 (7.5%)
CRS (according to EPOS criteria)	14 (35%)
History of ENT surgery	19 (47.5%)
Chronic inflammation (elevated IgG)	17 (42.5%)
Acute inflammation (elevated CrP and ESR)	6 (15%)
Chronic colonization with *P. aeruginosa*	
UAW	12 (30%)
LAW	18 (45%)
Chronic colonization with *S. aureus*	
UAW	11 (27.5%)
LAW	18 (45%)
Therapy	
Inhaled steroids	20 (50%)
Topical steroids	13 (32.5%)
Azithromycin	23 (57.5%)

* Mean calculated for adult study population, BMI calculated for patients under 18 y (n = 9) according to Kromeyer-Hauschild, mean percentile 30.9 (3–90).

1. Introduction

Equilibrated regulation of proteases and cytokines facilitates effective host defense against pathogens without affecting host tissues. In Cystic Fibrosis (CF), a common, recessively-inherited disease in western countries, an imbalance of protease–antiprotease networks in the airways contributes to tissue injury and chronic progressive lung disease as primary reason for premature death. The pathological vicious circle of defective ion channel function, viscous mucus in the airways, chronic bacterial infections, e.g. by gram-negative bacteria like *Pseudomonas* (*P.*) *aeruginosa* and chronic inflammation, is a hallmark of the disorder [1,2]. The resulting overexpression as well as enhanced and prolonged activity is known for several proteases in CF lower airways (LAW), whereas upper airways (UAW) and paranasal sinuses were out of focus. Recent studies provided evidence that upper airways and the paranasal sinuses in particular can be sites of primary pathogen acquisition and persistence [3,4].

The serine protease neutrophil elastase (NE) is released by azurophilic granules of neutrophils stimulated by cytokines and chemoattractants, especially interleukin 8 (IL8) and TNF [5]. As an important host defense mechanism, NE cleaves cell surface structures, especially the flagella of gram-negative bacteria like *P. aeruginosa* [6]. NE can be detected in nasal secretions [7], and elevated concentrations have been found in nasal secretions and serum of non-CF patients with RSV infections [8]. In the lungs of CF patients, NE is overexpressed and overactive in comparison to healthy probands.

Interestingly, besides inactivating pathogens elevated NE levels impair the innate and adaptive immune systems by cleavage of host defense proteins like secretory leukoprotease inhibitor (SLPI) [9] and tissue inhibitor of metalloproteinase (TIMP)1 [10] in the airway surface liquid (ASL), Fig. S2. Additionally, NE cleaves T cell surface receptors like CD2, CD4 and CD8. The activity of NE is strictly controlled by a complex network of other proteases and anti-proteases [11]. Within the latter, the serine protease SLPI is the major anti-protease of NE, especially in the upper respiratory tract [12]. SPLI levels are found to be increased during infection or in lungs of smokers. It is postulated that SLPI disrupts the microbial cell membrane via its cationic charge and is therefore an important player in host defense mechanisms against *P. aeruginosa* and *Staphylococcus* (*S.*) *aureus*. Additionally, as anti-inflammatory protein, SLPI decreases matrixmetalloproteinase (MMP)1 and 9 expressions in monocytes. SLPI itself is cleaved by proteases like cathepsins and it also can be downregulated by cytokines like IFNγ [9].

CTSS is a cysteine protease. Cathepsins are synthesized as inactive zymogens and released by lysosomes after stimuli. Cathepsins play an important role in immune responses against microbiological pathogens. Furthermore, especially CTSS is involved in major histocompatibility complex (MHC) class II maturation and antigen presentation. CTSS also influences the innate immunity of the lung. Surfactant protein A can be cleaved by CTSS, which can impair antibacterial activity [13]. CTSS cleaves and inactivates SLPI, which impairs inhibition of NE activity by SLPI [14], Fig. S2. Additionally, CTSS plays a role in extracellular matrix (ECM) degradation, which leads to lung destruction and emphysema. Therefore, the development of cathepsin inhibitors is an important target for new therapeutic approaches [15]. There is very little knowledge about cathepsins in CF. In the sputum of CF patients with chronic *P. aeruginosa* infection, significant correlations between CTSS expression levels and markers of inflammation like NE, IL8 and TNF were observed [16]. To our knowledge, CTSS in the upper airways has not been investigated.

MMP9 affiliates to a cluster of enzymes, which are involved in physiological processes like embryological development and tissue remodeling, due to breakdown of extracellular matrix proteins like collagen and elastin [17]. In healthy organisms, MMP function is strictly under the control of TIMPs. TIMP1 binds to both the precursor and active form of MMP9 in a ratio of 1:1 and inhibits its activation [17]. In the sputum and bronchoalveolar lavage (BAL) of CF patients, MMP9 levels are reported to be elevated when compared to healthy controls [18]. Furthermore, during acute exacerbations, MMP9 activity is enhanced in CF patients, whereas TIMP1 is degraded and therefore, the MMP9/TIMP1-ratio is elevated [19]. It is postulated that NE cleaves and activates MMP9 and degrades TIMP1 [19,20], Fig. S2. In the upper airways, MMP9 mRNA was detected in higher levels in non-CF patients with chronic rhinosinusitis (CRS) with polyps compared to CRS without polyps, while TIMP1 mRNA was not detected in either group [21]. MMP9/TIMP1 levels were also investigated in nasopharyngeal secretions of RSV-infected infants, but no correlation between exacerbation and mediator levels was observed [22]. In vitro nasal epithelial