Chitinase activation in patients with fungus-associated cystic fibrosis lung disease

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Background: Chitinases have recently gained attention in the field of pulmonary diseases, particularly in asthma and chronic obstructive pulmonary disease, but their potential role in patients with cystic fibrosis (CF)–associated lung disease remains unclear.

Objective: The aim of this study was to assess chitinase activity systemically and in the airways of patients with CF and asthma compared with healthy subjects. Additionally, we assessed factors that regulate chitinase activity within the lungs of patients with CF.

Methods: Chitinase activities were quantified in serum and bronchoalveolar lavage fluid from patients with CF, asthmatic patients, and healthy control subjects. Mechanistically, the role of CF airway proteases and genetic chitinase deficiency was assessed.

Results: Chitinase activity was systemically increased in patients with CF compared with that in healthy control subjects and asthmatic patients. Further stratification showed that chitinase activity was enhanced in patients with CF colonized with *Candida albicans* compared with that in noncolonized patients. CF proteases degraded chitinases in the airway microenvironment of patients with CF. Genetic chitinase deficiency was associated with *C albicans* colonization in patients with CF.

Conclusion: Patients with CF have enhanced chitinase activation associated with *C albicans* colonization. Therefore chitinases might represent a novel biomarker and therapeutic target for CF-associated fungal disease. (J Allergy Clin Immunol 2016;138:1183-9.)

Key words: *Cystic fibrosis, lung disease, chitinases, chitotriosidase, fungi,* Candida *species, neutrophils, proteases*

Chitin, the second most abundant polysaccharide in nature, is a component of insects, crabs, and fungi.[1] Chitin is degraded by chitinases, enzymes that protect lower life forms from chitin-bearing organisms. Unexpectedly, chitinase activities are readily detectable in several body fluids in human subjects, particularly in patients with metabolic or pulmonary diseases characterized by chronic inflammation.[2] Human subjects express 2 active chitinases, chitotriosidase and acidic mammalian chitinase (AMCase), with chitotriosidase regarded as the main active chitinase at host-pathogen interaction sites, such as the lung.[3-6]

Chitinases have recently gained attention in the field of pulmonary disease.[1,7,8] Studies provided evidence for a potential role of AMCase in asthmatic patients[9] and chitotriosidase in patients with chronic obstructive pulmonary disease (COPD).[10] A recent study further demonstrated that chitotriosidase levels were increased in both asthmatic patients and patients with COPD.[11] Chitotriosidase activity is regulated by genotype,[6] and *in vitro* studies provided evidence that innate immune cells, mainly neutrophils and macrophages, represent the key source of chitotriosidase.[12,13] We demonstrated previously that the chitinase-like protein YKL-40 accumulates in airway fluid from patients with cystic fibrosis (CF)–associated lung disease,[14] but the potential role of true enzymatically active chitinases in patients with CF remains to be determined.

CF-associated lung disease is characterized by neutrophilic inflammation and chronic infections with bacterial and fungal pathogens.[15-17] On the basis of chronic colonization with chitin-bearing fungi,[18] we hypothesized that chitinase activities might reflect the extent of neutrophilic inflammation and fungal colonization in patients with CF-associated lung disease. Therefore we studied activities of the main human chitinase (chitotriosidase) in the circulation and in airway fluids from patients with CF-associated lung disease and allergic asthma. Functionally, we studied the effect of CF airway proteases on chitinases and the relevance of genetic chitinase deficiency in a large cohort of patients with CF. For the first time, our studies demonstrate that chitinase activity is increased in patients with CF and associated with fungal colonization.

METHODS

Additional methods are described in detail in the Methods section in this article's Online Repository at www.jacionline.org.

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Abbreviations used
AMCase: Acidic mammalian chitinase
BALF: Bronchoalveolar lavage fluid
CF: Cystic fibrosis
CFTR: Cystic fibrosis transmembrane conductance regulator
COPD: Chronic obstructive pulmonary disease
RCSI: Royal College of Surgeons in Ireland

Patient cohorts

Chitinase activities were analyzed in sera and bronchoalveolar lavage fluid (BALF) from patients with CF, asthmatic patients, and healthy control subjects (Table I). Patients with CF were from the CF Center Tübingen (n = 56), Germany, and the CF center at the Royal College of Surgeons in Ireland (RCSI), Dublin (n = 49), Ireland. Asthmatic patients, patients with Gaucher disease, and control subjects were from the German center. All patients were recruited from the respective outpatient clinics. Inclusion criteria for CF were the diagnosis of CF based on clinical symptoms and positive sweat test results (sweat Cl^- concentration >60 mmol/L) or the presence of disease-causing mutations in the cystic fibrosis transmembrane conductance regulator *(CFTR)* gene, FEV_1 of greater than 30% of predicted value, and being clinically stable and receiving concomitant therapy at least 4 weeks before the study. Fungal colonization was defined as described previously in detail.[19] Colonization was defined as intermittent or chronic, according to Chotirmall et al.[19] The asthma group included patients with allergic asthma. The inclusion criteria were recurrent episodes of wheezing and objective evidence of asthma, as indicated by β_2-agonist–reversible airflow obstruction (≥12% and at least 200-mL improvement in FEV_1 percent predicted), bronchial hyperresponsiveness (exercise challenge), and 20% or greater intraday peak flow variability. All asthmatic patients used inhaled bronchodilators, and 9 asthmatic patients used inhaled corticosteroids. Spirometry and flow-volume curves were performed according to American Thoracic Society guidelines.[20] Seventeen control subjects without pulmonary diseases were selected as the control group. These subjects had no suspected or proved pulmonary disease and were free of respiratory tract infections. BALF was obtained, processed, and stored, as described previously.[21,22] The obtained BALF was filtered through 2 layers of sterile gauze. Sample processing was performed immediately on ice. After centrifugation (200*g* for 10 minutes), the supernatant was stored at −80°C until analysis. This study was approved by the institutional review boards/ethical committees of the University of Tübingen and the RCSI Dublin. Informed consent was obtained from all participants or, in the case of children, provided by their parents. For genotyping, DNA samples from 521 patients with CF from a previously described European cohort of patients with CF were used, which is described in detail in previous publications (see Table E1 in this article's Online Repository at www.jacionline.org for details).[23,24]

Chitinase substrate assays

Chitinase activities were quantified by using a commercially available substrate assay (Sigma-Aldrich, St Louis, Mo).

RESULTS

Chitinase activity in patients with CF

Chitinase activities were significantly increased in sera from patients with CF compared with those in healthy control subjects, as well as compared with asthma subjects (Fig 1, *A*). As a positive control, we included patients with a deficiency of glucocerebrosidase (glucosylceramidase; Gaucher disease), a lysosomal storage disease with chitinase activity used clinically as a serum biomarker.[25] Because we observed a broad range of chitinase activities in patients with CF, we searched for factors that were associated with chitinase activities in our CF cohort. Correlation analyses revealed that among all CF-related factors, including age, sex, genotype, bacterial/fungal colonization status, body mass index, and CF medications, only colonization with *Candida albicans* showed a statistically significant association with serum chitinase activities ($P < .01$). Based on this correlation, we stratified our patients with CF into those who were and were not colonized with *C albicans*, according to previously established criteria.[19] These studies demonstrated that chitinase activities were significantly increased in *C albicans*–colonized patients with CF compared with those in noncolonized patients with CF (Fig 1, *B*).[19] In contrast to *C albicans*, patients colonized with the fungus *Aspergillus fumigatus* did not show increased chitinase activities compared with those in patients without *A fumigatus*. Next, we analyzed chitinase activities in BALF from patients with CF. In line with previous reports in patients with COPD,[6] these studies showed that chitinase (chitotriosidase) activities were readily detectable in BALF from patients with CF (Fig 1, *C*). Consistent with serum activities, chitinase activities in BALF were also increased in *C albicans*–colonized compared with noncolonized patients with CF (Fig 1, *C*). In contrast and consistent with our serum results, BALF chitinase activities did not differ significantly between *A fumigatus*–colonized and noncolonized patients with CF (Fig 1, *C*). Neither antibiotic nor steroid use showed a statistically significant correlation with systemic ($P > .05$ and $P > .05$, respectively) or BALF ($P > .05$ and $P > .05$, respectively) chitinase activities. When viewed in combination, these data illustrate that chitinase activity is increased in patients with CF and is associated with fungal *C albicans* colonization.

Proteases cleave chitinases in the airway microenvironment of patients with CF

Because chitinase release is a protective mechanism to defend against chitin-containing organisms, such as fungi, we sought to investigate the factors in the airway microenvironment of patients with CF that modulate chitinase activities. Airway neutrophils in patients with CF release proteases that degrade a variety of proteins[26,27] and are proposed to be predictive biomarkers in early CF-associated lung disease.[28] Because neutrophilic inflammation in the microenvironment of patients with CF leads to an accumulation of both proteases and chitinases, we analyzed their interaction by assessing whether proteases, which were typically found in the airways of patients with CF, have an effect on recombinant chitinases. These studies demonstrated that neutrophil elastase, cathepsin L, and cathepsin B degraded recombinant chitinase protein *in vitro* in a time-dependent manner (Fig 2, *A*, upper panel). To assess the relevance of this proteolytic effect for airway fluids of patients with CF *ex vivo*, we incubated BALF from patients with CF with recombinant chitinase (Fig 2, *A*, lower panel). These studies confirmed our findings illustrating that BALF from patients with CF degraded chitinase protein, particularly BALF obtained from adults with CF with a high elastolytic activity (>0.03 μmol/min/mL) compared with pediatric BALF with a low elastolytic activity (<0.001 μmol/min/mL). Pretreatment of BALF from patients with CF with serine protease or cathepsin inhibitors was able to rescue chitinases from BALF-mediated degradation of patients with CF (Fig 2, *A*, lower panel). To further evaluate the *in vivo* relevance of this mechanism, we quantified both chitinase and elastase activities in BALF from patients with CF (Fig 2, *B*). We focused on elastase because it upregulates other proteases,