Matrix metalloprotease-9 dysregulation in lower airway secretions of cystic fibrosis patients

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Matrix metalloproteases (MMPs) are proteolytic enzymes that regulate extracellular matrix turnover and aid in restoring tissue architecture following injury. There is an emerging role for extracellular matrix destruction in the pathogenesis of chronic neutrophilic lung diseases. In this study, we examined the expression and activity profiles of MMPs in lower airway secretions from cystic fibrosis (CF) patients, patients with acute respiratory failure (ARF), and normal controls. A discrete repertoire of MMP isoforms was found in the CF samples, with robust MMP-9 expression compared with normal controls and ARF. CF samples possessed increased levels of active MMP-9, as well as decreased amounts of tissue inhibitor of metalloprotease-1 (TIMP-1), a natural inhibitor of MMP-9. The CF inpatient samples demonstrated fully active MMP-9 activity compared with CF outpatients, ARF, and normal controls. CF samples also demonstrated increased human neutrophil elastase (HNE) levels compared with ARF and normal controls. To examine potential mechanisms for the protease dysregulation seen in the CF clinical samples, in vitro studies demonstrated that HNE could activate pro-MMP-9 and also degrade TIMP-1; this HNE-based activation, however, was not seen with MMP-8. A strong correlation was seen between HNE and MMP-9 activity in CF inpatient samples. Finally, the dysregulated MMP-9 activity seen in CF inpatient sputum samples could be significantly reduced by the use of MMP-9 inhibitors. Collectively, these findings further emphasize the proposed pro tease/antiprotease imbalance in chronic neutrophilic lung disease, providing a potential mechanism contributing to this proteolytic dysregulation.

A limited number of studies have examined MMPs in CF lung disease. An earlier study suggested an increase in MMP-9 activity in CF sputum compared with asthmatic subjects and normal control subjects (8). Dunsmore et al. (12) reported that MMP-7 was elevated in CF airway epithelium compared with normal controls and that this was localized predominantly to alveolar type II cells. Ratjen and colleagues (24) examined bronchoalveolar lavage fluid from CF patients and found increased MMP-8, MMP-9, and MMP-9-to-TIMP-1 ratios in children with CF compared with healthy controls. A recent report by Sagel (27) demonstrated excessive MMP-9 expression in the lower airway secretions of CF patients, and a secondary regulation provided by direct interactions with proteins, such as tissue inhibitors of MMPs (TIMPs) and α1-antitrypsin. MMP-8 and -9 are stored and released from neutrophil granules (32), and MMP-7 is expressed and released from the airway epithelium, where it serves an important role in epithelial repair (20). Left unchecked, MMPs have the capacity to degrade essentially all components of the extracellular matrix, potentially contributing to the tissue destruction and dysfunc tion seen in a variety of chronic lung diseases (29). Structural airway changes are a prominent feature of cystic fibrosis (CF), a chronic genetic disorder in which abnormal ion transport predisposes patients to infection and neutrophilic inflammation (26). The widening of airways and loss of normal mucociliary clearance mechanisms leads to the development of bronchiectasis (7). These structural changes are a direct cause of the pulmonary morbidity and mortality seen in the vast majority of CF patients and are felt to be a consequence of unrelenting airway inflammation in the disease (19). Previous studies have demonstrated increased sputum neutrophil accumulation in CF patients vs. normal and disease controls, with inverse relationships between neutrophil counts and lung function (21, 23, 28). Neutrophil influx occurs early in CF lung disease, with a significant burden seen in many CF infants before established infection with CF pathogens (7, 19, 21). Neutrophils are the predominant source of human neutrophil elastase (HNE), a well-described serine protease that contributes to CF lung disease. HNE expression and activity are elevated in CF sputum with reduced levels of compensatory antiproteases, such as secretory leukoproteinase inhibitor and α1-antitrypsin (19, 28). This excessive and untempered protease activity can disrupt bacterial clearance and degrade elastin and contributes to a perpetuation of airway inflammation and destruction (3, 34).

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negative correlation between MMP-9 levels and lung function (forced expiratory volume in 1 s (FEV₁)).

In this report, we examined the protease/antiprotease balance with regard to MMP activity in CF lung disease compared with normal subjects and subjects with acute respiratory failure (ARF). We hypothesized that CF patients had a unique sputum MMP profile compared with ARF and normal control individuals, and that CF inpatient samples demonstrated increased proteolytic activity compared with CF outpatient samples. A unique MMP profile, including neutrophil-derived MMP-8 and -9, was identified in the majority of CF samples, with particularly elevated MMP-9 activity demonstrated. HNE activity was also high in CF samples relative to control, with a notable correlation demonstrated between HNE and MMP-9 activity in CF inpatient samples. Investigating potential relationships between these proteases, our studies demonstrate HNE is sufficient to activate MMP-9 and degrade TIMP-1 at physiological concentrations seen in CF secretions. Finally, MMP-9 activity in CF samples could be reduced by specific and nonspecific small molecule inhibitors. Together, the results provide support for an important regulatory relationship between HNE and MMP-9 and provide mechanistic evidence for the dysregulated MMP-9 activity observed in CF.

METHODS

Patient Samples

University of Alabama at Birmingham Institutional Review Board approval was obtained before all studies involving human subjects and samples. Informed consent was obtained from all subjects or caregivers of subjects with CF and/or ARF. Samples from normal controls were collected from normal saline and centrifuged at 1,000 rpm for 10 min with separation of pellet from supernatant. The supernatant was collected, protein concentration was measured, and then separate aliquots were saved for measurements (−80°C).

Protein Detection

All samples were electrophoresed through SDS-polyacrylamide gels (both reducing and nonreducing conditions) and electroblotted onto Immobilon-P polyvinylidene failure membranes. Membranes were blocked in Tris buffer (pH 7.4) containing 5% powdered milk.

Table 1. Demographic information of study groups

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<th>Parameter</th>
<th>Normal Controls</th>
<th>Acute Respiratory Failure</th>
<th>CF Inpatient</th>
<th>CF Outpatient</th>
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<tr>
<td>n</td>
<td>14</td>
<td>26</td>
<td>33</td>
<td>11</td>
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<tr>
<td>Mean age (range)</td>
<td></td>
<td>7.1±1.3 yr (1 mo-19 yr)†</td>
<td>22.1±1.3 yr (9–34 yr)</td>
<td>25.8±2.21 yr (15–40 yr)</td>
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<td>13:20</td>
<td>4:7</td>
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<td>FEV₁ = 41.7 (3.60) A*</td>
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<td>(P = 0.006)</td>
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<td></td>
<td>FEV₁ = 54.3 (6.41) D</td>
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<td>PA⁺ (5)</td>
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for 1 h. Once washed, they were incubated with primary antibody (anti-MMP or anti-TIMP) overnight at 4°C. After incubation, samples were washed with borate saline (100 mM boric acid, 25 mM sodium borate, 75 mM NaCl) and incubated with secondary antibody for 1 h. Species-specific IgG horseradish peroxidase conjugates were used as secondary antibody at dilutions of 1:5,000. Immunoblots were then developed using enhanced chemiluminescent kits (GE Healthcare, Piscataway, NJ). Unless otherwise stated, 16 μg of sample were loaded for each immunoblot lane.

MMP Activity and TIMP Quantification Assays

Briefly, MMP-8- and MMP-9-specific ELISA-based activity assays were used to quantify specific MMP activity (R&D Systems, Minneapolis, MN). Samples were diluted to fit manufacturer’s sensitivity for individual kits (0–25 ng/ml for MMP-8 and 0–16 ng/ml for MMP-9 kits). Both samples and recombinant enzyme standards were prepared and incubated for 2 h at room temperature in 96-well plates coated with monoclonal antibodies for the MMP(s) (MAB 908 for MMP-8 and MAB 911 for MMP-9) of interest. After incubation, samples and standards were activated with 1 mM aminophenylmercuric acetate (APMA), a chemical activator of MMPs, and further incubated for 2 h at 37°C. After incubation, a fluorogenic substrate (Fluor-Pro-Leu-Gly-arginine methyl ester, a chemical activator of MMPs, and further incubated for 2 h at 37°C. After incubation, a fluorogenic substrate (Fluor-Pro-Leu-Gly-arginine methyl ester) was then added, and the plate was incubated at 37°C for 18 h. The plate was then read on a spectrofluorometer (SpectraMax Gemini, Molecular Devices, Sunnyvale, CA; excitation and emission wavelength of 320 and 405 nm, respectively), and data were quantified using standard curves provided with the kits. Standardized kits for measuring the activity and concentration of other MMPs identified during this study were not available.

For the studies of TIMP-1, samples were diluted to fit manufacturer’s sensitivity for ELISA (0–10 ng/ml). Both samples and recombinant TIMP-1 standards were prepared and incubated for 2 h at room temperature in 96-well plates coated with TIMP-1 monoclonal antibodies. Bound TIMP-1 was then conjugated with a horseradish peroxidase-based secondary antibody for 1 h. A colorimetric substrate (hydrogen peroxide and chromagen) was placed in each well, and color change was assessed after 30 min. Color changes were quantified on a colorimeter (Bio-Rad, Hercules, CA) via standard curves provided with the kits.

HNE Assays

Briefly, HNE-specific ELISA activity kits were used to quantify HNE concentrations in clinical samples (Calbiochem, San Diego, CA). Samples were diluted to fit manufacturer’s kit specificity (0–20 ng/ml). Both samples and recombinant HNE standards were prepared and incubated for 2 h at room temperature in 96-well plates coated with a monoclonal antibody for HNE. A fluorogenic substrate (MeO-Succ-Ala-Ala-Pro-Val-AMC) was then added, and the plate was incubated for 2 h. Finally, the plate was read on a spectrofluorometer (SpectraMax Gemini, Molecular Devices; excitation and emission wavelength of 360 and 40 nm, respectively), and data were quantified using standard curves provided with the kits.

MMP-8 and MMP-9 Activation

For in vitro studies involving activation of pro-MMPs with APMA, 1 mM APMA (R&D Systems) was incubated with recombinant pro-MMPs (R&D Systems) in a 37°C incubator for 2 h. For pro-MMP activation with HNE, recombinant pro-MMPs were incubated with specified concentrations of recombinant HNE (Calbiochem) at 37°C for 2 h.

Statistical Analysis

Descriptive statistics, including mean and SE, were determined for all continuous data. An exponential correlation was generated between HNE and MMP-9 activity. Paired and unpaired t-tests were used for comparisons of MMP, HNE, and TIMP activities utilizing SigmaStat statistical software (Jandel). A P value ≤0.05 was used to determine statistical significance. As this was an exploratory study to examine MMP expression and activity in the three study groups, no formal power calculations were performed in advance of sample collection.

Material

All primary monoclonal antibodies and recombinant MMPs were purchased through either R&D Systems or Chemicon (Temecula, CA). HNE, HNE-specific inhibitor N-[2-[4-(2,2-dimethylpropionyl)oxy]phenylsulfonylamino]benzoyl amine-2-carboxylic acid N-[o-(p-ivaloxybenzene)sulfonylamino]benzoyl l-arginine methyl ester (catalog no. 324759), and MMP-9 inhibitor (C27H33N3O5S anthranilic acid) were purchased via Calbiochem.

RESULTS

Demographic information summarizing the study groups is shown in Table 1. CF samples were derived from patients who were hospitalized for uncomplicated pulmonary exacerbations (n = 33) and patients seen in outpatient clinics as part of routine (“well”) follow-up visits (n = 13). ARF samples (n = 26) included pooled samples during days 1–5 of intubation, and control samples (n = 26) were obtained from subjects undergoing routine outpatient surgical procedures (no known lung disease). As expected, the CF study groups were significantly older than the pediatric ARF subjects (P < 0.05). Both inpatient and outpatient CF groups had a female predominance. The inpatient CF group was enriched for severe mutations (particularly delta F508 homozygous mutation) and had significantly reduced lung function [FEV1, forced vital capacity (FVC)] at the beginning of hospitalizations relative to the CF outpatient controls (P < 0.01). All CF inpatient and outpatient subjects were pancreatic insufficient, as judged by their treating physician.

MMP-9 Activity is Increased in the Airway Secretions of CF Inpatients

Using specific antibodies to detect various MMP isoforms (MMP-1, -2, -3, -7, -8, -9, -10, -11, -12, -13, -19, -20, -26, -27, -28), screening Western blots from CF and ARF samples were performed. Positive controls with recombinant enzymes were utilized for all screened MMPs. CF patient samples demonstrated detectable MMP-8, MMP-9, MMP-11, MMP-12, and TIMP-1 expression; ARF samples demonstrated a similar pattern with detectable MMP-2, -8, -9, -11, and TIMP-1. Normal controls had only sporadic staining for MMP-8, MMP-9, and TIMP-1. Together, the results point toward a limited repertoire of detectable MMP isoforms in the lower airways of the two disease states with MMP-8 (Fig. 1A) and MMP-9 (Fig. 1C), demonstrating increased staining relative to other isoforms in both CF and ARF. To quantify MMP activity, we performed MMP-9 and MMP-8 activity assays. MMP-9 activity was in general higher in CF inpatient samples compared with ARF (Fig. 1D), and all disease groups demonstrated high activity (three- to sevenfold increases) relative to the normal controls. Parallel studies were performed to compare MMP-8 activity in CF and control samples (Fig. 1B). MMP-8 activity was elevated in the ARF samples compared with normal controls, and both populations had elevated MMP-8 activity compared with CF patients. Of note, MMP-9 activity was elevated relative to MMP-8 activity within each disease group.
**MMP-9 Activity Does Not Correlate With Clinical Disease Measures**

Among CF subjects, no clear change in sputum MMP-9 activity could be detected within the inpatient CF group across the period of hospitalization (mean MMP-9 activity = 1,213.5 ± 369 ng/mg on day 0–1 samples, mean MMP-9 activity = 1,376.8 ± 314.1 ng/mg on days 10–14), despite clear improvements in lung function (FEV₁ or FVC) (Table 1). There was also no correlation with MMP-9 activity and lung function in CF outpatient samples (data not shown). In addition, no clear association between sputum microbiology, gender, age, or disease treatment and MMP-9 activity was identified in either of the CF groups (data not shown). A positive correlation between MMP-9 and the pulmonary inflammatory biomarker HNE was seen (and is discussed in further detail below).

**MMP-9 in CF Inpatient Sputum is Constitutively Active**

Since MMP-9 activity was increased in CF sputum, we examined whether MMP-9 regulation was maintained in CF lower airway secretions. MMP-9 activity is normally limited in part by secretion of its zymogen form, followed by conversion from proenzyme to active MMP-9 in vivo. Utilizing 1 mM APMA, a typical activator of pro-MMP-9 for in vitro studies, the data showed that MMP-9 was fully and constitutively active in CF inpatient samples relative to clinically stable CF outpatients and controls (Fig. 2). Specifically, MMP-9 function was enhanced by 57 ± 29.1% and 52 ± 8.2% following chemical stimulation with APMA in normal controls and ARF patients, respectively. In contrast, less APMA-induced activation of MMP-9 was seen in CF outpatient samples (39 ± 18.0%), and this activation was absent in samples from CF inpatients (2.6 ± 5.9% over basal activity, *P* < 0.01 compared with normal controls).

**HNE Activates MMP-9 But Not MMP-8 In Vitro**

To examine if relationships between HNE and MMP-8 or MMP-9 were present in CF sputum, we compared HNE activity across the study populations and confirmed that HNE levels were elevated in CF inpatient and outpatient sputum samples compared with normal controls (Fig. 3A). We next examined whether HNE had stimulatory effects on
pro-MMP-9 and pro-MMP-8zymogen proteins. In functional studies, purified pro-MMP-8 was readily activated by the nonspecific chemical stimulus APMA, but failed to demonstrate activation following incubation with increasing concentrations of HNE (Fig. 3B). In contrast, pro-MMP-9 was activated by all concentrations of HNE, with maximal activation seen following incubation with 2.5 ng/ml of HNE (Fig. 3C). In additional control experiments, activation of pro-MMP-9 by HNE was reduced by 47 ± 3.2% following cotreatment with an HNE-specific inhibitor (P < 0.01), while APMA activation of pro-MMP-9 was insensitive to similar treatment (supplemental figure 1; the online version of this article contains the supplemental data). The concentrations of HNE capable of activating pro-MMP-9 were within the range detected in CF inpatient sputum samples (Fig. 3A). HNE incubation had little effect on pro-MMP-8, as all protein remained in its inactive zymogen form (Fig. 3D, top). In contrast, increasing concentrations of HNE selectively depleted the pro-MMP-9 band (92 kDa) relative to active MMP-9 enzyme (78 kDa) (Fig. 3D, bottom).

MMP-9 and HNE Activity Correlates In Vivo

Our in vitro (Fig. 3) results demonstrated that HNE alone was sufficient to activate pro-MMP-9 in vitro at physiological concentrations of HNE. Figure 4 demonstrates a close exponential correlation between HNE and MMP-9 in CF inpatient samples (n = 16), with a correlation coefficient (R^2) of 0.74. In contrast, neither MMP-9 nor HNE activity correlated with measures of lung function (FEV1, FVC) in both CF inpatient and outpatient samples (R^2 for MMP-9/FEV1 = 0.01, MMP-9/FVC = 0.01 for CF inpatient samples; R^2 for HNE/FEV1 = 0.1, HNE/FVC = 0.1 for CF outpatient samples).

TIMP-1 Concentration is Reduced in CF Lung Disease

MMP-9 is also known to be negatively regulated by TIMP-1, a naturally occurring MMP inhibitor. Figure 5A compares TIMP-1 immunoblots of CF and control sputum, confirming variable TIMP-1 detection in all conditions. TIMP-1 concentrations in the CF specimens were depleted relative to control specimens, as quantified by ELISA (Fig. 5B). When a ratio of total active MMP-9 to TIMP-1 concentration [an established measure of MMP-9 regulation (27, 36)] was calculated for samples in each group, ratios from CF inpatients and outpatients were significantly increased compared with ARF (Fig. 5C). Together, these findings suggest that loss of MMP-9 inhibition by TIMP-1 may contribute to the increased MMP-9 activity seen in CF sputum samples.

TIMP-1 is Degraded by HNE In Vitro

TIMP-1 has been previously shown to serve as a substrate for HNE activity (17, 22). To confirm this as another possible mechanism for MMP-9 dysregulation in CF, recombinant TIMP-1 was incubated with HNE for increasing time periods (Fig. 6). HNE (27 kDa) and TIMP-1 (28 kDa) are shown in Fig. 6 (lanes H and T, respectively). No change in HNE bands was seen through the course of the experiments (24-h coinubation). In contrast, TIMP-1 bands were rapidly degraded by coinubcation with HNE, directly demonstrating that active HNE is sufficient to degrade TIMP-1.

MMP-9 in CF Sputum is Sensitive to MMP Inhibitory Molecules

The results shown in Figs. 1–4 indicate that MMP-9 is expressed at high levels and is dysregulated in CF sputum, with constitutive activity seen relative to both ARF and normal control samples. To examine whether excessive MMP-9 activity in CF sputum could be reduced or normalized, samples were treated with various established MMP-9 inhibitory molecules (31). MMP-9 activity in CF sputum was blocked following treatment with EDTA (1 mM), doxycycline (an orally available antimicrobial, 100 µg/ml), and MMP-9 inhibitor [C2H33NiO5S (Calbiochem, catalog no. 444278), a commercially available specific inhibitor of MMP-9, 50 ng/ml] (Fig. 7). The results confirm that the MMP-9 inhibitory effects of these small molecules are retained in CF sputum samples, providing a potential avenue to restore regulation to this protease in vivo.

DISCUSSION

In this paper, we examined the expression of MMP isoforms in CF lower airway secretions compared with pediatric patients with ARF and normal controls. A distinct pattern of MMP isoform expression was observed in both CF and ARF patient populations, with neutrophil-derived MMPs (MMP-8 and -9) demonstrating prominent expression. MMP-9 was the predominant MMP isoform identified in CF samples, with threefold increased activity in CF outpatient and sevenfold increased activity in CF inpatients compared with normal controls. Interestingly, MMP-9 was constitutively active in CF inpatient samples, demonstrating little inducible zymogen in these samples relative to the other disease conditions, and pointing...
toward an important difference in the proteolytic profile seen in CF inpatients.

HNE, a well-established proinflammatory serine protease, demonstrated 40-fold increased activity in CF inpatient samples compared with normal controls (n = 10) (*P < 0.01 CF inpatients vs. normal control). To examine possible contributions of high HNE activity to increased MMP-9 activity seen in vivo, we tested whether regulatory relationships between HNE and pro-MMP-9 could be demonstrated in vitro. Our experiments showed that HNE, at physiological concentrations seen in CF sputum, was sufficient to cleave and activate pro-MMP-9 and degrade TIMP-1. CF inpatient sputum demonstrated a strong correlation between HNE and MMP-9 activity. Small-molecule inhibitors of MMP-9 were active in CF sputum, reducing protease activity by ~80%. Together, our results identify markedly elevated MMP-9 activity in CF sputum and describe two potential mechanisms that contribute to this dysregulation, which are mediated by HNE.

HNE Activates MMP-9

In vivo mixing experiments with purified MMP-9 zymogen and HNE demonstrated that HNE treatment was sufficient to activate MMP-9, converting proenzyme to active protease. This effect was specific, as parallel experiments with pro-MMP-8 showed no activating effects via HNE. HNE targets a variety of substrates and has also been shown to have activating effects on MMP-2 and cathepsin B (4, 25). Previous work by Ferry et al. (13) has suggested that HNE, a serine protease well known to contribute to CF lung inflammation and damage (3, 11, 14), can enhance MMP-9 activity, both in vitro and in a murine model. In that study, activation of MMP-9 was performed in a murine model of acute lung injury (following LPS administration), demonstrating an increased 75-kDa gelatinolytic band. Additionally, the 92-kDa proenzyme was depleted relative to the 78-kDa active enzyme (open arrowhead) over the HNE concentrations of 2.5–10 ng/ml.
mice. This study also demonstrated conversion of recombinant pro-MMP-9 to active enzyme with HNE incubation. Our studies extend these observations by examining the effects of various physiological concentrations of HNE found in CF sputum on the conversion of pro-MMP-9 to active protease. Additionally, we utilized an HNE inhibitor to block activation of pro-MMP-9 by HNE, further demonstrating specificity of HNE effects for MMP-9. We then examined HNE and MMP-9 activity in CF inpatient samples, reporting a strong, positive correlation between the two proteases in vivo. Together, the results provide strong support for the hypothesis that increased HNE activity in CF sputum can disrupt MMP-9 regulation via proenzyme activation.

**HNE Degrades TIMP-1**

TIMP-1 is a 184-residue protein containing two N-glycosylated sites (5) and five disulfide linkages (10). Previous work by Nagase suggests that TIMP-1 is degraded by HNE. In these studies, the degraded TIMP-1 was unable to inhibit active MMP-3 in a dose-dependent manner (17, 22). Additionally, Shapiro et al. (30) have shown in chronic, smoke-exposed mice that HNE can cleave and inactivate TIMP-1 and that MMP-12 can cleave and inactivate α1-antitrypsin, the natural inhibitor of HNE in vivo.

Our findings demonstrate a similar pattern of TIMP degradation by HNE. Based on known HNE substrate specificity, at least six potential cleavage sites can be mapped in TIMP-1. Although the crystal structure of the MMP-9/TIMP-1 complex has not been fully characterized, it has been postulated that the interaction is similar to that of MMP-3/TIMP-1 (1).

**Clinical Implications of Dysregulated MMP-9 Activity**

Previous work has led to the protease/antiprotease model of disease, in which antiprotease inactivation by proteases generates a pro-proteolytic environment seen in chronic inflammatory lung diseases (30, 34). One important observation from our study was the increased protease activity (both total and constitutive) measured in CF inpatients compared with CF outpatients (Figs. 1–3), with little change in MMP-9 activity seen over the course of standard inpatient pulmonary care. These results suggest that, during periods of acute pulmonary decline, MMP-9 dysregulation is accentuated, possibly due to...
increased HNE activity. We speculate that this excessive proteolytic activity during inpatient exacerbations may contribute to an augmentation of structural lung damage and airway remodeling in CF patients. Additionally, MMP-9 activity failed to decline in association with treatments shown to reduce other inflammatory markers in the CF airway (23). One could speculate, therefore, that constitutive MMP-9 activity may serve a proinflammatory role in CF. MMP-9 can clip the amino terminus from IL-8, creating a peptide product with chemotactic potency that is enhanced approximately 10-fold above the parent molecule (37). More recently, our group has characterized a novel collagen-derived peptide in chronic obstructive pulmonary disease and other diseases of the airways, proline-glycine-proline, that has prominent neutrophil chemotaxis activity (38). Based on the substrate specificity of MMP-9, it is plausible that high levels of active enzyme may play a proinflammatory role in CF and other neutrophilic lung diseases through cleavage of structural proteins, leading to a variety of neutrophil chemotactic molecules. A model of this hypothesis is summarized in Fig. 8. Clearly, more work is needed to clarify the importance of MMP-9 activity in CF airway disease, as high levels of unregulated enzyme activity have the potential to increase inflammatory signaling, neutrophil load, and to accelerate bronchiectasis.

While this study examined important proteases, which are present in CF lung disease, we acknowledge limitations of our study. First, there were differences in the study populations. The CF samples were from significantly older subjects compared with the ARF population; additionally, the CF inpatients had an increased proportion of delta F508 homozygous individuals compared with CF outpatients [although no evidence of milder disease (e.g., pancreatic sufficiency) was observed in either group]. Second, the samples collected in CF patients were spontaneously expectorated compared with samples from ARF and control patients, which were obtained through endotracheal suctioning. In addition, the ARF samples were pooled over a 5-day period for a given patient, as opposed to CF and control samples, which were obtained at single time points. While extensive qualitative bacteriology was available for the CF samples, quantitative cultures were not performed. Finally, while our sputum processing ensured that TIMPs are maintained in the supernatant, it is possible that other nonspecific MMP inhibitors may have been depleted during processing. Each of these potential confounders could influence the protease measurements of the airway and should be considered in future studies of MMP biology and pathophysiology in CF airway disease.

In summary, our studies demonstrate a unique profile of MMPs expressed in CF sputum. MMP-9 was found to be the predominant active MMP isofrom and demonstrated elevated activity compared with its antiprotease, TIMP-1. HNE may disrupt normal regulation of MMP-9 through direct activation of pro-MMP-9zymogen and inactivation of TIMP-1 in CF airway disease. Restoration of the protease/antiprotease balance in chronic CF airway inflammation may represent a viable strategy to reduce the excessive inflammatory phenotype and potentially impact tissue remodeling and destruction produced by unopposed MMP-9 proteolytic activity.
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