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SHORT REPORT

Targeting Airway Immunity in Lung Disease

A novel DNase assay reveals low DNase activity in severe asthma

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Abstract

Secreted deoxyribonucleases (DNases), such as DNase-I and DNase-IL3, degrade extracellular DNA, and endogenous DNases have roles in resolving airway inflammation and guarding against autoimmune responses to nucleotides. Subsets of patients with asthma have high airway DNA levels, but information about DNase activity in health and in asthma is lacking. To characterize DNase activity in health and in asthma, we developed a novel kinetic assay using a Taqman probe sequence that is quickly cleaved by DNase-I to produce a large product signal. We used this kinetic assay to measure DNase activity in sputum from participants in the Severe Asthma Research Program (SARP)-3 ($n = 439$) and from healthy controls ($n = 89$). We found that DNase activity was lower than normal in asthma [78.7 relative fluorescence units (RFU)/min vs. 120.4 RFU/min, $P < 0.0001$]. Compared to patients with asthma with sputum DNase activity in the upper tertile activity levels, those in the lower tertile of sputum DNase activity were characterized clinically by more severe disease and pathologically by airway eosinophilia and airway mucus plugging. Carbamylation of DNase-I, a post-translational modification that can be mediated by eosinophil peroxidase, inactivated DNase-I. In summary, a Taqman probe-based DNase activity assay uncovers low DNase activity in the asthma airway that is associated with more severe disease and airway mucus plugging and may be caused, at least in part, by eosinophil-mediated carbamylation.

NEW & NOTEWORTHY We developed a new DNase assay and used it to show that DNase activity is impaired in asthma airways.

asthma; carbamylation; deoxyribonuclease; DNase; eosinophil

INTRODUCTION

The secreted deoxyribonucleases (DNases) (DNase-I and DNase-IL3) are blood endonucleases responsible for degradation of extracellular DNA (eDNA) that is a component of neutrophil extracellular traps (NETs) or that is released by apoptotic and necrotic cells (1). DNase-I preferentially cleaves protein-free DNA and is inhibited by G-actin, whereas DNase-IL3 cleaves DNA-protein complexes and is not inhibited by G-actin (2–7). The activity of both DNases is dependent on the presence of calcium and magnesium (5–8). Degradation of intravascular DNA released after cellular disruption prevents formation of clots that obstruct blood vessels and cause organ damage (1, 2, 9, 10). Loss of function of DNase-I is associated with systemic lupus erythematosus (SLE), a disorder caused by disturbed clearance of nuclear DNA-protein complexes after cell death (9–11).

DNases also have roles in airway biology. For example, in cystic fibrosis (CF), eDNA levels in sputum are detectable in milligram quantities and contribute significantly to the

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abnormal biophysical properties of CF mucus (12, 13). Recombinant human DNase-I (rhDNase-I; Pulmozyme, Genentech) has clinically important mucolytic effects in patients with CF (14, 15), and has also been proposed to have anti-inflammatory effects in neutrophilic asthma (16). A case study reported that nebulized endotracheal rhDNase-I resulted in rapid improvement in a patient with asthma, who was unresponsive to all other treatments, including nebulized bronchodilators, intravenous magnesium sulfate, intravenous corticosteroids, and antibiotics (17).

Activity of DNase-I can be influenced by carbamylation (18), a post-translational modification in which protein lysine residues are converted to homocitrulline (19, 20). Prior studies of bovine pancreatic DNase-I show that completely carbamylated DNase-I is inactive (18). Carbamylation is relevant in asthma because eosinophil peroxidase uses the pseudohalide thiocyanate as substrate to form isocyanic acid that can then carbamylate lysine residues and protein N-termini (21, 22). Digestion of DNase-I by proteases may also modulate its activity, and a candidate protease for this effect is neutrophil elastase that is present in high levels in the CF airway and in a subset of patients with asthma (23, 24).

Despite the potential for DNases to regulate airway mucus gel properties or airway inflammation, there is little published literature in human subjects about DNase activity in the airways in health and disease. We set out here to develop a bioassay to quantify DNase activity in sputum and to use this assay to characterize airway DNase activity in health and in patients with asthma. Our approach to the human studies was to measure DNase-I levels in a sputum biobank from the Severe Asthma Research Program (SARP)-3, a longitudinal cohort study that is enriched in patients with severe forms of asthma (25).

MATERIALS AND METHODS

Participants

Healthy controls and adult patients with asthma had been recruited to the Severe Asthma Research Program (SARP)-3 by seven clinical research centers in the United States. Additional healthy controls had been recruited to research studies in the UCSF Airway Clinical Research Center. All subjects had provided a sample of induced sputum and aliquots of the sputum supernatant were stored in a tissue bank. A subset of subjects underwent computed tomography (CT) lung scanning, and these scans had been scored for mucus plug burden in each bronchopulmonary segment, as previously described (26). Study procedures were approved by the IRB at each institution and an independent Data Safety Monitoring Board. All subjects provided written informed consent and/or assent.

Healthy controls.

Healthy control participants had been recruited to research studies in the UCSF Airway Clinical Research Center between January 1, 2005, and December 31, 2014. All studies included one or two baseline visits, which used standardized protocols for clinical characterization and collection, processing, and storage of induced sputum. Healthy participants had no history of pulmonary disease, no history of atopic disease or allergic rhinitis, and had normal airway responses to inhaled methacholine.

Patients with asthma.

Adult patients with asthma were recruited to the Severe Asthma Research Program (SARP)-3 between November 1, 2012, and October 1, 2014, by seven clinical research centers (including UCSF) in the United States. The SARP protocol is a longitudinal cohort study in which 60% of participants have severe asthma as defined by the European Respiratory Society/American Thoracic Society (ERS/ATS) criteria (25). The SARP protocol included two baseline visits in which patients underwent detailed characterization studies and provided samples of induced sputum. The data reported here are from these two baseline visits.

Induced Sputum

Induced sputum (entire expectorate) was homogenized using methods previously described (27). Sputum induction was conducted using an ultrasonic nebulizer with 3% saline. Sputum was kept on ice or refrigerated and processed within 1–4 h from collection. Sputum was homogenized in a 10% solution of Sputolysin (EMD Millipore, Temecula, CA) and centrifuged to yield a supernatant that was aliquoted for storage at −80°C. Each center shipped two aliquots to the UCSF SARP-3 center for measurement of analytes.

Kinetic Assay for DNase Activity

In initial experiments, we evaluated whether DNase levels could be measured in sputum using a commercial assay. We found that the available assay had limitations (detailed in the Supplemental, including Supplemental Fig. S1) that precluded its application for measures of DNase in sputum. We therefore developed an assay that utilized Taqman probes (Biosearch Technologies) as reporters of DNAse activity. Taqman probes have a fluorophore (5′FAM) and a quencher (3′BHQ) on their single DNA strand, and cleavage of the probe causes it to fluoresce (Fig. 1*A*). DNase-I has sequence bias for cleavage sites (28–30), and eight probes were screened to increase the probability of identifying a probe sequence that would yield a fast reaction speed and large product signal (in relative fluorescence units; RFU). Each probe was diluted to 0.2 μM (1.7 μg/mL) in DNase buffer (50 mM Tris-HCl + 5 mM $MgCl_2$ + 5 mM $CaCl_2$, pH 7.5), and rhDNase-I (Pulmozyme, Genentech, South San Francisco, CA) was diluted to 34 nM in PBS, for a final concentration of 17 nM (1.25 U) in the assay. Fifty microliters of 0.2 μM DNA Taqman probe in DNase buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM $CaCl_2$, pH 7.5) was mixed with 50 μL of rhDNase-I to start the reaction. Fluorescence was read using a BioTek H1 Plate Reader and BioTek Gen 5 Software at abs/em 490/520 nm every minute for 90 min. The maximum slope (Max *V*) was calculated over 20 time points and reported as relative fluorescence units per minute (RFU/min). The Taqman probe selected for the assay was mixed with 50 μL of sputum supernatant diluted 2- or 10-fold in PBS with or without 10 mM EDTA (known DNase inhibitor) (31).

Testing of the commercial DNase activity assay, and additional validation of the DNase kinetic assay in sputum, using a DNA degradation assay, can be found in the Supplement.

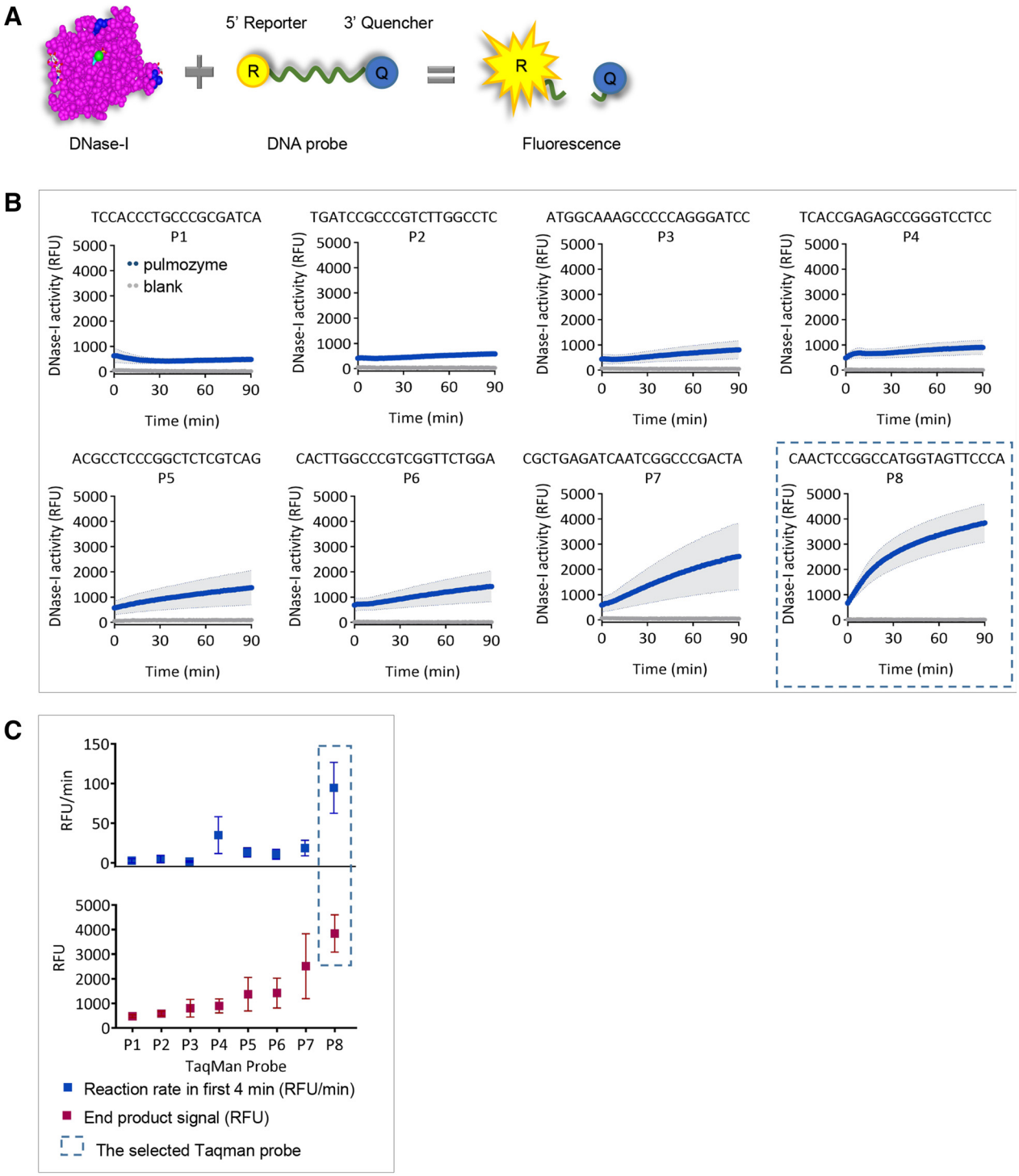

Figure 1. Kinetic assay for deoxyribonuclease (DNase) activity. *A*: schematic showing a Taqman probe with a fluorophore (5′FAM) and a quencher (3′BHQ) on its single DNA strand. Cleavage of the probe by DNase causes it to fluorescence. *B*: eight Taqman sequence probes were used as assay substrate for rhDNase, and probe cleavage was measured in relative fluorescence units (RFU) ($n = 3$ for each probe). *C*: the CAACTCCGGCCATGGTAGTTCCCA Taqman probe (P8) was chosen as the optimal probe, because it yielded the fastest reaction speed and the largest product signal ($n = 3$).

DNase Activity Assay with Magnesium Chloride

To test the effect of magnesium chloride on DNase activity in sputum, 50 μL of frozen sputum supernatant or 17 nM (1.25 U) of rhDNase-I (Pulmozyme) was mixed with 50 μL of DNA Taqman probe 0.2 μM, in three dilutions of magnesium chloride, 0, 2.5, and 10 mM in Tris-HCl 50 mM + $CaCl_2$ 2.5 mM. Fluorescence was read at abs/em 490/520 every 1 min for 90 min, and the maximum slope (Max *V*) was calculated over 20 times points and reported as RFU/min.

DNase-I ELISA

DNase-I protein levels were measured in a subset of 57 healthy and 93 asthma sputum samples using a commercially available DNase-I ELISA (Antibodies Online, Cat. No. ABIN6574255) in sputum supernatants that were diluted 10-fold. These samples were selected based on sufficient volume available following assay for DNase.

DNase-I Carbamylation

Recombinant human DNase-I of 17 μM (1250 U) was incubated with 1 M potassium cyanate (Acros Organics) at 37°C for 1, 2, 3, 4, 8, and 12 h and the reaction was stopped by running the protein three times against PBS through a centrifugal filter with a molecular weight cutoff of 10 kDa. The recovered concentrated protein was then reconstituted to its original concentration of 17 μM and stored at 4°C before performing the DNase activity assay. A sham set of noncarbamylated 17 μM DNase tubes was also processed in the same way. Carbamylation was determined by immunoblotting using a rabbit anti-carbamyl-Lysine polyclonal antibody 1:2,000 (CellBiolabs, Cat. No. STA-078).

Measures of Extracellular DNA and Eosinophil Peroxidase in Sputum

Data for these analytes were available in the SARP-3 database because of prior publications in the cohort. Specifically, the method of measurement for eDNA is detailed in the study by Lachowicz-Scroggins et al. (32) and for eosinophil peroxidase (EPX) is in the study by Dunican et al. (26).

Statistical Analysis

All statistical analyses were performed using GraphPad Prism software (v.8.4.2). Two-group comparisons were analyzed using Mann–Whitney *t* test for variables with nonparametric distributions. DNase activity in the presence of varying doses of magnesium chloride was analyzed using repeated-measures one-way ANOVA with Geisser–Greenhouse correction, followed by Tukey's post hoc analysis for multiple comparisons. DNase activity with and without carbamylation was analyzed using repeated-measures two-way ANOVA. Correlations between continuous variables were assessed using Spearman's correlation. DNase activity in asthma was grouped into tertiles and the clinical characteristics were compared using χ^2 for categorical variables, ANOVA for parametric continuous variables, and Kruskal–Wallis for nonparametric continuous variables. Data are presented as means ± SE

RESULTS

A Novel Taqman Probe-Based DNase-I Activity Assay Detects DNase Activity in Sputum

We screened eight Taqman probes with the following sequences in the DNase activity assay: *1*) ATGGCAAAGCCCCCAGGGATCC; *2*) ACGCCTCCCGGCTCTCGTCAG; *3*) CGCTGAGATCAATCGGCCCGACTA; *4*) TCACCGAGAGCCGGGTCCTCC; *5*) CACTTGGCCCGTCGGTTCTGGA; *6*) TCCACCCTGCCCGCGATCA; *7*) TGATCCGCCCGTCTTGGCCTC; *8*) CAACTCCGGCCATGGTAGTTCCCA. The CAACTCCGGCCATGGTAGTTCCCA sequence was selected as the optimal assay probe because rhDNase-I cleaved it quickly to produce the largest product signal (Fig. 1, *B* and *C*). Using this probe in the DNase assay, we measured DNase activity in sputum. In initial experiments, we found that DNase activity was measurable in sputum, dilution dependent, inhibited by EDTA, and augmented by magnesium (Fig. 2, *A* and *B*). Furthermore, when a DNA plasmid was incubated in either rhDNase-I or in sputum, the plasmid was digested (Supplemental Fig. S2).

DNase Activity Is Lower than Normal in Patients with Asthma

DNase activity was measured in induced sputum from 88 healthy subjects and 439 patients with asthma whose clinical characteristics are described in Table 1. We found significant overlap in the range of sputum DNase activity levels in health and in asthma, but the mean sputum DNase activity was significantly lower in asthma than in health (Fig. 2*C*). In contrast, although sputum DNase-I protein levels (measured in a subset of subjects) largely overlapped, mean sputum DNase-I protein levels were slightly higher in asthma than in health (Fig. 2*D*).

Pathological and Clinical Correlates of Sputum DNase Activity in Asthma

To explore the pathological and clinical traits associated with low sputum DNase activity in asthma, we first examined correlation coefficients for sputum DNase activity and sputum granulocytes. We found that sputum DNase activity was inversely correlated with sputum eosinophil percentage and eosinophil peroxidase (EPX) levels (Fig. 2, *E* and *F*) but not with neutrophil percentage (data not shown). We also found that sputum DNase activity was positively correlated with extracellular DNA (eDNA) levels in sputum (Fig. 2*G*). We next examined the correlation of sputum DNase activity and airway mucus plugging. In the subset of asthma who had undergone CT lung scanning, we found that sputum DNase activity correlated inversely and significantly with their airway mucus plug scores (Fig. 2*H*).

To explore the clinical traits associated with low sputum DNase activity in asthma, we compared the clinical features of patients with asthma in three tertiles of sputum DNase activity. Compared to patients with asthma who had sputum DNase activity levels in the highest tertile, those whose DNase activity was in the lower tertile had a higher frequency of having asthma classified as severe according to the ATS/ERS consensus definition (33), a higher frequency of use of high dose inhaled corticosteroids and oral corticosteroids, and a higher frequency of nasal polyps (Table 2). In addition, and consistent with the correlation data aforementioned, sputum and blood eosinophil levels, sputum EPX levels, and blood neutrophil levels, were significantly higher in patients in the low DNase activity subgroup, and the mucus plug scores showed a strong trend for difference (Table 2). Conversely, sputum extracellular DNA (eDNA) concentrations were higher in the high DNase activity subgroup.

Carbmamylation of DNase-I Decreases Its Activity

The finding that DNase activity in asthma sputum was low but DNase-I protein levels were not, prompted us to consider mechanisms by which DNase activity might be

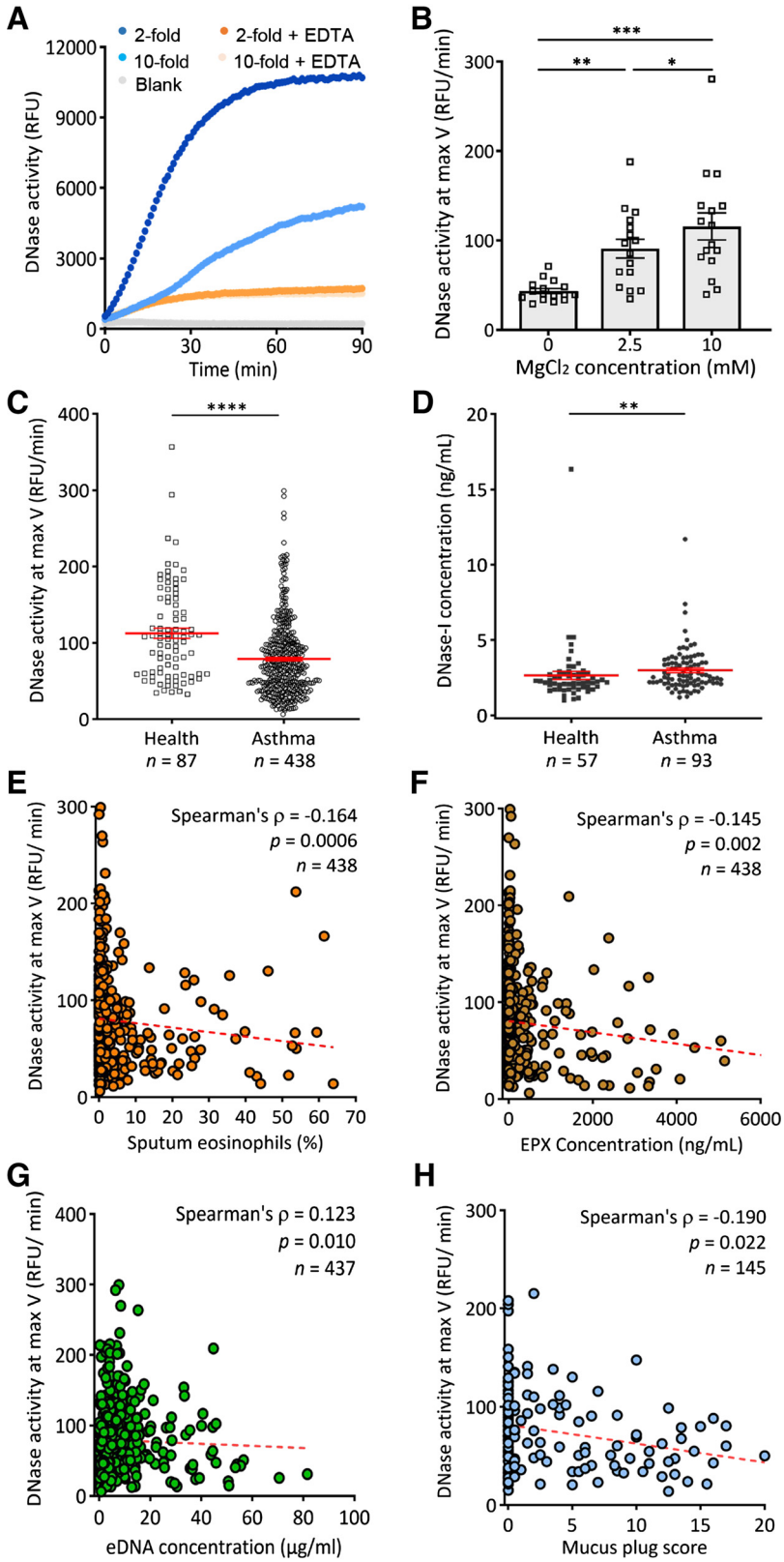

Figure 2. Performance of the Taqman-based deoxyribonucleases (DNase) assay in sputum. *A*: representative examples of DNase activity in a sputum sample from a healthy donor. Data are shown at two- and 10-fold dilutions and in the presence and absence of EDTA (a known DNase-I inhibitor). *B*: DNase-I activity in healthy sputum supernatant samples ($n = 16$) increases in the presence of $MgCl_2$. *C*: DNase activity in sputum samples from healthy donors ($n = 87$) and asthma donors ($n = 438$). *D*: DNase concentration in health ($n = 57$), and asthma ($n = 93$). *E*: DNase activity in sputum from asthma donors ($n = 438$) correlates inversely with the percentage of sputum eosinophils. *F*: DNase activity in sputum from asthma donors ($n = 438$) correlates inversely with sputum eosinophil peroxidase (EPX) levels. *G*: DNase activity in sputum from asthma donors ($n = 437$) correlates positively with extracellular DNA (eDNA) levels in sputum. *H*: DNase activity in sputum from asthma donors ($n = 145$) correlates inversely with the mucus plug score determined from radiologist evaluation of computed tomography (CT) lung scans. Data are presented as means ± SEM, and statistical significance was reported as $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; $^{****}P \leq 0.0001$.

suppressed in asthma. A clue was the significant inverse correlation of sputum DNase activity levels and sputum eosinophil percentage. Eosinophil peroxidase is the most abundant eosinophil granule protein and it can generate isocyanic acid to carbamylate lysine residues as a post-translational protein modification. Previous studies have shown that bovine DNase-I activity is decreased by carbamylation (18), and we explored if human DNase-I activity is similarly decreased by

Table 1. *Clinical and demographic features of the healthy subjects and patients with asthma*

	Healthy (n = 87)	Asthma (n = 438)
Age, mean (SD)	37.86 (12.57)	44.17 (16.42)
Female, *n* (%)	50 (56.2)	280 (63.9)
Race, *n* (%)		
White	57 (64.0)	273 (62.3)
Black	9 (10.1)	110 (25.1)
Asian	14 (15.7)	14 (3.2)
Other/more than one	9 (10.1)	41 (9.4)
Hispanic, *n* (%)	5 (5.6)	18 (4.1)
BMI, mean (SD)	25.87 (5.38)	32.01 (8.75)
FEV1 % predicted, mean (SD)	98.24 (12.06)	73.78 (19.41)
FVC % predicted, mean (SD)	99.71 (13.22)	86.62 (17.47)

BMI, body mass index; FEV1, forced expiratory volume in one second; FVC, forced vital capacity.

carbamylation. Exposure of DNase-I to potassium cyanate resulted in the formation of carbamyl-lysine (Fig. 3*A*) and loss of DNase-I activity in a time-dependent manner with complete activity loss after 4 h (Fig. 3*B*).

DISCUSSION

We developed a reliable and efficient assay for measuring DNase activity in sputum using Taqman DNA probes as the DNase substrate. In screening different Taqman probes as assay substrates, we found that rhDNase-I was more likely to cleave probes in regions of mixed sequence and less likely to cleave in regions rich in one base. This is consistent with prior reports that, although DNase-I is not sequence or base-specific, its cutting rate is sequence-dependent, so that regions of DNA consisting of runs of consecutive As, Ts, Gs, or Cs are less likely to be cleaved than regions of mixed sequence (28, 29, 34, 35). DNase-I cleaves both double- and single-stranded DNA, but is 100–500 times more active in hydrolysis of double-stranded than single-stranded DNA (36). For the DNase assay, we selected the TaqMan probe that was cleaved the fastest by DNase-I and produced the greatest end product, to optimize the assay for sensitivity to detect relatively low concentrations in sputum. This provided a sensitive assay that, as expected, was potentiated by magnesium chloride in a dose-dependent manner (37). Although we used DNase-I as a key reagent in assay development our assay was not designed to be DNase-I specific and should detect other DNase isoforms as well.

In applying the DNase assay to measure DNase activity levels in sputum in asthma and in health, we found that sputum DNase activities were significantly decreased in asthma. The clinical traits associated with low sputum DNase activity in asthma included medication requirements typical of patients with severe disease. Specifically, the DNase low subgroup was characterized by requirement for high doses of inhaled steroids and more frequent use of daily oral corticosteroids. Other clinical and pathological features also marked this DNase low asthma subgroup as severe, including more prominent airway eosinophilia and higher mucus plug scores on CT lung scans. This latter finding is intriguing because it raises the possibility that low DNase activity could be a pathophysiologic mechanism of airway mucus plugging in asthma. Because NETs increase the viscoelasticity of mucus gels (38), a loss of DNase activity in the airway could impact turnover of pathologic mucus gels and promote mucus plugging.

Although DNase activity was significantly lower in asthma than in health, the concentration of DNase-I protein was not, so that low protein levels are unlikely to explain low DNase activity in asthma. A possible mechanism of decreased

Table 2. *Characteristics of subjects with asthma stratified by tertiles of sputum DNase activity*

	DNase Tertiles			
	Tertile 1 Low DNase Activity (6.3–49.2 RFU/min)	Tertile 2 Medium DNase Activity (49.3–90.1 RFU/min)	Tertile 3 High DNase Activity (90.6–299.3 RFU/min)	*P*
n	146	146	146	
DNase-1 activity, median (IQR)	34.80 [25.75, 42.90]	69.13 [59.43, 79.44]	121.09 [100.83, 146.55]	<0.001
Age, mean (SD)	46.63 (15.58)	42.50 (16.23)	43.66 (17.34)	0.106
Female, *n* (%)	97 (66.4)	95 (65.1)	88 (60.3)	0.515
BMI, mean (SD)	30.39 (7.83)	32.40 (8.87)	33.23 (9.30)	0.017
Severe asthma (%)*	95 (65.1)	92 (63.0)	75 (51.7)	0.044
High dose ICS (%)†	97 (68.3)	95 (65.5)	79 (54.9)	0.015
Daily OCS (%)	28 (19.2)	13 (8.9)	13 (9.0)	0.009
Cumulative exacerbations over 3 yr, median (IQR)	2.00 [0.00, 4.00]	1.00 [0.00, 3.00]	1.00 [0.00, 4.00]	0.154
Pre-BD FEV1 % pred, mean (SD)	70.76 (19.89)	76.31 (18.66)	74.27 (19.39)	0.047
Pre-BD FVC % pred, mean (SD)	84.53 (17.60)	88.49 (17.00)	86.63 (17.71)	0.151
Blood eosinophils (cells/μL), median (IQR)	240.0 [130.0, 378.0]	249.5 [160.5, 440.3]	211.0 [111.5, 370.5]	0.019
Sputum eosinophils (%), median (IQR)	1.00 [0.20, 5.50]	0.75 [0.05, 3.72]	0.50 [0.00, 1.70]	0.011
Sputum EPX, median (IQR)	48.00 [0.00, 350.00]	44.00 [14.00, 147.00]	24.00 [2.00, 100.50]	0.073
Blood neutrophils (cells/μL), median (IQR)	4160.0 [3156.0, 5583.8]	4100.0 [3024.8, 5256.8]	3736.5 [2694.8, 4931.8]	0.031
Sputum neutrophils (%), median (IQR)	51.60 [33.90, 69.10]	48.30 [32.10, 71.92]	56.60 [32.35, 74.03]	0.647
Sputum eDNA, median (quartiles)	3.08 [1.52, 6.74]	4.96 [2.57, 9.95]	5.04 [2.64, 9.00]	0.004
History of nasal polyps (%)	42 (28.8)	28 (19.2)	22 (15.2)	0.014
Mucus plug score¶, median (IQR)	2.250 [0.00, 8,88]	0.50 [0.00, 8.25]	0.00 [0.00, 2.50]	0.048

DNase, deoxyribonucleases; eDNA, extracellular DNA; EPX, eosinophil peroxidase; ICS, inhaled corticosteroids; OCS, oral corticosteroids; RFU, relative fluorescence units. *Severe asthma: Asthma which requires treatment with high-dose inhaled corticosteroids plus a second controller (and/or systemic corticosteroids) or which remains uncontrolled despite this therapy; †High dose inhaled corticosteroid: ≥880 mcg fluticasone equivalents per day; ¶Mucus plug score data were available for 145 patients with asthma who had undergone CT lung scanning during their characterization visits.

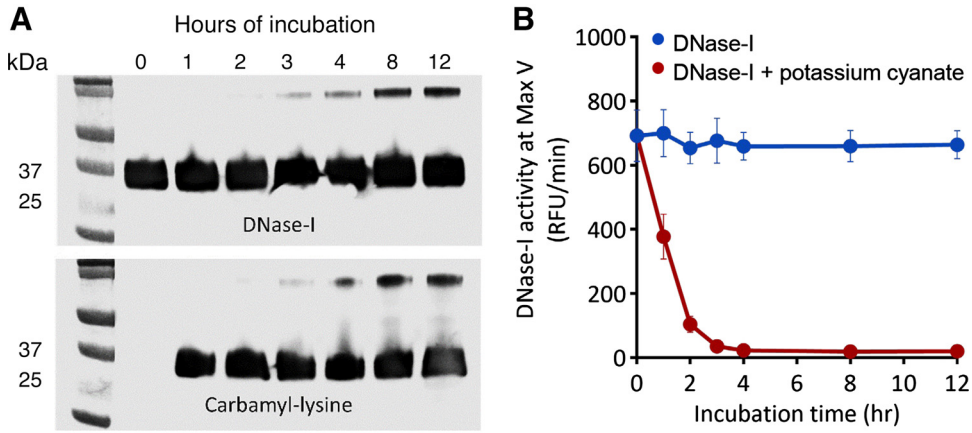

Figure 3. Effect of carbamylation or protease digestion on deoxyribonucleases (DNase)-I activity. *A:* Western blots showing that Recombinant human DNase-I (rhDNase-I) is carbamylated after 1 h incubation with 1 M potassium cyanate. Antibodies probed for DNase-I (*top*) or for carbamyl-lysine (*bottom*). *B*: measurement of the activity of DNase-I in the presence of potassium cyanate (red) and in its absence (blue), show that rhDNase-I activity decreases in a time-dependent manner with carbamylation ($n = 3$ experiments per experimental condition).

DNase activity in the airway is carbamylation. Bovine DNase-I activity is decreased by carbamylation (18) and eosinophil peroxidase-mediated carbamylation occurs during asthma exacerbation (21). Our data provide evidence that eosinophil-related carbamylation may mediate low DNAse activity in the asthma airway. First, we show that patients with low sputum DNAse activity have high sputum eosinophil percentages. Second, we show that exposure of DNase-I to potassium cyanate to form carbamylysine results in loss of enzyme activity. These data mirror prior data showing that bovine pancreatic DNase-I is susceptible to inactivation by carbamylation in a cyanate-rich environment (16). Bovine pancreatic DNase-I has one α-amino group and nine lysines and the amino acid sequences of bovine and human DNase-I share 77% identity. Bovine DNase-I requires 6 h to carbamylate seven of its 10 residues, and 12 h to be fully carbamylated and inactive (18). Human DNase-I has only six lysines and one α carbon (39), and we show here that carbamylation decreases its activity in a time-dependent manner at 1, 2, and 3 h with complete inactivation by 4 h. Carbamylation may not be the only mechanism of decreased DNase-I activity in the asthma airway. Other possible mechanisms include the presence of G-actin, a known inhibitor of DNase-I (40, 41), or the activity of proteases such as neutrophil elastase, Pr3, and cathepsin G (23, 42–44).

In conclusion, we developed a novel assay for measuring DNase activity in sputum, and used it to show that DNase activity is decreased in asthma. Patients with low DNase activity have more severe disease and are characterized pathologically by airway eosinophilia and airway mucus plugging. The mechanisms of decreased DNase-I in asthma are not revealed by our study, but we provide evidence to implicate carbamylation as one possible mechanism. Loss of airway DNase activity may impact the ability of the airway to resolve airway inflammation or turn over pathological mucus and it could promote autoimmune responses to nucleotides. Our data should prompt consideration of whether rhDNase-I treatment in patients with low DNase activity might lessen disease severity.

DATA AVAILABILITY

All data generated during this study are included in this article. The SARP-3 cohort database is available through dbGaP (https://www.ncbi.nlm.nih.gov/gap/) under the accession no. phs002788.v1.p1.

SUPPLEMENTAL MATERIAL

Supplemental Figs. S1 and S2: https://doi.org/10.6084/m9.figshare.25511536.v2.

GRANTS

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

A.R.C., W.W.R., M.C.T., and J.V.F. conceived and designed research; A.R.C. performed experiments; A.R.C., M.A.L., W.W.R., M.C.T., and J.V.F. analyzed data; A.R.C., M.C.T., and J.V.F. interpreted results of experiments; A.R.C. prepared figures; A.R.C., M.A.L., W.W.R., M.C.T., and J.V.F. drafted manuscript; A.R.C., M.A.L., W.W.R., S.A.A.C., M.W.J., A.T.H., E.R.B., M.F., M.C., K.S., S.C.E., E.I., N.N.J., D.T.M., W.C.M., S.E.W., P.G.W., B.D.L., M.C.T., and J.V.F. edited and revised manuscript; A.R.C., M.A.L., W.W.R., S.A.A.C., M.W.J., A.T.H., E.R.B., M.F., M.C., K.S., S.C.E., E.I., N.N.J., D.T.M., W.C.M., S.E.W., P.G.W., B.D.L., M.C.T., and J.V.F. approved final version of manuscript.

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