anti-tumor necrosis factor treatment. If previous *Mtb* exposure is diagnosed, chemoprophylaxis may be indicated to prevent active TB.

Regarding the research implications, the host-pathogen interaction is finely balanced, with only a small subset of *Mtb*-exposed individuals developing pulmonary disease to continue transmission (1, 4). The clinical observations that are emerging from the biologic treatment era provide entirely novel and highly relevant insights into human immune function and host-pathogen interactions. The increase in active TB after immune checkpoint inhibition suggests that excessive immunity may be just as harmful as insufficient immunity. Supporting this concept, Comstock and colleagues demonstrated in a study of 82,000 individuals that a strong response to TB antigens, which should be considered protective, actually associates with progression to active TB (8). Infection of mice deficient in PD-1 results in rapidly lethal inflammation (9, 10), consistent with a protective role in TB. Perhaps the most sobering implication is that it reinforces the finely balanced knife edge of the human-*Mtb* interaction. Simply driving an exaggerated immune response, without first defining determinants of progression versus protection in TB, risks inadvertently accelerating transmission and worsening the pandemic in the longer term (4). ■

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Mucin Concentrations and Peripheral Airway Obstruction in Chronic Obstructive Pulmonary Disease

To the Editor:

Recent studies have revealed that increased airway mucin concentrations in chronic obstructive pulmonary disease (COPD) slow mucociliary clearance and produce mucus adhesion with mucus plug formation (1). Furthermore, data from SPIROMICS (Subpopulations and Intermediate Outcomes in COPD Study) demonstrated an association between induced sputum (IS) mucin concentrations and measures of airflow obstruction, e.g., FEV₁ and FEV₁/FVC (2). Peripheral airways are the earliest and dominant sites of airflow obstruction in COPD, and Hogg and colleagues demonstrated that intraluminal mucus plugs contribute a major component to peripheral airway obstruction (3). For development of targeted therapies, it is important to understand whether there are associations between mucus concentrations and peripheral airway obstruction in COPD.

We hypothesized that sputum mucin concentrations would reflect small airway mucus concentrations and hence the likelihood of small airway mucus plaques/plug-mediated obstruction. This hypothesis was based on mucus transport measurements reporting peripheral-to-central mucus transport (4) and on analyses of sputum that identified mucus plugs, which, based on their small size, may be of peripheral airway origin. Accordingly, we tested for associations between sputum mucin concentrations and characteristics with complementary spirometric and computed tomography (CT) measures of peripheral airway obstruction in the SPIROMICS cohort.

Methods

A complete description of the demographics of the SPIROMICS cohorts and methods can be found in previous publications (2, 5). For studies of mucus/peripheral airway associations, 884 subjects from whom IS was collected through SPIROMICS were analyzed. For IS analyses, total mucin concentrations were measured by gel-filtration chromatography/refractometry (6). Technicians who were blinded to

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Figure 1. Relationships between total mucin concentrations and mucus properties and peripheral airway spirometric and computed tomography indices. (A) Total mucin concentration versus $FEF_{25-75\%}$ (POST $FEF_{25-75\%}$). (B) Mucus plug density versus $FEF_{25-75\%}$. (C) Total mucin concentration versus residual volume/total lung volume (RV/TLV). (D) Sputum mucus consistency versus RV/TLV. (E) Total mucin concentration versus parametric response mapping–functional small airway disease (PRM-FSAD). (F) Sputum consistency versus PRM-FSAD. The horizontal line in the boxes represents the median, and the bottom and top of the boxes represent the 25th and 75th percentiles, respectively. The whiskers represent the upper adjacent value (75th percentile plus 1.5 times the interquartile range) and the lower adjacent value (25th percentile minus 1.5 times the interquartile range), and the dots represent outliers.

the spirometric/CT outcomes scored sputum samples based on plug density and watery versus mucoid properties. These technicians were also blinded as to airway structure and function measurements. Forced expiratory flow, midexpiratory phase ($FEF_{25-75\%}$) spirometry measurements and CT measures of peripheral airway obstruction, including measures of residual volume/total lung volume (RV/TLV) and parametric response mapping–functional small airway disease (PRM-FSAD), were obtained according to SPIROMICS protocols (5, 7). Associations were studied with ANOVA and Tukey-Kramer tests using the mucin concentration variable divided into quartiles (2). In addition, airway dimensions, stratified by airway anatomic level (8), were related to IS total mucin concentrations and mucoid characteristics.

Results

Analyses of mucin and peripheral airway spirometric parameters revealed that $FEF_{25-75\%}$ was inversely related to total mucin concentrations and mucus plug density (Figures 1A and 1B). Analyses of correlation between mucus properties and CT measures of peripheral airway function revealed that both increased total mucin values and mucoid sputum were also associated with increased RV/TLV (Figures 1C and 1D) and PRM-FSAD (Figures 1E and 1F) values.

Significant associations between total sputum mucin concentrations and airway wall dimensions were also observed (Table 1). Higher mucin concentrations were associated with greater relative airway wall thickness, as defined by the percent

wall area relative to the total airway area. This association was evident at the more peripheral subsegmental and sub-subsegmental levels, but not in the more centrally located airways. Partitioning the measure of percent wall area into absolute wall and lumen areas revealed that higher mucin concentrations were associated with thinner walls and even narrower lumens, resulting in a higher mucin–greater percent wall area association. A 1-SD-unit higher mucin concentration was associated with a 0.34-mm²-smaller subsegmental airway luminal area (3.6% narrower). Similar associations with subsegmental and sub-subsegmental airway dimensions were observed for mucoid versus watery mucus consistency (not shown).

Discussion

SPIROMICS sputum total mucin concentrations, mucus consistency, and plug density were associated with spirometric and CT measures of peripheral airway disease. A strong association was noted in the relationship between mucin concentration and a spirometrically determined measure of peripheral airflow, i.e., $FEF_{25-75\%}$. Associations with total mucin concentrations and consistency were also observed with CT measures of RV/TLV and PRM-FSAD.

Of particular relevance to the relationships between airway mucins and regional airway obstruction, analyses of mucin concentrations and airway wall dimensions revealed that the relationships were strongest in the more peripheral CT-resolved airways. Although we speculate that mucus hyperconcentration produces mucus stasis and narrowed lumens, longitudinal studies

Table 1. Airway Dimension Analyses Relative to Sputum Mucin Concentration

	Mean (SD)	Mean Difference in Airway Dimension (β) per SD Increment of Mucin Concentration			
		Unadjusted		Adjusted	
		β	P Value	β	P Value
Percent wall area					
Lobar airways	50.3 (5.4)	0.0	0.790	0.0	0.879
Segments	60.2 (5.2)	0.1	0.576	0.1	0.413
Subsegments	63.8 (4.5)	0.2	0.028	0.2	0.003
Sub-subsegments	65.8 (4.8)	0.2	0.014	0.2	0.002
Wall area, mm ²					
Lobar airways	69.2 (27.8)	-0.4	0.419	-0.3	0.434
Segments	35.1 (11.9)	-0.2	0.225	-0.2	0.228
Sub-segments	22.3 (8.7)	-0.3	0.018	-0.3	0.032
Sub-subsegments	17.2 (7.8)	-0.3	0.006	-0.2	0.053
Lumen area, mm ²					
Lobar airways	72.4 (43)	-0.1	0.900	0.1	0.895
Segments	25.1 (16.3)	-0.2	0.391	-0.2	0.372
Subsegments	13.5 (8.7)	-0.3	0.049	-0.3	0.030
Sub-subsegments	9.7 (7.0)	-0.3	0.008	-0.2	0.009

Definition of abbreviation: CT = computed tomography.

Mean differences in airway dimensions were calculated using generalized estimating equations with exchangeable covariance matrix and robust standard errors to account for multiple airway measures per participant ($N = 884$) and linear regression to adjust for covariables (age, sex, height, race/ethnicity, CT dose, CT model, and lung volume achieved at CT). Percent wall area = $100 \times$ (cross-sectional airway wall area/total cross-sectional airway area).

will be required to test the converse notion, i.e., that narrowed lumens produce mucus hyperconcentration/stasis. Nevertheless, because reductions in airway area produce squared reductions in airflow, the 1-SD-unit higher mucin concentration/0.34-mm²-smaller airway subsegmental airway luminal airway relationship suggests that the magnitude of mucus obstruction is pathophysiologically relevant.

Data from theoretical, *in vitro*, and animal model studies have established strong relationships among mucin concentrations, mucus transport rates, and, in the event of hyperconcentrated mucus, plaque/plug formation within airway lumens (1). More recently, measures of mucus adhesive interactions with airway surfaces as a function of mucus concentration have been reported, and again revealed strong mucus concentration–airways adhesion relationships (9). Analyses have been conducted that juxtapose mucus adhesive forces with the shear forces generated by peripheral airways (low flow) versus central airways (high airflow). These studies confirmed the notion that it is difficult to clear mucus adhered to small airway surfaces by cough as compared with the relative ease of disadhering and expectorating mucus from central airways (9). Thus, it appears likely that mucin concentrations in the ranges measured in these studies, coupled with minimal peripheral airway cough clearance, will produce mucus accumulation and plug formation in peripheral COPD airways.

Notably, our measurements of mucin concentrations were obtained from IS, which likely represents material expectorated from central airways. Despite mucociliary clearance studies showing peripheral-to-central mucus transport (4), we cannot

unambiguously assume that the mucins/mucus plugs collected by sputum induction reflect peripheral airway materials. Mitigating this concern is the likelihood that the increased mucin concentrations measured in IS reflects a global airway disease process that produces mucus hyperconcentration both centrally and peripherally. However, direct bronchoscopic measurements of peripheral airway mucus concentrations will be required to establish the relationship between large and small airway mucus concentrations.

Quantifying and elucidating the pathogenesis of peripheral airway disease in COPD is a key requirement for future therapeutics development and patient care. Sputum mucin concentrations may prove to be a useful biomarker in smoking subjects at risk for COPD (2) and, in view of the reported relationship between small airway mucus obstruction and early death, in COPD subjects with later disease at risk of premature death (10). ■

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Cigarette Smoking Acutely Decreases Serum Levels of the Chronic Obstructive Pulmonary Disease Biomarker sRAGE

To the Editor:

Chronic obstructive pulmonary disease (COPD) is caused by a combination of genetic susceptibility and chronic exposure to

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inhaled noxious gases such as cigarette smoke. To monitor progression and classification of this complex disease, reliable biomarkers are needed. One of the most promising biomarkers for COPD, especially reflecting the presence and progression of emphysema, is sRAGE (soluble receptor for advanced glycation end-products) (1).

The RAGE receptor is a multiligand pattern recognition receptor that is mainly expressed by type I alveolar epithelial cells. RAGE activation induces NF- κ B-mediated proinflammatory responses and is involved in alveolar tissue damage (2). RAGE signaling is inhibited by the endogenous decoy receptor sRAGE, which binds freely circulating RAGE ligands and prevents homodimerization of RAGE, which is necessary for activation. Production of sRAGE takes place by alternative splicing or proteolytic cleavage. In patients with COPD, expression of RAGE was found to be increased in lung tissue, whereas circulating concentrations of sRAGE were decreased (1, 3). The cause of the lower circulating sRAGE levels in patients with COPD is currently unknown. Furthermore, little is known about the effect of smoking on circulating levels of sRAGE. In the current study, we investigated the effect of smoking on serum sRAGE levels, which should be taken into account when investigating sRAGE as a biomarker for COPD.

Serum samples from patients with COPD (Global Initiative for Chronic Obstructive Lung Disease [GOLD] stage I, $n = 25$; GOLD stage II, $n = 12$; GOLD stage III, $n = 20$; and GOLD stage IV, $n = 16$) (ClinicalTrials.gov Identifier: NCT00850863) were used. Additionally, serum samples were obtained from light-social-smoking control subjects without airway obstruction (young: 18–40 yr, $n = 49$; old: >40 yr, $n = 26$) with age- and smoking history-matched patients with mild COPD ($n = 13$) (ClinicalTrials.gov Identifier: NCT00807469) (4). Serum samples were collected from the young and old healthy control subjects and matched patients with COPD after they went at least 2 days without smoking, and 2 hours after they smoked three cigarettes within 1 hour. Exhaled carbon monoxide was analyzed using a Micro+ Smokerlyzer at baseline to determine whether the individuals had not smoked recently, and after smoking to determine whether they had inhaled a sufficient amount of cigarette smoke. Serum sRAGE levels were measured using a simplified immunoprecipitation in 96-well ELISA format-coupled liquid chromatography mass spectrometry assay (5), a novel and fully validated method to measure sRAGE with high specificity and selectivity, and a commercially available sRAGE ELISA (Human RAGE DuoSet DY1145; R&D Systems). All study protocols were approved by the Medical Ethics Committee of the University Medical Center Groningen, Groningen, the Netherlands, and all subjects provided written informed consent. Furthermore, all clinical procedures were performed according to the standards set by the latest Declaration of Helsinki.

Patients with COPD of all stages of severity had significantly lower serum sRAGE levels than control subjects (Figure 1A). Furthermore, patients with severe COPD (GOLD stage IV) had lower circulating sRAGE levels than patients with mild (GOLD stage I) COPD. The serum sRAGE levels were independent of age, as the levels in young (<40 yr old) control subjects were similar to those observed in old (>40 yr old) control subjects. Next, we investigated the acute effect of smoking