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Measuring Airway Mucin 2 in Patients with Severe Chronic Obstructive Pulmonary Disease with Bacterial Colonization

To the Editor:

Sibila and colleagues reported an analysis of the levels of the secreted polymeric mucins MUC2, MUC5AC, and MUC5B in the sputum and bronchoalveolar lavage fluid of patients with severe chronic obstructive pulmonary disease (COPD) (1). We are writing because they found a very high level of MUC2 in both specimens, whereas most recent studies by others find little or no MUC2 in the airway. Therefore, this report raises the possibility of an error in measurement that could cause confusion in the field.

Specifically, the authors found levels of MUC2 in lavage fluid 1.3-fold higher than MUC5B and 101-fold higher than MUC5AC. Similarly, they found levels of MUC2 in sputum 4.3-fold higher than MUC5B and 77-fold higher than MUC5AC. In contrast, a quantitative comparison of mucin transcripts in mouse lungs in healthy conditions found Muc2 levels only 0.35% those of Muc5b and 10% those of Muc5ac, which is expressed in mice at a low level at baseline (2). During allergic inflammation, Muc5ac increased 43-fold, whereas Muc2 did not increase significantly. Mouse proximal airways resemble human distal rather than proximal airways, but these data suggest that Muc2 is a minor polymeric mucin in the mammalian airway if it is present at all. Supporting this, mass spectrometry analysis of horse tracheal mucus showed no Muc2 peptides, and reverse transcriptase–polymerase chain reaction of tracheal epithelium showed no Muc2 transcripts (3).

In human specimens, mass spectrometry analysis of sputum from healthy subjects and apical secretions from normal bronchial epithelial cell cultures identified MUC5AC and MUC5B but no MUC2 (4). Quantitative immunoblotting of sputum from normal subjects and patients with asthma, cystic fibrosis, or COPD using mucin-specific antisera detected MUC2 in only 6 out of 44 samples (5). In that study, the levels of MUC2 ranged from 0.2 to 2.5% of the total mucin content in the six positive samples and were obtained from one normal subject, two subjects with COPD, and three subjects with asthma.

In a subsequent study by the same group, quantitative immunoblotting of sputum specimens from 15 patients with COPD and 17 smokers without airflow obstruction showed MUC5AC and MUC5B at substantial levels in all samples, but MUC2 in only two COPD samples and one smoker sample, and only at very low levels (6). In unpublished work from the SPIROMICS (Subpopulations and Intermediate Outcome Measures in COPD Study) study, one of us (M.K.) performed a quantitative (label-free) mass spectrometry analysis of sputum samples from 20 healthy subjects and 46 subjects with severe COPD and found that the mean level of MUC5B is higher than that of MUC5AC by approximately 10-fold in healthy subjects and 3-fold in subjects with COPD, and MUC5B is higher than MUC2 by more than 1,000-fold in both groups.

In their Methods section, Sibila and colleagues state that MUC2, MUC5AC, and MUC5B were measured by validated ELISA kits from USCN Life Science, Inc., Wuhan, China, but neither this (1) nor their previous manuscript cited therein provide experimental details other than the limits of detection and that the manufacturer's instructions were followed. The authors note that "previous studies have suggested concerns about accurate measurements of MUC2, indicating that some results should be considered with caution," with which we agree. Possible causes of inaccurate measurement include cross-reacting antibodies or the degradation of mucin epitopes precluding immunodetection. The most reliable data at the protein level are probably obtained by quantitative immunoblotting or mass spectrometry, and these indicate that MUC2 is at most a minor component of airway mucus in health and in the inflammatory diseases examined to date. In contrast to these findings in mammalian airways, MUC2 is the major polymeric mucin in the intestinal tract.

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Reply

From the Authors:

We thank Dickey and colleagues for their interest in our recently published article on the secreted polymeric mucins levels in the sputum and bronchoalveolar (BAL) fluid of patients with severe chronic obstructive pulmonary disease (COPD) with and without bacterial airway colonization (1). Dickey and colleagues expressed surprise at the high levels of MUC2 in the airways of our patients with COPD, which is contrary to their previous studies (2, 3). We agree this was a surprising finding, and we discussed this extensively in the article, in which we mentioned previous studies in asthma, cystic fibrosis, and COPD that suggested higher levels of MUC5AC and MUC5B relative to MUC2 prior studies. As ours was a surprising finding in a relatively small cohort, we make clear in the article that low sample size limits the generalizability of the results and that the findings need to be confirmed in other cohorts.

The aim of our study was to compare the secreted mucin levels among colonized and noncolonized patients with COPD (study design registered at www.clinicaltrials.gov as NCT01976117) rather than to quantify different mucin levels in the COPD airway. For that purpose, we used commercial ELISA kits (USCN Life Science, Inc., Wuhan, China) previously validated by our group (4) and others (5).

Experimental details were: (*1*) 100 μl of seven standard dilutions; blank and each sample were incubated into single precoated well for 2 hours at 37°C. (*2*) Liquid was removed and 100 μl of detection reagent A were added and incubated for 1 hour at 37°C. (*3*) Detection reagent A was removed and wells were washed three times with 300 μl of 1× wash solution per time. (*4*) 100 μl of detection reagent B were added and incubated for 30 minutes at 37°C. (*5*) Detection reagent B was removed and washed five times with 300 μl of 1× wash solution per time. Then 90 μl of substrate solution was added to each well an incubated for 15 minutes at 37°C. (*6*) 50 μl of stop solution was added to each well, and optical density was immediately measured at 450 nm. To avoid degradation of mucin protein during incubation at 37°C, supernatant was supplemented with protease inhibitor cOmplete ethylenediaminetetraacetic acid–free (Roche, Indianapolis, IN) before analysis. To discard cross-reacting antibodies, three recombinant proteins, MUC2, MUC5AC, and MUC5B, supplied by the manufacturer, were used as quality control (positive/negative, respectively) on every ELISA test. This would make inaccurate measurement due to cross-reactivity of antibodies unlikely, and we made appropriate efforts to reduce the risk of degradation of mucins.

Using this methodology, MUC2 was detected in 42 BAL fluid samples and in 43 sputum samples from 45 stable patients with severe COPD (1). In addition, a positive correlation was identified among MUC2 levels from sputum and BAL samples from stable patients with COPD (1). Furthermore, sputum MUC2 levels had a direct relationship with FEV_1% predicted in COPD (1).

Although all of these findings suggest a proper mucin detection, we agree with Dickens and colleagues that some results should be considered with caution due to previously reported concerns in MUC2 measurements (6), as we stated in our limitations section. Further studies are needed to confirm these results that, in our opinion, suggested a potential association of MUC2 with bacterial colonization in chronic airway respiratory diseases.

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